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Special Issue Reprint

The Prevention, Treatment, and Complications of Diabetes Mellitus

Edited by
Manuel Aguilar-Diosdado

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The Prevention, Treatment, and Complications of Diabetes Mellitus

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Editor

Manuel Aguilar-Diosdado

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Editorial

Special Issue "The Prevention, Treatment, and Complications of Diabetes Mellitus"

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Diabetes mellitus (DM) is a world health problem of global repercussion. It is expected that, in the next 20 years, the number of patients with DM will increase to 642 million people [1]. Type 1 diabetes mellitus (T1DM) responds to a multifactorial pathogenesis essentially linked to an autoimmune aggression mediated by T cells and autoantibodies that generate a progressive loss of insulin producing cells in the pancreas [2]. On the other hand, the pathogenesis of type 2 diabetes mellitus (T2DM) is essentially linked to the development of a state of resistance to the actions of insulin [3]. Both types are the consequence of an interaction of environmental, epigenetic, and genetic factors [4]. Genetic factors promote a special susceptibility to the development of the disease and epigenetics is considered as the link between the environment and genetics, altering gene and protein expression that could affect in autoimmunity and in the vulnerability of beta cells of pancreatic islets to the external factors.

In fact, clinical practice guidelines state that good metabolic—glycemic—control contributes to the reduction of complications associated with T1DM [5] and with T2DM [6] and that the best parameter to define the degree of glycemic control is glycosylated hemoglobin (HbA1c) which provides the average blood glucose levels in the last 2–3 months [5,6]. In addition, the development of new technologies to improve the management of DM, such as continuous glucose monitoring systems (CGMS) that measure glucose in interstitial fluid, show that they are the best way to monitor glucose levels to avoid hypoglycemia and to reduce glucose excursions. In fact, different studies have shown that these devices significantly reduce the number and intensity of hypoglycemia and improve HbA1c levels in both T1DM [7] and T2DM patients receiving insulin therapy [8].

However, the main challenge to clinicians is to reduce morbidity and mortality linked to DM, since complications associated with DM progression can arise both in the short and long term of the disease evolution. Hyperglycemia is a frequent finding in both hospitalized [9] and outpatients [10] and the management of glucose levels is the target on the most clinical trials. The use of modern insulin pumps offer a great variety of possibilities to which is added the incorporation of sensors-augmented insulin-pump, thus allowing its regulation and a higher and better level of safety in the device [11]. In T2DM, the treatment with non-insulin agents [12] contributes to improve the efficiency of insulin on glycemia levels and reflects the need to find a specific and personalized therapies for the profile of each patient. Actually, there is an increasing need to identify and provide evidence about the efficiency and safety of new therapy modalities and some of them have been included in this Special Issue.

DM is a life-threatening disease that causes complications and is considered as a serious disorder that doubles the risk of premature death [13]. Some aspects related with different fields into the DM progression have also been addressed in this Special Issue, such as the obstructive sleep apnea due to hypoxia implications [14], microvascular [15] and macrovascular [16] complications, elevated low-density lipoprotein (LDL) and cholesterol [17], and different oral processes [18]. A specifically complication associated with

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gestational diabetes mellitus (GDM) is the increased risk of hypertensive disorders; in this regard, new models based on biomarker parameters allow us to detect patients with GDM and these disorders and start earlier preventive strategies [19].

Upon the improvement of DM management, the scientific knowledge of the different underlying processes is very important. The implication of molecular mechanisms that could justify an adequate treatment option or the optimal technologies for an early diagnosis have to be included into the structural organigram of the study and follow-up of DM. The potential new biochemical targets, such as IL-1 in T1DM [20] or insulin receptor in T2DM [21], involve a deep analysis of the intracellular signaling and its potential implication in the physiopathology of the disease.

The prevention of complications of DM is relevant and requires an early diagnosis together with adequate treatment and follow-up of the patient. The most effective preventive strategy to avoid and/or delay the onset and development of DM complications is early diagnosis and early intervention aimed at mitigating symptoms and reducing sequelae and costs.

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Article

Neutrophil-to-Lymphocyte Ratio Adds Valuable Information Regarding the Presence of DKA in Children with New-Onset T1DM

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Abstract: Diabetic ketoacidosis (DKA) is an acute life-threatening complication occurring mainly at the onset of type 1 diabetes mellitus. The neutrophil-to-lymphocyte ratio (NLR), a marker for systemic inflammation, has recently generated increasing interest in many chronic diseases. The aim of this cross-sectional study was to determine the value of the neutrophil-to-lymphocyte ratio (NLR) in association with DKA severity across these cases. A total of 155 children with new-onset type 1 DM from one large center were included in the study. Total and differential leukocyte counts were measured upon admission and calculation of the NLR was performed. Patients were classified into four groups: without DKA, mild, moderate, and severe DKA at disease onset. Total WBCs, neutrophils, and monocytes increased with DKA severity (p-value < 0.005), while eosinophiles displayed an inverse relationship (p-value < 0.001). Median NLR scores increased from those without ketoacidosis (1.11) to mild (1.58), moderate (3.71), and severe (5.77) ketoacidosis groups. The statistical threshold value of the NLR in predicting DKA was 1.84, with a sensitivity of 80.2% and a specificity of 80%. Study findings indicate that a higher NLR score adds valuable information regarding the presence of DKA in children with new-onset T1DM.

Keywords: new-onset T1DM; diabetic ketoacidosis; children; NLR score

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1. Introduction

Diabetic ketoacidosis (DKA) is an acute life-threatening complication occurring mainly at the onset of type 1 diabetes mellitus [1,2], with an incidence rate that spans from 13 to 80% [3–5]. Being a form of systemic inflammatory state [6,7], inflammatory markers such as blood leukocytes and C-reactive protein (CRP) play a key role in the pathogenesis [8,9]. Although complete blood counts (CBCs) are a part of the routine evaluation in diabetic patients, white blood cell (WBC) fractions did not receive significant attention from diabetes specialists in the past [10]. In recent years, however, there has been growing interest regarding the neutrophil-to-lymphocyte ratio (NLR) as a marker of systemic inflammation in cardiac diseases, neoplasms, and obesity, as well as in diabetes-related complications such as diabetic foot ulcers and retinopathy [11–14]. Against this background, our aim was to study the association between the NLR and DKA severity among children with new-onset T1DM.

2. Materials and Methods

2.1. Patient Recruitment

This cross-sectional study included data from one of the largest Romanian reference centers for pediatric T1DM. We reviewed 181 consecutive T1DM patients charts from the

Pediatric Emergency Hospital "Louis Turcanu" in Timisoara, Romania, between 1 January 2015 to 30 June 2022, in accordance with the principles of the Declaration of Helsinki (1975, revised in 2013). Ethical approval was obtained from the ethics committee.

Inclusion criteria for cases were noted as new-onset T1DM in children aged 0 to 18 years, with or without diabetic ketoacidosis. Diagnosis of type 1 DM was established according to the American Diabetes Association (ADA) criteria of 2021. Exclusion criteria were as follows: infectious states, any other medical conditions that could alter hematological parameters, and patients with other types of diabetes.

Patients with DKA had a plasma glucose level > 11 mmol/L, a urine ketone level defined as moderate to high (+ to +++), and an arterial pH value < 7.30 at the time of admission. The ADA (American Diabetes Association) criteria for DKA severity were used: mild DKA, $7.20 \leq pH < 7.30$; moderate DKA, $7.10 \leq pH < 7.20$; and severe DKA, pH < 7.10 [15].

2.2. Biochemical Assays

Laboratory tests, including routine biochemistry tests and arterial gas analysis, were performed in the hospital laboratory. Blood samples were drawn at admission before the initial therapy, to avoid posttreatment changes in CBC parameters, and collected for differential WBC counts in tubes with EDTA and processed using a Sysmex XN-550 (Sysmex Corporation, Kobe, Japan) automatic blood counting system. Glycated Hb (HbA1c) was measured using a high-performance liquid chromatography kit supplied by Cobas E 411–Roche, Japan. Peptide C was evaluated using automated chemiluminescent assay (Cobas E 411–Roche, Tokyo, Japan). Neutrophil-to-lymphocyte ratios (NLR) were calculated.

2.3. Statistical Analysis

All data analysis was performed using the standard computer program Statistical Package for the Social Sciences (SPSS) for Windows, version 28 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism9. The Shapiro–Wilk test was used to test the normality of the data distribution. Normally distributed variables were expressed as mean $\pm$ standard deviation (SD) and non-normally distributed variables were expressed as medians with interquartile ranges. Intergroup comparisons were performed by using an independent-sample t test and one-way ANOVA for normally distributed continuous data and Chi-Square tests for categorical variables. Non-normally distributed data were compared among multiple groups using the Kruskal–Wallis test. GraphPad Prism version 9 was used for univariate analysis with a post hoc procedure regarding NLR scores in DKA patients. Multiple regression analysis was performed to evaluate the association between the NLR or WBC parameters and the occurrence of DKA in T1DM patients. Receiver operating characteristic (ROC) curve analysis was plotted to compare the discrimination performance of HbA1c, C peptide, and CBC parameters in predicting DKA severity. The optimal threshold values were obtained using Youden's index (sensitivity + specificity $-$ 1, ranging from 0 to 1) and the maximized area under the curve (AUC). A p value (two-tailed) < 0.05 was considered statistically significant.

3. Results

3.1. Patient Characteristics Stratified by DKA Grade

Following the retrospective revision of T1DM electronic charts, there were 181 newly diagnosticated children. We excluded 26 patients due to concomitant acute infections. The study group included 155 children (76 males, 79 females), with a mean age of 9.00 ± 4.39 years (range 0–18 years).

According to the onset characteristics, fasting blood glucose, islet autoantibodies, serum C-peptide, ketone bodies, and blood gas analysis results [16], children with new-onset T1DM were divided into four groups: the non-DKA (n = 35), mild DKA (n = 25), moderate DKA (n = 33), and severe DKA (n = 62) group.

There were no significant differences in terms of age among the four groups. Regarding gender, there were more female patients in the severe DKA group. HbA1c levels were approximately equal in the four groups (mean = 11.40 ± 2.01).

3.2. Differential WBC Counts

As shown in Table 1, there was a significant difference in the total and differential WBC counts regarding the four groups, especially regarding total WBCs, neutrophils, and monocytes which increased with DKA severity ($p < 0.0005$). Eosinophiles displayed an inverse relationship to DKA severity (p-value < 0.001), decreasing with DKA severity. Lymphocytes were statistically lower in severe DKA patients compared to those with mild and moderate DKA.

Table 1. Demographic data and laboratory findings of all patients.

Parameters	Non-DKA (n = 35)	Mild DKA (n = 25)	Moderate DKA (n = 33)	Severe DKA (n = 62)	p
Age (years)	10 (5–13)	9 (6.5–13)	7.00 (3.50–11)	9.00 (5–12)	0.381
Males% (n)	42 (15)	76 (19)	48 (16)	41 (26) [b]	**0.028**
HbA1c (%)	11.37 ± 1.95	11.68 ± 1.94	11.37 ± 2.16	11.52 ± 2.04	0.931
C-peptide (ng/mL)	0.639 (0.41–0.94)	0.481 (0.35–0.67)	0.533 (0.29–0.77)	0.330 (0.18–0.47) [a, c]	**<0.001**
Blood pH	7.36 (7.34–7.37)	7.28 (7.23–7.29)	7.17 (7.13–7.20) [a]	6.97 (6.89–7.03) [a, b, c]	**0.000**
WBCs ($\times 10^3/mm^3$)	8.53 (6.64–10.13)	8.12 (6.68–8.90)	12.27 (9.92–15.47) [a, b]	18.78 (14.06–24.52) [a, b, c]	**<0.001**
Neutrophils ($\times 10^3/mm^3$)	3.79 (2.99–5.24)	4.58 (3.38–5.21)	8.97 (6.24–12.6) [a, b]	14.63 (11.06–18) [a, b, c]	**0.000**
Lymphocytes ($\times 10^3/mm^3$)	2.92 (2.50–4.66)	2.71 (1.96–3.49)	2.86 (2.04–3.96)	2.33 (1.59–3.2) [a]	**0.003**
Thrombocytes ($\times 10^3/mm^3$)	299 (229–327)	259 (223–344)	347 (283–405) [a]	342 (388–422) [a, b]	**<0.001**
Monocytes ($\times 10^3/mm^3$)	0.60 (0.49–0.74)	0.68 (0.51–0.77)	0.87 (0.70–1.39) [a]	1.71 (1.15–2.40) [a, b, c]	**<0.001**
Eosinophiles ($\times 10^3/mm^3$)	0.12 (0.05–0.22)	0.09 (0.03–0.13)	0.06 (0.02–0.20)	0.00 (0–0.02) [a, b]	**<0.001**
NLR	1.11 (0.80–1.80)	1.58 (1.17–1.93)	3.71 (1.98–4.85) [a, b]	5.77 (4.04–9.63) [a, b, c]	**0.000**

One-way ANOVA, Kruskal–Wallis H-test, and Chi-Square test. Data are expressed as mean ± standard deviation, median (interquartile range, IQR) or percentage (n, %). ICU, Intensive Care Unit; HbA1c, glycated hemoglobin; WBC, white blood cell count; NLR, neutrophil-to-lymphocyte ratio. Statistically significant differences, with a probability value of $p < 0.05$, are represented in bold. Compared with the non-DKA group, [a] $p < 0.05$. Compared with the mild DKA group, [b] $p < 0.05$. Compared with the moderate DKA group, [c] $p < 0.05$.

3.3. NLR Score

A Kruskal–Wallis H test was performed to determine if there were significant differences in NLR scores between children without ketoacidosis and those with mild, moderate, or severe ketoacidosis. The distributions of NLR scores were not similar for all groups, as assessed by visual inspection of a boxplot. Median NLR scores increased from those without ketoacidosis (1.11) to mild (1.58), moderate (3.71), and severe (5.77) ketoacidosis groups (Figure 1). The distributions of NLR scores were significantly different between groups: $X^2(3) = 97.681$, $p = 0.000$. Subsequently, multiple comparisons were performed through post hoc analysis using Dunn's (1964) procedure with a Bonferroni correction. Adjusted p-values are presented. This post hoc analysis revealed statistically significant differences in median NLR scores between those with severe DKA and those with moder-

ate (p = 0.002), mild (p = 0.000), or no DKA (p = 0.000); between mild and moderate DKA (p = 0.012), but not between those with mild DKA and no DKA (p = 1.000).

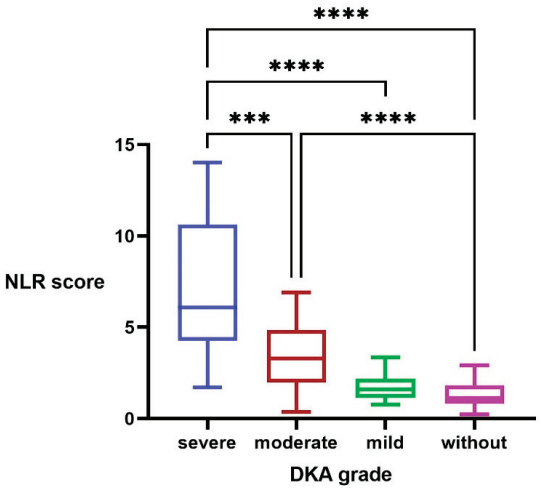

Figure 1. Univariate analysis with post hoc procedure regarding NLR scores in DKA patients; **** p = 0.000, *** p = 0.002.

3.4. Correlation and Regression Analyses

A multiple regression analysis was performed to determine the correlation between blood pH and age, gender, HbA1c, C peptide, and NLR. The multiple regression model was statistically associated with blood pH: $F(5, 130) = 41.485$, $p < 0.001$, adj. $R^2 = 0.600$. NLR score and age added significantly to the association, $p < 0.001$. Regression coefficients and standard errors are listed in Table 2.

Table 2. Linear regression analysis of factors related to blood pH in new-onset T1DM patients.

pH	B	95% CI for B		SE B	ß	R^2	ΔR^2
		LL	UL				
Model						0.654	0.640
Age	0.010 ***	0.004	0.015	0.003	0.239 ***		
Gender	0.003	−0.036	0.042	0.020	0.007		
HbA1c	0.001	−0.009	0.011	0.005	0.008		
C peptide	0.074 *	−0.014	0.135	0.030	0.145 *		
NLR	−0.038 ***	−0.044	−0.032	0.003	−0.770 ***		

B = unstandardized regression coefficient; CI = confidence interval; LL = lower limit; UL = upper limit; SE B = standard error of the coefficient; ß = standardized coefficient; R^2 = coefficient of determination; ΔR^2 = adjusted R^2. * p< 0.05, *** p < 0.001.

3.5. Receiver Operating Characteristics (ROC) Curve Analysis

The diagnostic ability of HbA1c, C peptide, WBCs, monocytes, and NLR in predicting DKA was analyzed by the ROC curve (Figures 2 and 3). The AUCs and cut-off values were calculated according to their specificity and sensitivity as predictive factors. The most influential indicators for DKA patients were WBCs (AUC 0.800; 95% CI: 0.723–0.877, p < 0.000), monocytes (AUC 0.815; 95% CI: 0.742–0.887, p < 0.000), NLR (AUC = 0.903; 95% CI: 0.854–0.952, p < 0.000), and, to a lesser extent, C peptide (AUC = 0.690; 95% CI: 0.591–0.789, p = 0.001), as opposed to HbA1c (Table 2).

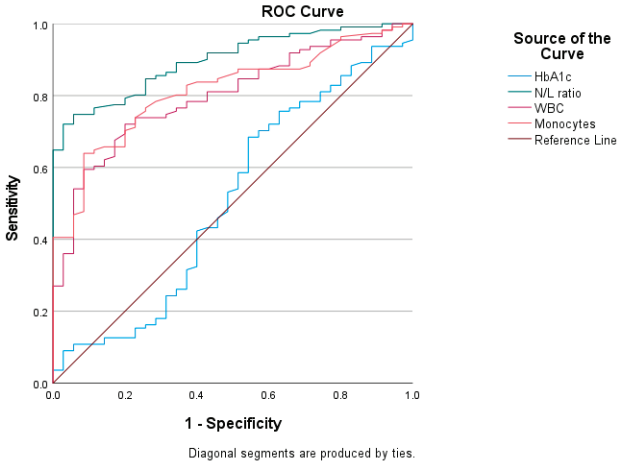

Figure 2. ROC curve analysis of HbA1c, WBCs, monocytes, and NLR; ROC, receiver operating characteristic. Significant differences were found ($p < 0.000$, respectively, for NLR, WBCs, and monocytes).

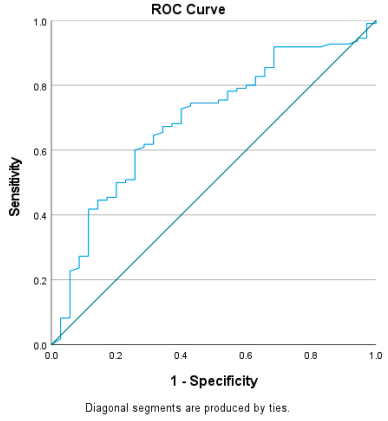

Figure 3. ROC curve analysis of C peptide; ROC, receiver operating characteristic.

The statistical threshold value of the NLR in predicting DKA was 1.84, with a sensitivity of 80.2% and a specificity of 80% (Table 3).

Table 3. ROC curve area and cut-off Values for predicting DKA. AUC = area under the curve; S.E. = standard error; CI = confidence interval.

Variable	AUC	S.E.	95% CI	Cut-Off	Sensitivity %	Specificity %
HbA1c	0.504	0.060	0.386–0.622	11.38	49.5	51.4
C peptide	0.690	0.050	0.591–0.789	0.554	68.2	60.0
WBC	0.800	0.039	0.723–0.877	8.860	79.2	57.1
Monocytes	0.815	0.037	0.742–0.887	0.675	80.2	62.9
NLR	0.903	0.051	0.854–0.952	1.84	80.2	80.0

4. Discussion

Type 1 diabetes mellitus (T1DM) represents one of the most frequent chronic illnesses affecting children [17]. Previous studies [16–23] have indicated an increase in both the frequency and severity of DKA cases in recent years. In our research, 81% of cases with T1DM presented with DKA, almost half of which were severe.

WBC counts, fractions, and indices, among which the NLR has received attention in recent years, were correlated with inflammation-associated diseases such as systemic hypertension [24], intracranial atherosclerosis [25], neoplasia [26], obesity [14], and type 2 diabetes [27–29].

The shifts in the percentage formula of white blood cells (increase in total WBCs, neutrophils, and monocytes; decrease in lymphocytes and eosinophiles) were similar to those cited in the literature [17,30].

Aside from systemic inflammation [31–33], the NLR, a well-characterized systemic inflammatory response marker [34], can also reflect both innate and adaptive immune (dys)function [9,35,36]. This simple ratio, which combines the predictive power of both increased neutrophil and decreased lymphocyte counts, has the advantage of being ubiquitous, cost effective, and also more stable compared with the absolute count [9,30,37]. Results from the present study are consistent with previous publications [10,30,38], in that WBC count and the NLR were found to be higher in patients with DKA.

Median NLR scores in our case were significantly different between groups, increasing from those without ketoacidosis (1.11; 0.80–1.80) to mild (1.58; 1.17–1.93), moderate (3.71; 1.98–4.85), and severe (5.77; 4.04–9.63) ketoacidosis groups. Our results regarding pediatric patients are consistent with a previous study addressing adults with DKA, which regards the NLR as a possible marker of the underlying severity of acute systemic inflammation in uninfected DKA patients [6]. Aside from the obvious effect of hemoconcentration on the NLR, the potential relationship between hyperglycemia and an increased NLR has been addressed in previous studies [39]. One possible explanation is that WBCs that are activated by advanced glycation end-products produce pro-inflammatory cytokines [29]. However, our study did not reveal statistical differences among the four groups in terms of mean HbA1c levels. This is consistent with some studies regarding children with DKA [40–42], and in opposition with other studies [17]. Another explanation is the fact that, in DKA, acute hyperglycemia promotes the accumulation of reactive oxygen species (ROS) which can damage peripheral blood lymphocytes' DNA. This in turn may cause the apoptosis of lymphocytes and affect their proliferation [6,43,44].

In the present study, with new-onset T1DM children grouped according to blood pH, multivariate logistic regression analysis was performed in order to assess whether confounding exists between age, sex, HbA1c, C peptide, and NLR regarding blood pH. The NLR displayed a good discriminatory power regarding association with DKA, through correlation with blood pH.; age at onset, and, to a lesser extent, C peptide added statistically significantly to the prediction. This is consistent with a previous published study regarding adult T1DM patients [10] but, to our knowledge, was not yet reported in children. An upside to examining children is their lack of many confounding factors that can affect NLR levels, such as common medications and comorbidities present in adult patients with diabetes.

Assessing the ROC curve, the presence of DKA in our study lot was associated with an elevated NLR, monocytes, and WBCs. The area under the curve was largest for the NLR, with values above 1.84 being most frequently present in children with DKA (sensitivity of 80.2% and specificity of 80%). Regarding C peptide, plasma values were negatively correlated with the presence of DKA, mainly values below 0.690 ng/mL (sensitivity of 68.2% and specificity of 60%).

There were some limitations in the present study. Firstly, the sample size was relatively small, which could limit the power of the analyses. Secondly, our patients are only from one hospital, so that selection bias cannot be ruled out. Additionally, only one measurement of CBC and subsequent NLR calculation were used in the analysis: those upon admission.

As such, there was no monitoring of the dynamic trend of the NLR. We look forward to additional multicenter studies with large samples.

5. Conclusions

This study adds complementary laboratory data regarding children with DKA at onset of T1DM [10,45]. It underlines the fact that higher NLR levels were associated with an increased prevalence of DKA in children with new-onset T1DM, and positively correlated with the DKA grade.

To the authors' knowledge, it represents the first study to evaluate the NLR based on DKA severity in children with new-onset T1DM. This finding has clinical significance, especially in pre-hospital settings, where blood gas analysis is usually not part of routine investigations, because it may improve the early diagnosis of DKA in children with elevated glucose level and thereby facilitate proper care.

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Increased Presentation of Diabetic Ketoacidosis and Changes in Age and Month of Type 1 Diabetes at Onset during the COVID-19 Pandemic in Spain

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Abstract: Objective: To assess the impact of the COVID-19 pandemic and lockdown measures on the presenting characteristics (age at diagnosis, severity, monthly distribution) of newly diagnosed type 1 diabetes in Spanish children. **Research Design and Methods**: An ambispective observational multicenter study was conducted in nine Spanish tertiary-level hospitals between January 2015 and March 2021. Inclusion criteria: new cases of type 1 diabetes in children (0–14 years) recording age, sex, date of diagnosis, presence of diabetic ketoacidosis (DKA) at onset, and severity of DKA. Data were compared before and during the pandemic. **Results**: We registered 1444 new cases of type 1 diabetes in children: 1085 in the pre-pandemic period (2015–2019) and 359 during the pandemic (2020–March 2021). There was a significant increase in the group aged ≤4 years in the pandemic period (chi-squared = 10.986, df 2, $p = 0.0041$). In 2020–2021, cases of DKA increased significantly by 12% (95% CI: 7.2–20.4%), with a higher percentage of moderate and severe DKA, although this increase was not significant. In 2020, there was a sharp decrease in the number of cases in March, with a progressive increase from May through November, higher than in the same months of the period 2015–2019, highlighting the increase in the number of cases in June, September, and November. The first three months of 2021 showed a different trend to that observed both in the years 2015–2019 and in 2020, with a marked increase in the number of cases. **Conclusions**: A change in monthly distribution was described, with an increase in DKA at onset of type 1 diabetes. No differences were found in severity, although there were differences in the age distribution, with an increase in the number of cases in children under 4 years of age.

Keywords: type 1 diabetes; COVID-19; diabetes onset; DKA

1. Introduction

Epidemiological studies have an important role in type 1 diabetes, since they enable estimation of the necessary resources for its management, as well as providing insight into its etiology and risk factors.

The estimated incidence of type 1 diabetes in the Spanish population under 15 years of age is 17.69 cases/100,000 inhabitants/year [1]. In Spain, incidence figures are lower in autonomous communities located in the north of the country, and higher in the south and center of the country, suggesting that the "north–south" gradient in the incidence of the disease described in Europe does not apply [2].

On 31 January 2020, the first case of coronavirus disease 2019 (COVID-19) was diagnosed in Spain [3]. From this time onwards, the incidence of the virus skyrocketed, leading to the declaration of a state of alarm and home lockdown on 14 March 2020. An association between COVID-19 and an increase in the number of hyperglycemia or diabetes cases has been suggested based on findings from different observational studies around the world. Numerous hypotheses have been proposed, although the mechanism that links them is not clearly defined [4]. Angiotensin-converting enzyme receptor 2 (ACE2) is the binding site for SARS-CoV-1 and -2 and is strongly expressed in pancreatic endocrine cells. Therefore, some studies postulate that the exposure to SARS-CoV-2 contributes to the observed increase of cases by precipitating or accelerating the onset of type 1 diabetes [5,6]. Another suggested mechanism would be the direct action of COVID-19 on pancreatic beta cells, by increasing proinflammatory cytokines and acute phase reactants leading to inflammation and direct cell damage.

A number of studies examining the presentation of new cases of type 1 diabetes in children and adolescents after the declaration of the pandemic, reported an increase in the frequency severity of diabetes ketoacidosis (DKA) presentation [6–12]. On the other hand, some studies have described an increase in the frequency of new case presentations [6,10–16], while others reported no increase or even a decrease in the number of type 1 diabetes [9,17].

The change in the frequency and severity in type 1 diabetes forms presentation after the emergence of the pandemic, raises the hypothesis that variations in the circulation of seasonal viruses, which could have been acting as triggers for onset or the delay in diagnosis due to individuals postponing consultation because of the fear of infection, may have contributed to this new scenario [9].

The aim of the present study was to assess the impact of the COVID-19 pandemic and the resulting lockdown measures on the presenting characteristics of newly diagnosed type 1 diabetes in Spanish children with respect to age at diagnosis, severity, and monthly distribution.

2. Research Design and Methods

This was a multicenter, observational, ambispective study conducted in nine Spanish tertiary-level hospitals between January 2015 and February 2021. We included all new diagnoses of type 1 diabetes in the pediatric population (0–14 years) in each of the participating centers. Age, sex, date of diagnosis, presence of DKA at onset, and severity of DKA (blood pH, serum bicarbonate) were recorded. Data were collected in two periods and compared before the pandemic (2015–2019) and during the pandemic (2020–2021).

The study was undertaken with the approval of the Ethics Committee of the Regional Hospital of Malaga. The diagnostic criteria for type 1 diabetes were those established by the American Diabetes Association [18]. The criteria used to define the severity of DKA followed the recommendations of the International Society for Pediatric and Adolescent Diabetes (ISPAD) [19] as follows: mild if pH < 7.3 or bicarbonate < 15 mmol/L, moderate if pH < 7.2 or bicarbonate < 10 mmol/L, and severe DKA if pH < 7.1 or bicarbonate < 5 mmol/L.

The statistical analysis was performed using R Commander Version 2.7-1 software (Chapman & Hall, Boca Raton, FL, USA). To evaluate the differences between the different study years, the chi-squared test was used, with $p < 0.05$ being considered statistically significant. The resulting data are presented in tables and figures to illustrate the distribution of the data.

3. Results

We collected data from 1444 new cases of type 1 diabetes in the pediatric age group during the period 2015–2021: 1085 in the pre-pandemic period (2015–2019) and 359 during the pandemic (2020–March 2021). Regarding the distribution of cases by age, an increase in the group aged ≤4 years was evident in the pandemic period, with a chi-squared value of 10.986 with 2 degrees of freedom and a *p*-value of 0.0041 (Table 1), indicating that the age distribution of new cases was not the same in these periods. This significant difference was at the expense of more cases (25%) in children under 4 years of age during the pandemic rather than in the pre-pandemic period (19%). The opposite occurred in the group aged 5–9 years, where there was a higher percentage in the first period (39%) rather than in the second (30%). During the period 2015–2019, 589 boys (54%) and 494 girls (46%) presented with type 1 diabetes; meanwhile, during 2020–2021, 205 boys (57%) and 154 girls (43%) did. No differences in gender distribution were found according to the period when diabetes onset occurred (X-square 0.754, df 0.38).

Table 1. Distribution of new cases by period and by age group.

	Age (Years)		
Period	**≤4 Years**	**5–9 Years**	**≥10 Years**
2015–2019	204 (19%)	424 (39%)	456 (42%)
2020–2021	88 (25%)	108 (30%)	163(45%)

During 2020–2021, the number of DKA cases increased significantly by 12% (95% CI: 7.2 to 20.4%) (Figures 1 and 2). In this period, 48% of new-onset cases presented DKA (26% mild, 38% moderate, and 36% severe), whereas during the 2015–2019 period, a lower percentage of DKA was reported, 36% (33% mild, 32% moderate, and 34% severe). A higher percentage of moderate and severe DKA cases were seen in this period, although this increase was not significant (Figure 2).

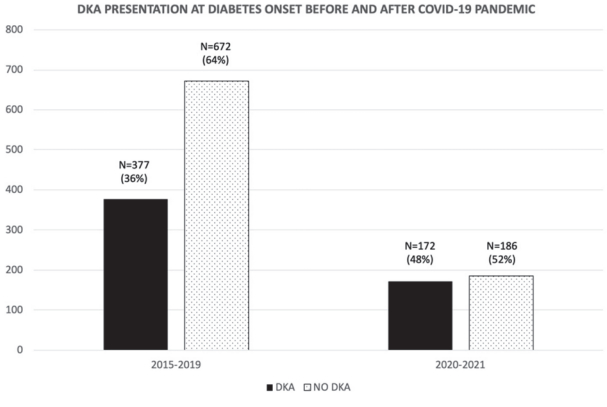

Figure 1. Percentage of DKA as a form of presentation during time periods 2015–2019 versus 2020–2021. Episodes are expressed in number and percentage (%).

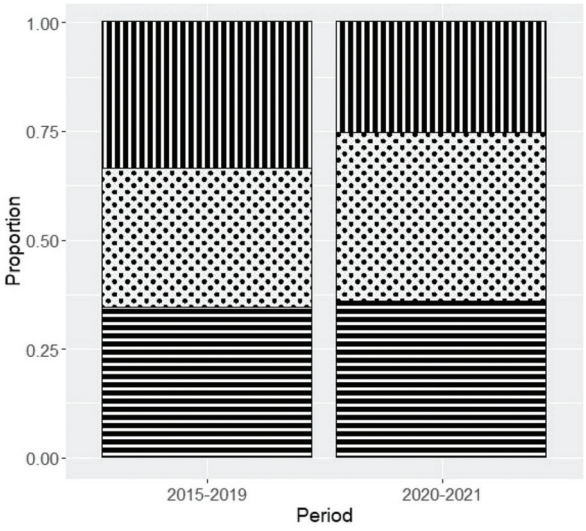

Figure 2. Proportion of cases by DKA severity during periods 2015–2019 and 2020–2021. Each square represents DKA severity cases. Squares filled with vertical black bars represent mild cases; Squares with black dots represent moderate cases. Squares filled with horizontal black bars represent severe cases.

The monthly distribution of new type 1 diabetes cases, as well as cases with DKA as a form of presentation, are shown in Figure 3. From 2015 to 2019, a dip in the number of cases from March through September was noted. In 2020, a sharp decrease in the number of cases diagnosed in March was observed, with an ulterior progressive increase from May through November, which was higher than in the same months of the period 2015–2019, with a notable increase in the number of cases during the months of June, September, and November. The first three months of 2021 showed a very different trend than the observed during the period 2015–2019 and in 2020, respectively, with a marked increase in the number of cases (Figure 3).

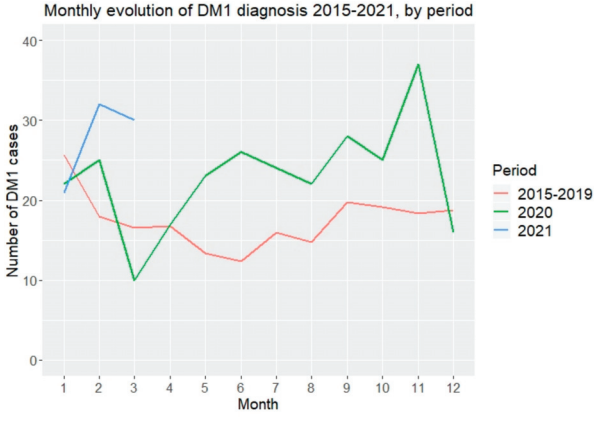

Figure 3. Monthly distribution of newly diagnosed type 1 diabetes cases by periods. Black line represents the cases by month for the period 2015–2019. Dotted line represents the cases by month during the year 2020. Dashed line represents the cases during the months of January, February, and March 2021.

4. Discussion

The COVID-19 pandemic has had a major impact on our society, generating changes that may have influenced the epidemiological situation of other diseases such as the onset of type 1 diabetes. Our study describes an increase in cases of new-onset type 1 diabetes with DKA presentation in children and adolescents under 14 years of age in the period 2020–2021, following the declaration of the COVID-19 pandemic by the WHO, compared to previous years. No differences in severity were found over the entire 2020–2021 period. This differs from observations made by other authors from Germany, Italy, Australia, and Canada during the first months after the start of the pandemic [6–13] who described an increase in moderate and severe forms of DKA. The fact that the first descriptions in this regard refer to periods immediately after lockdown may have conditioned these findings due to delayed use of health care during the beginning of the pandemic and the initial restrictions. We can speculate that this finding may lay on the fact that many patients delay or avoid visits to hospitals due to fear of getting infected with SARS-CoV-2. We can also speculate with a higher awareness of diabetes onset symptoms among primary care pediatricians from Spain that was quickly reinforced by the announcement of the International Society for Pediatric and Adolescent Diabetes (ISPAD) statement on COVID-19 and the increased risk of developing severe forms of DKA at onset [20] may have influenced this fact.

We also found significant differences in the distribution by age group, with an increase in 2020–2021 of children diagnosed under 4 years of age. A study carried out in Germany showed a significant increase in both the incidence of type 1 diabetes and severe forms of DKA, with these results being more striking in children under 6 years of age [7]. The latter leads us to consider that SARS-CoV-2 may potentially be acting as a trigger for the autoimmune destruction which accelerates the process rather than inflicting direct damage to the beta cell, as younger age groups tend to have a more rapid and intense beta cell loss [21]. In addition, we can hypothesize that a modification in the presentation pattern of other seasonal infective agents, such as syncytial respiratory virus of flu due to the pandemic, may be altering the diabetes age at diagnosis.

A study conducted by the Italian Society of Pediatric Endocrinology and Diabetes assessing the epidemiological change during the Italian lockdown (20 February to 14 April 2020), in comparison with the same period in 2019, observed a decrease in the incidence of type 1 diabetes in the confinement period, with the overall incidence being similar in both years [9]. A subsequent analysis, limited to the Lombardy region [13], which included the presentation of new-onset cases during 2020, reported an increase in the number of cases with respect to 2017 and 2018 but not 2019. An evaluation of the monthly distribution in our data shows a different distribution in both 2020 and 2021, which does not correspond to the monthly distribution of recent years in Spain. In the second quarter of 2020, coinciding with the lockdown period in Spain, there was a striking decrease in the number of cases in the months of March and April. These results are aligned with those from Germany, Italy, and Finland [9,12,17]. In contrast, there was an increase from May to November 2020. This rapid recovery of new-onset cases was both due to an interruption of the plateau of cases that usually occur during the summer months and a peak of the occurrence of new cases at the end of the year. This phenomenon has been also described more recently by Kamrath et al. in the DPV registry [22].

For the first quarter of 2021, our data show an increase in the expected number of cases in this period with respect to previous years. We cannot determine whether this increase in the actual number of cases in the first quarter of 2021 is the result of an increase in incidence or whether it is due to a change in monthly distribution as observed in previous months. We can speculate that impact that different COVID waves and associated protective measures may be having in terms of altering other seasonal infections such as flu, respiratory syncytial virus, rhinovirus, or coxsackie, would play a role in these epidemiological abnormalities observed for new diabetes cases. It is still unclear the involvement of COVID in the genesis or progression of type 1 diabetes. A recent report by the. Centers for Disease Control and Prevention (CDC) of the US reported that persons under 18 y with COVID-19 were more

likely to receive a new diabetes diagnosis >30 days after the infection than were those without COVID-19 and those with pre-pandemic acute respiratory infections [23].

A limitation of our study is that we cannot provide incidence rates. The representative sample limited to nine tertiary-level hospitals in Spain showed an increase in DKA as a form of presentation, with a higher presentation in children under 4 years of age and changes in the monthly distribution. In addition, our study collects cases only under the age of 14 years old as the majority of pediatric diabetes centers in Spain take care of patients up to this age group. This might be responsible for the differences observed in the findings of other European countries such as Italy or Germany. Further studies are needed to determine whether these epidemiological changes are directly related to SARS-CoV-2, with the modification of hygiene measures, or other factors that are not being considered at the present time.

In conclusion, a change in monthly distribution was noted, as well as an increase in DKA at onset of type 1 diabetes without differences in DKA severity during the first year after the pandemic commencement. Differences in age distribution were observed with an increase in the number of cases in children under 4 years of age. The final causes of these epidemiological changes remain unknown.

Author Contributions: I.L.-G. designed the study, collected data, participated in data interpretation, and wrote the manuscript. R.C.-H. collected data, participated in data interpretation, reviewed, and edited the manuscript. J.P.S. collected data, participated in data interpretation, reviewed the manuscript, and contributed to the statistical analysis. C.A.F., M.F.L., P.B.-C., J.A.-D., M.C.L., M.M.-F., S.C.B. and A.M.D. collected cases, participated in data interpretation, and reviewed the manuscript. All coauthors approved the final version of the manuscript. I.L.-G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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Comparison of Glycemic Variability and Hypoglycemic Events in Hospitalized Older Adults Treated with Basal Insulin plus Vildagliptin and Basal–Bolus Insulin Regimen: A Prospective Randomized Study

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Abstract: Background: The basal–bolus insulin regimen is recommended in hospitalized patients with diabetes mellitus (DM), but has an increased risk of hypoglycemia. We aimed to compare dipeptidyl peptidase 4 inhibitors (DPP4-i) and basal–bolus insulin glycemic outcomes in hospitalized type 2 DM patients. Methods and patients: Our prospective randomized study included 102 elderly T2DM patients (82 ± 9 years, HbA1c 6.6% ± 1.9). Glycemic control: A variability coefficient assessed by continuous glucose monitoring (Free Style® sensor), mean insulin dose and hypoglycemia rates obtained with the two treatments were analyzed. Results: No differences were found between groups in glycemic control (mean daily glycemia during the first 10 days: 152.6 ± 38.5 vs. 154.2 ± 26.3 mg/dL; $p = 0.8$). The total doses Kg/day were 0.40 vs. 0.20, respectively ($p < 0.001$). A lower number of hypoglycemic events (9% vs. 15%; $p < 0.04$) and lower glycemic coefficient of variation (22% vs. 28%; $p < 0.0002$) were observed in the basal–DPP4-i compared to the basal–bolus regimen group. Conclusions: Treatment of inpatient hyperglycemia with basal insulin plus DPP4-i is an effective and safe regimen in old subjects with T2DM, with a similar mean daily glucose concentration, but lower glycemic variability and fewer hypoglycemic episodes compared to the basal bolus insulin regimen.

Keywords: diabetes mellitus; vildagliptin; inpatient hyperglycaemia; older adults

1. Introduction

Hyperglycemia is a frequent finding in hospitalized patients (12.4–25%) [1]. Remarkably, more than 30% of patients with hyperglycemia detected during a hospitalization episode do not have a previous diagnosis of diabetes mellitus (DM) [2]. Hyperglycemia in hospitalized patients was consistently associated with a poor prognosis, especially in patients without a known history of diabetes [2]. A lack of adequate control of glycemia during hospitalization leads to a longer in-hospital stay, an increased incidence of infections and more hospital complications than patients without DM [1,3], which accounts for an increased necessity of healthcare resources in these patients [4,5].

Currently, different scientific societies recommend the administration of subcutaneous insulin as the treatment of choice for glycemic control in non-critical hospitalized patients

using a basal–bolus regime [4,6,7]. Treatment with oral hypoglycemic agents in this context is discouraged due to theoretical limitations regarding their effectiveness and safety. Among them, a slow onset of action and, for some oral agents, a long duration of action were found, implying an insufficient flexibility to adapt to quickly changing requirements throughout the day and to other circumstances, such as fasting requirements associated with medical therapeutic and exploratory procedures [1,8]. In addition to these potential inconveniences, there are some limitations of efficacy and safety in hospitalized patients with type 2 DM (T2DM) regarding the number of randomized studies with oral drugs, which have conditioned its full implementation as a standard of care.

Several recent randomized trials demonstrated the potential effectiveness of dipeptidyl peptidase 4 inhibitors (DPP4-i) in specific groups of hospitalized patients. DPP4-i are well-tolerated oral drugs that demonstrated their efficacy and safety in association with other oral hypoglycemic agents and/or insulin [9]. DPP4-i do not cause hypoglycemia or weight gain, nor do they present significant drug interactions, which make them a very attractive therapeutic option for the treatment of T2DM, particularly in the elderly. Among this group of antidiabetic oral agents, there is currently only one safety study in subjects older than 75 years, performed with vildagliptin to support its use in elderly patients [10].

With the aim of simplifying and facilitating the implementation of an effective and safe treatment for hospital hyperglycemia, Umpierrez et al. recently published the results of an alternative to a basal–bolus regime for patients with T2DM admitted to non-critical units (conventional hospitalization). This regime is based on the administration of a daily dose of basal insulin and a single dose of the DPP4-i sitagliptin (Sita-Hospital study) [11]. The results of the study showed a non-inferiority efficacy in relation to a full regime of insulin. Similar findings were reported in a study of non-cardiac surgical and medical patients with T2DM, in which linagliptin was used [12,13].

Glycemic variability (GV) was proposed as a novel marker of glycemic control [14,15]. A large multicenter study concluded that GV was a much stronger predictor of intensive care unit (ICU) mortality than mean glucose concentrations [16]. The coefficient of variation (CV) was also demonstrated as a strong independent index for measuring GV as it corrects for mean glucose levels [17–19].

The aim of the present study was to compare the efficacy, safety and glycemic variability outcomes of a combined basal–DPP4-i regime compared to a conventional basal–bolus insulin regimen in internal medicine patients with T2DM.

2. Materials and Methods

A prospective randomized study was set up in a tertiary university Hospital Germans Trias i Pujol, in Badalona, Spain, in which a total of 102 patients were included (Figure 1).

Patients with T2DM aged $\geq$65 years old, for whom all of the following concurrent conditions were present, were included in the study: (1) plasma glycemia on admission at less than 400 mg/dL and (2) treatment at home with any combination of oral antidiabetic drugs or with insulin therapy at a daily dose less 0.6 IU/Kg/day. Exclusion criteria were any of the following: (1) HbA1c >9%, (2) glycaemia at admission $\geq$400 mg/dL, (3) treatment with glucocorticoids (dose >5 mg/day of prednisone or equivalent), (4) previous treatment with insulin at a total daily dose $\geq$0.6 IU/Kg, and (5) any of the following active clinical situations or previous antecedents: acute myocardial infarction, acute pancreatitis, diagnosis of type 1 diabetes mellitus and/or hepatic cirrhosis. In all of the latter, the usual basal–bolus regime was administered, and the patients were not included in the study.

All participants provided written informed consent before the start of the protocol. Patients were randomly assigned to one of two treatment regimens according to the side of the room they were hospitalized in (hallway A or hallway B). The dose of insulin was started in both groups based on previous treatment of DM and capillary blood glucose (CBG) concentration at admission as indicated in Supplementary Tables S1–S3. In the basal–bolus group, half of the insulin dose was prescribed as basal insulin (glargine) once daily at bedtime and half as rapid-acting insulin (aspart) divided into three equal doses

before meals. For patients in the basal–DPP4-i group, the complete calculated dose of insulin was administered as long-acting analogue once daily at bedtime, and vildagliptin dose was calculated according to GFR: 100 mg/day if GFR ≥50 mL/min per 1.73 m^2 and 50 mg/day if GFR <50 mL/min per 1.73 m^2.

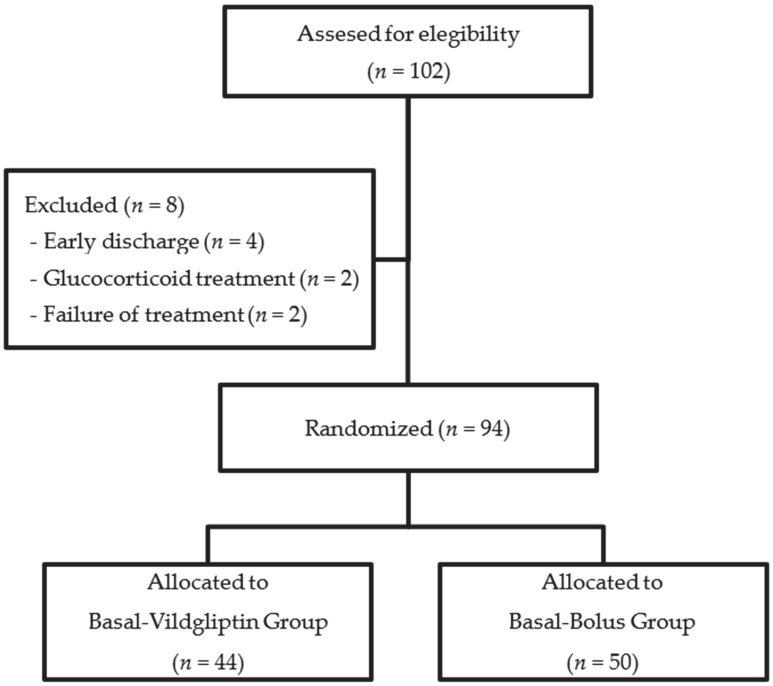

Figure 1. Flowchart of study population.

The goal of the treatment was to maintain glycemic control between 140 and 180 mg/dL. In both groups, CBG was measured before meals and bedtime. In the case of hypoglycemia (defined as grade 1 between 56 and 70 mg/dL, grade 2 < 56 mg/dL and grade 3 < 56 mg/dL plus neuroglycopenia symptoms), insulin treatment was reduced as indicated in Supplementary Tables S1 and S2. If CBG was >300 mg/dL, a correction dose of rapid-acting insulin was administered, as also indicated in Supplementary Table S1. Failure of treatment in the basal–DPP4-i group was defined as CBG > 300 mg/dL in two consecutive measures even after dose upload correction, and in these cases, the patient was switched to a basal–bolus regime.

In a subset of patients (n = 20), a FreeStyle® glycemic sensor was used to assess glycemic variability. Hypoglycemia detected by the sensor was confirmed by a CBG measurement, which was used for therapeutic decisions. The percentages of time in the various glycemic ranges were assessed according to the International Consensus on Time in Range [20] for older/high risk T2DM patients. Hyperglycemia was defined as: grade 1 (>50% of time between 181 and 250 mg/dL) and grade 2 (>10% of time >250 mg/dL). Hypoglycemia in this group was defined as a glycemic level below 70 mg/dL >1% of time. Acceptable time (time in range) in range was defined as >50% of time between 70 and 180 mg/dL.

HbA1c was measured within 24 h of randomization if the patient did not have a determination in the last 3 months. The degree of glycemic control, glycemic CV, mean insulin dose and hypoglycemia rates observed with the two therapeutic modalities were used for statistical analyses.

For the statistical analysis purpose, the first ten days of hospital stay were considered. Continuous variables are presented as mean ± standard deviations (SD). Comparisons between both therapeutic strategies were made using the Student's test for independent samples, the Fisher test or χ^2 (categorical variables). All the statistical analyses were performed with IBM SPSS Statistic software version 26 (IBM Corporation, New York, NY, USA).

3. Results

A total of 102 patients were eligible for the study. Of these, eight patients were excluded (four of them due to early discharge, two due to glucocorticoid treatment initiation after recruitment and two due to the failure of treatment). We analyzed 94 patients, 50 of which were included in the basal–bolus regime and 44 in the basal–DPP4-i regime (Figure 1).

Among all patients, the main reasons for admission were heart failure (30.8%), followed by respiratory infections (20.2%) and non-respiratory infections (16%). The causes for other patients were mostly part of geriatric syndrome and included consumptive syndrome, renal failure, confusional syndrome and falls, although none of them were significantly different regarding their frequency in both treatment arms. Those in the basal–bolus group compared to those in the basal–DPP4-i group, showed significant differences in sex (female 56% vs. 34%, $p = 0.04$, respectively) and weight (71.8 ± 16 Kg vs. 81.3 ± 18 Kg, $p = 0.008$, respectively). No significant differences were observed regarding HbA1c (6.7 ± 1.2% vs. 6.6 ± 0.9%) or any other baseline variable such as age, admission blood glucose, previous outpatient antidiabetic treatment, length of hospital stay and biochemical parameters (Table 1).

Table 1. Baseline characteristics of all the patients.

Variable	Basal–Bolus ($n = 50$)	Basal–DPP4-i ($n = 44$)	p-Value
Age (years)	78.2 ± 15	80.5 ± 7	0.35
Gender			
Male, *n* (%)	22 (44)	29 (66)	0.04 *
Female, *n* (%)	28 (56)	15 (34)	
Weight	71.8 ± 16	81.3 ± 18	0.008 *
BMI (kg/m^2)	28.53 ± 5.8	30.7 ± 6	0.05
Duration of diabetes (years)	15.4 ± 6	13.6 ± 6	0.13
Admission diabetes therapy			0.91
1 OAD, *n* (%)	18 (36)	19 (43.2)	
2 or more OAD, *n* (%)	7 (14)	12 (27.3)	
OAD + basal insulin, *n* (%)	11 (22)	8 (18.2)	
Basal insulin, *n* (%)	9 (18)	2 (4.5)	
Basal–bolus ± OAD	4 (8)	2 (4.5)	
Admission blood glucose (mg/dL)	171.9 ± 69	181.5 ± 68	0.5
HbA1c (%)	6.7% ± 1.2	6.6 ± 0.9	0.85
GFR (mL/min/1.73 m^2)	48.8 ± 24	48.1 ± 25	0.87
Length of hospital stay (days)	11.4 ± 9.3	11.9 ± 10	0.78

Abbreviations: OAD, oral antidiabetic agent; HbA1c, glycated hemoglobin, GFR, glomerular filtration rate. * Differences between groups <0.05. Data are mean ± standard deviation.

There were no statistical differences o → n any day of mean blood glucose measurements during the study period between the basal–bolus and basal–vildagliptin groups (157 ± 36.9 mg/dL vs. 145 ± 29.5 mg/dL, $p = 0.103$, respectively) (Figure 2). As expected,

the mean basal insulin dose requirements were significantly higher for the basal–bolus group, and a lower number of grade 1 hypoglycemia was observed in the basal–DPP4-i group. Regarding CV, the mean was <36% in both groups, being statistically lower in the basal–vildagliptin group (Table 2).

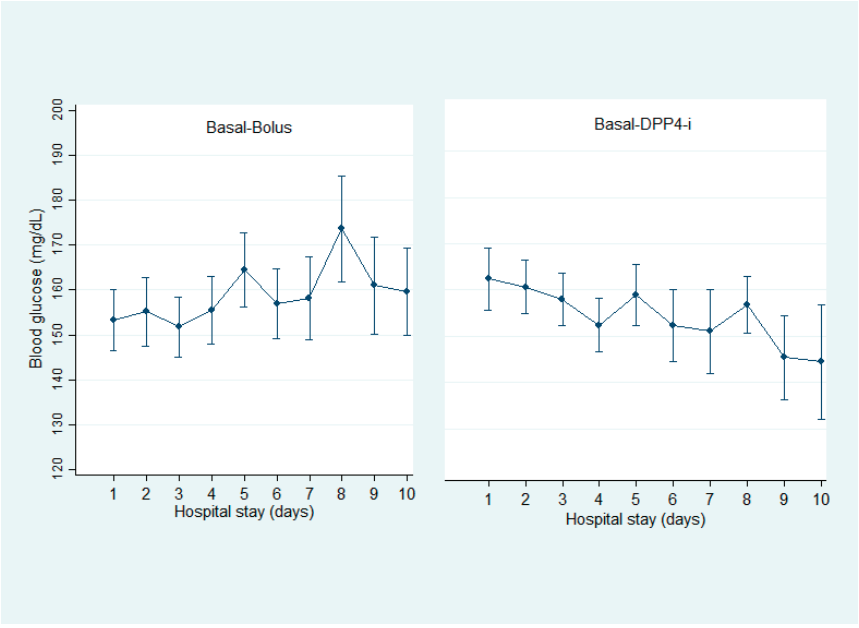

Figure 2. Mean daily blood glucose concentrations during hospital stays. Values are shown as mean ± standard deviations.

Table 2. Variables associated with glycemic control.

Variable	Basal–Bolus (*n* = 40)	Basal–Vildagliptin (*n* = 34)	*p*-Value
Mean CBG	157 ± 36.9	145 ± 29.5	0.103
Insulin dose			
Total mean insulin dose, IU/day	29.1 ± 11.8	15.3 ± 5.1	0.001 *
Total mean insulin dose, IU/kg/day	0.4 ± 0.17	0.2 ± 0.1	<0.001 *
Total glargine insulin dose, IU/day	14.1 ± 6	15.4 ± 5.1	0.105
Total aspart insulin dose, IU/day	14.4 ± 7.6	-	-
Hypoglycaemic events			
Patients with any blood glucose < 70 mg/dL, *n* (%)	8 (20)	1 (3.4)	0.023 *
Patients with any blood glucose < 54 mg/dL, *n* (%)	1 (2.5)	-	-
Hyperglycaemic events			
BG > 180 mg/dL, *n* (%)	10 (25)	5 (14.7)	0.386
CV (%)	28	22	<0.001 *

Abbreviations: CBG, capillary blood glucose; BG, blood glucose; CV, coefficient of variation. * Differences between groups <0.05. Data are mean ± standard deviation.

In patients who used the FreeStyle® glycemic sensor, when comparing the basal–bolus group with the basal–vildagliptin group, we found no statistical differences in glycemic

parameters, with a mean interstitial glycemia of 157 ± 43.6 mg/dL vs. 132 ± 23.8 mg/dL (p = 0.133), time in range 74.3 ± 18.8% vs. 82.3 ± 17.7% (p = 0.341), and CV 29.2 ± 6.8% vs. 23.7 ± 3.8% (p = 0.135). In both groups, there was no grade 1 hyperglycemia. Grade 2 hyperglycemia was found in two patients in the basal–bolus group and one patient in the basal–vildagliptin group. Hypoglycemia events were present in four patients in the basal–bolus group and in two patients in the basal–vildagliptin group.

4. Discussion

The present study confirms the feasibility of implementing an easier, safer and equally effective treatment modality using basal insulin plus DPP4-i, compared to the classic basal–bolus insulin regimen for the management of hyperglycemia in hospitalized older adults. In addition to demonstrating that good glycemic control can be achieved with both treatment modalities with a mean glycemic value of about 150 mg/dL, our study indicates that the number of hypoglycemic episodes is lower, less intense and particularly fewer in absolute terms with a basal–DPP4-i combination compared to a basal–bolus regimen. Moreover, the glycemic variability was lower with basal–DPP4-i treatment.

Hypoglycemia has serious consequences in terms of hospital outcomes, in-hospital length of stay, and in general, it increases the health resources consumed in one hospital episode stay. Hypoglycemia was associated with cardiovascular events, myocardial infarction and stroke due to the impact of the sharp increase in circulating catecholamines induced by the decrease in glycemic circulating levels [21]. In addition, it has recently been confirmed that glycemic variability is a very important treatment target in every diabetic patient. Consistent data indicate that glycemic variability is associated with increased oxidative stress [22] and other deleterious biologic processes that affect general health, particularly in older adults admitted with cardiovascular or infectious episodes.

In addition to efficacy and safety issues, this alternative regime using DPP4-i is more convenient for the patient and the nursing staff who provide care in hospital conventional beds. The decrease in insulin injections per se, as well as the advantage of the non-activity of DPP4-i molecules at low–normal glycemic levels, provides comfortability and safety in old and frail patients who may be unable to complete their intake of a given hospital meal due to increased anorexia or inappetence for hospital food.

The present study has some weaknesses, including a relative low number of subjects and a small subsample of patients using continuous glucose monitoring. In addition, the group of previously studied patients had an overall good control according to the HbA1c value at hospital entry. However, as for the potential strengths of the present study, the prototype of the included patients was typical of those mostly admitted in the hospital. In this regard, it seems logical that patients with relatively well-controlled DM did not require heavy dosing with a full basal–bolus insulin regime, which in fact may be more harmful than a single dose of long-acting insulin plus a DPP4-i for covering the prandial glycemia in a patient with an otherwise reasonable beta cell reserve. Additionally, the single-center nature of our study warranted the homogeneity of the procedures and patient inclusion.

5. Conclusions

The basal–bolus insulin therapy regimen remains a useful treatment for many inpatients, especially those with symptomatic hyperglycemia, poor glycemic control prior to admission, and those who fail to maintain glucose control with basal insulin, plus DPP4-i. However, the high rate of hypoglycemia represents a major limitation, and the active daily review of insulin dosage is mandatory, which is quite time consuming and requires certain expertise. Our results indicate that an alternative regime with the combination of basal insulin plus DPP4-I, was effective and safer than a basal–bolus regime as less hypoglycemic episodes were detected and with the added value of reduced glycemic variability in older well-controlled diabetic patients. Moreover, improved convenience for patients and the nursing staff may contribute to recommending this kind of treatment modality as the standard of care for controlling inpatient glycemia in most of adults with type 2 diabetes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11102813/s1, Table S1: Treatment regime glargine-DPP4i group; Table S2: Treatment regim basal-bolus group and Table S3: Aspart insulin scale.

Author Contributions: S.B. and A.R. collected data, performed the statistical analysis and interpretation and drafted the manuscript. A.P.-M.d.O., N.F., S.M., J.R., X.P., C.T., P.M. and B.S. collected data, contributed to the interpretation and provided input to the manuscript. N.A., G.U. and M.P.-D. designed the study, performed the statistical analysis and interpretation, and provided input to the manuscript. All authors have read and agreed to the published version of the manuscript.

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Systematic Review

Prevention of Peripheral Distal Polyneuropathy in Patients with Diabetes: A Systematic Review

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Abstract: Background: Diabetic peripheral neuropathy (DPN) is the most frequent chronic complication and is that which generates the highest disability and mortality in diabetes mellitus (DM). As it is currently the only microvascular complication of DM without a specific treatment, prevention is essential. The aim of this study was to determine the most effective preventive strategy to avoid or delay the appearance and/or development of DPN in patients with DM. Methods: A systematic search was carried out in the main health science databases (PubMed, Scopus, CINAHL, PEDro and The Cochrane Library) from 1 January 2010 to 31 August 2020. The study selection was conducted by two independent reviewers and data extraction was performed by the author. The eligibility criteria included randomized clinical trials (RCTs) and cohort studies from RCTs. Results: Eleven studies were selected that included 23,595 participants with DM. The interventions evaluated were intensive or standard glycemic control, the use of drugs to achieve glycemic control, and the promotion of a healthy lifestyle and exercise. Intensive glucose control achieved a significant reduction in the development of DPN in TIDM patients, and lifestyle modifications and exercise achieved it moderately in TIIDM patients. Conclusions: The main preventive strategy for DPN is intensive glycemic control with a target HbA1c < 6% in patients with TIDM and standard control of 7.0–7.9 in patients with TIIDM, incorporating lifestyle modifications.

Keywords: diabetes mellitus; diabetic complications; diabetic neuropathy; prevention and control; evidence; systematic review

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1. Introduction

Diabetic neuropathy (DN) is the most frequent chronic complication in diabetes mellitus (DM) [1–4], and is considered the most important predictor of mortality in patients with type II diabetes (TIIDM), being currently the only microvascular complication of DM without specific treatment [5]. Diabetic peripheral neuropathy (DPN) is the most common cause of diabetic foot complications, with chronic sensorimotor symptoms and signs [1]. There are several forms of DPN. The most common type is distal symmetric polyneuropathy, which causes neuropathic pain symptoms. Atypical forms of DPN include mononeuritis multiplex, radiculopathies, and treatment-induced neuropathies. Other diabetic neuropathies include autonomic neuropathies that affect the cardiovascular, gastrointestinal, and urogenital systems [5,6]. Due to the lack of treatments targeting the underlying nerve damage, prevention is the key component in this complication of DM, and for this reason it is essential to emphasize special attention paid to the feet, as these patients are at risk of injury due to a lack of sensation [6–8].

In this sense, diabetic foot is considered one of the conditions that generates more disability, economic costs in health systems and mortality [9]. It may be considered as a

supercomplication of several complications. Thus, patients with DM have a high rate of lower limb amputation, which increases when DN is present, and consequently the risk of foot ulceration is three times higher in patients with DN [10–13]. This complication in the lower extremities can be life-threatening in patients with foot ulceration, and can lead to subsequent infection. In this sense, since most amputations are preceded by foot ulceration, infection must be avoided. More extensive research is necessary for determining more precisely the need for amputation. It is important to avoid non-painful foot injuries by wearing well-fitting footwear and by performing regular inspections [4,6]. Health education is essential. DPN is the most common form of DN; its presentation is slow and progressive, usually distal and symmetrical. There is a progressive loss of sensitivity as well as motor weakness of the affected muscles, and dysfunction of the peripheral nerves of the autonomic nervous system, acting mainly on the lower limbs. Patients often report a sensation of "numb" feet, have altered distal vibratory sensation as well as altered joint position and sensations of tactile pressure and abnormal reflexes [12]. Normally, none of these alterations are painful, although it is reported that up to 25% of these patients may experience symptoms of neuropathic pain. It is described as numbness, paresthesias, hyperesthesias, allodynia, loss of sensation, muscle weakness, or loss of temperature sensation, risk of the complications of diabetic ulceration and non-traumatic amputation [3]. Amputation decisions are determined by patient comorbidities, performance, imaging studies, and clinical examination results [7,8]. In this sense, more extensive research is necessary to determine more precisely the need for amputation.

The most important risk factor for the development of this complication, apart from the duration of the disease, is hyperglycemia [14]. Intensive control is associated with a reduction in the prevalence of DN and painful symptomatology, especially in patients with type I DM (TIDM). In the case of patients with type II DM (TIIDM), good glycemic control is recommended in addition to the control of cardiovascular risk factors and lifestyle modifications [15–19].

Some studies reported that screening for symptoms and signs is very important, as it allows for early diagnosis in the early stages of DN [20]. It is estimated that about half of patients with DM are undiagnosed [21], and it is also established that the group of patients with glucose intolerance and prediabetes may also develop neuropathies, mainly DPN, as this is the most common form of presentation [11]. In addition, it is stated that up to 50% of patients with DPN may be asymptomatic [8]. DPN affects at least 20% of patients with TIDM, 20 years after disease onset, and 10–15% of newly diagnosed patients with TIIDM, increasing to 50% 10 years after diagnosis [20]. Of these patients, 10–15% may develop painful DPN, and symptomatic treatment may be necessary. Painful symptoms, as well as other types of complications derived from DPN, can have a significant impact on the quality of life of these patients. In addition, patients with DM with pain have three times the expenditure on medication, so in this sense, prevention is essential [14], considering that the expenditure on medication is expensive to health systems [1,9].

On the other hand, early diagnosis, prevention and treatment of symptoms help to reduce sequelae, costs and improve the quality of life of patients with DN. Despite a large body of evidence, current medication prescribing patterns are inconsistent. Previous studies reported first-line drugs for the treatment of neuropathic pain in painful DPN, including the α-2-delta subunit voltage-gated calcium channel blockers gabapentin and pregabalin, the selective serotonin and norepinephrine reuptake inhibitors (SNRIs) duloxetine, and the tricyclic antidepressant (TCA) amitriptyline. The most studied drug, and with the most beneficial results, is pregabalin [15,22]. Thus, the American Diabetes Association (ADA) recommends starting symptomatic treatment of neuropathic pain in DM with pregabalin or duloxetine, although gabapentin can also be used, but the patient's socioeconomic status, comorbidities, and possible drug interactions must be taken into account [7]. Opioid and atypical opioid analgesics are associated with a high risk of addiction and safety concerns and numerous serious adverse effects such as abuse or mortality. To date, prevention of DN has focused primarily on glycemic control [19,22]. Although studies have been published

that point out other types of preventive strategies to avoid the onset, development and evolution of this complication of DM, these lack great scientific evidence due to the poor quality of the studies, and on numerous occasions provide confusing results [7]. In this sense, this research attempts to shed light on the existing preventive alternatives for DN, not only highlighting the role of glycemic control as a preventive factor, but also revealing other options.

In view of these considerations, the aim of the present review was to determine which was the most effective preventive strategy to avoid or delay the appearance and/or development of DPN in patients with DM.

2. Materials and Methods

2.1. Protocol and Registration

This systematic review was carried out according to the general guidelines and recommendations made by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and was registered in the PROSPERO database (CRD:42020206120).

2.2. Eligibility Criteria

The study population consisted of patients with DPN. Documents published up to 30 September 2021 were included. We excluded documents that did not meet the eligibility criteria and those dealing with the diagnosis of DPN, studies on gestational diabetes and on the treatment of painful DPN, and investigations related to any neuropathy other than DPN. Documents that were not published in English, Spanish, French or Portuguese were excluded. Cohort studies and RCTs carried out from 1 January 2010 to 31 August 2020, following the PICO strategy.

1. Participants: Patients with DM, aged $\geq$ 18 years.
2. Interventions: Any strategy that entailed prevention or delay of DPN onset.
3. Comparisons: Placebo substances, any other alternative or natural progression of the disease in the control group.
4. Outcomes or results: The effectiveness of the intervention in terms of the prevention of DPN at the end of the studies in patients who did not present this condition at the beginning, or the improvement of this condition if they presented it at the beginning of the study, should be evaluated. Other outcomes may include quality of life measurements, adverse events, related costs, changes in neuropathic pain symptoms, presence of foot ulcerations and/or amputations, and events that prevented continuation of clinical trials.

2.3. Sources and Search

The databases used were Scopus, Cochrane, PubMed, PEDro, EMBASE, SciELO and CINAHL. PubMed was used as a free access tool for the search in Medline and Premedline. The search and the free search were done via Mesh terms. The following search terms were used, together with the operators "OR" and "AND". According to each database, the following search strategy was used. The key words used for the search were "diabetic neuropathies", "prevention", "control", "wound", "randomized controlled trial", "diabetic nephropathy", "case control studies", "quality of life", "cerebrovascular accident", "cardiovascular disease", "diabetic nephropathies", "peripheral occlusive artery disease", "autonomic neuropathy", "coronary artery disease", "depression", "neuropathic pain", "healthcare cost", and "diabetic retinopathy". The search strategy used can be consulted in Appendix A.

2.4. Study Selection

Two blinded reviewers (XXX) (XXX) participated in each stage of the study selection. First, they screened by titles and abstracts of the references identified through the search strategy. The authors assessed whether the studies collected through the literature search met the eligibility criteria, excluding those that were irrelevant and/or whose level of methodological quality was questionable. Full reports of all potentially relevant documents were then assessed for eligibility based on the eligibility criteria of this review. Disagreements were resolved by discussion between the two evaluators, or if consensus was not possible, further opinion was sought (XXX) (XXXX).

2.5. Data Extraction and Synthesis of Results

For the data extraction process, review authors used a standardized template containing information related to the eligibility criteria of the publications and the exclusion reasons for the selection of articles, and full title, country, and year of publication. After carrying out the first evaluation of the reports, the results obtained were discussed between the investigators, as well as the inclusion or exclusion of incompatible papers and, if necessary, the intervention of a third independent investigator. Finally, a form was designed for the extraction of data from the articles ultimately selected. This task was carried out by a single researcher. The data extracted were synthesized in an evidence table (including study design and setting, population characteristics, risk of bias assessment).

2.6. Risk of Bias Assessment

The assessment of the risk of bias in the studies was carried out using the Review Manager tool (RevMan) of the Cochrane Collaboration, version 5.3.77. This software evaluates the risk of bias of individual studies as well as among the studies included in the review by generating graphs, tables and percentages from the following domains.

The risks of bias criteria are classified as: "low risk", "high risk" or "unclear risk", assessing the risks of selection, conduct, detection, attrition, reporting and other possible biases. This task was carried out by the review author and is currently the main tool used for the assessment of risk of bias in studies and for the evaluation of methodological quality [23]. Thus, studies without a high risk of bias in any category were considered to be of high quality (1++), and those with a high risk or two unclear risks were considered to be of medium quality (1+). The rest were considered low quality (1−).

In addition, the STROBE [24] and CASPe [25] checklists were used to assess the quality of cohort studies and RCTs, respectively. These two methodological quality assessment scales are expressed as a numerical score based on the number of items completed. A statistical assessment was performed by two independent assessors using the IBM SPSS Statistics 22 80 software. The data were analyzed using the intraclass correlation coefficient (ICC), the purpose of which is to assess the agreement between two or more continuous measurements carried out repeatedly in a sample. The ICC takes values between 0 and 1. A significance level of less than 0.04 would indicate poor reliability, and values above 0.75 would indicate excellent reproducibility; intermediate values are considered adequate.

3. Results

The flow diagram summarizes the study selection processes, and each stage for the studies included in this review (see for details the PRISMA flow diagram in Figure 1) [26]. In total, 11 documents were included in our systematic review. Table 1 shows the studies excluded and the reasons after the application of the quality appraisal filter.

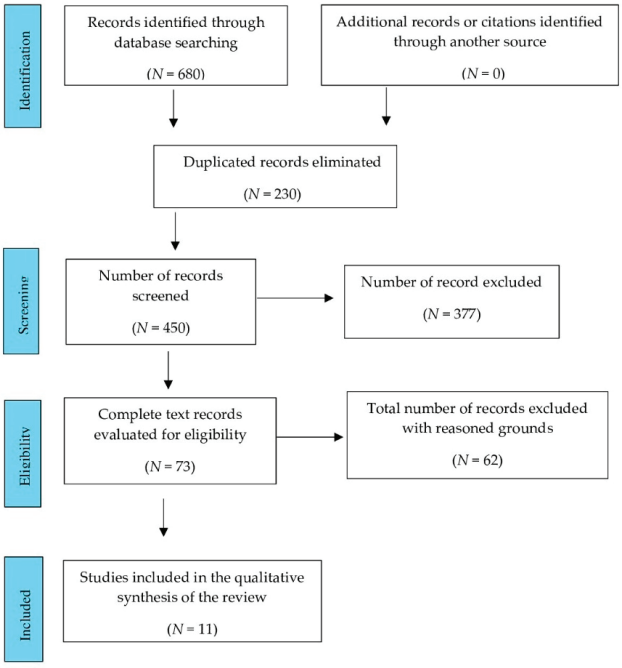

Figure 1. PRISMA flow diagram adapted with permission from the PRISMA group, 2020.

Table 1. Potential studies excluded.

Reason for Exclusion	Authors
RCTs that specifically address treatment rather than prevention of DPN	Farvid et al., 2011 [27] Song et al., 2011 [28] Rizzo et al., 2012 [29] Lavery et al., 2012 [30] Mueller et al., 2013 [31] Ulbrecht et al., 2014 [32] Dixit et al., 2016 [33] Ziegler et al., 2016 [34] Sharoni et al., 2018 [35] Venkataraman et al., 2019 [36] López-Moral et al., 2019 [37] Stubbs et al., 2019 [38] Ahmad et al., 2019 [39] Shu et al., 2019 [40] Sari et al., 2020 [41]
Cohort studies not from RCTs	Müller-Stich et al., 2013 [42] Hur et al., 2013 [43] Cho et al., 2014 [44] Ishibashi et al., 2018 [45] O'Brien et al., 2018 [46] Yang et al., 2020 [47] Cárdenas et al., 2019 [48]
Cohort studies that do not specifically address the prevention of DPN, but from RCTs	Aroda et al., 2016 [49] Gaede et al., 2016 [50] Abraham et al., 2018 [51] Braffett et al., 2020 [52]

3.1. Risk of Biases among the Studies Included

Figures 2 and 3 show the risk of biases of the study included in this systematic review.

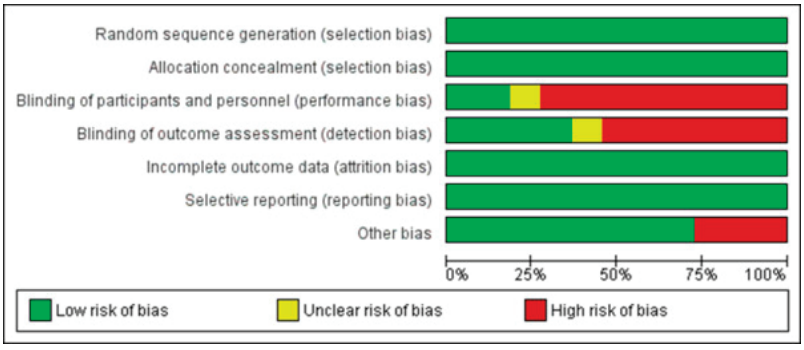

Figure 2. Risk of biases of included studies, overall analysis.

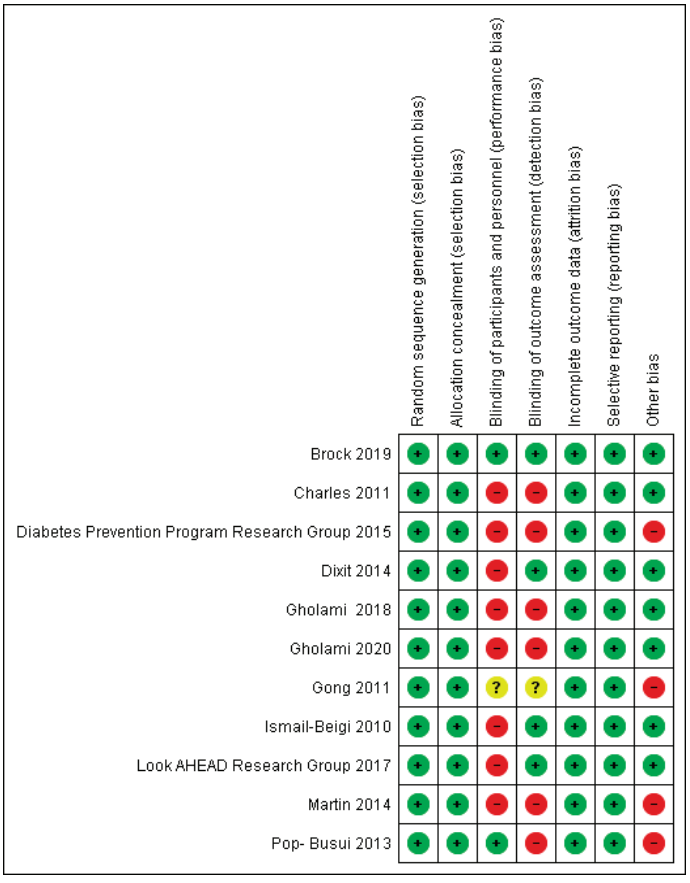

Figure 3. Risk of biases of the included studies [15,53–62], individual analysis. Green: low risk, yellow: unclear risk, red: high risk.

Allocation concealment and random sequence generation was evident in 100% of the studies. Blinding of participants and staff was present in less than 25%, and blinding of assessors was present in less than 50% of the included articles. Due to the nature of some included studies, such as cohort studies, 25% of the included studies were considered to be at high risk of other biases.

The levels of evidence evaluated according to the quality of the selected articles received a score of 1++ in 9.2% (n = 1) [53] qualifying it as high quality, 27.3% of the studies received a score of 1+ or medium quality (n = 3) [54–56], and the rest of the articles were scored as low quality, 1−, representing 63.5% (n = 7) [15,57–62].

3.2. Statistical Analysis of the Quality of the Included Studies

Detailed assessment ICC is summarized in Table 2. Table 3 summarizes the scores of the quality scales of the studies included in this review. The limitations of the review are summarized in Table 4.

Table 2. Intraclass correlation coefficient. Evaluation of agreement between continuous measurements.

	Intraclass Correlation [a]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Lower Bound	Value	df1	df2	Sig.
Single Measures	0.997 [b]	0.995	0.995	687.400	10	10	0.000
Average Measures	0.999 [c]	0.995	1.000	687.400	10	10	0.000

Two-way mixed effects model where people's effects are random and measures' effects are fixed. [a] Type C intraclass correlation coefficients using a consistency definition—the between measure variance is excluded from the denominator variance. [b] The estimator is the same, whether the interaction effect is present or not. [c] This estimate is computed assuming the interaction effect is ab-sent, because it is not estimable otherwise.

Table 3. Scores of the investigators on the quality scales of the included studies.

Authors	Scale	Review 1	Review 2
Ismail-Beigi et al., 2010	CASpe	10/11	10/11
Charles et al., 2011	CASpe	6/11	6/11
Gong et al., 2011	STROBE	16/22	16/22
Pop-Busui et al., 2013	STROBE	17/22	17/22
Dixit et al., 2014	CASpe	11/11	11/11
Martin et al., 2014	STROBE	16/22	16/22
Diabetes Prevention Program Research Group et al., 2015	STROBE	17/22	17/22
Look AHEAD Research Group et al., 2017	CASpe	9/11	9/11
Gholami et al., 2018	CASpe	9/11	9/11
Brock et al., 2019	CASpe	11/11	11/11
Gholami et al., 2020	CASpe	9/11	9/11

3.3. Limitations of Included Studies

Table 4 shows some of the studies with their limitations. Some of the reasons for its limitation were the sample size, number of dropouts or that not all patients were evaluated with all the measures, among other reasons.

Table 4. Limitations of the review.

Authors	Limitations
Ismail-Beigi et al., 2010	Early termination of the RCT due to increased mortality among participants.
Charles et al., 2011	Not all patients were evaluated with all measurements. Patients in the CASE IV subgroup were younger than the rest, so microvascular complications may have been lower in this group.
Gong et al., 2011	No results were obtained for 25% of the participants who died. Low incidence of nephropathy and neuropathy due to short duration of diabetes in participants.
Pop-Busui et al., 2013	Study not designed to detect an effect of the groups on DPN. A lower incidence of neuropathy was found in the IS group; however, the authors were unable to identify whether the benefit was specific to biguanides or thiazolidinediones. Small fiber neuropathy was not evaluated, as only the Michigan Neuropathy Screening Instrument (MNSI), which evaluates large fibers, was used. Subjectivity of the MNSI.
Dixit et al., 2014	The effect of aerobic exercise to halt or interrupt the natural course of DPN was not studied. The study had a large number of dropouts.
Martin et al., 2014	Intentional exclusion at the start of Diabetes Control and Complications Trial (DDCT) of participants with severe neuropathy. Patients in the conventional insulin therapy (CON) group were switched to intensive insulin therapy (INT) group because of the benefits of intensive glycemic control in patients with TIDM.
Diabetes Prevention Program Research Group et al., 2015	The combination of three different microvascular outcomes in the aggregate microvascular outcome.
Look AHEAD Research Group et al., 2017	Relationship of biguanide use with vitamin B12 depletion and the development of DPN. Levels of this vitamin were not recorded. Diagnosis of DPN by questionnaire, MNSI physical examination and Semmes-Weinstein (SW) monofilament.
Gholami et al., 2018	Small sample size, large number of dropouts, and only male participation.
Brock et al., 2019	Severe irreversible neuropathy, more male representation.
Gholami et al., 2020	Small sample size.

3.4. Synthesis of Results

3.4.1. Studies Included

Of the 11 included studies, seven were parallel-group RCTs [59,61], of which one was placebo-controlled [53]. The remaining four studies were cohort studies from RCTs, [58,60], of which one was placebo-controlled. The total follow-up period of the studies ranged from 8 weeks to 20 years. Table 5 summarizes the characteristics of the included studies.

Table 5. Main characteristics of the studies included.

Authors	Design	Participants (*N*)	Groups	Diabetes Type	Average Age (Years)	Duration of the Study	Interventions	Measured Results
Brock et al. (2019)	RCT, double-blind, placebo-controlled	39	IG (Liraglutide) *N* = 19 CG (placebo) *N* = 20	TIDM	50.4	32 weeks	Liraglutide Placebo	Changes in nerve potentials, proinflammatory cytokines, autonomic function and peripheral neurophysiological tests. MNSI
Charles et al. (2011)	RCT with parallel groups	1161	Routine Care (RC) *N* = 459 Intensive multifactorial treatment (IT) *N* = 702	TIIDM	59.9	6 years	IT: Education, medication and promotion of healthy lifestyle. CR: Danish recommendations for diabetes care.	AAI Vibration detection threshold (tuning fork) Light touch (SW)
Diabetes Prevention Program Research Group et al. (2015)	Cohort study of a parallel-group placebo-controlled RCT	2776	Placebo *N* = 935 Metformin *N* = 926 Lifestyle *N* = 915	TIIDM	51	15 years	Metformin Placebo Lifestyle	Diagnosis of diabetes HbA1c Albuminuria (Nephropathy) Fundus evaluation (Retinopathy) SW light touch (Neuropathy)
Dixit et al. (2014)	RCT of parallel groups	87	CG *N* = 47 (10 lost) EG *N* = 40 (11 lost)	TIIDM	CG: 59.45 EG: 54.40	8 weeks	EG: Moderate aerobic exercise, foot care education, healthy diet CG: Standard medical care, education	Motor and sensory nerve conduction studies in peroneal and sural nerves MDNS

Table 5. *Cont.*

Authors	Design	Participants (*N*)	Groups	Diabetes Type	Average Age (Years)	Duration of the Study	Interventions	Measured Results
Gholami et al. (2018)	RCT of parallel groups	24	Exercise $N = 12$ Control $N = 12$	TIIDM	CG: 43 ± 6.4 EG: 42 ± 4.6	12 weeks	Exercise: Running, walking or treadmill 3 times/week for 20–45 min. Control: Maintain usual level of physical activity.	Weight, BMI, % fat HbA1c Nerve conduction velocity (NCV) and nerve action potential amplitude (APAN) peoneal, tibial and sural nerves
Gholami et al. (2020)	RCT of parallel groups	31	CG $N = 15$ EG $N = 16$	TIIDM	52.8 ± 9.6	12 weeks	EG: Cycling exercises CG: Maintaining the usual level of physical activity	HbA1c Fasting glucose Flow mediated dilation (FMD), changes in intima-media thickness and basal diameter in superficial femoral artery, MDNS
Gong et al. (2011)	Cohort study of parallel-group RCTs	577	CG = $N = 136$ (42 lost) EG = $N = 441$ (135 lost)	TIIDM	CG 66.7 ± 9.2 EG 64.7 ± 9.3	20 years	EG: diet, exercise or diet + exercise CG: Regular medical care	Plasma glucose HbA1c, oral glucose tolerance test, Examination ocular fundus Inspection extremity lower limb AAI Light touch (SW)
Ismail-Beigi et al. (2010)	RCT of parallel groups	10,251	Intensive therapy $N = 5128$ Standard therapy $N = 5123$	TIIDM	62.2 ± 6	3.5 years	Intensive therapy: HbA1c < 6.0% Standard therapy: HbA1c 7.0–7.9%	Albuminuria Creatinine Fundus examination MNSI Vibratory sensitivity (tuning fork), light touch (SW)

Table 5. *Cont.*

Authors	Design	Participants (*N*)	Groups	Diabetes Type	Average Age (Years)	Duration of the Study	Interventions	Measured Results
Look AHEAD Research Group et al. (2017)	RCT of parallel groups	5145	Intensive lifestyle intervention (ILI) *N* = 2570 Diabetes support and education (DSE) *N* = 2575	TIIDM	58.7	11 years	ILI: 7% weight loss, reduced caloric intake, and increased physical activity DSE: Diabetes education focused on diet and exercise	MNSI Light touch (SW)
Martin et al. (2014)	Cohort study of a parallel-group RCT	1345	Intensive insulin therapy (INT) *N* = 687 Conventional insulin therapy (CON) *N* = 688	TIDM	33.6 ± 7	14 years	INT: insulin treatment aimed at near-normal glycemia. CON: insulin treatment according to current standards	Vibratory sensitivity Light touch (SW) MNSI Nerve conduction studies HbA1c
Pop-Busui et al. (2013)	Cohort study of a parallel-group RCT	2159	Insulin-sensitizing treatments (IS) *N* = 1080 Insulin-providing treatments (IP) *N* = 1079	TIIDM	62 ± 9	4 years	Insulin-sensitizing treatments Insulin-providing treatments	HbA1c, Duration of DM, Albuminuria Retinopathy Alcohol and tobacco consumption Blood lipids, Blood pressure, MNSI Prevalence of DPN

3.4.2. Participants

The total number of participants in all studies was 23,595, with ages ranging from 33.6 ± 7 to 66.7 ± 9.2 years, including 1834 patients with TIDM and 21,761 patients with TIIDM [54–59,61,62]. All studies divided participants into two groups, except the 2015 Diabetes Prevention Program Research Group et al. [57] study, which randomized participants into two intervention groups and one control group.

3.4.3. Interventions and Comparisons

Interventions included drugs such as liraglutide [53] for the reduction in the neuroinflammatory component that appears in DPN in patients with TIDM, intensive glucose control with a glycosylated hemoglobin (HbA1c) < 6% in the case of patients with TIDM [15], or in patients with TIIDM [55,62]. Another strategy employed was the comparison of insulin-sensitizing treatments and insulin-providing treatments for standard glycemic control in patients with TIIDM [60]. Moderate aerobic exercise was evaluated in two of the included articles [54,61], as well as cycling [59]. The most employed intervention among the included studies was the promotion of a healthy lifestyle through education, medication for the control of diabetes and cardiovascular risk factors in addition to diet in patients with TIIDM [56–58]. Comparisons were made with placebo [53,57], standard recommendations for diabetes care [60,62], maintaining usual physical activity level [59,61], diabetes education focused on exercise and diet control [56].

3.4.4. Analysis of Results

The presence of DPN was mainty evaluated. Other variables were taken into account, such as ankle arm index (AAI) [58,62], albuminuria and creatinine (nephropathy), fundus examinations [58] (retinopathy), glucose levels [59], oral glucose tolerance test [58], HbA1c [62], lower limb inspection [58], weight, body mass index (BMI), fat percentage [61], diagnosis of DM [57] or changes in intima media thickness and basal diameter of the superficial femoral artery [59]. In the case of neuropathy identification, the measurements used were nerve conduction velocity (NCV) studies [15,53,54,59,61], tests for vibration detection threshold assessment with a 128 Hz tuning fork, and light touch with the SW monofilament [58,62], and questionnaires such as the Michigan Diabetic Neuropatic Score (MDNS) [54,59] or the MNSI [55,56,60]. For all the results obtained in the studies, the significance level was $p < 0.05$.

3.4.5. Summary of Results

The drug liraglutide reduced the neuroinflammatory component interleukin-6 in adults with TIDM, but did not improve established DPN [53]. Intensive glycemic control significantly reduced the development of neuropathy in patients with TIDM, but this effect was not observed in patients with TIIDM [55]. Intensive lifestyle intervention in patients with TIIDM had negative effects in two of the studies [57,58], and positive effects in one [56]. Moderate-intensity aerobic exercise had a positive outcome for the improvement of established DPN and prevention in two of the included studies [54,61], as did cycling in patients with TIIDM [59]. Glycemic control therapy with insulin sensitizers significantly reduced the incidence of DPN compared with insulin-providing therapy, with more benefits for men [60]. The effect of glycemic control therapy with insulin sensitizers in patients with TIIDM was not observed [61,62].

4. Discussion

The aim of this systematic review was to determine which is the most effective preventive strategy to avoid or delay the appearance and/or development of DPN in patients with DM. Most studies seem to indicate that glycemic control is currently the most effective preventive strategy. Our literature search identified 11 studies examining patients with the variables related to diabetic neurophaties [15,53–61]. These aims were achieved in the review.

4.1. Intensive Glycemic Control

DPN has a multifactorial origin, in which different metabolic, inflammatory, autoimmune and vascular processes take place, leading to nerve degeneration [62]., Therefore, the prevention of these alterations is fundamental, with the control of maintained hyperglycemia being the main one [63]. In this sense, large studies have been carried out in which the effect of intensive glucose control with a target HbA1c of less than 6% in patients with TIDM were evaluated [64].

The Epidemiology of Diabetes Interventions and Complications (EDIC) study was performed to record the long-term effects of therapy on the development and progression of myocardiovascular complications and cardiovascular disease. Data published in 2010 by Albers et al. [65] from the EDIC follow-up demonstrated that intensive glucose control significantly delayed the development and progression of DPN. The prevalence of neuropathy increased from 9 to 25% in the INT group and from 17 to 35% in the conventional CON insulin therapy group ($p = 0.001$) and the incidence also remained lower in the INT group (22%) relative to the CON group (28%); ($p = 0.0125$). The effect was maintained in the article included in our 2014 systematic review of Martin et al. [15] in which the prevalence and incidence of DPN and Cardiovascular autonomic neuropathy (CAN) remained significantly lower in the Diabetes Control and Complications Trial (DCCT) intensive therapy group compared to the DCCT conventional therapy group up to year 13/14 of EDIC. This is in addition to being maintained in other smaller European cohorts, such as the Oslo study [66], and the one published by Ziegler et al. [67] in 2015, as well as in the EURO-DIAB study [68]. In contrast, the results presented by Holman et al. [69] in 2008 of the 10-year follow-up of participants in the United Kingdom Prospective Diabetes Study (UKPDS) in the sulfonylureas-insulin group, relative risk reductions persisted for microvascular disease ($p = 0.04$), but this effect was not seen in the metformin group of patients with TIIDM. Along the same lines, the Steno-2 study, according to data published by Gaede et al. [70] in 2003, did not have a significant effect on the progression of DPN after a follow-up of 13.3 years in patients with microalbuminuria, although it did reduce the development of CAN by 57% (Relative risk; RR 0.37; Confidence interval, 95% CI 0.18–0.79). With similar results, the 2008 ADVANCE study [71], which included 11,140 patients with TIIDM, also with two groups, one intensive therapy and one conventional glycemic therapy, demonstrated a decrease in the incidence of combined major macrovascular and microvascular events ($p = 0.01$), as well as in major microvascular events ($p = 0.01$), mainly due to the reduction in the incidence of nephropathy ($p = 0.006$), but did not demonstrate a significant difference in the groups in terms of relative risk reduction for the occurrence of DPN.

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial [72] was an RCT published in 2008 that studied the relationship between diabetes and cardiovascular disease, concluding that, compared with standard therapy, the use of intensive therapy to achieve target HbA1c levels for 3.5 years increased mortality and did not significantly reduce major cardiovascular events, which is why standard glycemic therapy rather than intensive therapy is advised in patients with TIIDM. In the ACCORD results for the development of microvascular complications presented in the 2010 study by Ismail-Beigi et al. [55], positive results were obtained for intensive therapy in terms of DPN prevention, but due to the increase in mortality and the number of cardiovascular events recorded, this study advises against intensive glycemic control in patients with TIIDM. Similarly, in the 2009 Veterans Affairs Diabetes Trial (VADT) RCT [73], no difference was found between the intensive or standard glucose control groups for microvascular complications of DPN after a median follow-up of 5.6 years.

In addition, the multicenter Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen-Detected Diabetes in Primary Care (ADDITION-Denmark) study by Charles et al. [62] published in 2011 did not find that screening followed by intensive glycemic control intervention led to a statistically significant difference in the prevalence of DPN and peripheral arterial disease (PAD) 6 years after diagnosis. However, positive results have been obtained for intensive control in patients with TIIDM in a Japanese

RCT with a small sample size, significant improvement in NCV ($p < 0.05$) and vibration thresholds ($p < 0.05$) at 6 years from the baseline [74]. In this line, in 2013, Hur et al. [43], performed a cohort study where they identified that HbA1c levels predict nerve degeneration and regeneration of myelinated fibers in patients with TIIDM and DPN. Therefore, maintaining optimal blood glucose control is likely to be essential to prevent nerve injury. Abraham et al. [51] 2017, Ishibashi et al. [45] 2019 and Cho et al. [44] 2014 further added the importance of dyslipidemia control, as high cholesterol and triglycerides seem to be found to be related to the future development of DPN in patients with TIIDM.

In 2012, a Cochrane review and meta-analysis by Callaghan et al. [75] was published that aimed to examine the evidence for intensive glucose control in the prevention of DPN in patients with TIDM and TIIDM. Revealing a significant decrease in the relative risk of developing clinical neuropathy in those who had intensive glucose control, RR of −1.84% (95% CI −1.11 to −2.56). For patients with TIIDM, the relative risk of developing neuropathy was −0.58% (95% CI 0.01 to −1.17). Most of the secondary outcomes were significantly in favor of intensive treatment in both populations. However, both types of participants had a significant increase in serious adverse events, including hypoglycemic events.

The results of this review demonstrate that tight glycemic control is effective in preventing the development of DPN in patients with TIDM, but the data were not significant for patients with TIIDM ($p = 0.06$), although improved glucose control has been shown to significantly reduce nerve conduction and vibratory threshold abnormalities. The authors noted that this intervention significantly increases the risk of severe hypoglycemic episodes and should be taken into account when assessing risk/benefit. Buehler et al. [76], in 2013 published a systematic review and meta-analysis on the effect of tight glucose control compared to standard control, in this case in patients with TIIDM. It was determined that intensive glucose control significantly reduced the progression of retinopathy (RR 0.80; 95% CI 0.71–0.91), the incidence of DPN (RR 0.94; 95% CI 0.89–0.99), as well as the progression of nephropathy (RR 0.55; 95% CI 0.37–0.80) but had no significant effect on the incidence of nephropathy (RR 0.69; 95% CI 0.42–1.14). In agreement, Fullerton et al. [64] in 2014 conducted a systematic review in which it was observed that intensive glycemic control reduces the risk of developing microvascular complications compared to conventional treatment, in the case of neuropathy by 4.9% versus 13.9%; RR 0.35 (95% CI 0.23–0.53); $p < 0.00001$. Hasan et al. [77] in 2016 conducted a systematic review and meta-analysis evaluating the efficacy and safety of intensive control compared to standard glycemic control in preventing the development of diabetic foot. Intensive control with an HbA1c target of 6.0–7.5% was associated with a significant decrease in the relative risk of amputation (RR, 0.65; 95% CI, 0.45–0.94; I(2) = 0%). Intensive control was associated with a slower decrease in the sensitive vibration threshold (mean difference, L8, 27; 95% CI, L9, 75 to L6, 79). No effect on neuropathic changes (RR, 0.89; 95% CI, 0.75–1.05; I(2) = 32%) or ischemic changes (RR, 0.92; 95% CI, 0.67–1.26; I(2) = 0%) was found in nine RCTs of patients with TIIDM.

The management of glycemic control suggested an optimal therapeutic approach depending on the patients with TIDM and TIIDM. Despite adequate blood glucose control, patients with TIIDM are likely to develop neuropathy [72]. This is why, in patients with TIDM, glycemic control with an HbA1c target of less than 6% is advised to prevent DPN and in the case of patients with TIIDM, glycosylated hemoglobin could range from 7.0–7.9%.

4.2. Use of Drugs

Pop-Busui et al. [60], in 2013, conducted a study where it was observed that glycemic control therapy with insulin sensitizers (IS) with metformin and thiazolidinediones (TZD) significantly reduced the incidence of DPN compared to insulin-providing therapies (IP) such as sulfonylureas, meglitinide or insulin. This result could be due to the anti-inflammatory, oxidative stress, lipid profile and weight improvement effects of TZDs and metformin, which would be coupled with the reduction in glycemia. However, no other studies have been published comparing the efficacy of the different drugs used for the treatment of DM in terms of the prevention and development of DPN.

With respect to liraglutide, Brock et al. [53], did not find a significant effect in terms of DPN prevention, although a decrease in proinflammatory cytokines was observed.

4.3. Lifestyle Modification

The most important and largest study on the prevention of the development of TI-IDM was the Diabetes Prevention Program (DPP) [78], where participants at high risk of developing DM were divided into two groups, and both were compared with placebo groups. One group was metformin, with an administration of 850 mg twice daily, and the other group was lifestyle modification through programs of at least 7% weight loss and 150 min of physical activity per week. The intervention reduced the incidence of DM by 58% (95% CI, 48 to 66%) in the lifestyle modification group and by 31% (95% CI 17 to 43%t) in the metformin group compared with placebo, highlighting the greater benefit of lifestyle modification.

Supporting these results, an RCT, "China Da Qing Diabetes Prevention" [79], divided participants into three subgroups: diet, exercise, and diet plus exercise. Participants in the combined intervention group obtained a 51% (hazard ratio (HR) 0.49; 95% CI 0.33–0.73) lower incidence of diabetes during the active period and 43% (0.57; 0.41–0.81) during the subsequent 20 years of follow-up.

The relationship of these interventions in terms of preventing vascular microcomplications in DM was detailed in the studies of Diabetes Prevention Program Research Group et al. [57] in 2015 and Gong et al. [58] in 2011. In both studies, negative results were obtained for the prevention of DPN development by not preventing the advancement of microvascular complications: However, in the study by Gong et al., it did decrease the incidence of severe retinopathy by 47%.

In contrast, in the case of the 2017 Look AHEAD Research Group et al. [56] study, it was determined that the intensive lifestyle intervention group demonstrated a significant decrease in DPN.

4.4. Practice of Physical Exercise

Balducci et al. [80] in 2006 examined the effects of long-term physical training on the development of DPN in patients with TIDM and TIIDM through an RCT. Significant differences were found in the improvement of nerve conduction in the peroneal and sural nerves for the group that performed physical activity, so the study suggests that long-term aerobic exercise could prevent or modify the onset of the natural history of DPN. This improvement in peroneal nerve conduction velocity and an improvement in neuropathic symptoms was observed in the longitudinal observational study by Azmi et al. [81].

Singleton et al. [82], in 2014, demonstrated increased intraepidermal nerve fiber density (IENFD) (1.5 ± 3.6 vs. -0.1 ± 3.2 fibers/mm, $p = 0.03$) of the leg in a cohort of 100 patients with DM and without neuropathy who received a weekly structured and supervised exercise program ($n = 60$) compared to patients who only received lifestyle counseling ($n = 40$), followed for 1 year.

Several RCTs have been published with positive results in terms of improved DPN with physical exercise, such as those conducted by Song et al. [28] in 2011, Mueller et al. [31] in 2013, Dixit et al. [33] in 2016, Ahmad et al. [39] in 2019, Stubbs et al. [38] in 2019, Dixit et al. [54] in 2014, Gholami et al. [61] in 2018 and Gholami et al. [59] in 2020.

However, several systematic reviews and meta-analyses have been published in favor of exercise as a preventive factor in DPN in patients with TIIDM, although it is unclear whether this effect is due to the associated decrease in HbA1c percentage, or whether other currently unidentified factors are involved.

In 2017, Villafaina et al. [83] published a systematic review determining that improved heart rate variability during exercise may be an important factor to consider as prevention in DN and associated mortality in patients with TIIDM. In the same vein, Bhati-Pooja et al. [84] in 2018 conducted a systematic review on physical exercise practice and autonomic cardiac function in patients with TIIDM ascertaining that this strategy significantly improves nerve

conduction. Gu et al. [85] in 2019 observed a positive influence of aerobic exercise on nerve function. In the case of DM associated with obesity, patients with DM who have to undergo bariatric surgery show an improvement in neuropathic symptoms [86].

4.5. Limitations of the Study

The review presents several limitations. Firstly, many of the studies analyzed present heterogeneity in outcome measures, while others studies report small sample size and short duration of follow-up. The authors have found that there is little evidence, and many knowledge gaps persist in the use of preventive alternatives; this should be considered. Furthermore, the risk of detection in eight included studies. In addition, in terms of the neuropathy evaluation technique and according to the literature consulted, there is variability, which is why it should be considered as another limitation.

5. Conclusions

According to the present review, DPN cannot be cured, so preventive measures are essential, with glycemic control being the main strategy. The preventive interventions studied included intensive or standard glycemic control, the use of drugs for glycemic control, lifestyle modifications and the practice of physical exercise. In the case of patients with TIDM, a clear benefit of intensive glycemic control with an HbA1c < 6% in the prevention of microvascular complications. In patients with TIIDM, standard glycemic control with an HbA1c between 7.0 and 7.9% is recommended and lifestyle modifications based on the practice of physical exercise, dietary control and control of cardiovascular risk factors are emphasized. Intensive glycemic control with insulin-sensitizing drugs is recommended in patients with TIDM, as well as lifestyle modifications in patients with TIIDM. The practice of moderate aerobic physical exercise is emerging as an important preventive factor in the development of neuropathy. More consistent studies are needed and with unification in the evaluation techniques that allow for consolidating some aspects of the knowledge of DPN. Therefore, the main principles of treatment for peripheral neuropathy are glycemic control, foot care, and pain management.

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Data Availability Statement: Details of the literature search are reported in Appendices A and B. Extracted data and details of the risk of bias assessments are available from the authors upon request.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Search Strategy

Appendix A.1. PubMed

Appendix A.1.1. Clinical Trials

Free search: (diabet* neuropath*) AND (“prevention” OR “control”) NOT (“ulcer” OR “wound” OR “cardiovascular” OR “pain” OR “nephropath*” OR “retinopath*” OR

"protocol")—Filters "Clinical trial", "10 years" and "human" were applied: 74 results were obtained.

Search by descriptors: "Diabetic Neuropathies/prevention and control" [Mesh].

Clinical trial", "10 years" and "human" filters were applied: 48 results were obtained.

Unified search: "Diabetic Neuropathies/prevention and control" [Mesh] OR [(diabet* neuropath*) AND ("prevention" OR "control") NOT ("ulcer" OR "wound" OR "cardiovascular" OR "pain" OR "nephropath*" OR "retinopath*" OR "protocol")].

The filters "Clinical trial", "10 years" and "human" were applied: In this PubMed search, we obtained a total of 122 results, and with the unified search we obtained 84, eliminating 38 duplicate records.

Appendix A.1.2. Cohort Studies

Free search: (diabet* neuropath*) AND ("prevention" OR "control") NOT ("ulcer" OR "wound" OR "cardiovascular" OR "pain" OR "netinopathy*" OR "retinopathy*" OR "systematic review" OR "meta-analysis") AND ("cohort stud*" OR "cohort analysis").

The filters "10 years" and "human" were applied: 40 results were obtained.

Search by descriptors: "Diabetic Neuropathies/prevention and control" [Mesh] AND ("cohort stud*" OR "cohort analysis").

The filters "10 years" and "human" were applied: 20 results were obtained.

Unified search: (diabet* neuropath*) AND ("prevention" OR "control") NOT ("ulcer" OR "wound" OR "cardiovascular" OR "pain" OR "netinopathy*" OR "retinopathy*" OR "systematic review" OR "meta-analysis") OR ["Diabetic Neuropathies/prevention and control" [Mesh]] AND ("cohort stud*" OR "cohort analysis").

The filters "10 years" and "human" are applied: in this search, we obtained a total of 60 results, and with the unified search 56 were obtained, so four duplicate records were eliminated.

Appendix A.2. Scopus

Free search: ("diabet* neuropath*") AND ("prevention" OR control).

This is limited to the last 5 years, from 2015 to 2020, with "article", in the "keywords" section. We limited the search to "randomized controlled trial", "Cohort Studies" and "human".

The terms "diabetic nephropathy", "case control studies", "qualitfy of life", "incidence", "cerebrovascular accident", "cardiovascular risk", "cardiovascular disease", "diabetic nephropathies", "peripheral occlusive artery disease", "child", "autonomic neuropathy", "case report", "diagnostic imaging", "coronary artery disease", "depression", "practical guideline", "neuropathic pain", "healthcare cost", "pilot study" and "diabetic retinopathy" were excluded.

Search strategy: KEY(("diabet* neuropath*") AND ("prevention" OR control)) AND DOCTYPE (ar) AND PUBYEAR > 2014 AND (LIMIT-TO (EXACTKEYWORD,"Human") OR LIMIT-TO (EXACTKEYWORD,"Cohort Studies") OR LIMIT-TO (EXACTKEYWORD," Randomized Control Trial")) AND (EXCLUDE (EXACTKEYWORD,"Case Control Study") OR EXCLUDE (EXACTKEYWORD,"Diabetic Nephropathy") OR EXCLUDE (EXACTKEYWORD,"Case-Control Studies") OR EXCLUDE (EXACTKEYWORD,"Cardiovascular Disease") OR EXCLUDE (EXACTKEYWORD,"Quality Of Life") OR EXCLUDE (EXACTKEYWORD,"Incidence") OR EXCLUDE (EXACTKEYWORD,"Cerebrovascular Accident") OR EXCLUDE (EXACTKEYWORD,"Diabetic Nephropathies") OR EXCLUDE (EXACTKEYWORD,"Peripheral Occlusive Artery Disease") OR EXCLUDE (EXACTKEYWORD,"Child") OR EXCLUDE (EXACTKEYWORD,"Autonomic Neuropathy") OR EXCLUDE (EXACTKEYWORD,"Cardiovascular Risk") OR EXCLUDE (EXACTKEYWORD,"Case Report") OR EXCLUDE (EXACTKEYWORD,"Diagnostic Imaging") OR EXCLUDE (EXACTKEYWORD,"Coronary Artery Disease") OR EXCLUDE (EXACTKEYWORD,"Depression") OR EXCLUDE (EXACTKEYWORD,"Practice Guideline") OR EXCLUDE (EXACTKEYWORD,"Neuropathic Pain") OR EXCLUDE (EXACTKEYWORD,"Health Care Cost")).

In this database, the unified search could be performed directly, since filters were added. A total of 233 results were obtained.

Appendix A.3. The Cochrane Library

Search strategy in "advanced search": "diabetic neuropathy" AND ("prevention" OR "control") NOT ("ulcer*" OR "wound*" OR "cardiovascular" OR "pain*" OR "nephropath*" OR "retinopath*" OR "treatment*" OR "protocol").

The filters from 2010 to present and "trials" are added: 154 results were obtained.

Cohort studies could not be found in this search engine, since it only indexes RCTs and systematic reviews.

In this database, we manually selected the duplicates that appeared, since it collects records from other databases, and, consequently, there were eight duplicates.

Appendix A.4. CINAHL

Appendix A.4.1. Clinical Trials

Search strategy: ("diabet* neuropath*") AND ("prevention" OR "control").

Filters applied: "Search all my search terms" "apply related words" "apply equivalent subjects", limit publication date from 2010 to 2020, publication type "clinical trial", "excludes pre-CINAHL", and gender "all": 26 results were obtained.

Appendix A.4.2. Cohort Studies

Strategy: ("diabet* neuropath*") AND ("prevention" OR "control")) AND ("cohort study" OR "cohort analysis").

Filters applied: limit publication date from 2010 to 2020, "Search all my search terms" "apply related words" "apply equivalent subjects", "exclude pre-CINAHL", and gender "all": 13 results were obtained.

Subsequently, all the references resulting from the search in all the databases were added to the bibliographic manager to eliminate possible duplicates between them, obtaining 13 more duplicates, which were eliminated. Finally, a total of 203 duplicates were eliminated.

Appendix B. Individual Characteristics of the Studies Included in the Review

- Study 1: Brock et al., 2019 [53]

Methods: Double-blind, parallel-group, placebo-controlled RCT.

Participants: Adults with TIDM and confirmed symmetrical polyneuropathy. Thirty-nine participants were randomized to receive liraglutide ($N = 19$) or placebo ($N = 20$).

Interventions: To test whether long-term treatment with liraglutide (an injectable drug used for the treatment of diabetes and obesity, acting in the same way as incretins), induces a decrease in inflammation, thus improving neuronal function, and consequently diabetic neuropathy. The duration was 6 weeks with a dose of 1.2 mg/day, continuing until 26 weeks, for a total of 32 weeks.

Results: The primary endpoint was change in latency of early brain evoked potentials. Secondary endpoints were changes in proinflammatory cytokines, cortical evoked potentials, autonomic function and peripheral neurophysiological tests. Compared to placebo, liraglutide reduced interleukin-6 ($p = 0.025$) with similar reductions in other proinflammatory cytokines. However, neuronal function was not altered at the central, autonomic or peripheral levels. Treatment was associated with 3.38 kg ($p < 0.001$) of weight loss and a decrease in urine albumin/creatinine ratio ($p = 0.02$).

Conclusions: The study concluded that treatment with liraglutide reduced interleukin-6 in adults with TIDM but did not improve established DPN. Lowering the systemic level of proinflammatory cytokines could lead to the prevention or treatment of the neuroinflammatory component in the early stages of diabetic neuropathy.

- Study 2: Charles et al., 2011 [62]

Methods: Parallel-group RCT examining the effects of early detection and intensive multifactorial treatment (IT) of patients with TIIDM in primary care on the prevalence of DPN and PAD over 6 years.

Participants: The study sample of 1161 participants was divided into two groups, the routine care group, RC (N = 459) and the intensive multifactorial treatment group, IT (N = 702).

Interventions: The interventions employed were different for the groups, consisting in the IT group of physician and patient education, medication use and promotion of healthy lifestyle, control of hyperglycemia, blood pressure and cholesterol, according to the regimen used in the Steno-2 Study [70], and in the CR group, patients received the standard pattern of diabetes care according to the Danish national recommendations.

Results: No statistically significant effect of IT on the prevalence of DPN and PAD was found compared to CR. The prevalence of an AAI $\leq$ 0.9 was 9.1% (95% CI 6.0–12.2) in the CR group and 7.3% (5.0–9.6) in the IT group. In participants evaluated for vibration detection threshold and light touch sensation the prevalence of at least one abnormal test was 34.8% (26.7–43.0) in the CR group and 30.1% (24.1–36.1) in the IT group.

Conclusion: It was determined that in a population with patients with type 2 diabetes screen-detected, screening followed by IT was not found to lead to a statistically significant difference in the prevalence of DPN and PAD 6 years after diagnosis. Additional information: also called "ADDITION-Denmark"study.

- Study 3: Diabetes Prevention Program Research Group et al., 2015 [57]

Methods: Study of the 3-year Diabetes Prevention Program (DPP) [87] RCT surviving cohort.

Participants: All participants were offered lifestyle training at the end of DPP. Overall, 2776 (88%) of the surviving DPP cohort were followed in the DPP Outcomes Study (DPPOS 2002–2013) and were analyzed by intention-to-treat.

Interventions: The 1996–2001 DPPOS was an RCT comparing an intensive lifestyle intervention or masked metformin with placebo in a cohort selected to be at high risk of developing diabetes. During DPPOS, the lifestyle group received a semiannual booster and the metformin group received unmasked metformin. This research aimed to determine the long-term extent of the beneficial effects of the lifestyle intervention or metformin on diabetes prevention originally demonstrated in the DPP and whether diabetes-associated microvascular complications would be reduced.

Results: During 15 years of follow-up, lifestyle intervention and metformin reduced diabetes incidence rates by 27% ($p < 0.0001$) and 18% ($p = 0.001$), respectively, compared to the placebo group. At year 15, the cumulative incidence of DM was 55%, 56% and 62%, respectively. The end-of-study prevalence of the aggregate microvascular outcome, composed of nephropathy, neuropathy and retinopathy, was not significantly different between treatment groups (11–13%) compared to the overall cohort. However, in women (n = 1887), the lifestyle intervention was associated with a lower prevalence (8.7%) than in the placebo (11%) and metformin (11.2%) groups, with a 21% ($p = 0.03$) and 22% ($p = 0.02$) reduction in the lifestyle group compared to placebo and metformin, respectively. Compared to participants who progressed to DM, those who did not do so had a 28% lower prevalence of microvascular complications ($p < 0.0001$).

Conclusion: This study claims that lifestyle intervention or metformin significantly reduced the development of DM over 15 years in predisposed cohorts, although there were no overall differences in aggregate microvascular outcome between treatment groups. However, those who did not progress to DM had a lower prevalence of microvascular complications than those who did.

- Study 4: Dixit et al., 2014 [54]

Methods: Parallel group RCT. The authors proposed evaluating the effect of moderate-intensity aerobic exercise (40–60% of heart rate reserve) on DPN. Participants: Patients with TIIDM and clinical neuropathy, defined with a minimum score of 7 on the Michigan

Diabetic Neuropathy Scale (MDNS). An experimental group (N = 47) and a control group (N = 40) were included.

Interventions: The experimental group (N = 47) received guidelines for moderate-intensity aerobic exercise, accompanied by standard medical care, foot care education and individual dietary recommendations. The control group (N = 40) received only standard medical care, foot care education and dietary recommendations.

Results: The groups suffered losses of 10 and 11 participants, respectively. Measurements were performed at baseline and at 8 weeks, including nerve conduction studies in the peroneal motor and sural sensory nerve, as well as the MDNS score. For the peroneal nerve, regarding nerve conduction velocity there was a significant difference in the two groups at 8 weeks (p = 0.03). This difference was also observed at 8 weeks in the sural sensory nerve, (p = 0.00). Significant differences were observed in the mean MDNS scores in the two groups at 8 weeks ($p < 0.05$).

Conclusion: It was established that moderate-intensity aerobic exercise may play a valuable role in interrupting the normal progression of MDNS in patients with TIIDM.

- Study 5: Gholami et al., 2018 [61]

Methods: Parallel group RCT. The study set out to examine the effects of aerobic training on nerve conduction velocity and action potential amplitude in lower limbs.

Participants: Men patients with TIIDM and DPN, 24 volunteers randomized into two groups: exercise group (N = 12) and control group (N = 12).

Interventions: Aerobic training consisted of 20–45 min walking or running at 50–70% of the heart rate reserve in three sessions per week for 12 weeks. Before and 48 h after the experimental period, nerve conduction studies were performed and blood samples were taken to analyze HbA1c, and fasting and 2 h postprandial glucose concentration.

Results: Sural nerve sensory conduction velocity (SNV) in the exercise group was significantly increased (from 35.2 $\pm$ 4.3 m/s to 37.3 $\pm$ 6.2 m/s) compared to the control group (p = 0.007). Changes in motor NCV in peroneal and tibial nerves and action potential amplitude (APAN) in all nerves studied were not significant between groups ($p > 0.05$). In addition, HbA1c decreased to a greater extent in the exercise group compared to the control (p = 0.014).

Conclusion: It was determined that aerobic exercise training may have the potential to hinder DPN progression by improving NCV. Given the scarce evidence in this domain, related to exercise, the mechanisms should be studied in the future.

- Study 6: Gholami et al., 2020 [59]

Methods: Parallel-group RCT. In relation to the previous study, in this case, the investigators evaluated the effect of physical training on superficial femoral artery (SFA) measurements and neuropathic symptoms in patients with DPN to observe the relationship of DPN with PAD.

Participants: Thirty-one volunteers with established DPN randomly assigned to the experimental (N = 16) and control (N = 15) groups.

Interventions: The experimental group performed cycling exercise (50–70% of heart rate reserve, 30–45 min, three sessions/week) for 12 weeks. Before and 48 h after the experimental period a 5-min flow-mediated dilation (FMD) response, changes in intima media thickness and basal diameter in SFA using color Doppler ultrasound and neuropathic score in MDNS were assessed as primary outcomes, and fasting glucose level, HbA1c and neuropathic score as secondary outcomes.

Results: The FMD percentage increased significantly in the experimental group (from 3.2 $\pm$ 1.1% to 5.7 $\pm$ 1.2%) compared to the control condition (p = 0.0001). However, there were no significant alterations in the basement membrane diameter and intima media thickness ($p < 0.05$). Significant improvements in fasting glucose, HbA1c and Michigan diabetic neuropathy score (MDNS) after exercise intervention (all $p < 0.05$) were also observed. Linear regression analysis indicated that the change in MDNS was significantly associated with change in HbA1c (p = 0.001) and FMD (p = 0.001).

Conclusion: This finding may be clinically of great importance, as metabolic and vascular factors have been indicated to be involved in the development of DPN.

- Study 7: Gong et al., 2011 [58]

Methods: Cohort study of participants in a parallel-group RCT. A 20-year follow-up study of the original participants was conducted to compare the incidence of microvascular complications in the combined intervention group versus the control group.

Participants: The original RCT involving 577 adults with impaired glucose tolerance (IGT) who were randomly assigned to a control group or the lifestyle intervention group (divided into three subgroups: diet, exercise, and diet plus exercise). Follow-up information was obtained on 542 (94%) of the original 577 participants.

Interventions: The aim of the diet intervention was to increase vegetable intake and reduce alcohol and sugar consumption of the participants and, in those who were overweight or obese, to reduce total calorie intake in order to lose weight. In the case of the exercise intervention, this consisted of increasing leisure time physical activity. The interventions were carried out over 6 years.

Results: The cumulative incidence of severe retinopathy was 9.2% in the combined intervention group and 16.2% in the control group ($p = 0.03$). After clinical and age adjusting, the incidence of severe retinopathy was 47% lower in the intervention group than in the control group ($p = 0.048$). No significant differences were found in the incidence of severe nephropathy ($p = 0.96$) or in the prevalence of neuropathy ($p = 0.89$) among survivors after 20 years.

Conclusion: Lifestyle intervention over 6 years in persons with IGT was associated with a 47% reduction in the incidence of severe vision-threatening retinopathy over a 20-year interval, mainly due to the lower incidence of diabetes in the intervention group. However, no similar benefits were observed for nephropathy or neuropathy. Additional information: also called "China Da Qing Diabetes Prevention Outcome Study".

- Study 8: Ismail-Beigi et al., 2010 [55]

Methods: A parallel-group RCT, called the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study [72], aimed to determine whether lowering blood glucose levels reduced the rate of microvascular complications in patients with TIIDM.

Participants: Patients with DM and with high HbA1c concentrations (>7.5%) and cardiovascular disease (or two or more cardiovascular risk factors) were randomized by central randomization to the intensive (target HbA1c of <6.0%) or standard (7.0–7.9%) glycemic control group. 10,251 patients were randomized, ($N = 5128$) to the intensive glycemic control group and ($N = 5123$) to the standard group.

Interventions: Intensive glycemic control compared with standard glycemic therapy. In this analysis, the predefined composite outcomes were: dialysis or renal transplantation, high serum creatinine (>291.7 μmol/L) or retinal photocoagulation or vitrectomy (first composite outcome), or peripheral neuropathy plus the first composite outcome (second composite outcome). Thirteen secondary measures of renal, ocular, and peripheral nerve function were also assessed. Investigators and participants were aware of the treatment group assignment. An analysis was performed for all patients who were evaluated for the microvascular outcome on the basis of the treatment assignment, regardless of treatments received or adherence to therapies.

Results: Intensive therapy was discontinued before the end of the study due to higher mortality in that group, and patients transitioned to standard therapy. At transition, the first composite outcome was recorded in 443 of 5107 patients in the intensive group versus 444 of 5108 in the standard group (HR 1.00, 95% CI 0.88–1.14; $p = 1.00$), and the second composite outcome was observed in 1591 of 5107 versus 1659 of 5108 (0.96, 0.89–1.02; $p = 0.19$). The results were similar at the end of the study, first composite outcome 556 of 5119 vs. 586 of 5115 (HR 0.95, 95% CI 0.85–1.07, $p = 0.42$); and the second 1956 of 5119 vs. 2046 of 5115, respectively (0.95, 95% CI 0.89–1.01, $p = 0.12$)). Intensive therapy did

not reduce the risk of microvascular outcomes, but delayed the onset of albuminuria. Six secondary end-of-study measures favored intensive therapy ($p < 0.05$).

Conclusion: The research concludes that the microvascular benefits of intensive therapy must be weighed against increased total and cardiovascular disease-related mortality, weight gain, and high risk of severe hypoglycemia, so as ACCORD proved, intensive glycemic control therapy does not provide significant benefits in patients with TIIDM.

- Study 9: Look AHEAD Research Group et al., 2017 [56]

Methods: The study Look AHEAD (Action for Health in Diabetes) [88] was a parallel-group RCT. It examined whether the intensive lifestyle intervention weight loss decreased cardiovascular morbidity and mortality in overweight or obese adults with TIIDM. Due to the nature of the study, patients and center investigators were not blinded. In addition, the coordinating center staff members responsible for data management and statistical analysis were also not blinded.

Participants: Beginning in 2001, a total of 5145 overweight and obese individuals with TIIDM were randomized to intensive intervention (ILI) (N = 2570) or diabetes support and education (DSE) control group (N = 2575) using a web-based management system at the study coordinating center at Wake Forest School of Medicine (Winsto-Salem, NC, USA). Randomization was stratified by clinical center and was not disclosed to clinical staff responsible for obtaining data on study outcomes.

Interventions: Intensive intervention (ILI) or diabetes support and education (DSE) control group. Interventions ended in September 2012, 9–11 years after randomization, but both groups continued to be followed for primary and secondary outcomes. Neuropathy assessments included MNSI completed at baseline in all participants, 5145 (ILI N = 2570; DSE N = 2575) and repeated annually thereafter, as well as SW monofilament testing performed in 3775 participants (ILI N = 1905, DSE N = 1870) at 1 and 2.3 years after intervention discontinuation.

Results: At baseline, the MNSI questionnaire scores were 1.9 ± 0.04 and 1.8 ± 0.04 in the ILI and DSE groups, respectively (difference not statistically significant). After 1 year, when weight loss was maximal in the ILI group ($8.6 \pm 6.9\%$) compared with DSE ($0.7 \pm 4.8\%$), the respective MNSI scores were 1.7 ± 0.04 and 2.0 ± 0.04 ($p \leq 0.001$). Subsequently, scores increased gradually in both groups, but remained significantly lower in the ILI group during the first 3 years and at the end of follow-up. In both groups, there was a significant association between the MNSI scores and changes in body weight, HbA1c and plasma lipids. There was no significant difference between groups in participants with MNSI physical examination scores $\geq$2.5, considered indicative of DN. Light tactile sensation measured separately in the right and left great toes did not differ between ILI and DSE, but when the data were combined for both toes, a light touch was better preserved in the ILI group.

Conclusion: It was determined that the ILI group had a significant decrease in DPN based on questionnaire diagnosis, which was associated with the magnitude of weight loss. In both the ILI and DSE groups, changes in MNSI score were also associated with changes in HbA1c and lipids. There were no significant effects of ILI on DPN physical examination measures performed 1–2.3 years after completion of the active intervention, except for light touch sensation, which was significantly better in the ILI group when measures were combined for both toes.

- Study 10: Martin et al., 2014 [15]

Methods: Surviving cohort study from the RCT Diabetes Control and Complications Trial and its follow-up study Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) [87,88]. The authors described the development and progression of neuropathy and related findings among patients with TIDM at 14 years after intervention.

Participants: Patients with TIDM. There were a total of 1441 (100%) DCCT participants, of whom 1375 (95.4%) agreed to participate in EDIC, of whom 1274 (92.7%) were active

in year 13 in EDIC, of whom 1226 (96.2%) were evaluated for CAN and 1186 (93.1%) for DPN [89].

Interventions: Intensive glycemic control vs. standard control. The primary outcome of DPN was assessed by clinical symptoms, signs and results of nerve conduction studies during DCCT and repeated in EDIC in year 13/14. CAN was assessed by the R-R response to stimulated breathing, Valsalva ratio and blood pressure response during years 13/14 and 16/17. In addition, symptoms reflecting neuropathic pain and autonomic function (including hypoglycemia awareness) were collected annually in EDIC using standardized questionnaires; peripheral neuropathy was also assessed annually using the MDNS. Genitourinary function assessments were collected in EDIC year 10.

Results: Intensive therapy during DCCT significantly reduced the risk of DPN and CAN at the end of DCCT (64% and 45%, respectively, $p < 0.01$). The prevalence and incidence of DPN and CAN remained significantly lower in the intensive therapy DCCT group compared to the conventional therapy DCCT group until year 13/14 of EDIC [90].

Conclusion: It was established that the persistent effects of prior intensive therapy on neuropathy measures over 14 years of EDIC largely mirror those observed for other complications of DM. DCCT/EDIC provides important information on the influence of glycemic control and the clinical course of DN and, most importantly, on how to prevent neuropathy in patients with TIDM.

- Study 11: Pop-Busui et al., 2013 [60]

Methods: Cohort study from the parallel-group RCT Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) [91] published in 2009. This trial demonstrated similar long-term clinical effectiveness of insulin-sensitizing (IS) versus insulin-providing (IP) treatments for patients with TIIDM on cardiovascular outcomes in a cohort with documented coronary artery disease.

Participants: A total of 2159 participants with TIIDM with documented coronary artery disease. IS ($N = 1080$), IP ($N = 1079$).

Interventions: Randomized glycemic control strategy of insulin-sensitizing (IS) versus insulin-providing (IP) treatments for TIIDM on the prevalence and incidence of DPN. DPN (defined as Michigan Neuropathy Screening Questionnaire (MNSI) > 2 clinical examination score) was assessed at baseline and annually for 4 years. Prevalence and incidence of DPN were compared by intention-to-treat models using generalized estimating equations logistic models for prevalence and Kaplan–Meier estimates and Cox regression models for incidence rates.

Results: The results were obtained for 2159 participants in the BARI 2D study (70% male) with baseline values and at least one follow-up MNSI score (mean age 62 $\pm$ 9 years, mean HbA1c 7.7 $\pm$ 1.6%, duration of diabetes 10 $\pm$ 9 years). There was no difference in the prevalence of DPN between the IS and IP groups during the 4 years of follow-up. In 1075 BARI 2D study participants without DPN at baseline, the 4-year cumulative incidence rate of DPN was significantly lower in the IS (66%) than in the IP strategy group (72%) ($p = 0.02$), which remained significant after adjusting for HbA1c ($p = 0.04$). In subgroup analyses, the IS strategy had a greater benefit in men (Hazard Ratio 0.75 [99% 95% CI 0.58–0.99], $p < 0.01$).

Conclusion: Among patients with TIIDM followed for up to 4 years in BARI 2D, a glycemic control therapy with IS significantly reduced the incidence of DPN compared to IP therapy and may provide more benefits for men.

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Decreased Need for Correction Boluses with Universal Utilisation of Dual-Wave Boluses in Children with Type 1 Diabetes

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Abstract: Insulin pumps offer standard (SB), square and dual-wave boluses (DWB). Few recommendations exist on how to use these dosing options. Several studies suggest that the DWB is more effective for high-fat or high-carbohydrate meals. Our objective was to test whether time in range (TIR) improves in children with type 1 diabetes (T1D) using the universal utilization of the dual-wave boluses for all evening meals regardless of the composition of the meal. This was a 28-day long prospective randomized open-label single-center crossover study. Twenty-eight children with T1DM using a Medtronic 640G pump and continuous glucose monitoring system were randomly assigned to receive either DWB or SB for all meals starting from 6:00 p.m. based solely on the food carbohydrate count. DWB was set for 50/50% with the second part extended over 2 h. After two weeks patients switched into the alternative treatment arm. TIR (3.9–10 mmol/L), time below range (TBR) (<3.9 mmol/L) and time above range (TAR) (>10 mmol/L) and sensor glucose values were measured and compared between the groups. Twenty-four children aged 7–14 years completed the study according to the study protocol. There were no statistically significant differences in mean TIR (60.9% vs. 58.8%; $p = 0.3$), TBR (1.6% vs. 1.7%; $p = 0.7$) or TAR (37.5 vs. 39%; $p = 0.5$) between DWB and SB groups, respectively. Subjects in the DWB treatment arm administered significantly less correction boluses between 6 p.m. and 6 a.m. compared to those in the SB group (1.2 ± 0.8 vs. 1.7 ± 0.8, respectively; $p < 0.01$). DWB for evening meals in which insulin is calculated solely on the food carbohydrate content did not improve TIR compared to standard bolus in children with T1D. However, DWB enabled to use significantly less correction boluses to achieve euglycemia by the morning compared to the SB.

Keywords: type 1 diabetes; insulin pump therapy; CGM; meal bolus; dual wave bolus; standard bolus

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1. Introduction

Despite of the wide use of modern diabetes technology among the pediatric population in the last decade [1] metabolic control still remains suboptimal in many cases [2]. One of the main obstacles preventing the achievement of treatment goals is controlling postprandial hyperglycemia. Information about how to manage glycemic excursions after meals is relatively limited. It is well known that the timing of the meal boluses is essential [3], but when high-fat or high-carbohydrate meals are consumed, optimal postprandial glycaemia is still hard to achieve [4].

Modern insulin pumps offer a variation of preprogrammed boluses: standard bolus (SB) square and dual-wave boluses (DWB). However, only few recommendations exist on how to benefit from the use of different bolus administration types [5]. The International Society for Pediatric and Adolescent Diabetes states that the impact of dietary fat and protein should be considered when determining the insulin bolus dose and delivery [5], but the optimal insulin bolus dose for meals high in fat and protein is undefined [4].

The use of a fat-protein unit has been previously proposed [6], but the method seems too complex for everyday use for most patients and was associated with a higher rate of

hypoglycemia [7]. According to previous research the prolonged bolus administration methods might have certain advantages for high-fat or high-carbohydrate meals [3,8–11]. For example, Jones et al. showed that the 8-h DWB was superior compared to the single wave bolus and provided the best glycemic control after a pizza meal [9]. Chase et al. demonstrated that the dual wave option where 70% is administered as a standard bolus and 30% as a square-wave over 2 h provided the most effective method of insulin administration for a meal high in carbohydrates, fat and protein [10]. O'Connell et al. confirmed the superiority of the dual-wave bolus (50% and 50% over 2 h) for low glycemic index foods as well [11]. These data suggest the DWB utilization might be the preferred insulin delivery mode as it resembles the physiologic postprandial biphasic insulin secretion described first by Curry et al. [12]. Optimal distribution of the DWB parts and duration of the extended part seems to be dependent on specific food content. Lopez et al. proposed that meals with a high fat and protein content require at least 60% of the total insulin upfront to control the initial postprandial glucose rise [13]. To our best knowledge there have been no controlled randomized studies investigating the benefit of one bolus type over the other in a real-life setting over an extended period. Previous studies have been mostly conducted in hospital-based settings and limited to analyze the effect of specific foods on postprandial hyperglycemia in very few individuals [8–10].

Therefore, we decided to perform a randomized controlled crossover study comparing SB to the DWB in a real-world setting. Considering that carb counting is difficult to master for many of our patients and substantial inter-individual differences exist in insulin dose requirements for fat and protein [14–16], high glycemic index and low glycemic index carbs [4], we decided to calculate the insulin dose solely based on the carbohydrate content in the food. Therefore, we decided to use DWB with a 50%/50% proportion with the second part extended for 2 h in order to achieve optimal insulin coverage for a typical Western type meal consumed for dinner. The main objective of the study was to test the hypothesis that the universal utilization of the DWB for all meals starting from 6:00 p.m. for 2 weeks improves time in range (TIR) in children with type 1 diabetes (T1D) compared to the SB use. The decision to limit the intervention period only to evening meals was carried out from the practical reason as this is the time when parents could supervise their children and follow the study plan.

2. Materials and Methods

This was a 28-day long prospective randomized open-label single-center crossover clinical study with a 14-day long run-in phase. Recruitment was carried out during an outpatient visit. All subjects and parents signed an informed consent form approved by the Research Ethics Committee of The University of Tartu before entering the study. The trial was registered in ClinicalTrial.gov under the identifier: NCT04668612. We used the following inclusion criteria: age 7–18 years, T1D duration over a one year, CGM and insulin pump treatment for at least 3 months prior to the recruitment, estimated HbA1c based on the 14-days CGM report below 8.5%, and a daily insulin dose of more than 0.5 international units per kilogram. A power-analysis was conducted and according to Rigby et al. the minimum sample size to demonstrate a clinically relevant change in TIR (10%) would have been 16 subjects [17]. All patients used the Medtronic MiniMed 640G pump with the Enlite sensors, because this system was most commonly used in our diabetic center at that time and equipped with the basal insulin auto suspend before low function. This was turned on in all study participants at the same level: insulin administration was temporarily suspended when the blood sugar of 3.9 mmol/L was predicted. Subjects with known diabetes complications or with elevated tissue transglutaminase IgA antibodies in the last two years and children who developed acute viral infections during the week preceding the recruitment were excluded. During the first study visit, which was the only on-site visit, we collected clinical data of subjects (age, gender, height, body weight, body mass index, pubertal stage according to Tanner scale) and evaluated the eligibility for the study. We titrated insulin doses, set up a bolus wizard in their pump and instructed patients

accordingly. After the first study visit patients entered the run-in phase for two weeks. During the run-in period we optimized treatment as much as possible and titrated insulin doses twice, based on the patients CGM reports of the preceding week sent to us via e-mail. Thereafter patients were re-evaluated for eligibility according to the study inclusion and exclusion criteria. We implemented two additional exclusion criteria: patients with a basal insulin proportion of more than 55% or who developed an acute viral infection during the run-in phase, were excluded.

After that, patients were randomly assigned into DWB or SB arms. After 14 days subjects switched into the alternative treatment arm. The study structure is shown in Figure 1. In the subsequent 2 weeks subjects received either DWB (50% of the insulin delivered as a bolus 10–15 min prior to the meal and 50% delivered over two hours) or a SB (100% of the insulin delivered as a bolus 10–15 min prior to the meal) for every meal after 06:00 p.m. Repeated meals were allowed provided mealtimes took place at least two hours apart. Eating with a shorter interval was only allowed in case of hypoglycemia <3.9 mmol/L. In case of hyperglycemia above 12 mmol/L for longer than 2 h after the start of the food bolus administration a correction bolus via standard bolus for both arms was recommended to the target value of 7.0 mmol/L. Prior to the study participants were not provided with specific instructions about CGM arrow trend management. Participants were encouraged to stick to their regular meal schedule and typical diet during the different treatment periods. During the 28-day long intervention period patients and parents were discouraged to change their basal doses on their own.

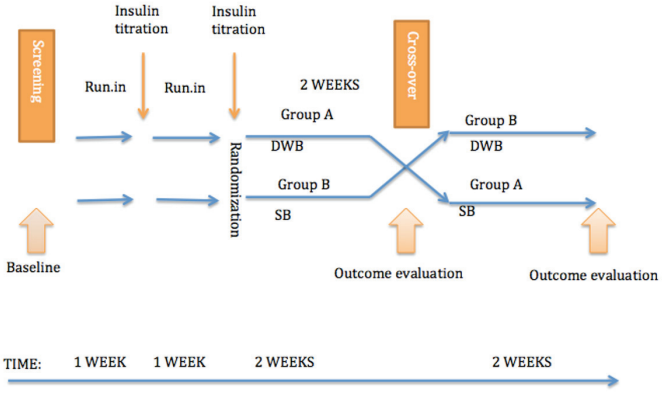

Figure 1. The structure of the study. DWB (dual-wave bolus); SB (Standard bolus).

The use of temporary basal was not recommended during 06:00 p.m. to 06:00 a.m. Within the intervention period correction bolus doses and food bolus coefficients were not modified, basal rate was adjusted only in the case of definite necessity as judged by the patient's physician. After receiving two weeks of SB or DWB CGM data from the pump were downloaded at home and sent to the investigators. Time in range (TIR), (>3.9 mmol/L and below 10 mmol/L), time below range (TBR) (<3.9 mmol/L) and time above range (TAR) (>10 mmol/L) range were recorded from the pump after finishing 2 weeks of each bolus type. Compliance to the study terms was evaluated before the data analysis. Data of the study subject was only included to our analysis if at least 80% of boluses were administered according to the bolus type the patient was randomized to CGM data were analyzed with the Care Link Professional software, which is provided by the manufacturer for the Medtronic system users. Comparison of TIR, TBR and TAR between the two groups was performed using Student's *t*–test. *p*-value 0.05 or less was considered as significant. The mean area under the curve (AUC) for the period from the first evening meal bolus after 6:00 p.m until 6:00 a.m. was calculated by the trapezoidal method [18]. The Statistical analysis was performed with R version 4.1.0 for Windows.

3. Results

Out of the screened 32 patients, 31 were enrolled and gave written consent, 1 did not meet the inclusion criteria. In total 31 participants entered the run-in-period, 3 patients had problems uploading sensor data at home and thus 28 patients completed the run-in and underwent randomization. Four participants cancelled the study's active arm due to the failure of following the study plan. Twenty-four patients (10 boys) with T1D, 7–14 years completed the study and their data was included into the analysis. The average age of the study subjects was 10.8 ± 2.0 years (Table 1). Before the run-in period, estimated HbA1c provided by the sensor CGM report was 7.6 ± 0.7%. Mean basal insulin proportion was 37.8 ± 10.7% at study entry. The average daily insulin dose per kilogram of the participants was 0.8 ± 0.1 IU/kg/d. After run-in and before randomization the estimated HbA1c of the study participants reduced to the 7.4 ± 0.5% (57.4–5.2 mmol/mol) and average basal insulin proportion remained almost unchanged on the level of 39 ± 10.5%. Mean TIR, mean TBR and mean TAR was not significantly different between the treatment arms as shown in Figure 2. The mean AUC calculated for the period from the first evening meal bolus after 6:00 p.m. until 6:00 a.m. was not statistically different between the SB and DWB study groups (98.73 ± 12.4 mmol/L × h vs. 101,63 ± 14.8 mmol/L × h, respectively; $p = 0.243$). Mean estimated HbA1c after two weeks of DWB or SB usage did not differ either and was 7.4 ± 0.5% for both treatment arms.

Table 1. Clinical characteristics of study subjects [1].

Number of Study Subjects = 24	
Gender (boys/girls)	10/14
Age (years)	10.8 ± 2.0
Weight (kg)	44.2 ± 12.6
BMI (kg/m^2)	18.7 ± 3.4
Predicted glycated hemoglobin A1c (%)	7.6 ± 0.7
Mean sensor glucose (mmol/L)	9.5 ± 1.1
Daily insulin dose per kg (IU/kg/d)	0.8 ± 0.1
Basal insulin (%)	37.8 ± 10.7
Bolus insulin (%)	62.2 ± 10.7

[1] Data is given as mean ± standard deviation.

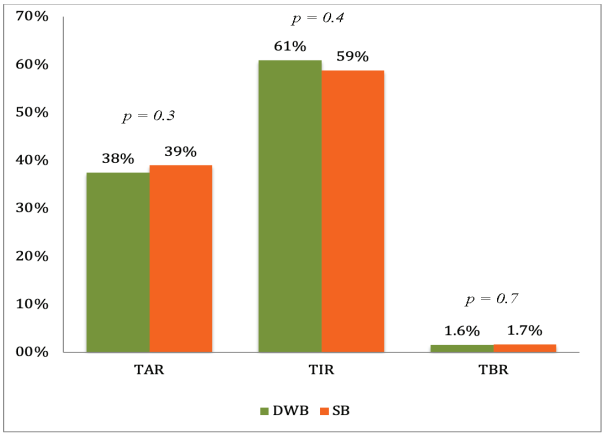

Figure 2. Comparison of mean TIR (Time in Range), TAR (Time above Range) and TBR (Time Below Range) between the treatment arms. DWB (dual-wave bolus); SB (Standard bolus).

Patients in the DWB treatment arm administered significantly less correction boluses between 6 p.m. and 6 a.m. than in the SB arm (1.2 ± 0.8 vs. 1.7 ± 0.8; $p < 0.01$). Eight patients in the SB had to use at least 2 correction boluses during the night, whereas in the DWB

group only four. The mean glucose profiles in both treatment arms are shown in Figure 3. The only statistically significant difference in mean glucose levels was seen 1 h after administering the bolus when it was significantly higher in the DWB arm compared to the SB arm (9.8 ± 1.6 mmol/L vs. 8.9 ± 1.8 mmol/L; $p = 0.01$), but with no differences at 2–6 h after the bolus. The mean number of basal suspension episodes from 6:00 PM to 06:00 AM did not differ between the DWB and SB treatment arms (0.99 ± 0.5 vs. 1 ± 0.6, respectively $p = 0.89$), neither were different the mean cumulative duration of suspension (2 h 27 min vs. 2 h 28 min, respectively; $p = 0.887$). Four patients in the DWB treatment arm improved their TIR be 14% to 23% whereas two patients increased their TIR by 13% and 14% in the SB treatment arm.

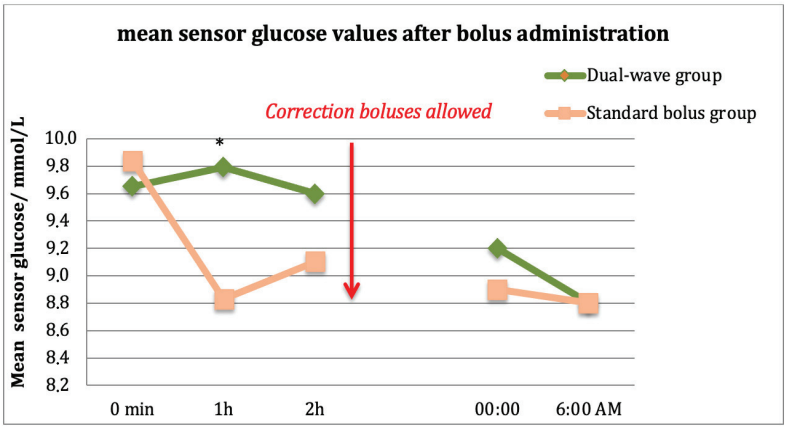

Figure 3. Comparison of mean sensor glucose values after main evening meal bolus administration between treatment arms. * Statistically relevant difference in sensor glucose between treatment arms, $p = 0.01$.

4. Discussion

We described the effect of the systematic use of the DWB on the TIR, TBR and TAR parameters in pediatric T1D patients in a real-life setting. This study was not able to demonstrate the direct benefit of such universal usage of the DWB instead of the SB for the evening meals. These results might indicate the non-superiority of one bolus type compared with the other, but could also derive from the small effect of the evening boluses on the TIR, which is affected by other glycemic and bolusing events during the day as well. In order to identify the preferred bolus type we also calculated the mean AUC for postprandial blood sugar excursions, but this was not different between the treatment arms. However, it should be taken into an account that in the current study design where additional meals and correction boluses were allowed and used 2 h after the main evening meal the mean AUC is also not an ideal parameter to compare treatment arms. In this study we showed a significantly reduced need for correction boluses in the DWB treatment arm.

Many authors have proposed that postprandial hyperglycemia is controlled more effectively when the insulin dose is calculated for both the carbohydrates and fat-protein nutrients [4–7] yet calculating the fat protein unit has not become standard practice since today although suggested a while ago. The main cause for this might be the complexity of this calculation and unwillingness of the patients to use this formulation for their prandial insulin dose. Until today the specific instructions are lacking for the accurate fat-protein adjustments and therefore we did not cover fat and proteins in our study. We hypothesized that the DWB, when calculated solely on the carbohydrate content of food, might be more effective than a SB in controlling late postprandial hyperglycemia, because it also covers the late effects of fats and proteins on blood sugar [4]. Several previously published studies support our suggestion, showing that postprandial hyperglycemia is more effectively

controlled, when prolonged methods of insulin administration are used instead of the SB [8–10,13]. However, these conclusions are largely based on blood glucose analyses in very few individuals after single meal analysis in relatively controlled settings and the long-term benefit of the prolonged bolus types' utilization has not been demonstrated. Bell et al. proposed that to achieve target glucose control following a high fat and high protein meal in adults a 30%/70% split over 2.4 h is required [14]. Lopez et al., when studying different split variations after a meal high in carbohydrates, calories and fat, demonstrated that a SB controlled the blood glucose excursion for the first 120 min only, after which there was a progressive rise in glucose level [13]. We also found that the SB provides significantly better control over the first hour of postprandial hyperglycemia, but in contrast to some previous studies [6,12], no difference in sensor glucose level was evident between the treatment arms at 2–6 h after the main evening meal bolus. Bell et al. showed that the optimal combination bolus split to maintain postprandial glycemia for a high-fat and high-protein meal was 60/40% or 70/30% delivered over 3 h [14], therefore our 50/50% split choice over 2 h might explain these differences in the results. Still in our study late hyperglycemia might have been controlled more effectively in the DWB arm, because they required a significantly lower number of correction boluses compared to the SB treatment arm to achieve normoglycemia by the morning. The difference in the number of correction boluses could be explained by the direction of CGM trend arrows 2 h after main evening meal. Patients in the SB treatment arm saw a mean blood sugar value of 9.1 mmol/L with a trend arrow up and therefore probably did a decision in favor to give an additional correction bolus whereas those in the DWB treatment arm saw a mean blood sugar value of 9.0 mmol/L with the trend arrow down and therefore decided to delay or skip the correction bolus. We can only speculate about the possible late postprandial blood sugar rise in SB treatment arm without additional corrections and actual late postprandial blood sugar control in the DWB group in the case of more active usage of correction boluses. Campell et al. also clearly showed that an additional 30% insulin administered 3 h after the high fat and protein meal provided additional benefit to the postprandial glucose control and made it comparable with a meal without any fat [19]. Future studies may reveal if universal utilization of the DWB with a higher initial insulin proportion as for example 70 percent initially and 30 as a extended bolus may provide better coverage for the first hour of postprandial hyperglycaemia as well as for late hyperglycaemia. Four patients benefitted from the DWB scheme for the dinner as their TIR improved more than 10% during the two weeks of DWB administration whereas in the SB arm there were two patients who improved their TIR more than 10%. Such result may be related to some unidentified random factors, but could also indicate, that personal food content preferences might play a role in this. The analysis of food content and its relation to postprandial glucose excursion in relation to different bolus types was beyond the scope of the current work as we attempted to prove the superiority of the dual-wave bolus regardless of food composition. However, it seems that the universal utilization of only one bolus type is not justified and that the choice of the bolus type should be based on the fat and protein content in the food. Nevertheless, the universal use of DWB did not increase the incidence of hypoglycemia as TBR, basal insulin suspension duration and frequency did not differ between the treatment arms. This can be postulated with a reservation as the data about prevention of hypoglycaemia by additional food was lacking, but the systematic use of the DWB for dinner seems to be a safe option in patient using insulin pump with auto suspension function.

The major limitation of our study is the small number of participants for the randomized trial and the risk for type 1 error, when making conclusions. However, to our best knowledge this is the only randomized trial comparing different types of boluses in real-life settings. Many confounders could have influenced the change in TIR and the need for correction boluses, but their impact of the confounders was diminished due to the randomized design of our study. Another significant limitation is the absence of a meal composition analysis, which should significantly influence the pattern of postprandial hyperglycemia, but this was beyond the scope of our study. Meal content analysis could

have given addition insight into the benefits and disadvantages of one or the other bolus type, but our intention was to test whether the DWB is suitable for all meals typical for a Western diet [20].

5. Conclusions

In conclusion, the present study demonstrated that the universal DWB utilization for evening meals in which insulin is calculated solely on the food carbohydrate content did not improve TIR compared to standard bolus. The DWB did not result in lower late postprandial hyperglycemia, but DWB users used fewer correction boluses to achieve euglycemia by the morning. Future studies are needed to find the most effective bolusing type for optimal postprandial blood sugar control.

Author Contributions: A.P. conceived the idea for the study; A.P. is responsible for funding acquisition; A.P., V.T. and M.L. worked on the methodology for the study; M.L. and A.P. conducted the study; M.L. is responsible for the formal analysis. Original draft preparation was carried out by M.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Research Ethics Committee of the University of Tartu (protocol code 307/T-17 and the date of approval was 20 April 2020).

Informed Consent Statement: All subjects provided informed consent to participate in the study.

Data Availability Statement: The trial was registered in https://clinicaltrials.gov/ct2/show/NCT04668612 under the identifier: NCT04668612.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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The Diabetes Transition of Hospital Care (DiaTOHC) Pilot Study: A Randomized Controlled Trial of an Intervention Designed to Reduce Readmission Risk of Adults with Diabetes

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Abstract: Hospital readmission within 30 days of discharge (30-day readmission) is a high-priority quality measure and cost target. The purpose of this study was to explore the feasibility and efficacy of the Diabetes Transition of Hospital Care (DiaTOHC) Program on readmission risk in high-risk adults with diabetes. This was a non-blinded pilot randomized controlled trial (RCT) that compared usual care (UC) to DiaTOHC at a safety-net hospital. The primary outcome was all-cause 30-day readmission. Between 16 October 2017 and 30 May 2019, 93 patients were randomized. In the intention-to-treat (ITT) population, 14 (31.1%) of 45 DiaTOHC subjects and 15 (32.6%) of 46 UC subjects had a 30-day readmission, while 35.6% DiaTOHC and 39.1% UC subjects had a 30-day readmission or ED visit. The Intervention–UC cost ratio was 0.33 (0.13–0.79) 95%CI. At least 93% of subjects were satisfied with key intervention components. Among the 69 subjects with baseline HbA1c >7.0% (53 mmol/mol), 30-day readmission rates were 23.5% (DiaTOHC) and 31.4% (UC) and composite 30-day readmission/ED visit rates were 26.5% (DiaTOHC) and 40.0% (UC). In this subgroup, the Intervention–UC cost ratio was 0.21 (0.08–0.58) 95%CI. The DiaTOHC Program may be feasible and may decrease combined 30-day readmission/ED visit risk as well as healthcare costs among patients with HbA1c levels >7.0% (53 mmol/mol).

Keywords: rehospitalization; transition care; pilot study; prospective randomized trial

1. Introduction

Hospital readmission within 30 days of discharge (30-day readmission) is a high-priority quality measure and cost target [1]. People living with diabetes are at higher 30-day readmission risk than those without diabetes [2–4]. Several interventions have shown promise for reducing the readmission risk of diabetes patients in mostly observational studies [4]. Selecting patients at high readmission risk for intervention may enable a more efficient use of resources than applying interventions broadly without regard to readmission risk. No previously published randomized controlled trials (RCTs) have tested an

intervention designed to reduce readmission risk in patients with diabetes and high readmission risk as predicted by a validated tool. We previously reported on the development and validation of the Diabetes Early Readmission Risk Indicator (DERRI™) [5–7], which predicts 30-day readmission risk in diabetes patients. The aim of the current pilot RCT was to explore the feasibility and potential efficacy of a novel, multi-component intervention, the Diabetes Transition of Hospital Care (DiaTOHC) Program, on 30-day readmission risk in adult patients with diabetes at high risk based on the DERRI™.

2. Materials and Methods

2.1. Study Design, Setting, and Ethics

This was a non-blinded pilot RCT with two parallel arms that compared usual care (UC) to the DiaTOHC Program (Intervention) at Temple University Hospital, an urban, academic, safety-net hospital in Philadelphia, PA. The protocol was registered in the National Clinical Trials Registry (NCT03243383) and approved by the Temple University Institutional Review Board (#24306). This study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects. The study was registered on ClinicalTrials.gov (NCT03243383), where the protocol is accessible.

2.2. Participants and Randomization

Inclusion criteria were an established diagnosis of diabetes, defined by preadmission use of a diabetes-specific medication and/or documentation of the diagnosis in the medical record, age ≥18 years, high predicted risk of 30-day readmission (≥27%) based on the DERRI™ [6], and hospital admission to a non-critical care unit. Exclusion criteria were pregnancy, binge drinking (at least 5 alcoholic drinks for males or 4 alcoholic drinks for females on the same day), drug abuse within 3 months before admission, receiving palliative care during the hospitalization, participation in another readmission risk reduction program, planned or actual transfer to another hospital or subacute facility, discharge expected within 12 h, lack of access to a phone, living more than 30 miles away from the hospital, HbA1c <5.7% (39 mmol/mol), and inability to speak English. After enrollment, subjects were excluded upon transfer to another hospital or subacute facility, discharge to hospice or a long-term care facility, signing out against medical advice, or inpatient death. We screened a computer-generated list of patients who were admitted to non-critical care units with orders for routine point-of-care blood glucose testing. If the primary hospital team approved, then potentially eligible patients were approached in their hospital room for further screening and informed consent.

Subjects were randomly assigned with a computer-generated randomization scheme 1:1 in randomly permuted blocks of 2, 4, or 6 to receive either the Intervention or UC. The study statistician (H.Z.) generated the random allocation sequence. Group assignments were placed in sealed envelopes and revealed sequentially as subjects were randomized. Study coordinators enrolled participants and assigned them to interventions based on the allocation sequence.

2.3. Usual Care and Intervention

Subjects in the UC group received the standard discharge instructions, education, medication reconciliation, and follow-up according to routine practice. Discharge instructions were generated using the Epic Hyperspace® (Verona, WI, USA) electronic health record (EHR), which is integrated between the inpatient and outpatient settings. Education was provided by bedside nurses and hospital providers at their discretion using stock materials in the EHR (ExitCare Clinical References). Subjects received training by a bedside nurse on using a glucometer and insulin as needed. Diabetes therapy upon discharge was determined by the primary team. Discharge instructions were routinely sent to the primary care provider (PCP) either by fax or EHR. Subjects received a phone call within 4 days after discharge from a hospital-employed community health worker that included checking on the health of the subject, confirming follow-up appointments and access to medications,

and answering questions. Problems were referred to a nurse navigator, all of whom had a nurse practitioner degree, for further management. A transition-of-care appointment was scheduled for all patients with a PCP within 10 days of discharge. This appointment focused on medication reconciliation, review of discharge instructions, and updating care needs since hospital discharge.

Subjects in the Intervention group received the DiaTOHC Program in addition to the UC described above. The DiaTOHC Program has three components: (1) patient-centered discharge education, (2) HbA1c-based adjustment of diabetes therapy upon discharge, and (3) post-discharge support.

2.3.1. Patient-Centered Discharge Education

The education consisted of two parts delivered by one of three study team navigators over the phone before discharge or 1 to 3 days after discharge according to subject availability. The first part was focused, customizable, diabetes discharge instructions and education using a 19-page booklet based on American Diabetes Association (ADA) guidelines that includes information on diet, physical activity, and self-care guidance, such as how to recognize and treat hypoglycemia and hyperglycemia [8]. The instructions addressing post-discharge use of diabetes medications were adapted from previously published work [9]. All the concepts tested in the revised Diabetes Knowledge Test (DKT2) are covered in the booklet [10]. Subjects who had not completed a formal outpatient diabetes education program in the prior 12 months were referred to a Diabetes Care and Education Specialist at the Temple Diabetes Center. The second part of discharge education was a comprehensive review of the discharge plan. A navigator reviewed the discharge plan with subjects, covering the treatment plan, how to take medications, reasons for and importance of follow-up appointments and testing, and how to reach post-hospital providers.

2.3.2. HbA1c-Based Adjustment of Diabetes Therapy upon Discharge

Diabetes therapy upon hospital discharge was determined by a study endocrinologist (C.V. or D.R.) using an algorithm based on previously published work and ADA guidelines (Supplementary Table S1) [8,11]. For subjects with baseline HbA1c <7.0% (53 mmol/mol), the preadmission treatment regimen was continued unless adjustments were needed for safety. For subjects with baseline HbA1c >7.0% (53 mmol/mol), preadmission non-insulin diabetes therapy was optimized, defined as using the next higher dose up to the maximum tolerated dose. Only FDA-approved diabetes therapies were used in the study. Metformin was started in subjects with Type 2 Diabetes who did not have a contraindication to using it. Depending on the baseline HbA1c level, insulin was adjusted or added to the preadmission regimen.

2.3.3. Post-Discharge Support

One to three days after discharge, a navigator called subjects to assess their status, confirm receipt of and compliance with medications, verify follow-up appointments, assess barriers to following the discharge plan, determine the need for a community health worker, and review BG levels. Similar phone calls were made weekly for four weeks following discharge or until the first unplanned readmission. Subjects who were discharged on non-insulin regimens were asked to check their BG levels at least once a day, and subjects discharged on insulin at least twice a day. If a subject reported BG levels <70 or >240 mg/dL (3.9 or 13.3 mmol/L), then the navigator notified a study physician, who contacted the subject by phone to adjust diabetes therapy per protocol (Supplementary Tables S2 and S3). In addition, all intervention subjects received a referral for a nursing visit in the home to assess medical needs for support at home. Referral to a community health worker was made if subjects were found to have non-medical needs and/or obstacles to maintaining self-care and attending follow-up appointments, including transportation, food, housing, financial, and legal issues.

2.4. Data Collection, Measures, and Sample Size

The primary outcome was all-cause unplanned hospital readmission within 30 days of discharge. Secondary outcomes assessed at 30 days after discharge were rate of any emergency department (ED) visit not associated with a hospital admission, composite rate of unplanned readmission or ED visit, cost of post-discharge acute care and the intervention (details below), daily frequency of self-monitored blood glucose (SMBG) testing, and three categories of hypoglycemia defined as any SMBG level <70, <54, or <40 mg/dL (3.9, 3, or 2.2 mmol/L, Intervention group only). Data on hypoglycemia in the Intervention group were obtained during the follow-up navigator phone calls. Because the UC group did not receive navigator calls, comparable data on hypoglycemia were not available. Baseline characteristics were recorded based on self-report and review of the medical record.

Approximately 5 weeks after discharge, a study coordinator called subjects in both groups to assess readmissions, ED visits, and the frequency of SMBG testing. In addition, a novel patient experience questionnaire was administered to Intervention subjects. Subjects were asked to respond to each of the following statements with either Agree, Neutral, or Disagree: (1) "I understood my discharge instructions," (2) "The diabetes teaching in the booklet was helpful," and (3) "I was happy with the support I got after leaving the hospital." Healthcare encounters were confirmed in the EHR, which is integrated with several other healthcare systems in the region by Epic Care Everywhere. Readmissions that could not be confirmed in the EHR were confirmed by obtaining discharge records. Change in HbA1c level from baseline (hospital admission) was assessed 3 months after discharge.

The cost of post-discharge acute care was based on the sum of the estimated cost of all planned and unplanned readmissions and ED visits within 30 days of discharge. The cost of each readmission was based on the observed length of stay and a unit cost of USD 3045 per hospital day in 2017 among patients with diabetes [12]. The cost of each ED visit was based on a unit cost of USD 1110 [12]. The cost of the intervention was based on the value of time spent by the navigators and study physicians. Based on the average annual income of a navigator working 2000 h per year, navigator time was valued at USD 58 per hour. Similarly, based on the fiscal year 2018 median income of an assistant professor in endocrinology working 2300 h per year in the United States, physician time was valued at USD 101 per hour [13].

A target of 60 subjects per group was the largest deemed feasible. The last date of enrollment was determined by navigator availability. As a pilot trial, this study is not powered to detect statistically significant differences in outcomes.

2.5. Analysis

Distributions of the data were assessed by descriptive statistical and graphical methods. Summary statistics are reported as mean ± standard deviation or median (interquartile range). Because of skewed distributions, the ratio of estimated costs between groups was calculated using log-transformed gamma regression [14]. The primary analyses for all outcomes were performed in the intention-to-treat (ITT) population, defined as having been randomly assigned to a study group and not meeting post-enrollment exclusion criteria. In prespecified analyses, outcomes were assessed in the ITT subgroup of subjects with a baseline HbA1c >7.0% (53 mmol/mol). No statistical testing was performed for this pilot trial.

3. Results

3.1. Participant Flow

Between 16 October 2017 and 30 May 2019, a total of 3915 patients were assessed for eligibility and 3822 were excluded (Figure 1). The remaining 93 patients were randomized, and 47 were allocated to Intervention, 46 to UC. Because two subjects withdrew consent, the analyzed ITT cohort had 45 Intervention subjects and 46 UC subjects.

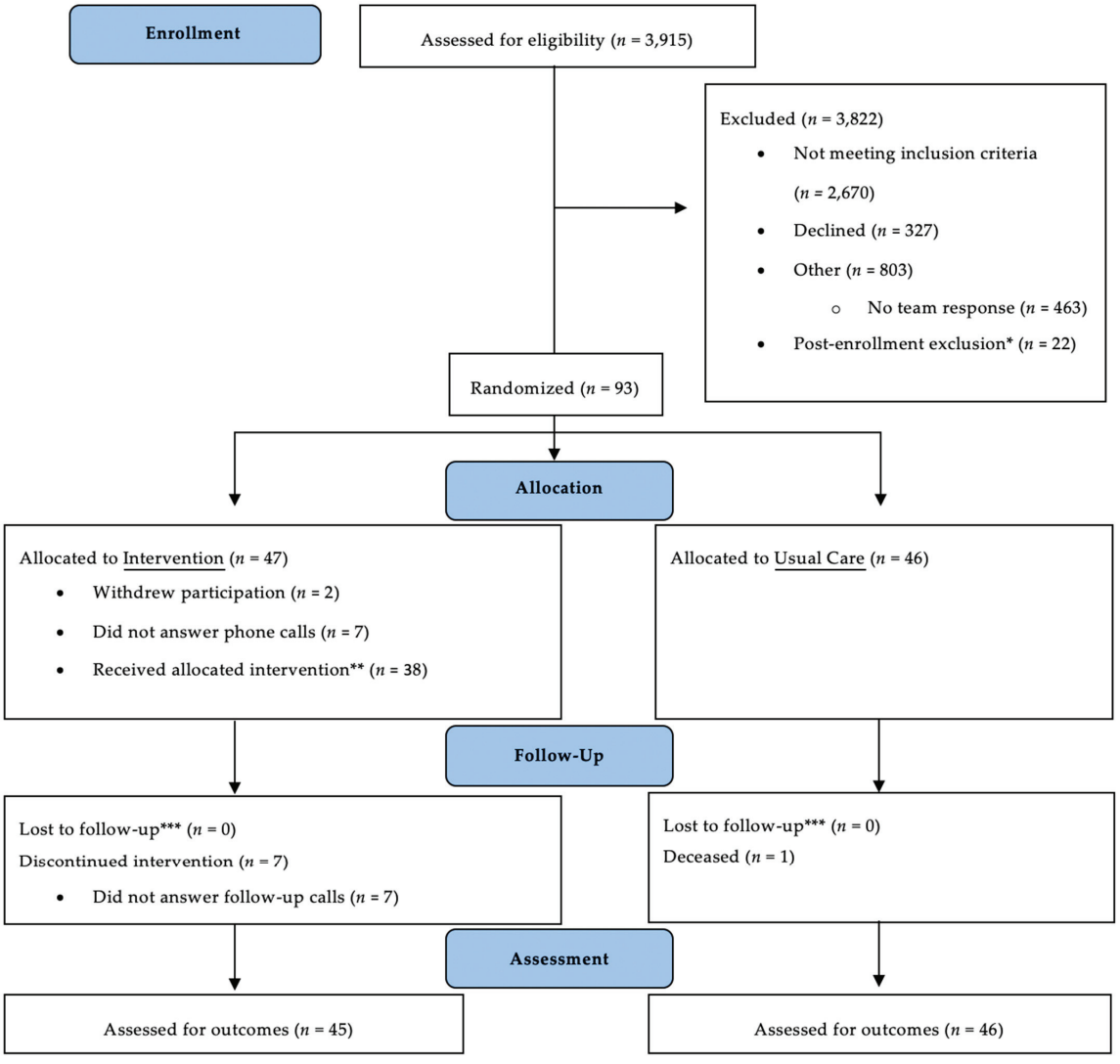

Figure 1. Flow of patients in trial. * Post-enrollment exclusion criteria were transfer to another hospital or subacute facility, discharge to hospice or a long-term care facility, signing out against medical advice, or inpatient death; ** Subject received education, adjustment of diabetes therapy upon discharge, and at least 1 follow-up phone call; *** Electronic health record used for follow-up if subject could not be contacted.

3.2. Baseline Characteristics

Mean age was 58.7 ± 12.7 years, duration of diabetes 15.1 ± 10.0 years, and median HbA1c 8.7% (7.1–10.6%), 72 mmol/mol (54–92 mmol/mol) (Table 1). The cohort was 71% Black, 28% White, 14% Hispanic and mostly low-income (86%). Most patients (95%) had Type 2 Diabetes. Predicted 30-day readmission risk was similar between groups (38.4 ± 7.6% Intervention, 37.5 ± 7.5% UC).

Table 1. Baseline characteristics of Intervention and Usual Care groups.

Variable	All Patients $N = 91$	Intervention $n = 45$	Usual Care $n = 46$
Age, years	58.7 ± 12.7	58.5 ± 13.7	58.9 ± 11.7
Female	47 (51.6)	21 (46.7)	26 (56.5)
Income, USD			
Less than $12,060	25 (27.5)	9 (20.0)	16 (34.8)
$12,060–$16,239	16 (17.6)	8 (17.8)	8 (17.4)
$16,240–$24,599	15 (16.5)	9 (20.0)	6 (13.0)
$24,600–$49,999	22 (24.2)	11 (24.4)	11 (23.9)
$50,000 or more	13 (14.3)	8 (17.8)	5 (10.9)
Race			
Black	65 (71.4)	29 (64.4)	36 (78.3)
Other	1 (1.1)	0 (0.0)	1 (2.2)
White	25 (27.5)	16 (35.6)	9 (20.0)
Hispanic	13 (14.3)	9 (20.0)	4 (8.7)
Education, years	12.6 ± 2.5	13.0 ± 3.0	12.1 ± 1.8
Employment Status			
Disabled	64 (70.3)	32 (71.1)	32 (69.6)
Employed	1 (1.1)	1 (2.2)	0 (0.0)
Retired	16 (17.6)	6 (13.3)	10 (21.7)
Unemployed	10 (11.0)	6 (13.3)	4 (8.7)
Insurance			
Medicaid only	16 (18.0)	9 (20.9)	7 (15.2)
Medicare and Medicaid	17 (19.1)	9 (20.9)	8 (17.4)
Medicare only	24 (27.0)	10 (23.3)	14 (30.4)
None	3 (3.4)	2 (4.7)	1 (2.2)
Private	29 (32.6)	13 (30.2)	16 (34.8)
Smoking			
Current smoker	18 (19.8)	9 (20.0)	9 (19.6)
Former smoker	40 (44.0)	20 (44.4)	20 (43.5)
Never	33 (36.3)	16 (35.6)	17 (37.0)
Body mass index (kg/m^2)	35.2 ± 10.9	36.2 ± 11.7	34.2 ± 10.0
Type of Diabetes			
Type 1	5 (5.5)	3 (6.7)	2 (4.3)
Type 2	86 (94.5)	42 (93.3)	44 (95.7)
Diabetes duration, years	15.1 ± 10.0	13.6 ± 8.5	16.6 ± 11.2
A1c at admission	8.7 (7.1–10.6)	8.9 (7.2–11.1)	8.5 (7.1–10.0)
A1c at admission >7.0% (53 mmol/mol)	69 (76.7)	34 (77.3)	35 (76.1)
Preadmission Home Medication Route			
Insulin only	52 (57.1)	27 (60.0)	25 (54.3)
No medications	7 (7.7)	1 (2.2)	6 (13.0)
Oral & insulin	19 (20.9)	13 (28.9)	6 (13.0)
Oral only	11 (12.1)	3 (6.7)	8 (17.4)
Other	2 (2.2)	1 (2.2)	1 (2.2)
Preadmission sulfonylurea use	8 (8.8)	3 (6.7)	5 (10.9)
Preadmission metformin use	19 (20.9)	9 (20.0)	10 (21.7)
Preadmission insulin use	73 (80.2)	42 (93.3)	31 (67.4)
Preadmission statin use	64 (70.3)	28 (62.2)	36 (78.3)
Preadmission glucocorticoid use	18 (19.8)	8 (17.8)	10 (21.7)
Preadmission blood pressure medications			
None	14 (15.4)	9 (20.0)	5 (10.9)
ACE-i or ARB and Non-ACE/ARB	25 (27.5)	13 (28.9)	12 (26.1)
Only ACE-i or ARB	23 (25.3)	6 (13.3)	17 (37.0)
Only non-ACE or ARB	29 (31.9)	17 (37.8)	12 (26.1)
History of severe hypoglycemia	34 (37.8)	17 (37.8)	17 (37.8)
Current or prior DKA or HHS	9 (9.9)	5 (11.1)	4 (8.7)

Table 1. *Cont.*

Variable	All Patients $N = 91$	Intervention $n = 45$	Usual Care $n = 46$
Microvascular complications			
0	35 (38.5)	15 (33.3)	20 (43.5)
1	35 (38.5)	20 (44.4)	15 (32.6)
2	15 (16.5)	7 (15.6)	8 (17.4)
3	6 (6.6)	3 (6.7)	3 (6.5)
Macrovascular complications			
0	25 (27.5)	13 (28.9)	12 (26.1)
1	38 (41.8)	20 (44.4)	18 (39.1)
2	21 (23.1)	9 (20.0)	12 (26.1)
3	6 (6.6)	2 (4.4)	4 (8.7)
4	1 (1.1)	1 (2.2)	0 (0.0)
Anemia diagnosis	62 (68.1)	33 (73.3)	29 (63.0)
Discharged within 90 days before index admission	81 (89.0)	45 (100.0)	36 (78.3)
ED visit within 90 days before index admission	24 (30.4)	10 (26.3)	14 (34.1)
Admission priority			
Emergent	75 (82.4)	37 (82.2)	38 (82.6)
Planned	4 (4.4)	2 (4.4)	2 (4.3)
Urgent	12 (13.2)	6 (13.3)	6 (13.0)
Home zip code within 5 miles of hospital	78 (85.7)	40 (88.9)	38 (82.6)
Discharge status			
Against medical advice	1 (1.1)	0 (0.0)	1 (2.2)
Home with nursing care	28 (30.8)	14 (31.1)	14 (30.4)
Home without additional services	56 (61.5)	29 (64.4)	27 (58.7)
Subacute facility (rehabilitation or skilled nursing)	5 (5.5)	2 (4.4)	3 (6.5)
No discharge within prior year	1 (1.1)	0 (0.0)	1 (2.2)
Predicted risk of readmission within 30 days, %	38.4 ± 7.6	39.2 ± 7.8	37.5 ± 7.5
Admission blood glucose, mg/dL	208.1 ± 107.7	188.7 ± 95.6	227.1 ± 116.4
Admission blood glucose, mmol/L	11.6 ± 6.0	10.5 ± 5.3	12.6 ± 6.5
Admission serum sodium, mmol/L	136.0 ± 4.9	136.3 ± 4.9	135.7 ± 5.0
Admission serum potassium, mmol/L	4.3 ± 0.8	4.3 ± 0.9	4.2 ± 0.7
Admission serum creatinine, mg/dL	1.7 (1.1–3.2)	2.0 (1.1–3.2)	1.5 (1.1–3.2)
Admission eGFR, mL/min	39.8 ± 20.6	39.8 ± 20.5	39.8 ± 20.9
Admission hematocrit, %			
High	2 (2.2)	2 (4.4)	0 (0.0)
Low	69 (75.8)	30 (66.7)	39 (84.8)
Normal	20 (22.0)	13 (28.9)	7 (15.2)
Brief Health Literacy Screen Score	11.9 ± 2.9	12.3 ± 3.0	11.6 ± 2.7
PHQ–2 Score	1.0 (0.0–2.0)	1.0 (0.0–2.0)	2.0 (1.0–3.0)
Diabetes Knowledge Test Score	57.3 ± 15.6	59.1 ± 15.7	55.5 ± 15.5
Problem Areas in Diabetes Score	30.6 ± 24.3	36.3 ± 25.1	25.1 ± 22.4
Predicted risk of readmission within 30 days, % *	38.4 ±7.6	39.2 ± 7.8	37.5 ± 7.5

Values are mean ± SD, median (IQR), or *n* (%) unless otherwise stated. * Predicted risk based on Diabetes Early Readmission Risk Indicator (DERRI™). IQR (interquartile range), ACE-i (angiotensin-converting enzyme inhibitors), ARB (angiotensin II receptor blockers), DKA (diabetic ketoacidosis), HHS (hyperosmolar hyperglycemic state), eGFR (estimated glomerular filtration rate), PHQ (patient health questionnaire).

3.3. Outcomes

The 30-day readmission rate was 31.1% in the Intervention group and 32.6% in the UC group (Table 2). The combined 30-day readmission or ED visit rate was 35.6% in the Intervention group and 39.1% in the UC group. The number of SMBG tests was 2.4 ± 1.6 per day in the Intervention group and 1.8 ± 1.4 per day in the UC group. Costs in the Intervention group were 33% of the costs in the UC group. Only 11% of Intervention participants reported having at least one BG level <70 mg/dL (3.9 mmol/L) during follow-up. Change in HbA1c was similar at 3 months between the two groups. Among survey respondents in the Intervention group, 97% understood their discharge instructions, 93%

believed the diabetes teaching was helpful, and 93% were happy with the support they received after leaving the hospital.

Table 2. Outcomes in Intervention and Usual care groups.

Intention-to-Treat Cohort			
Variable [a]	**All Patients** ***N*** **= 91**	**Intervention** ***n*** **= 45**	**Usual Care** ***n*** **= 46**
Readmission	29 (31.9)	14 (31.1)	15 (32.6)
ED visit	8 (8.8)	4 (8.9)	4 (8.7)
Readmission or ED visit	34 (37.4)	16 (35.6)	18 (39.1)
Costs, USD	–	5542 ± 10,970	6657 ± 16,969
Costs, USD	–	172 (127–5546)	0 (0–5667)
Costs, Intervention:Usual Care ratio [b] (95%CI)		0.33 (0.13–0.79)	
Hypoglycemia			
-Blood glucose <70 mg/dL (3.9 mmol/L)	–	5 (11)	–
-Blood glucose <54 mg/dL (3.0 mmol/L)	–	2 (4)	–
-Blood glucose <40 mg/dL (2.2 mmol/L)	–	1 (2)	–
Number of daily SMBG tests	2.1 ± 1.5	2.4 ± 1.6	1.8 ± 1.4
Change in HbA1c at 3 months, %	−0.9 (−1.6–0.2)	−1.0 (−1.6–0.2)	−0.9 (−1.4–0.2)
Change in HbA1c at 3 months, mmol/mol	−10 (−18–2)	−11 (−18–2)	−10 (−15–2)
Subgroup with baseline HbA1c >7.0%	All Patients *N* = 69	Intervention *n* = 34	Usual Care *n* = 35
Readmission	19 (27.5)	8 (23.5)	11 (31.4)
ED visit	7 (10.1)	3 (8.8)	4 (11.4)
Readmission or ED visit	23 (33.3)	9 (26.5)	14 (40.0)
Costs, USD	–	3657 ± 8230	6967 ± 18,863
Costs, USD	–	154 (126–1246)	0 (0–5661)
Costs, Intervention:Usual Care ratio [b] (95%CI)		0.21 (0.08–0.58)	
Hypoglycemia			
-Blood glucose <70 mg/dL (3.9 mmol/L)	–	5 (14.7)	–
-Blood glucose <54 mg/dL (3.0 mmol/L)	–	2 (5.9)	–
-Blood glucose <40 mg/dL (2.2 mmol/L)	–	1 (2.9)	–
Number of daily SMBG tests	2.2 ± 1.6	2.5 ± 1.6	2.0 ± 1.5
Change in HbA1c at 3 months, %	−1.0 (−2.2–0.0)	−1.1 (−2.2–0.0)	−0.9 (−2.3–0.1)
Change in HbA1c at 3 months, mmol/mol	−11 (−24–0)	−12 (−24–0)	−10 (−25–1)

Values are mean ± SD, median (IQR), or *n* (%) unless otherwise stated. IQR, interquartile range; CI, confidence interval. [a] Within the 30 days after hospital discharge. [b] Costs of 30-day readmissions, ED visits, and the intervention. SMBG, self-monitored blood glucose.

3.4. Ancillary Analysis

Among the 69 subjects with baseline HbA1c >7.0% (53 mmol/mol), the 30-day readmission rate was 23.5% in the Intervention group and 31.4% in the UC group (Table 2). The combined 30-day readmission or ED visit rate was 26.5% in the Intervention group and 40.0% in the UC group. Among the Intervention participants, 15% reported having at least one BG level <70 mg/dL (3.9 mmol/L) during follow-up. The number of SMBG tests was 2.5 ± 1.6 per day in the Intervention group and 2.0 ± 1.5 per day in the UC group. Costs in the Intervention group were 21% of the costs in the UC group. Change in HbA1c was similar at 3 months between the two groups.

4. Discussion

This pilot RCT suggests the DiaTOHC intervention, with which participants were overwhelmingly satisfied, may be feasible at an urban, academic, safety-net hospital. Readmission rates in the Intervention and UC groups were similar. However, the trial raises the possibility that the intervention may decrease readmission/ED visit risk among patients with a baseline HbA1c >7.0% (53 mmol/mol). In this subgroup, Intervention subjects experienced a 34% relative risk reduction in readmission/ED visit risk and absolute risk

reduction of 13.5%. Additionally, costs were substantially lower in the Intervention group. Furthermore, hypoglycemia during the intervention was uncommon, with 11% of Intervention participants reporting any SMBG <70 mg/dL (3.9 mmol/L). Other trials with similar HbA1c-based discharge treatment algorithms reported post-discharge hypoglycemia rates of 23–29% [11,15].

Several mostly observational studies have investigated the effect of various interventions on readmission risk in diabetes patients [4], categorizable as inpatient diabetes education only, inpatient diabetes management by a dedicated service, and multi-component programs consisting of education, transition-of-care support, and/or outpatient follow-up. The relative risk reductions of these interventions vary considerably from 0 to 71%, with most studies showing benefit.

The current study adds to the small number of related published RCTs with a novel approach: combining multi-component intervention with selection of high-risk patients using a validated tool. This pilot study, however, is limited by lacking power for detecting differences between groups. Because observation of the UC group was limited, we were unable to compare hypoglycemia rates or office visits between groups. Given the nature of the intervention, blinding was not feasible. In addition, the statisticians were not blinded. Lastly, the findings may not generalize to other sites and settings.

In conclusion, the possible reduction in 30-day readmission/ED visit risk in the higher HbA1c subgroup merits further investigation in a larger, multi-center RCT.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11061471/s1, Table S1: HbA1c-based adjustment of diabetes therapy; Table S2: Outpatient basal insulin dose adjustment; Table S3: Outpatient prandial/pre-meal insulin dose adjustment based on subsequent mealtime/HS BG values [16].

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Temple University (protocol code 24306, approved on 24 May 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available on request due to restrictions on sharing of protected health information.

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Blood Pressure Monitoring and Perinatal Outcomes in Normotensive Women with Gestational Diabetes Mellitus

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Abstract: Alterations in ambulatory blood pressure detected by monitoring (ABPM) have been associated with perinatal complications in hypertensive pregnant women. Aim: To establish the relationships between the blood pressure (BP) profiles detected by ABPM and adverse perinatal outcomes in normotensive women with gestational diabetes mellitus (GDM). Methods: A prospective study of normotensive women in whom 24 h ABPM was performed at 28–32 weeks of pregnancy. The obstetric and perinatal outcomes were evaluated. Results: Two hundred patients were included. Thirty-seven women with GDM and obesity had significantly higher mean systolic BP (SBP) and nocturnal SBP and diastolic BP (DBP) compared to women with only GDM (n = 86). Nocturnal SBP (OR = 1.077; p = 0.015) and obesity (OR = 1.131; p = 0.035) were risk factors for the development of hypertensive disorders of pregnancy (HDPs). Mothers of newborns with neonatal complications (n = 27) had higher nocturnal SBP (103.8 vs. 100 mmHg; p = 0.047) and DBP (62.7 vs. 59.4; p = 0.016). Women who delivered preterm (n = 10) had higher BP and a non-dipper pattern (p = 0.005). Conclusions: Nocturnal SBP was a predictor of HDPs in normotensive women with obesity or GDM. Alterations in ABPM in these patients were associated with poor obstetric and perinatal outcomes.

Keywords: gestational diabetes mellitus; ambulatory blood pressure monitoring; hypertensive disorders of pregnancy; perinatal outcomes

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1. Introduction

Hypertensive disorders of pregnancy (HDPs) imply an increase in maternal and neonatal morbidity and mortality as well as an increased risk of obstetric and perinatal complications [1–3]. HDPs include [3] gestational hypertension, defined as hypertension (systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) $\geq$ 90 mmHg) after the 20th week of gestation in women who were normotensive at baseline, and whose BP return to normal by 12 weeks after delivery; preeclampsia, the new onset of hypertension after the 20th week of gestation and develops proteinuria or end-organ dysfunction; chronic hypertension, defined as documented prepregnancy hypertension, use of antihypertensive medication before pregnancy or hypertension before 20 weeks of gestation and persisting 12 weeks after delivery; and preeclampsia over chronic hypertension, when preeclampsia appears in women with pre-existing chronic hypertension. These disorders affect 5–10% of pregnancies worldwide [4,5], and the presence of some comorbidities, such as gestational diabetes mellitus (GDM), can increase the risk of developing HDPs [6]. Furthermore, the

prevalence of GDM has been increasing worldwide; this is related to the current obesity epidemic [7]. Therefore, it is necessary to design specific models that allow us to detect the development of HDPs in patients with GDM early in order to start preventive strategies in these women who are at increased risk.

Although isolated office blood pressure (BP) measurement remains the most commonly used method to detect hypertension in pregnancy in clinical practice, ambulatory blood pressure monitoring (ABPM) provides more reliable records and informs clinicians about BP variability over a 24 h period and the circadian rhythm [8]. In a healthy population, nocturnal BP physiologically falls by 10–20% compared to daytime BP values, which is known as a dipper pattern. The absence of this nocturnal BP fall (known as a non-dipper pattern) [9] and nocturnal hypertension have been associated with the development of HDPs in pregnant women [10–12]. These observations have led some authors, such as Salazar et al. [12,13], to recommend using ABPM in high-risk pregnancies (including women with pregestational diabetes) to detect early BP profile alterations in patients who subsequently develop HDPs. In pregnant women with GDM, there is insufficient evidence, but in a previously published study, we reported that high nocturnal SBP levels increase the risk of developing HDPs in pregnant women with GDM [14].

Previous studies have described an association between BP profile alterations in hypertensive pregnant women and obstetric and perinatal complications, such as preterm delivery, lower birth weight, and fetal growth restriction (FGR) [15–17]. However, few studies have related maternal and neonatal outcomes to BP variability in normotensive pregnant women [18,19].

The aim of the present study was to analyze the relationships between BP profiles (detected by ABPM) in normotensive pregnant women and risk factors for HDPs such as GDM and adverse obstetric and perinatal outcomes.

2. Materials and Methods

2.1. Study Design and Study Population

We conducted a prospective observational study of 255 normotensive pregnant women attending a joint Endocrinology and Obstetrics clinic of the Puerta del Mar University Hospital (Cadiz, Spain), who were selected consecutively between August 2014 and December 2018. Women with GDM were divided according to their pregestational BMI (with or without obesity) and compared to non-diabetic women with normal weight (control group) and women with only obesity (without GDM). Only women with singleton pregnancies who delivered at the Puerta del Mar University Hospital were included. The following exclusion criteria were applied: women with pre-existing chronic hypertension or taking antihypertensive drugs at the time of recruitment, with a diagnosis of placental insufficiency, prepregnancy diabetes, morbid obesity (body mass index (BMI) > 40 kg/m^2), smoking, and underlying chronic or acute systemic disease. The study protocol was approved by the Ethics Committee of the hospital (code number 1507-N-16) and conformed to the principles of the Declaration of Helsinki. Informed consent was obtained from all the participants.

Pregnant women at high risk of preeclampsia were advised to take 100 mg of aspirin daily from 12 weeks until 37 weeks, according to our hospital protocol based on NICE guidelines [20]. ASA prophylaxis was indicated in women at high risk with any of the following high-risk factors: hypertensive disease during a previous pregnancy, chronic kidney disease, autoimmune disease such as systemic lupus erythematosus or antiphospholipid syndrome, type 1 or type 2 diabetes, and chronic hypertension. ASA prophylaxis was also indicated in pregnant women with more than one moderate risk factor for preeclampsia: first pregnancy, age of 40 years or older, pregnancy interval of more than 10 years, BMI $\geq$ 35 kg/m^2 at first visit, family history of preeclampsia, and multi-fetal pregnancy. Obesity was designated as having a prepregnancy BMI $\geq$ 30 Kg/m^2. GDM was defined according to the criteria of the National Diabetes Data Group [21], and women with GDM were initially managed with diet. The glycemic targets during pregnancy were fasting glucose < 95 mg/dL and postprandial glucose at 1 h < 140 mg/dL. If blood glucose targets

were not achieved with dieting in two weeks, insulin therapy was initiated. None of the women received oral hypoglycemic drugs.

Between 28 and 32 weeks of pregnancy, ABPM was performed on the nondominant arm during a 24 h period using a Spacelabs 90207 monitor (Spacelabs, Redmond, WA, USA). Measurements were scheduled every 20 min during the day and every 30 min at night. Patients were instructed to maintain normal daily activities, and sleep time was defined as the period between going to bed and rising the next morning. Only ABPMs with at least 66% successful measurements and at least one record per hour were considered valid. The following ABPM circadian patterns were established: the dipper pattern (BP decrease between 10–20% in the nocturnal period compared to the daytime period) and the non-dipper pattern (BP decrease < 10% in the nocturnal period compared to the daytime period).

On the day of the insertion of the ABPM device, fasting blood samples were collected at that moment for biochemical analysis, and maternal clinical data, method of conception, and obstetric history (including parity, previous history of GDM or macrosomia, history of gestational hypertension and/or preeclampsia) were obtained. Office BP was measured with an automated BP monitor (Omron HEM-7200-E (Kyoto, Japan)) in a sitting position twice on the same day and before ABPM.

The following obstetric and perinatal data were recorded after delivery: gestational age at delivery (gestational age was determined by first trimester crown–rump length measurements and was corrected in the case of a discrepancy of ±3 days with the last menstrual period), type of delivery, delivery route (eutocic, instrumental extraction, or cesarean section) delivery complications, birthweight, and the Apgar score. We documented complications of pregnancy such as HDPs, gestational hypertension (BP > 140/90 mmHg after the 20th week of gestation and return to normal BP levels in the postpartum period), preeclampsia (BP > 140/90 mmHg associated with proteinuria >300 mg/24 h or end-organ dysfunction), miscarriage, and intrauterine fetal death. Preterm delivery was considered as a delivery that occurred before 37 gestational weeks. FGR was defined as a birthweight less than the 5th percentile on a customized pediatric curve, small for gestational age (SGA) was designated as a birthweight below the 10th percentile, and large for gestational age (LGA) was when birthweight was greater than the 90th percentile for gestational age. Neonatal composite adverse outcomes were defined by the presence of hypoglycemia, hyperbilirubinemia, congenital malformations, or admission to the neonatal intensive care unit.

2.2. Statistical Analysis

Data were processed and analyzed using IBM SPSS version 24.0 software for MS Windows. Descriptive statistics of the variables measured are presented as the mean and standard deviation (SD) for quantitative variables, and frequencies and percentages for the qualitative variables. We used the Shapiro–Wilk test to monitor the normality of the distributions. The X2 test (or Fisher's exact test, as required) was used to compare qualitative variables, which does not allow us to specify the statistical significance between the four groups. The magnitude of association was calculated using the odds ratio (OR) with the precision described by the 95% confidence interval (95% CI).

Comparisons between quantitative variables and groups were performed with the Student's t-test and one-way analysis of variance (ANOVA) for the parametric variables. Bonferroni post hoc tests were used to identify significant differences between specific groups in case of a significant F value from an ANOVA. Correlations among variables were evaluated using Pearson's correlation test.

A multivariate analysis was performed using non-conditional logistic regression. The stepwise technique was used to select the independent variables introduced into the model. The goodness of fit of the final model was assessed using the Hosmer–Lemeshow test. p values less than 0.05 were considered statistically significant.

3. Results

Two hundred and fifty-five pregnant women were recruited, and fifty-five women were excluded: 11 who gave birth elsewhere and 44 with ABPM readings that were not valid. We observed no significant difference in maternal age at enrollment, maternal pre-pregnancy BMI or gestational age between women who had valid ABPM data and completed the study when compared with the 55 women lost to follow-up. The remaining 200 women were divided into four groups (Figure 1): Group 1 (n = 37) were women with GDM and obesity, Group 2 (n = 86) were women with GDM without obesity, Group 3 (n = 13) were women with obesity without GDM, and Group 4 (n = 64) were women with neither obesity nor GDM (control group).

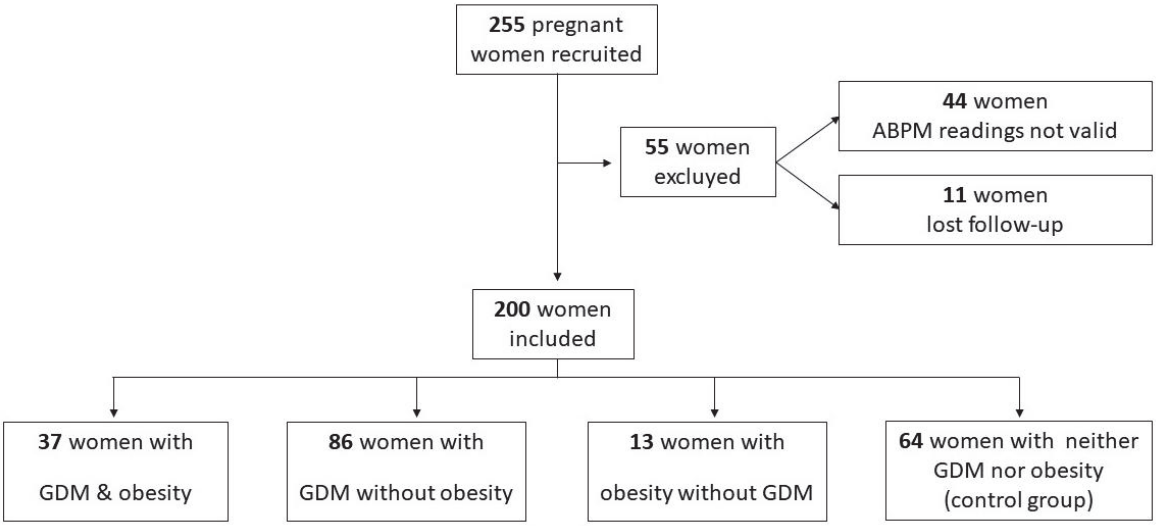


Figure 1. Study flow chart: algorithm for the identification and classification of eligible women. ABPM: ambulatory blood pressure monitoring; and GDM: gestational diabetes mellitus.

Baseline clinical characteristics and laboratory features from each group are shown in Table 1. Maternal age was significantly higher in women with GDM. There was no significant difference in the family history of diabetes mellitus or hypertension, method of conception, history of polycystic ovarian syndrome, parity, or obstetric history (including antecedent of miscarriage, previous macrosomia, history of gestational hypertension, and/or preeclampsia) between groups.

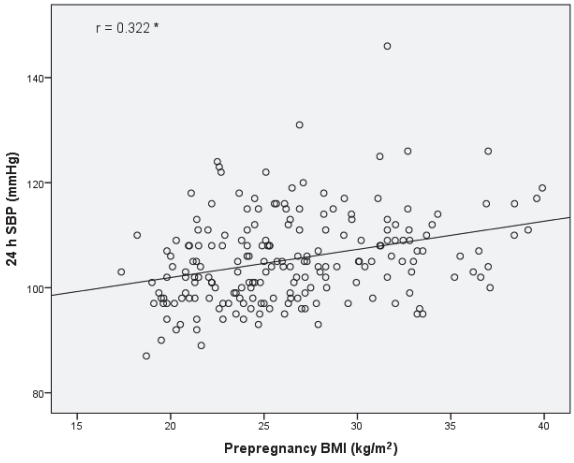
Concerning the characteristics of pregnancy, office SBP and DBP were significantly greater in the group of women with GDM and obesity than in the group of women with only GDM, but there were no significant differences compared to women with obesity without GDM or between the other groups (Table 1). In relation to the laboratory variables, women with GDM and obesity showed higher levels of triglycerides and HbA1c. The rest of the measured variables were not significantly different between groups.

Table 1. Demographic, clinical, and laboratory variables, and ABPM parameters according to presence of GDM and/or obesity.

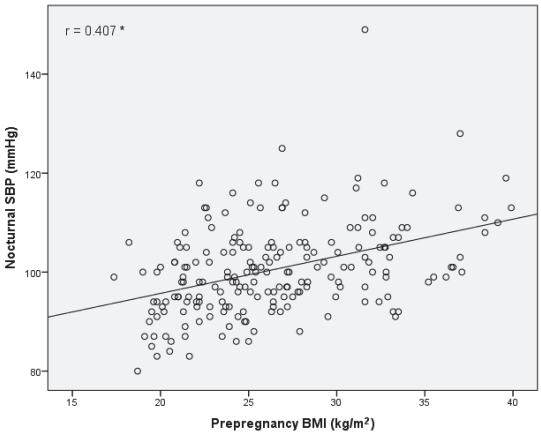
Variables	GDM and Obesity (*n* = 37)	GDM without Obesity (*n* = 86)	Obesity without GDM (*n* = 13)	Control (*n* = 64)	*p*-Value
Maternal age (y)	33.97 ± 3.956	34.96 ± 4.182	30.85 ± 5.414	33.36 ± 4.876	0.009 [a]
Prepregnancy BMI (kg/m^2)	33.81 ± 2.51	24.27 ± 3.02	32.97 ± 3.15	23.80 ± 2.79	<0.001 [b]
Family history DM	19 (51.45%)	38 (44.2%)	6 (46.2%)	22 (34.4%)	0.4
Family history AHT	16 (43.2%)	38 (44.2%)	5 (38.5%)	23 (35.9%)	0.8
Parity					0.5
Nulliparous	16 (43.2%)	33 (38.4%)	7 (53.8%)	28 (43.8%)	
Multiparous	21 (56.8%)	53 (61.6%)	6 (46.2%)	36 (56.2%)	
Previous history of GDM	9 (24.3%)	21 (24.4%)	0	5 (7.8%)	0.01
Office SBP (mmHg)	114.7 ± 12.2	107.6 ± 15.6	116.2 ± 12.4	110.6 ± 11.3	0.02
Office DBP (mmHg)	70.9 ± 8.8	65.2 ± 8.6	67.6 ± 7.7	65.2 ± 8.5	0.005 [c]
Total cholesterol (mmol/L)	6.13 ± 1.21	6.47 ± 1.22	6.27 ± 1.33	6.46 ± 0.99	0.5
LDL-cholesterol (mmol/L)	3.75 ± 1.48	3.77 ± 1.28	3.29 ± 1.15	3.46 ± 0.97	0.5
HDL-cholesterol (mmol/L)	1.91 ± 0.55	1.92 ± 0.43	1.89 ± 0.47	1.99 ± 0.54	0.9
Triglycerides (mmol/L)	2.55 ± 1.15	2.16 ± 0.72	2.46 ± 0.88	2.01 ± 0.7	0.012 [d]
HbA1c (mmol/mol)	33.3 ± 2.62	30.4 ± 2.03	29.3 ± 2.03	29.2 ± 1.87	<0.001 [e]
24 h SBP (mmHg)	109.19 ± 9.61	104.27 ± 8.46	108.15 ± 7.58	104.19 ± 7.45	0.009 [f]
24 h DBP (mmHg)	66.73 ± 5.45	64.53 ± 6.66	64.38 ± 4.42	64.69 ± 5.45	0.3
Daytime SBP (mmHg)	110.89 ± 9.51	106.64 ± 9.15	109.15 ± 8.57	106.55 ± 8.04	0.06
Daytime DBP (mmHg)	68.54 ± 5.53	66.97 ± 6.90	65.77 ± 5.31	66.97 ± 5.86	0.46
Nocturnal SBP (mmHg)	105.84 ± 10.90	99.15 ± 8.68	105.31 ± 7.67	98.39 ± 7.60	<0.001 [g]
Nocturnal DBP (mmHg)	62.70 ± 7.04	59.28 ± 7.08	60.38 ± 5.63	59.03 ± 5.49	0.034 [h]
Non-dipper pattern	22 (59.5%)	42 (48.8%)	9 (69.2%)	20 (31.3%)	0.01

Values are expressed as means ± standard deviation. Categorical variables are given as the number of subjects (*n*) with the percentage in parenthesis. GDM = gestational diabetes mellitus; BMI = body mass index; DM = diabetes mellitus; AHT = Arterial hypertension; SBP = systolic blood pressure; DBP = diastolic blood pressure; LDL = low-density lipoprotein; HDL = high-density lipoprotein; and HbA1c = glycated hemoglobin. [a] GDM without obesity vs. Obesity without GDM $p = 0.01$. [b] GDM and obesity vs. GDM without obesity $p < 0.001$; GDM with obesity vs. control $p < 0.001$; Obesity without GDM vs. GDM without obesity $p < 0.001$; Obesity without GDM vs. control $p < 0.001$. [c] GDM and obesity vs. GDM without obesity $p = 0.005$; GDM and obesity vs. control $p = 0.009$. [d] GDM and obesity vs. control $p = 0.01$. [e] GDM and obesity vs. GDM without obesity $p = 0.001$; GDM and obesity vs. Obesity without GDM $p = 0.007$; GDM with obesity vs control $p < 0.001$. [f] GDM and obesity vs. GDM without obesity $p = 0.018$; GDM and obesity vs. control $p = 0.02$. [g] GDM and obesity vs. GDM without obesity $p = 0.001$; GDM and obesity vs. control $p < 0.001$. [h] GDM and obesity vs. GDM without obesity $p = 0.05$; GDM and obesity vs. control $p = 0.04$.

With regard to ABPM parameters, there were some significant differences between groups (Table 1). The GDM with obesity group had significantly higher mean SBP, nocturnal SBP and DBP than women in the GDM without obesity group. We observed a significantly positive correlation between BMI and ABPM parameters (24 h SBP and nocturnal SBP and DBP), as shown in Figure 2.



(a)



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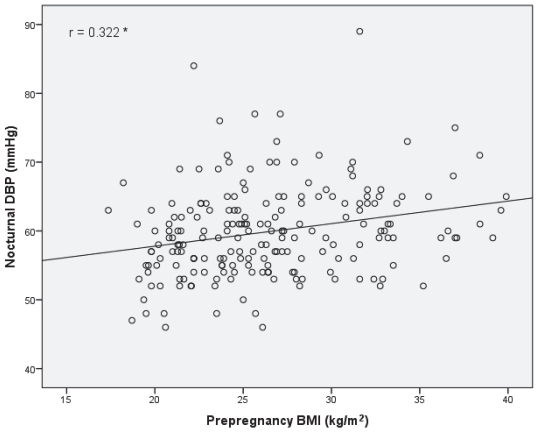

(c)

Figure 2. (**a**) Correlation between 24 h SBP and prepregnancy BMI. (**b**) Correlation between nocturnal SBP and prepregnancy BMI. (**c**) Correlation between nocturnal DBP and prepregnancy BMI. Linear correlation between ABPM parameters and prepregnancy BMI. Correlations were evaluated using Spearman's correlation test r. * $p < 0.01$. ABPM: ambulatory blood pressure monitoring; SBP: systolic blood pressure; DBP: diastolic blood pressure; and BMI: body mass index.

Table 2 shows the pregnancy and obstetric and perinatal outcomes data. Among women with GDM (n = 123), the use of insulin therapy was higher in women with obesity (59.5% vs. 41.5%), but the total insulin doses were similar between the two groups (20.95 ± 17.06 vs. 19.92 ± 14.21 UI). All the other women with GDM were controlled with diet and lifestyle modifications. Prophylaxis with ASA was higher in patients with obesity, particularly those with added GDM.

Table 2. Characteristics of pregnancy and obstetric and perinatal outcomes according to the presence of GDM and/or obesity.

Variables	GDM and Obesity (n = 37)	GDM without Obesity (n = 86)	Obesity without GDM (n = 13)	Control (n = 64)	p-Value
GDM treatment					0.05
Diet only	15 (40.5%)	50 (58.1%)	-	-	
Diet + insulin	22 (59.5%)	36 (41.9%)			
Total doses insulin (UI)	20.95 ± 17.06	19.92 ± 14.21			0.8
ASA prophylaxis	11 (29.7%)	7 (8.1%)	3 (23.1%)	3 (4.7%)	0.001
Development of HDPs	6 (16.2%)	5 (5.8%)	3 (23.1%)	2 (3.1%)	0.02
Preeclampsia	2 (5.4%)	2(2.3%)	0	1 (1.6%)	0.6
Gestational hypertension	6 (16.2%)	5 (5.8%)	3 (23.1%)	1 (1.6%)	0.007
Gestational age at delivery (week)	38.47 ± 1.30	36.16 ± 1.37	40.12 ± 0.92	39.54 ± 1.23	<0.001 [a]
Preterm delivery < 37 week	4 (10.8%)	6 (7.0%)	0	0	0.06
Weight gain (kg)	7.30 ± 4.32	8.40 ± 4.17	9.15 ± 5.05	10.86 ± 4.29	0.001 [b]
Instrumental delivery	10 (27%)	25 (29.1%)	1 (7.7%)	12 (18.8%)	0.1
Cesarean section	14 (37.8%)	19 (22.1%)	6 (46.2%)	16 (25%)	0.1
Childbirth complications	25 (67.6%)	39 (45.3%)	6 (46.2%)	22 (34.4%)	0.015
Birthweight (g)	3212 ± 615	3188 ± 465	3391 ± 421	3313 ± 475	0.3
FGR	3 (8.1%)	5 (5.8%)	1 (7.7%)	5 (7.8%)	0.9
SGA	7 (18.9%)	9 (10.5%)	2 (15.4%)	8 (12.5%)	0.6
LGA	5 (13.5%)	8 (9.3%)	0	8 (12.5%)	0.5
Neonatal adverse outcomes	8 (21.6%)	12 (14.2%)	2 (15.4%)	5 (7.8%)	0.3

Values are expressed as means ± standard deviation. Categorical variables are given as the number of subjects (n) with the percentage in parenthesis. GDM = gestational diabetes mellitus; ASA = acetylsalicylic acid; HDPs = hypertensive disorders of pregnancy; FGR = fetal growth restriction; SGA = small-for gestational age; and LGA = large-for-gestational age. [a] GDM and obesity vs. GDM without obesity p = 0.044; GDM and obesity vs. Obesity without GDM p = 0.001; GDM with obesity vs. control p = 0.001. [b] GDM and obesity vs. control p = 0.001; GDM without obesity vs. control p = 0.006.

Regarding the development of HDPs (including gestational hypertension and preeclampsia), the subgroup analysis showed no significant difference between the GDM and non-GDM groups (68.8% vs. 31.3%; p = 0.5). However, HDPs were higher in groups with obesity (56.3% vs. 43.8%; p = 0.005).

In the analysis of obstetric and perinatal outcomes (Table 2), delivery occurred earlier in diabetic groups, and the mean gestational age at delivery was lower in the GDM without obesity group. Weight gain was higher in non-GDM women, particularly those without obesity. No statistically significant differences were found in birthweight or neonatal composite adverse outcomes (hyperbilirubinemia, hypoglycemia, congenital malformations, or admission to the intensive care unit for any reason). Conversely, the rate of global delivery complications (including cesarean section during labor, instrumental delivery, and perineal tear) was significantly higher in GDM women with obesity than in the other groups.

To evaluate the relationship between the ABPM parameters and the development of HDPs, bivariate analysis was conducted, and the results are shown in Table 3. Pregnant women who developed HDPs had higher SBP (mean, daytime and nocturnal) and daytime DBP than normotensive women. Concerning the neonatal composite adverse outcomes, the nocturnal averages were significantly higher in women whose newborns had neonatal complications, as outlined in Table 3. We also identified a non-dipper pattern, and ABPM parameters of BP were significantly higher in women with preterm delivery than in mothers who had a term pregnancy. The remaining variables analyzed for obstetric and perinatal outcomes were not significantly different (results not shown).

Table 3. Bivariate analysis of the association between BP parameters, analyzed by ABPM, and develop of HDP, preterm delivery and the presence neonatal composite adverse outcomes.

Variables	Development of HDPs		Preterm Delivery		Neonatal Adverse Outcomes	
	Yes (n = 16)	No (n = 184)	Yes (n = 10)	No (n = 190)	Yes (n = 27)	No (n = 173)
24 h SBP (mmHg)	113.1 ± 13.5 *	104.7 ± 7.6 *	114.5 ± 14.5 *	104.9 ± 7.8 *	107.9 ± 11.1	105 ± 7.9
24 h DBP (mmHg)	68.8 ± 8.9	64.6 ± 5.5	72.1 ± 8.1 *	64.6 ± 5.6 *	67.0 ± 7.9	64.6 ± 5.5
Daytime SBP (mmHg)	115.6 ± 12.5 *	106.9 ± 8.2 *	115.9 ± 13.6	107.1 ± 8.4	109.7 ± 10.8	107.2 ± 8.5
Daytime DBP (mmHg)	71.1 ± 8.1 *	66.8 ± 5.9 *	73.6 ± 7.8 *	66.8 ± 5.9 *	68.9 ± 8.0	66.9 ± 5.9
Nocturnal SBP (mmHg)	107.8 ± 16.7 *	99.9 ± 7.9 *	112.0 ± 17.4 *	99.9 ± 8.1 *	103.8 ± 13.2 *	100 ± 8.3 *
Nocturnal DBP (mmHg)	62.9 ± 11.4	59.6 ± 5.9	68.9 ± 9.8 *	59.4 ± 6.1 *	62.7 ± 8.6 *	59.4 ± 6.1 *
Non-dipper pattern	7 (43.8%)	86 (46.7%)	9 (90.0%) *	84 (44.2%) *	15 (55.6%)	78 (45.1%)

Values are expressed as means ± standard deviation. Categorical variables are given as the number of subjects (n) with the percentage in parenthesis. * $p < 0.05$. HDPs = hypertensive disorders of pregnancy; SBP = systolic blood pressure; and DBP = diastolic blood pressure.

Multivariate analysis used the development of HDPs as the independent variable and all the variables that were statistically significant in the univariate model were used as dependent variables, with some independent variables considered as having possible clinical significance. Table 4 summarizes the results of these analyses in the final model. The outcomes indicated nocturnal SBP (OR = 1.077) and BMI (OR = 1.131) as risk factors for the development of HDPs.

Table 4. Final model of the multivariable regression analysis for the risk prediction of HDP. B: Beta; Exp (B): beta exponent; 95% CI for Exp (B): 95% confidence interval for beta exponent. GDM = gestational diabetes mellitus; BMI = body mass index; and SBP = systolic blood pressure.

Variables	B	p-Value	Exp (B)	95% CI for Exp (B)
Nocturnal SBP	0.074	0.015	1.077	1.015–1.143
Prepregnancy BMI	0.123	0.035	1.131	1.009–1.268
Dipper pattern	0.990	0.112	2.691	0.793–9.130
GDM	0.077	0.902	1.080	0.316–3.685
Age	−0.033	0.601	0.967	0.854–1.096

4. Discussion

In the present study, we found higher BP levels detected by ABPM in pregnant women who subsequently developed HDPs as well as adverse obstetric and perinatal outcomes. Our results suggest that ABPM could have clinical utility in the prediction of the risk for HDPs in pregnant women with comorbidities, such as GDM and prepregnancy obesity. To our knowledge, this is the largest sample size study on ABPM performed in women with GDM, and furthermore, we have included a group of patients with only obesity, without GDM, due to the close relationship between obesity and GDM [7] and the possibility that some of the results could be attributed to the presence of obesity, independently of the metabolic alterations present in GDM.

GDM and prepregnancy obesity are independent risk factors for maternal and neonatal complications [22–26]. In our cohort, the incidence of HDPs was 8% (n = 16), and no significant differences between groups were found with respect to the presence of GDM. However, obesity was observed to be an independent risk marker for developing HDPs, which is consistent with previous studies [23,27,28]. Moreover, when obesity is associated with GDM, the risk for HDPs increases compared to pregnant women with GDM without obesity, in agreement with other investigators [24,25,29,30]. On the other hand, while some authors found that the development of HDPs was even more frequent in pregnant women with obesity without GDM [24], others have not observed the same feature [30]. Either way, prepregnancy BMI seems to have a stronger influence than GDM on the development of HDPs [26].

Currently, there are no specific recommendations regarding the use of ABPM in pregnancy, except for the differentiation between true hypertension and the white-coat effect [31,32]. However, some authors recommend its usefulness in high-risk pregnancies [13]. In fact, Salazar et al. found a higher rate of masked hypertension (defined as a mean BP > 130/80 mmHg with normal office BP levels) and nocturnal hypertension in normotensive women with high-risk pregnancy, regarding these variables as predictors of the development of HDPs. In our cohort, we found higher 24 h, daytime, and nocturnal SBP and daytime DBP levels in pregnant women who developed HDPs. Furthermore, multivariate analysis identified nocturnal SBP as an independent risk factor for the development of HDPs, coinciding with the data published in relation to pregnant women with chronic hypertension or high-risk pregnancy [10,12]. In a cohort of normotensive pregnant women with GDM, we also observed that higher nocturnal SBP increased the risk of developing HDPs [14]. In addition, in this study, we found that patients in the GDM with obesity group had higher mean SBP, nocturnal SBP, and DBP than pregnant women with GDM without obesity. These data show that ABPM could have specific utility in this group with double risk, obesity and GDM. In this group, we also observed higher levels of triglycerides, in agreement with some authors [33,34], and postulated that this atherogenic profile could be related to endothelial dysfunction causing subclinical BP alterations, as has been described in subjects with type 1 diabetes mellitus [35]. Pregnant women with obesity and GDM also had higher HbA1c levels and received insulin therapy more frequently than GDM women without obesity, similar to findings reported by other investigators [24,29], but in our cohort, there was no difference in the total dose of insulin used between the two groups. Weight gain during pregnancy was significantly lower in the GDM groups than in the control group, which can be explained by specific dietary management and better compliance to recommendations in diabetic women. Nevertheless, inadequate weight gain has been related to the development of HDPs by other authors [29] as well as poor obstetric and perinatal outcomes [36].

In our study, the non-dipper pattern was more prevalent in the obesity groups, whereas women without obesity more frequently had a dipper circadian pattern. Although a significant relationship between the non-dipper pattern and the diagnosis of GDM in normotensive pregnant women has been described [14,37], these works do not analyze the influence of pre-pregnancy obesity as an independent factor in the ABPM pattern. In fact, there was a significant correlation between prepregnancy BMI and mean SBP and nocturnal SBP levels detected by ABPM (Figure 2) but not with blood glucose levels. These findings are consistent with the previous hypothesis reported [22,24,26] that considers prepregnancy obesity to have a more important role in the development of HDPs than GDM.

The incidence of HDPs in our patients was lower than expected, but it was higher in pregnant women with prepregnancy obesity even though ASA use was greater in this population. A possible explanation for these findings could be the use of low doses of ASA according to current recommendations in clinical practice guidelines [20,31] to reduce the risk of preeclampsia, preterm delivery, FGR, and miscarriage in pregnant women with chronic hypertension or risk factors for preeclampsia.

As described in the literature, gestational age at delivery was lower in pregnant women with GDM, probably due to the higher frequency of indications for induction in these patients compared to women with obesity without GDM. Most patients who had a preterm delivery showed a non-dipper pattern in ABPM, according to the data published by other authors, in pregnant women who develop gestational hypertension [16] and preeclampsia [38]. Furthermore, we observed significantly higher mean and nocturnal BP, both systolic and diastolic, and daytime SBP levels in these women compared to those with delivery at term, similar to that described previously in patients with preterm delivery associated with gestational hypertension [17].

The rate of delivery complications was higher in pregnant women with GDM and obesity, in agreement with other publications [24], but we did not find any differences with respect to birthweight, Apgar score, the rate of cesarean section, FGR, SGA, LGA or

perinatal complications (composite variable) between the different groups. An association between the presence of FGR or SGA and circadian variation in BP in both normotensive and hypertensive pregnant women has been described in the literature [15,16,18,19]. In our study, BP values in ABPM were higher in women who had FGR, but these findings were not statistically significant. Nevertheless, we detected nocturnal SBP and DBP values that were significantly higher in pregnant women whose newborns had neonatal complications (hypoglycemia, hyperbilirubinemia, congenital malformations or admissions to the intensive care unit), as has been reported by some authors in women who subsequently developed gestational hypertension [17,39].

The principal limitation of our study was the small size of the obesity group without GDM (13 women), which could have influenced our ability to detect any differences from the other groups, and the inclusion of more patients could have provided greater precision for our results. However, the sample size was adequate to detect statistically significant and clinically relevant results.

Nevertheless, the most important strength is that this study is the largest sample size study evaluating the relationship between ABPM parameters and obstetric and perinatal outcomes in pregnant women with GDM considering obesity, and the inclusion of non-diabetic women both with and without obesity.

5. Conclusions

We concluded that normotensive pregnant women with GDM and obesity have a high risk of maternal and perinatal complications, with a presumably greater impact of obesity than GDM. Subclinical alterations in the BP profile in women with preterm delivery and neonatal complications have been described, and nocturnal SBP predicts the development of HDPs. Thus, ABPM may be useful for identifying women at higher risk of adverse obstetric and perinatal outcomes who could benefit from preventive actions. Studies with larger sample sizes would be necessary to confirm our results and allow us to find greater differences.

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Prediabetes Is Associated with Increased Prevalence of Sleep-Disordered Breathing

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Abstract: Type 2 diabetes leads to severe nocturnal hypoxemia, with an increase in apnea events and daytime sleepiness. Hence, we assessed sleep breathing parameters in the prediabetes stage. A cross-sectional study conducted on 966 middle-aged subjects without known pulmonary disease (311 patients with prediabetes and 655 controls with normal glucose metabolism) was conducted. Prediabetes was defined by glycated hemoglobin (HbA1c), and a nonattended overnight home sleep

study was performed. Participants with prediabetes (n = 311) displayed a higher apnea–hypopnea index (AHI: 12.7 (6.1;24.3) vs. 9.5 (4.2;19.6) events/h, $p < 0.001$) and hypopnea index (HI: 8.4 (4.0;14.9) vs. 6.0 (2.7;12.6) events/h, $p < 0.001$) than controls, without differences in the apnea index. Altogether, the prevalence of obstructive sleep apnea was higher in subjects with prediabetes than in controls (78.1 vs. 69.9%, $p = 0.007$). Additionally, subjects with prediabetes presented impaired measurements of the median and minimum nocturnal oxygen saturation, the percentage of time spent with oxygen saturations below 90%, and the 4% oxygen desaturation index in comparison with individuals without prediabetes ($p < 0.001$ for all). After adjusting for age, sex, and the presence of obesity, HbA1c correlated with the HI in the entire population (r = 0.141, $p < 0.001$), and the presence of prediabetes was independently associated with the AHI (B = 2.20 (0.10 to 4.31), $p = 0.040$) as well as the HI (B = 1.87 (0.61 to 3.14), $p = 0.004$) in the multiple linear regression model. We conclude that prediabetes is an independent risk factor for an increased AHI after adjusting for age, sex, and obesity. The enhanced AHI is mainly associated with increments in the hypopnea events.

Keywords: apnea; glycated hemoglobin; hypopnea; prediabetes; obstructive sleep apnea

1. Introduction

In recent years, there has been growing evidence suggesting that type 2 diabetes can lead to the development of sleep breathing disorders (SBD) [1]. The deleterious effect of type 2 diabetes on nocturnal sleep breathing includes increased nocturnal awakenings, higher sleep fragmentation through higher rates of microarousals, changes in sleep architecture, sleep quality reduction and, consequently, excessive daytime sleepiness [2–4]. Furthermore, type 2 diabetes appears to be an independent risk factor for severe hypoxemia [5]. The Sweet Sleep study characterized obstructive sleep apnea (OSA) in patients with type 2 diabetes, providing evidence that the composition of their apnea–hypopnea index (AHI) is characterized by an increase in apnea events, with no differences or even reduction in hypopnea episodes [2]. Additionally, a small interventional study with 35 patients with type 2 diabetes and OSA has recently shown how the improvement of glycemic control without significant weight loss exerts beneficial effects on sleep breathing parameters [6]. This multifaceted relationship between diabetes milieu and sleep breathing is based on several pathophysiological mechanisms that include insulin resistance, inflammatory and oxidative stress-activated signaling pathways, leptin resistance and abnormalities in the autonomic nervous system [1,7]. A recent analysis of 151,194 participants from three prospective U.S. cohorts has showed that individuals with insulin-treated diabetes had 43% higher OSA risk when compared to those without diabetes [8]. These data possibly indicate that SBD appears in established diabetes with the worst metabolic control, which requires intensive glucose lowering.

Little information exists regarding the potential nighttime respiratory dysfunction in subjects with prediabetes. This prodromal stage in the hyperglycemia continuum that exists before the clinical diagnosis of type 2 diabetes affects 34.5% of all United States adults based on their fasting glucose or glycated hemoglobin (HbA1c) level [9]. Some of the etiopathogenetic mechanisms involved in the development of SDB in type 2 diabetes, such as insulin resistance and low-grade inflammation, are also part of the prediabetes environment. In fact, data from the National Health and Nutrition Examination Survey 2005–2008 showed that self-reported markers of SDB (sleep duration, snoring, snorting, and daytime sleepiness) were associated with prediabetes [10].

On this basis, our main goal was to test the impact of prediabetes in the sleep breathing pattern in a cross-sectional study of 989 middle-aged subjects without type 2 diabetes.

2. Materials and Methods

2.1. Ethics Approval

The protocol was approved by the Arnau de Vilanova University Hospital Ethics Committee (CEIC-1410). Moreover, the trial was conducted according to the ethical guidelines of the Helsinki Declaration and Spanish legislation regarding the protection of personal information was also followed. Written informed consent was provided by all individuals when they were included in the study. Informed consent was obtained from all individual participants included in the study.

2.2. Design of the Study and Report of the Study Individuals

The ILERVAS project is an ongoing randomized intervention study to assess the prevalence of subclinical vascular disease in the province of Lleida, Spain (ClinTrials.gov Identifier: NCT03228459, 25 July 2017) [11,12]. A total of 8330 middle-aged participants were recruited from diverse primary health care centers between January 2015 and December 2018. The inclusion criteria were women aged between 50 and 70, men aged between 45 and 65, and the presence of at least one cardiovascular risk factor (dyslipidemia, hypertension, obesity, smoking and/or having a first-degree relative with premature cardiovascular disease). The exclusion criteria were medical history of cardiovascular disease, type 2 diabetes, chronic kidney disease, active neoplasia, a life expectancy less than 18 months, institutionalized population (jail and penitentiary inmates, patients at psychiatric hospitals, persons in nursing homes, and persons in boarding schools) and pregnancy.

For the present study, 2411 consecutive subjects were assessed for eligibility between March 2017 and September 2018 and invited to perform a nonattended cardiorespiratory polygraphy. We excluded 1335 for the following reasons: unwillingness to participate in the study (n = 932), unable to locate by telephone (n = 285), patients under treatment with continuous positive airway pressure (CPAP) (n = 81), previously undiagnosed type 2 diabetes (n = 15), and unknown kidney disease (n = 22). Additionally, 110 subjects with a first unsatisfactory sleep study refused to repeat it a second time. Therefore, the investigation was finally performed with nine hundred and sixty-six individuals: 311 patients with prediabetes and 655 controls with normal glucose metabolism (Supplemental Figure S1).

2.3. Diagnosis of Prediabetes

Following the present American Diabetes Association guidelines, prediabetes was defined as an HbA1c between 39 and 47 mmol/mol (5.7 to 6.4%) and a normal glucose metabolism as an HbA1c < 39 mmol/mol (<5.7%) [13]. The HbA1c test was conducted on capillary blood using a point-of-care device (Cobas B 101®, Roche Diagnostics S.L., Sant Cugat del Vallès, Spain), based on a latex agglutination inhibition immunoassay technique that meets the generally accepted performance standards for HbA1c [14].

2.4. Nighttime Respiratory Function Assessment

All participants underwent a nonattended overnight home sleep study using a cardiorespiratory polygraphy (ApneaLink™ device, Resmed, Sydney, Australia) according to standard techniques [15]. Oronasal flow, thoracoabdominal movements and pulse oximetry were recorded. Cardiorespiratory polygraph records were scored manually according to standard criteria, and records with less than 3 h of sleep time were repeated. Apnea was defined as an absence of or reduction in nasal airflow of >90% with a duration of at least 10 s. A hypopnea was defined as a reduction of 30% to 90% in oronasal airflow for at least 10 s and associated with a drop in arterial oxygen saturation (SpO2) of at least 3.0%. The AHI was defined as the sum of apneas plus hypopneas recorded during the study per h of monitoring time, and participants were classified into non-OSA (AHI < 5 events/h), mild OSA (AHI between 5 and 14.9 events/h), moderate OSA (AHI between 15 and 29.9 events/h), and severe OSA (AHI $\geq$ 30 events/h) [16]. Four oxygen saturation measures were considered: the median and the minimum SpO_2 level, the cumulative percentage of time

spent with oxygen saturations below 90% (CT90), and the 4% oxygen desaturation index (ODI4%).

2.5. Excessive Daytime Sleepiness Assessment

Excessive daytime sleepiness was evaluated using the Epworth Sleepiness Scale (ESS), a widely used questionnaire based on one's likelihood to fall asleep unintentionally during eight daytime situations [17]. A score of 10 or more is considered sleepy. This questionnaire was completed by 80.2% of participants.

2.6. Covariates Assessment

Body weight and height were measured without shoes and slight clothing, and body mass index (BMI) was determined from kilograms divided by height in meters squared. Waist and neck circumferences were assessed using a nonstretchable tape with a precision of 0.1 cm. Waist circumference was measured midway between the iliac crest and the lowest rib on the horizontal plane with the individual in a standing position. Neck circumference was assessed in a plane as flat as possible, closely below the laryngeal prominence, while standing erect with eyes facing forward. Blood pressure was assessed in triplicate after five minutes' rest via an automated device (Omron M6 Comfort HEM-7221-E (Omron Healthcare, Kyoto, Japan)) at 2 min breaks, and the mean of the last two was calculated. Additionally, smoking habits (never, former or current smoker) were also known. Smokers who stopped smoking one or more years prior to visiting were considered former smokers.

2.7. Statistical Analysis

As the skewed distribution of all the variables was confirmed by the Shapiro–Wilk test, only nonparametric methods were used. Quantitative data were expressed as median (interquartile range) or as a percentage. The Mann–Whitney U test was used to compare continuous variables, while the Pearson's Chi-squared test was used to compare categorical records. The relationship between continuous variables was examined by the Spearman correlation test.

Three multiple linear regression models to explore the variables independently related to the AHI, the apnea index and the hypopnea index were used. Variables with a potential impact on sleep breathing function (i.e., age, sex, BMI) and the prediabetes stage were introduced in the models. The adequacy of the regression models was verified through submitting the residuals) to a normality test. All "p" values were based on a two-sided test of statistical significance. Significance was recognized at the level of $p < 0.050$. All statistical investigations were completed using the SSPS statistical package (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA).

3. Results

The main clinical characteristics of the study population according to the presence of prediabetes are displayed in Table 1. Subjects with prediabetes were older and presented a higher proportion of women, obesity, and hypertension than control ones.

When the sleep breathing was assessed, participants with prediabetes displayed a significantly higher AHI (12.7 (6.1;24.3) vs. 9.5 (4.2;19.6) events/h, $p < 0.001$) than controls (Table 2). The hypopnea index was also greater among participants with prediabetes (8.4 (4.0;14.9) vs. 6.0 (2.7;12.6) events/h, $p < 0.001$), without differences in the apnea index (Figure 1). Altogether, the prevalence of OSA was higher among participants with prediabetes than in controls (78.1 vs. 69.9%, $p = 0.007$). Additionally, patients with prediabetes presented a higher CT90 and ODI4%, as well as lower median and minimum SpO_2 levels ($p \leq 0.001$ for all) than patients without prediabetes. No difference in the daytime sleepiness was observed between groups.

Table 1. Main clinical characteristics of the study population according to the presence of prediabetes.

	Prediabetes (*n* = 311)	Control Group (*n* = 655)	*p*
Age (years)	59 (54;63)	56 (52;61)	<0.001
Women, *n* (%)	180 (57.8)	316 (48.2)	0.004
HbA1c (mmol/mol)	40 (39;42)	36 (33;37)	<0.001
HbA1c (%)	5.8 (5.8;6.0)	5.4 (5.2;5.5)	<0.001
Hypertension, *n* (%)	147 (47.2)	240 (36.6)	0.001
Systolic blood pressure (mm Hg)	132 (120;143)	128 (118;139)	0.008
Diastolic blood pressure (mm Hg)	81 (75;88)	81 (74;87)	0.569
Obesity, *n* (%)	124 (39.8)	177 (27.0)	<0.001
BMI (Kg/m^2)	29.7 (26.9;33.2)	27.9 (24.9;31.0)	<0.001
Waist circumference (cm)	103 (96;110)	98 (92;106)	<0.001
Neck circumference (cm)	37.5 (35.0;41.0)	37.5 (34.5;41.0)	0.261
Current or former smoker, *n* (%)	184 (59.1)	430 (65.6)	0.050

Information is displayed as a median (interquartile range) or *n* (percentage). HbA1c: glycated hemoglobin; BMI: body mass index.

Table 2. Nighttime respiratory characteristics of the study population according to the presence of prediabetes.

	Prediabetes (*n* = 311)	Control Group (*n* = 655)	*p*
Time of evaluation (hs)	7.2 (6.4;8.0)	7.2 (6.5;8.0)	0.684
AHI (events/h)	12.7 (6.1;24.3)	9.5 (4.2;19.6)	<0.001
Apnea index (events/h)	1.6 (0.4;5.8)	1.3 (0.4;4.3)	0.159
Hypopnea index (events/h)	8.4 (4.0;14.9)	6.0 (2.7;12.6)	<0.001
OSA, *n* (%)	243 (78.1)	458 (69.9)	0.007
Mild OSA, *n* (%)	108 (34.8)	227 (34.6)	0.142
Moderate OSA, *n* (%)	76 (24.4)	138 (21.0)	0.060
Severe OSA, *n* (%)	57 (18.5)	89 (13.6)	0.010
Median SpO_2 level (%)	92 (91;93)	93 (92;94)	<0.001
Minimum SpO_2 level (%)	82 (77;85)	83 (80;87)	<0.001
CT90 (%)	14 (4;33)	6 (1;24)	<0.001
ODI4% (events/h)	14 (8;27)	11 (5;21)	0.001
Epworth Sleepiness Scale score *	4 (2;5)	3 (2;5)	0.740

Information is displayed as a median (interquartile range) or *n* (percentage). AHI: Apnea–hypopnea index; OSA: obstructive sleep apnea; SpO_2: oxygen saturation; CT90: cumulative time percentage with SpO_2 < 90%; ODI4%: number of 4% oxygen desaturation index; * The Epworth Sleepiness Scale was completed by 80.2% of participants.

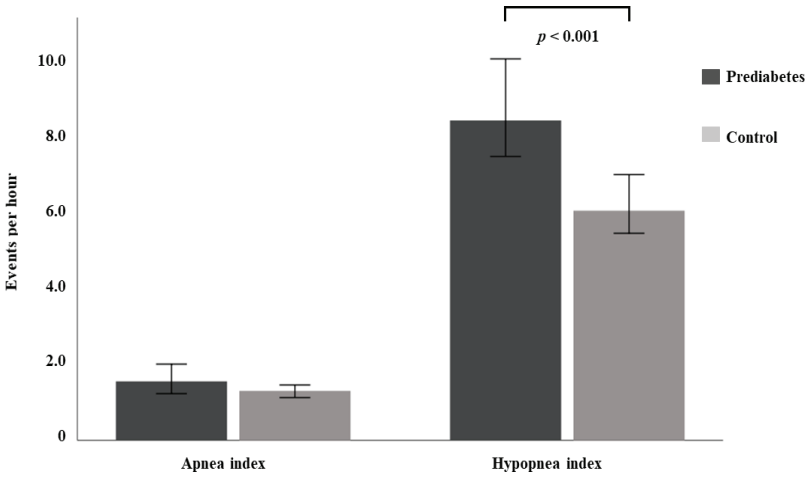

Figure 1. Plot displaying the number of apneas and hypopneas according to the presence of impaired glucose metabolism.

Both in the entire population and in those with prediabetes, HbA1c was slightly correlated with the hypopnea index (r = 0.141, $p < 0.001$ and r = 0.171, $p = 0.002$, respectively). HbA1c was also significantly correlated with others nighttime respiratory measurements in both groups (Table 3).

Table 3. Correlations of sleep respiratory measurements with glycated hemoglobin in the entire population and in participants with prediabetes.

	Prediabetes (n = 311)		Entire Population (n = 966)	
	r	p	r	p
AHI (events/h)	0.093	0.099	0.131	<0.001
Apnea index (events/h)	−0.039	0.492	0.065	0.042
Hypopnea index (events/h)	0.171	0.002	0.141	<0.001
Median SpO_2 level (%)	−0.166	0.003	−0.204	<0.001
Minimum SpO_2 level (%)	−0.126	0.025	−0.159	<0.001
CT90 (%)	0.144	0.010	0.209	<0.001
ODI4% (events/h)	0.123	0.028	0.150	<0.001
Epworth Sleepiness Scale score *	0.009	0.884	−0.035	0.322

AHI: Apnea–hypopnea index; SpO_2: oxygen saturation; CT90: cumulative time percentage with $SpO_2 < 90\%$; ODI4%: number of 4% oxygen desaturation index; * The Epworth Sleepiness Scale was completed by 80.2% of participants.

Finally, the multiple linear regression model (Table 4) showed that there was a significant and independent association between the presence of prediabetes and the AHI (B = 2.20 (0. 10 to 4.31), $p = 0.040$) as well as the hypopnea index (B = 1.87 (0. 61 to 3.14), $p = 0.004$), but no with the apnea index.

Table 4. The multiple linear regression model for the AHI, apnea index and hypopnea index.

AHI (Events/h) $R^2 = 0.12$	B (95% IC)	Standardized Regression Coefficients	p
Age (Years)	0.36 (0.19 to 0.52)	0.14	<0.001
Sex (Male)	8.29 (6.23 to 10.35)	0.24	<0.001
Obesity (BMI $\geq$ 30 kg/m^2)	8.54 (6.44 to 10.64)	0.26	<0.001
Prediabetes (HbA1c 5.7 to 6.4%)	2.20 (0. 10 to 4.31)	0.58	0.040
Apnea index (events/h) $R^2 = 0.06$			
Age (Years)	0.21 (0.13 to 0.30)	0.17	<0.001
Sex (Male)	3.58 (2.55 to 4.62)	0.20	<0.001
Obesity (BMI $\geq$ 30 kg/m^2)	1.73 (0.67 to 2.78)	0.11	0.001
Prediabetes (HbA1c 5.7 to 6.4%)	0.16 (−0. 90 to 1.22)	0.00	0.765
Hypopnea index (events/h) $R^2 = 0.12$			
Age (Years)	0.13 (0.03 to 0.23)	0.11	0.013
Sex (Male)	3.81 (2.57 to 5.05)	0.15	<0.001
Obesity (BMI $\geq$ 30 kg/m^2)	5.94 (4.68 to 7.21)	0.29	<0.001
Prediabetes (HbA1c 5.7 to 6.4%)	1.87 (0. 61 to 3.14)	0.82	0.004

B: unstandardized beta; AHI: Apnea–hypopnea index; BMI: body mass index. The adequacy of the regression models was verified through submitting the residuals to a normality test: in all cases $p < 0.001$.

4. Discussion

To the best of our knowledge, this is the first study to provide evidence that prediabetes (after adjusting for age, sex, and presence of obesity) presents with a distinctive sleep breathing pattern, with increased hypopnea episodes but with no differences in apnea events. In our study, and together with classical risk factors for sleep disorders such as age, BMI and male sex, the presence of prediabetes is independently associated with the hypopnea events. These results suggest that the previously evidenced deleterious impact of type 2 diabetes on nocturnal sleep breathing begins in the prediabetes stage, and that any

degree of disorder in glucose metabolism will influence this process. Additionally, patients with prediabetes also display a higher prevalence of OSA, as well as an impairment of nocturnal oxygen saturation. These data reinforce and extend previous reports, and run in accordance with the International Diabetes Federation, which recommends screening for OSA subjects with prediabetes [18,19].

Limited information exists on whether prediabetes increases the risk of developing OSA in the general population. A previous cross-sectional study evaluated 137 subjects with extremely obesity who underwent a portable sleep registration at home [19]. According to a standardized 2 h oral glucose tolerance test, the prevalence of OSA increased from 33% in subjects with normal glucose tolerance to 67% and 78% in patients with prediabetes and type 2 diabetes, respectively. Additionally, after adjustment for key clinical and systemic variables such as age, sex, BMI, systemic inflammation, insulin resistance, hypertension, smoking, alcohol consumption and medication, prediabetes was still associated with 3-fold increased odds of OSA compared with those subjects with normal glycaemia [19]. Our study, with a population with a mean BMI of 28.7 kg/m^2, allows us to analyze the results without the confounding factor of obesity, which directly affects OSA and glucose metabolism abnormalities. However, this study did not provide data related to the number of apneas and hypopneas added together resulting in the AHI. Therefore, our study spread these findings to a broader population with overweight and assessed the components of the AHI to better understand the impact of prediabetes on SBD.

A clear pathophysiology mechanism for the association between glucose abnormalities and sleep breathing has not yet been elucidated. However, insulin resistance, inflammation, visceral adiposity, autonomic dysfunction, and leptin resistance deserve to be commented on. In Sprague Dawley rats, *Ramadan* et al. showed the contribution of insulin resistance to apnea development. Additionally, oral treatment with metformin—an insulin-sensitizer drug—was able to avoid and reverse apnea episodes [20]. Data from 1780 men and 1785 women evaluated within the Epidemiologic Study on the Insulin Resistance Syndrome (DESIR) study showed that insulin resistance was related to a 6-year incident observed apnea during sleep [21]. The standardized odds ratios for fasting plasma insulin, HOMA-IR or triglycerides were 1.31, 1.31, and 1.24, respectively. More interestingly, the relation of insulin resistance and incident observed apnea was homogeneous across BMI classes for both men and women [21]. Similarly, women with polycystic ovary syndrome—characterized by insulin resistance—are more likely to have OSA and experience excessive daytime sleepiness than controls [22]. In addition, insulin resistance may mediate a blockage effect on the pharyngeal dilator muscle, just as the alterations in arterial muscle tone that are well recognized in prediabetes vascular disease [23,24]. Inflammatory processes associated with prediabetes might also affect the upper respiratory tract, reducing the lumen and favoring obstruction [19]. In fact, systemic inflammation measured by fibrinogen and C-reactive protein levels has been associated with nocturnal oxygen saturation parameters and the apnea–hypopnea index in snorers with compromised upper airway anatomy without type 2 diabetes [25]. In addition, other metabolic pathways that would explain the association between prediabetes and OSA have also been suggested, such as microvascular damage, lung microangiopathy, decreased muscle strength, nonenzymatic glycosylation of lung proteins, defects in the bronchiolar surfactant layer and the deficit in glucagon-like peptide 1 concentrations [1].

Our population with prediabetes exhibits a BMI near to 30.0 kg/m^2, with an increased prevalence of obesity and a higher waist circumference in comparison with control participants. Adiposity may appear as a source of proinflammatory factors as well as lead to the narrowing of the upper airways [26]. However, no differences in neck circumference between groups were observed in our study, making the second option less likely to be responsible for the higher prevalence of OSA in patients with prediabetes. As autonomic dysfunction is already present in prediabetes, both afferent and efferent motor pathway dysfunctions may influence the regulation of blood gases and oxygen delivery, contributing to a blunted ventilatory response to hypoxemia [1,27]. Finally, as leptin has been associated

with prediabetes in a dose-dependent manner, pathways related to leptin resistance in type 2 diabetes could also contribute to deficiencies in central respiratory control [28].

As patients with OSA have an almost two-fold higher risk of developing cardiovascular events and all-cause mortality than controls, its increased prevalence could participate in the milieu that favors cardiovascular disease in the prediabetes stage [29,30]. In fact, in a more extensive cohort from the ILERVAS project, subjects with prediabetes presented a higher prevalence of subclinical atheromatous disease than the control group, especially in the carotid territory [31]. Furthermore, nocturnal cycling hypoxia, together with female sex and fasting plasma glucose, has been demonstrated to be independently associated with an increased density of carotid vasa vasorum, an early event in atheromatous disease [32].

Only one study has, so far, focused on weight loss intervention in subjects with both prediabetes and obstructive sleep apnea, showing that changes in SpO_2 were associated with changes in insulin sensitivity but not with weight loss [33]. Similarly, our group has demonstrated that in type 2 diabetes the improvement of metabolic control achieves a significant reduction in sleep breathing parameters not related with weigh modifications [6]. These results point out a direction for the further improvement of metabolic control in individuals with prediabetes. Whether this optimization must be conducted only with lifestyle changes or medical treatment is not known. For example, glucagon-like peptide (GLP)-1, widely used as anti-diabetic drug, may be an effective therapy for patients with prediabetes and OSA [34–36]. Similarly, the combination of metformin and dapagliflozin for 24 weeks in patients with a newly diagnosed type 2 diabetes and OSA achieved a reduction in both the AHI and daily somnolence in comparison with a control group receiving metformin plus glimepriride [37].

Sleep quality, assessed using the Pittsburgh Sleep Quality Index (PSQI), has also been evaluated in subjects with prediabetes [38]. In this way, Iyegha et al. showed how 62% of subjects with prediabetes suffered from poor sleep quality, compared with less than half of normoglycemic subjects [38]. We have no data regarding sleep quality in the ILERVAS project, but no differences in the ESS were observed between participants with and without prediabetes. Our results are in concordance with those of Renko et al., in which daytime sleepiness was not linked with using sleep medication or impaired glucose regulation [39]. Altogether, this may suggest that sleep quality is more sensitive to the prediabetes stage than daytime sleepiness.

This study has a few limitations that need to be addressed. Currently, we have diagnosed prediabetes according only to HbA1c values, one of the three possibilities accepted by the American Diabetes Association. However, as different tools identify different populations, future studies using fasting plasma glucose (impaired fasting glucose) and 2 h plasma glucose (impaired glucose tolerance) are needed. Second, we have no information about the time of appearance of prediabetes in our population, a factor that might influence our results similarly to how the known evolution time in type 2 diabetes affects the incidence of classical chronic complications. Third, the gold standard diagnosis of OSA according to the American Academy of Sleep Medicine is through polysomnograms, not cardiorespiratory polygraphs. The latter shows a lower diagnostic performance compared to polysomnography that is related to the difficulty in identifying apneas and especially hypopneas. However, population-based studies assessing sleep breathing parameters in large populations are easily performed with at-home registers. In addition, this fact amplifies the significance of our results, in which a higher prevalence of hypopnea events in patients with prediabetes compared to the control group has been detected. Fourth, we have no circulating biomarkers of insulin resistance or systemic inflammation, and therefore, their potential role in this association could not be assessed in our study. Finally, Fifth, we do not have data on psychiatric illnesses in our population, which have a direct effect on the severity of OSA. Moreover, the cross-sectional nature of the study does not allow us to establish causality with the results, as well as characteristic of the ILERVAS population (Spanish middle-aged individuals with low-to-moderate cardiovascular risk) precludes us from generalizing our results to the global population. Finally, future studies

need to be designed to better evaluate the underlying mechanisms that link prediabetes with OSA and its clinical significance.

5. Conclusions

In summary, the prevalence of OSA was significantly higher in participants with prediabetes that in the control group in a large cohort study of Spanish subjects with low-to-moderate CV risk. After adjusting for age, sex, and the presence of obesity, the increased AHI in participants with prediabetes was mainly associated with increments in the hypopnea events, supporting the hypothesis that glucose abnormalities exert a lineal negative impact on SBD, from insulin resistance with normal fasting glucose to confirmed diagnosis of type 2 diabetes. Moreover, the presence of prediabetes independently predicted the AHI and the hypopnea events per hour. Additional studies to identify subjects with prediabetes more vulnerable to experiencing problems with nighttime respiratory function, and factors that accelerate its progression and severity, are needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11051413/s1, Figure S1: Flow chart in study population.

Author Contributions: Data curation: R.G, F.B., G.T., A.S., M.D., C.L.-C. and L.G.-C. Formal analysis: R.G., F.B., G.T., A.S., M.D., C.L.-C. and L.G.-C. Project administration: M.B.-L., E.F. and A.L. Investigation: F.P., E.C.-B. and C.F.-S. Methodology: F.P., E.C.-B. and C.F.-S. Software: F.P., E.C.-B. and C.F.-S. Resources: M.B.-L., E.F. and A.L. Supervision: R.P., D.M., C.H., R.S. and A.L. Validation: R.P., D.M., C.H., R.S. and A.L. Visualization: E.S. and E.S.-B. Writing—original draft: E.S. and E.S.-B. Writing—review and editing as well as final approval of the version to be published: all authors. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Arnau de Vilanova University Hospital (protocol code CEIC-1410; date approval: 2 January 2015).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the signed consent agreements around data sharing, which only allow access to the researchers of the study following the project purposes. Requestors wishing to access the data used in this study can make a request to M.B.-L. The request will be subjected to approval and formal agreements regarding confidentiality and secure data storage being signed the data would be the provided.

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A New Approach to Overcome Insulin Resistance in Patients with Impaired Glucose Tolerance: The Results of a Multicenter, Double-Blind, Placebo-Controlled, Randomized Clinical Trial of Efficacy and Safety of Subetta

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Abstract: Impaired glucose tolerance (IGT) is a common carbohydrate metabolism disorder worldwide. To evaluate the efficacy and safety of 12-week Subetta therapy in correcting 2-h plasma glucose in patients with IGT, a multicenter, double-blind, placebo-controlled, randomized clinical trial was performed. Derived by technological treatment of antibodies to insulin receptor β-subunit and endothelial NO synthase, Subetta increases the sensitivity of insulin receptors by activating the insulin signaling pathway. Oral glucose tolerance test (OGTT), fasting plasma glucose (FPG), and glycated hemoglobin (HbA1c) were examined at screening, after 4 and 12 weeks. In Per Protocol population, 2-h plasma glucose in the Subetta group decreased by 2.05 ± 2.11 mmol/L (versus 0.56 ± 2.55 mmol/L in the Placebo group) after 12 weeks. The difference between the two groups was 1.49 ± 2.33 mmol/L ($p < 0.0001$). After 12 weeks, 65.2% of patients had 2-h plasma glucose <7.8 mmol/L. FPG remained almost unchanged. HbA1c tended to decrease. The number of adverse events did not differ in both groups. Subetta treatment is beneficial for patients with IGT; it also prevents progression of carbohydrate metabolism disorders.

Keywords: impaired glucose tolerance; 2-h plasma glucose; glycated hemoglobin

1. Introduction

Impaired glucose tolerance (IGT) is an intermediate stage of carbohydrate metabolism disorders between normal glucose tolerance (NGT) and type 2 diabetes mellitus (T2DM) [1,2]. The average transition time from IGT to T2DM is 4 years [3]. IGT can be associated with

impaired fasting glucose (IFG) or normal fasting plasma glucose (FPG) [1,4,5]. IGT is considered as prediabetes, along with IFG and glycated hemoglobin (HbA1c) values in the range of 5.7–6.4% [1,2]. Progression from IGT to T2DM is associated with hyperglycemia, age, and weight [4].

The global prevalence of IGT was 7.5% of the adult population in 2019, and projected prevalence is expected to reach 8.0% in 2030, and 8.6% in 2045 [6].

The rates for the patients who have both IFG and IGT are 15.8% and 20.2% according to World Health Organization (WHO) and American Diabetes Association (ADA) data, respectively [1,5,7].

In patients with IGT, the risk of T2DM development is 6 times higher compared to people with NGT, the relative risk of mortality is 1.48 times more than in a healthy population, and frequency of fatal cardiovascular events is increased by 1.66 times [1]. According to the International Diabetes Federation, 1 in 10 adults are living with diabetes [8].

It was proposed that IGT is a manifestation of insulin resistance due to "lipid overspill from subcutaneous adipose into ectopic sites" [5] (i.e., liver, pancreas, and skeletal muscle) in individuals with a positive energy balance, excess lipid accumulation, and weight gain [9].

Intensive lifestyle/behavior change programs are the first-line interventions in patients with prediabetes for T2DM prevention [10–15]. If these approaches are insufficient to achieve weight loss and glycemic control, metformin therapy is recommended by ADA [12]. However, the U.S. Food and Drug Administration has yet to approve any medicines for diabetes prevention [12]. At the same time, in 2021 the European Medicines Agency approved liraglutide [16] and recommended the granting of marketing authorization for semaglutide [17] for weight management in patients with obesity or overweight in the presence of prediabetes and other comorbidities.

One of the approaches to overcome insulin resistance and prevent progression of the carbohydrate metabolism disorders is Subetta therapy. This drug is a biotechnological product containing two components based on affinity-purified antibodies to the β-subunit of the insulin receptor (INSR-β) and to endothelial NO synthase (eNOS). Subetta stimulates activation of the insulin receptor alone and in the presence of insulin increasing phosphorylation of INSR-β [18]. Subetta significantly enhances the insulin sensitivity of human muscle cells through stimulation of glucose transport to myocytes mediated by glucose transporter 4 [19,20].

In patients with T1DM and poor glycemic control in a basal-bolus insulin regimen, Subetta add-on therapy improved the glycemic profile without insulin dose intensification and without increasing the overall hypoglycemia rates [21].

The pharmacological activity of Subetta can be used in patients with IGT to increase the sensitivity of cells to endogenous insulin, which is reduced due to insulin resistance.

In this clinical trial, we evaluated the efficacy and safety of 12-week Subetta therapy in correcting 2-h plasma glucose in patients with IGT.

2. Materials and Methods

2.1. Study Overview

This multicenter, double-blind, placebo-controlled, randomized, parallel-group clinical trial was carried out between 10 October 2018 and 23 March 2020 in 44 medical institutions in the Russian Federation (see Supplementary Materials, Study overview). The protocol of the study and the study results are posted in the ClinicalTrials.gov results database (NCT03725033) [22].

The screening of patients was performed over 7 days, after which they were randomized into two groups and treated for 12 weeks. Patients visited medical centers three times—on day 1, after 4 weeks (visit 2), and after 12 weeks (visit 3) of the treatment, and they were examined at each visit. The observation period lasted for up to 13 weeks.

2.2. Patient Selection and Assessment

Patients of either gender, aged 18–70 years old, with prediabetes, obesity (especially visceral or abdominal obesity), dyslipidemia (with high triglycerides and/or low high-density lipoproteins), arterial hypertension, or high genetic burden of diabetes mellitus (diabetes in first-degree relatives) were considered as candidates for participation in the study.

The screening procedures included medical history, registration of concomitant conditions and diseases, physical examination, calculation of body mass index (BMI), and oral glucose tolerance test (OGTT) for measurement of FPG and 2-h plasma glucose. In addition, venous blood samples (for detection of HbA1c level and biochemical and clinical analyses) and urine samples were obtained from each patient.

All laboratory tests were carried out in the central laboratory, which provided the equipment for collection and sample preparation in the medical centers. Blood samples were collected after a night fast of no less than 10 h. A venous blood sample from the antecubital vein was drawn into two vacuum tubes for plasma and serum separation. The delivery of biological samples was performed within 6 h, in compliance with the requirements of transportation conditions. Measurement of FPG and 2-h plasma glucose was performed using the enzyme (hexokinase) method and photometry in a biochemical analyzer (ARCHITECT c16000, Abbot, Chicago, IL, USA). HbA1c was determined by a method certified in accordance with the National Glycohemoglobin Standardization Program and standardized in compliance with the reference values adopted in the Diabetes Control and Complications Trial. Hematology, blood chemistry and urinalysis were performed to assess the safety of the treatment. Blood chemistry included alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, creatinine, total cholesterol, total bilirubin, total protein, sodium, and potassium.

The inclusion criteria were as follows: plasma glucose level from 7.8 to 11.0 mmol/L two hours after a 75 g oral glucose load during an OGTT, while FPG < 7.0 mmol/L; HbA1c, 5.7–6.4%; BMI, 25.0–39.9 kg/m^2; consent to use contraceptive methods during the study (for men and women with reproductive potential).

The recommendations of the WHO and the International Diabetes Federation were used for the diagnosis of IGT and prediabetes.

One of the inclusion criterion was a BMI of 25.0–39.9 kg/m^2 (overweight, or 1/2 degree obesity). Measurement of body weight and height was carried out at the screening stage on standardized (calibrated according to the factory method) scales and a stadiometer. BMI was calculated according to the formula: weight/(height in meters)^2.

Candidates with normal BMI were not included in the study. After examination during the screening period, the presence of IGT in patients with a BMI of 25.0–39.9 kg/m^2 could be not confirmed if the parameters of carbohydrate metabolism did not meet the inclusion criteria.

The exclusion criteria were as follows: T1DM, T2DM, and other specific types of diabetes; acute disease or exacerbation/decompensation of a chronic disease; uncontrolled arterial hypertension; acute coronary syndrome, myocardial infarction, acute impairment of cerebral circulation during the previous 6 months; unstable or life-threatening arrhythmia during the previous 3 months; acute or chronic heart failure with functional class III or IV; respiratory failure; chronic kidney disease (classes C3–5 A3); hepatic insufficiency (class C according to Child–Pugh); oncology disease; an allergy/hypersensitivity to medication administered; mental illness or drug abuse; history of bariatric surgery; pregnancy, breastfeeding; childbirth less than 3 months before enrollment; use of any prohibited medications.

One of the exclusion criterion was uncontrolled arterial hypertension, defined as office systolic blood (BP) pressure $\geq$ 160 mmHg and/or diastolic BP $\geq$ 100 mm Hg (grade 2 hypertension or grade 3 hypertension according to ESH/ESC 2013 guidelines for the management of arterial hypertension). BP was measured in the office by a doctor at screening and at every visit using an automated validated upper-arm cuff BP devices based on the oscillometric technique. Measurement of BP was performed in accordance with 2017 American College of Cardiology/American Heart Association Guideline.

2.3. Randomization and Blinding

After screening, the patients were randomized into two groups to be assigned Subetta or Placebo. An interactive voice/web response randomization system (based on a random number generator) was used by physicians. Block randomization was performed in blocks of 4. A personal code documented in the chart and unchanged during the study was given to each patient in order to maintain confidentiality.

The studied drug was delivered to medical centers in boxes and packages that did not carry information regarding the active substance. Manufacturing, packaging, and labelling with unique identification codes of the double-blind medications (Subetta or placebo/identical in shape and taste tablet containing excipients) were performed by OOO NPF MMH. Neither participants nor physicians, investigators, trial centers staff, or the Sponsor's project team were aware of the treatment group assignment throughout the study and until the database lock.

2.4. Treatment

Subetta was administered for oral use, 2 tablets per intake twice a day, 15 min before a meal for 12 weeks. The tablet should be held in the mouth until complete dissolution occurred.

Each Subetta tablet contains affinity purified technologically-treated antibodies to β-subunit of the insulin receptor (6 mg) and antibodies to endothelial NO synthase (6 mg). The active substance is produced by patented technology (US Patent 8,617,555 B2) in accordance with European Pharmacopeia requirements [21].

Placebo was administered according to Subetta administration schedule.

The compliance with the study therapies was assessed at the 2nd and 3rd visits according to the count of tablets returned.

Patients were given advice on nutrition and physical activity. A pregnancy test was carried out for all fertile women.

Patients were allowed to use concomitant therapy, including agents acting on the renin-angiotensin system, beta blocking agents, and calcium channel blockers.

Blood glucose lowering drugs, insulins and analogues, corticosteroids, thyroid preparations, hormonal contraceptives, appetite stimulants, anti-obesity preparations, long-action organic nitrates, and diuretics were prohibited drugs.

2.5. Study End Points and Statistical Analysis

The primary efficacy end point was a change in 2-h plasma glucose (during OGTT) after 12 weeks of treatment. The secondary end points were as follows: percentage of patients with 2-h plasma glucose <7.8 mmol/L, change in FPG, and change in HbA1c after 12 weeks of treatment.

Statistical analysis was performed using SAS (Version 9.4) (SAS Institute, Cary, NC, USA) statistical software. Data of the full analysis set, excluding the failure to satisfy major entry criteria, were used for the intention-to-treat (ITT) analysis. The data of all patients who completed the therapy as per the study protocol without any missing scheduled visits were used for the Per-Protocol (PP) analysis of the efficacy (data are presented in square brackets).

The sample size was calculated assuming the difference in reduction of 2-h plasma glucose between Product and Placebo groups would be no less than $\varepsilon = 1.1$, while the standard deviation would be $\sigma = 3.11$. The power of statistical tests "$p = (1 - \beta)$" was assumed to be 80% (the probability of correct rejection of the null hypothesis was 0.8); the probability of a type I error "α" was allowed to be less than 0.05% (the probability of the erroneous acceptance of an alternative hypothesis was less than 0.05); the statistical criteria used were two-tailed. The minimum required size for each group was 143 patients; at least 842 patients had to be included, taking into account a dropout rate at 66% subjects (Cw = 0.66) during the study for various reasons. The dropout rate was based on the results of a blinded interim analysis.

Two interim analyses were planned and performed during the study:

(1) Blinded interim analysis to clarify population characteristics and adjust the sample size (only upward). As a result of this analysis, the sample was increased, and Version 2 of the study protocol was released.
(2) Unblinded interim analysis included data from more than 50% of the planned sample (n = 538). Unblinded interim analysis was planned for early trial stop due to efficacy (O'Brien–Fleming boundary) or null hypothesis acceptance (Pocock boundary).

According to O'Brien and Fleming [23], if the p-value for the primary efficacy endpoint is <0.00388 in the interim analysis of data, the study may be stopped due to evidence of efficacy.

After the inclusion of 538 patients, the study was terminated when interim analysis showed significant reduction in 2-h plasma glucose in patients of the Subetta group in comparison with placebo therapy. Interim analysis demonstrated that the result of the Subetta therapy was sufficient to stop the study due to the achievement of efficacy, because the type I error (0.0028 (<0.0001)) was below the critical value (0.00388) established by the O'Brien–Fleming rules for the interim analysis.

Analysis of continuous variables was carried out using the nonparametric Wilcoxon test and Student's t-test for normally distributed variables. Normality of variables was accessed using the Shapiro–Wilk test. Multivariate analysis was performed using analysis of variance for repeated measurements (repeated-measures ANOVA, PROC MIXED). The Holm method (PROC MULTTEST) was used as a correction for multiplicity. Fisher's exact test was used to compare the proportions.

3. Results

3.1. Patient Demographics and Baseline Characteristics

In total, 538 subjects with suspected IGT were enrolled. After passing the screening procedures, 336 subjects were excluded by the doctors as they did not meet inclusion criteria, or they met exclusion criteria. The remaining 202 subjects were randomized into two groups: 105 to the Subetta group and 97 to the Placebo group. The results of the treatment and observation of these patients (n = 202) were used to conduct an ITT analysis of efficacy and to assess the safety of the investigational therapy. The number of patients who received the full course of the therapy, completed all prescribed visits, and did not have significant deviations from the protocol was 174, including 92 in the Subetta group and 82 in the Placebo group. The data from these patients were used for the PP efficacy analysis. Data from 28 patients (13 patients in the Subetta group and 15 patients in the Placebo group) were not included in the PP analysis of efficacy for various reasons. Figure 1 presents the study design flow diagram.

Patients in both groups did not differ in demographic and baseline clinical characteristics, including age, gender, BMI, vital signs, and initial parameters of carbohydrate metabolism (2-h plasma glucose, FPG, and HbA1c). Baseline characteristics of the patients are presented in the Tables 1–3.

As can be seen from Table 1, the majority of the study participants were women. To assess the effect of gender on the results of the study, we conducted analysis that showed no statistical significance of gender, both in ITT and PP populations. The statistical analysis used involves mixed models for 2-h plasma glucose with gender covariate and CMH test with Breslow–Day test for percentage of patients with 2-h plasma glucose < 7.8 mmol/L after 12 weeks with gender as the main strata (see Supplementary Materials, Tables S1–S4).

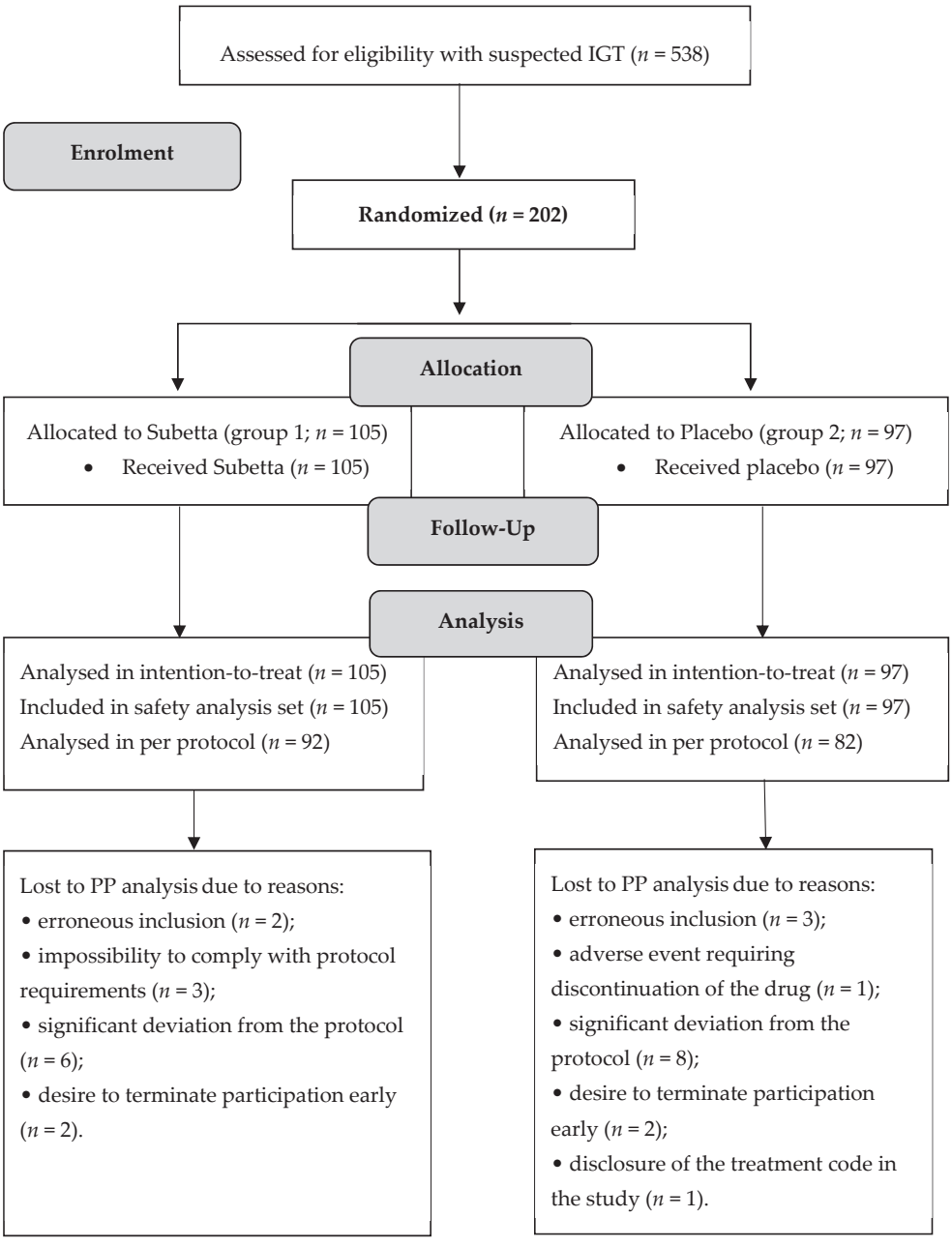

Figure 1. Study design flow diagram.

Table 1. Baseline demographic and anthropometric characteristics of the patients.

Characteristics	ITT Analysis (N = 202)		PP Analysis (N = 174)	
	Subetta	Placebo	Subetta	Placebo
Age, years				
Mean ± SD	56.6 ± 8.6	56.1 ± 8.6	57.0 ± 9.1	56.3 ± 8.6
Median	58	57	58.5	56.5
Minimum	28	33	28	38
Maximum	70	69	70	69
Q1–Q3	51–54	52–64	52.5–64	52–64
Statistics	Z = 0.64; p = 0.52		Z = 0.84; p = 0.40	
Male/female, %	25.7/74.3	22.7/77.3	27.2/72.8	25.6/74.4
	p = 0.63		p = 0.86	
Body weight, kg				
Mean ± SD	88.2±15.0	88.7±14.5	87.8±14.4	89.3 ± 14.8
Median	85	89	84.8	89
Minimum	58	62	58	62
Maximum	120	128.2	120	128.2
Q1–Q3	76.6–99.1	77–98	77.3 – 98.4	77–98.7
Statistics	Z = 0.30; p = 0.76		Z = 0.68; p = 0.50	
BMI, kg/m^2				
Mean ± SD	31.8 ± 4.2	32.0 ± 4.3	31.7 ± 4.0	32.0 ± 4.1
Median	31.2	32.1	31.2	31.9
Minimum	25.1	25.3	25.1	25.4
Maximum	39.7	39.5	39.7	39.5
Q1–Q3	28.4–35	28.2–35.5	28.6–34.7	28.3–35.5
Statistics	Z = 0.28; p = 0.78		Z = 0.30; p = 0.77	

Notes. Mean ± SD—mean and standard deviation. Q1–Q3—the first and third quartiles. N—number of patients. The age of the patients was analyzed using the Wilcoxon test; the result of the normality test using the Shapiro–Wilk test: ITT—Subetta—p = 0.0003, Placebo—p = 0.0019; PP—Subetta—p = 0.0002, Placebo—p = 0.0030. Gender was analyzed and compared using Fisher's exact test. The result of the normality test using the Shapiro–Wilk test: BMI—Subetta—p = 0.0006, Placebo—p = 0.0017; PP—Weight—Subetta—p = 0.1249, Placebo—p = 0.2565, BMI—Subetta—p = 0.0055, Placebo—p = 0.0008.

Table 2. Baseline blood pressure of the patients.

	ITT Analysis (N = 202)		PP Analysis (N = 174)	
	Subetta	Placebo	Subetta	Placebo
Systolic blood pressure, mm Hg				
Mean ± SD	127.5 ± 8.0	127.8 ± 8.5	127.4 ± 7.9	127.8 ± 8.3
Median	129	127	128.5	127
Minimum	100	98	100	98
Maximum	147	156	147	156
Q1–Q3	122–132	122–134	122–132	122–134
Statistics	Z = 0.48; p = 0.63		Z = 0.56; p = 0.58	
Diastolic blood pressure, mm Hg				
Mean ± SD	79.1 ± 5.6	79.4 ± 6.9	78.8 ± 5.7	79.4 ± 7.0

Table 2. *Cont.*

	ITT Analysis (N = 202)		PP Analysis (N = 174)	
	Subetta	**Placebo**	**Subetta**	**Placebo**
Median	80	80	80	80
Minimum	62	54	62	54
Maximum	90	97	90	97
Q1–Q3	75–83	75–84	74.5–83	75–84
Statistics	Z = 0.15; p = 0.88	Z = 0.41; p = 0.68	Z = 0.15; p = 0.88	Z = 0.41; p = 0.68

Notes. Mean ± SD—mean and standard deviation. Q1–Q3—the first and third quartiles. N—number of patients. Blood pressure indicators were analyzed using Student's t-test and Wilcoxon's test; the result of the normality test using the Shapiro–Wilk test: Systolic blood pressure (ITT analysis)—Subetta—p = 0.1900, Placebo—p = 0.0071; (PP analysis)—Subetta—p = 0.1205, Placebo—p = 0.0019; Diastolic blood pressure (ITT analysis)—Subetta—p = 0.0483, Placebo—p = 0.2262; (PP analysis)—Subetta—p = 0.0489, Placebo—p = 0.2045.

Table 3. Baseline parameters of carbohydrate metabolism of the patients.

Parameters	ITT Analysis (N = 193 *)		PP Analysis (N = 174)	
	Subetta	**Placebo**	**Subetta**	**Placebo**
2-h plasma glucose, mmol/L				
Mean ± SD	9.3 ± 0.9	9.1 ± 0.9	9.3 ± 0.8	9.1 ± 0.9
Median	9.2	9.0	9.2	9.0
Q1–Q3	8.5–9.9	8.4–9.6	8.5–9.9	8.4–9.6
95% CI	9.1–9.4	8.9–9.3	9.1–9.4	8.9–9.3
N *	101	92	92	82
Statistics	Z = 1.22; p = 0.22		Z = 1.47; p = 0.14	
Fasting plasma glucose, mmol/L				
Mean ± SD	5.8 ± 0.6	5.9 ± 0.6	5.9 ± 0.6	5.9 ± 0.6
Median	5.9	5.9	5.95	5.9
Q1–Q3	5.4–6.3	5.5–6.3	5.4–6.3	5.5–6.3
95% CI	5.7–6.0	5.7–6.0	5.7–6.0	5.7–5.9
N *	101	92	92	82
Statistics	Z = 0.29; p = 0.77		Z = 0.23; p = 0.82	
HbA1c,%				
Mean ± SD	6.0 ± 0.2	6.0 ± 0.20	6.0 ± 0.2	6.0 ± 0.2
Median	5.9	6.0	5.9	6.0
Q1–Q3	5.8–6.1	5.8–6.2	5.8–6.1	5.8–6.2
95% CI	5.9–6.0	6.0–6.0	5.9–6.00	6.0–6.0
N *	101	92	92	82
Statistics	Z = 1.92; p = 0.06		Z = 1.72; p = 0.08	

Notes. Mean ± SD—mean and standard deviation. Q1–Q3—the first and third quartiles. * N—number of patients. Blood samples were taken from 9 randomized patients (n = 4, Subetta group; n = 5, placebo group) who were fully treated and underwent all procedures according to the protocol, but the central laboratory did not provide carbohydrate metabolism values due to technical problems. Data were analyzed using the Wilcoxon test; the result of the normality test using the Shapiro–Wilk test: 2-h plasma glucose (ITT analysis)—Subetta—p = 0.0013, placebo—p = 0.0034; (PP analysis)—Subetta—p = 0.0047, Placebo—p = 0.0050; fasting plasma glucose (ITT analysis)—Subetta—p = 0.0002, Placebo—p = 0.0226; (PP Analysis)—Subetta—p = 0.0001, Placebo—p = 0.0490; HbA1c (ITT analysis)—Subetta—p = 0.0001, Placebo—p = 0.0009; (PP Analysis)—Subetta—p = 0.0001, Placebo—p = 0.0011.

Various comorbidities were found in 91.4 [91.3]% (n = 96 [n = 84]) of patients in the Subetta group and in 87.6 [89.0]% (n = 85 [n = 73]) of subjects of the Placebo group (p = 0.49 [p = 0.62]).

Sixty one percent of patients [60.9]% (n = 62 [n = 56]) in the Subetta group and 63.9 [63.4]% (n = 62 [n = 52]) of patients in the Placebo group (p = 1.00 [p = 0.76]) had metabolic and nutritional disorders (dyslipidemia, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, hyperuricemia, etc.); 57.1 [57.6]% (n = 60 [n = 53]) and 45.4 [47.6]% (n = 44 [n = 39]) of patients (p = 0.12 [p = 0.22]) had vascular diseases (arterial hypertension, atherosclerosis of vessels of various localization, chronic venous insufficiency, etc.); 40.0 [42.4]% (n = 62 [n = 56]) and 37.1 [37.8]% (n = 62 [n = 56]; p = 0.77 [p = 0.77 [p = 0.64]) had heart diseases (coronary heart disease, angina pectoris, cardiac arrhythmias, NYHA class I/II heart failure, etc.); 22.9 [25.0]% (n = 24 [n = 23]) and 22.7 [24.4]% (n = 22 [n = 20]; p = 1.00 [p = 1.00]) had diseases of muscle, skeletal, and connective tissue; 24.8 [22.8]% (n = 26 [n = 21]) and 23.7 [25.6]% (n = 23 [n = 21]; p = 0.87 [p = 1.00]) had diseases of the gastrointestinal tract; 19.0 [17.4]% (n = 20 [n = 16]) and 19.6 [18.3]% (n = 19 [n = 15]; p = 1.00 [p = 1.00]) had diseases of the liver and biliary tract; 21.0 [21.7]% (n = 22 [n = 20]) and 20.6 [20.7]% (n = 20 [n = 17]; p = 1.00 [p = 1.00]) had thyroid gland pathology. Other diseases were less common. Statistical analysis using Fisher's exact test did not reveal significant differences between groups of patients in the incidence of concomitant diseases.

Seventy two percent [71.7]% (n = 75 [n = 66]) of patients in the Subetta group and 72.2 [72.0]% (n = 70 [n = 59]) in the Placebo group received permitted concomitant therapy (p = 1.00 [p = 1.00]). Most patients in both groups took drugs for the treatment of cardiovascular diseases, including agents acting on the renin-angiotensin system (52.4 [53.3]% (n = 55 [n = 49]) in the Subetta group and 53.6 [52.4]% (n = 52 [n = 43]) in the Placebo group; p = 0.89 [p] = 1.00]), beta blocking agents (30.5 [29.3]% (n = 32 [n = 27]) and 26.8 [29.3]% (n = 26 [n = 24]); p = 0.64 [p = 1.00]), calcium channel blockers (13.3 [15.2]% (n = 14 [n = 14]) and 10.3 [9.8]% (n = 10 [n = 8]), respectively; p = 0.52 [p = 0.36]). Medications from other pharmacological groups were taken by a relatively small percentage of the study participants. Fisher's exact test did not show differences between groups for concomitant therapy.

Patients in the Subetta and the Placebo groups excluded from the PP analysis also did not differ (both among themselves and compared to patients whose data were included in the analysis) in baseline demographic, anthropometric, clinical characteristics, comorbidities, and concomitant therapy.

The adherence of patients to the therapy after 4 weeks was 100.5 ± 12.5 [99.7 ± 5.6]% in the Subetta group and 101.9 ± 13.6 [100.6 ± 4.8]% in the Placebo group, and after 12 weeks of treatment this was 98.1 ± 7.4% [98.3 ± 4.8]% and 100.5 ± 8.5 [98.8 ± 4.4]%, respectively (p = 0.76 [p = 0.72]).

3.2. Primary Efficacy Endpoint

Two-hour plasma glucose concentration in the Subetta group significantly decreased after 12 weeks of treatment. Figure 2 indicates the change from baseline of 2-h plasma glucose after 12 weeks of treatment in patients of the PP sample.

The difference in 2-h glucose level between baseline and after 12 weeks of treatment in the Subetta group was −1.9 ± 2.2 [−2.0 ± 2.1] mmol/L (versus −0.7 ± 2.2 [−0.5 ± 2.5] mmol/L in the Placebo group). The difference in the reduction of 2-h plasma glucose between the Subetta and the Placebo groups was 1.2 ± 2.3 [1.4 ± 2.3] mmol/L (p = 0.0028 [p < 0.0001]).

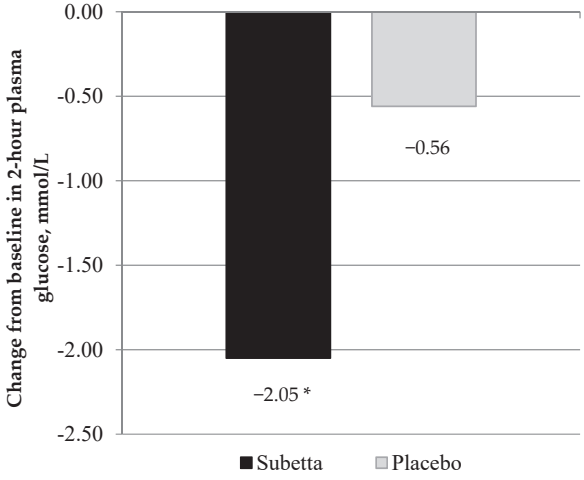

Figure 2. Change from baseline in 2-h plasma glucose after 12 weeks of treatment (PP analysis). Note. * $p < 0.0001$ vs. placebo.

3.3. Secondary Efficacy Endpoint

Figure 3 shows the percentage of patients with 2-h plasma glucose < 7.8 mmol/L after 12 weeks of treatment in patients of the PP sample, where the advantage of Subetta was 17.6% ([$p = 0.0219$]).

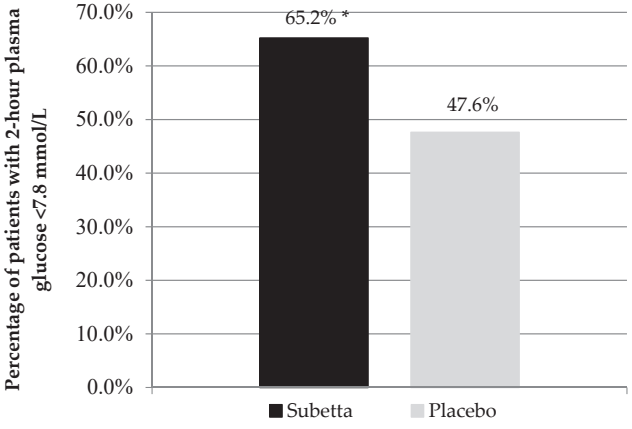

Figure 3. Percentage of patients with 2-h plasma glucose < 7.8 mmol/L after 12 weeks of treatment (PP analysis). Note. * $p = 0.0219$ vs. placebo.

The baseline FPG level was either within the normal range or slightly exceeded the normal threshold level of 6.0 mmol/L in most patients in both groups. The boundaries of the lower and upper quartiles were 5.4–6.3 [5.4–6.3] mmol/L in the Subetta group and 5.5–6.3 [5.5–6.3] mmol/L in the Placebo group ($p = 0.06$ [$p = 0.08$]).

FPG remained almost unchanged during 12 weeks of treatment in both groups. The boundaries of the lower and upper quartiles after 12 weeks were 5.4–6.5 [5.4–6.5] mmol/L and 5.4–6.4 [5.4–6.5] mmol/L in the Subetta and the Placebo group, respectively ($p = 0.99$ [$p = 0.74$]).

Within 12 weeks of treatment, there was a trend towards a decrease in HbA1c level in both groups. The boundaries of the lower and upper quartiles after 12 weeks were 5.5–6.0

[5.5–6.0]% and 5.6–6.0 [5.6–6.1]% in the Subetta and the Placebo group, respectively. This means that, after 12 weeks, 25% of patients in the Subetta group had HbA1c below 5.5%.

3.4. Safety Analysis

Subetta had no impact on the vital signs of patients, including blood pressure, heart rate, and respiratory rate. The mean values of the vital signs throughout the study were normal and well-controlled. There were no differences in these parameters during treatment between both groups.

In total, 16 adverse events (AEs) were reported in 15 (14.3%) patients of the Subetta group, and 27 AEs reported in 20 (20.6%) patients of the Placebo group (see Supplementary Table S5).

Frequency analysis (Fisher's exact test) did not reveal significant differences between the number of patients with AEs in the Subetta and the Placebo groups ($p = 0.27$).

The most frequent AEs were changes in laboratory and instrumental test results. In the Subetta group, this was an increase in the number of leukocytes in urine ($n = 1$), an increase in alanine aminotransferase (ALT) level ($n = 1$), an increase in blood pressure ($n = 1$), weight loss ($n = 1$), and in the Placebo group, an increase in the ALT ($n = 1$), an increase in aspartate aminotransferase ($n = 1$), an increase in HbA1c level ($n = 1$), an increase in blood pressure ($n = 3$), and a decrease in weight ($n = 1$).

Within 3 months, in 7 patients IGT progressed to T2DM, including 2 patients in the Subetta group and 5 patients in the Placebo group.

In the Subetta group, 13 (81.43%) AEs were mild and 3 (18.7%) were moderate. No AEs were reported with definite/possible relationship with the study drug. The frequency of distribution of AEs depending on the severity ($p = 0.42$) and the relationship with the drug ($p = 0.17$) did not differ in the two groups (see Supplementary Tables S6 and S7).

AEs classified as severe were not registered in the clinical trial. There was no evidence of drug-to-drug interaction with medications administered concomitantly with Subetta, nor were there any hypersensitivity events.

4. Discussion and Conclusions

This study demonstrated the efficacy of Subetta in patients with IGT. The therapeutic action of Subetta is manifested in the reduction of 2-h glucose, which prevents progression of disorders of carbohydrate metabolism. After 12 weeks of Subetta administration, 2-h glucose was restored to normal levels in most patients with IGT.

Administration of Subetta had no effect on FPG. On the one hand, this may indicate that the drug has (first of all) an antihyperglycemic effect, without affecting normal plasma glucose concentration. On the other hand, it was found that disorders of carbohydrate metabolism in IFG and IGT differ in development mechanisms. It is known that impaired insulin secretion and suppression of gluconeogenesis are the main mechanisms in the development of IFG [24]. Obviously, the pharmacological activity of Subetta, which consists of sensitizing the insulin receptor and increasing the sensitivity of cells to insulin, does not significantly affect the processes of hormone secretion and gluconeogenesis [20]. In this regard, the level of fasting glycemia in patients who took the study drug for 12 weeks remained unchanged, while 2-h (post-load) hyperglycemia was amenable to good correction (precisely by reducing insulin resistance).

Along with eliminating IGT in most patients, Subetta "initiated" HbA1c correction. Baseline "pre-diabetic" HbA1c values tended to decrease after 12 weeks of treatment. As is known, HbA1c is a long-term blood glucose control indicator; it reflects glucose concentration over the past 2–3 months [25]. Obviously, during the very first weeks after starting the treatment, there was no significant change in 2-h plasma glucose. Therefore, peaks of postprandial hyperglycemia in this initial treatment period contributed negatively and HbA1c was not decreased below 5.7% in most patients. However, it should be noted that 25% of patients in the Subetta group had HbA1c values below 5.5%. It is possible that the use of the drug for a longer period may normalize HbA1c in most patients with IGT.

Insulin resistance, which is leading in carbohydrate metabolism disorders in patients with IGT, is well corrected by Subetta over a 12-week course of treatment. The effect of the drug is realized by improving glucose utilization due to a decrease in insulin resistance. Obviously, longer therapy can contribute to a more significant normalization of carbohydrate metabolism and prevent the progression of disorders to T2DM.

The endothelioprotective effect of the second component of Subetta (technologically treated antibodies to eNOS) [19] is also important in therapy because vascular complications manifest already at the IGT stage [26]. The synergistic action of the two components of the drug has a positive effect on both insulin resistance and endothelial dysfunction, thereby preventing the progression of micro- and macroangiopathies.

This study has several limitations. It has a relatively small sample size due to a high drop-out rate during screening. Patients with serious concomitant diseases were not included in the study, and therefore the efficacy of Subetta in these patient categories was not investigated. Due to the short duration of treatment (12 weeks), the effects of Subetta on body weight, as well as waist circumference and lipid profile, were not evaluated. In addition, the therapeutic effects of various schemes of Subetta administration have not been evaluated.

In conclusion, the results of this study demonstrated the therapeutic potential of Subetta in patients with IGT. Obviously, longer studies need to be planned to prove the long-term effects of Subetta in patients with insulin resistance.

5. Patents

Subetta is a drug manufactured by OOO "NPF" MATERIA MEDICA HOLDING". Patents on Subetta: US 8,617,555 B2; MX 331246; RU 2509572, and RU2531048.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm11051390/s1: Study overview; Table S1: Level of 2-h plasma glucose with gender covariate (ITT analysis); Table S2: Level of 2-h plasma glucose with gender covariate (PP analysis); Table S3: Percentage of patients with 2-h plasma glucose <7.8 mmol/L after 12 weeks of treatment (ITT analysis); Table S4: Percentage of patients with 2-h plasma glucose <7.8 mmol/L after 12 weeks of treatment (PP analysis); Table S5: Adverse events; Table S6: Relationship between the drug and adverse events; Table S7: Adverse events severity.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Boards of all medical centers and the National Council for ethics of the Ministry of Health of the Russian Federation (protocol code 195 dated 9 July 2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study prior to enrolment.

Data Availability Statement: Study details are provided at (https://clinicaltrials.gov/ct2/show/NCT03725033 (accessed on 23 January 2022)) and can also be obtained by contacting the study sponsor OOO "NPF" MATERIA MEDICA HOLDING".

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Review

IL-1β Implications in Type 1 Diabetes Mellitus Progression: Systematic Review and Meta-Analysis

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Abstract: During Type 1 Diabetes Mellitus (T1DM) progression, there is chronic and low-grade inflammation that could be related to the evolution of the disease. We carried out a systematic review and meta-analysis to evaluate whether peripheral levels of pro-inflammatory markers such as interleukin-1 beta (IL-1β) is significantly different among patients with or without T1DM, in gender, management of the T1DM, detection in several biological fluids, study design, age range, and glycated hemoglobin. We searched PubMed, Embase, Web of Science, and Scopus databases, and 26 relevant studies (2186 with T1DM, 2047 controls) were included. We evaluated the studies' quality using the Newcastle–Ottawa scale. Meta-analyses were conducted, and heterogeneity and publication bias were examined. Compared with controls, IL-1β determined by immunoassays (pooled standardized mean difference (SMD): 2.45, 95% CI = 1.73 to 3.17; $p < 0.001$) was significantly elevated in T1DM. The compared IL-1β levels in patients <18 years (SMD = 2.81, 95% CI = 1.88–3.74) was significantly elevated. The hemoglobin-glycated (Hbg) levels in patients <18 years were compared (Hbg > 7: SMD = 5.43, 95% CI = 3.31–7.56; $p = 0.001$). Compared with the study design, IL-1β evaluated by ELISA (pooled SMD = 3.29, 95% CI = 2.27 to 4.30, $p < 0.001$) was significantly elevated in T1DM patients. IL-1β remained significantly higher in patients with a worse management of T1DM and in the early stage of T1DM. IL-1β levels determine the inflammatory environment during T1DM.

Keywords: IL-1β; type 1 diabetes mellitus; chronic inflammation; systematic review; meta-analysis

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1. Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease often diagnosed in childhood that progresses with pancreatic β-cell destruction and life-long insulin dependence. T1DM susceptibility involves a complex interplay between genetic and environmental factors and with the participation of adaptive immunity, although there is now growing evidence for the role of innate inflammation [1].

T1DM, in the early stage of the disease, is characterized by chronic inflammation that involves pancreatic islet degeneration. The maintenance activation of the innate immune system impairs insulin secretion and action, and inflammation also contributes to diabetes complications, such as diabetic retinopathy and nephropathy. Prior to the manifestation of the disease, a pre-diabetic period may last several years and is characterized by the detection of circulating autoantibodies against beta-cell antigens [2]. There is evidence that indicates a direct pathogenic effect of IL-1β on the islet during the development of T1DM. In pancreatic samples from adult living donors, the presence of IL-β and TNF-α has been detected, mainly in macrophages and dendritic cells [3]. However, despite strong preclinical evidence demonstrating that targeting inflammatory pathways can prevent

secondary complications, there are still no treatments for diabetes that target innate immune mediators [4].

In patients with T1DM, higher levels of proinflammatory cytokines have been detected that are physiological constituents of any inflammatory reaction, including interleukins (IL-1α, IL-1β, IL-10, IL-12), interferons (IFNα/β, IFNγ), transforming growth factor-β (TGF-β), tumor necrosis factors (TNFα, TNFβ), and nitric oxide (NO) [5]. The microenvironment is also enriched in anti-inflammatory cytokines including IL-4, IL-10, IL-13, and IL-22, which are generally associated with protective effects over β-cell survival [6].

The role of cytokines in the pathogenesis of autoimmune disorders, particularly T1DM, has been extensively investigated to determine their potential therapeutic value. Screening for the presence of cytokines during the early stages of T1DM can serve to identify immunological response-related soluble factors and a better diagnosis and treatment of the disease.

Interleukin 1 (IL-1) is a 17 kDa protein highly conserved through evolution and is a key mediator of inflammation [7], and it has been suggested as candidate for inducing beta-cell apoptosis in vitro and aggravating diabetes in vivo. Recently, a significant number of studies have given attention to the role of IL-1β in the pathogenesis of autoimmune and inflammatory diseases. There are numerous studies that relate the polymorphisms and gene variations in the IL-1β gene with the differences in the transcription and expression of the IL-1β gene that could correlate with the development of many autoimmune and inflammatory diseases, such as systemic lupus erythematosus [8], rheumatoid arthritis [9], and multiple sclerosis [10].

The genetic or pharmacological inhibition of IL-1 action has clinical efficacy in many inflammatory diseases, due to IL-1 acting on T-lymphocyte regulation. The adverse effects of IL-1β on human beta cells in vitro and in animal models have promoted recent clinical trials in volunteers with recent-onset type 1 diabetes, using strategies involving the systemic blockade of IL-1β or its receptors [7,11]. Genetic or pharmacological abrogation of IL-1 action reduces disease incidence in animal models of type 1 diabetes mellitus [12]. The modulating effect of IL-1 on the interaction between the innate and adaptive immune systems and the effects of IL-1 on the beta-cell point to this molecule being a potential interventional target in autoimmune diabetes mellitus.

Regarding the participation of other pro-inflammatory cytokines, such as TNF-α and IL6, in patients with T1DM, these were found to be linked to elevated level of serum IL-6 and TNF-α, on which the age, ethnicity, and disease duration [12,13] in T1DM patients had no effect on the serum IL-6 levels for promoting diabetes mellitus. The IL-1β level's modulation during different stages of T1DM could be a sensor of progression and good management of disease over time.

With this background, we conducted the first systematic review and meta-analysis to qualitatively and quantitatively evaluate the available scientific evidence on circulating IL-1β levels in T1DM. The aim of this work is to determine the modulated levels of IL-1β between patients with or without T1DM, and to explore their hypothetical influential variables (i.e., geographical area, age, sex, human tissues, biochemical parameters, research methods, and IL-1β determination techniques).

2. Material and Methods

This systematic review and meta-analysis complied with *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) and *Meta-analysis of Observational Studies in Epidemiology* (MOOSE) guidelines [14,15], and closely followed the criteria of the *Cochrane Handbook for Systematic Reviews of Interventions* [16].

2.1. Protocol

In order to minimize the risk of bias and improve the transparency, precision, and integrity of this study, a protocol on its methodology was a priori registered in the PROSPERO international prospective register of systematic reviews (www.crd.york.ac.uk/PROSPERO,

accessed on July 2020, registration code CRD42020180062) [17]. The protocol adhered to the PRISMA-P statement to ensure a rigorous approach [18].

2.2. Search Strategy

We searched PubMed, Embase, Web of Science, and Scopus databases for studies published before the search date (upper limit = October 2020), with no lower date limit. The searches were conducted by combining thesaurus terms used by the databases (i.e., MeSH and EMTREE) with free terms (Table S1, Supplementary Materials p. 1), and built to maximize sensitivity. We also manually screened the reference lists of retrieved studies for additional relevant studies. All references were managed using Mendeley Desktop v. 1.19.4 (Elsevier, Amsterdam, The Netherlands); duplicate references were eliminated using this software.

2.3. Eligibility Criteria

Inclusion criteria: (1) original research studies without language, publication date, follow up periods, study design, geographical area, sex, or age restrictions; (2) T1DM subjects compared to no T1DM as control group; (3) lL-1β determination by enzyme-linked immunosorbent assay (ELISA), quantitative real time polymerase chain reaction (qRT-PCR) and/or flow cytometry in human samples from any anatomical origin; (4) the names and affiliations of authors, recruitment period and settings were examined to determine whether studies were conducted in the same study population. In such cases, we included the most recent study or that which published more complete data.

Exclusion criteria: (1) retracted articles, reviews, meta-analyses, case reports, clinical trials, editorials, letters, abstracts of scientific meetings, personal opinions or comments, and book chapters; (2) in vitro and animal experimental studies; (3) studies that do not report the disease of interest (i.e., T1DM), do not assess IL-1β levels, or those without a control group; (4) studies reporting insufficient data to extract or estimate mean ± standard deviation (SD); (5) overlapping populations.

2.4. Study Selection Process

The eligibility criteria were applied independently by three authors (LGJ, FCC, and AIA). Discrepancies were resolved by consensus with a fourth author (PRG). Articles were selected in two phases, first screening the titles and abstracts of the retrieved articles in an initial selection, and then reading the full text of the selected articles, excluding those that did not meet the review eligibility criteria.

2.5. Data Extraction

Three authors (LGJ, FCC, and AIA) independently extracted data from the selected articles, completing a data collection form in a standardized manner using Excel v. Microsoft Office Professional Plus 2013 (Microsoft. Redmond, WA, USA). These data were additionally cross-checked in multiple rounds, solving discrepancies by consensus. The data were gathered on the first author, publication year, study country and continent, sample size, source of sample (i.e., type of tissue), IL-1β determination (extracting means ± SD and measuring units) in T1DM and controls (i.e., patients not affected by T1DM), age, year of diagnosis, sex of patients, Hbg levels, research methods analysis technique (e.g., ELISA or qRT-PCR), and type of study (i.e., cross-sectional, case-control, or cohorts).

2.6. Evaluation of Quality and Risk of Bias

We used the Newcastle–Ottawa quality assessment scale (NOS) to assess the risk of bias [19]. The evaluation was conducted by two independent reviewers who were knowledgeable about the content and methodology. The results were compared and conflicts resolved by agreement between the two reviewers, with input from a third reviewer if necessary. The studies that received a star in each domain were considered to be of high quality. The maximum score was 8, the minimum 0. It was decided a priori that a score of

7 reflected high methodological quality (i.e., low risk of bias), a score of 5 or 6 indicated moderate quality, and a score of 4 or less indicated low quality (i.e., high risk of bias).

2.7. Statistical Analysis

Mean (±SD) IL-1β levels were extracted to compare between T1DM patients and controls. Since variations in laboratory determination methods were expected (see protocol), the standardized mean difference (SMD) was chosen as an effect size measure, estimated by Cohen's d method with their corresponding 95% confidence intervals (CI). Data expressed as order statistics (i.e., median, interquartile range and/or maximum–minimum values) were computed and transformed into means (±SD) using the methods proposed by Luo et al. and Wan et al. [20,21]. If it was desirable to combine two or more different means (±SD) from subgroups into a single group, the method provided by the Cochrane Handbook was followed [16]. When the data were only expressed graphically, they were measured and extracted using Engauge Digitizer 4.1. In the meta-analyses, SMDs with 95% CIs were pooled using the inverse-variance method under a random-effects model (based on the DerSimonian and Laird method), which accounts for the possibility that there are different underlying results among study subpopulations (i.e., IL-1β variations among tissues, linked to geographical areas, or related to the inherent heterogeneity of the wide range of experimental methods). Forest plots were constructed to graphically represent the overall effect and for subsequent visual inspection analysis ($p < 0.05$ was considered significant). The heterogeneity between studies was evaluated applying the χ^2-based Cochran's Q test (given its low statistical power, $p < 0.10$ was considered significant) and quantified using Higgins I^2 statistic (values of 50–75% were interpreted as a moderate to high degree of inconsistency across the studies), which estimates what proportion of the variance in observed effects reflects variation in true effects, rather than sampling error [22,23]. Preplanned stratifications (by geographical area, type of tissue, age, Hbg levels, study design, matching, and type of analysis) and univariable meta-regression analyses (by sex and risk of bias) were conducted to identify potential sources of heterogeneity and to explore the potential variation of IL-1β levels on these subgroups [24]. For illustrative purposes, weighted bubble plots were also constructed to graphically represent the fitted meta-regression lines. Sensitivity analyses were additionally performed to test the reliability of our results, evaluating the influence of each individual study on the pooled estimations. For this purpose, the meta-analyses were repeated sequentially, omitting one study each time (the classic "leave-one-out" method). Finally, canonical and contour-enhanced funnel plots were constructed, and the Egger regression test ($p < 0.10$ considered significant) and the non-parametric trim-and-fill method were performed to evaluate small-study effects, such as publication bias [25–28]. Stata version 16.1 (Stata Corp., College Station, TX, USA) was employed for all tests, with the commands syntax being manually typed (PRG) [29].

3. Results

3.1. Results of the Literature Search

The flow diagram (Figure 1) depicts the identification and selection process of the studies. We retrieved a total of 3143 records published before October 2020: 626 from MEDLINE/PubMed, 817 from Embase, 826 from the Web of Science, 874 from Scopus, and one [30] from the reference lists screening. After eliminating the duplicates, 1666 studies were considered potentially eligible. After screening their titles and abstracts, 59 were selected for full-text reading. After excluding studies that did not meet all eligibility criteria (all of the studies excluded and their exclusion criteria are listed in the Supplementary Materials, pp. 2–6), 26 studies were finally included in the systematic review for qualitative evaluation (all of the studies included are listed in the Supplementary Materials, pp. 7–9) and 25 studies for quantitative meta-analysis. Due to the presence of a considerable degree of clinical, methodological, and statistical heterogeneity, only plasma and serum studies were meta-analyzed to obtain results derived from more homogeneous subpopulations and more

reliable results, while determinations from gingival fluid and vitreous humor were omitted from the meta-analysis.

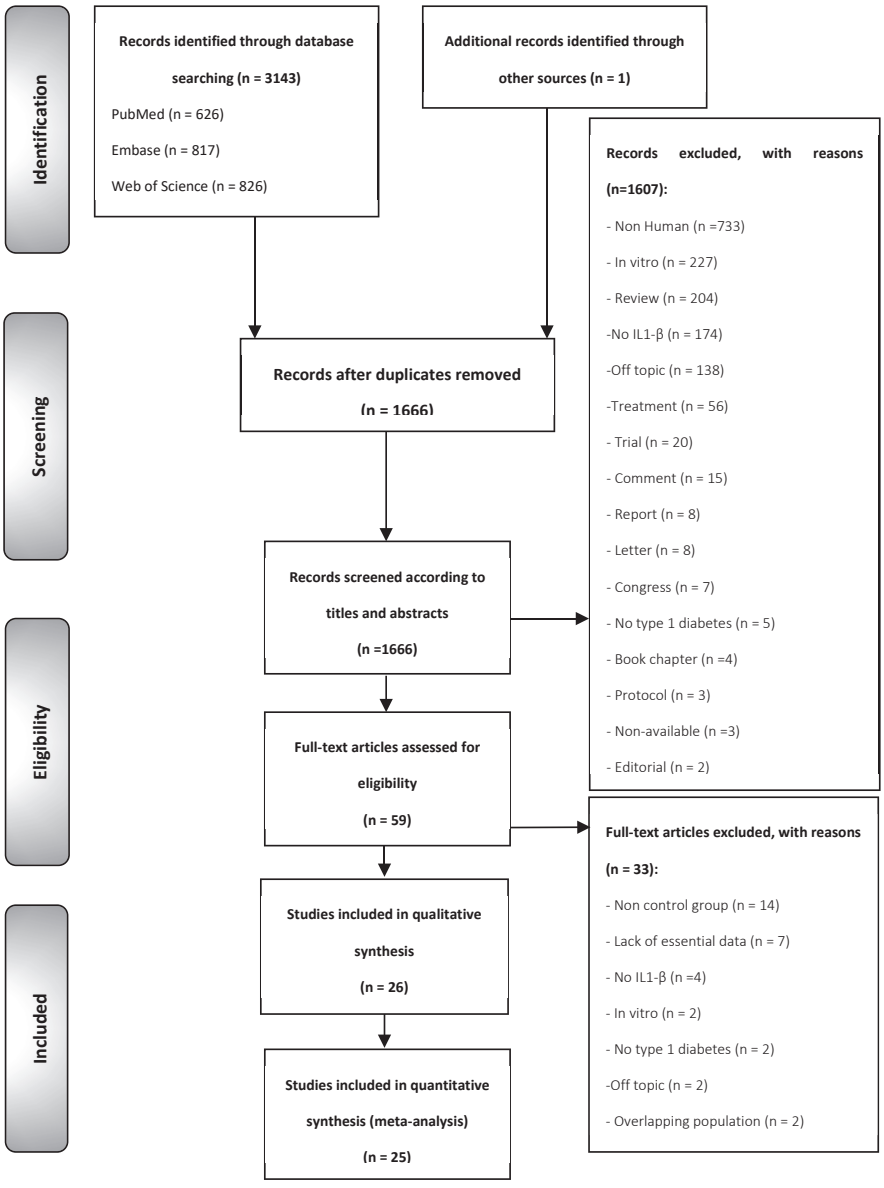

Figure 1. Flow diagram. Identification and selection process of relevant studies comparing IL-1β levels between T1DM patients and controls.

3.2. Study Characteristics

Table 1 summarizes the characteristics of the 26 selected studies comparing the changes in circulating IL-1β levels on a total of 4179 T1DM and control patients, and Table S2 (Supplementary Materials, p. 10) exhibits the variables gathered from each study in more detail. One study [31] analyzed IL-1β levels in two tissues (plasma and vitreous humor)

being considered as two different analysis units (i.e., n = 27 studies/4233 patients). Sample sizes ranged between 18 and 961 subjects. The studies were conducted in all continents except for Oceania and comprised 12 in Europe, 6 in Asia, 5 in South America, 3 in Africa, and 1 in North America. IL-1β determination was performed by immunoassays in 22 studies (18 by ELISA and 4 by panels; 15 in serum, 5 in plasma, and 1 in gingival crevicular fluid (not meta-analyzed) and 1 in vitreous humor (not meta-analyzed)), flow cytometry in 3 studies (2 in serum and 1 in cord blood plasma), and 2 studies in qRT-PCR (gingival tissue and peripheral blood mononuclear cells (PBMC)).

Table 1. Summarized characteristics of reviewed studies.

Total	26 studies *
Year of publication	2004–2019
Number of patients	
Total	4179 patients *
Cases with T1DM	2186 patients
Controls	2047 patients
Sample size, range	18–961 patients
IL-1β determination	
Immunoassays	22 studies (18 by ELISA, 4 by panels)
Flow cytometry	3 studies
qRT-PCR	2 studies
Source of samples	
Serum	17 studies
Plasma	5 studies
Gingival crevicular fluid	1 study
Vitreus humour	1 study
Cord blood plasma	1 study
Gingival tissue	1 study
Peripheral blood leukocytes	1 study
Geographical region	
Europe	12 studies
Asia	6 studies
South America	5 studies
Africa	3 study
North America	1 study

*—One study (Koskela et al., 2013) analyzed IL-1β levels in two tissues (plasma and humour vitreus), being considered as two different analysis units (i.e., n total = 27 studies/4233 patients).

3.3. Qualitative Evaluation

The qualitative analysis was conducted using the Newcastle–Ottawa Scale (NOS), which evaluates potential sources of bias in eight domains (Table 2).

Table 2. Summary of risk of bias assessment based on Newcastle–Ottawa Quality Assessment Scale. Two reviewers who had content and methodological expertise independently and in duplicate assessed and graded the risk of bias for the included studies with an adapted version of the Newcastle–Ottawa scale (NOS), which has been described elsewhere [8]. The assessments were compared and conflicts resolved by agreement between the two reviewers. The maximum score was 8, the minimum score 0. It was decided a priori that a score of 7 was reflective of high methodological quality (e.g., low risk of bias), a score of 5 or 6 indicated moderate quality, and a score of 4 or less indicated low quality (e.g., high risk of bias). A filled blue star indicates that a star has been awarded, and a blank star indicates that no star has been awarded and the study has been graded as poor quality in that category [8]. Wells GA (2010) The Newcastle–Ottawa Scale (NOS) For Assessing The Quality Of Non Randomised Studies In Meta-Analyses. Ottawa (ON): Ottawa Health Research Institute.

Study	Selection			Control		Outcomes			Overall Quality
	Representativeness of the T1DM patients	Selection of the non-T1DM subjects	Properly IL1b quantification	Glycemic control	Control of confounding factors	Assessment of T1DM progression	Appropriate follow up period	Adequacy of follow up	
Pérez-Bravo et al. (2004)	★	★	★	★	★	☆	★	★	High
Lo et al. (2004)	★	★	★	★	★	☆	★	★	High
Holm et al. (2006)	★	★	★	★	★	★	★	★	High
Dogan et al. (2006)	★	★	★	☆	★	★	★	★	High
Arabi et al. (2007)	★	★	★	★	★	★	★	★	High
Duarte et al. (2007)	★	★	★	★	★	★	★	★	High
Salvi et al. (2010)	★	★	★	★	★	☆	★	★	High
Meyers et al. (2010)	★	★	★	★	★	★	★	★	High
Gabbay et al. (2012)	★	★	★	☆	★	☆	★	★	Moderate
Svensson et al. (2012)	★	★	★	☆	★	★	★	★	High
Ururahy et al. (2012)	★	★	★	★	★	★	★	★	High
Fartushok et al. (2012)	★	★	★	★	★	☆	★	★	High
Koskela et al. (2013)	★	★	★	★	★	☆	★	★	High

Table 2. *Cont.*

Study	Selection			Control		Outcomes		Overall Quality	
	Representativeness of the T1DM patients	Selection of the non-T1DM subjects	Properly IL1b quantification	Glycemic control	Control of confounding factors	Assessment of T1DM progression	Appropriate follow up period	Adequacy of follow up	
Allam et al. (2014)	★	★	★	★	★	★	★	★	High
Farhan et al. (2014)	★	★	★	☆	★	☆	★	★	Moderate
Aguilera et al. (2015)	★	★	★	★	★	★	★	☆	High
Aravindhan et al. (2015)	★	★	★	☆	★	★	★	☆	Moderate
Alnek et al. (2015)	★	★	★	★	★	★	★	★	High
Mohamed et al. (2016)	★	★	★	☆	★	★	★	★	High
Fatima et al. (2016)	★	★	★	★	★	☆	★	☆	Moderate
Talaat et al. (2016)	★	★	★	★	★	★	★	★	High
Duque et al. (2017)	★	★	★	☆	★	☆	★	☆	Moderate
Abdel-Latif et al. (2017)	★	★	★	★	★	☆	★	★	High
Leiva-Gea et al. (2018)	★	★	★	★	★	★	★	★	High
Ziaja et al. (2018)	★	★	★	☆	★	☆	★	☆	Moderate
Thorsen et al. (2019)	★	★	★	★	★	☆	★	★	High

The most frequent biases could be the inadequate description of patient characteristics (age, sex, etc.), failure to report the study period or place of recruitment, and the inclusion of patients outside the population of interest. In our revision, we only included studies in which the groups of diabetic patients were adequately selected and matched between conditions with their respective controls. Studies without a non-T1DM comparator group were excluded.

However, 100% of the studies showed a representativeness of the T1DM patients, selection of the non-T1DM subjects, and proper IL-1β quantification. In relation to confounding factors, the analysis revealed the use of remarkably severe criteria, and no confounding factors were found. Moreover, there were no studies without T1DM patients, improperly diagnosed patients, or insulin-treated patients. The sum of all of these criteria contributes to avoidance of the overall risk of potential bias, increasing the quality of the evidence of the results reported in this systematic review. On the other hand, there were some parameters that introduced a higher possibility of bias. The absence of suitable glycemic control introduces potential bias into our research (30% of the studies were potentially biased). Concerning T1DM progression, this was increased in 46% of the reviewed studies due to the lack of information about the years of evolution of the disease. In regard to the follow up and attrition rate, the risk of bias was elevated in 19% of the studies due to participants being lost to follow-up, which means essential data to evaluate any differences with the characteristics of the final study sample were not fully obtained.

3.4. Quantitative Evaluation (Meta-Analysis)

3.4.1. IL-1β Determination by Immunoassays

The *IL-1β* levels were significantly higher in T1DM patients than in controls (SMD = 2.45, 95% CI = 1.73 to 3.17; $p < 0.001$). A significant degree of heterogeneity was observed ($p < 0.001$; $I^2 = 98.6\%$) (Figure 2, Table 3).

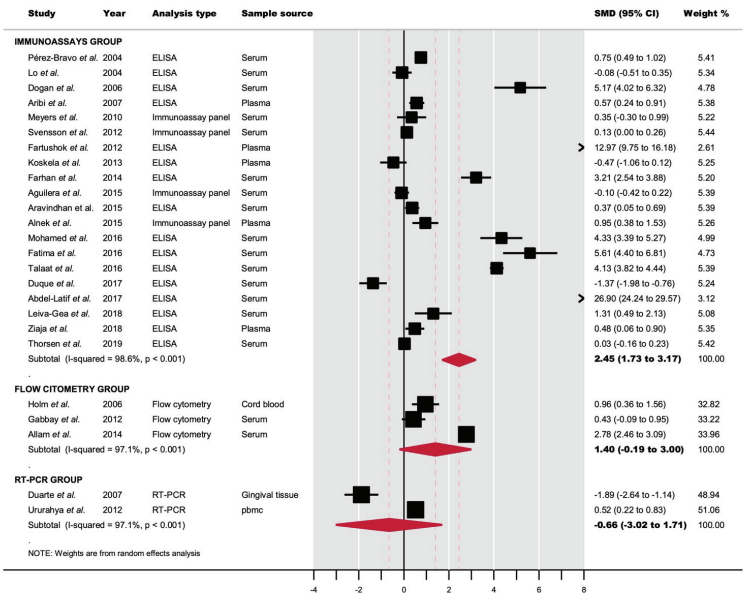

Figure 2. Forest plot graphically representing the meta-analyses evaluating the changes in circulating IL-1β levels between T1DM patients and controls (random-effects models, inverse-variance weighting based on the DerSimonian and Laird method). Standardized mean difference (SMD) was chosen as effect size measure. An SMD > 0 suggests that IL-1β levels are higher in T1DM. Diamonds indicate the overall pooled SMDs with their corresponding 95% confidence intervals (CI).

Table 3. Meta-analyses on circulating IL-1β levels in type 1 diabetes mellitus.

Meta-Analyses	No. of Studies	No. of Patients	Stat. Model	Wt	Pooled Data		Heterogeneity		*Supplementary Materials* [a]
					SMD (95% CI)	*p-Value*	P_{het}	I^2 (%)	
Determination by immunoassays									
All [b]	20	3490	REM	D-L	2.45 (1.73 to 3.17)	<0.001	<0.001	98.6	——
Subgroup analysis by geographical area [c]									Figure S1, p. 11
Africa	3	403	REM	D-L	10.41 (2.58 to 18.23)	0.01	<0.001	99.5	
Asia	5	885	REM	D-L	2.61 (0.56 to 4.66)	0.01	<0.001	99.0	
Europe	9	1875	REM	D-L	1.04 (0.49 to 1.59)	<0.001	<0.001	95.0	
North America	1	38	——	——	0.35 (−0.30 to 0.99)	0.29	——	——	
South America	2	289	REM	D-L	−0.29 (−2.37 to 1.79)	0.78	< 0.001	97.4	
Subgroup analysis by age [c]									Figure S2, p. 12
<18 years old	14	2870	REM	D-L	2.81 (1.88 to 3.74)	<0.001	<0.001	98.9	
>18 years old	6	620	REM	D-L	1.56 (0.48 to 2.65)	0.002	<0.001	96.5	
Subgroup analysis by HbAc1 levels in patients <18 years old [c,d]									Figure S3, p. 13
<7	2	79	REM	D-L	−0.04 (−2.67 to 2.58)	0.97	<0.001	96.2	
>7	8	1138	REM	D-L	5.43 (3.31 to 7.56)	0.001	<0.001	99.1	
Subgroup analysis by age matching [c]									Figure S4, p. 14
Matched	15	3172	REM	D-L	3.06 (2.19 to 3.94)	<0.001	<0.001	98.8	
Unmatched	5	318	REM	D-L	0.90 (−0.18 to 1.97)	0.10	<0.001	94.4	
Subgroup analysis by sex matching [c]									Figure S5, p. 15
Matched	11	2379	REM	D-L	0.55 (0.19 to 0.91)	0.003	<0.001	92.9	
Unmatched	3	224	REM	D-L	0.88 (−1.15 to 2.90)	0.40	<0.001	97.5	
NA	6	887	REM	D-L	8.66 (5.37 to 11.96)	<0.001	<0.001	98.9	
Subgroup analysis by sample source [c]									Figure S6, p. 16
Serum	15	3111	REM	D-L	2.73 (1.85 to 3.61)	<0.001	<0.001	98.9	
Plasma	5	379	REM	D-L	1.34 (0.28 to 2.41)	0.01	<0.001	94.3	
Subgroup analysis by type of analysis [c]									Figure S7, p. 17
ELISA	16	2235	REM	D-L	3.29 (2.27 to 4.30)	<0.001	<0.001	98.8	
Immunoassay panel	4	1255	REM	D-L	0.25 (−0.08 to 0.58)	0.14	0.02	70.5	
Subgroup analysis by study design [c]									Figure S8, p. 18
Case-control	16	2447	REM	D-L	2.77 (2.00 to 3.55)	<0.001	<0.001	98.1	
Cohort	1	398	—	—	0.03 (−0.164 to 0.23)	0.74	—	—	

Table 3. *Cont.*

Meta-Analyses	No. of Studies	No. of Patients	Stat. Model	Wt	Pooled Data		Heterogeneity		
					SMD (95% CI)	*p-Value*	P_{het}	I^2 (%)	*Supplementary Materials* [a]
Cross-sectional	3	645	REM	D-L	1.39 (−1.56 to 4.34)	0.36	<0.001	99.3	
Univariable meta-regression [e]									
Sex (% of T1DM males)	17	2928	Random-effects Meta-regression		Coef = 0.011 (−0.619 to 0.641)	0.97	——	——	Figure S9, p. 19
Risk of bias (NOS score)	20	3490	Random-effects Meta-regression		Coef = 0.195 (−3.209 to 3.598)	0.91	——	——	Figure S10. p. 20
Determination by qRT-PCR									
All [b]	2	216	REM	D-L	−0.66 (−3.02 to 1.71)	0.59	<0.001	97.1	——
Determination by Flow Citometry									
All [b]	3	455	REM	D-L	1.40 (−0.19 to 3.00)	0.08	<0.001	91.8	——

Abbreviations: Stat., statistical; Wt, method of weighting; SMD, standardized mean difference; CI, confidence intervals; REM, random-effects model; D-L, DerSimonian and Laird method; HbAc1, hemoglobin Ac1; T1DM, type 1 diabetes mellitus; NOS, Newcastle–Ottawa Scale; NA, not available. [a] More information in the Supplementary Materials; [b] meta-analyses; [c] subgroup meta-analyses; [d] the studies recruiting patients >18 years old or with missing data were excluded for this analysis; [e] effect of study covariates on circulating IL-1β levels among patients with T1DM compared with controls.

3.4.2. IL-1β Level Determination by Flow Cytometry

The *IL-1β* levels were higher in T1DM patients than in controls (SMD = 1.40, 95% CI = −0.19 to 3.00), close to significant ($p = 0.08$), and very probably underpowered (potentially yielding a non-significant result due to type II error) (n = 3 studies) (Figure 2, Table 3).

3.4.3. IL-1β mRNA Level Determination by qRT-PCR

We did not find significant differences ($p = 0.59$) between T1DM and controls (SMD = −0.66, 95% CI = −3.02 to 1.71). This result was derived from the meta-analysis of only two studies, with imprecise results (very wide confidence intervals) and the true direction of the effect is not yet estimable (Figure 2, Table 3).

3.4.4. Analysis of Subgroups

Subgroup meta-analyses were only performed for *IL-1β* determination by immunoassays, due to the considerable number of studies (n = 20) and high number of patients (n = 3490) being investigated (Table 3). The statistically significant association was maintained in the following subgroups by continents (Africa: SMD = 10.41, 95% CI = 2.58 to 18.23, $p = 0.01$; Asia: SMD = 2.61, 95% CI = 0.56 to 4.46, $p = 0.01$; Europe: SMD = 1.04, 95% CI = 0.49 to 1.59, $p < 0.001$), age (<18 years: SMD = 2.81, 95% CI = 1.88 to 3.74, $p < 0.001$; >18 years: SMD = 1.56, 95% CI = 0.48 to 2.65, $p = 0.002$), Hbg levels in patients <18 years (Hbg > 7: SMD = 5.43, 95% CI = 3.31 to 7.56, $p = 0.001$), sample source (serum: SMD = 2.73, 95% CI = 1.85 to 3.61, $p < 0.001$; plasma: SMD = 1.34, 95% CI = 0.28 to 2.41, $p = 0.01$), type of analysis (ELISA: SMD = 3.29, 95% CI = 2.27 to 4.30, $p < 0.001$), and study design variables (case-control design: SMD = 2.77, 95% CI = 2.00 to 3.55, $p < 0.001$, age matching: SMD = 3.06, 95% CI= 2.19 to 3.94, $p < 0.001$; sex matching: SMD = 0.55, 95% CI = 0.19 to 0.91, $p = 0.003$) (Table 3) (Figures S1–S8, Supplementary Materials, pp. 11–18).

3.4.5. Meta-Regression

The potential effect of sex and risk of bias on IL-1β levels determined by immunoassays was explored, but we did not find any significant association for the covariates under analysis ($p = 0.97$ and $p = 0.91$, respectively) (Table 3) (Figures S9 and S10, Supplementary Materials, pp. 19–20).

3.5. Quantitative Evaluation (Secondary Analyses)

3.5.1. Sensitivity Analysis

The general results did not substantially vary after the sequential repetition of meta-analyses, omitting one study each time. This suggests that the combined estimations reported do not depend on the influence of a particular individual study (Table S3, Supplementary Materials, p. 21).

3.5.2. Small-Study Effects Analysis

These analyses were only applied to the meta-analysis on *IL-1β* determination by immunoassays (n = 22 analysis units). The meta-analyses on *IL-1β* determination by flow cytometry (n = 3) and qRT-PCR (n = 2) harbored low sample sizes, and these methods lack statistical power when the number of primary studies is fewer than ten [28]. Egger's regression test indicates statistically significant asymmetry ($p_{Egger} = 0.014$). The funnel plot appears to be slightly asymmetric for the studies plotted at the bottom (Figure 3); however, due to a considerable degree of inter-study heterogeneity, its visual inspection analysis is complex. Consequently, a contour-enhanced funnel plot was constructed (overlaid on the "canonical" funnel plot; Figure 3) to help distinguish publication bias from other causes of asymmetry. This plot leads us to suspect that "missing" studies would be located in the symmetric counterparts with negative significance (i.e., outside of the white region), potentially ruling out publication bias. In addition, the non-parametric trim and fill method did not detect the presence of unpublished studies, confirming the reliability of

our results according to the studies published, so the final estimate was not adjusted based on imputation techniques for missing studies.

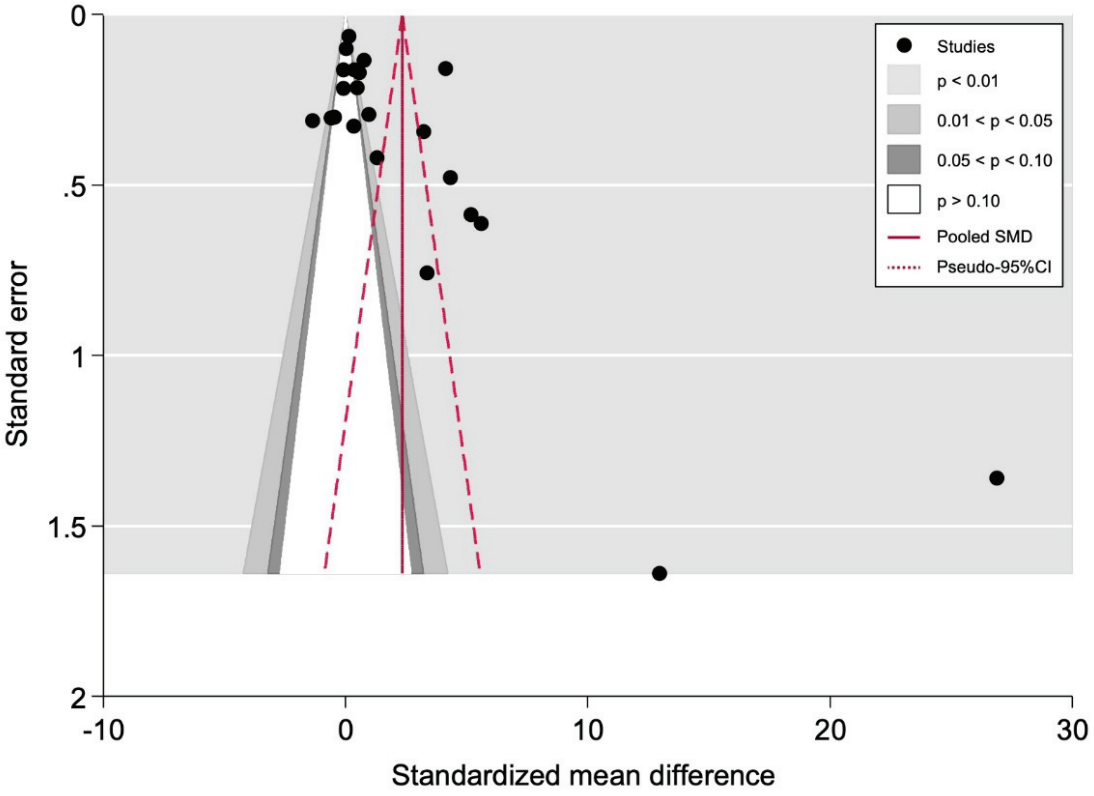

Figure 3. Canonical and contour funnel plots of the estimated circulating IL-1β levels (assessed across immunoassays) comparing type 1 diabetes mellitus and controls, expressed as standardized mean difference (SMD) against its standard error. The red vertical line corresponds to the pooled SMD estimated in the meta-analysis. The two diagonal intermittent lines represent their pseudo-95% CI. Contours represent the defined conventional levels of statistical significance (i.e., 0.01, 0.05, 0.10) accompanied by associated shaded regions. The black circles represent the 22 studies meta-analyzed.

4. Discussion

In this systematic review, significantly higher IL-1β peripheral levels in T1DM patients compared to healthy subjects were shown, according to the meta-analysis on the determination of IL-1β by immunoassays from serum or plasma (SMD = 2.45, 95% CI = 1.73 to 3.17; $p < 0.001$; n = 20 primary-level studies/3490 patients). Young T1DM patients remained significantly higher than T1DM adults. The present study determines an association between glycemic status and IL-1β peripheral levels, which is important in the methodological approach performed to determine them. Based on this window of opportunity, our meta-analysis supports further research on IL-1β as a therapeutic target in T1DM.

The increased peripheral IL-1β levels in childhood indicate a potent role during the first years of the disease, which can contribute to the cytokine storm [32] associated with the first stage of T1DM. Primary prevention strategies targeting inflammatory-mediated comorbidity may prevent secondary complications in the future for these patients [33–35]. Previous study revealed the potential therapeutic effects of anti-inflammatory treatments

by reducing the peripheral levels of pro-inflammatory cytokines in T1DM-associated complications [36]. Our results suggest that the peripheral pro-inflammatory marker IL-1β is more likely to be increased in the younger population with T1DM compared to adult patients. The different levels detected between the two age ranges studied is particularly significant: child T1DM patients (<18 years old) show a higher IL-1β level than adult T1DM patients (>18 years old), which could be related to the cytokine storming associated with early events in T1DM [37]. Usually, younger T1DM patients present a shorter evolution time of disease, and the immune alterations develop at the beginning of the onset of T1DM. Moreover, in this age group, insulin sensitivity is highly variable due to growth, sexual maturation, and self-care capacity at these ages. In this regard, and supporting these data, young T1DM patients with poor glycemic control present higher IL-1β levels compared with the same age range of T1DM patients with good glycemic management. Several studies have demonstrated an association between the low presence of cytokines and better insulin secretion [12]. The analysis of demographic and geographic area indicates a significant influence of medical assistance and management of T1DM progression care on the level of IL-1β detected.

This study is the first meta-analysis focusing on IL-1β implications in T1DM, different from previous meta-analytic studies based on other cytokines and only restricted to adult patients [12,13,38]. Many previous studies examining other blood cytokine levels during T1DM, such as TNF-α and IL6 [12,13], only included adult patients and this could possibly be attributed by lack of data on IL-1β expression along the T1DM or the inadequate methodological standardization of patient characteristics.

The importance of the methodological approach used for determining IL-1β, and its biological source, has been elucidated in the present work. We found a significantly increased number of analyses performed in serum compared to other biological fluids. The easy accessibility of serum and the periodical clinical testing of it could indicate serum as the principal biological fluid for the determination of inflammatory parameters. Regarding the methodological approaches, we found that the results obtained by ELISA assay are more consistent and with homogeneous groups than other actual immunoassay techniques. The precision of ELISA or the use of only one marker could contribute to a better determination. The exact determination of IL-1β levels is a critical point for determining the clinical standard value, and the results showed in the present analysis confirm that ELISA maintained the range of determination between different studies analyzed; however, other types of immunoassays present a higher range of variability.

IL-1 is a therapeutic target in T1DM patients [11]. Different clinical trials with IL1Ra (anakinra) for adult patients or human monoclonal anti-IL-1β antibody (canakinumab) in pediatric T1DM patients have not been effective in maintaining B-cell function; however, the present meta-analysis showed a critical time point during T1DM progression that could be important to keep in mind for the administration of treatments. A significant relationship between the inflammatory index and β-cell function was not observed in the TN-14 trial. As in consonance with the TN-14 study, our results validated that pediatric onset T1DM is characterized by a more aggressive disease process compared to adult onset T1DM [39], and the relationship identified age dependency in young patients (<18 years (Figure S3)), as shown in the Cabrera et al. article [40]. It is important to note that in the majority of trials, all of the studies focused on evaluating the function of the pancreas using insulin secretion/C-peptide levels; however, in the present study, we tried to elucidate the waves in T1DM-associated IL-1β levels, and determine the relationship with glycated hemoglobin, T1DM management, and age. Usually, the younger T1DM patients present a shorter evolution time of disease, with the immune alterations developing at the beginning of the onset of T1DM. Moreover, in this age group, insulin sensitivity is highly variable due to growth, sexual maturation, and self-care capacity at these ages. In this regard, and supporting these data, the young T1DM patients with a poor glycemic control present higher IL-1β levels compared with the same age range of T1DM patients with good glycemic management. Several studies have demonstrated an association between the

low presence of cytokines and better insulin secretion [12]. A positive correlation (change versus change) of plasma HbA1c and plasma IL-6, TNF-α, and IL-1β has been described in a diabetic animal model [41], and in our meta-analysis, we found an association between the glycated hemoglobin and the serum IL-1β levels detected.

According to our qualitative evaluation carried out using the Newcastle–Ottawa Scale, the included studies harbored a low overall risk of potential bias. This fact increases the quality of the evidence of the results reported in our meta-analysis [42]. We also showed that not all studies were conducted, in methodological terms, with the same rigor. Studies should more meticulously communicate the years of evolution of the disease, and control groups should be more carefully designed, being appropriately matched for age and sex. Future studies assessing the relationships between IL-1β levels among T1DM patients could consider the recommendations given in this systematic review and meta-analysis to improve and standardize future research.

Some potential limitations should also be discussed. First, our meta-analysis revealed a considerable degree of inter-study heterogeneity. Heterogeneity is a common finding in meta-analyses dealing with serum biomarkers—particularly cytokines—measured and expressed as continuous variable [12,13]. It must also be noted that a random-effects model was applied in all meta-analyses to account for heterogeneity. When considering the uses and limitations of meta-analytical techniques, a key strength is the ability to reveal patterns across the study results and identifying potential subpopulations (i.e., sources of heterogeneity) [43]. In this sense, our meta-analysis may have identified differences among geographical regions, age, Hbg levels, and analysis techniques, among other factors, that may constitute true sources of heterogeneity, potentially exerting an impact on IL-1β level variations in T1DM. Furthermore, only plasma and serum determinations were meta-analyzed to obtain results derived from more homogeneous clinical and methodological subgroups. Future studies are needed to obtain a higher quality of evidence on the determinations derived from other anatomical sites (e.g., crevicular gingival fluid or vitreous humor). Another element that can explain the heterogeneity is the lack of standardization of the assays used to measure IL-1β. Second, visual inspection analysis of the canonical funnel plot and statistical analyses detected the presence of asymmetry, pointing out small-study effects. Therefore, the random-effects model could be overestimating our results, giving more weight to the studies with a lower sample size, where sampling error may be influential [44]. Nevertheless, the enhanced-contour funnel plot and the trim and fill method allowed us to suspect that the reported asymmetry is artefactual, due to sampling variation or to chance [9], and not really to the presence of publication bias, which could be ruled out [26]. Third, another potential limitation could be related to our eligibility criteria, where clinical trials were excluded, in spite of the advantages in longitudinal associations derived from this study design. In order to meet our objectives, we first a priori designed our study protocol, and we only considered primary-level cohort studies/small case series, case control, and cross-sectional studies to be included, due to their observational study design. There are controversies on the integration of observational and interventional mixed primary-level studies in meta-analysis, particularly in the context of molecular biomarkers with clinical implications. Since our research was performed to better understand the natural history of the condition type 1 diabetes mellitus in the context of IL-1β levels, the inclusion of treatment/interventionist studies (which, by definition, try to decrease the chronic inflammation in diabetes or to eliminate risk factors) could potentially distort the reality of this disease, attenuate inflammation, modulate il-1β levels, introduce a new heterogeneity source, and, consequently, affecting the achievement of our goals. Finally, another potential limitation is the absence of an association between secondary complications, such as diabetic retinopathy, and the IL-1β levels. It was demonstrated that IL-1β increased in the diabetic mouse retina and IL-1β induced pericyte apoptosis via NF-κB activation under high glucose conditions, thereby increasing endothelial permeability in diabetic retinopathy [45]. However, we could not undertake a meta-analytical approach on the diabetic secondary complications due to the low number of articles with inclusion

criteria established. Despite the above limitations, the study strengths include our careful study design, a sensitive literature search strategy, the absence of restrictions by date limits or publication language, robust qualitative recommendations for the development and design of future studies on this topic, and the comprehensive meta-analytical approach, showing powerful statistical findings across many analyses.

5. Conclusions

In conclusion, this systematic review and comprehensive meta-analysis provides a deep exploration of the possible role of IL-1β as a tool cytokine in T1DM progression and management of disease. IL-1β is significantly increased in young T1DM patients, which can be used as a marker to initiate the administration of new therapeutic approaches for IL-1β modulation. The relationship between the status of T1DM and IL-1β levels measured by ELISA corroborate the strong affinity between the inflammatory context and T1DM glycemic status, determined by Hbg levels. Further analysis and validation are needed to establish a clinical standard value for IL-1β associated with different T1DM status. The results obtained allow for the hypothesis of a potential role of IL-1β as a therapeutic target in the early stages of T1DM, where the actual treatments are focused on the pharmacological abrogation of IL-1β action and reducing T1DM progression. The evaluation of IL-1β levels in the early stages of the disease could support the finding that inflammatory status is associated with glycemic control.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11051303/s1, Table S1: Search strategy for each database, number of results, and execution date; Table S2: Variables of study; Figure S1: Geographical area; Figure S2: Age; Figure S3: HbAc1 levels in patients <18 years; Figure S4: Age matching; Figure S5: Sex matching; Figure S6: Source of sample; Figure S7: Type of analysis; Figure S8: Study design; Figure S9: Effect of the covariate Sex; Figure S10: Effect of the covariate Risk of Bias; Table S3: Sensitivity analysis.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Metabolic Control of the FreeStyle Libre System in the Pediatric Population with Type 1 Diabetes Dependent on Sensor Adherence

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Abstract: Aims: To evaluate the relationship between daily sensor scan rates and changes in HbA1c and hypoglycemia in children. Methods: We enrolled 145 paediatric T1D patients into a prospective, interventional study of the impact of the FreeStyle Libre 1 system on measures of glycemic control. Results: HbA1c was higher at lower scan rates, and decreased as the scan rate increased to 15–20 scans, after which it rose at higher scan rates. An analysis of the change in hypoglycemia, based on the number of daily sensor scans, showed there was a significant correlation between daily scan rates and hypoglycemia. Subjects with higher daily scan rates reduced all levels of hypoglycaemia. Conclusions: HbA1c is higher at lower scan rates, and decreases as scan rate increases. Reductions in hypoglycemia were evident in subjects with higher daily scan rates.

Keywords: flash glucose monitoring; sensor scanning; hypoglycemia

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1. Introduction

The FreeStyle Libre 1 flash glucose monitoring system (Abbott Diabetes Care, Witney, UK) is an established technology that measures glucose in interstitial fluid (ISF). A sensor worn on the back of the upper arm takes a reading every minute that can be scanned using a hand-held reader or smartphone to receive a current glucose result, along with historic results with a 15-min frequency. The FreeStyle Libre sensors are calibrated in the factory and have a wear time of up to 14 days without the need for the user to perform daily calibration using finger-prick tests [1].

The FreeStyle Libre flash glucose monitoring system was proven in the IMPACT and REPLACE studies [2,3] to reduce time in hypoglycemia below 70 mg/dL by 38% (IMPACT) and 43% (REPLACE) over 26 weeks, for adults with type 1 (T1D) or type 2 diabetes (T2D) on insulin, compared with the finger-prick method for the self-monitoring of blood glucose (SMBG). Neither study showed a significant change in HbA1c observed with the flash glucose monitoring compared with SMBG. In the SELFY prospective interventional single-arm study [4], which used the FreeStyle Libre system in 76 children and adolescents with T1D, mean HbA1c was reduced from 7.9% to 7.5% over 8 weeks, compared with SMBG. Separate prospective observational and randomized control studies show that flash glucose monitoring is associated with significant improvements in HbA1c in adults with T1D [5], or with T2D on insulin [6]. Moreover, meta-analysis of up to 25 real-world clinical studies has confirmed that starting the FreeStyle Libre system is associated with a significant and sustained reduction in HbA1c for adults and children with T1D [7,8] and for adults

with T2D [7]. However, none of those studies correlated these improvements with the number of scans.

Flash glucose monitoring is now included in the Portfolio of Services of the Public Health System in Spain for T1D, and Andalucia has been a pioneer region in introducing flash glucose monitoring in the paediatric population in Spain. Here, we report on the impact of flash glucose monitoring over 6 months on measures of glycaemic control in a paediatric T1D population, being treated either with multiple daily injections of insulin (MDI), or with the continuous subcutaneous infusion of insulin (CSII).

The aim of the study is to understand the association between daily sensor scan rates and changes in HbA1c and in measures of hypoglycemia, in order to be able to establish from what number of scans per day an improvement can be seen, and from what point during the follow-up period (1 month, 3 months or 6 months) we will be able to give realistic recommendations to achieve the expected effect.

2. Methods

2.1. Study Design and Participants

We enrolled 145 pediatric T1D diabetes patients into a prospective, interventional study on the impact of the continuous use of FreeStyle Libre system on HbA1c levels, and on measures of hypoglycemia. The inclusion criteria were as follows: the presence of T1D with disease duration >1 year; an age of 4–18 years at study entry. Subjects were excluded if they did not adhere with the routine clinical review, or had used the FreeStyle Libre system prior to start of the study. Baseline characteristics are shown in Table 1. Mean age (±SD) of study subjects was 11.36 (±3.06) years, and the average duration of diabetes was 5.2 (±3.2) years. Patients were treated either with MDI (n = 119) or with CSII (n = 26).

Table 1. Baseline clinical characteristics of the study population.

Parameter		*n*
Age (years)	11.36 ± 3.06	145
Duration of diabetes (years)	5.2 ± 3.2	145
Mean HbA1c at baseline (%)	7.11 ± 0.80	142
Mean HbA1c for subjects <7.5% at baseline	6.73 ± 0.47	102
Mean HbA1c for subjects ≥7.5% at baseline	8.09 ± 0.71	40
Treatment type	MDI	119
Treatment type	CSII	26

Values are mean ± SD unless otherwise indicated. MDI, multiple daily injections of insulin; CSII, continuous subcutaneous insulin infusion.

All study subjects underwent group training, along with their main caregivers and diabetes team, to understand the functionality of the FreeStyle Libre flash glucose monitoring system, as well as the LibreView web platform which is used to view the glycaemic data collected by the FreeStyle Libre system.

The study was carried out in accordance with the regulations published in Boletín Oficial de la Junta de Andalucía (BOJA), with resolution on 17 April 2018, from the SAS management, for the inclusion of glucose monitoring systems in the Portfolio of Services of the Public Health System of Andalusia.

Data acquisition and analysis was performed in compliance with protocols approved by the Ethical Committee of the Provincial Ethics Committee of Malaga and Andalusian Ministry of Health and Family (ethical approval number: PIGE 0533-219).

Written informed consent was obtained from all participants prior to study.

All data is available through contacting the authors.

2.2. Outcomes Measures

Laboratory measurement of HbA1c was carried out at baseline, and at 1, 3, and 6 months. Measures of hypoglycemia were monitored using the LibreView® platform, (Abbott Diabetes Care, Witney, UK) using the most recent 14 days of glycaemic data at 1 month, 3 months and 6 months. Parameters measured included those accepted in international consensus guidelines for interpreting continuous glucose monitoring (CGM) data [9,10]. These were: the number of sensor scans per day; percentage of readings <70 mg/dL; number of Level 1 hypoglycemia events <70 mg/dL; number of Level 2 hypoglycemia events <54 mg/dL; percentage time in range (TIR) between 70–180 mg/dL. Across the study period, FreeStyle Libre users were divided into 4 groups based on their mean daily scan rates. These subgroups corresponded to the quartiles of the daily scan rates. These were: 0–6 scans/day; 7–8 scans/day; 9–11 scans/day; and >11 scans/day. Changes in reported glycemic measures were assessed in this context.

2.3. Statistical Analysis

Data were analysed using the established parametric method of comparing the means of normally distributed observations (paired Student's t statistic), X^2 for percentages. ANOVA was used for comparing different groups, as well as applying generalized mixed linear models, which can be more flexible in the analysis of data which incorporates multiple variables, such as metabolic control and insulin-treatment modality.

3. Results

3.1. Change in HbA1c in Relation to Daily Scan Rates with the FreeStyle Libre System

When we looked at the changes in HbA1c across the whole study group, there was a significant relationship between the change in HbA1c with the number of daily scans ($p < 0.001$), as demonstrated by the mixed linear modelling. Figure 1 shows that the change in HbA1c had a U-shaped relationship with the number of daily scans; HbA1c was higher at lower scan rates, and decreased as scan rate increased to between 15–20 scans, after which it raised at higher scan rates.

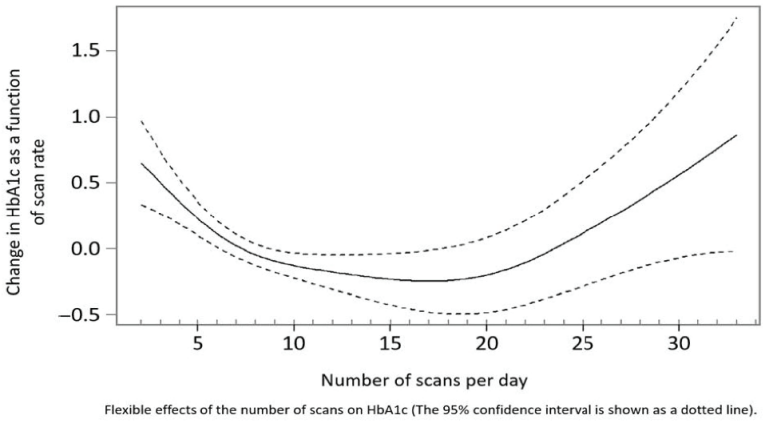

Figure 1. Relationship between number of daily scans with the FreeStyle Libre system and HbA1c.

It is interesting to observe that from 15–20 scans a day glycosylated hemoglobin was negatively influenced. This fact may be related to an infective adherence, which shows repeated ineffective acts. These data had already been published with the number of capillary glycemic controls per day, where it was seen that a greater number of capillary glucose controls were related to better control measures in hemoglobin; however, from a certain number it was related to caregiver anxiety, and/or the patient, without having a favorable impact on metabolic control.

The confidence interval, represented as a dotted line in the graph, shows the dispersion of the results.

3.2. Relationship between Daily Scanning Rates and Change in Hypoglycemia

Hypoglycemia was assessed using three parameters based on FreeStyle Libre sensor data uploaded to the LibreView platform for the preceding 14 days at each study point. These were: percentage time with glucose readings <70 mg/dL; the number of clinically relevant Level 1 hypoglycemic events <70 mg/dL; the number of clinically significant Level 2 hypoglycemic events <54 mg/dL.

An analysis of change in hypoglycemia, based on the number of daily sensor scans, showed that there was a significant correlation between daily scan rates and the change in all three measures of hypoglycemia over the intervention period (Table 2). At each timepoint after starting FreeStyle Libre, there was a pattern of increasing % time <70 mg/dL, as well as an increase in events <70 mg/dL and <54 mg/dL, for groups with scan rates rising to 9–11 scans/day, then reducing for the group at >11 scans/day. With increasing time using the FreeStyle Libre system, the number of events <70 mg/dL and events <54 mg/dL decreased consistently, as scan rates increased. For the number of events <70 mg/dL, there was a significant reduction from 19.29 events/day to 12.69 events/day by 6 months ($p < 0.001$), for people with 9–11 scans/day, and from 13.57 events/day to 9.82 events/day ($p = 0.01$) for users with >11 scans/day. The number of events <54 mg/dL fell from 6.22 events/day to 3.68 events/day ($p = 0.01$) over 6 months for users with 7–8 scans/day, and from 7.50 events/day to 5.03 events/day ($p = 0.04$) for users with 9–11 scans/day. For % time <70 mg/dL, there was a significant fall from 5.8% to 3.88% ($p = 0.023$) from 1 to 6 months with scan rates of >11 scans/day.

Table 2. Relationship between daily scanning rates and change in hypoglycemia.

% Time <70 mg/dL (±SE)				
No daily scans	Month 1	Month 3	Month 6	*p* value *
0–6	4.75 (0.55)	4.94 (0.37)	4.83 (0.41)	0.909
7–8	6.64 (0.69)	5.48 (0.51)	5.26 (0.41)	0.169
9–11	7.92 (0.81)	6.21 (0.47)	6.52 (0.47)	0.14
>11	5.89 (0.81)	4.53 (0.39)	3.88 (0.34)	0.03
Number of events <70 mg/dL (±SE)				
No daily scans	Month 1	Month 3	Month 6	*p* value *
0–6	8.22 (0.96)	7.74 (0.50)	5.97 (0.45)	0.06
7–8	13.30 (1.15)	9.81 (0.68)	11.16 (0.60)	0.1
9–11	19.29 (1.66)	13.27 (0.71)	12.69 (0.66)	<0.001
>11	13.57 (1.34)	12.37 (0.68)	9.82 (0.55)	0.01
Number of events <54 mg/dL (±SE)				
No daily scans	Month 1	Month 3	Month 6	*p* value *
0–6	1.93 (0.37)	2.73 (0.29)	2.28 (0.28)	0.455
7–8	6.22 (0.83)	3.00 (0.38)	3.68 (0.34)	0.01
9–11	7.50 (1.12)	4.54 (0.42)	5.03 (0.42)	0.04
>11	3.38 (0.65)	3.45 (0.35)	3.28 (0.32)	0.897

Data are means (±standard error) in each case. * *p* value for change in readings at 6 months compared to 1 month.

In the variable number of events for <70 mg / dL in the 7–8 scan group, the difference at 6 months, compared with the third, was not significant nor clinically relevant (6.21 versus 6.52), so it cannot be taken into account. This scan group (7–8) only shows significant and concordant differences in the reduction of events of less than 54 mg/dL. For scan group of

>11 scans/day there was a significant fell from 5.8% to 3.88% ($p = 0.023$) from 1 to 6 months, and for users with 9-11 scans/day fell from 7.50 events/day to 5.03 events/day ($p = 0.04$) at 6 months.

4. Discussion

This study, regarding a cohort of 145 children and young people aged 18 years or younger with T1D, shows that starting the FreeStyle Libre system is associated with improvements in glucose control for this group of people with diabetes. This includes reductions in HbA1c and improvements in hypoglycemia.

As they are part of other articles published in relation to glycosylated hemoglobin and the reduction of severe hypoglycemia, this article focuses on the role of wear time and adherence measured by the number of scans per day in metabolic control parameters.

This article has the purpose of being able to establish practical recommendations regarding the number of scans recommended to achieve the established objectives that do not enslave the patient or the caregiver, and allow realistic expectations for their use.

Moreover, we established that in case of more than 15–20 scans per day, an unfavorable impact was observed on the metabolic control valued in glycosylated hemoglobin. This may show an uneffective adherence and, it could be a diagnostic key of psycho-emotional exhaustion for those seeking positive results in the short and long term by raising the number of scans.

An important observation from our study is that there was a significant correlation between daily scan rates and the change in all three measures of hypoglycemia over the 6-month study period, including the number of hypoglycemic events < 54 mg/dL. Across the outcome timepoints after starting FreeStyle Libre, there was a pattern of decreasing % time < 70mg/dL, as well as a decrease in events <70 mg/dL and <54 mg/dL for groups with higher daily scan rates, which emphasizes again the importance of patient education and compliance with the use of the device, and acting according to the sensor readings revealed at higher scan rates. In the absence of masked baseline measures of hypoglycemia, the simplest explanation for this trend is that the different scan rates at month 1 are diagnostic of the level of glycemic control prior to starting the FreeStyle Libre. The lowest scanning group may correlate with those with poor prior control if they were previously low SMBG testers, and could likely have the least time with low glucose. Higher scan rates can indicate a desire for good control that is revealed by more events with glucose <70 mg/dL and <54 mg/dL in the early phase of using the FreeStyle Libre system.

However, as users become more experienced with the FreeStyle Libre system, at 3 months and at 6 months, the higher-scanning patients improve their glycemic performance compared to month 1, as they learn to use the full capabilities of the system. Thus, the number of low glucose events <70 mg/dL decreases significantly from month 1 to month 6, for the groups scanning 9 times per day or more, and the % time <70 mg/dL falls significantly for people with >11 scans/day. Since there was no masked baseline for measures of hypoglycemia, the interpretation for this might be that as scan rates increased, the FreeStyle Libre device provided diagnostic feedback on glucose levels, but above 11 scans/day the users were able to make therapeutic decisions themselves to avoid hypoglycemia.

Similarly, the number of clinically significant hypoglycemic events < 54 mg/dL fell significantly across the study period for people scanning between 7 and 11 times per day. Overall, this paints a picture of how the experience and engagement by the user makes an impact, such that they are able to interpret and act on their flash glucose data more effectively. By 6 months, the higher-scanning users were able to improve on their performance of the first month. This emphasises the value of the FreeStyle Libre system as a therapeutic tool in terms of reducing hypoglycemia at higher scan rates for experienced users. This observation is aligned with a real-world analysis of associations between FreeStyle Libre sensor-scanning frequency and a range of glycaemic measures [11].

These observations are aligned with data from the AWeSoMe real-world study [12] on 71 young people, aged from 1–25 years old with T1D, who had self-funded their FreeStyle Libre system; however, this latter study did not include a masked baseline set of readings. The SELFY single-arm study in 76 children and young people did not show a significant change in % time in hypoglycemia <70 mg/dL, despite a 14-day masked baseline period of wear [4], which appeared to differ from the overall pattern that we saw in our study. However, the SELFY study was an 8-week multicentre study, compared with our single-centre 6-month real-world study, and showed a significant reduction in the number of events <70 mg/dL, in common with our observations.

Reduction in the risk of severe hypoglycemia amongst children and young people with T1D has been reported in only one other study to date, which showed a 53% reduction in the rate of severe hypoglycemia for 278 subjects after 12 months, following a switch from SMBG testing to the use of the FreeStyle Libre system [13].

Although the reduction in hypoglycemia is present in other studies on monitoring systems, ref. [12] the Flash device has one peculiarity in comparison with other monitoring devices: it is easier to manage, because it is not necessary to calibrate it.

Regarding HbA1c, it is curious that after 20 scans HbA1c increased in our sample. This could be a result of ineffective repeated impulsive behaviors. These data had already been published with the number of capillary glycemic controls per day, where it was seen that a greater number of capillary glucose controls were related to better control measures of hemoglobin [12]; however, from a certain number, this was related to caregiver anxiety, and/or the patient, without having a favorable impact on metabolic control.

A limitation of our study is that the longitudinal changes in measures of hypoglycemia were not able to include a baseline reading for those derived from the masked use of the FreeStyle Libre system, prior to users becoming aware of their sensor-glucose readings. Thus, changes in these metrics across the 6-month study period can be argued to be a study effect, as the consequence of the users becoming better educated in the day-to-day management of their diabetes was due to their participation in the study.

5. Conclusions

This prospective observational report from a single centre in Spain underlines both the value of flash glucose monitoring with the FreeStyle Libre system, and also the importance of understanding individual glycaemic profiles. In our study, we show that adherence with the FreeStyle Libre system is important to fully realize the benefits of flash glucose monitoring. Our study also indicates that additional investigation is required to identify which children and young people with T1D are most likely to benefit from use of the FreeStyle Libre system.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Provincial Ethics Committee of Malaga (17th April 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data is available through contacting the authors.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Article

Validation of an Automated Screening System for Diabetic Retinopathy Operating under Real Clinical Conditions

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Abstract: Background. Retinopathy is the most common microvascular complication of diabetes mellitus. It is the leading cause of blindness among working-aged people in developed countries. The use of telemedicine in the screening system has enabled the application of large-scale population-based programs for early retinopathy detection in diabetic patients. However, the need to support ophthalmologists with other trained personnel remains a barrier to broadening its implementation. Methods. Automatic diagnosis of diabetic retinopathy was carried out through the analysis of retinal photographs using the 2iRetinex software. We compared the categorical diagnoses of absence/presence of retinopathy issued by family physicians (PCP) with the same categories provided by the algorithm (ALG). The agreed diagnosis of three specialist ophthalmologists is used as the reference standard (OPH). Results. There were 653 of 3520 patients diagnosed with diabetic retinopathy (DR). Diabetic retinopathy threatening to vision (STDR) was found in 82 patients (2.3%). Diagnostic sensitivity for STDR was 94% (ALG) and 95% (PCP). No patient with proliferating or severe DR was misdiagnosed in both strategies. The k-value of the agreement between the ALG and OPH was 0.5462, while between PCP and OPH was 0.5251 ($p = 0.4291$). Conclusions. The diagnostic capacity of 2iRetinex operating under normal clinical conditions is comparable to screening physicians.

Keywords: diabetic retinopathy; teleophthalmology; diagnostic accuracy; population-based screening; sight-threatening diabetic retinopathy

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1. Introduction

In 2019, the IDF (International Diabetes Federation) estimated that 463 million adults worldwide suffered from diabetes, and projected the number to rise to 700 million by 2045 [1]. The prevalence of diabetes in Andalusia, the most populated autonomous community in the south of Spain, is higher (15.3%) than in the rest of Spain (12.5%), in close relation to lifestyle and socioeconomic factors [2].

Retinopathy is the most common microvascular complication in patients with diabetes mellitus [3]. In developed countries, diabetic retinopathy (DR) is one of the leading causes of blindness among people of working age [4]. A recent meta-analysis has calculated that in diabetic patients aged 20−79 years, the overall prevalence of any DR is 35% [5]. While the global prevalence of DR and Diabetic Macular Edema (DME; potential complication of DR), for the period 2015 to 2019 were 27.0%, and this prevalence in Europe was estimated to be 20.6%, calculated from the results of population-based studies with retinography [6]. It has been known for decades that proper treatment of DR decreases the incidence of severe visual loss when early diagnosed [7]. Telemedicine systems enable the remote analysis of digital fundus photographs, thus detecting the presence of DR lesions. Based on this

technology, population-based screening programs have been developed in different countries [8]. The growing number of diabetic patients and their periodic medical monitoring entails an increase of these DR-detection digital analyses. Due to the limited number of ophthalmologists, other professionals are required to address DR screening. In particular, these range from family physicians, endocrinologists or nurses in high-income countries to trained non-medical personnel in middle-income countries [9–13].

In recent years, the research effort has focused on the development of automated diagnostic strategies that can complement or replace screening personnel, which would help reduce the workload and improve access for diabetic patients requiring early diagnosis [14,15].

The Andalusian Public Health System (APHS) provides universal health care to the 8.4 million inhabitants of Andalusia, which represent 18% of the Spanish population. The APHS encompasses an extensive network, with two levels of care (1500 primary healthcare centers and 49 hospitals) based on accessible, high-quality, patient-centered care, in a system with universal coverage and funded by taxation. The APHS, through the program for Early Detection of Diabetic Retinopathy (APDR), which is part of the Comprehensive Healthcare Plan for Diabetes (CHPD), provides a network of digital desktop fundus cameras placed in primary healthcare centers throughout the region. The screening system developed by APHS consists of two phases: in the first phase, digital fundus photographs of diabetic subjects with no previous diagnosis of DR are recorded with a non-mydriatic retinal camera (NMRC). Secondly, the primary care physicians (PCP) of each center assess these photographs, and then, both those displaying a probable DR diagnosis and inconclusive ones are sent via a specific intranet to a reference ophthalmologist for diagnostic confirmation. From January 2005 to June 2019, 888,318 examinations were performed, corresponding to 429,791 patients [2]. It should be noted that patients with a healthy retina are examined periodically, which increases the number of images under study every year.

To reduce this increasing workload of PCP performing the screening activity, the Lynch Diagnostics (Granada, Spain) (LD) computer-aided diagnostic platform, in collaboration with APHS, developed the 2iRetinex algorithm. The 2iRetinex software (Granada, Spain) lassifies the screening images according to their quality. Specifically, for the non-rejected images, it provides information on the number, type and location of the lesions and, ultimately, a final diagnostic decision.

The aim of this research study is the validation of the 2iRetinex software as a complement or substitute for the screening physician. The clinical diagnosis of the screening physician is formulated in terms of the absence or presence of DR. To perform the comparison of this categorical diagnostic strategy with the same categories provided by the algorithm, we introduced the algorithm into the APDR system. The algorithm extracted the diagnosis of the presence or absence of diabetic retinopathy from the system, obviating the additional information. The agreed diagnosis of three specialist ophthalmologists is used as the reference standard ("ground truth") to measure its clinical performance.

2. Materials and Methods

2.1. Study Participants from APDR

An analytical study of diagnostic tests was performed on the fundus images of diabetic patients regularly attending the Andalusian program for early diagnosis of diabetic retinopathy (APDR) circuit. The used protocols were approved by the Ethical Committee for Research and Clinical Trial of Hospital Puerta del Mar (Cadiz, Spain), number 62/16.

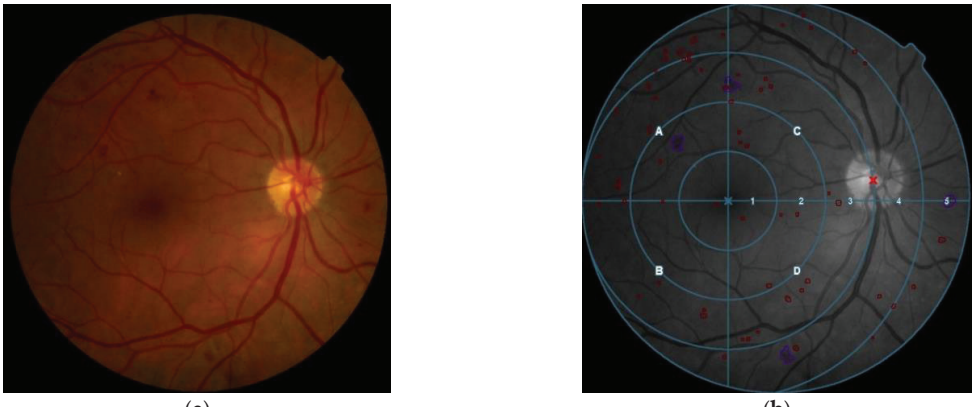
To compare the diagnostic capability of the 2iRetinex algorithm (ALG) with that of the screening PCP under usual clinical conditions, we calculated the required sample based on a previous APDR study with a prevalence of retinopathy in diabetic patients of 30% [16]. To obtain an average sensitivity of 85% and specificity of 82% for the screening PCP [16] and a sensitivity of 90% and a specificity of 93% for the ALG (internal unpublished data from the company, on a random sample of 575 patients in 2015), as well as, a type 1 and 2 error at 0.5 and at 0.2, respectively, the sample size estimated was 2421 patients. For a paired

sample, the required sample size calculation was 2297 patients. Furthermore, as the rate of ungradable (UNG) images was estimated to be 10%, an additional 242 and 230 patients were added, for independent and paired samples, respectively [16].

Retinographies were obtained from all diabetic patients attending ten primary care health centers in the region of Andalusia (Spain) included in the APDR patient flow circuit using a standardized protocol previously described [16–18]. Briefly, three photographs of each eye were recorded using a retinal camera. The following camera models were used: the Topcon NW 200 (Topcon, Tokyo, Japan) in seven of the ten study centers and in the remaining three centers, the Topcon NW100 (Topcon, Tokyo, Japan), the Zeiss VISUCAM (Carl Zeiss Meditec AG, Jena, Germany) and DRS retinography, respectively. Then, the PCP analyzed the images stored on the APDR server. The images of the patients classified as UNG and those that present findings of DR are assigned, through the intranet, to the reference ophthalmologist who issued a clinical judgment of confirmation. If no DR lesions were detected (NODR) the patient continued in the screening system for future examinations.

2.2. 2iRetinex Software

The 2iRetinex software (patent number RPI201499900601833) extracted different features from the retinographies. Specifically, 2iRetinex extracted the vascular tree through the isolation of the green channel and microaneurysms and hemorrhages (which are candidates for lesions by applying structural characteristics), through analysis of the dark residual objects. In addition, the 2iRetinex software superimposed a binary mask on the green layer of the images, which made it possible to locate the optic disc. Using a filter also highlighted the bright lesions (exudates and cotton spots). Furthermore, it was able to detect the position of the macula. Finally, a topographic reference system was established to locate the lesions by coordinates. After checking that the image was suitable for analysis, the original image (Figure 1a) was transformed into the resulting image shown in Figure 1b.



(**a**) (**b**)

Figure 1. Software analysis 2iRetinex: (**a**) A original APDR image of fundus photography of a patient with diabetic retinopathy; (**b**) Location and type of lesions. Superimposed the coordinates system. Red lesions in red, white lesions in blue.

2.3. Source of Images and Data

In the participating centers of this study, between April 2017 and June 2018, a capture system was installed between the retinography and the APDR network. This system allowed the original images in TIFF format to be sent to the LD diagnostic platform to obtain the patient's clinical diagnosis through the 2iRetinex software (ALG) using the same diagnostic categories as the PCP. These images, but not the diagnoses, were available on a server for the ophthalmic researchers (SJC, PAM, PAD). The latter were the ones who established the reference diagnosis (OPH). Simultaneously, the images were returned to

the APDR network for compression into JPG for final screening by the PCP, following the usual circuit. The PCPs were not aware of the parallel diagnostic systems. Moreover, at no time were the real graders aware of the diagnosis issued by the other study participants before issuing their clinical judgment.

To ensure that the study was double-blind, the LD platform provided an internal control code. Once the OPH and ALG diagnoses were established, the list of codes related to the original health identification was sent to the administrative management of the APDR. Likewise, the demographic data of the study sample and the PCP diagnoses were sent from the APDR for cross-checking.

For each patient, data were obtained for the categorical variables (gender and type of diabetes) and the quantitative variables (age and years of duration of the disease at the time the retinographies were taken; years from diagnosis). The database also recorded the use of pre-exploration mydriatics.

2.4. Diagnostic Criteria and Convention

For each patient, the PCP, ALG and OPH diagnosis were obtained, with the same diagnostic categories. The following criteria were used to classify the images:

If the image lacked sufficient quality to confirm or rule out diabetic lesions, it was classified as Ungradable (UNG).

If the image quality was sufficient and no diabetic retinopathy lesions were found, it was classified as No Diabetic Retinopathy (NODR)

Regardless of the quality of the image, if retinopathy lesions were observed, the image was classified as DR.

For the final diagnosis of each patient, as carried out in the APDR circuit, the following criteria were followed:

If at least one of the patient's two eyes was classified as UNG, the patient was diagnosed as UNG.

If DR lesions were detected in the images of at least one eye, the patient was diagnosed as DR.

If none of the images of the patients considered evaluable showed lesions of DR, the patient was diagnosed as NODR.

Research ophthalmologists (SJC, PAM, PAD) classified the stage of DR according to the International Clinical Diabetic Retinopathy Severity Scale [18]. This scale consists of five stages: no diabetic retinopathy (NODR), mild (MILD), moderate (MOD), and severe non-proliferative DR (SEV), and proliferative retinopathy (PROL). The diagnosis of diabetic macular edema (DME) was established by detecting the presence of any signs suggestive or evident of macular edema in at least one eye. Cases diagnosed as DME, SEV and PROL were considered as patients with sight-threatening DR lesions (STDR). If the patient had DR lesions in both eyes, the stage of diagnosis corresponded to that of the eye with the highest degree of DR.

2.5. Statistical Analyses

The study data were analyzed using descriptive statistics. Means and standard deviations were calculated for quantitative variables and proportions for qualitive ones. We used the Chi-squared test and the t-test to compare proportions and means, respectively. Sensitivity, specificity, predictive values, and likelihood ratios were used to assess the accuracy of diagnostic tests. Likewise, reliability and agreement were quantified using kappa coefficient. The kappa concordance values of each test (ALG and PCP) were established with respect to the OPH diagnosis and the similarity of the results were examined. The area of the simple ROC curve was calculated for each examiner with respect to ground truth and compared between them. Level of significance was estimated at $p < 0.05$.

Statistical analyzes performed using IBM SPSS Statistic v 24 software (Armonk, NY, USA). The sample calculation and the capacity of the diagnostic tests have been quantified using the Epidat 3.1 program (Xunta de Galicia, Galicia, Spain) (www.sergas.es/Saude-publica/EPIDAT).

3. Results

3.1. Analysis of the Original Sample. Gradable/Ungradable Concordance

During the download period, image folders of 3575 diabetic patients were obtained from the 10 primary healthcare centers. Due to duplication of files and empty downloads without files, 55 patients were removed. Therefore, the original sample consisted of 3520 image folders, of which 43,9% corresponded to women (Table 1). The average age of the patients was 64.4 ± 14.6 years and the mean of the years since the diagnosis of diabetes was 10.37 ± 7.5 years. The majority of patients (88.2%) had type 2 diabetes mellitus (DM2), while patients with type 1 diabetes mellitus (DM1) accounted for 11.4%. In contrast, a minimum proportion (0.4%) of the sample corresponded to patients either diagnosed with other categories or without detailed diagnosis (Others).

Table 1. Main characteristics of the study sample obtained from de APDR between April 2017 and June 2018. * Mean SD. DR = diabetic retinopathy. DME = diabetic macular edema. NDME = no diabetic macular edema. STDR = sight-threatening DR.

Variable of Interest			Study Sample (n = 3520)	
Gender (F/M)			43.9%/56.1% (1545/1975)	
Age (years)			64.4 ± 14.6 *	
Years from diagnosis			10.4 ± 7.5 *	
Mydriasis (before photograph)			97.8%	(3443)
Diabetes type	Type 2		88.2%	(3103)
	Type 1		11.4%	(402)
	Others		0.4%	(15)
Ungradable images (UNG)			11.6%	(407)
No diabetic Retinopathy (NODR)			69.9%	(2460)
Diabetic retinopathy (DR)	Mild		8.1%	(286)
	Moderate	NDME	8.1%	(285)
		DME	1.8%	(65)
	Severe	NDME	0.1%	(2)
		DME	0.2%	(8)
	Proliferative	NDME	0.1%	(3)
		DME	0.1%	(4)
STDR			2.3%	(82)

Six hundred and fifty-three patients (18,5%) had images with features of DR. Macular edema was detected in 77 patients (2.2%) and DR stage became STDR in 82 patients (2.3%). However, it was not possible to establish diagnostic criteria on the condition of the retina in 11.6% of the images (UNG).

Regarding the distribution by gender, the mean age and mean duration of diabetes were significantly higher among the diabetic females. In addition, in female patients, the proportion of DM1 was higher, and the proportion of DM2 was lower than in the male group. However, there was no significant difference in the prevalence of each stage of DR between genders (Table 2).

Table 2. Comparison of the study variables between female and male patients. DR = diabetic retinopathy. DME = macular edema diabetic. STDR = sight-threatening DR. * Results with statistically significant differences.

Variable of Interest		Female (n = 1545)	Male (n = 1975)	p-Value
Age (years)		65.4 ± 15.6	64 ± 13.8	0.0419 *
Years from diagnosis		11.1 ± 8.1	9.8 ± 7	<0.0001 *
Mydriasis		97.6%	98%	0.4196
Diabetes type	Type 2	85.8%	90%	=0.0001 *
	Type 1	13.7%	9.6%	=0.0001 *
	Others	0.5%	0.4%	0.6579
DR stage	Mild	8.1%	8.2%	0.9143
	Moderate	10%	9.9%	0.9216
	Severe	0.2%	0.4%	0.2913
	Proliferative	0.2%	0.2%	1
DME		2.1%	2.2%	0.8394
STDR		2.2%	2.4%	0.6952

Considering the gradable/non-gradable (GRAD/UNG) category of images (previously described in methods), we found that the group of patients with UNG images showed significant differences in the mean age and proportions of the types of diabetes compared to patients with GRAD images (Table 3). Specifically, our data showed that patients with UNG images were 9.3 years older than patients with GRAD images (72. 6 vs. 63. 4). We also found a statistically significantly higher proportion of type 2 diabetic patients and a lower proportion of type 1 diabetic patients, in the group of patients with UNG images (Table 3).

Table 3. Comparison of the common variables between patients with gradable and ungradable images. * Results with statistically significant differences.

Variable of Interest		Gradable	Ungradable	p-Value
Gender (F/M)		43.8%/56.2%	44.2%/55.8%	0.8785
Age (years)		63.4 ± 14.6	72.6 ± 11.8	<0.0001 *
Years from diagnosis		10.3 ± 7.3	10.9 ± 9.2	0.1450
Mydriasis		98%	96.6%	0.0678
Diabetes type	Type 2	87.2%	95.6%	<0.0001 *
	Type 1	12.4%	4.2%	<0.0001 *
	Others	0.4%	0%	0.2012

Regarding the graders used, the images of 407 patients (11.6%) were classified as UNG according to the consensus diagnosis of the OPHs (Tables 4 and 5). Likewise, the automatic analysis software considered the images of 927 patients (26.3%) as inadequate (Table 4), while the PCPs could not provide any diagnosis in 461 patients (13.1%; Table 5). A significant difference was detected in the proportions of UNG images between ALG and OPH classifier strategies and between ALG and PCP classifier strategies ($p < 0.0001$). The difference of 1.54% in the proportions of UNG images between OPH and PCP also reached the limit of significance ($p = 0.0494$).

Table 4. Number of patients in every pair of diagnostic categories comparing ALG vs. OPH.

	OPH-DR	OPH-NODR	OPH-UNG	Total
ALG-DR	455	379	33	867
ALG-NODR	80	1582	64	1726
ALG-UNG	118	499	310	927
Total	653	2460	407	3520

Table 5. Number of patients in every pair of diagnostic categories comparing PCP vs. OPH.

	OPH-DR	OPH-NODR	OPH-UNG	Total
PCP-DR	373	233	25	631
PCP-NODR	214	2005	209	2428
PCP-UNG	66	222	173	461
Total	653	2460	407	3520

The unweighted Kappa statistic was used to assess inter-rater reliability in classifying images into UNG or GRAD. The Kappa statistic shows the observed level of agreement adjusted for the level of agreement that could have occurred by simple chance. A value of 0.75–1.00 indicates an excellent agreement, 0.4–0.75 represents a moderate agreement, and lower values display the deficient agreement. We found that the kappa value was 0.3623 for the agreement between ALG and OPH classifier strategies. The agreement between PCP and OPH obtained a k-value of 0.3144. The kappa homogeneity test showed a Chi-square value of 2.7274 corresponding to a $p = 0.0986$. Therefore, the difference in agreement between the two diagnostic strategies was not statistically significant.

Description of the distribution of the diagnostic categories UNG, DR and NODR of the two diagnostic strategies compared to the criteria of the ophthalmologists:

The proportions of the GRAD/GRAD and UNG/UNG agreement categories for ALG versus OPH were 0.78 and 0.30, respectively, with a composite agreement ratio of 0.80. The proportions of the GRAD/GRAD and UNG/UNG agreement categories for PCP versus OPH were 0.84 and 0.25, respectively, with a composite ratio of 0.85.

Overall, these results demonstrated the proportion of UNG was higher in ALG, and the proportion of composite agreement was higher in PCP. It should be noted that the concordance between ALG and OPH was slightly better (0.3623 vs. 0.3144) because there was a greater coincidence when classifying UNG images (ALG 0.30 vs. PCP 0.25).

3.2. Comparison of DR/NODR Diagnostic Category in Unpaired Samples. Diagnostic Validity

The 407 patients considered as UNG by the OPHs were removed from the original sample. This new sample of 3113 patients was partially described when the comparison between GRAD vs. UNG samples was carried out (Table 3). Based on the results of Tables 4 and 5, two samples were selected. The first sample, called ALG-OPH, contained all patients considered GRAD by these two graders ($n = 2496$). The second sample, called PCP-OPH, included all patients considered GRAD by these other pairs of graders ($n = 2825$).

Our data showed that the PCP-OPH group had a mean age of 1.4 years, significantly higher than that of the ALG-OPH group ($p = 0.0003$). No significant differences were detected between the two samples for any of the other variables (Table 6).

Table 6. Comparison of the study variables ALG-OPH vs. PCP-OPH. NDME = no diabetic macular edema. DR = diabetic retinopathy. DME = diabetic macular edema. STDR = sight-threatening DR. * Results with statistically significant differences.

Variable of Interest		ALG-OPH (n = 2496)	PCP-OPH (n = 2825)	p Value
Gender (F/M)		42.9%/57.1%	43.6%/56.4%	0.6071
Age (years)		61.4 ± 14.7	62.8 ± 14.6	0.0003 *
Years from diagnosis		10.2 ± 7.3	10.3 ± 7.3	0.6522
Mydriasis		98.3% (2454)	98.6% (2785)	0.3753
Diabetes type	Type 1	14.5% (361)	12.8% (362)	0.0710
	Type 2	85.2% (2126)	86.8% (2451)	0.0928
	Others	0.3% (9)	0.4% (12)	0.5394
NODR		78.6% (1961)	79.2% (2238)	0.5924
DR		21.43% (535)	20.78 (587)	0.5619
DR stage	Mild	10.1% (251)	9.5% (267)	0.4622
	Moderate	10.9% (272) (NDME 217) (DME 55)	10.8% (305) (NDME 245) (DME 60)	0.9068
	Severe	0.3% (7) (NDME 1) (DME 6)	0.3% (9) (NDME 2) (DME 7)	1
	Proliferative	0.2% (5) (NDME 2) (DME 3)	0.2% (6) (NDME 2) (DME 4)	1
DME		2.6% (64)	2.5% (71)	0.8173
STDR		2.7% (67)	2.7% (75)	1

The frequencies of the matching of the diagnostic categories of each strategy with the criteria of the OPH and the values of indexes (sensitivity, specificity, positive and negative predictive values, likelihood ratios) that determine the validity of the diagnostic tests with the different classifier strategies are shown in Table 7. In particular, the ALG strategy showed a greater ability than the PCP to detect retinopathy in diabetic patients' retinographies (Sensitivity, 85.05 vs. 64.54). However, the PCP strategy better identified individuals without retinopathy as demonstrated by its specificity values (80.67 vs. 89.59). These results suggest that the diagnostic ability of the ALG was overall superior to that of the PCP.

Table 7. Unpaired sample. Diagnostic category pairs frequency and diagnostic validity indexes.

Variable of Interest	ALG-OPH (95% CI)	PCP-OPH (95% CI)
True Positive	455	373
False Positive	379	233
False Negative	80	214
True Negative	1582	2005
Prevalence	21.43% (19.80−23.06)	20.78% (19.26−22.29)
Sensitivity	85.05% (81.93−88.16)	63.54% (59.56−67.52)
Specificity	80.67% (78.90−82.45)	89.59% (88.30−90.88)
Positive Predictive Value	54.56% (51.12−58.00)	61.55% 57.60−65.51)
Negative Predictive Value	95.19% (94.13−96.25)	90.36% (89.11−91.61)
Likelihood Ratio +	4.40 (3.99−4.85)	6.10 (5.33−6.99)
Likelihood Ratio -	0.19 (0.15−0.23)	0.41 (0.37−0.45)

Four of the 80 DR patients classified as healthy by the ALG were positive for MOD plus DME and in the case of the PCP strategy it was 4 out of 214. The proportion of STDR patients incorrectly diagnosed was 5.9% (4/67) for the ALG and 5.3% (4/75) for the PCP. There was no statistically significant difference in the proportions of misdiagnosed

patients between the two strategies (ALG vs. PCP; $p = 0.8693$). In fact, the sensitivity of the ALG strategy to diagnose STDR was 94% and that of PCP 95%. No patient with severe or proliferative retinopathy was misdiagnosed by ALG or PCP. Both strategies had a low positive predictive value (PPV; Table 7), being even lower for ALG (PPV 54.56 vs. 61.55) but a high negative predictive value (ALG 95.19 vs. PCP 90.36). The ALG strategy not only had a higher sensitivity, but also a better negative predictive value than the PCP strategy.

Likelihood ratios (LR) are another alternative for calculating the diagnostic accuracy and summarizing the information endowed in both sensitivity and specificity. The LR is the probability that a particular test result would be expected among patients with the condition diagnosed compared to the likelihood that that same result would be expected in a patient without the condition. Good diagnostic tests have LR+ > 10 and their positive result has a significant input to the diagnosis. Good diagnostic tests have LR- < 0,1. The lower the LR- the more significant contribution of the test is in ruling-out (Table 8)

Table 8. Ranges of likelihood ratio values and their impact on diagnostic accuracy.

Likelihood Ratio +	Likelihood Ratio −	Usefulness
10	<0.1	Highly relevant
5–10	5–10	Good
2–5	2–5	Fair
<2	<2	Poor

In the ALG strategy, the modification of the previous probability for positive test results was small (LH + 4.40, Table 7). In fact, we calculated the post-test probability using Fagan's nomogram and obtained that the probability of a patient having a DR changed from 21% to 55%. About 1 in 1.8 positive tests corresponded to patients with retinopathy. The subsequent probability, if the test was negative, was modified to 5%, so approximately 1 of each negative result corresponded to an individual without DR. Likewise, the modification of the previous probability in the PCP test, for positive results, was moderate (LH + 6.10), changing the probability of a patient having a DR from 21% to 62% (Fagan's nomogram). About 1 in 1.6 positive tests corresponded to patients with retinopathy. The subsequent probability, if the test is negative, was modified from 21% to 10%, so approximately 1 in 1.1 negative results corresponded to an individual without retinopathy.

To determine the discriminative power of the different classifier strategies of DR diagnosis, our results were compared using the area under the curve (AUC) of the Receiver Operating Characteristics (ROC) curve (Figure 2).

The AUC for the PCP displayed a value of 0.7657 (95% CI 0.7452 to 0.7861), while the value of AUC for ALG was 0.8286 (95% CI 0.8111 to 0.8461). We found that there was a significant difference between the two curves ($p = 0.0000$). Therefore, these power results shown in Figure 2 indicate that the ALG was more efficient than the PCP.

Finally, to assess the intergrader reliability for DR diagnosis, we used the Kappa statistic. We found that the k-value of the agreement between the ALG and OPH was 0.5462 (95% CI 0.5109 to 0.5815), while between PCP and OPH was 0.5251 (95% CI 0.4865 to 0.5636). The kappa homogeneity test showed a Chi-square value of 0.6252 corresponding to a $p = 0.4291$. Therefore, the difference in concordance between the two diagnostic strategies was not statistically significant.

3.3. Comparison of Diagnostic Tests in Paired Samples

To confirm the previous results, we studied the patients classified as GRAD by the three diagnostic strategies, removing from the original sample all patients considered UNG for any of the strategies. In Table 9, the proportions and means of the paired sample obtained were described.

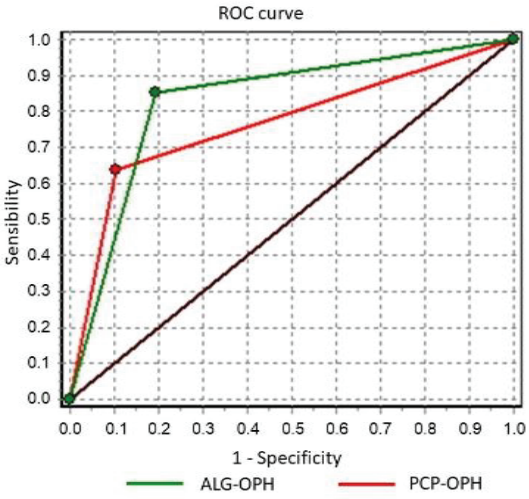


Figure 2. ROC curves comparison. Green line: Algorithm. Red line: Primary Care Physician.

Table 9. Main descriptors of the sample obtained from de APDR for the study between April 2017 and June 2018. * Mean SD. DME = diabetic macular edema. NDME = macular edema non-diabetic. STDR= sight-threatening DR.

Variable of Interest			Study Sample (n = 2335)
Gender (F/M)			42.7%/57.3% (997/1338)
Age (years)			61.1 ± 14.7 *
Years from diagnosis			10.1 ± 7.2 *
Mydriasis (before photograph)			98.8% (2306)
Diabetes type	Type 2		85% (1984)
	Type 1		14.7% (343)
	Others		0.3% (8)
No Diabetic Retinopathy (NODR)			78.9% (1842)
DR prevalence			21.1%
Diabetic retinopathy (DR)	Mild		10.2% (238)
	Moderate	NDME	8.3% (194)
		DME	2.2% (51)
	Severe	NDME	0.04% (1)
		DME	0.2% (5)
	Proliferative	NDME	0.04% (1)
		DME	0.1% (3)
STDR			2.6% (61)

The frequencies of pairs of diagnostic categories of each strategy compared to the criteria of the OPH, and the values of the diagnostic validity indexes for the sample described are displayed in Table 10.

Table 10. Paired sample. Diagnostic category pairs frequency and diagnostic validity indexes.

Variable of Interest	ALG-OPH (95% CI)	PCP-OPH (95% CI)
True Positive	418	300
False Positive	351	200
False Negative	75	193
True Negative	1491	1642
Prevalence	21.1% (19.44−22.79)	
Sensibility	84.8% (81.52−88.06)	60.9% (56.44−65.26)
Specificity	80.9% (79.12−82.77)	89.1% (87.69−90.59)
Positive Predictive Value	54.36% (50.77−57.94)	60% (55.61−64.39)
Negative Predictive Value	95.21% (94.12−96.30)	89.48% (88.05−90.91)
Likelihood Ratio +	4.45 (4.02−4.92)	5.60 (4.83−6.50)
Likelihood Ratio −	0.19 (0.15−0.23)	0.44 (0.39−0.49)

The pattern of the indexes shown in Table 10 is comparable to that found in the unpaired sample (Table 7). In fact, the prevalence of DR was the same in all the samples. In summary in the paired sample, the ALG showed a greater ability than PCP to detect DR lesions. However, PCP better identified patients without DR.

Similarly, as in the unpaired sample, we determined that the probability that an individual with a positive test would have retinopathy was small in both strategies (PPV 54.36% vs. 60%), being even lower for the ALG. Both tests presented a high negative predictive value. Therefore, for the unpaired sample, we can conclude that the 2iRetinex software is a good predictor of the absence of DR and a moderate predictor of the presence of DR. In contrast, the diagnostic utility of PCP screening is considered good for positive results and fair for negative.

The AUC for the PCP showed a value of 0.75, while for the ALG was 0.8287. We found that there was a significant difference between the two curves (Chi-square value 28.0575, $p = 0.0000$). Consequently, these results shown in Figure 3 indicate a greater diagnostic power of the ALG.

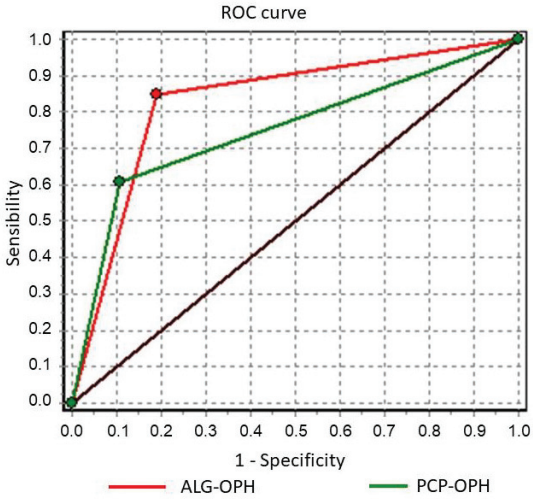


Figure 3. ROC curves comparison of the diagnostic validity. Paired sample. Red: Algorithm. Green: Primary Care Physician.

The k-value of the concordance between the ALG and OPH was 0.5455, while between PCP and OPH it was 0.4974. The homogeneity test for the difference in k-values was not significant (Chi-square 2.7898 $p = 0.949$).

4. Discussion

Digital fundus photography is considered an acceptably accurate procedure for detecting DR [13]. Image processing and interpretation in DR screening programs require initial training and ongoing updating for all personnel involved. This entails personal effort and resource consumption. Population-based screening programs for detecting DR help reduce visual loss by identifying sight-threatening cases and referring them to specialists for treatment. A recent meta-analysis of 33 studies worldwide concludes that teleophthalmology has a moderate sensitivity and high specificity for detecting the absence of DR. However, the results obtained for the diagnosis of the diseased retina show widespread variations [19]. In recent years, there has been increasing interest in applying automation processes in ophthalmic telemedicine to reduce the need for screening professionals and to homogenize diagnostic criteria regardless of the origin and composition of the sample being evaluated [20]. For that reason, in this study, we assessed the use of the 2iRetinex software as a complement or substitute for the screening physician.

In this assessment of images from 3520 patients from ten APDR primary care centers, the prevalence of any form of DR was 18.5%, while STDR cases accounted for 2.3% of the study subjects. Similar results were obtained in the first-year screening of the Scottish program, where the prevalence of DR and STDR was 19.3% and 1.9%, respectively. These values increased in the subjects reviewed one year (20.5% and 2.3%, respectively) [21]. Likewise, other studies with similar characteristics showed a percentage of STDR ranging from 2.57% to 4% [8,22,23].

According to the American Telemedicine Association Validation Level, the first phase of our APDR screening system can be classified as a category 1 program, with an on-disease/non-disease diagnostic criterion issued by the PCP [24]. Meanwhile, the second phase, characterized by the review of pathological retinographies by the referring ophthalmologist is classified as a category C2 program. The ophthalmologist establishes the stage and the time frame for the patient's review or for initiating treatment. Previous studies indicated that artifacts may be present in 3−30% of the photographs without mydriasis [25]. Of note, widespread use of tropicamide (97.8% of patients) does not pose any additional risk [26]. In this regard, the proportion of non-valuable retinal images in published studies is highly dependent on the age distribution of the sample, which in turn is related to the presence of eyelid and corneal abnormalities and especially cataracts. In our study, despite the use of tropicamide to dilate the pupil, the proportion of patients with UNG images was 11. 6%. Moreover, PCPs were unable to make a judgement on 13. 1% of patients. Likewise, the automatic analysis of the algorithm considered 26.3% to be unclassifiable. Our data are consistent with those of other studies. In particular, in a validity study, the iGrading automatic assessment system found 26.16% of 2309 patients to be ungradable [27]. The Italian multicenter study NO BLIND classified 23.4% of the telediagnostic images as "poor quality non-diagnostic images" [28]. Following this line in another study, the ratio of non-gradable patients classified by the automatic system was twice that of the human classification [22]. Indeed, in this study, 404 patients by the criteria of the automatic system were discarded, and 197 patients by the criteria of the ophthalmologist. Only in two other cases did they agree in classifying them as non-gradable [22]. Similarly, in our study, we found the same lack of diagnostic agreement in image grading between ALG and PCP with respect to OPH (k = 0.3623 and 0.3144, respectively). This suggests that the mechanisms underlying decision-making are different in algorithms and clinicians. The grading criteria of the automatic systems may have been more demanding than those of the humans, but what is evident is that their implementation is invariable and not affected by the inherent inconsistency of human subjectivity [29].

Our results on the diagnostic accuracy of screening PCP for any grade of DR showed a good mean specificity (89.6%) and a reduced sensitivity (63.5%). However, for STDR the sensitivity increased to 95%. These values are similar to those reported in other studies [8,11,26,30]. Noteworthy, our findings are consistent with a previous partial study about the diagnostic ability of screening PCP at three APDR primary care centers in a small

sample of patients [17]. Similarly, the concordance in the diagnosis of DR with respect to the clinical judgment of the ophthalmologist was k = 0.408 and sensitivity was 97% and specificity 80%.

The second level of APDR development, which would make it a screening and follow-up program to monitor patients with mild and moderate retinopathy without macular edema within the program, has not yet been implemented [30]. It could be that the screening physicians want to reduce the workload of ophthalmologists at the second and third levels of care by under-diagnosing cases that do not require referral.

Regarding the results related to the diagnostic accuracy of the 2iRetinex software analysis test (ALG), an overall sensitivity of 85% was obtained for any degree of retinopathy and a specificity of 81%. For STDR cases, sensitivity increased to 94%. It should be noted that the agreement with the diagnosis of the reference ophthalmologists was similar to that of PCP and the area of the simple ROC curve (0. 8286) was slightly similar to that of PCP. Cost-effective ALG screening programs to identify DR lesions on digital images have been reported previously [31]. In particular, Fleming et al. [31] stated that this inclusion of algorithms increased the sensitivity to 100% in DR detection and reduced manual screening by more than 35%.

EyeArt, Retmaker, iGrading or IDx are commercially available systems that have been used in teleophthalmic screening programs for DR diagnosis [20]. The functionality of iGrading is comparable to that of 2iRetinex by combining an image quality system and a DR identification criterion. In the validation study conducted in Valencia [23], iGrading showed excellent sensitivity values of 97.4% and specificity values of 98.3% for patients with STDR. For its part, iGrading has been used as a level 1 grading in the Scottish screening program after extensive validation since 2010 with a sensitivity of 97.8% and specificity of 41.2% for referable DR [32]. The other two systems, Eyeart and Retmaker showed good diagnostic accuracy with a sensitivity for STDR of 94.7% and 85%, respectively [33]. These methods qualified as Automated Retinal Image Analysis System (ARIAS) have not yet developed a sufficient level of autonomy to establish a classification of the patient's damage and recommend treatment [34].

ARIAS have been changed with the development of artificial intelligence systems and Deep learning (DL), a subtype of machine learning (ML) that does not require image engineering. Early ML techniques for detecting DR used mathematical image transformation techniques and image engineering [35]. Moreover, DL develops its own pattern recognition representations after being fed raw data [36,37].

In relation to this, it is known that IDx has updated its ARIAS with an artificial intelligence system. The new version IDx-DR v2 is designed to identify DR referable to a specialist without human supervision. IDx-DR v2 achieved 100% sensitivity and 81.82% specificity for derivable DR and 100% sensitivity and 94.64% specificity for STDR in the sample examined [38]. A meta-analysis demonstrated that ML algorithms have a high diagnostic accuracy for the diagnosis of DR on color fundus photographs suggesting that they may also be ready for clinical application in screening programs [29]. However, early results published in relation to this ML had methodological inconsistencies, such as lack of external validation and the presence of biases. Most of these methods do not provide full interpretations of the relevant findings of retinal pathological signs. Furthermore, it should be noted that, at present, the full implementation of this "black box classification system" presents difficulties for acceptance by clinicians and patients [39].

The main limitation of our study is the dichotomous diagnosis made by the screening PCP. At least at this stage, the APDR screening program is not designed for PCP to classify the DR stage. Indeed, this limitation has prevented us from obtaining full diagnostic validity indexes results in patients with STDR.

The diagnostic capability for STDR stages is excellent for both diagnostic strategies (PCP and ALG). In the case of the overall diagnostic ability for any form of diabetic retinopathy with our algorithm, the 2iRetinex and PCP strategies are comparable. Moreover, their agreement with respect to the re-evaluation of ophthalmologists does not show

significant differences. Therefore, the introduction of this real-time software into the APDR workflow would allow knowing whether the images taken of the patient's fundus are of sufficient quality before the patient leaves the examination center. Thus, a re-examination could be recommended if necessary. This strategy would reduce the delay of repeat examinations and consequently, less disrupt the patient's social and work activity. All this suggests that the automatic examination system could be introduced in a real way by integrating it with the screening physician in the first phase.

In this study, we have validated our DR prediction software (2iRetinex) on a sample of patients under real clinical conditions in the routine APDR circuit. Although further assessment is needed to validate the system, it has been confirmed as a tool that could be integrated into DR screening programs. This could improve the quality of screening models in the future. Due to this, studies of the combined use of algorithms and manual classification emerge as an urgent need to achieve better performance. Thereby, the workload of manual classification could be minimized. We plan to conduct studies that focus on extending the algorithm using the 2iRetinex software to detect other common comorbid eye diseases such as age-related macular degeneration (AMD) and glaucoma.

Author Contributions: S.J.-C. and P.A.-M. were responsible for all aspects of the project including conceptualization and design of the study. S.J.-C., P.A.-M. and P.A.-R. reviewed all the images and stablished the reference diagnosis; E.M. acquired and analyzed APDR data. S.J.-C. and P.A.-M. prepared figures and tables. S.J.-C. and P.A.-M. provided the first draft, prepared final figures, and discussed the results. E.M. and M.A.-D. provided critical review; S.J.-C. and P.A.-M. provided the final version and financial support for the project leading to this publication. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Anonymized data processed in an excel file are available on reasonable request. Restrictions apply to the availability of APDR data. These data are subject to ethical restrictions (Data Protection Act).

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Review

State of Evidence on Oral Health Problems in Diabetic Patients: A Critical Review of the Literature

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Abstract: Diabetes mellitus (DM) is a global health problem, having recognized that in the next 20 years the number of diabetic patients in the world will increase to 642 million. DM exerts enormous repercussions on general health diabetic (especially derived from vascular, cardiac, renal, ocular, or neurological affectation). It entails in addition a high number of deaths directly related to the disease, as well as a high health care cost, estimated at $673 billion annually. Oral cavity is found among all the organs and systems affected in the course of DM. Important pathologies are developed with higher prevalence, such as periodontitis (PD), alterations in salivary flow, fungal infections, oral cancer, and oral potentially malignant disorders (OPMD). It has been proven that PD hinders the metabolic control of DM and that the presence of PD increases the possibility for developing diabetes. Despite the relevance of these oral pathologies, the knowledge of primary care physicians and diabetes specialists about the importance of oral health in diabetics, as well as the knowledge of dentists about the importance of DM for oral health of patients is scarce or non-existent. It is accepted that the correct management of diabetic patients requires interdisciplinary teams, including dentists. In this critical review, the existing knowledge and evidence-degree on the preventive, clinical, diagnosis, prognosis, and therapeutic aspects of oral diseases that occur with a significant frequency in the diabetic population are developed in extension.

Keywords: diabetes mellitus; oral health; oral medicine; oral pathology; periodontitis; dental caries; oral cancer

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1. Introduction

Diabetes mellitus (DM) is a health problem of global importance that affects a large number of patients around the world. According to data reported by relevant international organizations (http://diabetesatlas.org/es/sections/worldwide-toll-of-diabetes.html, accessed on 15 December 2020), in the next 20 years, the number of worldwide diabetic patients will increase to 642 million people. DM exerts enormous repercussions on general health diabetic (especially derived from vascular, cardiac, renal, ocular, or neurological affectation). It entails in addition a high number of deaths directly related to the disease, as well as a high health care cost, estimated at $673 billion annually. Current scientific evidence indicates that DM is the consequence of an interaction of environmental, epigenetic, and genetic factors [1]. Among the environmental factors are fundamentally infections and the microbiota involved—of particular importance is the microbiota affecting the oral and intestinal cavities—diet and others. Epigenetics is currently considered as the link between the environment and genetics, altering gene and protein expression. Epigenetic factors—including DNA methylation, histone modification, and microRNAs (e.g., miR-15b, miR-29, or miR-122 [2])—regulate gene expression. These key events are implicated in autoimmunity and in the vulnerability of beta cells of pancreatic islets. Finally, genetic factors promote a special susceptibility to the development of the disease [3]. In this aspect, more than 60 genes, altered chromosomal loci and polymorphisms (e.g., rs12255372 and

rs7903146 variants of *TCF7L2* [4]), have been implicated. Type 1 diabetes mellitus (DM1) responds to a multifactorial pathogenesis essentially linked to an autoimmune aggression mediated by autoantibodies that generates a progressive loss of insulin-producing β-cells in the pancreas [5]. On the other hand, the pathogenesis of type 2 diabetes mellitus (DM2) is essentially linked to the development of a state of resistance to the actions of insulin [6].

Oral cavity is found among the organs and systems affected in the course of DM (Table 1). Nevertheless, the information on many of the diabetic related oral diseases—with regard to diagnosis, treatment, and prevention—is limited among health care providers in diabetic patients, especially endocrinologists and family doctors. Likewise, the knowledge that dentists have on the relationships between oral health and DM, and the information on the implications of the dentist in the control of diabetic patients seem limited. Furthermore, knowledge about the aforementioned aspects is frequently based on scant scientific evidence. Undoubtedly, the prevention and control of oral pathology in diabetic patients improves their quality of life and most likely facilitates the long-term control of DM and consequently improves their prognosis. The health care providers for the treatment and follow-up of diabetic patients should be well informed of oral pathologies frequently associated with DM in order to be able to prevent, diagnose, and treat them, or if necessary, refer patients to specialized centers for their management. This most likely requires the implementation of educational programs that convey evidence-based information. In this critical review, the existing knowledge and its degree of evidence, the preventive, clinical, diagnosis, prognosis, and therapeutic aspects of oral diseases occurring with a significant higher frequency in the diabetic population are developed in extension, attending to both type 1 and type 2 DM.

Table 1. Oral manifestations that may be present in patients with Diabetes Mellitus.

Oral Manifestations	Description	References
Periodontitis	The concept of periodontitis refers to a chronic inflammatory process characterized by microbially-associated, host-mediated inflammation that results in loss of periodontal attachment (i.e., by loss of marginal periodontal ligament fibers, apical migration of the junctional epithelium, and apical spread of the bacterial biofilm along the root surface of teeth). Initially, bacterial biofilm formation begins gingival inflammation (i.e., dental-biofilm induced gingivitis) with periodontitis initiation and progression. Furthermore, a multifactorial origin influenced by additional risk factors, such as smoking, is now supported on the immunoinflammatory bases of periodontitis. The relationship between periodontitis and DM (i.e., high prevalence and magnitude of association) is based on a solid evidence level.	[7,8]
Oral candidiasis	Fungal infections, particularly by species of the genus *Candida* sp. Common clinical manifestations are the presence of extensive reddened areas (erythematous candidiasis) along the oral mucosa, generally associated with patchy lingual depapillation and commissural cheilitis. DM patients may present whitish lumps, similar to milk or yogurt clots (speudomembranous candidiasis). Oral candidiasis is usually symptomatic, causing discomfort, burning, or frank pain.	[9,10]
Oral cancer	Oral cancer is the malignant neoplasm affecting lips, oral cavity, or oropharynx. Oral squamous cell carcinoma represents around 90% cases and has a 5-year mortality rate of 50%. The reasons for the increased development of oral cancer in diabetics are not well known, although clinical, biochemical, and molecular reasons have been proposed.	[11,12]
Oral potentially malignant disorders (OPMD)	OPMDs are a significant group of mucosal disorders that may have an increased susceptibility to develop oral cancer, which are essentially oral leukoplakia, oral lichen planus (OLP), proliferative verrucous leukoplakia, erythroplakia and actinic cheilitis. Leukoplakia and OLP—both prevalent OPMDs, associated with considerable malignant transformation rates—have a higher prevalence in subjects with DM than in general population.	[12,13]
Dental caries	Dental caries also known as tooth decays are caused by a breakdown of the tooth tissues. This breakdown is the result of dental plaque's bacterias on teeth that produce acid destroying tooth tissues (e.g., enamel) and results in tooth decay. Diabetic patients, as a consequence of a series of associated oral conditions—xerostomia, high levels of dental plaque, etc.—would be more predisposed to the development of dental caries.	[14,15]

Table 1. *Cont.*

Oral Manifestations	Description	References
Burning mouth syndrome (BMS)	BMS is an atypical chronic pain essentially characterized by the presence of a burning sensation, stinging, or frank pain that is located mainly on the tongue, lips, and palate, although it can spread to any other location, without that there are recognizable mucosal lesions that may justify this condition. BMS seems to present an increased prevalence in patients with DM compared to healthy subjects. It could be associated with the peripheral neuropathy frequently reported in diabetic patients.	[16,17]
Salivary secretion alterations	Alterations in salivary secretion are generically known by the term "dry mouth", which however refers to two different processes, the first related to an objective reduction of salivary flow due to salivary hypofunction (i.e., unstimulated whole saliva flow rate of <0.1 mL/min); and the second, the subjective sensation of lack of saliva in the absence of flow disorders. Dry mouth is one of the most common complaints in diabetic patients, with a partially unknown pathophysiology, which could be related to diabetic neuropathy of parotid gland, pathologic alterations in the salivary glands structure (e.g., vacuolization or acinar atrophy), or hyperglycemia and poorly controlled DM.	[18,19]
Taste perception alterations	Taste perception alterations, mainly hypogeusia (i.e., a partial loss of taste) have been reported in patients with DM. The reasons for the increased development of taste perception alterations in diabetics are not well known, although it has been proposed that it could be associated with the peripheral neuropathy frequently reported in diabetic patients.	[20,21]
Halitosis	Halitosis is a symptom where a person has bad breath. It can be caused by bad oral health, singularly dental care, caries, or periodontitis. Patients with DM are predisposed to halitosis, probably related to the frequent prevalence of these diseases in diabetic patients.	[22,23]
Delayed wound healing	Delayed wound healing is a complication in diabetics after oral surgery, especially in patients with poorly controlled DM. The probable cause of delayed wound healing is damaged small terminal vessels, responsible of reduced blood flow, with an insufficient supply of cellular nutrients through the blood circulation, decreased inflammatory and immune response.	[24–26]

2. Scientific Framework

We chose the critical narrative review design as the scientific framework of this paper on the basis that this type of design covers a wide range of aspects in a given topic. Furthermore, this study design offer the reader global information on a health problem with different facets (not easily achievable through other designs such as systematic reviews and meta-analyses). In this paper, we follow the concept of critical review used by Grant and Booth [27]. A critical review aims to demonstrate that the writer has extensively researched the literature and critically assessed its quality. It goes beyond merely describing the articles identified and includes some degree of analysis and conceptual innovation. An effective critical review presents, analyzes, and synthesizes material from a variety of sources. This concept is widely accepted in the international literature as evidenced by the high number of citations their work has received (5198 citations to date). The main strength of a critical review is based on offering an opportunity to "take stock" and evaluate what is the previous body of a health problem while at the same time making it possible to contribute the authors' own opinions and experiences.

We searched MEDLINE through PubMed (as main electronic database) and Web of Science (for bibliometric analysis purposes) for studies published before the year 2021 (upper limit), with no lower date limit. Search strategy was conducted by combining thesaurus terms used by the databases (i.e., MeSH) with free terms, constructed to maximize sensitivity. In a first line general search, the root keywords and synonyms combined were "diabetes mellitus", "oral health", "periodontal diseases", "oral candidiasis", "oral cancer", "oral potentially malignant disorders", "caries", "burning mouth syndrome", and "salivary secretion alterations". In addition, several more specific searches were conducted by combining relevant aspects of the goals to be reviewed (i.e., relationships between oral diseases and diabetes mellitus, prevention, diagnosis, prognosis, and therapeutic implications). We also manually screened the reference lists of the handled studies for additional relevant

studies. Most of the revised studies were included or excluded according to an exhaustive analysis of the title, abstract, year of publication, impact of the journal, and number of citations received. Although these last two criteria may introduce a potential selection bias, its application is necessary when handling a large number of records (e.g., in the first line general search context, simply using the following syntax: ("Diabetes Mellitus"[mh] OR "type 1 diabetes"[all] OR "T1DM"[all] OR "type 2 diabetes"[all] OR "T2DM"[all] OR "diabetes"[all]) AND ("Oral Health"[mh] OR "oral health"[all] OR "mouth diseases"[all] OR "Periodontitis"[mh] OR "periodontitis"[all] OR "periodontal diseases"[all] OR "Mouth Neoplasms"[mh] OR "Mouth Neoplasms"[all] OR "oral squamous cell carcinoma"[all] OR "oral cancer"[all] OR "oral potentially malignant disorders"[all] OR "OPMD"[all] OR ("oral"[all] AND precancer*[all]) OR "Leukoplakia, Oral"[mh] OR "leukoplakia"[all] OR "erythroplakia"[all] OR "Lichen Planus, Oral"[mh] OR "oral lichen planus"[all] OR "Oral Submucous Fibrosis"[mh] OR "oral submucous fibrosis"[all] OR "Dental Caries"[mh] OR "caries"[all] OR "carious"[all] OR "dental decay"[all] OR "Burning Mouth Syndrome"[mh] OR "Burning Mouth Syndrome"[all] OR "BMS"[all] OR "Salivary Gland Diseases"[mh] OR "xerostomia"[mh] OR "xerostomia"[all] OR "dry mouth"[all] OR "hyposalivation"[all]), more than 7000 registers were retrieved). We would also like to clarify that a potential selection bias would only affect to the identification of primary-level studies. Given our effort to develop this review from an evidence-based scientific context, we applied optimal search filters designed for retrieving systematic reviews and meta-analyses (i.e., *Centre for Reviews and Dissemination-CRD* filter; sensitivity = 99.5%, 95%CI = 97.3–99.9 [28,29]). This approach should overcome the potential selection bias, decreasing the rate of missing systematic reviews.

3. Periodontitis

The concept of periodontitis (PD)—according to the new classification scheme for periodontal and peri-implant diseases and conditions [7]—is characterized by microbially-associated, host-mediated inflammation that results in loss of periodontal attachment [8]. PD disease drives the activation of host-derived proteinases with loss of marginal periodontal ligament fibers, apical migration of the junctional epithelium, and apical spread of the bacterial biofilm along the root surface of teeth [8]. Initially, bacterial biofilm formation begins gingival inflammation (i.e., dental-biofilm induced gingivitis [7]); nevertheless, PD initiation and progression is dependent on dysbiotic ecological changes in the microbiome. It occurs in response to nutrients from gingival inflammation and tissue breakdown products with the enrichment of some species and anti-bacterial mechanisms that attempt to contain the microorganisms within the gingival sulcus area once inflammation has initiated [8]. Furthermore, a multifactorial origin influenced by additional risk factors, such as smoking, is now supported on the immunoinflammatory response that trigger the dysbiotic microbiome changes, and also likely influence severity of PD for such individuals [8]. PD is an important health problem because of its prevalence and the systemic repercussions that it entails. Epidemiological studies have reported that 10–15% of the worldwide population suffers from advanced PD [30]. Likewise, the association between PD and some systemic disorders including cardiovascular and metabolic diseases is well known [31–33].

DM is the most prevalent systemic disease in which it has been shown, after extensive research, that it predisposes to the development of PD [34–37]. A recent meta-analysis [38] that collected information from 27 studies (3092 diabetic patients and 23,494 controls) has reported a prevalence of PD of 67.8% in patients with DM and 35.5% in controls (odds ratio [OR] = 1.85; 95%CI = 1.61–2.11), results that in an unappealable way give an idea of the magnitude of the problem. Furthermore, cohorts that include patients with DM1 and DM2 report a higher prevalence of PD in DM1 (78.8% compared to DM2 (70.5%); OR = 2.60 vs. OR = 1.71). A recent systematic review and meta-analysis [39] has also confirmed that DM1 is a relevant risk factor for the development of PD, with a proportion of patients affected more than double for DM1 compared to non-diabetic individuals. In addition, another recent systematic review [40] has also reported an evident bidirectional epidemiological

relationship between DM2 and PD, such that the prevalence of DM2 was significantly higher in patients with PD (OR = 4.04, $p < 0.001$), and vice versa (OR = 1.58, $p < 0.001$). The association of DM and PD has recently been considered a comorbidity [41,42].

3.1. Mechanisms Linking DM and PD

Poorly controlled DM generates sustained hyperglycemia, which in turn induces an increase in the inflammatory response in the periodontal tissue; this stimulates the receptor activator of nuclear factor κB (RANK)/RANK-Ligand (RANKL) axis with an increase in osteoclastogenesis and destruction of the alveolar bone, which will conclude with the clinical attachment loss, one of the PD hallmarks. The existing scientific evidence on the biological mechanisms linking DM and PD is detailed below (Figure 1).

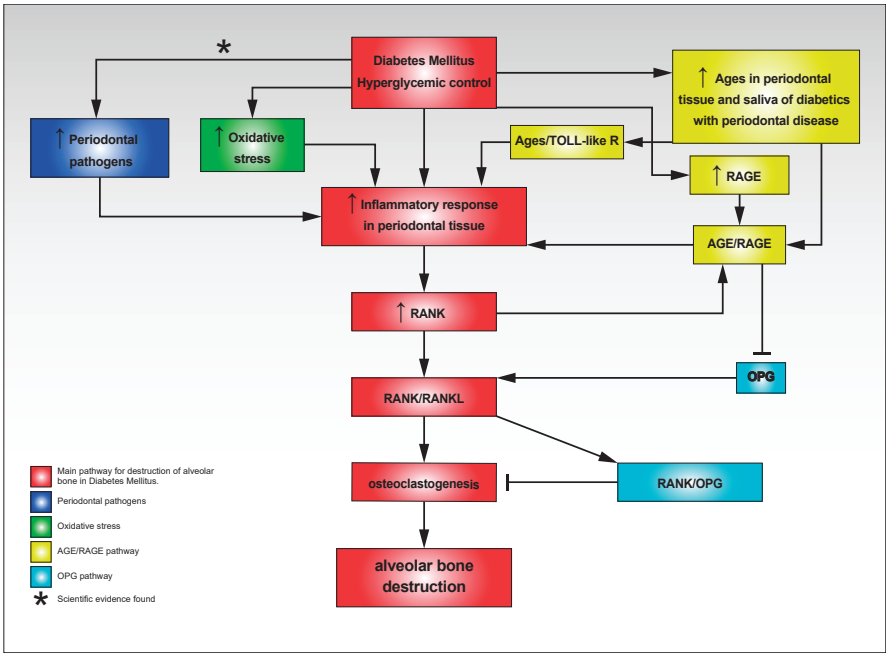

Figure 1. Diabetes Mellitus with a poor control generates sustained hyperglycemia, which in turn induces an increase in the inflammatory response in the periodontal tissue; this in turn stimulates the RANK/RANKL axis with an increase in osteoclastogenesis and destruction of the alveolar bone, which will conclude with the loss of teeth—one of the hallmarks in periodontitis. The increase in the number of periodontal pathogens, the increase in reactive oxygen species (ROS), and the increase in the expression of role of advanced glycation end products (AGE) and its receptor (RAGE) also activate the inflammatory response in the periodontal tissue. The receptor activator of nuclear factor κB (RANK)/RANK-Ligand (RANKL)-RANK/osteoprotegerin (OPG) balance will be important in maintaining periodontal bone homeostasis.

3.1.1. Impact of DM on the Oral Microbiota

It should be recognized that there is very limited and contradictory scientific evidence on the possible impact that DM may exert on the oral microbiota [41,43]. A narrative review [44] has reported that DM1 and DM2 do not have a significant effect on the composition of the periodontal microbiota and that glycemic control level does not seem to significantly influence the composition of the subgingival biofilm. On the contrary, some studies [45–48] indicate that in patients with DM, poor glycemic control could translate into a high number of periodontal pathogens. Currently, we know that these periodontal pathogens are related to the onset and exacerbation of PD [49]. However, the main limitation presented by the evidence on this subject is related to most of these studies are cross-sectional. This study design makes difficult to determine if the more than fre-

quent concomitance of PD and DM responds to a causal relationship or is the result of the presence of common risk factors [41].

3.1.2. Pro-Inflammatory Mediators in Patients with PD and DM

It is currently known that the penetration of periodontal pathogens into the periodontal connective tissue triggers an inflammatory response linked to the development and progression of PD [50]. Evidence from clinical studies supports that DM with poor glycemic control is associated with significantly high levels of pro-inflammatory mediators in gingival tissue [43]. The pathway that best documents the comorbidity between DM with poor glycemic control and PD is inflammation, having shown that an evident local and systemic inflammatory process underlies both conditions that determines its evolution and severity [41]. In vitro and in vivo studies in humans strongly indicate that DM is associated, in a proportional way to glycemic control, with a higher expression of pro-inflammatory mediators in periodontal tissue (TNF-α, IL-6, -8, -10, -12, α1β, substance P, eotaxin, macrophage inflammatory protein 1a, GM-CSF, MMP-1, ICAM1, RANKL, PGE2, Toll-like receptor-2, -4, and -9, caspase 3) and with the activation of the Th-17 pathway [44,51–67]. These observations have also been reported in animal models that have evidenced a significantly greater inflammatory response in diabetic vs. non-diabetic, having suggested that periodontal bacteria induce the upregulation of several pro-inflammatory and pro-apoptotic genes in diabetes [68,69].

It has also been pointed out that hyperglycemia and the conditions associated with DM can promote oxidative stress [70] through different pathways with the consequent influence on the inflammatory response. Reactive oxygen species (ROS) has been reported to stimulate the production of pro-inflammatory cytokines through the activation of MAPK, NF-Kβ, Wnt, NALP3 inflammosome pathways, and the activation of the transcription factor FoxO [71–74].

3.1.3. Role of Advanced Glycation End Products (AGE) and Its Receptor (RAGE) in the Development of PD in Diabetes

An important effect of chronic hyperglycemia in uncontrolled DM is related to the non-enzymatic glycation of proteins and lipids, which results in the formation of AGEs. Higher levels of AGEs have been reported in the serum of patients with DM2 in relation to the extent of their PD [75]. The accumulation of AGEs can lead to cellular stress exerting pro-inflammatory and oxidative effects directly or through their interaction with RAGEs. RAGE is a multiligand receptor belonging to the immunoglobulin superfamily of cell-surface molecules [76] that is overexpressed in DM and has been shown to play a role in the development and progression of some complications of diabetes [77] and also in PD in these patients. In this sense, in diabetic mice it has been shown that the loss of bone linked to the infection by *Porphyromonas gingivalis* was mediated by the overexpression of AGE and RAGE [44]. It has also been reported that RAGE contributes to impaired tissue repair in surgical wounds in a diabetic mouse model, and that inhibition of RAGE-mediated signaling increased the rate of tissue healing and repair [78]. Likewise, it has been shown that the AGE-RAGE interaction delays bone healing in the absence of infection, both in osteoblast cultures and in craniotomies in animal models [79]. Finally, AGE could also bind to Toll-like receptors [44]. A significant increase in the expression of these receptors has been observed in the gingival tissue of patients with DM and PD [80], having reported that their activation exerts a pro-inflammatory effect in diabetics similar to that displayed by RAGE, which is especially significant for the Toll-like receptor 4 [81]. Through this pathway, the AGE-Toll-like receptors interaction can increase inflammation and tissue destruction in diabetic PD.

3.1.4. Role of Hyperglycemia in Bone Destruction in PD

The final biological event with the greatest clinical implications in PD is tissue destruction, including destruction of the alveolar bone with consequent tooth loss. The destruction of the alveolar bone is essentially due to the stimulus of the RANK for its

ligand (RANKL). RANK is mainly expressed in the membrane of osteoclasts and preosteoclasts and binds to RANKL which is secreted by T cells, indicating that the inflammation inherent in PD induces destruction of the alveolar bone through the stimulation of osteoclastogenesis related to the pathway RANK/RANKL [41]. The natural antagonist of RANKL is osteoprotegerin (OPG), in such a way that the RANK/OPG binding induces the inhibition of osteoclastogenesis. The RANK/OPG ratio is therefore a determining factor in the metabolism and homeostasis of the alveolar bone [41]. Several studies have indicated that DM with poor glycemic control favors the destruction of the alveolar bone in patients with PD mediated by the activation of the RANK/RANKL axis [82–85]. Increased levels of RANKL have been reported in periodontal tissue and crevicular fluid from diabetic patients with poor glycemic control [44,86,87], as well as increased levels of soluble RANKL [82,88] and an increase in the RANK/OPG ratio in poorly controlled DM [82,83]. Studies on animal experimentation also indicate an increase in osteoclastic activity linked to an increase in RANKL levels [89–92]. Finally, it has been interestingly pointed out that the AGE/RAGE axis can also contribute to osteoclastogenesis via increased expression of RANKL and downregulation of OPG in various cell types [93,94]. In an animal model, an increase in osteoclastic activity linked to overexpression of AGEs has been reported, while animals lacking RAGEs exhibited an increase in bone mass and a decrease in the number of osteoclasts [94,95].

3.2. DM Increases the Severity of PD

The existing evidence in this regard indicates that patients with DM are at greater risk of developing more severe PD [96–102]. The parameters most commonly used to measure the severity of PD are the probing depth or pocket depth, the bacterial plaque index, the level of clinical anchorage, which constitutes an important indicator of tissue damage, the number of missing teeth, and the rate of bleeding on probing. A systematic review and meta-analysis [38] has indicated that all these severity indicators are significantly more altered in DM compared to controls. Probing depth was significantly deeper in diabetics compared with controls (mean difference [MD] = 0.23 mm, 95% CI = 0.17–0.29, $p < 0.001$) [38]; plaque index was significantly elevated in the diabetic group (MD = 0.20 mm, 95% CI = 0.18–0.23, $p < 0.001$) [38]; clinical attachment level also reflected higher degree of damage to periodontal tissue in diabetics (MD = 0.39 mm; 95% CI = 0.28–0.50, $p < 0.001$) [38]; diabetics with periodontitis had on average less teeth than the non-diabetic group with periodontitis (MD = −2.14 teeth, 95% CI= −2.87 to −1.40, $p < 0.001$) [38]; bleeding on probing was found affecting more teeth in the diabetic group compared with the control group (MD = 7.90 teeth; 95% CI, 4.24–1.56, $p < 0.001$) [38]. In summary, this systematic review shows with the higher quality of evidence to date that severity of periodontitis is greater in patients with diabetes than in non-diabetic populations. This is relevant for clinical practice and confirms that oral cavity assessment should form a routine part in the clinical evaluation of patients with DM [38].

3.3. PD Worsens the Control and Prognosis of DM

Several studies provide evidence on the negative effect that PD has on the prognosis of diabetes both in terms of mortality and the appearance of DM typical complications [103–108]. A study carried out in Pima Indians—an ethnic group that lives in the state of Arizona (USA) and in the states of Sonora and Chihuahua (Mexico) that shows a high prevalence of DM—reported a significant increase in mortality adjusted for sex and age directly related to the control of their PD. Thus, in diabetic patients without PD or with PD with good control, mortality was 3.7 deaths/1000 inhabitants/year, while in diabetic patients with poor control of PD, mortality amounted to 28.4 deaths/1000 inhabitants/year [103]. Likewise, a large study [104] has reported an increase in cardiovascular mortality in diabetic patients with PD and chronic kidney disease.

Diabetic patients with PD also have a higher risk of complications typical of DM [105,106]; it was published after a joint consensus meeting between the International Diabetes Feder-

ation and the European Federation of Periodontology [43], derived from the analysis of 14 studies that included 31,988 patients, so diabetic retinopathy is significantly associated with PD (OR = 1.2–2.8) and the severity of PD is correlated with the severity of retinopathy. Likewise, in patients with DM1 and DM2 with PD there is a higher frequency of kidney complications. Furthermore, a significant association was also reported between DM with PD and the risk of neuropathic foot ulcers development (OR = 6.6); finally, the risk of cardiovascular complications (coronary heart disease, cerebrovascular events and subclinical heart disease) is also significantly increased in diabetic patients with PD.

There is sufficient evidence to support that adequate periodontal treatment generates an improvement in glycemic control in type 2 diabetic patients, evidenced by a reduction in glycated hemoglobin (HbA1c) levels between 0.29% and 0.48% that remains for at least three months after treatment. Although there is insufficient evidence on whether this reduction is maintained after six months of periodontal treatment [14,43,109–111]. This result has also been corroborated by other studies with a moderate quality of evidence [112–118] and by a Cochrane review [117]. The beneficial effect of periodontal treatment in diabetics also seems to translate into a reduction in inflammatory mediators evidenced through a reduction in serum levels of TNF-α and CRP [119,120]. However, it does not seem that the different types of periodontal treatment (surgical, non-surgical, accompanied or not by antibiotics, antiseptics, or with oral hygiene instructions) exert different effects on glycemic control in patients with DM.

Finally, there is reasonable evidence that indicates that PD could increase the risk of developing diabetes, since HbA1c levels have been increased in people with PD without diabetes [107,108]. The joint consensus meeting between the International Diabetes Federation and the European Federation of Periodontology [43] analyzed six representative studies from USA, Japan, and Taiwan populations, (n = 77,716 patients) showing a greater probability of developing prediabetes and diabetes (hazard ratio [HR] = 1.19–1.33) in patients with PDs.

3.4. Dental Implants, Peri-Implantitis, and DM

As mentioned, one of the fundamental consequences of PD in patients with DM is the loss of teeth, which occurs more markedly in elderly patients [69], and thus, one of the more subtle effects of the DM, especially DM2, could be the decrease in quality of life associated with tooth loss and compromised mastication function [121]. Modern dentistry restores lost teeth essentially through dental implants, which has been a real revolution in this field. However, questions have arisen regarding the feasibility and safety of dental implants in the diabetic population. On this issue there is scant and sometimes confusing evidence on how poorly controlled DM affects the prevalence of peri-implant disease, a process equivalent to PD [122,123], which implies bone loss around the implant [124]. In addition, there is also no consistent evidence about whether in patients with DM there is a significantly greater loss of dental implants after their placement [125–127], although apparently there is a delay in osseointegration of the implants related to the poor glycemic control [50,124,128]. A recent systematic review and meta-analysis [129] has indicated that there were statistically significant differences between the groups of DM and non-DM with regard to marginal bone loss ($p < 0.001$), probing depth ($p < 0.001$), and bleeding around dental implants ($p < 0.001$), obtaining the non-DM group the lower complication rates. Finally, in some studies it has been suggested that poorly controlled DM constitutes a relative contraindication for implant therapy [69], although on the contrary, numerous studies support the use of dental implant therapy in diabetic patients even with poor control of the glycaemia [130–133].

4. Oral Candidiasis

The relationship between fungal infections, and in particular infection by species of the genus *Candida* sp., with DM has been widely studied [10,134–136]. It has been clearly established that diabetic patients have an increased susceptibility to fungal infections

compared to non-diabetics [137,138]. These susceptibility requires predisposing factors that decisively alter the balance between the host and the yeasts, allowing the passage of *Candida* sp. from its usual commensal state to pathogen, causing infection.

Among the different types of fungal infections that can occur in diabetic patients, oral candidiasis [6] stands out due to its higher frequency and clinical consequences. Significantly higher rates of colonization of the oral mucosa by *Candida* sp. have been described in patients with DM1 (85%) and DM2 (68%) compared with non-diabetics (27%) [138]. One study revealed that 66% of the yeasts isolated from DM patients were *C. albicans* [139]. However, fungal colonization of the oral mucosa is not equivalent to infection, requiring some pathophysiological conditions and associated factors for the infection to finally occur [9,140–144]. These factors are firmly established in diabetic patients and are as follows: (a) Maintained hyperglycemia with increased levels of HbA1c and high levels of glucose in saliva favors the multiplication of *Candida* sp., the increase in the number of receptors available for *Candida* sp., decreased neutrophil activity and increased adherence of *Candida* sp. to the epithelial cells of the oral mucosa [145–151]; (b) the decrease in salivary pH favors the growth of *Candida* sp., the increase in phospholipase and extracellular acid protease levels and the increase in the levels of yeast adhesion to epithelial cells [152–154]; (c) in DM there is a diminished response of the tissue to the injury favoring the colonization of the oral mucosa by *Candida* sp. even in the absence of clinical manifestations [155,156]; (d) and finally, poor oral hygiene, advanced age, female gender and xerostomia are also factors that can appear in DM and have been shown to be associated with a greater tendency to develop fungal infections in diabetics [157–160].

The common clinical manifestations of oral candidiasis are the presence of extensive reddened areas (erythematous candidiasis) along the oral mucosa, which are generally associated with patchy lingual depapilation and commissural cheilitis. Diabetic patients may present also speudomembranous candidiasis characterized by the presence of whitish lumps, similar to milk or yogurt clots, on an erythematous mucosa. These lumps are easily dislodged when scraped off with gauze leaving an erythematous mucosa. Oral candidiasis is usually symptomatic, causing discomfort, burning, or frank pain. Examination of the oral mucosa usually reveals, together with the events described, an absence of salivation or thick and pasty saliva. Diabetic patients may also develop a type of candidiasis associated with the use of removable dental prostheses called prosthetic stomatitis. It is characterized by the appearance of a reddened area under the prosthesis resin, being the mucosa not covered by the prosthesis respected. This form of candidiasis is usually asymptomatic, although a degree of discomfort may also occasionally occur.

5. Oral Cancer and Oral Potentially Malignant Disorders

Oral cancer is a global oral health problem. The most recent data published by prestigious entities (Global cancer incidence, mortality and prevalence [GLOBOCAN] project, International Agency for Research on Cancer [IARC], World Health Organization [WHO]) indicate the appearance of 354,864 new cases and 177,384 patients death per year [161], and a five-year-mortality rate of 50% directly related to this tumor [11]. A fact of great concern is that mortality from oral cancer has not decreased substantially in recent years, despite the fact that the oral cavity is explored by multiple specialists (otolaryngologists, maxillofacial surgeons, dermatologists, dentists, and family doctors). A systematic review and meta-analysis recently published by our research group indicates that diabetic patients have a significantly higher prevalence and risk of developing oral cancer compared to the general population [12]. Worldwide studies on oral cancer incidence and prevalence [162] indicate a strong geographical predisposition for the development of oral cancer, with India and Southeast Asian countries showing the highest figures. This geographical distribution seems to depend on the high levels of tobacco consumption in these countries as this habit is the most relevant etiological factor for the development of oral cancer. However, in our meta-analysis, the subgroup analysis showed the increased risk of development of oral cancer in the diabetic population not dependent on the geographical area studied. In our

opinion, and based on these results, the predisposition to the development of cancer in the diabetic population depends directly from conditions associated with DM.

The reasons for the increased development of oral cancer in diabetics are not well known, although clinical, biochemical, and molecular reasons have been proposed. Furthermore, oral cancer and DM share some epidemiological facts and etiological factors, among which are obesity, sedentary lifestyle, advanced age, and diet [163]. On the other hand, hyperinsulinemia due to insulin resistance, through the activation of EGF1R, gives rise to the upregulation of some pro-proliferative and antiapoptotic pathways that have also been documented activated in oral cancer in non-diabetics (PI3K-akt-mTor, MAPK [Ras-Raf-MEK-Erk], and Bcl-2) [164]. Upregulation of these pathways conclusively concludes with the upregulation the *CCND1* gene [165,166]. Our research group has recently pointed out that the upregulation of *CCND1* and the overexpression of its product (cyclin D1) play a determining role in the cascade of molecular events that occur in the malignant transformation of the oral epithelium [167,168]. Therefore, it could be hypothesized that the link between DM and the development of oral cancer is hyperinsulinemia and insulin resistance [12]. Furthermore, as previously mentioned in this paper, hyperglycemia by generating oxidative stress with the release of ROS could cause DNA damage [169]. Hyperglycemia could also be accompanied by an increase in glucose consumption by tumor cells, also known as Warburg effect. This is a well-known hallmark of cancer proposed by Hanahan and Weinberg [170], which seems to induce an increase in cell proliferation associated with an activation of GLUT-1 and GLUT-3, and EGF, EGFR, and PKC-α [171–173].

We have also documented in our research line an increase in oral cancer-related mortality 2.09 times higher in the diabetic population compared to the non-diabetic (95%CI = 1.36–3.22, $p = 0.001$). This fact, which has also been observed in other types of cancers (liver, pancreas, ovarian, colon, lung, bladder, and breast carcinomas [174,175]) could be due to the phenotype more aggressive—proliferative and invasive—that develops cancer in diabetics as well as the deterioration of the general health of the diabetic related with complications (kidney disease, ischemic disease, etc. [176]) as well as the limitations for surgical treatment linked to postoperative risks, together with higher postoperative mortality [177].

Our research group has also reported the increased risk of development of oral potentially malignant disorders (OPMD) experienced by diabetic patients compared to the general population [12]. OPMDs are a significant group of mucosal disorders that may precede the diagnosis of oral squamous cell carcinoma (OSCC) [13,178], among which are essentially oral leukoplakia [179,180], oral lichen planus (OLP) [181–183], proliferative verrucous leukoplakia [184–186], erythroplakia, and actinic cheilitis [187,188]. Patients diagnosed with OPMDs may have an increased susceptibility to develop cancer anywhere in their mouth during their lifetime [13]. Our previous meta-analysis has shown that oral leukoplakia occurs with a prevalence of 2.49% in the diabetic population (2490 per 100,000 patients with DM) being the risk of developing oral leukoplakia in a diabetic 4.34 times higher compared to the general population. (95%CI = 1.14–16.55, $p = 0.03$; 10 studies, 7440 patients). A recent study by the WHO collaborative group for the study of cancer and OPMD [179] has reported a risk of oral cancer in oral leukoplakia close to 9%, which indicates the concern of the diagnosis of leukoplakia in a diabetic patient. The risk of developing oral leukoplakia in diabetics does not depend on the geographical area, nor does it depend on tobacco consumption, which indicates that it is probably related to factors exclusively associated with diabetes.

Our results are also strong for OLP, another important and highly prevalent OPMD [189] and associated with considerable malignant transformation rates [181–183]. Patients with DM present a prevalence of OLP of 2.72% (2720 per 100,000) with a chance of developing OLP 1.87 times higher than in the non-diabetic (95%CI = 1.37–2.57, $p < 0.001$; 22 studies, 5830 patients) [12,190]. Nowadays, the premalignant character of OLP and its progression to cancer high rate have been clearly documented [181], so it is reasonable to hypothesize that in a considerable number of patients diabetics, the appearance of oral cancer may come from the malignant evolution of a previous OLP.

6. Other Oral Conditions Associated with DM

6.1. Dental Caries

The analyses on the prevalence of dental caries in the diabetic population present contradictory and conflicting results [14]. It would seem logical to think that diabetic population, as a consequence of a series of associated oral conditions (xerostomia, high levels of dental plaque, etc.) would be more predisposed to the development of dental caries [191]. A recent systematic review and meta-analysis [15] has reported DM1 patients having significantly higher caries prevalence compared to controls. Although no significant differences were found between DM2 and controls and between well-controlled and poorly controlled diabetics. On the contrary, a study [192] with a large sample (300 diabetics vs. 300 controls) reported a higher prevalence of dental caries in non-diabetics, which the authors attribute to the fact that perhaps the diet of patients with DM generally contains less fermentable carbohydrates and more protein [193]. Another analysis did not find differences in the prevalence of crown caries, although significant differences were found for the prevalence of root caries [194].

6.2. Burning Mouth Syndrome (BMS)

It is an atypical chronic pain essentially characterized by the presence of a burning sensation, stinging or frank pain that is located mainly on the tongue, lips and palate, although it can spread to any other location, without that there are recognizable mucosal lesions that may justify this condition. BMS usually appears in women over 30 years of age and is frequently associated with a history of various emotional disorders [195]. It is a common disease, with an estimated prevalence ranging from 0.7% to 4.6% of the general population [16]. BMS is a process with an impact on the patients quality life, although, despite its frequency and relevance, the pathogenesis is unknown to a large extent [196]. In general, there is an absence of epidemiological primary-level studies focused on the association between BMS and DM. Increased prevalence of BMS in patients with DM compared to healthy subjects has been reported [197,198], while others did not find any differences in prevalence of BMS [199]. A significant association between BMS and peripheral neuropathy has been reported in diabetic patients [17]. It could indicate that BMS in diabetic patients constitutes another manifestation of diabetic neuropathy, although this is an unconfirmed theory.

6.3. Salivary Secretion Alterations

Alterations in salivary secretion are generically called by the term "dry mouth", which however refers to two different processes, the first related to an objective reduction of salivary flow due to salivary hypofunction, defined by an unstimulated whole saliva flow rate of <0.1 mL/min, collected for 5 to 15 min, or chewing-stimulated whole saliva flow rate of <0.7 mL/min, collected for 5 min [200]; and secondly, "dry mouth" can also refer to the subjective sensation of lack of saliva in the absence of flow disorders [18]. The prevalence of salivary hypofunction with decreased salivary flow is estimated to range widely from 1% to 65% of the general population [201].

Dry mouth is one of the most common complaints in diabetic patients. Numerous cross-sectional studies have reported decreased salivary flow from both DM1 [19,202–207] as in DM2 [19,205,208–218]. The pathophysiology of the lack of salivary flow in DM is partly unknown. It has been hypothesized that the parotid innervation involvement in the context of diabetic neuropathy could somehow be involved in the decrease of salivary flow in these patients, although the studies present contradictory results [19,202,219–221]. It should also be noted that the tricyclic antidepressant, frequently used in the treatment of this disorder associated with diabetic neuropathy, produce dry mouth [222]. Some studies have reported alterations in the structure of the salivary glands in patients with DM, including vacuolization or acinar atrophy [223,224]. Likewise, in patients with DM it is common to find asymptomatic parotid enlargement that has been interpreted as a compensatory mechanism for salivary hypofunction [225].

Hyperglycemia seems to be another of the mechanisms responsible for the lack of saliva in diabetics. Significant decreases in salivary flow have been shown in poorly controlled DM compared to those with good glycemic control [19,202,211,212]. In this sense, the overexpression of AGE and RAGE, secondary to hyperglycemia, has been increased in the lacrimal gland tissue in diabetic animal models and associated with dry eyes [226]. Although something equivalent has not been investigated in lacrimal glands, at least theoretically this mechanism could also be operating to salivary hypofunction. RAGE overexpression has been observed in the submaxillary gland of diabetic rats [227]. It is also known that polyuria and osmotic diuresis secondary to hyperglycemia frequently appear in DM, which can trigger dehydration and compensatory hyposalivation [228,229].

6.4. Taste Perception Alterations

Taste perception alterations, mainly hypogeusia, have been reported both in patients with DM1 and DM2, in a significantly higher proportion than in controls [192]. These alterations have also been related to the development of obesity [21] secondary to hyperphagia [20]. Although the alterations in taste perception at the moment are of unknown cause, it has been hypothesized that the disorders of diabetic neuropathy and salivary hypofunction could be in the background of these alterations [192].

6.5. Halitosis

Patients with diabetes are predisposed to halitosis [22], having been reported that approximately 25% of patients with diabetes mellitus suffer from halitosis [230]. The pathogenesis of this disorder is probably related to the frequent presentation of gingivitis, periodontitis, dental caries and xerostomia, which prevents adequate self-cleaning of the oral mucosa. In addition, some of the bacteria that are frequently isolated in the infections of diabetic patients are anaerobes that contribute to the production of volatile products that increase halitosis [23]. In this sense, under the background of periodontitis, bacterial putrefaction and the generation of volatile sulfur compounds could lead to sulfide compound odor [22,231]. On the other hand, under the background of xerostomia, Koshimune et al. [232] found higher concentrations of methyl mercaptan and hydrogen sulfide in patients with salivary secretion alterations. Another study found an association between halitosis and increased HbA1c levels among type 2 diabetic subjects [233]. It was hypothesized that this relationship could be related to the phenomenon of ketoacidosis associated with poorly controlled diabetes [233]. Further studies are needed to explain the nature of this association.

6.6. Delayed Wound Healing

A tendency towards delayed wound healing has been described, especially in patients with poor control of their diabetes in whom long-term complications occur [24]. Probably these long-term complications affect the small terminal vessels, damaging them [25], which produces an insufficient supply of cellular nutrients through the blood circulation, decreasing the inflammatory and antibacterial response [26]. Elevated HbA1c levels $\geq$ 6.5% significantly increase the risk of developing infections after dental interventions and complications of surgical wound healing. For this reason, it is advisable to obtain better control of glycosylated hemoglobin figures [234]. However, those pathological processes in which it is suspected that their presence is contributing to poor diabetes control, and in which surgical treatment is required, should not be delayed in order to achieve a better metabolic control of the disease [234]. In these cases, post-surgical wound care should be maximized and clinical considerations should be made on the convenience of using antibiotics in each specific case [234]. Regarding the type of antibiotic to be used in diabetic patients, the basic rules of antibiotherapy should be respected, i.e., cultures should be performed in these patients in order to select the most effective antibiotic [235]. If necessary, the administration of a broad-spectrum antibiotic should be initiated pending

the results of the sensitivity study, and this should be maintained if the study demonstrates its efficacy [235].

Finally, DM is frequently related to other pathological processes, such as hypertension, that require drugs that could also cause decreased salivary flow [19].

7. Need for an Interdisciplinary Team in the Care of Diabetic Patients in Relation to Their Oral Health. Information to the Diabetic Patients about Their Oral Health

From the foregoing it is deduced the importance of oral health in diabetic patients and the reciprocal relationships that exist between good metabolic control of DM and oral health. From this derives the need to establish interdisciplinary teams in the management of diabetic patients, among which dentists should necessarily be. The information available in this regard indicates, however, that at least half of primary care physicians and diabetes specialists do not have adequate knowledge about the importance of oral health in general and about PD in particular in diabetic patients. Furthermore, those clinicians who claim to have knowledge on the subject do not transfer it to their clinical practice and only a third of the professionals refer their patients for a dental consultation [236]. In fact, some studies conclude that active collaboration between dentists, primary care physicians and diabetes specialists does not exist, and the referral of patients to share their care according to competencies is absent. In this way, diabetic patients in many cases are receiving neither the information nor the adequate treatment in relation to their oral health problems [237].

7.1. Attitude of Primary Care Physicians and Specialists Involved in the Management of Diabetic Patients in Relation to Their Oral Health Care

- Clinicians should discuss with diabetic patients the importance of oral health in their disease in relation to the influence it exerts on the metabolic control of the disease and on the reduction of the risk of developing some of the potential complications of DM. Likewise, diabetic patient should be advised to periodically go to the dental clinic for review their oral status [69];
- Clinicians should screen for the main oral conditions that occur in diabetes. This screening should include the evaluation of the periodontal status through simple questions about the existence of spontaneous gingival bleeding or during mastication and brushing, the appearance of mobility or displacement of teeth, the loss of teeth, the presence of halitosis, and the existence of suppuration or periodontal abscesses. Likewise, the presence of erythematous or pseudomembranous candidiasis should be evaluated both through the presence of its symptoms (itching or oral pain) and its signs (oral mucosa affected by extensive red areas and imprecise limits or white areas in the form of lumps that come off easily when scraped with gauze);
- Clinicians should screen the main OPMDs that appear in diabetic patients with a higher prevalence than in the general population (essentially oral leukoplakia and OLP), as well as oral cancer (delimited red areas, ulcers or overgrowths of the oral mucosa older than 15 days) [13,178];
- Clinicians should perform a scrutiny of salivary flow alterations, essentially questioning the patient about the presence of dry mouth symptoms and examining the oral mucosa (obvious absence of saliva or thick saliva, with a parchment-like appearance of the oral mucosa);
- Clinicians should refer diabetic patients to the dental office in the event of any oral health problem detected during control and follow-up visits;
- Clinicians should seek basic training in oral health that allows them to detect the presence of oral disorders that appear in diabetes.

7.2. Attitude of Dentists in the Management of Diabetic Patients in Relation to Their Oral Health Care

- Dentists should discuss with their patients the mutual influences between oral health and diabetes by seeking information on how diabetes can affect oral health [238–243];

- Dentists should promote lifestyle changes on the habits of diabetic patients in order to exert a favorable impact on their oral and general health;
- Dentists must promote attitudes aimed at obtaining the maximum efficiency of oral care in diabetics [43,244]:
  - The medical history should be meticulous and detailed;
  - Communication with primary care physicians and other specialists involved in the care of diabetics should be fluid;
  - The intraoral examination should be meticulous looking for the frequent oral alterations in diabetics, with special reference to the signs and symptoms of PD, oral candidiasis, dry mouth and the presence of OPMD and oral cancer.
- The dental treatment of diabetics should focus on the control of acute infection, offering a therapy plan that is as less complex as possible. Likewise, emergencies in the dental clinic (hyperglycemia, hypoglycemia) must be recognized early and adequately managed. Considerations should be given to which are the most appropriate times to perform dental treatments and what should be the optimal duration of appointments, planning the treatment according to difficulties. Deep anesthesia and good pain and stress control should be provided during treatment;
- Dentists should advise and promote the replacement of missing teeth, the restoration of decayed teeth, and the implementation of preventive oral health habits;
- The dentist must be aware of the existence of the growing number of diabetics in the world [245], many of whom are undiagnosed [246,247]. Dental clinics could act as linkers involved in diabetes screening. In this sense, the suspicion of diabetes in a dental patient should prompt the dentist to request a check of glucose levels in venous blood and in case of alteration, the referral of the patient to his primary care physician for study and treatment if necessary [248];
- Dentists should seek basic training on DM and its complications.

7.3. Information Diabetic Patients Should Receive about Their Oral Health

- Diabetic patients should be given information about their oral health and its relationship to diabetes;
- Diabetic patients should receive information from dentists on the higher prevalence of PD in DM and on the negative consequences this has for the metabolic control of diabetes and on the presentation of complications of diabetes;
- Diabetic patients should receive information from dentists on habits and lifestyle that prevent the development of oral complications of diabetes;
- Diabetic patients should know that they are at risk of developing oral candidiasis;
- Diabetic patients should know that they are at risk of developing oral cancer and OPMD, through accurate, evidence-based information;
- Diabetic patients should know that they could develop alterations in salivary flow with dryness related to their disease;
- Diabetic patients should know the importance of making regular visits to the dental clinic;
- Diabetic patients must make commitments to their oral care.

7.4. Practical Measures and Recommendations to Follow in a Routine Dental Care Session

- Prior to dental treatment, a comprehensive medical history should be performed, singularly recording the type of diabetes, complications, treatment, and control status [249];
- International consensus guidelines state HbA1c levels <6.5% as the main parameter to measure and confirm an appropriate metabolic control [250];
- Pre-prandial blood glucose levels ranging between 70 and 130 mg/dL and post-prandial blood glucose levels < 180 mg/dL also should be confirmed to ensure an adequate metabolic control [250];
- Although well-controlled DM patients could be treated similarly to non-diabetics, short appointments in the morning are preferably to reduce stress of patients [248,251];

- At the beginning of each appointment, the dentist should make sure that the diabetic patient has eaten (fasting must be imperatively avoided) and taken their medications as usual, to avoid a hypoglycemic episode [248,251].

8. Conclusions

Diabetic patients present a notable predisposition to the development of oral pathologies, among which PD stands out, which reaches a prevalence of 67.8%. DM patients have a special predisposition to the development of fungal infections, especially of the *Candida* sp. genus, with significantly higher rates of oral mucosa colonization by *Candida* sp. both in patients with DM1 (85%) and DM2 (68%) compared to non-diabetics (27%). A higher prevalence of oral cancer and OPMD in diabetics has been reported, including oral leukoplakia, with a prevalence of 2.49% in patients, and oral lichen planus with a prevalence of 2.72%. Dental caries, burning mouth syndrome, alterations in saliva secretion, altered taste perception, halitosis, and delayed wound healing are also conditions associated with DM. All these disorders generate important complications that notably worsen the already deteriorated health status of diabetic patients. The frequent involvement of the oral cavity in these patients requires an interdisciplinary approach to its management and adequate guidelines for informing patients about these aspects. It is also essential to increase the training of diabetes care providers as well as patients in relation to their oral health.

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Good Metabolic Control in Children with Type 1 Diabetes Mellitus: Does Glycated Hemoglobin Correlate with Interstitial Glucose Monitoring Using FreeStyle Libre?

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Abstract: Background: Good metabolic control of Type 1 diabetes (T1D) leads to a reduction in complications. The only validated parameter for establishing the degree of control is glycated hemoglobin (HbA1c). We examined the relationship between HbA1c and a continuous glucose monitoring (CGM) system. Materials and methods: A cohort prospective study with 191 pediatric patients with T1D was conducted. Time in range (TIR), time below range (TBR), coefficient of variation (CV), number of capillary blood glucose tests, and HbA1c before sensor insertion and at one year of use were collected. Results: Patients were classified into five groups according to HbA1c at one year of using CGM. They performed fewer capillary blood glucose test at one year using CGM ($-6 +/- 2$, $p < 0.0001$). We found statistically significant differences in TIR between categories. Although groups with HbA1c < 6.5% and HbA1c 6.5–7% had the highest TIR (62.214 and 50.462%), their values were highly below optimal control according to CGM consensus. Groups with TBR < 5% were those with HbA1c between 6.5% and 8%. Conclusions: In our study, groups classified as well-controlled by guidelines were not consistent with good control according to the CGM consensus criteria. HbA1c should not be considered as the only parameter for metabolic control. CGM parameters allow individualized targets.

Keywords: type 1 diabetes mellitus; pediatric diabetes; continuous glucose monitoring; time in 28 range; HbA1c; capillary blood glucose test

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1. Introduction

Type 1 diabetes mellitus (T1D) is one of the most common chronic diseases in children. Due to its onset early in life and the lack of a definitive treatment, those affected live with the disease for a long time and, therefore, have a high burden of morbidity and mortality, since complications can arise both in the short and long term [1].

It has been shown that good metabolic control of the disease leads to a reduction in these complications [2,3]. The only currently validated parameter for establishing the degree of control of the disease is glycated hemoglobin (HbA1c), which, although providing very useful information, has a number of important limitations. It is an analytical parameter that reflects the average blood glucose values in the preceding two to three months. The main limitations are that it does not consider acute hypoglycemic and hyperglycemic events or the frequency and magnitude of intraday and interday blood glucose variability. Similarly, HbA1c values may be affected in situations such as anemia, hemoglobinopathies, or transfusions, among others [4,5].

In the 1980s, the Diabetes Control and Complications Trial (DCCT) demonstrated that those patients with T1D who were able to maintain HbA1c levels closer to those without diabetes had a lower incidence of microvascular and cardiovascular complications, both avoiding or delaying their onset (primary prevention) and slowing their progression (secondary prevention). In addition, it was found that initial metabolic control had a long-term influence on the subsequent clinical course, which was termed "metabolic memory". These data have been validated 30 years after the initial study with the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study at 30 years (DCCT-EDIC) [6].

Targets for good disease control based on HbA1c levels vary depending on the consensus used [1,7]. All these guidelines agree that targets should be individualized for each patient (Table 1).

Table 1. Reference values for blood glucose and HbA1c according to different societies.

	NICE	ISPAD	ADA
Preprandial blood glucose (mg/dL)	70–126	70–130	90–130
Postprandial blood glucose (mg/dL)	90–162	90–180	
Bedtime glucose (mg/dL)	70–126	80–140	90–150
HbA1C (%)	≤6.5	<7	<7.5

NICE: The National Institute for Health and Care Excellence. ISPAD: International Society for Pediatric and Adolescent Diabetes. ADA: American Diabetes Association. HbA1c: glycated hemoglobin.

Since the advent of continuous glucose monitoring (CGM) systems, we have gained more information on the variability of blood glucose and on acute hypoglycemic and hyperglycemic events, all while decreasing the number of capillary blood glucose tests required.

From the data obtained from the CGM system downloads, efforts were made to identify a set of data that would serve as criteria for good or poor control of the disease. Accordingly, the ATTD (Advanced Technologies and Treatments for Diabetes) congress, reached a new consensus in which 10 parameters were defined [4,8] (Table 2).

Table 2. Standardized CGM parameters in the ATTD 2019 consensus and target values for T1D.

Variable	Target
1. Number of days of CGM	14 days
2. Percentage of time CGM was active	>70%
3. Mean glucose/standard deviation	<154 mg/dL/<29%
4. Glucose management indicator/estimated HbA1C	<7%
5. Glucose variability (coefficient of variation) (%)	<36%
6. Time in range from 70 to 180 mg/dL (% of time)	>70%/>16 h 48 min
7. Time above range >180 mg/dL (% of time) Hyperglycemia level 1	<25%
8. Duration hyperglycemia level 1	<6 h
9. Time above range >250 mg/dL (% of time) Hyperglycemia level 2	<5%
10. Duration hyperglycemia level 2	<1h 12 min
11. Time below range <70 mg/dL (% of time) Hypoglycemia level 1	<4%
12. Duration hypoglycemia level 1	<1 h
13. Time below range <54 mg/dL (% of time) Hypoglycemia level 2	<1%
14. Duration hypoglycemia level 2	<15 min

CGM: continuous glucose monitoring. ATTD: Advanced Technologies and Treatments for Diabetes. HbA1c: glycated hemoglobin. T1D: type 1 diabetes.

The FreeStyle Libre flash glucose monitoring system 1 (Abbott Diabetes Care, Witney, UK) is an established technology that measures interstitial fluid glucose levels. A sensor

worn on the back of the upper arm takes a reading every minute that can be scanned using a hand-held reader or smartphone to receive a current glucose result along with historic results with a 15-min frequency. The FreeStyle Libre sensors are calibrated in the factory and have a wear time of up to 14 days without the need for the user to perform daily calibration using finger-prick tests [9].

The IMPACT and REPLACE studies [10,11] conducted with the FreeStyle Libre 1 sensor in adults with T1D or type 2 diabetes (T2D) on insulin therapy showed no decrease in HbA1c compared to SMBG. The SELFY study [12], performed in children and adolescents with T1D, reported a decrease in HbA1c from 7.9% to 7.4% after 8 weeks of sensor use compared to SMBG ($p < 0.001$). A meta-analysis [13] performed in 2019 analyzing results from 271 studies found a 0.55% (95% CI −0.70, −0.39) decrease in HbA1c 2–4 months after initiation of FreeStyle Libre 1 use in patients with T1D and T2D. In the 447 children and adolescents included, the mean decrease in HbA1c was 0.54% (95% CI −0.84, −0.23), and this improvement was maintained at 12 months. It was concluded that initiation of sensor use as part of diabetes management resulted in a decrease in HbA1c in adults and children with T1D and in adults with T2D.

The objective of our study is the evaluation of pediatric patients with T1D after one year of use of the Free Style Libre system, categorizing them by their HbA1c, in order to be able to know the differences in the number of capillary blood glucose controls before and after implantation, as well as CGM parameters that presents each category of HbA1c.

2. Material and Methods

2.1. Study Design and Participants

The funding of this study was in accordance with the regulations of the Official Gazette of the Andalusian Government (BOJA), resolution of 17 April 2018, regarding the organization of the Andalusian Health Service (SAS) to include CGM systems among the benefits provided by the Andalusian Public Health Service and a research project funded by the Andalusian Ministry of Health and Family (PIGE 0533-219).

This study was carried out from June of 2018 to September of 2019, following approval by the Ethics Committee of the Regional Hospital of Malaga. This prospective study was undertaken following insertion of the FreeStyle Libre 1 sensor in 191 pediatric patients with T1D. The inclusion criteria were presence of T1D with disease duration of more than one year, age between 4 and 18 years at the start of the study, and no previous experience using the FreeStyle Libre 1. Furthermore, only those patients who had more than 80% use of the sensor were included.

The exclusion criteria were having previously used interstitial blood glucose monitoring or having anemia or hemoglobinopathy that could constitute a bias.

Finally, we subdivided all patients in different groups according to cut-off points of HbA1c:

Group 1 HbA1c $\leq$ 6.5%
Group 2 HbA1c 6.5–7% (more than 6.5 and less than or equal to 7)
Group 3 HbA1c 7–7.5% (more than 7 and less than or equal to 7.5)
Group 4 HbA1c 7.5–8% (more than 7 and less than or equal to 8)
Group 5 HbA1c $\geq$ 8%

2.2. Variables

The data were extracted using the LibreView® platform (Abbott Diabetes Care, Witney, UK) one year after insertion of the FreeStyle Libre 1 sensor (Abbott Diabetes Care, Witney, UK), taking into account the last 14 days of use prior to the office visit. The parameters collected were those accepted in the consensus guidelines on the interpretation of CGM [4,5,7]: time in range (TIR), percentage of time below range (TBR), and coefficient of variation (CV), as well as average number of scans per day. In addition to these variables, we also analyzed sex, age, and the number of capillary blood glucose tests performed before sensor insertion and one year later. HbA1c values were also obtained before sensor insertion

and at one year of use. The determination was made through a capillary blood sample using the DCA Vantage analyzer system (immunoassay technique) in the laboratory of the Regional Hospital of Malaga.

Capillary blood glucose measurements were collected after downloading the last 14 days of the glucometer in use prior to sensor insertion. Collection of capillary blood glucose readings at one year was performed by downloading the glucometer in use. For all patients, this could be done through the LibreView® platform, as they were using the reader with a glucometer function with FreeStyle Optium® capillary blood glucose strips.

Clinical data were collected through a written questionnaire completed by the primary caregiver and supervised by the healthcare team.

3. Statistical Analysis

All analyses were performed with R (R Core Team, 2020, University of Auckland, CAL, USA). Normality and homoscedasticity were tested using the Anderson–Darling and Fligner–Killeen tests, respectively. For quantitative variables, the statistics (mean and standard deviation) were reported, and for categorical variables, the absolute and relative frequencies were reported. To study the relationship between each of the quantitative variables of two groups or samples, the *p*-value associated with the Student's *t*-test or nonparametric test such as Wilcoxon test, when normality was not proven were conducted. To study the relationship between each of the quantitative variables of several groups or samples, the p-value associated with the Kruskall–Wallis test was used. In the case of two categorical variables, Fisher's test or the chi-square test was used.

4. Results

4.1. Difference in the Number of Capillary Blood Glucose Tests Performed per Day

The patients were classified into five groups according to their HbA1c at one year of using the FreeStyle Libre 1 sensor. The differences in the effect of the sensor were explored between the different groups and within the same group, before using the sensor and at one year of use (Table 3).

In most of the groups, the patients performed fewer capillary blood glucose tests one year after insertion of the sensor (mean: −6.0, standard deviation: 2.0). For statistical analysis, the Wilcoxon signed-rank test for paired samples was used. Statistically significant differences were found between the number of capillary blood glucose tests before and after the use of the sensor within each group (V = 23, *p*-value < 0.0001).

To assess the difference in the number of capillary blood glucose tests between the different groups, we used the Kruskal–Wallis nonparametric test. There were no significant differences between the groups (Kruskal–Wallis, chi-squared = 4.5977, standard deviation = 4.0, *p*-value 0.3311) (Figure 1).

4.2. Monitoring Parameters

TIR is defined as the time during which blood glucose levels are between two points, usually in the range 70–180 mg/dL or 70–140 mg/dL. Statistically significant differences were found in both TIRs 70–180 mg/dL and 70–140 mg/dL between the different groups (Table 3).

According to the aim of TIR $\geq$ 70%, 33.33% of group 1 achieved time in range, while 12% of group 2 and 9.37% of group 3 achieved time in range.

TBR is defined as the percentage of time in which blood glucose is $\leq$70 mg/dL. We found no statistically significant differences between the different HbA1c categories (Table 3).

CV is a measure of glucose variability derived from the standard deviation and the interquartile range. No statistical significance was observed between the different HbA1c categories (*p*-value 0.054) (Table 3).

Table 3. Patients categorized by level of glycated hemoglobin one year after sensor use with results of different variables.

		HbA1c ≤ 6.5% (*n* = 58)	HbA1c 6.5–7% (*n* = 49)	HbA1c 7–7.5% (*n* = 38)	HbA1C 7.5–8% (*n* = 28)	HbA1c ≥ 8% (*n* = 18)	*p*-Value (Kruskal–Wallis)
Sex	Boys	28 (48.3%)	28 (57.1%)	18 (47.4%)	15 (53.6%)	10 (55.6%)	NS
	Girls	30 (51.7%)	21 (42.9%)	20 (42.9%)	13 (46.4%)	8 (44.4%)	
Age (years) Mean (SD)		10.8 (3.3)	11.7 (3)	10.8 (3.4)	10.6 (2.5)	12.2 (2.3)	NS
No. capillary blood glucose/day baseline Mean (SD)		7.0 (1.4)	7.2 (1.4)	6.7 (1.1)	7.3 (1.6)	6.4 (1.1)	NS
Miss		9	5	9	1	2	
No. capillary blood glucose/day after a year Mean (SD)		1.3 (1.9)	0.9 (1.5)	0.7 (1.2)	1 (1.6)	1.4 (2.2)	NS
Miss		5	2	0	1	0	
% TIR (Glucose 70–180 ng/mL) Mean (SD)		62.214 (11.584)	50.462 (10.856)	47.625 (13.995)	39.385 (6.104)	32.636 (7.953)	<0.001
Miss		2	0	0	0	0	
% TIR (Glucose 70–140 ng/mL) Mean (SD)		40.923 (12.114)	30.885 (9.253)	26.781 (9.797)	22.538 (3.843)	17.636 (5.143)	<0.001
Miss		3	0	0	0	0	
% TBR Mean (SD)		5.397 (5.474)	4.271 (4.321)	3.789 (3.699)	3.667 (4.010)	5.167 (4.890)	NS
Miss		0	1	0	1	0	
CV Mean (SD)		38.562 (10.315)	38.983 (8.899)	40.170 (7.359)	37.544(6.224)	44.078 (8.917)	0.054
Miss		0	1	0	1	0	
Scanning frequency (SD)		9.857 (3.035)	9.120 (3.113)	10.875 (7.129)	11.538 (4.612)	7.545 (3.830)	0.238
Miss		2	1	0	0	0	

TIR: time in range measured in percentage. TBR: percentage of time below 70 mg/dL. CV: coefficient of variation measured in percentage. SD: standard deviation. NS: Not significant. HbA1c: glycated hemoglobin.

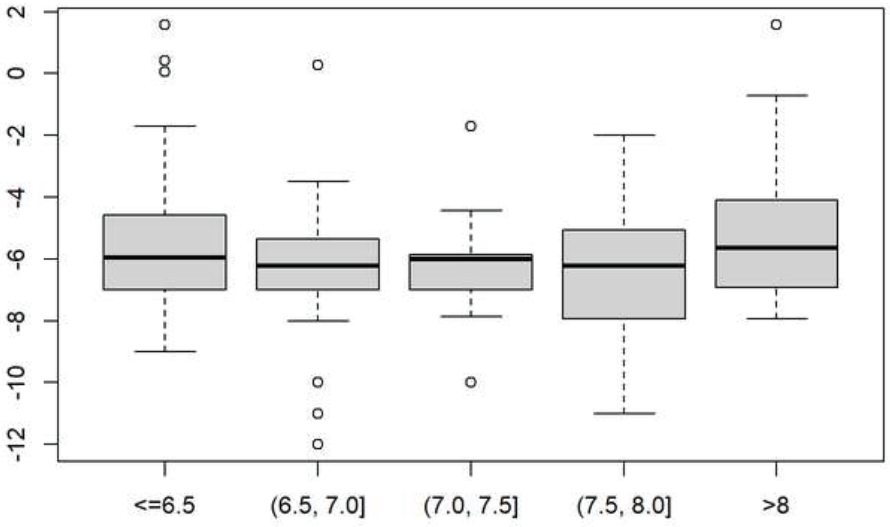

Figure 1. Difference in the lowest number of capillary blood glucose readings per day between the different capillary glycated hemoglobin groups one year after sensor insertion.

Table 3 reports TBR, which allowed us to observe that the lower TIR was not due to the higher TBR, since in most categories it was close to the recommended one (less than 5%); thus, it is the highest time above range which would explain the shortest time in range that we observed in the data.

5. Discussion

According to the data extracted from the latest ISPAD consensus, good metabolic control based solely on HbA1c is considered to have a value less than 7% [1,7]. However, although group 1 (HbA1c $\leq$ 6.5%) had the highest TIR between 70 and 180 mg/dL, the mean of this percentage was 62.214%, below the value accepted by the CGM Consensus [4,8] for optimal control, which is set at TIR $\geq$ 70%. Group 2 (HbA1c 6.5–7%) (which would also be within the good control group per the ISPAD) had a mean TIR of 50.462%. Thus, we can conclude that the HbA1c accepted as optimal for good metabolic control ($\leq$7%) does not correspond to an adequate TIR value ($\geq$70%). The data obtained from our study indicate that the groups classified as well-controlled by the NICE (The National Institute for Health and Care Excellence, UK), ISPAD (International Society for Pediatric and Adolescent Diabetes, Berlin, Germany), and ADA (American Diabetes Association, Virginia, USA) guidelines ($\leq$6.5%, $\leq$7%, and $\leq$7.5%, respectively) [1,7] are not consistent with good control according to the CGM consensus criteria. With these results, data are presented that support a new paradigm for metabolic control of T1D, in which the validity of HbA1c is not considered as the only parameter for metabolic control of the disease. The incorporation of CGM parameters allows for clear and individualized metabolic control targets in terms of hyperglycemia, hypoglycemia, and variability, underscoring the need for scalability of therapies that allow these targets to be achieved. Longer studies to correlate the recommended monitoring parameters with long-term macrovascular and microvascular complications are needed.

Several studies to date have attempted to correlate HbA1c with TIR in T1D patients. In 2019, two meta-analyses [14,15] were performed that sought evidence of this association. The first of these [14] studied 545 adult patients with T1D and compared HbA1c with different CGM parameters. It was concluded that CGM measures relevant to hyperglycemia (including TIR and mean glucose) were highly correlated with each other but moderately correlated with HbA1c, meaning that a particular TIR or change in a patient's TIR could be associated with a wide range of HbA1c values. The second [15] included a total of 1137 adult patients with T1D in whom data correlating percentage of TIR and HbA1c were analyzed. It was concluded that there was a strong relationship between the two, with every 10% change in TIR resulting in a 0.8% change in HbA1c.

The percentage of TBR considered optimal is $\leq$5%. We found that the groups meeting this parameter were those with HbA1c 6.5–7%, 7–7.5%, and 7.5–8%, while the groups with HbA1c $\leq$ 6.5% and over 8% had higher values than those recommended.

We also noted a sharp decrease in the number of daily capillary glucose tests in all groups with the use of the FreeStyle Libre system 1, which is associated with greater convenience for the patient. Pediatric patients with T1D had a mean of 7–8 capillary blood glucose tests per day for metabolic control, with physical deterioration (skin of the hands) and the social stigmatization associated with the continuous handling of blood.

6. Conclusions

Harmonization of the recommendations for glycated hemoglobin and for time-in-range is lacking, as patients with glycated hemoglobin considered to be adequately controlled have lower time-in-range averages than those recommended for the pediatric population. Long-term studies correlating monitoring parameters with long-term complications are needed to identify monitoring targets that reduce macrovascular and microvascular complications.

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Institutional Review Board Statement: The study was conducted according to the guidelines of Declaration of Helsinki, and approved by Ethics Committee of the Regional Hospital of Malaga.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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Review

Heart Failure in Type 1 Diabetes: A Complication of Concern? A Narrative Review

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Abstract: Heart failure (HF) has been a hot topic in diabetology in the last few years, mainly due to the central role of sodium-glucose cotransporter 2 inhibitors (iSGLT2) in the prevention and treatment of cardiovascular disease and heart failure. It is well known that HF is a common complication in diabetes. However, most of the knowledge about it and the evidence of cardiovascular safety trials with antidiabetic drugs refer to type 2 diabetes (T2D). The epidemiology, etiology, and pathophysiology of HF in type 1 diabetes (T1D) is still not well studied, though there are emerging data about it since life expectancy for T1D has increased in the last decades and there are more elderly patients with T1D. The association of T1D and HF confers a worse prognosis than in T2D, thus it is important to investigate the characteristics, risk factors, and pathophysiology of this disease in order to effectively design prevention strategies and therapeutic tools.

Keywords: type 1 diabetes; heart failure; cardiovascular disease; diabetic myocardiopathy

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1. Introduction

Heart failure (HF) is one of the most frequent causes of hospital admission and has a poor prognosis in most cases, despite great pharmacological advances developed in recent decades for heart failure with reduced ejection fraction (HFrEF; left ventricular ejection fraction < 40%) [1]. In addition, there is a group of patients with an ejection fraction > 50% (heart failure with preserved ejection fraction or HFpEF), who also have a poor prognosis, but for whom there are still no proven effective therapies [2]. In the field of diabetes, thanks to the role of several pharmacological groups in the prevention of cardiovascular events, among which is hospitalization for HF, this condition has acquired a central role as one of the most frequent complications of type 2 diabetes (T2D) [3]. However, it is also gaining more interest in patients with type 1 diabetes (T1D), especially due to the longer life expectancy that makes it easier to find older patients with long-standing T1D. In fact, epidemiological evidence shows us that diabetes increases the risk of HF twice in men and up to five times in women [4]. Among patients with diabetes, an estimated 40% have HF, with higher mortality and risk of hospitalization than patients without diabetes [5].

Although due to the development of sodium-glucose cotransporter 2 inhibitors (iSGLT2) and glucagon-like peptide 1 analogues (aGLP1), the evidence on heart failure in T2D is growing and extensive, in T1D there is a paucity of data. In this review, we will focus on the evidence about HF in T1D with a special interest in the epidemiology, risk factors, and pathophysiology of this complication.

2. Epidemiology and Risk Factors

2.1. Epidemiology

Until a few years ago, most epidemiological and long-term follow-up studies on diabetes were focused on classic cardiovascular complications with an atherosclerotic profile. However, thanks to the data from the cardiovascular safety studies of new antidiabetic drugs, more and more studies with epidemiological data are being published also on T1D, though its incidence and prevalence are not well established. In a 10-year retrospective study performed by McAllister et al. of over 3.25 million people without DM and with T2D and T1D, there were 1313 events of HF among patients with T1D. The crude incidence rate of hospitalization for HF in the T1D group was 5.6 per 1000 person-years compared to 2.4 in patients without diabetes and 12.4 in patients with T2D. However, the case-fatality rate was higher in patients with T1D than people without diabetes mellitus; the difference was larger for men (OR, 1.91; 95% CI, 1.68–2.18) than for women (OR, 1.31; 95% CI, 1.05–1.65) [6].

In a recent observational study, Kristófi et al. analyzed a population of 59,331 patients with T1D and 484,241 patients with T2D in Sweden and Norway, looking for the prevalence and event rates of myocardial infarction, HF, stroke, chronic kidney disease, all-cause death, and cardiovascular death. They observed that patients with T1D had a higher risk of HF and renal disease in different age groups than patients with T2D. The age-adjusted risk for patients 65–79 years showed that the risk of heart failure was 1.3 to 1.4 times higher in patients with T1D than with T2D. They also found greater cardiovascular mortality in T1D in patients above 55 years [7]. Similarly, in a recent meta-analysis carried out by Cai et al. that included 10 observational studies with 166,027 patients, a relative risk of heart failure of 4.29 (95% CI 3.42–4.86) was observed in patients with T1D compared with healthy controls. This meta-analysis suggests that T1D is associated with an increased risk of several cardiovascular diseases, among them HF [8].

In another recent paper, Chadalavada et al. investigated the effect of diabetes in mortality and incident HF with the entire population of the UK Biobank. They included a total population of 493,167 participants, of which 22,685 had diabetes (4.6%). They found a hazard ratio (HR) for HF of 1.9 (CI 95% 1.7–2) among patients with diabetes compared to healthy controls. Interestingly, they found that women with T1D had an 88% increased risk of HF compared to men (HR 4.7 (CI 95% 3.6-6.2) vs 2.5 (CI 95% 2.0–3.0), respectively) and this association was independent of confounding factors. In T2D, the risk of HF was also greater in women but to a lesser extent [9]. On the incidence of HF in T1D, Avogaro et al. performed a systematic review and meta-analysis, including 6 studies published between 1990 and 2018. In their age-adjusted model, the incidence rate of HF in patients with T1D was 3.18 ($p < 0.001$) compared to the general population [10]. Finally, in a nationwide retrospective study performed in Korea, Lee et al. explored HR for cardiovascular disease and early death in people with T1D compared with people with T2D and healthy controls. During more than 93,300,000 person-years of follow-up, they found an HR of hospitalization for HF of 2.105 (CI 95% 1.901–2.330) in T1D compared to T2D and an HR of 3.024 (CI 95% 2.730–3.350) compared to the non-diabetes group. This greater risk for HF in T1D remained after adjustment for fasting plasma glucose and some cardiovascular risk factors, such as smoking, dyslipidemia, hypertension, physical activity, or body mass index, among others [11].

2.2. Risk Factors

Cardiovascular risk factors are well established and data from the Diabetes Control and Complications Trial (DCCT) and its observational follow-up Epidemiology of Diabetes Interventions and Complications (EDIC) demonstrated that an early period of 6–7 years of intensive glycemic control significantly reduced the risk for cardiovascular disease. Although the effect tends to decrease over the years, it remains highly significant 30 years later (a reduction of 30% compared to conventional treatment; $p = 0.016$) [12,13]. However, the presence of diastolic dysfunction has been demonstrated even in adolescent and young adult patients with T1D, as potential early markers of heart failure [14,15]. Moreover,

some studies suggest that multiple risk factor control reduces the risk for myocardial infarction or stroke but has little association with the risk for HF in T1D [16]. Therefore, the study and identification of risk factors for HF in people with T1D are key points to improve the detection, management, and prognosis of this complication.

Regarding glycemic control, the data from DCCT/EDIC studies showed that glycemic control represented as HbA1c was the strongest modifiable risk factor for congestive heart failure after 29 years of follow-up in 1441 patients with T1D. Each 1% increase in HbA1c produced an incidence ratio of 3.15 ($p < 0.01$) for congestive heart failure [12]. Therefore, early intensive therapy seems to have an effect in reducing HF risk in the long-term (five-fold difference among intensive therapy group and conventional treatment), though in the analysis at 30 years, the number of events related to HF were too small to establish a definitive conclusion [13]. In line with these results, Rawshani et al. assessed the relative prognostic importance of 17 risk factors on cardiovascular outcomes in a nationwide register of patients with diabetes in Sweden. For HF, they found that the most important predictors were albuminuria (β-coefficient 3.63 (3.05–4.31)), HbA1c (β-coefficient 1.025 (1.020–1.030)), and systolic blood pressure ((β-coefficient 1.35 (1.25–1.44)) [17]. Kristófi et al. [7], in line with previously published data that identified albuminuria and chronic kidney disease (CKD) as risk factors for cardiovascular disease in T1D [18,19], found that prevalent and incident CKD are more common in T1D than in T2D. These higher levels of kidney impairment may play a role in higher rates of cardiovascular disease, reinforcing the importance of cardiorenal syndrome.

A possible explanation for this greater risk of renal and cardiovascular disease, and among them HF, is that the disease duration is often longer in T1D than T2D. Therefore, the probability of microvascular complications and the effects of hyperglycemia in cardiovascular outcomes are greater in T1D [7]. In fact, in the meta-regression analysis performed by Avogaro et al., age was significantly associated with the incidence ratio of HF [10]. Another study by McAllister and colleagues of over 3.25 million people among which there were 18,240 subjects with T1D, found that by the age of 20 years the prevalence of HF is similar among patients with T1D and T2D, but by the age of 80 years the prevalence of HF is higher in T1D and the same occurs with case-fatality rate, suggesting that the accumulation of risk factors and more prevalent microvascular complications in this group may contribute to higher incident and prevalent HF. Another hypothesis suggested in this study was that lower rates of prescription drugs known to reduce the risk of HF, such as antihypertensives, drugs acting on the renin-angiotensin system, or lipid lowering drugs, may also contribute to higher incident and prevalent HF. However, differences remained despite the older mean age of patients with T2D and after adjusting for individual risk of HF according to baseline characteristics. Though these are data from a retrospective study and some information was taken from clinical recordings with a risk of missing information, it is a very interesting hypothesis to consider [6].

Another emerging field of investigation in HF in T1D that may be intimately linked to glycemic control is cardiac autoimmunity. In an analysis derived from DCCT/EDIC, Sousa et al. measured the prevalence and profiles of cardiac autoantibodies in samples from DCCT and divided them in two groups, patients with HbA1c > 9% (n = 83) and patients with HbA1c < 7% (n = 83) at 26 years of follow-up. The same analysis was performed in similar groups of patients with T2D. They found that the DCCT HbA1c > 9% group had significantly higher levels of cardiac autoantibodies than the DCCT HbA1 < 7% group, while glycemic control was not related to cardiac autoimmunity in T2D. Moreover, positivity for two or more autoantibodies during DCCT was associated with a greater risk of cardiovascular disease (HR 16.1 [95% CI 3.0–88.2]) and coronary artery calcification (OR 60.1 [95% CI 8.4–410.0]) [20]. The same authors recently published a study showing that cardiac autoimmunity is related to subclinical myocardial dysfunction, independent of classical cardiovascular disease risk factors. They observed in a sample from DCCT that patients with two or more cardiac autoantibodies had greater left ventricular end-diastolic volume, end-systolic volume, left ventricular mass, and lower left ventricular

ejection fraction [21]. These observations suggest that there are different mechanisms for cardiovascular disease and thus for HF in T1D and T2D and those mechanisms may be tightly related to long exposure to hyperglycemia in T1D.

Finally, regarding risk factors, there is an interesting study carried out by Khedr et al. on 78 adolescents with T1D of at least 6 years of duration, in whom they analyzed some lipid biomarkers as predictors of diastolic dysfunction. They found diastolic failure to occur in 50% of the females and 66.6% of the males, and described that lower high-density lipoproteins (HDL) (OR 0.93, 95% CI 0.88–0.99) and a higher total cholesterol/HDL ratio (OR 2.55, 95% CI 1.9–5.45) and triglycerides/HDL ratio (OR 2.74, 95% CI 1.12–6.71) were associated with diastolic failure [22].

Considering that classical risk factors seem to be important, but it also seems that HF in T1D has differential characteristics, it is necessary to continue investigating the most important risk factors for the development of this complication (Figure 1).

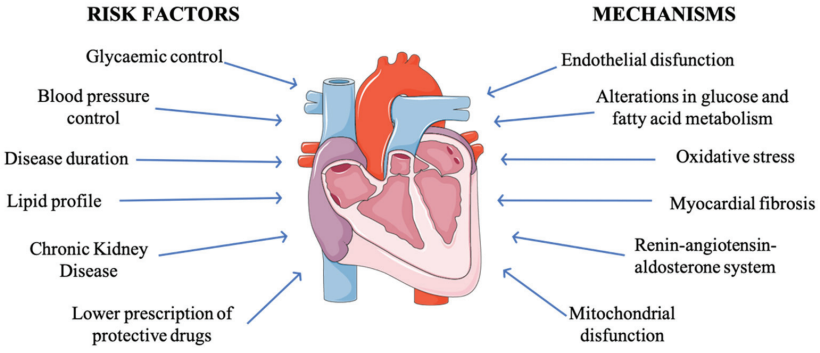

Figure 1. Risk factors and pathophysiology of heart failure in diabetes.

3. Pathophysiology

The mechanisms responsible for the association between diabetes and heart failure are not entirely clear, although a great variety of them have been proposed, such as endothelial dysfunction, alterations in glucose and fatty acid metabolism at the myocardial level, myocardial fibrosis, the increase in oxidative stress, or the activation of local neurohormonal systems, such as the renin-angiotensin-aldosterone system, endothelin, or the sympathetic nervous system [23] (Figure 1). It has also been proposed that some of these mechanisms can cause systolic or diastolic ventricular dysfunction even in the absence of coronary artery disease or structural disease [5]. There is a multitude of preclinical data, but they are still to be clarified.

Diabetic cardiomyopathy pathophysiology is widely studied in T2D, while its mechanisms in T1D are less clear. Hyperglycemia and chronic inflammation present in both types, promoting cardiac hypertrophy and fibrosis, increasing myocardial stiffness, and resulting in diastolic and systolic dysfunction. Increased levels of glucose lead to a higher production of advanced glycation end products (AGEs), which have been suggested to trigger deleterious effects on ventricular function through the formation of crosslinks between collagen molecules in the extracellular matrix, impairing its degradation and leading to myocardial stiffness and diastolic dysfunction [24]. Activated endothelial cells also contribute by promoting the uncoupling of endothelial nitric oxide synthase (NOS) resulting in diminished nitric oxide (NO) levels. This decreases soluble guanylate cyclase (sGC) activity and cyclic guanosine monophosphate (cGMP) content in the myocardium, which impairs the protective effects of protein kinase G (PKG) [25].

Due to the insulinopenia in T1DM, fatty acid β-oxidation is increased to maintain adenosine triphosphate (ATP) producti; however, this process becomes ineffective during diabetes evolution, resulting in intracellular lipid accumulation and lipotoxicity [26]. Increased intracellular fatty acid concentration and mitochondrial dysfunction lead to an

increased production of reactive oxygen species (ROS). Excess ROS production causes the activation of cellular and mitochondrial nitrogen oxides (NOX), which leads to the generation of superoxide and hydrogen peroxide [27]. These effects result in cardiomyocyte loss, cardiac hypertrophy, and inflammation with fibrosis of the extracellular matrix [28]. N-acetylcysteine (NAC) has been used as an antioxidant in mouse models of T1D to normalize oxidative stress and therefore prevent the development of cardiomyopathy [29].

Mitochondrial dysfunction is usually found in cardiac tissue in T1D patients. Decreased mitochondrial oxidative capacity is caused by altered mitochondrial ultrastructure, proteomic remodeling, and oxidative damage to proteins and mitochondrial DNA [30].

Concerning cardiac inflammation, the infiltration of macrophages and lymphocytes is usual in DM. These inflammatory cells secrete cytokines, such as tumor necrosis factor (TNF), interleukin 6 (IL-6), interleukin 1β (IL-1β), interferon-γ, and transforming growth factor β (TGFβ) that can produce profibrotic responses, leading to further adverse remodeling. Studies in mice have detected higher T-cell infiltration in the myocardium in T1D [31] and some attempts to reduce cardiac fibrosis by decreasing T-cell trafficking have been successful [32]. Regarding the immune system, as we mention before, Sousa et al. observed higher levels of cardiac autoantibodies in patients with T1D and poor glycemic control, and patients positive for ≥2 cardiac autoantibodies were more likely to have subclinical myocardial dysfunction as well as a higher cardiovascular disease risk. Chronic hyperglycemia may cause subclinical myocardial injury favoring the exposure of heart muscle proteins as α-myosin to the immune system. In patients with T1D and poor glycemic control, the immune system is dysregulated and may overreact to these proteins, producing an expansion of proinflammatory CD4 T-cells specific to α-myosin and the development of autoantibodies [20,21].

Another mechanism implicated in the pathophysiology in T1D mouse models is increased cardiomyocyte intracellular Ca^{2+} due to lower sarcoplasmic reticulum Ca^{2+} pump activity because of the decreased glucose transporter type 4 (GLUT 4) recruitment to the plasma membrane, mediating this disturbance in contractile dysfunction and arrhythmia [33].

Renin-Angiotensin-Aldosterone activity is increased under diabetic conditions. Angiotensin-II receptor type 1 (AT1R) density and synthesis are increased in T1D hearts, and the increase in fibrosis is partially inhibited following treatment with ACE inhibitors and AT receptor blockers [34]. Moreover, a frequent complication related to sustained hyperglycemia is cardiac autonomic neuropathy which includes abnormalities in heart rate control, vascular hemodynamics, and cardiac structure and function. An early characteristic of cardiac autonomic neuropathy is the reduction of parasympathetic activity with an imbalance toward higher sympathetic activity [35].

Among the new fields that are opening in the pathophysiology of heart failure, the intestinal microbiota and some of its metabolites stand out [36]. In some models, *Akkermansia*, *Prevotella 9*, *Paraprevoltella*, and *Phascolarctobaterium* have been associated with changes in cardiac structure and function [37]. The "intestinal hypothesis" of heart failure postulates that the reduction in cardiac output causes damage to the intestinal barrier that generates dysbiosis, favoring the proliferation of pathogenic species such as *Candida* and the reduction of anti-inflammatory bacteria such as *Faecalibacterium prausnitzii.* Similarly, the microbiota can promote heart failure through the modulation of intestinal immunity. Segmented filamentous bacteria favor the production of IL-6 and interleukin 23 [38] and *Bacteroides Fragilis* favors the production of anti-inflammatory cytokines that, in murine models, have been shown to reduce ventricular remodeling after myocardial infarction [39]. Bacterial metabolites also seem to have a role; for example, the reduction of short-chain fatty acids can favor the damage of the intestinal barrier and promote dysbiosis and the translocation of endotoxins to the bloodstream [40]. Trimethylamine N-oxide (TMAO) also seems to act as a risk factor for heart failure, since it has been observed in animal models to facilitate the release of calcium in the heart muscle by altering contractility and may also increase myocardial fibrosis [41,42]. It has also been observed that higher levels of TMAO in blood appear to be associated with a worse prognosis [43].

4. Diagnosis

The pathophysiological timeline of diabetic cardiomyopathy seems to follow the trend observed in other non-structural heart diseases, with the initial development of left ventricular diastolic dysfunction followed by subclinical systolic dysfunction with preserved ejection fraction and finally progressing to HFrEF [44,45]. In advanced stages, the diagnosis of HF is based on a combination of clinical data of the patient—compatible signs and symptoms based on the classic Framingham criteria—supported by diagnostic tests. Diagnostic confirmation is necessary in all cases, given its prognostic implication and the need to carry out an adequate therapeutic adjustment [46].

However, in the population with T1D, it is important to diagnose diastolic dysfunction and subclinical systolic dysfunction, to do an early diagnosis of the disease using sensitive cardiac markers that are easy to incorporate in routine clinical practice. Type B natriuretic peptides (BNP, NT-ProBNP) are plasmatic biomarkers, which are released in response to ventricular stretching and volume overload within the cardiac chambers, and can be affected by parameters such as age, sex, BMI, or renal function. These markers are a useful tool to guide the diagnosis of HF in the acute setting, in either diabetic or non-diabetic patients. Data from the multinational Breathing Not Properly trial suggest that diabetes is not a confounding variable in the interpretation of BNP levels in this situation [47]. A recent study [48] determined that higher NT-ProBNP levels were independently associated with HF in 664 subjects with T1D [HR 1.7 (95% CI: 1.1–2.4), $p = 0.01$]. The latest guidelines for the diagnosis and treatment of HF recommend the use of these natriuretic peptides both in acute and non-acute settings to rule out HF, given its high negative predictive value, but not to establish its diagnosis. Thus, the diagnosis in diabetic patients in the non-acute setting should follow the diagnostic algorithm that emphasizes that patients with a high probability of HF may have an echocardiogram to confirm or rule out the diagnosis [49]. Echocardiography is postulated as a central tool in the diagnosis of HF, given its safety, easy access, and highly informative character (cardiac chamber volumes, ventricular and valve function, and myocardial wall thickness, among other aspects) [50].

For the study of diastolic dysfunction in young people with T1D [51], it is recommended to follow the general indications of the American Society of Echocardiography and the European Association of Cardiovascular Imaging, through indices that include involving pulse Doppler transmitral inflow velocities (E and A waves) and tissue Doppler early and late mitral annular diastolic velocities (e' and a'), atrial size measurements, and pulmonary venous flow evaluation [52]. Thus, in recent years, more sensitive ultrasound techniques have been incorporated to detect the more subtle abnormalities of cardiac function that would go unnoticed with conventional techniques and measurements (such as ventricular deformation and desynchrony indices). Although left ventricular diastolic dysfunction is the earliest manifestation of HF in the diabetic population [53], recently, the role of left atrial dysfunction as an active contributor to the initial diastolic dysfunction suffered by these patients has been revealed [54]. Ifuku M et al. [14] observed left atrial dysfunction (such as left Atrial phasic strain) in adolescents and young people with T1D ($n = 53$) compared to non-diabetic controls ($n = 53$) and assert that it could constitute an early and sensitive marker of diastolic dysfunction in T1D. The E/e' ratio is frequently used as a marker of diastolic dysfunction (Yoldaş T, 2018). Bradley TJ et al. [55] observed an E/e' ratio (7.3 ± 1.2 vs. 6.7 ± 1.3; $p = 0.0003$) increased in patients with T1D ($n = 199$) compared to non-diabetic subjects ($n = 178$). However, not all the findings are consistent in this regard [56].

Kaushik A et al. [57], in a recent study, found the presence of preclinical ventricular dysfunction echocardiographic alterations in the population with T1D. Specifically, they found lower left ventricular strain indices [basal lateral LV (21.39 ± 4.12 vs. 23.78 ± 2.02; $p = 0.001$), mid-lateral LV (21.43 ± 4.27 vs. 23.17 ± 1.92 $p = 0.02$), basal septum (20.59 ± 5.28 vs. 22.91 ± 2.00; $p = 0.01$), and mid septum (22.06 ± 4.75 vs. 24.10 ± 1.99; $p = 0.01$] in children and adolescents with T1D ($n = 50$) compared to non-diabetic controls ($n = 25$), despite the absence of manifest heart failure and normal ejection fraction. In addition, greater endothe-

lial dysfunction was detected by flow-mediated dilatation (FMD) in subjects with T1D compared to non-diabetic patients (8.36 ± 4.27 vs. 10.57 ± 4.12, $p = 0.04$). These myocardial alteration parameters correlated with HbA1c levels ($r = -0.327$, $p = 0.017$). These findings reinforce the hypothesis of the possible early effect of the diabetic metabolic environment on myocardial function.

Some studies that evaluate systolic function in T1D with HFrEF expose a parallel reduction in ultrasound parameters such as longitudinal tension and global ventricular circumference, as well as a reduction in the systolic strain rate using speckle-tracking echocardiography [58], although not all studies have reported changes in this regard [59,60]. Different studies have also reported subclinical cardiac dysfunction in young subjects with T1D [61–63] although other studies have not reached this conclusion [60,61]. This controversy could probably be due to differences in the characteristics of the subjects with T1D -glycemic control, time of evolution of the disease [64], and the use of different ultrasound protocols for the determination of ultrasound parameters in the comprehensive evaluation of cardiac dysfunction, which calls for standardized approaches to facilitate their interpretation.

These basic and central examinations based on clinical, analytical, and mainly ultrasound parameters can be completed with other modalities such as cardiac magnetic resonance imaging. Cardiac MRI allows for calculating improved rates of myocardial deformation of diastolic incoordination, including biventricular desynchrony and incoordination. The EMERALD study, carried out in a young population with T1D, reports alterations in the diastolic pressure of the ventricular septum and the diastolic relaxation fraction, which reflects an uncoordinated and energetically less favorable myocardial relaxation compared to non-diabetic subjects [65].

Although the identification of this underlying heart problem in T1D can be very important to delay or prevent the development of manifest HF, it is necessary follow-up with these patients, through longitudinal studies, to accurately determine the clinical importance of the preclinical myocardial changes detected in this population.

5. Treatment

According to the latest European guidelines for the treatment and diagnosis of HF [49], in patients with HFrEF, interventions that reduce morbidity and mortality confer a similar benefit in the presence or absence of diabetes. In addition to the control of classic cardiovascular risk factors, the use of beta-blockers, angiotensin-converting enzyme inhibitors (ACEIs), spironolactone, or eplerenone is proposed. Meanwhile, other drugs are recommended only in selected patients with symptomatic HFrEF, both diabetics and non-diabetics, as is the case with the use of diuretics, sacubitril/valsartan, ivabradine, hydralazine, isosorbide dinitrate, or angiotensin II type I receptor blockers.

The identification of asymptomatic T1D patients with cardiac dysfunction may favor the development of useful therapeutic strategies in diabetic cardiomyopathy, to optimize the treatment of these patients and improve the prognosis of the disease. As we mentioned before, McAllister et al. [6] and Kristófi et al. [7] found that the total age-adjusted CVRD burden and risks were greater among patients with T1D compared with those with T2D and HF rates were significantly higher in T1D patients depending on the age group. They also highlighted that the use of antihypertensive, antiplatelet, and statin drugs was much higher in T2D than in T1D, although these differences could be explained by differences in age and comorbidities. These findings highlight the need to improve preventive strategies beyond glycemic control in the T1D population from an early age.

The gold-standard treatment in T1D is the use of basal-bolus insulin therapy and the early intensive therapy is a fundamental aspect to reduce HF risk in the long-term [13]. Currently, new strategies to measure glucose levels, including the detection of interstitial glucose through Continuous Glucose Monitoring (iCGM) or Flash Glucose Monitoring (FGM), allow the adjustment of insulin therapy to improve metabolic control and achieve optimal control, as well as a more accurate assessment of glycemic variability and its

reduction [66,67]. Besides, because glycemic variability is an independent risk factor for developing long-term complications in diabetic patients, continuous glucose monitoring might be a valuable tool in this context [67].

Certain drugs approved for the treatment of T2D such as metformin, aGLP1, and iSGLT2 are being evaluated as potential complementary drugs to insulin therapy in T1D [68]. Reflections in this regard underline the importance of the proper selection of patients with T1D and a close follow-up of them, in the case of the use of iSGLT2 due to the associated risk of developing "normoglycemic" diabetic ketoacidosis (DKA) [69]. Based on recent positive results from the DEPICT study [70], dapagliflozin 5 mg was the first iSGLT2 to have its marketing authorization in Europe in March 2019 as an additional drug to insulin therapy in patients with T1D with a body mass index (BMI) $\geq$27 kg/m^2 [71]. It also recently received Scottish Medicines Consortium (SMC) approval [72] as well as National Institute for Health and Care Excellence (NICE) approval following health economic analysis, in which dapagliflozin was found to be a highly cost-effective treatment option in people with T1D inadequately controlled by insulin alone [73]. However, its non-authorization in other places such as the U.S., and the BMI restrictions reflect safety concerns regarding the "normoglycemic" DKA risk. Since the available data are unclear, it is important to proceed on an individual basis for people that fall into these categories. Selecting appropriate people with T1D for iSGLT2 treatment is critical for minimizing the DKA risk and maximizing the potential benefits associated with this treatment. Those most likely to benefit from dapagliflozin treatment include overweight/obese people, established on stable optimized insulin therapy (i.e., not recently diagnosed), with high insulin needs (i.e., > 0.5 units/kg of body weight/day), and a low DKA risk profile, who have demonstrated adherence to their insulin regimen and the ability to understand and utilize relevant education relating to DKA risk [74].

In recent years, the development of these hypoglycemic molecules such as aGLP1 or iSGLT2 and the performance of cardiovascular safety studies for their commercialization have shown that they are not only beneficial in glycemic control, but also have cardioprotective effects in both T2D and non-diabetic patients. This opens the door to a clinical entity with important clinical repercussions, highly prevalent as we have seen in the general diabetic population and T1D. Furthermore, in certain stages, the lack of therapeutic options stands out, and although the studies show promising results, there are no specific data on the use of these drugs in the T1D population.

Metformin is the first-line treatment in T2D. In recent years, cohort studies and systematic reviews have analyzed its role in cardiovascular disease, finding that metformin seems to be associated with a reduction in mortality from all causes in T2D patients with HF, as well as with a reduction in readmissions by HF [75,76], so it is recommended in the current guidelines of the European Society of Cardiology [49] as a first-line drug in patients with T2D and HF. In T1D, REMOVAL a placebo-controlled trial to Metformin, data suggest that it might have a wider role in cardiovascular risk management, but do not support the use of metformin to improve glycemic control in adults with long-standing T1D [77].

Relative to iSGLT2, in the DECLARE-TIMI 58 trial, dapagliflozin treatment was associated with a lower rate of HF-related death and hospitalization than the placebo [78]. Likewise, dapagliflozin treatment has also been associated with a reduction in HF-related hospitalization rates in patients with or without HFrEF and a reduction in cardiovascular mortality and all-cause mortality compared to the placebo in patients with T2D and HFrEF [79], as well as in patients with T2D and previous myocardial infarction [80]. These benefits are the same in patients without diabetes with HFrEF [81], so its cardiovascular benefit would be independent of the hypoglycemic effect. The cardioprotective effects of empagliflozin are very similar [82]. These data are reinforced by later trials such as EMPRISE, where empagliflozin showed greater efficacy in the incidence of HF compared to sitagliptin, in reducing hospitalization for HF in T2D patients with and without cardiovascular disease [83]. Moreover, in the EMPEROR-Reduced trial, the use of empagliflozin reduced the risk of hospitalization for HF and cardiovascular mortality, regardless of the presence or absence of diabetes [84].

In this line, the EMPA-TROPISM (ATRU-4) study supports the benefit of empagliflozin in the treatment of HF regardless of its glycemic status, by demonstrating significant improvement in the key parameters of cardiac dysfunction, such as left ventricular (LV) volume, LV mass, LV systolic function, functional capacity, and quality of life of non-diabetic HFrEF patients [85]. Finally, results from the EMPEROR-preserve trial have been recently published, showing a reduction of the combined risk of cardiovascular death or hospitalization for HF with 10 mg empagliflozin in patients with HFpEF, regardless of the presence or absence of diabetes [86]. Other molecules of this pharmacological group have also shown benefits concerning cardiovascular mortality and hospitalization for HF (canagliflozin, CANVAS) [87], (sotagliflozin, SOLOIST-WHF) [88] or (ertugliflozin, VERTIS) [89]. In the case of sotagliflozin, they found benefits in HFpEF as well.

Regarding aGLP1, trials have shown heterogeneous information with favorable results in the reduction of cardiovascular mortality events for some molecules (Table 1): LEADER (liraglutide) [90], SUSTAIN-6 (semaglutide) [91], REWIND (dulaglutide) [92]; and neutral effects for other: ELIXA (lisixenatide) [93] and EXSCEL (long-acting exenatide) [94], without finding favorable specific results on HF. Subsequently, more specific trials have been conducted with liraglutide in patients with or without diabetes and HFrEF, which have further increased the uncertainty about the use of this molecule in subjects with established HF. The FIGHT trial, carried out in 300 patients recently hospitalized for HF, found that the use of liraglutide did not lead to greater clinical stability after hospitalization. Likewise, in the LIVE study (n = 241), it was found that the use of liraglutide did not affect left ventricular systolic function (LVEF) compared to the placebo in patients with stable HF, although it was associated with an increase in heart rate and serious adverse cardiac events, such as sustained ventricular tachycardia, atrial fibrillation, or worsening ischemic heart disease (10% vs 3%, p = 0.04). A meta-analysis published in recent years showed encouraging results regarding cardiovascular safety with the use of aGLP1, suggesting that they can reduce major adverse cardiovascular events, cardiovascular mortality, and all-cause mortality risk; no significant effect was identified in relation to hospitalization for HF [95] or even with reduced risk of hospitalization for HF [96]. A double-blind clinical trial [97] performed on T2D patients (n = 49) showed that treatment for 26 weeks with liraglutide versus a placebo reduced early diastolic LV filling and LV filling pressure to normal levels, pathogenic characteristics of HFpEF. However, future studies are needed to investigate these potential effects of aGLP1 in HF in its early stages and its benefits in other populations such as non-diabetic, obese, or T1D subjects.

Table 1. Evidence on hospitalization for heart failure and cardiovascular mortality with glucagon-like peptides 1 agonist (aGLP1) and sodium-glucose cotransporter 2 inhibitor (i-SGLT2) from randomized controlled trials.

		Study	Hospitalization for HF	CV Mortality
GLP1 receptor agonists	Liraglutide	LEADER [90]		↓
	Semaglutide	SUSTAIN-6 [91]		↓
	Dulaglutide	REWIND [92]		↓
SGLT2 inhibitors	Dapagliflozin	DECLARE-TIMI 58 [78]	↓	
	Empagliflozin	EMPRISE [83]	↓	
		EMPEROR-Reduced [84]	↓	↓
		EMPEROR-Preserve [86]	↓	↓
	Canagliflozin	CANVAS [87]	↓	↓
	Ertugliflozin	VERTIS [89]	↓	

GLP1: glucagon-like peptide 1; SGLT-2: sodium-glucose cotransporter 2; HF: heart failure; CV: cardiovascular.

Regarding the position of the different international cardiology and endocrinology societies in the use of iSGLT-2 and aGLP1, both are the recommended therapies in cases of T2D with cardiovascular disease, preferably leaning towards the use of the former in HF cases without ruling out the use of aGLP1 [68,98–101]. However, the American Heart Failure Society specifies the precaution of its use in situations of acute decompensation [102].

6. Conclusions

HF is a complication of increasing concern in diabetes, and given the high incidence of HF and the risk of hospitalization for HF in the population with T1D, more studies should be developed in this regard to clarify pathophysiological aspects, determine specific risk factors to control, and develop standardized protocols to establish specific precision biomarkers for the diagnosis of this entity in T1D patients from early stages.

The relationship between classic cardiovascular risk factors—such as hyperglycemia, hypertension, or dyslipidemia—and the cardiac and vascular abnormalities seen in people with T1D is not fully understood, so further research is required to identify potential treatment targets allowing for the development of therapeutic agents in this field. Some therapeutic groups, such as iSLGT2 and aGLP1, have shown a clear benefit in preventing cardiovascular complications in T2D. In particular, iSGLT2 have shown to be very effective in reducing HF-related deaths and hospitalization for HF in both T2D and non-diabetic patients. However, it remains to be determined if they are useful and safe in patients with HF and T1D.

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Effects of Weight Gain after 20 Years of Age and Incidence of Hyper-Low-Density Lipoprotein Cholesterolemia: The Iki Epidemiological Study of Atherosclerosis and Chronic Kidney Disease (ISSA-CKD)

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Abstract: The aim of this study was to investigate the effects of long-term weight gain from the age of 20 on incidence of hyper-low-density-lipoprotein (LDL) cholesterolemia in the general population of Japanese people. Methods: We conducted a population-based retrospective cohort study using annual health checkup data for residents of Iki City, Nagasaki Prefecture, Japan. A total of 3179 adult (≥30 years old) men and women without hyper-LDL cholesterolemia at baseline, who underwent two or more health checkups were included in the analysis. Information on weight gain (≥10 kg) after 20 years of age was obtained using questionnaire. The outcome of this study was development of hyper-LDL cholesterolemia defined as LDL-cholesterol level ≥3.62 mmol/L and/or initiation of lipid-lowering medications. Results: During a mean follow-up period of 4.53 years, 665 of the 3179 participants developed hyper-LDL cholesterolemia (46.5/1000 person-years). The incidence of hyper-LDL cholesterolemia was higher in participants with a weight gain of ≥10 kg (55.3/1000 person-years) than among those with a weight gain of <10 kg (41.8/1000 person-years). This association remained statistically significant even after adjustment for age, sex, smoking, daily drinking, exercise, obesity, hypertension, and diabetes (multivariable hazard ratio 1.31, 95% confidence interval 1.08–1.58, $p = 0.006$). Conclusion: A weight gain of ≥10 after 20 years of age affected the development of hyper-LDL cholesterol regardless of age, sex, and obesity in a general population of Japanese.

Keywords: weight gain; LDL cholesterol; hyper-LDL cholesterolemia; longitudinal study; general population

1. Introduction

Cardiovascular disease is one of the leading causes of death in Japan as well as worldwide, with 18 million fatalities accounting for 32% of total deaths worldwide [1]. The risks of cardiovascular disease are associated with lifestyle factors such as smoking, diet,

and exercise habits, and metabolic factors such as obesity, serum lipid levels, hypertension, and diabetes [2,3]. Of these, elevated low-density lipoprotein (LDL) cholesterol levels are among the most important [4]. Randomized controlled trials have demonstrated the short-term effects of interventions to reduce body weight on LDL cholesterol levels [5]. However, the effects of long-term weight changes on LDL cholesterol levels are not clear. The objective of this study was to investigate the effects of long-term weight gain from the age of 20 on incidence of hyper-low-density-lipoprotein (LDL) cholesterolemia in the general population of Japanese.

2. Materials and Methods

2.1. Study Design and Participants

The Iki Epidemiological Study of Atherosclerosis and Chronic Kidney Disease (ISSA-CKD) project was a retrospective open cohort study of the general population of Iki City, Nagasaki Prefecture, Japan. The details of the study methods have been previously reported [6–11]. In brief, Iki Island is in the north of Nagasaki Prefecture and the population is approximately 27,000. In Iki City, medical examinations are conducted annually for residents over 30 years of age. A total of 7895 people who participated in these medical examinations at least once during study period between 2008 and 2017. We excluded 1879 individuals who underwent a health checkup only once, 2289 individuals who had previously been diagnosed with hyper-LDL cholesterolemia (LDL-C levels $\geq$3.62 mmol/L and/or use of lipid-lowering medications), and 548 individuals with missing information on weight gain after 20 years of age. Therefore, this study enrolled 3179 individuals. The study was approved by the Fukuoka University Clinical Research Ethics Center (approval number: 2017M010).

2.2. Data Collection

Information on weight change after 20 years of age was collected using a standardized yes/no questionnaire ("has your weight increased by 10 kg or more since 20 years of age?"). The cut-off point of 10 kg was defined based on recommendation from the Ministry of Health, Labour and Welfare to detect people who gained weight by 10 kg or more after 20 years of age because they are at high risk of diabetes and hypertension [12]. We also used a standardized questionnaire regarding smoking, daily alcohol intake, and regular exercise habits, as well as the use of blood pressure, lipid-lowering, and glucose-lowering drugs. A current smoker was defined as a participant who smoked $\geq$100 cigarettes or had smoked continuously for $\geq$6 months. A current alcohol drinker was defined as a participant who drank daily. A regular exercise habit was defined as exercise performed for $\geq$30 min at least twice weekly. Height and weight were measured without shoes and body mass index (BMI; kg/m^2) was calculated. Obesity was defined as a BMI $\geq$ 25 kg/m^2 [13]. Blood pressure (BP) was measured by trained staff using a mercury, automatic, or aneroid sphygmomanometer with an appropriately sized cuff, measured with the right upper arm according to standard guidelines after at least 5 min of rest in a sitting position [14]. Hypertension was defined as BP $\geq$ 140/90 mmHg, or use of BP-lowering medications [15]. Casual blood samples were also collected. LDL cholesterol levels were determined using a direct enzymatic method. High-density lipoprotein (HDL) cholesterol and triglyceride levels were also measured using the enzymatic method. Blood glucose and HbA1c levels were measured enzymatically, and diabetes was defined as a fasting blood glucose level $\geq$7.0 mmol/L, non-fasting blood glucose $\geq$11.1 mmol/L, HbA1c (National Glycohemoglobin Standardization Program) $\geq$6.5%, or use of glucose-lowering drugs [16].

2.3. Outcome

During the follow-up period from 2008 to 2017, we defined the first time when each participant received a medical examination as the baseline and followed the patients up to 2017. The outcome of this study was incidence of hyper-LDL cholesterolemia. The onset of hyper-LDL cholesterolemia was defined as an LDL-C level of $\geq$3.62 mmol/L or the

initiation of lipid-lowering drugs during the follow-up period [17], which was confirmed at the end of follow-up.

2.4. Statistical Analysis

We applied Wilcoxon test for continuous variables and Chi-square tests for categorical variables to compare baseline characteristics between the two groups: those whose weight had increased by ≥10 kg after 20 years of age and those who did not. The incidence of hyper-LDL cholesterolemia was calculated in person-years. The effect of weight gain of ≥10 kg after 20 years of age on the development of hyper-LDL cholesterolemia was estimated using univariable and multivariable Cox proportional hazard models. The multivariable analysis was adjusted for age, sex, smoking, drinking, exercise, obesity, hypertension, and diabetes. We conducted a subgroup analysis to stratify the effect of weight gain on the development of hyper-LDL cholesterolemia by subgroup (under 65 years or over 65 years of age, male or female sex, non-obese or obese). The differences between subgroups were tested by adding an interaction term to the statistical model. Statistical Analysis System (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA) was used to perform the statistical analyses. All *p*-values reported were two-sided, and the significance level was set at $p < 0.05$.

3. Results

Table 1 shows the baseline characteristics according to weight gain after 20 years of age. Participants with a weight gain of ≥10 kg were more likely to be men, obese, hypertensive, and diabetic, as well as higher levels of BMI, blood pressure, HbA1c, HDL-C, LDL-C, and triglycerides. During a mean follow-up period of 4.53 years, 665 of the 3179 participants developed hyper-LDL cholesterolemia (46.5/1000 person-years).

Table 1. Participant baseline characteristics according to weight gain after 20 years of age.

	Weight Change after 20 Years of Age		
	<10 kg (*N* = 2146)	**≥10 kg (*N* = 1033)**	***p* Value**
Age	58.9 ± 11.7	58.9 ± 10.4	0.977
Female	1111 (51.8%)	413 (40.0%)	<0.0001
Current smoker	474 (22.1%)	232 (22.4%)	0.810
Daily drinking	574 (26.9%)	331 (32.3%)	0.0018
Exercise	1549 (73.3)	769 (75.2%)	0.250
Body mass index	22.0 ± 2.63	26.1 ± 3.18	<0.0001
Obesity	256 (11.9%)	635 (61.5%)	<0.0001
Systolic blood pressure, mmHg	127 ± 18.9	132 ± 18.4	<0.0001
Diastolic blood pressure, mmHg	73.7 ± 11.0	77.0 ± 11.3	<0.0001
Hypertension	787 (36.7%)	534 (51.7%)	<0.0001
LDL-cholesterol, mmol/L (mg/dL)	2.74 ± 0.54 (106 ± 21.0)	2.90 ± 0.50 (112 ± 19.5)	<0.0001
HDL-cholesterol, mmol/L (mg/dL)	1.67 ± 0.44 (64.6 ± 16.9)	1.46 ± 0.39 (56.5 ± 15.0)	<0.0001
Triglyceride, mmol/L (mg/dL)	1.15 ± 0.87 (102 ± 76.9)	1.56 ± 1.21 (138 ± 107)	<0.0001
HbA1c, %	5.30 ± 0.63	5.50 ± 0.80	<0.0001
Diabetes	133 (6.2%)	106 (10.7%)	<0.0001

LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c, glycated hemoglobin.

Table 2 shows the risks of hyper-LDL cholesterolemia according to weight gain after 20 years of age. The incidence of hyper-LDL cholesterolemia was higher in participants with a weight gain of ≥10 kg (55.3/1000 person-years) than among those with a weight gain of <10 kg (41.8/1000 person-years). This association remained statistically significant even after adjustment for age, sex, smoking, daily drinking, exercise, obesity, hypertension, and diabetes (multivariable-adjusted hazard ratio 1.31, 95% confidence interval 1.08–1.58,

p = 0.006). Similar results were obtained after adjustment for more detailed categories for alcohol intake (no drinkers, occasional drinkers or daily drinkers [<22 g/day, 22–43 g/day or ≥44 g/day]): multivariable hazard ratio 1.31, 95% confidence interval 1.08–1.58, p = 0.005. Sensitivity analysis with adjustment of BMI instead of obesity demonstrated that the hazard ratio of weight gain ≥10 kg after 20 years of age for development of hyper-LDL cholesterolemia was 1.18 (95% confidence interval 0.97–1.43) but the association was not statistically significant (p value = 0.100). Likewise, sensitivity analysis with adjustment of waist circumference instead of obesity demonstrated that the hazard ratio of weight gain ≥10 kg after 20 years of age for development of hyper-LDL cholesterolemia was 1.16 (95% confidence interval 0.96–1.41) but the association was not statistically significant (p value = 0.122).

Table 2. Risks of hyper-LDL cholesterolemia according to weight gain after 20 years of age.

	Weight Change after 20 Years of Age		
	<10 kg (*N* = 2146)	**≥10 kg (*N* = 1033)**	***p* Value**
Number of events	406	259	
Person-years	9721.38	4679.49	
Incidence/1000 person-years	41.8	55.3	
Crude hazard ratio (95%CI)	Reference	1.33 (1.14–1.55)	<0.001
Multivariable-adjusted * hazard ratio (95%CI)	Reference	1.31 (1.08–1.58)	0.006

* Adjusted for age, sex, smoking, daily drinking, exercise, obesity, hypertension, and diabetes. CI, confidence interval.

Table 3 shows the results of subgroup analyses. There were no clear differences in the effects of weight gain of ≥10 kg after 20 years of age for the development of hyper-LDL cholesterolemia between subgroups defined by age (<65 vs. ≥65 years), sex, and obesity (all p > 0.1 for interaction).

Table 3. Subgroup analyses.

	Adjusted * Hazard Ratio	***p* for Interaction**
Age (years)		
<65	1.40 (1.10–1.77)	
>65	1.12 (0.82–1.54)	0.13
Sex		
Male	1.26 (0.96–1.65)	
Female	1.33 (1.02–1.72)	0.90
Obesity		
Absent	1.26 (0.99–1.59)	
Present	1.38 (1.00–1.91)	0.63

* Adjusted for age (except for subgroup analysis by age), sex (except for subgroup analysis by sex), smoking, daily drinking, exercise, obesity (except for subgroup analysis by obesity), hypertension, and diabetes.

4. Discussion

The results of this large-scale longitudinal study of the general Japanese population showed that a weight gain of ≥10 kg after 20 years of age was significantly associated with the incidence of hyper-LDL cholesterolemia. This outcome remained significant even after adjusting for age, sex, smoking, daily drinking, exercise, obesity, hypertension, and diabetes. A similar association was observed between subgroups defined by age, sex, and obesity.

Previous studies have investigated the effects of body weight changes on serum LDL cholesterol levels. A meta-analysis of 73 randomized trials including 32,496 participants (mean age 48 years, weight 102 kg, BMI 36.3 kg/m^2) reported that short-term body weight reduction due to lifestyle-related interventions was associated with decreased LDL cholesterol levels in both men and women [4]. A prospective observational study of

3388 overweight Polish individuals aged 45–64 years reported was no significant association between a two-year change in body weight and serum LDL cholesterol levels [18]. Regarding the effects of long-term body weight change, a cross-sectional study investigating the association between body weight gain after 20 years of age and the prevalence of hyper-LDL cholesterolemia in 1715 Chinese participants in the general population aged 45–60 years reported that women with a weight gain of ≥10 kg after 20 years of age had a higher prevalence of hyper-LDL cholesterolemia than those without; however, this association was not observed in men [19]. In the present large-scale, longitudinal study of the general Japanese population, long-term body weight change age 20 years of age was associated with increased risks of the future development of hyper-LDL cholesterolemia. The effects of long-term body weight change on hyper-LDL cholesterolemia were comparable between sexes. Based on these findings, long-term weight gain is likely to affect the future development of hyper-LDL cholesterolemia.

The mechanisms underlying the association between long-term weight gain and hyper-LDL cholesterolemia have not been fully elucidated. Weight gain from the age of 20 years appears to mainly reflect an increase in visceral fat volume owing to decreased muscle mass volume and basal metabolic rate after this age [20–22]. Increased visceral fat volume causes insulin resistance [23–25], which is associated with an increased pool of LDL precursors including very-low-density lipoprotein (VLDL) [26,27]. Insulin resistance is also associated with a reduction in the number of LDL receptors, a decrease in the LDL-binding affinity of these receptors, and a subsequent reduction in the clearance of LDL particles [26–28]. Increased pooled LDL precursors and decreased clearance of LDL particles, which are associated with visceral fat, might be attributable to the development of hyper-LDL cholesterolemia.

This is the first longitudinal study of the association between a weight gain of ≥10 kg after 20 years of age and hyper-LDL cholesterolemia in the general population including adult Japanese men and women. The limitations of this study were its retrospective design, the use of a recall-based questionnaire, and the analysis of data from medical examinations of the general public, which may have resulted in a bias toward health-conscious participants.

5. Conclusions

In conclusion, in adult Japanese men and women, a weight gain of ≥10 kg after 20 years of age affected the development of hyper-LDL cholesterol regardless of age, sex, and obesity. A population strategy with interventions to maintain a proper weight is important to prevent the subsequent occurrence of cardiovascular events, increased risk of death, and medical costs.

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Data Availability Statement: The data are not publicly available in order to preserve the anonymity of the subjects involved in the study.

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Review

A Practical Guide for the Management of Steroid Induced Hyperglycaemia in the Hospital

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Abstract: Glucocorticoids represent frequently recommended and often indispensable immunosuppressant and anti-inflammatory agents prescribed in various medical conditions. Despite their proven efficacy, glucocorticoids bear a wide variety of side effects among which steroid induced hyperglycaemia (SIHG) is among the most important ones. SIHG, potentially causes new-onset hyperglycaemia or exacerbation of glucose control in patients with previously known diabetes. Retrospective data showed that similar to general hyperglycaemia in diabetes, SIHG in the hospital and in outpatient settings detrimentally impacts patient outcomes, including mortality. However, recommendations for treatment targets and guidelines for in-hospital as well as outpatient therapeutic management are lacking, partially due to missing evidence from clinical studies. Still, SIHG caused by various types of glucocorticoids is a common challenge in daily routine and clinical guidance is needed. In this review, we aimed to summarize clinical evidence of SIHG in inpatient care impacting clinical outcome, establishment of diagnosis, diagnostic procedures and therapeutic recommendations.

Keywords: steroid induced hyperglycaemia; hospital; practical guide

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1. Introduction

Steroidal therapies in particular glucocorticoids (GC), represent therapeutic agents of great importance in the treatment and prophylaxis of various acute and chronic inflammatory as well as autoimmune disorders [1]. Despite their efficacy, the use of steroids is associated with a variety of side effects that can be pragmatically divided into three categories: (1) Immediate side effects include the occurrence of fluid retention with oedema, blurriness of vision, impairments of mood, immune response modulation and the development of steroid induced hyperglycaemia (SIHG). (2) Idiosyncratic side effects summarize the development of avascular necrosis, cataract formation, glaucoma and psychosis. (3) More gradual side effects affecting the endocrine system and inducing the development of bone disease, dyslipidaemia, obesity and adrenal suppression [2]. As GCs decrease peripheral insulin sensitivity, increase hepatic gluconeogenesis, trigger insulin resistance on the level of the lipid metabolism and adipose tissue, as well as inhibit pancreatic insulin production and secretion, they represent a drug class with the highest risk of provoking the development of hyperglycaemia and overt diabetes mellitus (DM) [3–5].

2. Prevalence of Steroid Induced Hyperglycaemia in the Hospital

The prevalence of SIHG is dependent on the dose, indication and setting of use. Individual conditions such as age, baseline body mass index (BMI) and family history of diabetes are known to impact the risk of SIHG development. Older observational data indicate that 2% of incident diabetes cases in a primary care population are associated with GC therapy and the odds ratios for presenting with new-onset diabetes after introduction of GCs in various studies has been described to range from 1.36–2.31 [6]. Patients without

history of diabetes developed in-hospital hyperglycaemia (≥10 mmol/L; ≥180 mg/dL) in 70% when relevant doses of GCs were administered [7]. A meta-analysis summarized studies in which patients without pre-existing diabetes who received systemic GCs and showed a rate of SIHG development in 32.3% and in further 18.6% diabetes was sustainable during the follow up [8]. In patients who received solid organ transplantation and GC therapy the prevalence was described to be between 17% and 32% [9,10] and in a high-risk population of people who received high dose systemic therapy for acute Graft-versus-Host disease, two thirds of the cohort showed median glucose readings in the hyperglycaemic range (defined as fasting glucose ≥7 mmol/L or ≥126 mg/dL) [11]. As SIHG is suggested to be a transient problem resolving after the discontinuation of GCs, data indicate that diabetes can persist and GCs just unmasked a pre-existing glucose metabolism disorder [12,13]. While mainly systemic steroids were identified to expose the patient to an increased risk for hyperglycaemia, recently, topically used GCs were also shown to be associated with an elevated risk of diabetes [14].

3. Impact of Steroid Induced Hyperglycaemia

It has been shown that acute and chronic hyperglycaemia that are present in many cases in the hospital setting are important risk factors for prolonged hospital stays, infectious complications, poorer surgical outcomes and increased mortality [15–17]. Data of people with SIHG without previously known DM are scarce. Some studies reported an association of reduced response to chemotherapeutics and increased mortality in patients with haematological disorders as well as in patients with solid cancer [11,18–21]. Similar outcome with inferior prognosis is reported for patients who underwent kidney transplantation [22] or in case of being hospitalized for acute exacerbated chronic obstructive pulmonary disease [23]. However, the question whether elevated blood glucose is just a surrogate parameter for severe illness and adverse outcome [24] or if blood glucose might be a modifiable risk factor has not been answered yet. Given the fact that most of the studies yet performed employed observational designs and data derived from randomized controlled clinical studies are still substantially lacking, uncertainties whether asymptomatic and transient inpatient hyperglycaemia should be treated, remain [25].

4. Definition of Steroid Induced Hyperglycaemia

SIHG is defined as abnormally elevated blood glucose associated with the use of GCs in patients with or without pre-existing DM. The diagnostic criteria for SIHG do not differ from other types of diabetes and include a confirmed fasting blood glucose ≥7 mmol/L (≥126 mg/dL), a glucose level of ≥11.1 mmol/L (≥200 mg/dL) at 2 h following ingestion of 75 g glucose in an oral glucose tolerance test (OGTT), an HbA1c ≥6.5% (≥48 mmol/mol) or a random blood glucose ≥11.1 mmol/L (≥200 mg/dL) [26]. However, in patients with SIHG, diagnosis can be more challenging: fasting blood glucose might be normal especially when short- or intermediate-acting GCs are administered in single morning doses. Apart from its difficulties in implementation of oGTT in hospitalized patients, hyperglycaemia might be absent after glucose exposure in an oGTT, especially when it is performed in the morning when the diabetogenic effect of the GCs is not yet present. HbA1c might be inconspicuous especially in those with new-onset GC therapy as it reflects the glycaemic situation in the weeks prior to the time point of measurement. In addition, several conditions such as chronic kidney disease or hemoglobinopathies, that are frequently present in people requiring steroids, affect the reliability of HbA1c measurements. Nevertheless, determination of HbA1c can be useful to evaluate glycaemic control in patients who are on long-term GC therapy or to distinguish between new-onset diabetes and pre-existing DM in a situation of hyperglycaemia after GC initiation. Due to the mentioned limitations of the usual diagnostic approach to detect SIHG, it is recommended to perform frequent (capillary) glucose monitoring in those who receive high doses of GCs (defined as >20 mg prednisolone or equivalent). This approach is particularly recommended in people with a high risk to develop SIHG (e.g., advanced age, higher BMI, previously present impaired

glucose tolerance, prediabetes or family history of diabetes). Then, a random glucose value ≥11.1 mmol/L (≥200 mg/dL) can be utilized to establish the diagnosis of SIHG [27].

5. Treatment Targets

No clear evidence is available for the establishment of therapeutic goals for patients with SIHG [28]. According to the American Diabetes Association (ADA) glucose targets for patients with SIHG do not differ from those with any other type of diabetes and should be individualized according to specific factors such as life expectancy, comorbidities, patient compliance and risk of hypoglycaemia [29]. In hospitalized patients a target glucose range of 7.8–10.0 mmol/L (140–180 mg/dL) is recommended for the majority of critically and non-critically ill patients. More stringent goals such as 6.1–7.8 mmol/L (110–140 mg/dL) may be appropriate for selected patients, if this goal can be achieved without relevant hypoglycaemia [30]. However, when aiming to achieve lower target glucose levels it has to be considered that people with SIHG often suffer from severe underlying disease (e.g., cancer), are in the perioperative care setting (e.g., recently transplanted patients or those requiring steroids as supportive therapy [31]), receive concomitant complex therapies (chemotherapy, immunosuppressants, antimicrobial therapy, etc.) and thus, might be prone to larger glucose fluctuations. In the course of treatment, GCs need frequent dose adaptions that result in altered requirements of glucose lowering therapies. As a consequence, the risk for hypoglycaemia is increased when stringent glucose targets were chosen. Therefore, in specific patient populations (incurable disease with short life expectancy, advanced age and comorbidities, susceptibility for hypoglycaemia and impaired awareness of hypoglycaemia) the major aim will be the avoidance of hypoglycaemia and hyperglycaemic symptoms [29,32,33].

6. Admission to the Hospital

HbA1c should be assessed in all people with previous DM in order to evaluate glycaemic control prior to GC initiation. In people who were not previously diagnosed with DM and who require relevant amounts of GCs (>20 mg prednisolone or equivalent) or who are at high risk to develop diabetes or SIHG (criteria see Figure 2), HbA1c should be assessed at admission [34]. This helps to distinguish whether a pre-existing unrecognized DM is present which would result in a more pronounced glycaemic excursion following GC therapy initiation. It can also be assumed that hyperglycaemia is self-limiting after cessation of GC treatment and glucose levels return to normal, given that HbA1c levels were inconspicuous prior to GC treatment [35]. A position statement released by the Joint British Diabetes Societies (JBDS) has defined an algorithm for glucose monitoring in hospitalized patients requiring GC treatment. They postulate that determinations of glucose should be performed at least once daily, preferably prior to lunch or 1–2 h post lunch or before the evening meal in people without diabetes in whom GC therapy was initiated. Once daily glucose measurements should be continued and if glucose readings exceed 11.1 mmol/L (200 mg/dL) repeatedly, frequency of testing should be increased to 4 times daily (before each meal and at bedtime) which is mandatory in patients with pre-existing diabetes treated with GC [33]. In the course of hospitalization and scheduling of GC initiation, patients should be well informed about potential side effects of GCs including SIHG and its therapeutic consequences.

7. Initiation of Glucose Lowering Therapy

A practical approach when the implementation of glucose lowering therapy should be initiated was published by Suh et al. who recommend initiating therapy when pre- or post-prandial glucose repeatedly exceed 7.8 (140 mg/dL) or 11.1 mmol/L (200 mg/dL), respectively [28,33]. Similar to the management strategies to lower glucose in patients with type 2 diabetes (T2DM), stepwise intensification of antihyperglycaemic therapy and frequent re-evaluation should be performed in SIHG. The glucose lowering agents of

choice should match daily glucose profiles and the mechanism of action should fit to the corresponding GC agent.

8. Treatment of Steroid Induced Hyperglycaemia in the Hospital

8.1. Oral Antihyperglycaemic Agents

In the outpatient setting some oral hypoglycaemic agents (OHA) might have the potential to improve glycaemic control and prevent or delay the development of SIHG [36,37]. There is only very little evidence available showing clinical efficacy of using OHA for in-hospital hyperglycaemia caused by GCs. Insulin sensitizers such as metformin and pioglitazone might be used to enhance insulin sensitivity and reduce insulin resistance [38–41] and can be continued in preexisting T2DM unless contraindications exist. However, in hospitalized patients, specifically in those who are acutely ill, susceptibility to hypoxia or acute kidney injury as well as fluid retention can limit the use of these agents. In addition, in particular pioglitazone, needs an expanded time to exert full action which disqualifies it to be applied for acute SIHG. Insulin secretagogues, stimulating endogenous insulin production might be suitable to tackle mild SIHG in the inpatient setting, specifically in inpatients who are non-severely ill and who receive short-acting steroids once daily in the morning [33]. However, insulin secretagogues should be used with caution as there is an increased risk of hypoglycaemia especially when steroid doses are tapered or meals are skipped. The safe side effect profile of incretin mimetics such as DPP4 inhibitors might support their application in hospitalized patients with SIHG, their acute glucose lowering effect is of moderate extent and mostly they are mostly used as an adjunct to insulin therapy. The use of GLP1-receptor agonists bears the risk of gastrointestinal adverse effects in particular during the initiation phase which limits their broad usage for acutely ill, hospitalized patients with SIHG [28]. The use of the sodium-glucose co transporter-2 (SGLT2) inhibitor dapagliflozin has shown to be safe in patients hospitalized for chronic obstructive pulmonary disease (COPD) developing SIHG, but did not improve glycaemic control or clinical outcomes [42].

OHAs might be an adequate choice in inpatients with stable and non-critical disease and mild hyperglycaemic excursions. In those with significant hyperglycaemia and severe illness, insulin remains the treatment of choice in the hospital setting as also suggested by the current guidelines for inpatient diabetes management [30].

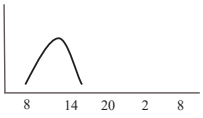
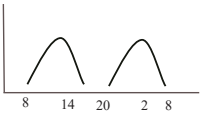
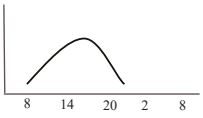
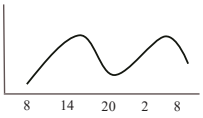
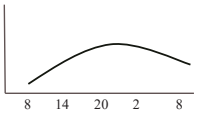
8.2. GC Dependent Glucose Increase and the Choice of Insulin Therapy

The hyperglycaemic effect of different GCs can be pragmatically transferred to the pharmacokinetic profiles of different GCs. Thus, the insulin therapy chosen for SIHG has to take the used agent, the current dose, the time point and interval of the GC administration into account. Table 1 summarizes the pharmacokinetics of available GCs adapted from the literature [43,44], Table 2 indicates potential glucose profiles according to the administered GC agent.

Table 1. Different corticosteroids and their equivalent doses, steroidal kinetics and potential to trigger hyperglycaemia.

Glucocorticoids		Approximate Equivalent Dose (mg)	Plasma Peak Concentration (minutes)	Elimination Half-Life (hours)	Duration of Action (hours)	Hyperglycaemic Effects (hours)		
						Onset	Peak	Resolution
Short-acting	Hydrocortisone	20	10	2	8–12	1	3	6
Intermediate-acting	Predniso(lo)ne	5	60–180	2.5	12–36	4	8	12–16
	Methylprednisolone	4	60	2.5	12–36	4	8	12–16
Long-acting	Dexamethasone	0.75	60–120	4	36–72	8	variable	24–36

Table 2. Schematic illustration of different glucocorticoids and their potential effect on glycaemia. Long-acting agents are usually administered only once daily. These examples are presuming people with normal glucose homeostasis prior to start of glucocorticoid therapy. X-axis: time of the day; y-axis: potential influence on glucose.

Glucocorticoids		Hyperglycaemic Effects (hours)			Glucose Profiles (GC Given Once Daily [8 a.m.])	Glucose Profiles (GC Given Twice Daily [8 a.m. and 20 p.m.])
		Onset	Peak	Resolution		
Short-acting	Hydrocortisone	1	3	6		
Intermediate-acting	Predniso(lo)ne	4	8	12–16		
	Methylprednisolone	4	8	12–16		
Long-acting	Dexamethasone	8	variable	24–36		n.a.

The upcoming paragraphs describe different scenarios of patients with normal glucose homeostasis under regular conditions as well as patients with T2DM well-controlled under dietary recommendations or treated with OHA in whom relevant hyperglycaemia is a consequence of GC administration who subsequently require insulin therapy. The paragraphs contain recently available recommendations which were given for people with new-onset hyperglycaemia or previously known T2DM.

8.2.1. Scenario 1: Short-Acting Glucocorticoids (Hydrocortisone)

Short-acting hydrocortisone has a considerably high mineralocorticoid activity and is therefore suitable as first-line agent in the therapy of adrenal insufficiency. In its usual application as hormone replacement therapy, hydrocortisone should not cause relevant hyperglycaemia if the substance is administered in physiological doses. For these reasons, no data of SIHG induced by short-acting GCs are available and the recommendations arise from speculations. However, the required physiological doses are often overestimated and exogenous Cushing syndrome including SIHG can occur [45]. In addition, in specific conditions such as acute illness, stress or during surgery substantial dose increases can be required that might induce SIHG. Hydrocortisone is characterized by a fast onset and short duration of the intended effect. Simultaneously, the expectable glucose profile in selected patients will show to have a fast and strong increase but only of short duration. Hence, these commonly transient and mostly self-limiting glucose peaks remain often unrecognized. Whether these short-term hyperglycaemic episodes require glucose lowering therapy has to be decided on an individual basis. In patients with significant hyperglycaemia or impaired health status, the agent of choice is short-acting insulin (rapid-acting insulin analogues or regular insulin) which should be injected at the time or shortly after GC administration. As hydrocortisone is usually administered twice or thrice daily, multiple rapid-acting insulin doses might be suitable to improve glycaemic control. However, it has to be taken into account that morning doses during replacement therapy are usually higher than doses throughout the day and insulin requirements thus might be lowered subsequently. Initiation of the dose can be recommended with 0.1 IU/kilogram (kg) bodyweight (BW) [46]. In addition, insulin therapy can be intensified by including insulin corrections in case of higher subsequent glucose values or persisting post-prandial hyperglycaemia

assuming that the intensification requires pre/post-prandial glucose assessments. In these cases, schematic increments of 0.04 IU/kg for pre-prandial values from 11.1–16.7 mmol/L (200–300 mg/dL) or 0.08 IU/kg for values ≥ 16.7 mmol/L (≥300 mg/dL) can be added to the scheduled insulin dose. It is important to mention that insulin requirements are GC dose-dependent; hence, reduction of GC is usually related to an improvement of glycaemia. Reduction of rapid-acting insulin should be performed proportionally to the reduction in GC dose, vice versa rapid-acting insulin dose can be increased when doses of GCs are recommended to be increased [2,46].

8.2.2. Scenario 2: Intermediate-Acting Glucocorticoids (Predniso(lo)ne and Methylprednisolone)

Intermediate-acting glucocorticoids represent the most commonly prescribed steroid agents. Their high glucocorticoid activity makes them useful for long-term anti-inflammatory and immunosuppressant treatment especially in solid-organ transplant patients and those with COPD. Considering a single dose administration in the morning, which corresponds to the typical prescription, hyperglycaemia develops slowly, but continuously, mostly lasts until the evening and gradually recovers until the next morning simultaneously following the peak and duration of action of the steroid agent. To best fit this glucose pattern short- or intermediate-acting basal insulins such as insulin detemir or NPH (neutral protamine Hagedorn) insulin is recommended. A clinical recommendation to initiate insulin was issued by Clore et al. who suggest initiating a weight-dependent scheme with 0.4 IU/kg of NPH insulin [47]. Another study described clinical efficacy when lower doses of NPH (0.2–0.3 IU/kg) dependent of the GC dose were administered and whether patients were fasting or not [48]. While the kinetics of intermediate-acting glucocorticoids appear to fit best to the glucose lowering property of NPH insulin, two randomized studies with insulin glargine U100 at a fixed starting dose of 0.5 IU/kg [49] or initiated according to admission glucose (0.3 or 0.4 IU/kg) [50] demonstrated non-inferiority compared to NPH insulin in regards of efficacy and safety, including nocturnal hypoglycaemia. A sufficient performance was also confirmed in a study which used insulin glargine U100 incorporated in a clinical decision support system for the treatment of in hospital SIHG [51]. Probably a reasonable and simple approach is to initiate basal insulin in a GC dose-dependent dose, starting with 0.1 IU/kg BW if patients receive 10 mg of prednisone or equivalent and 0.2 IU/kg BW in case GC dose is 20 mg, 0.3 IU/kg BW when dose was set at 30 mg and so on [47,52]. Insulin dose finding based on patient age and kidney function has been proposed, indicating that initial doses should be lower in those with impaired kidney function (eGFR < 30 mL/min/1.73 m^2) or older than 70 years [17,33]. Subsequent dose adjustments should be based on achievement of glycaemic targets assessed by glucose measurements performed the next morning given that the GC is taken in the morning. Multiple daily administrations of intermediate-acting GCs are more complex as hyperglycaemia might overlap and persistent hyperglycaemia can occur (see glucose profile in Table 2). In this case, NPH insulin once daily will not be sufficient and NPH twice daily or a switch to longer-acting insulin (e.g., glargine) is required. If necessary, additional rapid-acting insulin boluses might be added. This can be established by either correctional bolus insulin (correction factor see scenario 1) or by switching to premixed insulin with a mixture of 70% rapid-acting and 30% basal insulin administered simulously to the GC intake [46].

8.2.3. Scenario 3: Long-Acting Glucocorticoids (Dexamethasone)

Dexamethasone, as the most potent GC agent, is characterized by a prolonged duration of action lasting for more than 24 h. It is clinically used in various scenarios such as in inflammatory diseases, as an analgesic or for the reduction of brain pressure in cerebral cancer or cerebral edema. In the recent severe acute respiratory syndrome coronavirus type 2 (COVID-19) pandemic, dexamethasone has been recommended for those with impairments in gas exchange due to viral pneumonia [53], irrespective of diabetes status. This approach needs to be further investigated in people with diabetes as deterioration of glycaemic control and new-onset hyperglycaemia were associated with inferior outcome

in people with COVID-19 [54–57]. For hyperglycaemia during dexamethasone treatment for COVID-19, Rayman et al. have recently published a guidance article. In insulin naïve patients, they recommend to start NPH insulin when glucose exceeds a threshold of 12 mmol/L (~216 mg/dL) in a dose of 0.3 IU/kg/day while 2/3 should be administered in the morning and the remaining third in the evening. They also propose a dose reduction to 0.15 IU/kg in case of age >70 years or eGFR below 30 mL/min. Insulin doses are recommended to be titrated according to morning or evening glucose vales in a manner of a reduction of 20% if glucose falls below 4.1 mmol/L (~70 mg/dL) or decreased by 10% in case of glucose between 4.1–6.0 mmol/L (~70–110 mg/dL). Vice versa, insulin dose should be up-titrated by 20% if glucose values exceed 18 mmol/L (~320 mg/dL) and by 10% if glucose values are between 12.1 and 18 mmol/L (~220–320 mg/dL) [58]. In general, hyperglycaemia in association with long-acting GCs, which are usually administered in the morning, develops slowly, peaks during the day (varying time point) and is sustained for 24 h after intake. Thus, intermediate-acting basal insulins (NPH insulin, insulin detemir) should be prescribed twice daily (initial dose 0.3 IU/kg BW). Alternatively, long- or ultralong-acting basal insulin analogues (insulin glargine U100/U300 or insulin degludec) might be the most appropriate insulin to control hyperglycaemia in this situation (initial dose 0.2 IU/kg BW). Insulin dose should be adjusted according to glucose 24 h after GC intake and onset of hyperglycaemia. To date, to the best of our knowledge, not a single study has been conducted to test new generation ultra-long-acting basal insulin analogues for the treatment of SIHG.

8.3. Insulin Intensification and Adjustments

Especially in those without pre-existing diabetes prior to GC treatment, it is of utmost importance for insulin titration to know current GC dose and GC dose changes (tapering or increase). In a pragmatic approach, insulin dose can be adjusted by half the percentage of the GC dose change. For example, when GCs are increased or tapered by 50%, insulin dose is suggested to be increased or reduced by 25%, respectively. In patients with pre-existing DM a deterioration of glycaemic control secondary to GC therapy can be expected. In this regard, type of GC agent as well as time point and interval of GC application have to be taken into account.

8.3.1. Adjustment of Basal Insulin Therapy

When basal insulin therapy was already initiated, up-titration by 10–20% should be performed in case of sustained hyperglycaemia (fasting glucose exceeding 11.1 mmol/L [200 mg/dL]) on 2–3 subsequent days [17,33]. Alternatively, adjustments can be performed in 2 IU increments (conservative approach) to reach the individual glucose target; however, a steady dose adjustment must be warranted. Persisting hyperglycaemia despite basal insulin titration with predominantly postprandial hyperglycaemia requires additional rapid-acting insulin administrations either as rapid-acting insulin injection or incorporated in premixed insulins.

8.3.2. Adjustment of Rapid-Acting Insulin Therapy

Rapid-acting insulins should be primarily administered at the time point of GC administration and can be initiated with 0.1 IU/kg BW. In addition, rapid-acting insulin should be used to correct pre-prandial and spontaneous hyperglycaemia. In such cases add-on of 0.04 IU/kg for pre-prandial values from 11.1–16.7 mmol/L (200–300 mg/dL) or 0.08 IU/kg for values $\geq$ 16.7 mmol/L ($\geq$300 mg/dL) can be additionally added to the scheduled insulin dose. It is important to mention that insulin requirements depend on GC dose; hence, reduction of GC is usually accompanied by an improvement of glycaemia. Reduction of rapid-acting insulin should be performed proportionally to the reduction in GC dose, vice versa rapid-acting insulin dose can be increased when GC doses are increased [2,46].

8.3.3. Adjustment of Basal-Bolus Insulin

In patients with pre-existing basal-bolus insulin therapy doses of basal and bolus insulin should be adjusted according to the above recommendations. However, those with endogenous insulin deficiency (as people with type 1 diabetes) are more prone to hypoglycaemia which has to be considered when doses are increased [59]. A specific approach how to adjust insulin in people with preexisting type 1 diabetes is given in Section 8.3.5.

A schematic algorithm for the initiation and intensification of glucose lowering therapy in SIHG is illustrated in Figure 1. This algorithm is not valid for patients with preexisting type 1 diabetes.

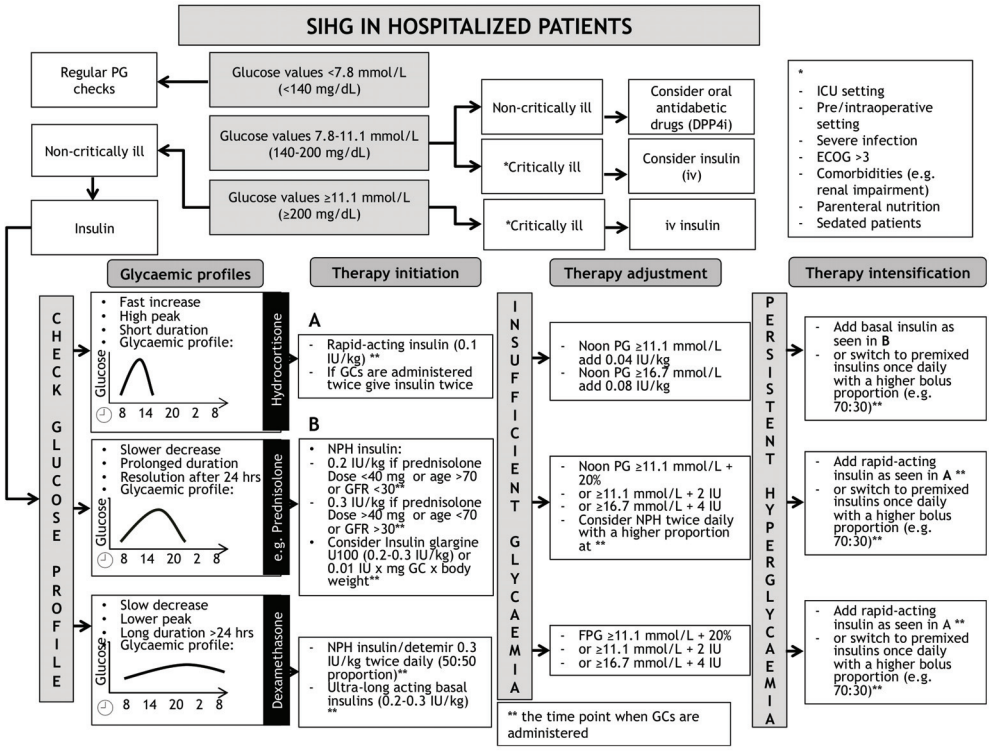


Figure 1. Opinion-based schematic algorithm for initiation, adjustment and intensification of insulin therapy for treatment of SIHG. DPP4i = Dipeptidyl-Peptidase4-inhibitor, ECOG = Karnofsky index, FPG = Fasting plasma glucose, GC = Glucocorticoid, ICU = Intensive Care Unit, IU = International Units, NPH = Neutral Protamine Hagedorn, SIHG = Steroid induced hyperglycaemia. * = definition of critical illness, ** = indicating the time point when glucocorticoids are administered. (**A**) indicates recommendations for initiation of rapid-acting insulin. (**B**) indicates recommendations to initiate basal in-sulin.

8.3.4. Adjustment of Insulin Therapy in Patients with Type 1 Diabetes (T1DM)

It has been shown that relevant doses of transiently administered GCs (in the referenced study 60 mg prednisone/day) lead to an increase in insulin requirements of 70% on average with considerable inter-individual variation to normalize blood glucose levels in patients with previously known well controlled T1DM. This glucose increase was sustained the day after GC therapy was discontinued, indicating a longer lasting hyperglycaemic effect despite the use of an intermediate-acting GC agent. Interestingly, the GC induced additional insulin requirements to achieve reasonable glycaemic control varied considerably and independently from previous insulin dose (30–100% increase) which makes recommendations for adjustments challenging. [60]. Dashora et al. described a 50%

increase in insulin requirements in females with T1DM requiring variable doses of GC therapy for treatment of hyperemesis gravidarum [61].

Due to the heterogeneity of the effect of GC on glucose metabolism in patients with T1DM it is recommended to intensify frequent monitoring of glucose upon initiation of GC therapy, as deterioration of glycaemic control has to be expected. As patients with T1DM are more prone to hypoglycaemia in comparison to patients with T2DM, initial dose adjustments have to be taken very carefully and in an iterative manner [33]. GCs are a well-known trigger for diabetic ketoacidosis in patients with deficiency or absence of endogenous insulin secretion, thus proper insulin dose adjustments to the GC therapy are recommended and transient hyperglycaemia should not be trivialized in these patients. Clinical evidence for insulin dose adjustments for patients with T1DM on GC therapy both, in the inpatient or in the outpatient setting, is largely lacking and not described in detail in any treatment guideline [62]. Moreover, the present article discusses SIHG in the hospital setting where besides GC therapy, numerous other factors such as acute disease and altered daily routine additionally influence glucose control. As there is only very little evidence available we suggest a cautious increase in total daily insulin dose (TDD) according to prednisolone (or prednisolone equivalent [PE]) dose, a suggestion that needs further scrutiny in clinical practice:

- PE of 20 mg → 10% increase in TDD
- PE of 40 mg → 20% increase in TDD
- PE of 60 mg → 30% increase in TDD

Taking these estimations into account the following considerations are important:

- Adjustments of insulin therapy when short-acting steroids (hydrocortisone) are used:
  - If short acting GCs are used, then an increase of rapid-acting insulin at the time point of GC intake might be sufficient. A correctional rapid-acting insulin dose can be administered in case of persistent hyperglycaemia after 3–4 h when the rapid-acting insulin action has tapered off. As a consequence, the ratio of rapid-acting to basal insulin will exceed the usual 50:50 ratio.
- Adjustments of insulin therapy when intermediate-acting steroids (e.g., prednisolone) are used:
  - Approach A: An increased dose of rapid-acting insulin at the time of intermediate-acting prednisolone administration might be appropriate aiming to achieve glucose control at noon.
  - Approach B: In case of pre-existing therapy with intermediate-acting basal-insulins (NPH insulin or insulin detemir) that are usually injected twice daily, a dose increases at the time point of GC intake (usually in the morning) is recommended.
  - Approach C: In patients previously using (ultra-)long acting basal-insulins (insulin glargine U100/U300 or insulin degludec), approach A might be sufficient; in case of an expected long-term GC treatment, these patients might benefit most from a switch to intermediate-acting basal insulins (NPH insulin, insulin detemir. In such case, the basal insulin should be injected twice daily with a proportionally higher dose at the time point when the GC agent is administered.
- Adjustments of insulin therapy when long-acting steroids (e.g., dexamethasone) are used:
  - Long-acting GCs will trigger continuous and long-lasting hyperglycaemia over 24 h, thus it might be suitable to adjust the total daily basal-insulin dose according to the GC dose as outlined above.

Of note, the continuation of using preexisting insulin pump therapy (continuous subcutaneous insulin infusion [CSII]) in the hospital is not recommended in the majority of cases especially in those who are acutely hospitalized and severely ill. In patients without physical or mental disorders, the self-managed continuation of CSII therapy might be justified [63,64]. CSII systems provide adjustable basal rates, programming of different basal rate profiles as well as a temporary % increase/decrease of the current basal rate.

Moreover, bolus dosing can be performed more frequently to administer correctional insulin when required without an additional injection as in pen-based therapy. Thus, in insulin pump users the continuation of insulin pump therapy with according to adjustment of insulin dose might be a considerable option if deemed practicable by the physicians in charge. However, clinical evidence supporting this presumption is not available yet.

In summary, the adjustment of insulin doses in patients with complex previous insulin therapies (i.e. T1DM) can be performed according to the above recommendations, which are quite carefully elaborated, but still require additional individualization, frequent glucose monitoring and close-meshed therapy adjustments.

8.3.5. The Critically Ill Patient

Hyperglycaemic derailments as well as severe and subsequent hypoglycaemia might complicate the clinical course in patients hospitalized on intensive medical care units and might impact on adverse outcomes. During critical illness, factors such as stress, inflammation, failure of kidney function or administered therapeutics, specifically GCs, detrimentally impact on glucose metabolism in people with and without previously known diabetes. In most of patients with critical illness and hyperglycaemia, insulin therapy should be introduced as continuous intravenous application [30]. Intravenous insulin provides the advantage of more rapid insulin adjustments to hyperglycaemic levels. Rapid-acting human insulin or analogues should be prepared by 50 IU rapid-acting insulin mixed with 50 mL sodium chloride (0.9%) with a starting dose of 0.1 IU/kg/h [64]. The switch to subcutaneous insulin is recommended when patient status improves (e.g., uptake of oral nutrition, scheduled transfer to general ward) and metabolic status is balanced. Basal insulin can be started at a dose of 50% of the previous 24 h insulin dose as administered intravenously in an overlapping manner (basal insulin application 2 h prior to cessation of intravenous insulin) in order to prevent rebound hyperglycaemia or acidosis [64]. Of course, also critically ill patients with SIHG should be treated with intravenous insulin, however, no specific recommendations for the treatment with intravenous insulin differing from the recommendation in "usual" critical care hyperglycaemia are available. The used insulin dose of the intravenous insulin application can help to estimate the appropriate dose of subcutaneously administered insulin.

9. Discharge from the Hospital

GCs frequently need to be continued after the inpatient stay and hence, hyperglycaemia also might persist [12]. Of note, hyperglycaemic state remains often also in those where GC therapy was discontinued indicating that people with SIHG are prone to develop T2DM.

Based on recommendations of a guideline published by the Joint British Diabetes Societies [33], it is necessary that all patients should be informed about the nature of SIHG, symptoms of hypo- and hyperglycaemia and its consequences if not properly treated. If applicable, patients should be trained in the use of insulin pens and self-monitoring of blood glucose (SMBG). Patients should be advised in the frequency of necessary SMBG and recommended to document glucose values and if applicable insulin doses. Optimally, all patients, irrespective of diabetes therapy at hospital discharge should have access to adequate glucose monitoring technology at home in order to avoid subsequent relevant hyperglycaemia. Adequate and individualized treatment plans should be made available to patients to avoid consecutive presentations at emergency departments potentially resulting in hospital readmissions. Individual therapy regimens should be prepared which contain recommendations for insulin dosing and which give a chance of self-adjustments especially taking into account possible dose changes in the GC therapy. Patients should be offered the possibility to regularly contact the medical staff of the outpatient clinic in case of concerns or problems regarding current glycaemic control. The general practitioner should be introduced in the case and preferentially take the lead concerning the management of the hyperglycaemic state. In addition, HbA1c should be measured every three months [65]. A possible admission and discharge algorithm is illustrated in Figure 2.

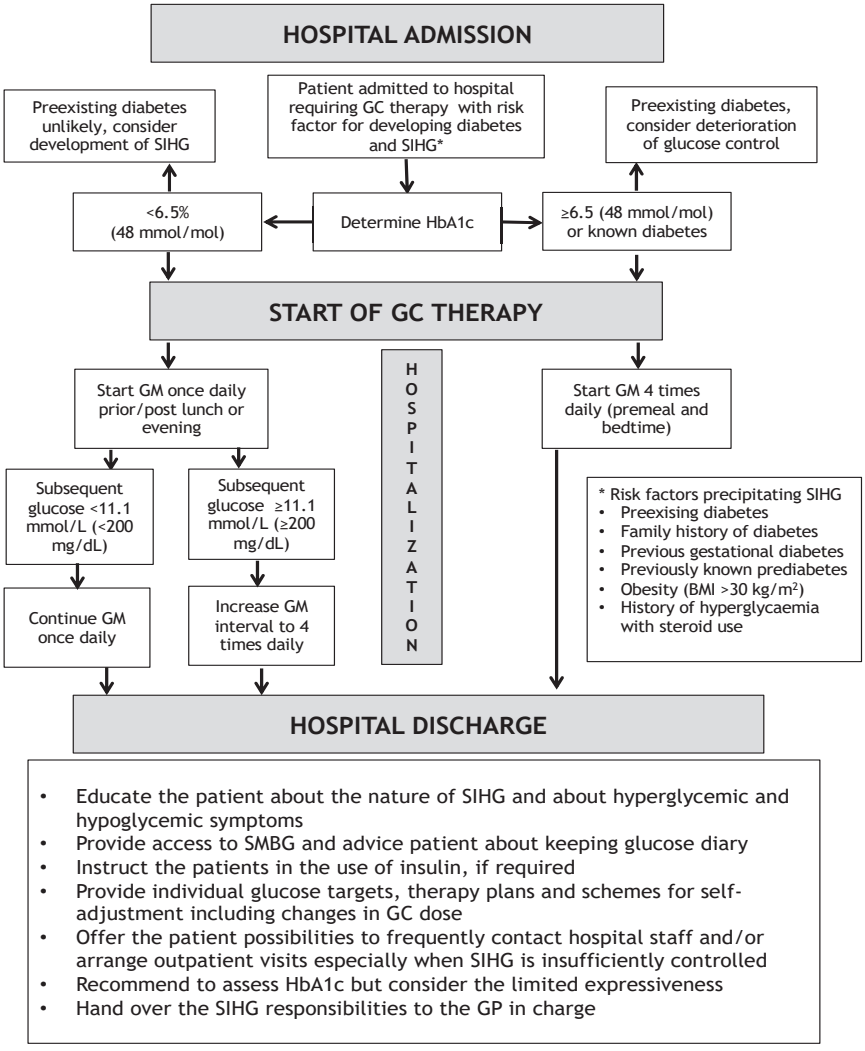


Figure 2. Opinion based admission and discharge algorithm for hospitalized patients with SIHG modified from [33]. BMI = Body mass index, GC = Glucocorticoids, GM = Glucose Monitoring, GP = General practitioner, SIHG = Steroid induced hyperglycaemia, SMBG = self-monitored blood glucose. * = risk factors for steroid induced hyperglycaemia.

10. Discussion

GCs are frequently prescribed as they have been confirmed to potentially improve outcomes in various autoimmune and inflammatory diseases, as well as recently also in COVID 19 [66]. Relevant doses of GC therapy potentially lead to hyperglycaemia in both hospitalized patients and patients in outpatient care exposing them to a higher risk of acute and chronic complications.

Diagnosis, monitoring and in particular the management of SIHG represents an everyday challenge and often physicians not specifically working in the field of diabetes and endocrinology have to deal with the management. Therefore, this review aims to provide summary figures that can be used by clinicians for the management of SIHG in routine care. There is a large amount of data available which confirmed the potential burden of chronic hyperglycaemia in both type 1 and type 2 diabetes. In contrast, whether

mild, asymptomatic and mostly transient hyperglycaemia, specifically in hospitalized patients impacts on outcome, has not been systematically investigated, specifically not in those with SIHG [25]. Observational data identified elevated glucose as potential biomarker for adverse surgical outcomes, longer hospital stays and an increased mortality [67–69]. Vice versa, aggressive glucose lowering therapy in hospitalized patients, in particular using insulin with the risk of causing hypoglycaemia has also shown to negatively impact mortality [70]. For SIHG, which represents a different but important entity, clinical data, both from observational and interventional trials, is largely lacking.

Certainly, there is an increasing need to provide more evidence which, first, identifies the most safe and efficient therapy modalities to treat SIHG, secondly, sets a basis for defining recommended glucose targets and thirdly, allows to answer the question whether improved glycaemia translates to superior outcomes. In addition, novel diabetes technology such as continuous glucose monitors, electronic decision support systems and automated insulin delivery systems might be beneficial to better control SIHG and provide more outcome data in the near future.

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Eating Speed and Incidence of Diabetes in a Japanese General Population: ISSA-CKD

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Abstract: Background: We investigated whether eating speed was associated with the incidence of diabetes in a Japanese general population. Methods: A total of 4853 Japanese individuals without diabetes at baseline were analyzed. Self-reported eating speed was categorized as slow, medium, and fast on the basis of questionnaire responses. The study outcome was the incidence of diabetes. Results: After an average follow-up period of 5.1 years, 234 individuals developed diabetes. The incidence of diabetes per 1000 person-years was 4.9 in the slow eating speed group, 8.8 in the medium eating speed group, and 12.5 in the fast eating speed group, respectively (*** $p < 0.001$ for trend). The HRs were 1.69 (95%CI 0.94–3.06) for the medium eating speed and 2.08 (95%CI 1.13–3.84) for the fast eating speed, compared to the slow eating speed (* $p = 0.014$ for trend) after adjustment for age, gender, smoking status, drinking, exercise, obesity, hypertension, and dyslipidemia. Conclusion: Faster eating speed increased a risk for the incidence of diabetes in a general Japanese population.

Keywords: diabetes; eating speed; primary prevention; lifestyle

1. Introduction

Diabetes is a life-threatening disease that causes microvascular and macrovascular complications [1–6]. Diabetes is considered as a serious disorder that doubles the risk of premature death [7]. A longitudinal study demonstrated that the incidence rate of coronary artery disease per 1000 person-years in Japanese patients with type 2 diabetes was 9.59, which is approximately three times higher than the general population [8]. In Japan, diabetic kidney disease is the leading cause (43.5%) among new dialysis patients [9]. The number of people with diabetes and impaired glucose tolerance in Japan is estimated at 20 million, and this number has been increasing since 1997 [10]. According to the 2016

National Health and Nutrition Survey by the Japanese Ministry of Health, Labor and Welfare, the prevalence rate of type 2 diabetes in Japan was 12.1%. The effective prevention of type 2 diabetes requires up-to-date knowledge of risk factors for the disease.

It has been shown that interventions seeking to impact lifestyle behaviors, including improving dietary and exercise habits, can prevent the onset of type 2 diabetes [11–13]. Obesity [11–14] and insufficient exercise have been implicated as established modifiable risk factors for type 2 diabetes [15–17], as well as impaired glucose tolerance, smoking [18], alcohol intake [19,20], and inadequate diet (calorie intake and content) [11–13,21–23]. Several studies have shown that fast eating is associated with the increased risk of type 2 diabetes [24–26]. Previous studies have used questionnaires to classify eating speed. For example, the question was "Do you eat faster than people who eat together at the same table?" or "Do you eat faster than people of the same generation?" In one study, the eating speed was classified into five groups (very slow, relatively slow, medium, relatively fast, and very fast) [25]. However, the evidence on this topic is mainly derived from case–control studies or studies conducted among special populations (e.g., worksite populations), and it is unclear to what extent this evidence is generalizable to general populations. The aim of this large-scale population-based study was to examine the effect of eating speed on the development of diabetes in a general population in Japan.

2. Materials and Methods

2.1. Study Design

The Iki City Epidemiological Study of Atherosclerosis and Chronic Kidney Disease (ISSA-CKD) is a population-based retrospective cohort study that uses annual health checkup data for the citizens of Iki City, Nagasaki Prefecture, Japan. ISSA-CKD has been described in the accompanying literatures [27–30]. The present study was conducted according to the guidelines of the Declaration of Helsinki of 1975, revised in 2013, and approved by the Fukuoka University Clinical Research and Ethics Center (No.2017M010).

2.2. Participants

A total of 7895 individuals received annual health checkups from 2008–2017. Of these people, 3042 (38.5%) were excluded: 1881 dropped out from consecutive follow-up annual medical checkups, and 1161 had diabetes at baseline. Thus, 4853 citizens were analyzed in this study.

2.3. Data Collection

At baseline, we collected information on eating speed, using a questionnaire with the following question: "How fast is your eating speed compared with others?" The response categories were slow, medium, and fast. Information on smoking, alcohol drinking, regular exercise, family history of diabetes, and current use of medications for hypertension, dyslipidemia, and diabetes was also collected via questionnaire. We defined obesity as a BMI $\geq$ 25 kg/m^2. Participants who had smoked 100 cigarettes or more, or who had smoked regularly for 6 months and more were defined as currently smoking. Drinking behavior was defined as drinking on 5 days or more per week. Regular exercise was defined as exercising $\geq$30 min/day at least twice a week. Hypertension was defined as a systolic blood pressure of 140/90 mmHg or more or use of blood pressure-lowering medicine. Fasting or casual blood and urine samples were collected. Plasma glucose levels were measured by an enzymatic method, and glycated hemoglobin (HbA1c) levels (National Glycohemoglobin Standardization Program value) were determined by a high-performance liquid chromatography method. The diagnosis of diabetes was determined by a fasting glucose level $\geq$ 6.99 mmol/L, casual blood glucose level $\geq$ 11.10 mmol/L, HbA1c $\geq$ 6.5%, or the use of glucose-lowering therapies. Serum low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride concentrations were measured enzymatically. Dyslipidemia was defined as LDL cholesterol $\geq$ 3.62 mmol/L, HDL cholesterol < 1.03 mmol/L, triglycerides $\geq$ 1.69 mmol/L or the use of lipid-lowering medication.

2.4. Outcome

The incidence of diabetes (fasting glucose level ≥ 6.99 mmol/L, casual blood glucose level ≥ 11.10 mmol/L, HbA1c ≥ 6.5%, or the use of glucose-lowering therapies) at the end of follow-up.

2.5. Statistical Analysis

Continuous variables were expressed as means ± SD. Simple regression models were used to determine trends across tertile groups of eating speed. Categorical variables were expressed as the number (percentage) of participants. Logistic regression models were used to test trends across groups. Incidence rates of diabetes were expressed by person-year. We estimated crude and multivariable-adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs) of the effect of eating speed on the development of diabetes by the use of Cox proportional hazards models. Then, we next adjusted for age, sex, smoking status, alcohol drinking, exercise, obesity, hypertension and dyslipidemia. A two-tailed p value of less than 0.05 was considered statistically significant. Analyses were performed using SAS, Version 9.4.

3. Results

The average age of the participants at baseline was 59.6 years, 55.5% were women, and the average BMI was 23.6 kg/m^2. The mean baseline fasting blood glucose level was 5.1 ± 0.5 mmol/L, and the mean HbA1c level was 5.1 ± 0.4%. A total of 1350 people (27.8%) were classified in the fast eating speed group, 2993 (61.7%) were classified in the medium eating speed group, and 510 (10.5%) were classified in the slow eating speed group. Table 1 shows the baseline characteristics. A self-reported faster eating speed was associated with younger age, higher BMI, higher triglycerides, and lower levels of HDL-cholesterol.

Table 1. Baseline characteristics by self-reported eating speed.

	Self-Reported Eating Speed			p Value for Trend
	Slow	Medium	Fast	
	(N = 510)	(N = 2993)	(N = 1350)	
Age, mean (SD), years	61.6 (±10.7)	59.8 (±10.5)	58.5 (±10.8)	*** < 0.001
Male, N/total N (%)	180/510 (35.3%)	1271/2993 (42.5%)	709/1350 (52.5%)	*** < 0.001
Smoking status, N/total N (%)				
Never smoker	423/510 (82.9%)	2275/2993 (76.0%)	975/1350 (72.2%)	*** < 0.001
Ex-smoker	19/510 (3.7%)	151/2993 (5.0%)	89/1350 (6.6%)	
Current smoker, <20 cigarettes/day	17/510 (3.3%)	134/2993 (4.5%)	72/1350 (5.3%)	
Current smoker, ≥20 cigarettes/day	22/510 (4.3%)	226/2993 (7.6%)	129/1350 (9.6%)	
Current smoker, missing information on the number of cigarettes/day	29/510 (5.7%)	207/2993 (6.9%)	85/1350 (6.3%)	
Alcohol intake †, N/total N (%)				
No	305/505 (60.4%)	1609/2970 (54.2%)	649/1342 (48.4%)	** 0.004
Occasional alcohol drinking	100/505 (19.8%)	680/2970 (22.9%)	347/1342 (25.9%)	
Daily current alcohol drinking, <20 g/day	43/505 (8.5%)	221/2970 (7.4%)	97/1342 (7.2%)	
Daily current alcohol drinking, 20–39.9 g/day	39/505 (7.7%)	318/2970 (10.7%)	182/1342 (13.6%)	
Daily current alcohol drinking, ≥40 g/day	18/505 (3.6%)	142/2970 (4.8%)	67/1342 (5.0%)	
Regular exercise ‡, N/total N (%)	120/510 (23.5%)	809/2993 (27.0%)	357/1350 (26.4%)	0.451
Body mass index, mean (SD), kg/m^2	22.7 (±3.3)	23.3 (±3.3)	24.5 (±3.6)	*** < 0.001
Obesity §, N/total N (%)	101/510 (19.8%)	815/2993 (27.2%)	554/1350 (41.0%)	*** < 0.001
Systolic blood pressure, mean (SD), mmHg	128.7 (±19.6)	129.0 (±18.3)	128.9 (±19.0)	0.987
Diastolic blood pressure, mean (SD), mmHg	73.8 (±10.8)	74.8 (±11.1)	75.6 (±11.3)	** 0.002
High-density lipoprotein cholesterol, mean (SD), mmol/L	1.63 (±0.41)	1.62 (±0.42)	1.55 (±0.41)	*** < 0.001
Low density lipoprotein cholesterol, mean (SD), mmol/L	3.10 (±0.82)	3.18 (±0.81)	3.20 (±0.82)	0.06
Triglyceride, mean (SD), mmol/L	1.29 (±0.93)	1.28 (±0.84)	1.44 (±1.03)	*** < 0.001
Dyslipidemia ¶, N/total N (%)	194/510 (38.0%)	1244/2993 (41.6%)	642/1350 (47.6%)	*** < 0.001
Hypertension ††, N/total N (%)	209/510 (41.0%)	1272/2993 (42.5%)	588/1350 (43.6%)	0.308
HbA1c, mean (SD),%	5.1 (±0.3)	5.1 (±0.4)	5.1 (±0.4)	0.669
Fasting blood glucose (SD), mmol/L †††	5.0 (±0.5)	5.0 (±0.5)	5.1 (±0.6)	** 0.0013

† Habitually drinking on 5 or more days per week. ‡ Habitually exercising ≥ 30 min per day twice or more per week. § Body mass index ≥ 25 kg/m^2. ¶ Low-density lipoprotein cholesterol ≥ 3.62 mmol/L, high-density lipoprotein cholesterol < 1.03 mmol/L, triglycerides ≥ 1.69 mmol/L, or use of lipid-lowering medication. †† Systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or use of blood pressure-lowering medication. ††† Available for 381 participants in the slow group, 2230 in the medium group, and 1017 in the fast group. ** $p < 0.05$, *** $p < 0.001$.

During an average follow-up of 5.1 years (24,745 person-years), 234 individuals developed diabetes (incidence rate: 9.4 per 1000 person-years). Table 2 shows the risks of diabetes by reported eating speed. The incidence rates (per 1000 person-years) were 4.9 for the slow eating speed group, 8.8 for the medium eating speed group, and 12.5 for the fast eating speed group ($p < 0.001$ for trend). These associations remained statistically significant even after adjustment for age, gender, smoking status, drinking habits, exercise habits, obesity, hypertension, and dyslipidemia: The multivariable-adjusted HRs (95% CIs) were 1.69 (0.94–3.06) for medium eating speed, and 2.08 (1.13–3.84) for fast eating speed, compared with the reference group of slow eating speed (* $p = 0.014$ for trend). When BMI (instead of obesity), systolic blood pressure (instead of hypertension), HDL-c and triglycerides (instead of dyslipidemia) were included in multivariable analysis as covariates, the hazard ratios were 1.72 (95% CIs 0.95–3.11) for medium eating speed and 1.94 (95% CIs 1.05–3.58) for fast eating speed compared with slow eating speed. When waist circumference (instead of BMI) was included in multivariable analysis as covariate, the hazard ratios were 1.72 (95% CIs 0.94–3.08) for medium eating speed and 2.05 (95% CIs 1.15–3.78) for fast eating speed compared with slow eating speed.

Table 2. Risk of diabetes mellitus by self-reported eating speed.

	Self-Reported Eating Speed			***p* Value for Trend**
	Slow	**Medium**	**Fast**	
	(N = 510)	**(N = 2993)**	**(N = 1350)**	
N of events/person-years	12/2468	134/15,234	88/7034	
Incidence rate (per 1000 person-years)	4.9	8.8	12.5	
Crude hazard ratio	1	1.82	2.61	*** < 0.001
(95% Confidence interval)	(Reference)	(1.01–3.29)	(1.43–4.77)	
Adjusted hazard ratio †	1	1.69	2.08	** 0.014
(95% Confidence interval)	(Reference)	(0.94–3.06)	(1.13–3.84)	

† Adjusted for age, sex, smoking status, alcohol drinking, exercise, obesity, hypertension and dyslipidemia. ** $p < 0.05$, *** $p < 0.001$.

Table 3 shows the results of the subgroup analysis. The effect of reported eating speed on the development of diabetes was comparable across the subgroups defined by age, gender, obesity, hypertension, dyslipidemia, smoking, drinking habits, and regular exercise (all $p > 0.1$ for the interactions).

Table 3. Subgroup analysis.

	Self-Reported Eating Speed			***p* Value for Interaction**
	Slow	**Medium**	**Fast**	
	(*N* = 510)	**(*N* = 2993)**	**(*N* = 1350)**	
Age				
<65 years	1 (reference)	1.04 (0.48–2.29)	1.52 (0.68–3.36)	0.105
≥65 years	1 (reference)	2.64 (1.06–6.55)	2.61 (1.01–6.79)	
Sex				
Male	1 (reference)	2.48 (0.91–6.80)	3.03 (1.09–8.42)	0.617
Female	1 (reference)	1.28 (0.61–2.68)	1.57 (0.72–3.43)	
Obesity				
Yes	1 (reference)	1.35 (0.54–3.37)	1.94 (0.77–4.87)	0.462
No	1 (reference)	1.97 (0.91–4.29)	2.02 (0.88–4.61)	
Hypertension				
Yes	1 (reference)	1.91 (0.83–4.40)	2.31 (0.98–5.45)	0.895
No	1 (reference)	1.47 (0.63–3.41)	1.74 (0.73–4.17)	
Dyslipidemia				
Yes	1 (reference)	1.77 (0.71–4.41)	2.39 (0.95–6.02)	0.402
No	1 (reference)	1.76 (0.80–3.84)	1.88 (0.82–4.31)	

Table 3. *Cont.*

	Self-Reported Eating Speed			p Value for Interaction
	Slow	Medium	Fast	
	(N = 510)	(N = 2993)	(N = 1350)	
Current smoking				
Yes	1 (reference)	1.26 (0.38–4.12)	1.10 (0.31–3.84)	0.349
No	1 (reference)	1.82 (0.92–3.61)	2.48 (1.23–5.00)	
Daily alcohol intake				
Yes	1 (reference)	5.20 (0.71–37.88)	5.33 (0.71–39.81)	0.298
No	1 (reference)	1.33 (0.71–2.49)	1.81 (0.94–3.46)	
Regular exercise				
Yes	1 (reference)	1.63 (0.50–5.31)	2.63 (0.79–8.70)	0.662
No	1 (reference)	1.63 (0.82–3.25)	1.81 (0.89–3.69)	

Values are hazard ratios (95% confidence intervals) adjusted for age (except for the subgroup analysis by age), sex (except for the subgroup analysis by sex),obesity (except for the subgroup analysis by obesity), hypertension (except for the subgroup analysis by hypertension), dyslipidemia (except for the subgroup analysis by dyslipidemia), current smoking (except for the subgroup analysis by current smoking), daily alcohol drinking (except for the subgroup analysis by alcohol drinking) and regular exercise (except for the subgroup analysis by regular exercise). Obesity: body mass index $\geq$ 25 kg/m^2. Hypertension: systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure $\geq$ 90 mmHg or use of blood pressure-lowering medication. Dyslipidemia: low-density lipoprotein cholesterol $\geq$ 3.62 mmol/L, high-density lipoprotein cholesterol < 1.03 mmol/L, triglycerides $\geq$ 1.69 mmol/L, or the use of lipid-lowering medication.

4. Discussion

In this large-scale observational study of a general Japanese population, a self-reported faster eating speed was associated with a higher risk of developing diabetes. This association remained significant in the multivariable analysis, including age, sex, smoking status, drinking, regular exercise, obesity, hypertension and dyslipidemia as covariates. The correlation of eating speed with incidence of diabetes was comparable across subgroups defined by age, sex, obesity, hypertension, dyslipidemia, current smoking and drinking.

Previous evidence on the relationship between eating speed and the risk of type 2 diabetes is mainly derived from case–control studies. A case–control study conducted in Lithuania compared 234 individuals with newly diagnosed type 2 diabetes with 468 controls, demonstrating that the risk of type 2 diabetes was more than doubled for people who ate quickly compared with others [31]. In Japan, Sakurai et al. [25] reported that eating quickly increased the risk of diabetes among 2050 middle-aged Japanese male workers undergoing medical examinations. In a 3-year longitudinal study of 172 people in Japan who underwent medical examinations in a single hospital, Totsuka et al. [32] found that self-reported fast eating speed was associated with the incidence of impaired glucose tolerance, which was confirmed using a 75 g glucose tolerance test. One large-scale population-based study of a Japanese population who underwent annual health checkups reported a 1.12-fold higher risk of diabetes in the group of fast eating speed than in the combined group of medium and slow eating speed during 1-year to 3-year follow-up [26]. The present large-scale population-based longitudinal study with long-term follow-up (average 5.1 years) confirmed the findings of previous studies and clearly demonstrated a strong, linear relationship between self-reported eating quickly and the development of diabetes (multivariable-adjusted HRs 1.69 for medium eating speed and 2.08 for fast eating speed compared with the reference group of slow eating speed, * p = 0.014 for trend) among a general Japanese population.

The precise mechanisms by which eating speed increases the incidence of diabetes have not been clearly defined, but one possible explanation for the effect is the development of insulin resistance through weight gain. Fast eating has been shown to lead to weight gain, obesity [25,32–39], and the subsequent development of insulin resistance [24,25,32,39]. Second, fast eating may cause postprandial hyperglycemia. It has been reported that, in healthy subjects, thorough mastication was associated with lower levels of postprandial blood glucose compared with normal mastication [40]. Therefore, fast eating, which is associated with lower mastication, may cause postprandial hyperglycemia. Over time,

postprandial hyperglycemia may gradually cause pancreatic β-cell exhaustion, leading to a decrease in insulin secretion [41]. Third, a decrease in mastication may lead to an increase in food intake. An animal study found that, in rats, thorough mastication activated histamine in the hypothalamus and binding of histamine to H1 receptors in the paraventricular nucleus and ventromedial lobe of the hypothalamus resulted in food intake suppression [42]. Thus, fast eating, which is associated with decreased mastication, may increase food consumption. Fourth, decreases in the secretion of peptide YY and glucagon-like peptide 1 (GLP-1) by fast eating may cause postprandial hyperglycemia [43]. Fifth, fast eating may be associated with a delayed feeling of fullness and satiety, which leads to over-eating. A previous study reported that slow eating speed reduced ghrelin secretion in response to carbohydrate load in obese adolescents [44]. Furthermore, Rigamonti et al. reported that slow feeding rates increased peptide YY and GLP-1 secretion [43,45]. Taken together, fast eating may cause these changes in hormone secretion, leading to a delay in the feeling of fullness and satiety, which leads to over-eating.

The strengths of the present study were its relatively large sample size and population-based longitudinal design. In addition, the onset of diabetes was evaluated by blood glucose and HbA1c levels at annual medical examinations. Some previous studies have evaluated the onset of diabetes based only on self-reported information. The present study has several limitations. First, eating speed was self-reported and was not objectively evaluated. The accuracy of evaluating eating speed based on self-report is controversial. Woodland et al. demonstrated that the match rate of self-reported eating speed and the objective measure of eating rate was 47.4% [46]. A future study using a reliable method to assess eating speed will be required to obtain more objectivity. Second, a detailed nutritional survey was not conducted in this study. Third, people who are interested in their own health are more likely to undergo medical examinations than those who are not. Our findings obtained from participants of the ISSA-CKD study do not always apply to the general population. Further study will be interesting to elucidate whether or not similar results can be observed in the general Japanese population. Fourth, no information was available on the etiological type of diabetes, although most onsets after age 40 are type 2 diabetes [47,48]. Fifth, a detailed amount of exercise was not available. However, previous studies have shown that exercise ($\geq$4 METs/h/week) of at least 30 min per week on at least 2 days a week is the minimum required to improve physical fitness and musculo-skeletal function [49]. We created the questionnaire about exercise habits on this basis. A future study using a reliable method to assess exercise habits and physical fitness index will be required.

5. Conclusions

In conclusion, a self-reported faster eating speed was clearly associated with a higher risk of developing diabetes in this large-scale observational study of a general Japanese population. A feasible strategy in the future is to work with physicians and registered dietitians to provide nutrition therapy to improve eating speed during medical examination. The population strategy to reduce eating speed appears to provide further protection against the emerging burden of diabetes.

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