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Nutritional Deficiency in Celiac Disease

Current Perspective

Edited by
Anil K. Verma

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Editor

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About the Editor

Anil K. Verma

Anil K. Verma holds a Ph.D. degree in Biomedical Sciences from Università Politecnica delle Marche (UNIVPM), Italy. Currently, he is a postdoctoral fellow at UNIVPM, Italy. He has been a visiting scholar at the Massachusetts General Hospital, Boston USA. Dr. Verma is a budding researcher with more than 10 years of research experience in the field of Gastroenterology with main interest in celiac disease and other gluten-related disorders. His research expertise is focused on; epidemiology of celiac disease, finding non-invasive biomarkers for diagnosis of celiac disease, HLA-DQ distribution, gluten contamination. He was involved in various landmark studies related to the epidemiology of celiac disease, HLA-DQ distribution, and gluten-contamination. He has received a number of research awards, including Società Italiana di Gastroenterologia Epatologia e Nutrizione Pediatrica (SIGENP) award for young researcher, Italy (2016) and First award for oral presentation in SIGENP congress Rome, Italy (2017). He is an active member of SIGENP, and the International Society for the study of Celiac Disease (ISSCD). Until 2021, Dr. Verma has published more than 30 important articles in these fields.

Preface to "Nutritional Deficiency in Celiac Disease: Current Perspective"

Adherence to a strict gluten-free diet is the only effective treatment for celiac disease to date, resulting in absolute regression in celiac-associated symptoms. A gluten-free diet is also recommended in other gluten-related disorders. Gluten is a mixture of storage proteins found in wheat and related grains that contains certain immune-potent fragments (prolamins) sufficient to inflict a toxic effect in the intestinal mucosa by triggering an immunological response in genetically susceptible individuals. Gluten is a vital source not only of protein but also various macro- and micronutrients. Complete prevention of gluten from the diet causes alterations in the level of macro- and micronutrients, which eventually lead to nutritional imbalance both in adult and pediatric individuals with celiac disease.

Naturally gluten-free (grains, fruits, vegetables, etc.) and processed gluten-free products (commercial products) are advised as a substitute for gluten-containing food for patients afflicted with celiac disease. Although gluten-free products are enriched with nutrients, they do not however fulfill the nutrition requirements as sufficiently as gluten-containing products. It is reported that in comparison to gluten-containing products, gluten-free products contain a low amount of needed micronutrients (iron, folate, and vitamin B), fiber, and a high amount of carbohydrate and lipid.

In recent years, nutritional complications have been reported in patients with celiac disease, which is a serious health issue especially in growing children, where it needs immediate attention. This Special Issue is focused on nutritional deficiencies related to celiac disease.

Collectively, this Special Issue includes articles on various topics of nutrition and celiac disease and provides updated knowledge on in addition to discussing new prospects of nutritional deficiencies in celiac disease. I am extremely thankful to all the contributors to this Special Issue, which would not have been possible without the precious contribution and technical support of the Nutrients editorial team.

Anil K. Verma
Editor

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Editorial

Nutritional Deficiencies in Celiac Disease: Current Perspectives

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Gluten-induced T-cell-mediated immune response damages the villous structure that significantly affects the functioning of the small intestinal mucosa. Due to continuous mucosal damage, the villous cannot sufficiently absorb the nutrients from the food, ultimately leading to severe nutritional deficiencies in patients with celiac disease (CD) [1]. A complete gluten-free diet (GFD), the only accepted treatment of CD, gradually neutralizes the immune response and regenerates the damaged villi [2]. In comparison with gluten-containing food that is a major source of vitamins, iodine, dietary fiber, proteins, and minerals, the gluten-free alternative grains (rice, maize, etc.) and processed gluten-free food are inadequately nutritious and cannot compensate the necessary nutritional requirements [3,4]. Hence, strict adherence to GFD causes a continuous nutritional imbalance in CD patients, abolishes their quality of life (QOL), and eventually leads to moderate to severe disorders such as iron deficiency anemia and depression [5].

This Special Issue was planned to gather information about new prospects of nutritional deficiencies in CD. Articles related to the nutritional deficiencies in CD were invited. In total, 12 articles (8 original and 4 review articles) were published.

A substantial number of studies already correlated the consumption of Ultraprocessed food (UPF) with different diseases. However, very few studies assessed the side effects of UPF on oxidative/antioxidants balance. In the first original article, Nestares et al. evaluated the influence of UPF consumption on oxidative stress, antioxidant capacity, and inflammatory signaling in celiac children consuming a GFD. The authors reported that CD children who consume more UPF and perform less physical activity display higher levels of oxidative stress and some pro-inflammatory cytokines, regardless of the duration of GFD [6].

A considerable number of CD patients are often diagnosed with autoimmune and non-autoimmune thyroid disorders [7,8]. Iodine is an essential micronutrient for the synthesis of thyroid hormones, and its absorption occurs in the small intestine. Iodine malabsorption contributes to non-autoimmune thyroid disorders in CD. However, this hypothesis has never been investigated. In the second original study, probably the first study in the CD literature, Delvecchio et al. investigated the iodine excretion after proper dietary recommendations. The authors reported that the school-age CD patients have iodine deficiency during the diagnosis that partially recovers after one year of GFD [9].

A strict GFD limits the food choices for CD patients influencing them to choose foods with a high caloric content and a higher proportion of fat and protein. It is well known that commercial gluten-free products often have compromised nutritional quality, compared with their gluten-containing equivalents [3,4]. In the third original contribution, a cross-sectional age- and gender-matched study, Ballestero-Fernández et al., probably in a first study of its kind, investigated the nutritional status of the Spanish CD patients. The authors had a complete evaluation of the nutritional status of the Spanish adults diagnosed with CD and found an equivalent nutritional status in celiac adults, as compared with healthy volunteers, with the dietary deviations found similar to those of the Spanish population [10].

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-related coronavirus disease of 2019 (COVID-19) pandemic has terribly been plaguing humankind since

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December 2019 [11]. CD has also not been untouched from this extremely challenging situation [12]. Regardless, a strict GFD reduces the QOL of CD patients [5]. However, following a GFD may have been more difficult during this pandemic. In an important original study (online survey), using an online ad hoc and validated questionnaire, Bascuñán et al. investigated perceptions about the general pandemic effects, current clinical conditions and dietary characterization, adherence to GFD, and mental health (anxiety and depression) in patients with gluten-related disorders including CD. The authors reported a higher level of depression and anxiety in celiac patients compared with the general population. However, this study does not clarify if the observed differences were due to the presence of disease or the pandemic [13].

In another timely needed original study, Falcomer et al. evaluated the QoL of Brazilian celiac patients during the course of the COVID-19 pandemic. The authors concluded that although the COVID-19 pandemic has historically posed a challenge for the Brazilian population, this period was not associated with a negative impact on Brazilian CD individuals' QoL [14].

The most frequent extra-intestinal manifestation of CD is iron deficiency anemia (IDA). The iron absorption occurs primarily in the proximal duodenum section that is typically destroyed in CD. This destruction reduces iron absorption [15], although oral options such as products containing ferrous sulfate (FS) as an iron replacement are available. Poor tolerability of such oral supplements is frequent in patients with CD. In a review article based on iron deficiency anemia in CD, Talarico et al. comprehensively discussed oral ferrous supplements options and concluded that iron-based products (bivalent or trivalent) are well tolerated, compared with FS, but they may be less effective in correcting IDA, especially with CD patients [16].

Autoimmune thyroid diseases (AITDs) such as Hashimoto's thyroiditis (HT) and Graves' disease (GD) are the major causes of hypo and hyperthyroidism and frequently coexist with CD [17]. In contrast to iron deficiency, AITDs worsen preexisting thyroid dysfunction due to the decreased activity of the heme-dependent thyroid peroxidase [18]. In the second review article, Starchl et al. rigorously reviewed the literature to establish knowledge of CD and its relation to AITDs, as well as the influence of iron, vitamin D, and the gut microbiota on their onset and progression. The authors found the existence of a significant thyroid–gut axis, indicating effects of the gut microbiome on the immune system and the absorption of micronutrients, as well as on thyroid function [19].

Replacement of wheat and other related grains with gluten-free equivalents is associated with the increased consumption of trans fats, salt, sucrose, and phosphorus. These unhealthy food options can lead to the development of metabolic disorders and other cardiovascular complications [20].

In another original article, Gładyś et al. assessed the nutritional value of a GFD in adult CD patients before and after one year of standard dietary education. The authors found that 38% of CD patients do not adhere to a standard GFD. The authors also reported that CD subjects consume more unhealthy food; therefore, the role of dietitians in the treatment of CD must be increased [21].

On the other hand, it is important for healthcare workers, researchers, and dieticians to have enough knowledge about CD. In recent years, insufficient knowledge of CD among healthcare workers has been reported [22,23]. However, only a limited number of studies have been reported thus far on this important issue. In an original study, using an online survey in Poland, Dembiński et al. assessed the knowledge of healthcare professionals concerning nutritional deficiencies in patients with CD. The authors found that healthcare professionals and medical students in Poland have insufficient knowledge of the risk of nutritional deficiencies in patients with CD [24].

Following a long-term strict GFD often causes nutritional deficiencies [25]. In the third review article, Cardo et al. gave an updated vision on nutritional deficiencies in adult celiac patients following a GFD. The authors concluded that it is vital to carry out a

continuous and personalized follow-up of celiac patients from the moment of diagnosis. For this supervision, the role of celiac expert nutritionists is essential [26].

Although IDA usually reverts with a GFD, some patients show persistent IDA. Recent studies suggest an association between the rs855791 polymorphism in the TMPRSS6 gene and persistent IDA in adults with CD [27,28]. In an interesting original study, Urbaszek et al. assessed the potential link between rs855791 and persistent IDA in pediatric patients with CD. The authors, probably for the first time, reported the prevalence of rs855791 genotypes in children with CD. The study suggests that persistent IDA is uncommon in pediatric patients with CD [29].

Iron deficiency (ID) with or without anemia is a common complication in both children and adults with CD, causing significant morbidity and impairment of health-related quality of life (HRQL) [5]. In a fourth comprehensive review article, Montoro-Huguet et al. remarkably covered all the important aspects of ID and CD. The authors expansively discussed the prevalence of CD among patients with anemia and vice versa. This review article provides healthcare professionals an algorithm based on the severity of symptoms, its impact on the HRQL, the tolerance and efficiency of oral iron, and other factors that predict a poor response to oral iron, such as the severity of histological damage, poor adherence to GFD, and blood loss due to mucosal lesions [30].

Collectively, this Special Issue includes articles on various topics of nutrition and CD and provides updated knowledge and new prospects of nutritional deficiencies in celiac disease.

Conflicts of Interest: The author declares no conflict of interest.

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Article

Influence of Ultra-Processed Foods Consumption on Redox Status and Inflammatory Signaling in Young Celiac Patients

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Abstract: The current study was designed to assess the influence of consumption of ultra-processed (UPF) on oxidative/antioxidant balance and evoked inflammatory signaling in young patients with celiac disease (CD). The study included 85 children. The celiac group (n = 53) included children with CD with a long (>18 months, n = 17) or recent (<18 months, n = 36) adherence to a gluten-free diet (GFD). The control group (n = 32) included healthy children with a significantly lower consumption of UPF compared to the CD group, both expressed as kcal/day (p = 0.043) and as percentage of daily energy intake (p = 0.023). Among children with CD, the group with the lowest consumption of UPF (below the 50% of daily energy intake) had a greater Mediterranean diet (MD) adherence and higher moderate physical activity levels. In addition, CD children with the lowest consumption of UPF had healthier redox (lower soluble superoxide dismutase-1 and 15-F2t-isoprostanes) and inflammatory profiles (lower macrophage inflammatory protein-1α) compared to the group with the highest consumption of UPF (all, $p < 0.05$) regardless of the time on a GFD. These findings highlight the importance of a correct monitoring of the GFD. An unbalanced GFD with high consumption of UPF and an unhealthy pattern with less physical activity and worse adherence to MD results in a worse inflammatory profile, which could act as a parallel pathway that could have important consequences on the pathophysiology of the disease.

Keywords: celiac disease; ultra-processed foods; gluten-free diet; inflammatory signaling; oxidative stress; children

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1. Introduction

Celiac disease (CD) occurs in about 1–1.4% of people in most populations [1]. It is a multifactorial, autoimmune disorder, caused by an immune reaction which is triggered by the ingestion of gluten and related proteins. This occurs in people who carry the DQ2 and/or DQ8 Human Leukocyte Antigen (HLA) class II haplotypes. Aa variable combination of high CD-specific antibody titers, an inflammatory enteropathy with different degrees of severity and a wide range of digestive and/or systemic symptoms [2] characterize this disease.

The pathogenesis of CD is complicated and still not fully explained. Gluten causes an abnormal innate and adaptive immune response in patients, generating autoantibodies [3] that can affect not only the intestine but the entire organism. Oxidative stress has an

important function in the pathogenesis of many intestinal manifestations of CD; the main processes possibly involved in gliadin toxicity are a higher concentration of reactive oxygen species (ROS), a lower antioxidant capacity and a pro-inflammatory state [4]. Gliadin gene sequence contains regions responsible for triggering oxidative stress and inducing the release of proinflammatory cytokines like interleukin 1β (IL1β) [5] and interleukin 15 (IL-15) [6]. Due to an adaptive immune response, various epitopes of gliadin can stimulate tumor necrosis factor (TNF) and other proinflammatory cytokines. Th1 response increases interferon gamma (IFNγ), that leads to intraepithelial lymphocyte toxicity and onset of CD [7]. On the other hand, gut microbiota seems to play an important role in the pathogenesis of the disease, and changes in its composition have been observed in celiac children [8].

The only available treatment for CD is a lifelong strict gluten-free diet (GFD) is. The improvement and resolution of symptoms typically occur within days or weeks and often precedes the normalization of serological markers and duodenal villous atrophy [9]. A GFD in children must be suitable for growth and pubertal development [10]. However, the nutritional adequacy of the GFD remains controversial and some evidence suggests that GFD is nutritionally unbalanced [11–13]. The scientific literature raises questions about the food choices of individuals with CD because it is a restrictive diet which substitutes naturally gluten-containing products with their ultra-processed gluten-free analogs [14].

Ultraprocessed foods (UPF) are products of food technology, formulated from 5 or more industrial ingredients and containing little or no whole food [15]. They are considered harmful to human health due to their high caloric content (up to 500 kcal/100 g) and low nutritional quality, high glycemic load, because of being rich in sodium, simple sugars and saturated and trans fats, low in fiber, proteins and various micronutrients, and because they contain a large number of additives in order to resemble natural foods as closely as possible [14,16–18]. Its production and consumption have increased throughout the world [14]. They are appetizing which makes them able to displace more nutritionally interesting foods and to interfere with the ability to control eating habits [18].

Diverse studies correlated the consumption of UPF with the increase in chronic non-communicable diseases in children and adults [14,16,19–21]. However, there are no studies that associate the consumption of UPF with oxidative stress and evoked inflammatory signaling in celiac children in a GFD. Nonetheless, lifestyle changes and including exercise might help in the regulation of redox state and decrease inflammation in children [22,23].

Until now, most studies on the nutritional value of GFD have focused on studying its deficiencies, and an increase in body weight has been described as a consequence of excessive consumption of dietary products rich in vegetable fats (e.g., rapeseed, palm and coconut oil) [24]. However, there have been no large case-control studies on the effect of UPF consumption in celiac children. Therefore, the aim of the present study was to evaluate the influence of UPF consumption on oxidative stress, antioxidant capacity and inflammatory signaling in celiac children on a GFD.

2. Materials and Methods

2.1. Subjects

The research was carried out following the principles of the Declaration of Helsinki and its later amendments and it was approved by the Ethics Committee of the University of Granada (Ref. 201202400000697). The study included 85 children aged 7–18 years old, who attended the Gastroenterology, Hepatology and Child Nutrition Service from the "Virgen de las Nieves" University Hospital in Granada, Spain.

32 healthy children were included in the control group. Their serological screening was negative and they had no history of any chronic disease. These children attended this Service due to minor symptoms related to chronic functional constipation, according to the Rome IV criteria [25]. They were included in the control group once it was verified that it was due to transitory gastrointestinal symptoms (functional constipation). The inclusion criteria for the control group were the following: (a) age between 7 and 18 years,

(b) absence of serum IgA and IgG anti-transglutaminase (tTG) antibodies, (c) normal weight for the age, (d) absence of gastrointestinal disorders in the previous year and (e) normal appetite. Children with diagnosed CD according to the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) [2] were included in the CD group (n = 53). The exclusion criteria for both groups were acute and chronic inflammation, liver or kidney diseases, diabetes, chronic asthma, inflammatory bowel disease, and consumption of dietary supplements that contain substances with antioxidant activity. Obese patients were also excluded (following the criteria of the International Task Force) [26] as well as those who did not sign the informed consent. Written informed consent was obtained from all parents.

2.2. Clinical and Socio-Demographics

The same group of researchers assessed participants' clinical and socio-demographic characteristics (i.e., age, household composition, parents' marital status, educational level and smoking habit).

The International Physical Activity Questionnaire [27] was used to register physical activity.

2.3. Anthropometric Measures

In both the control and the CD groups, Anthropometric characteristics (weight, height) were assessed. A stadiometer (Seca200, Hamburg, Germany) was used to measure height to the nearest 5 mm. The same mechanical balance was used to measure body weight (Seca200, Hamburg, Germany).

2.4. Blood Sampling

Venous blood samples from fasting patients were collected into anticoagulated tubes with sodium heparin during morning hours. Blood samples were centrifuged at 2500× g at 4 °C for 10 min to obtain plasma. Plasma samples remained frozen (−80 °C) until measurements.

2.5. Soluble Superoxide Dismutase (SOD) 1

The HND3MAG-39K Milliplex MAP Human Neurological Disorders Magnetic Bead Panel 3 (Millipore Corporation, Missouri , Saint Louis, MO, USA), based on immunoassays on the surface of fluorescent-coded beads (microspheres), was used to determine Soluble isoforms of SOD1 in plasma following the specifications of the manufacturer (50 events per bead, 50 µL sample, gate settings: 8000–15,000, time out 60 s). A LABScan 100 analyzer (Luminex Corporation, Texas, Austin, TX, USA) with a xPONENT software for data acquisition was used to reads the plate. The average values for each set of duplicate samples or standards were within 15% of the mean. Standard curve: SOD1: 0.04–30 ng/mL. Soluble enzyme concentrations in plasma samples were determined by comparing the mean of duplicate samples with the standard curve for each assay.

2.6. 15-F2t-Isoprostanes

The isoprostanes are prostaglandin-like compounds formed in vivo from the free radical-catalyzed peroxidation of essential fatty acids. To measure the isoprostanes in urine, a commercial kit Enzyme Immunoassay for Urinary Isoprostane (Oxford Biomedical Research, Oxford, UK) was used, which is a competitive enzyme-linked immunoassay (ELISA) for determining levels of 15-F2t-Isoprostane (the best characterized isoprostane) in urine samples. In order to eliminate interference due to non-specific binding, the urine samples were mixed with an enhanced dilution buffer. The 15-F2t-Isoprostane in the samples or standards competes with the 15-F2t-Isoprostane conjugated to horseradish peroxidase (HRP) for binding to a polyclonal antibody specific for 15-F2t-Isoprostane coated on the microplate. When the substrate is added, the HRP activity results in color development; the intensity of the color is proportional to the amount of 15-F2t-Isoprostane-HRP bound and inversely proportional to the amount of unconjugated 15-F2t-Isoprostane

in the samples or standards. The plate was read spectrophotometrically (Bio-tek, Vermont, Winooski, VT, USA) at 450 nm.

2.7. Total Antioxidant Status (TAS)

Peripheral blood was placed in pre-cooled test tubes on the examination day in order to determine plasma TAS levels. The plasma was immediately separated in a refrigerated centrifuge, aliquoted and stored at −20 °C until further use. A TAS Randox kit (Randox Laboratories, Ltd., Crumlin, UK) was used to analyze freshly thawed batches of plasma. Results were expressed in mM of Trolox equivalents. The reference range for human blood plasma is given as 1.30–1.77 mmol/L by the manufacturer. The linearity of calibration extends to 2.5 mmol/L of Trolox. We used measurements in duplicate in order to determine intra-assay variability.

2.8. Inflammatory Parameters

The HCYTMAG-60K-PX29 Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Corporation, Missouri, Saint Louis, MO, USA) was used following the specifications of the manufacturer to determine in plasma IFN-γ, Interleukin (IL)-10, IL-12P40, IL-12P70, IL-13, IL-15, IL-17A, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, Interferon-inducible protein (IP)-10, Monocyte chemoattractant protein (MCP)-1, Macrophage inflammatory protein (MIP)-1α, MIP-1β, TNF-α, TNF-β and Vascular endothelial growth factor (VEGF). The plate was read on a LABScan 100 analyzer (Luminex Corporation, Texas, Austin, TX, USA) with a xPONENT software for data acquisition. Standard curve: 3.2–10.000 pg/mL. Cytokines concentrations in plasma samples were determined by comparing the mean of duplicate samples with the standard curve for each assay.

2.9. Dietary Assessment

A three-day food record, two on weekdays and one on the weekend was used to assess the dietary intake. The same trained nutritionist carefully explained face to face the diary to the children and their parents and accompanied with a photographic atlas including different portion-size food pictures and a set of household measures and detailed instructions for its compilation [28]. All foods consumed during the different meals (breakfast, morning snack, lunch, afternoon snack, dinner) were included in the survey. For each meal, participants were requested to report an exhaustive description of the food and the recipes (including cooking methods and sugar or fats added during the meal preparation), food amount (according to the atlas) and the brands of packaged foods consumed.

Then foods were classified according to the NOVA classification, which has been employed in many studies conducted in several countries and is the most frequently used method to examine diets according to food processing, [17]. International bodies including PAHO, WHO and FAO also recognize and use it [18,29]. NOVA is a food classification based on the extent and purpose of industrial food processing, which classifies foods into four groups: unprocessed and minimally processed foods, processed culinary ingredients, processed foods and ultra-processed foods [30]. The latter category encompasses a group of industrial formulations that are manufactured using several ingredients and a series of processes. In order to classify the foods as unprocessed or minimally processed, culinary ingredients, processed and ultra-processed foods based on the NOVA classification, the three-day food records were analyzed [30]. The total dietary energy intake was calculated for each individual. Subsequently, the energy (kcal/day) and percentage of calories (% of the total daily energy intake) derived from each category of the NOVA classification food item was calculated. The same trained nutritionist analyzed all diaries using the *Evalfinut* software that includes the Spanish Food Composition Database [31]. The specific composition of gluten and gluten-free UPF (from labels) were introduced in the software

when calculating dietary balance. The mean of the three-day food records was employed in the present analyses.

Moreover, the Mediterranean diet Quality Index in Children and Adolescents (KIDMED) survey was employed to assess adherence to the Mediterranean diet (MD) [32]. The KIDMED index ranges from 4 (no adherence to the MD) to 12 (complete adherence to the MD). This index was determined using a 16-point questionnaire, which assesses various dietary habits. Each answer was scored according to its consistency with habits associated with the Mediterranean dietary pattern. Then the scores were added up to quantify the total index of the participant's adherence to the MD.

2.10. Data Analyses

Descriptive statistics (mean standard deviation) for quantitative variables and percentage of participants (%) for categorical variables were employed to describe the baseline characteristics of the study sample. To explore differences in the continuous variables, the Student-*t* test was conducted. Furthermore, differences in categorical variables were assessed by using the Chi-squared test.

We employed a one-way analysis of covariance (ANCOVA) after adjustment for age, sex and following a GFD for at least 18 months to assess differences in food groups consumption and NOVA food classification of children by CD (celiac group vs healthy group). The MD adherence by percentage of energy consumed from UPF in celiac children (below 50% of daily energy intake vs. above 50% of daily energy intake) was compared by ANCOVA after adjusting for age, sex and following a GFD for at least 18 months. Moderate physical activity (METS/week) by percentage of energy consumed from UPF in celiac children (below 50% vs. above 50% of daily energy intake) was compared by ANCOVA after adjusting for following a GFD for at least 18 months. An ANCOVA after adjusting for moderate physical activity, following a GFD for at least 18 months and the MD adherence was also employed to assess differences in levels of oxidative/antioxidant biomarkers and inflammatory profiles between celiac children consuming UPF below 50% of daily energy, above 50% of daily energy and control children. Post-hoc multiple comparisons (Bonferroni's correction) were applied to examine pairwise differences between groups (e.g., celiac disease with daily energy intake from UPF below 50% vs. controls). In those outcomes where outliers were/remained influential, we employed a subtle variation of winsorizing (convert back from a z-score: replacing extreme scores ($z > 2.58$) with a score equivalent to ± 2.58 standard deviations from the mean) in order to handle these outliers.

Differences in dietary habits in CD children by percentage of energy intake from ultra-processed foods (below 50% vs. above 50%) were assessed with an ANCOVA after adjusting for following a GFD for at least 18 months (Supplementary Table S1).

We conducted the data analyses with the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, Version 22. IBM Corp.: Armonk, NY, USA), and the statistical significance was set at $p \leq 0.05$.

3. Results

The baseline sociodemographic, dietary and anthropometric characteristics and physical activity levels of the study sample are shown in Table 1. A total of 53 children with CD participated in the study (mean age 9.6 $\pm$ 3.8 years). More than half of the participants with CD followed a GFD (68%) for less than 18 months and had a medium-high MD adherence (88%). The control group included 32 healthy children (mean age 10.7 $\pm$ 4.0 years). There were differences in height ($p = 0.048$) and weight ($p = 0.018$) between groups.

Table 1. Sociodemographic characteristics of the study participants.

Variable	Celiac Group (*n* = 53)	Control Group (*n* = 32)	*p*
Age (years)	9.6 (3.8)	10.7 (4.0)	0.187
Sex (female, n [%])	37 (69.8)	13 (40.6)	0.008
Weight (kg)	32.9 (12.3)	40.1 (15.0)	0.018
Height (cm)	134.6 (21.1)	144.3 (22.7)	0.048
Physical activity levels (METS/week)			
Moderate physical activity (*n* = 81)	981 (1061)	937 (825)	0.842
Vigorous physical activity (*n* = 81)	1425 (1548)	1599 (1644)	0.632
Mediterranean diet adherence n (%) (*n* = 81)			
Low	6 (11.8)	1 (3.1)	0.199
Medium	23 (45.1)	19 (63.3)	
High	22 (43.1)	11 (33.3)	
Following a gluten-free diet for at least 18 months n (%)			
Yes	17 (32.1)	-	
No	36 (67.9)	-	
Parents' marital status (% married) (*n* = 68)	39 (97.5)	28 (100)	0.339

SD, standard deviation; Values shown as mean ± SD unless otherwise indicated. METS, Metabolic equivalents.

Differences in food groups consumption and NOVA food classification of children by CD (celiac vs. control group) are shown in Table 2. The celiac group had a significantly higher consumption of UPF compared to the control group, both expressed as kcal/day ($p = 0.043$) and as percentage of daily energy intake ($p = 0.023$). After further adjusting the model for following a GFD for at least 18 months, there were no statistically significant differences but CD children showed a trend close to signification indicating a higher percentage of energy intake from UPF among celiac compared to controls ($p = 0.056$).

Table 2. Differences in food groups consumption and NOVA food classification of children by celiac disease (celiac vs. control group).

	Celiac Group (*n* = 53)	Control Group (*n* = 32)	*p* [a]	*p* [b]
Energy (kcal/day)	1905 (69.4)	1839 (90.4)	0.571	0.818
NOVA food classification				
Unprocessed or minimally processed foods (kcal/day)	654 (42.0)	733 (55.6)	0.272	0.325
Unprocessed or minimally processed foods (%E)	35.5 (1.9)	40.6 (2.6)	0.129	0.273
Processed culinary ingredients (kcal/day)	113 (12.8)	98 (16.7)	0.490	0.289
Processed Foods (kcal/day)	213 (28.8)	278 (37.5)	0.182	0.117
Ultra-processed food and drink products (kcal/day)	920 (54.9)	730 (71.6)	0.043	0.119
Ultra-processed food and drink products (%E)	47.0 (2.2)	38.6 (2.8)	0.023	0.056

E, energy. [a] Model adjusted for age and sex. [b] Model additionally adjusted for following a gluten free diet for at least 18 months. Values shown as mean (standard error).

The Mediterranean diet adherence and moderate physical activity levels by percentage of daily energy consumed from UPF in celiac children is shown in Figure 1. The group with the highest intake of energy from UPF (above the 50% of total energy) showed a lower MD adherence than the group with the lowest intake (below the 50% of total energy) in both, the unadjusted model ($p = 0.041$) and after adjusting the model for age, sex and following a GFD for at least 18 months ($p = 0.046$). The group with the highest intake of energy from UPF (above the 50% of total energy) showed lower levels of moderate physical activity than the group with the lowest intake (below the 50% of total energy) in both, the unadjusted model ($p = 0.037$) and after adjusting for following a GFD for at least 18 months ($p = 0.040$).

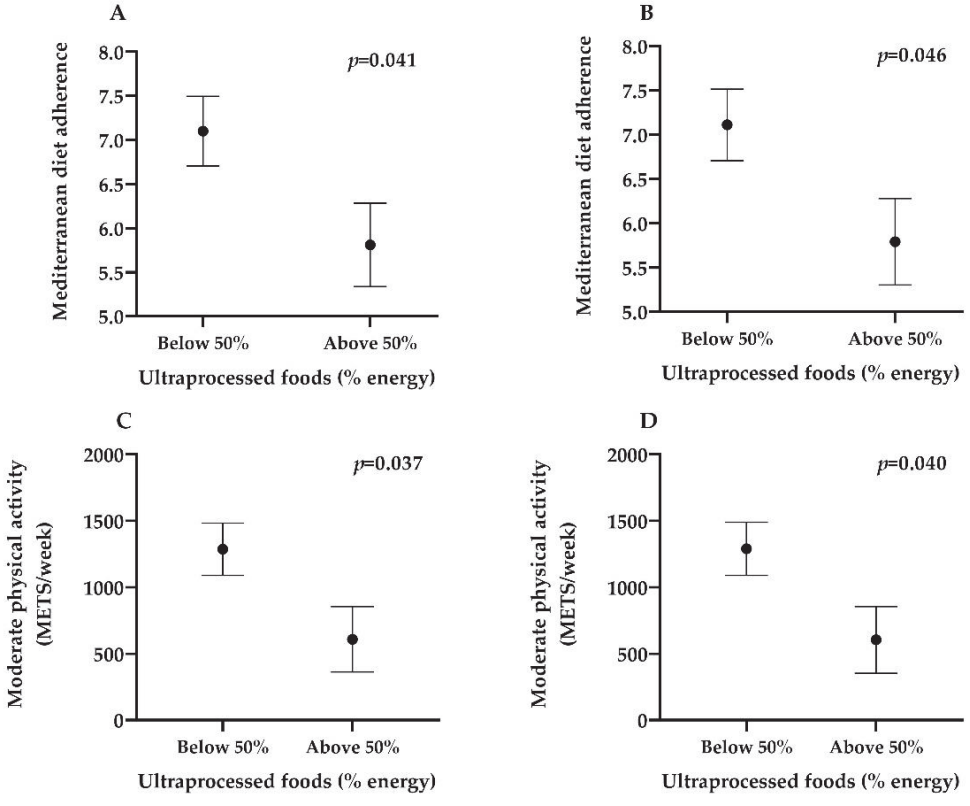

Figure 1. Mediterranean diet adherence and moderate physical activity levels (METS/week) by percentage of energy consumed from ultra-processed foods. Dots represent mean and bars represent standard error. (**A**) Mediterranean diet adherence by energy intake from ultra-processed foods below 50% vs. energy intake from ultra-processed foods above 50%. Model unadjusted. (**B**) Mediterranean diet adherence by energy intake from ultra-processed foods below 50% vs. energy intake from ultra-processed foods above 50%. Model adjusted for age, sex and following a gluten free diet for at least 18 months. (**C**) Moderate physical activity (METS/week) by energy intake from ultra-processed foods below 50% vs. energy intake from ultra-processed foods above 50%. Model unadjusted. (**D**) Moderate physical activity (METS/week) by energy intake from ultra-processed foods below 50% vs. energy intake from ultra-processed foods above 50%. Model adjusted for following a gluten free diet for at least 18 months.

Differences in levels of oxidative/antioxidant biomarkers and inflammatory profiles between CD children by percentage of energy from UPF (below 50% vs. above 50%) and the control group are shown in Table 3. After adjusting the model for physical activity levels, following a GFD for at least 18 months and MD adherence, levels of SOD1 (oxidative stress-mediated antioxidant), 15-F2t-isoprostanes (biomarker related to damage to the prostaglandins) and MIP-1α (pro-inflammatory cytokines) were lower in CD children consuming UPF below 50% of daily energy intake compared to CD children consuming UPF above 50% of daily energy intake (all, $p < 0.05$). In addition, CD children consuming UPF above 50% of daily energy had higher levels of SOD1, IFN-γ and MIP-1α compared to healthy controls (all, $p < 0.05$).

Table 3. Differences in oxidative/antioxidant biomarkers and inflammatory profiles in celiac children by percentage of energy intake from ultra-processed foods (below 50% vs. above 50%) and control children.

	Celiac Children Below 50%	Celiac Children Above 50%	Control Children	p [a]	p [b]
Oxidative/antioxidant biomarkers					
SOD1 (pg/mL)	87.7 (13.4) (n = 16) [a]	148.2 (16.6) (n = 13) [a,b]	83.7 (12.6) (n = 18) [b]	0.014	0.020
15-F2t-isoprostanes (pg/mL)	8.3 (0.3) (n = 22) [a]	9.9 (0.4) (n = 18) [a]	9.2 (0.3) (n = 27)	0.008	0.004
TAS (mmol/L)	1.7 (0.1) (n = 22)	1.5 (0.1) (n = 18)	1.6 (0.1) (n = 26)	0.154	0.144
Inflammatory markers					
IFN-γ (pg/mL)	45.8 (8.1) (n = 22)	69.6 (9.2) (n = 15) [a]	38.8 (7.6) (n = 26) [a]	0.043	0.047
IL-10 (pg/mL)	11.8 (2.0) (n = 22)	16.5 (2.3) (n = 18)	12.3 (1.9) (n = 26)	0.265	0.239
IL-12P40 (pg/mL)	32.9 (5.2) (n = 16)	44.3 (6.1) (n = 12)	40.6 (4.9) (n = 19)	0.331	0.478
IL-12P70 (pg/mL)	8.5 (1.1) (n = 22)	10.5 (1.3) (n = 18)	8.8 (1.0) (n = 26)	0.456	0.415
IL-13 (pg/mL)	39.5 (18.1) (n = 16)	54.2 (22.4) (n = 10)	52.7 (18.1) (n = 16)	0.842	0.953
IL-15 (pg/mL)	4.9 (0.9) (n = 17)	6.5 (1.0) (n = 14)	5.9 (0.9) (n = 21)	0.529	0.721
IL-17A (pg/mL)	6.9 (1.1) (n = 20)	8.6 (1.2) (n = 17)	6.3 (1.0) (n = 24)	0.334	0.364
IL-1α (pg/mL)	33.5 (5.9) (n = 21)	43.9 (6.8) (n = 17)	32.5 (5.6) (n = 24)	0.376	0.425
IL-1β (pg/mL)	4.6 (0.5) (n = 22)	4.4 (0.6) (n = 18)	4.8 (0.5) (n = 26)	0.903	0.913
IL-2 (pg/mL)	3.4 (0.4) (n = 21)	3.5 (0.5) (n = 17)	3.4 (0.4) (n = 23)	0.994	0.994
IL-3 (pg/mL)	8.9 (1.3) (n = 20)	7.8 (1.4) (n = 17)	9.7 (1.1) (n = 25)	0.576	0.644
IL-4 (pg/mL)	24.2 (5.5) (n = 15)	18.1 (5.4) (n = 16)	25.9 (4.6) (n = 22)	0.556	0.494
IL-5 (pg/mL)	3.2 (0.6) (n = 20)	4.1 (0.7) (n = 15)	2.9 (0.5) (n = 25)	0.451	0.492
IL-6 (pg/mL)	10.9 (5.4) (n = 16)	22.4 (6.6) (n = 10)	18.2 (5.6) (n = 15)	0.385	0.544
IL-7 (pg/mL)	20.1 (1.9) (n = 21)	18.5 (2.2) (n = 18)	21.5 (1.8) (n = 26)	0.591	0.658
IL-8 (pg/mL)	7.2 (1.6) (n = 22)	9.1 (1.8) (n = 18)	7.4 (1.5) (n = 25)	0.708	0.812
IP-10 (pg/mL)	519.8 (48.6) (n = 22)	552.9 (55.3) (n = 18)	528.2 (45.8) (n = 26)	0.901	0.967
MCP-1 (pg/mL)	379.4 (25.9) (n = 22)	305.7 (29.4) (n = 18)	317.3 (24.3) (n = 26)	0.116	0.142
MIP-1α (pg/mL)	4.3 (0.9) (n = 10) [a]	11.7 (1.2) (n = 6) [a,b]	6.9 (1.0) (n = 9) [b]	<0.001	0.001
MIP-1β (pg/mL)	32.5 (1.9) (n = 22)	28.6 (2.2) (n = 18)	29.0 (1.8) (n = 26)	0.324	0.350
TNF-α (pg/mL)	24.8 (1.6) (n = 22)	21.8 (1.8) (n = 18)	24.6 (1.5) (n = 26)	0.387	0.392
TNF-β (pg/mL)	32.1 (15.2) (n = 17)	40.2 (17.2) (n = 13)	40.4 (14.9) (n = 18)	0.913	0.941
VEGF (pg/mL)	88.6 (8.3) (n = 22)	95.4 (9.3) (n = 18)	88.3 (7.8) (n = 25)	0.825	0.799

[a] Model adjusted for moderate physical activity (METS/week) and following a gluten free diet for at least 18 months. [b] Model additionally adjusted for the Mediterranean diet adherence. [a,b] superscripts in the same row indicate a significant difference ($p < 0.05$) between groups with the same letter. Values shown as mean (standard error). SOD1 superoxide dismutase 1; TAS, total antioxidant status; IL, interleukin; IP-10, Interferon-inducible protein; IFN-γ, Interferon-γ; MCP-1, Monocyte chemoattractant protein-1; METS, Metabolic equivalents; MIP, Macrophage inflammatory protein; TNF, Tumour necrosis factor; VEGF, Vascular endothelial growth factor.

Differences in dietary habits in CD children by percentage of energy intake from UPF (below 50% vs. above 50%) are shown in Supplementary Table S1. After adjusting for following a GFD for at least 18 months, CD children with more than 50% of daily energy intake from UPF showed a lower intake of whole dairy products ($p = 0.006$) and a trend to higher intake of poultry ($p = 0.076$) and a lower intake of vegetables ($p = 0.077$).

4. Discussion

Our results suggest that the consumption of UPF was higher in the CD children compared to the control group. Among CD children, those with a lower intake of UPF showed better inflammatory signaling and oxidative status (i.e., lower values for oxidative biomarkers and pro-inflammatory cytokines).

The only effective treatment for CD is a life-long strict GFD which, apart from maintaining the safe limit of gluten intake, must also be nutritionally balanced to ensure a healthy life. However, a GFD is a restrictive diet whose nutritional adequacy remains controversial with some evidence suggesting that GFD is nutritionally unbalanced [11–13]. The scientific literature raises questions concerning the food choices of individuals with CD because they substitute natural gluten-containing products with their ultra-processed gluten-free analogs [14]. This could have a negative effect on health, and it should be seriously taken into account, since the limited choice of food products in the diet of chil-

dren with CD could induce a high consumption of UPF, such as snacks and biscuits. Our results showed that, in general, CD children had a greater percentage of daily energy intake from UPF and drink products than the control children. This could be partially explained because, within the range of gluten-free foods, CD children usually choose those that are made gluten free through a process of purification instead of those that are naturally gluten free, leading to a diet higher in fat and carbohydrates concentration [12,13]. However, after adjusting UPF consumption for time of GFD (18 months), we observed that the differences disappeared, which may be due to a better adherence to the GFD and a better balance of macro and micronutrients among these patients, which highlights the need for close monitoring diet quality after the establishment of a GFD [33].

The consumption of UPF has been directly related to increased mortality and the appearance of chronic non-communicable diseases, especially cardiovascular diseases, but also obesity, cancer or depression in the adult population [34]. There are several mechanisms which might explain this relationship including a lower consumption of vitamins A, B_{12}, C, E, calcium, zinc, fiber and polyunsaturated fatty acids, and a higher intake of trans fats, sodium and sugars [20,21,35–39]. Furthermore, this eating pattern negatively affect the gut microbiota through the appearance of intestinal dysbiosis, which can trigger a pro-inflammatory immune response and an increase in intestinal permeability [14].

Despite the fact that there are numerous studies showing the defects of GFD, only a few have assessed its effect on the clinical course and there are hardly any studies that assess its role in the physiopathology of the disease. It is known that gut microbiota disturbance [40] plays a key role in the pathogenesis of CD, as colonizing gut bacteria are critical for the normal development of host defense [41] for its metabolic and protective function of the host [42]. Various studies have revealed an alteration in the microbiota of celiac patients compared to the healthy controls, observing a reduction in the population of *Lactobacillus* and *Bifidobacterium* and an increase especially in *Bacteroides*, *Escherichia coli* and *Clostridium leptum* [8,43]. Furthermore, a profile of bacterial proteases, capable of hydrolyzing gliadin, has been described in celiac patients that was absent in healthy ones, conforming a different bacterial proteolytic activity [44]. In this sense, the fact that the CD children in our study had a higher intake of UPF may aggravate the pathophysiology of the disease. On the other hand, the fact that CD children consume more UPF could be because of, but not only, the characteristics of a poorly completed GFD and, although less likely, because of a less healthy consumption pattern in this group prior to the onset of the disease, due to the relationship of UPF with the development of intestinal pathologies [14].

Contrarily, unprocessed and minimally processed food-based diets have shown the capacity to promote gut microbiota eubiosis, anti-inflammatory response, and epithelial integrity through bacterial butyrate production [45]. Indeed, a higher consumption of UPF by CD children in our study leads to a less favorable redox and inflammatory profile (compared to CD children consuming less than 50% of daily energy intake from UPF and controls), which altogether could also aggravate the pathophysiology of their disease. It is known that the oxidative status present in certain chronic diseases such as CD, especially at diagnosis, is caused by the interaction of gluten in the lamina propria, acting at the local and genomic level with an intracellular oxidative imbalance, characterized by increased levels of lipid peroxidation products [46,47] which can induce the formation of oxidative DNA lesion products (8-OHdG) [48]. Additionally, CD patients present a severe reduction of antioxidant capacity (including antioxidant vitamins) [49]. It is known that ROS signaling can enhance the synthesis of inflammatory mediators such as TNF-α and IL-1 [50]. In fact, we previously [4] reported the increase in these inflammatory molecules in children with CD who followed a GFD. Interestingly, these changes are maintained after adjusting for GFD time, which could be due to a strong enough response to induce an inflammatory state which is maintained and probably a parallel pathway in the pathogenesis of chronic inflammation and the oxidative status of uncontrolled disease. In this sense, we can assume that there is another source of the deleterious pro-inflammatory cytokines. Present results suggest that a higher UPF consumption and lower physical

activity levels could be responsible. A healthy lifestyle includes meeting the recommendations regarding minimum levels of physical activity. It is well established that adequate physical activity levels promote better inflammatory and redox profiles in the general adult population [51,52]. Notwithstanding, there are specific ways in which the balance between pro- and anti-inflammatory, oxidant-antioxidant factors associated with exercise can influence health and growth in children [22]. Therefore, the influence of physical activity and exercise on inflammatory signal and redox status in children remains unclear. In fact, SOD concentrations were lower among our CD children with UPF below 50% of daily energy intake, which may suggest a lower need of release of this relevant antioxidant enzyme in this group of patients. It should be taken in account that SOD levels usually increase with exercise among adult populations [52]. However, oxidative stress responses to exercise and the underlying mechanisms in the pediatric population are still unclear [53]. Therefore, not only diet matters, and it might be mandatory that CD children exercise for a better prognosis of the disease [23]. Studies exploring the influence of objectively measured physical activity levels and different exercise programs on CD patients are warranted in order to better design effective lifestyle interventions.

In our study, the adherence to the MD was inversely related to the intake of UPF, in a similar way to that described previously [54]. Notwithstanding, small differences were found regarding dietary habits between CD children according to UPF intake. In fact, CD children with more than 50% of daily energy intake from ultra-processed foods showed a lower intake of whole dairy products and a trend to higher intake of poultry and lower of vegetables. As a result, although a tendency towards healthier habits has been observed in CD children with less than 50% of daily energy intake from UPF, the differences might not be strong enough to reflect changes in the overall dietary pattern. Consequently, the global index of adherence to the MD may not be sensitive enough. Indeed, even after adjusting our results for MD adherence, patients who consumed less than 50% of their daily energy intake from UPF presented a more favorable oxidative-antioxidant and inflammatory profiles than those with greater UPF consumption. The MD is characterized by being a diet with high antioxidant power and with nutrigenomic modulation capacity, showing itself as a protective factor against various diseases [55], but in recent years there has been a greater consumption of processed and ultra-processed foods of animal origin in the pediatric population [56]. In this sense, other studies have correlated the consumption of UPF and the development of diseases adjusting for adherence to the MD and they failed to confirm the present results [57].

5. Strengths and Limitations

There are some limitations that must be underlined. Firstly, the present study sample size was relatively small and it should be noted that since it is a cross-sectional study we cannot establish causal relationships, and consequently the present results must be interpreted with caution. Secondly, the assessment of physical activity levels was self-reported (through the IPAQ) instead of objectively measured through accelerometry, which constitute the gold standard. Based on the present findings, it is advisable to recruit a higher number of participants to confirm or contrast the present findings.

6. Conclusions

Overall, our results indicate that CD children who consumed more UPF and performed less physical activity presented higher levels of oxidative stress and some pro-inflammatory cytokines, regardless of the time on a GFD. This suggests that for a better redox and inflammatory profile it is necessary to promote healthy lifestyle habits which include improvements in the quality of the diet, regardless of CD. An unhealthy dietary pattern it is shown as a parallel pathway that could have important consequences in terms of cellular aging and long-term prognosis in CD children. Among the healthy living patterns, the adherence to a MD might be a key factor, which was inversely associated with the consumption of UPF.

Supplementary Materials: The following are available online at https://www.mdpi.com/2072-6643/13/1/156/s1, Supplementary Table S1. Differences in dietary habits in celiac children by percentage of energy intake from ultra-processed foods (below 50% versus above 50%).

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Iodine Absorption in Celiac Children: A Longitudinal Pilot Study

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Abstract: Background: non-autoimmune thyroid disorder is a common finding in celiac patients, more frequent than in the general population. An impairment of iodine absorption has been hypothesized, but it has never been investigated so far. We aimed to evaluate the iodine absorption in children and adolescents with newly diagnosed celiac disease. **Methods:** 36 consecutive celiac patients (age 7.4 years, range 2.4–14.5 years) before starting a gluten-free diet (GFD) were enrolled. We assayed the urinary iodine concentration (UIC) in a 24-h urine sample, at baseline (T0) after 3 (T1) and 12 months (T2) of GFD. **Results:** UIC at T0 was 64 µg/L (IQR 45–93.25 µg/L) with an iodine deficiency rate of 77.8%. UIC was not different according to histological damage, clinical presentation (typical vs atypical); we found no correlation with the thyroid function tests and auxological parameters. UIC was not statistically different at T1 (76 µg/L) and T2 (89 µg/L) vs T0. UIC at T2 was similar between patients with positive and negative anti-transglutaminase antibodies at T2. No patients presented overt hypothyroidism during the study. **Conclusions**: We found that iodine absorption in celiac children is impaired compared to the general population; it increases slightly, but not significantly, during the GFD. We should regularly reinforce the need for a proper iodine intake in celiac disease patients to reduce iodine deficiency risk.

Keywords: celiac disease; iodine; thyroid; urinary iodine concentration; endocrine consequences

1. Introduction

Celiac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals [1]. The critical genetic elements (human leukocyte antigen DQ2 and DQ8), the auto-antigen involved (tissue transglutaminase, tTG), and the environmental trigger (gluten) are well known. The intestinal mucosal damage induced by gluten determines villous atrophy and activation/expansion of B cells responsible for autoantibodies production [2].

Autoimmune thyroid disorders (ATD) and non-autoimmune thyroid disorders (NATD) occur in 5–12% of celiac patients [3–7], a figure that is higher than in healthy controls [7–9].

In particular, NATD has been reported to be 3-times more frequent in CD than in general population [6,7]. It has been hypothesized that NATD in CD is secondary to a decreased thyroid hormones synthesis, induced either by an iodine organification defect or by a functional hypothalamic-pituitary disturbance consequent to isolated malnutrition [10,11]. Indeed, gluten withdrawal is often followed by the normalization of thyroid function [5,7].

Iodine is an essential micronutrient for the synthesis of thyroid hormones, and the first step is the absorption in the small intestine [12]. The observation that the mucosal recovery secondary to the gluten-free diet (GFD) is followed by normalization of thyroid function has reinforced the idea that iodine malabsorption contributes to NATD in CD [6]; however, this hypothesis has never been investigated so far.

In the present study, we aimed to evaluate iodine absorption in children and adolescents newly diagnosed with celiac disease before starting the GFD and up to one year later.

2. Materials and Methods

2.1. Study Design

We performed a longitudinal study between February 2017 and May 2019 at the Pediatric Department of the University Hospital of Bari (Italy), a tertiary referral centre for the diagnosis and follow-up of endocrinological and gastroenterological disorders in our region. We recruited children and adolescent with a new diagnosis of CD. The celiac patients were followed-up for 12 months, and symptoms, auxological data, thyroid function tests, urinary iodine concentration (UIC), and anti-tTG were recorded at baseline (T0), after 3 (T1), and 12 months (T2) of GFD.

2.2. Subjects

We recruited 36 children diagnosed with CD based on serologic tests and a duodenal biopsy according to the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) criteria [13]. The inclusion criteria were: 1. Diagnosis of CD according to ESPGHAN guidelines; 2. Willingness to join the study; 3. Age at recruitment <18 years. The exclusion criteria were 1. GFD before the diagnosis of CD; 2. Previous diagnosis of thyroid disease; 3. ATD at recruitment or during the 12-month follow-up; 4. Any disease that could affect thyroid function (i.e., chronic liver or renal disease, autoimmunity or malignancy); 5. Medication that could influence serum thyroid-stimulating hormone (TSH), free thyroxine (fT4), and free triiodothyronine (fT3).

The study adhered to the Declaration of Helsinki and the protocol was approved by the local ethical committee in Bari (Study number: 5200; protocol number: 26989CE); all patients/guardians/controls gave their informed consent prior to inclusion in the study.

2.3. Methods

Data collection included clinical history, growth assessment and thyroid function tests. Height (H) and weight (W) in underwear were measured and the nutritional status evaluated by the body mass index (BMI).

Serum TSH, fT4, fT3 and antibodies against thyroperoxidase (anti-TPO) and thyroglobulin (anti-TG) were assayed. Biochemical assays were performed using commercial kits (Dimension EXL integrated chemistry system LOCI Module Siemens, Erlangen, Germany) with immunoenzymatic (TSH, fT4, and fT3; TSH normal range 0.3/3.6 μg/L, fT4 normal range 0.70/1.80 ng/dL, fT3 normal range 2.2/4.2 pg/mL) and immunoradiometric techniques (anti-TPO and anti-TG antibodies). Hypothyroidism was defined as TSH above the normal values, and classified as subclinical if TSH $\leq$10 μg/L (levothyroxine replacement not required) or overt if TSH >10 μg/L; levothyroxine replacement was started if TSH was confirmed >10 μg/L after 4 weeks.

Serum IgA concentrations, tTG-IgA, and endomysial antibodies (EMA) were tested. Quantitative detection of tTG was assessed by an ELISA test (ORGENTEC Diagnostika; Mainz, Deutschland; cut-off value: >10 AU) and EMA-IgA by indirect immunofluorescence, using monkey's oesophagus sections as substrate (Euroimmun Italia Diagnostica Medica

SRL; Padova, Italia; cut-off >1:10). IgA levels were assayed by nephelometry in all subjects. No patients showed selective IgA deficiency (defined as serum IgA <0.05 g/L). Human leukocyte antigen (HLA) class II typing (DQA1*02:01, DQA1*03, DQA1*05, DQB1*02, DQB1*03:01/03:04, DQB1*03:02/03:05, DRB1*03, DRB1*04, DRB1*07, DRB1*11, DRB1*12) was performed by PCR sequence-specific oligonucleotide using DQ-CD Typing Plus (DiaGene, Palermo, Italy) [14].

Patients with positive serological tests for CD underwent upper endoscopy with multiple duodenal biopsies according to ESPGHAN criteria to confirm the diagnosis [13]. The same pathologist graded all biopsies specimens [15]. After recruitment, all celiac patients started GFD.

Iodine absorption was assessed by the UIC in 24 h-urine samples. The urinary iodine excretion is a very sensitive indicator of iodine intake since iodine is absorbed by the small intestine and mostly excreted by the kidney. All subjects recruited in the study were instructed by an experienced dietitian to guarantee a well-balanced diet with an adequate amount of iodized salt (daily intake of 3–5 g of salt containing 30 ppm of iodine) in keeping with the World Health Organization (WHO) program [16] for at least ten days before the measurement. Dietary recall about iodine intake and GFD was performed at each visit. Patients and controls received a proper container and were invited to collect urine from the second nicturition of the day to the first of the following morning. When the collection was returned, the urine was shaken, and two samples were obtained and immediately stored and frozen at −20 °C for later analysis. The assay was performed in the Chemistry and Clinical Biochemistry Laboratory, Catholic University School of Medicine, Rome, Italy. Urine iodine levels were analyzed by colorimetry (LTA s.r.l., Milan, Italy) using a spectrophotometric procedure based on the Sandell-Kolthoff reaction, in which iodate ion acts as a catalyst in the reduction of ceric ammonium sulphate (yellow color) to the cereus form (colorless) in the presence of arsenious acid. The specimens were treated using ammonium persulfate in advance to eliminate interfering contaminants.

In patients >6 years of age at recruitment, the iodine intake was classified as: insufficient if UIC <100 μg/L; adequate between 100 and 199 μg/L; above requirements between 200 and 299 μg/L; and excessive when ≥300 μg/L [16].

On the basis of WHO guidelines, the patients were classified on the basis of age at recruitment as 0–5 years (suggested minimal iodine intake 90 μg/day), 6–12 years (recommended iodine minimal intake 120 μg/day), and >12 years (suggested minimal iodine intake 150 μg/day) [16].

2.4. Statistical Analysis

Height and BMI were expressed as standard deviation score (SDS) [17]. The statistical analysis was performed with IBM SPSS Statistics v20.0 computer software for Mac. Data were reported as median and interquartile range (IQR) and analyzed by non-parametric tests. The differences between frequencies were evaluated by the Chi-Square (χ^2) test and the differences among groups by the Mann-Whitney U test or the Kruskal-Wallis H test as appropriate. The difference between paired groups was evaluated by the Wilcoxon test. Correlations were evaluated by Spearman's correlation coefficient. Finally, patients were categorized into tertiles for UIC and BMI to compare the other continuous variables. A p-value < 0.05 (2-sided level) was considered statistically significant.

3. Results

3.1. Patients Features

We recruited ten males (27.8%) and 26 females (72.2%) with a median age of 7.4 years (IQR 4.6/9.8 years), height −0.45 SDS (−1.21/0.98 SDS), and BMI −0.25 SDS (−0.96/0.32 SDS). All the enrolled patients showed mucosal atrophy [15 patients with grade B1 (villous to crypt ratio less than 3:1 with still detectable villi), and 21 with grade B2 (villi no longer detectable) according to Corazza-Villanacci classification] [15]. Twenty-one patients (58%) presented with typical symptoms (diarrhea, and/or bloating and/or weight loss) while

fifteen with atypical symptoms or were asymptomatic. Two patients (5.5%) had short stature (H < −2 SDS) and 2 patients (5.5%) were underweight (BMI SDS < −2 SDS). TSH was 2.31 μg/L (1.85/3.06 μg/L), fT4 1.03 ng/dL (0.99/1.13 ng/dL), and fT3 4.18 pg/mL (3.61/4.22 pg/mL). Seven patients (19.4%) presented subclinical hypothyroidism at recruitment, and none of them overt hypothyroidism (highest TSH 8.47 μg/L, fT4 normal in all patients) (Table 1).

Table 1. Features of the patients at diagnosis of celiac disease (T0), after 3 (T1) and 12 (T2) months of gluten-free diet

	Study Group		
	T0 (36 Patients)	**T1 (28 Patients)**	**T2 (23 Patients)**
Age (years)	7.4 (4.6/9.8)	7.6 (5/9.8)	8.5 (5.6/11.6)
Gender	10 M, 26 F	8 M, 20 F	6 M, 17 F
UIC (μg/L)	64 (45/93.25)	76 (60.25/105)	89 (48/124)
TSH (μg/L)	2.31 (1.85/3.06)	2.19 (1.70/3.46)	2.03 (1.52/3.50)
fT4 (ng/dL)	1.03 (0.99/1.13)	1.05 (0.97/1.14)	1.00 (0.95/1.19)
fT3 (pg/mL)	4.18 (3.61/4.22)	4.23 (3.60/4.66)	4.08 (3.72/4.38)
tTG-IgA (titer range, AU)	Positive in all patients (31.9/>200)	Positive in 14 patients (50%) (10.1/>200)	Positive in 10 patients (43.5%) (12.1/>200)
EMA-IgA	Positive in all patients	n.a.	n.a.

Data are reported as median and interquartile range. n.a.: not available; UIC: urinary iodine concentration; tTG-IgA: anti-transglutaminase IgA; EMA-IgA: anti-endomysial IgA. Data are displayed as median (interquartile range).

Fifteen patients were 0–5 years old, seventeen 6–12 years old, and four older than 12 years old.

3.2. Findings at T0

The UIC was 69 μg/L (45/93.25 μg/L) in the study group and 72 μg/L (45/120 μg/L), 68 μg/L (47/89.5 μg/L) and 80 μg/L (41.75/130.25 μg/L) in patients 0–5, 6–12 and older than 12 years, respectively (p = ns among the 3 age groups). Seventeen patients out of 21 who were older than 6 years at recruitment (80.9%) were iodine insufficient. No significant correlations were found between UIC and TSH, fT4, fT3, age at recruitment, H SDS, and BMI SDS in the study group.

Celiac patients with grade B1 had similar UIC, 72 μg/L (45/88 μg/L), to patients with grade B2, 63 μg/L (44.5/105 μg/L) (Figure 1; p = ns). TSH, fT4, fT3, H SDS, and BMI SDS were not different between patients with grade B1 and B2, but the formers were younger [5.2 (4.4/7.5) years] than the latter [9.3 (6.25/11.1) years] (p = 0.028).

The UIC at diagnosis was not different between patients with typical symptoms and patients with atypical symptoms [72 μg/L (44.5/101.0 μg/L) vs 66.5 μg/L (45.2/86.5 μg/L); p = ns].

No statistical difference was found between the 28 patients with iodine deficiency and the 8 patients with adequate iodine intake as regards TSH [2.27 μg/L (1.70/3.00 μg/L) vs. 2.62 μg/L (2.04/4.00 μg/L), respectively], fT4 [1.02 ng/dL (0.99/1.11 ng/dL) vs. 1.07 ng/dL (0.99/1.46 ng/dL), respectively], and fT3 [4.18 pg/mL (3.69/4.35 pg/mL) vs. 3.78 pg/mL (3.25/4.25 pg/mL), respectively].

H, BMI SDS, and thyroid hormone levels were not statistically different among the three tertiles for UIC. Similarly, UIC, H, and thyroid hormone levels were not statistically different among the three tertiles for BMI SDS.

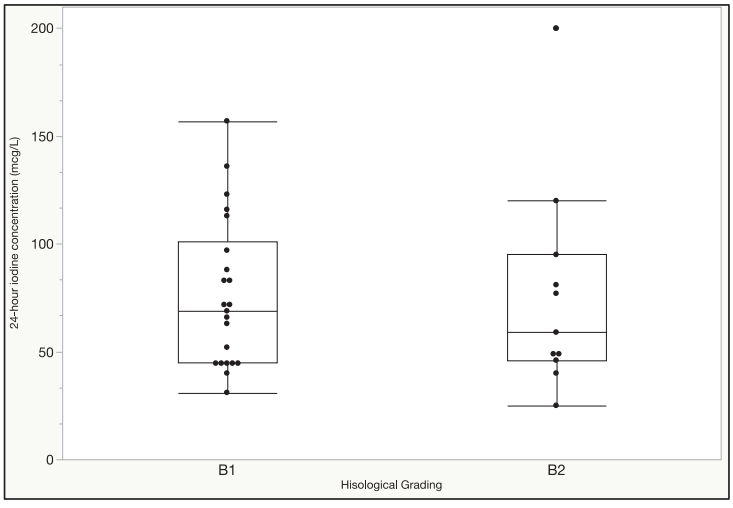

Figure 1. 24-h urinary iodine concentration (UIC) boxplot and scatter plot in patients with B1 [72 (45/88) μg/L] and B2 [63 (44.5–105) μg/L]) grading at T0 (p = ns).

3.3. Findings at T1

Data were available for 28 patients (two patients did not attend the visit, three patients asked to drop out from the study, three patients did not complete the urine collection). The UIC was 76 (60.25/105) μg/L, not different from the value at T0 of 69 (45.25/93.25) μg/L.

The UIC was 97 μg/L (74/167 μg/L) and 63 μg/L (39/82 μg/L) in patients 0–5 and 6–12 years old, respectively (only 2 patients older than 12 years). Fourteen patients out of 17 older than 6 years of age at recruitment (82.3%) were iodine insufficient. No significant correlations were found between UIC and TSH, fT4, fT3, age at recruitment, H SDS, and BMI SDS in the study group.

TSH was 2.19 μg/L (1.70/3.46 μg/L), fT4 1.05 ng/dL (0.97/1.14 ng/dL), and fT3 4.23 pg/mL (3.60/4.66 pg/mL). Six patients (20.7%) presented subclinical hypothyroidism and one hypothyroidism (TSH 10.6 μg/L) without treatment as TSH decreased spontaneously after one month. Thyroid function tests were not statistically different from the values at T0. The TSH and fT3 levels were not different between the 20 patients with iodine deficiency and the 8 patients with adequate iodine intake, while fT4 was lower in patients with iodine deficiency (p = 0.033).

UIC at T1 did not correlate with UIC at T0 and was not different between celiac patients with grade B1 and B2 at diagnosis.

3.4. Findings at T2

Data were available for 23 patients (1 patient did not attend the visit, one patient asked to drop out from the study, three patients did not complete the urine collection). The UIC was 89 μg/L (48/124 μg/L), not statistically different from the value at T0 of 59 μg/L (44/95 μg/L) and at T1 of 81 μg/L (62/118 μg/L).

UIC was 112 μg/L (75/136 μg/L) and 60 μg/L (37/123 μg/L) in patients 0–5 and 6–12 years old, respectively (only 2 patients older than 12 years). UIC was not statistically different as compared to T0 and T1 in each age subgroups. Ten patients out of 14 older than 6 years of age at recruitment (71.4%) were iodine insufficient.

TSH was 2.03 μg/L (1.52/3.50 μg/L), fT4 1.00 ng/dL (0.95/1.19 ng/dL), and fT3 4.08 pg/mL (3.72/4.38 pg/mL). Four patients (16%) presented subclinical hypothyroidism and none overt hypothyroidism. Thyroid function tests were not different from the values

at T0 and at T1. The TSH, fT4 and fT3 levels were not different between the 16 patients with iodine deficiency and the 7 patients with adequate iodine intake.

UIC at T2 was correlated with UIC at T0 (r2 = 0.427, p = 0.042) and at T1 (r2 = 0.516, p = 0.017) and was not different between celiac patients with grade B1 and grade B2 at diagnosis.

Anti-tTG tests were still positive in 10 patients (43.5%) and UIC was not different between patients with positive and negative tests. In patients with positive celiac tests, UIC was 68 µg/L (44.5/101.2 µg/L) at T0 and 102 µg/L (34.5/135.7 µg/L) at T2 (p = ns), while in patients with negative celiac tests it was 52 µg/L (44.0/93.0 µg/L) at T0 and 76 µg/L (54.0/117.5 µg/L) at T2 (p = ns). Figure 2 displays the UIC at T0, T1, and T2.

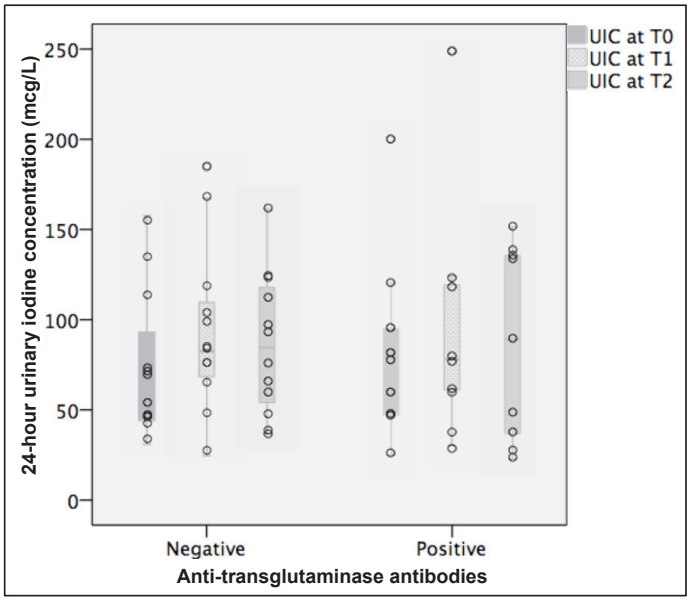

Figure 2. 24-h urinary iodine concentration (UIC) boxplot and scatter plot in patients with positive (10 patients) and negative (13 patients) celiac serological tests at T2. p-value was not significant in both groups. In patients with negative serological celiac tests UIC was 60 µg/L (44/103.5 µg/L) at T0, 82.5 µg/L (65.5/113.3 µg/L) at T1, and 84.5 µg/L (51/120.3 µg/L) at T2. In patients with positive serological celiac tests UIC was 77 µg/L (46.5/107.5 µg/L) at T0, 78 µg/L (50/121.5 µg/L) at T1, and 89 µg/L (32/136.5 µg/L) at T2.

Dietary recall showed an appropriate iodine intake [16] and strict compliance to the GFD [18] at each time point.

Age, UIC, TSH, fT4 and BMI were not different between patients who dropped out and who did not drop out of the study (Table S1).

4. Discussion

The present study, first in the CD literature, shows that children and adolescents newly diagnosed with CD present iodine deficiency and that this state improve, even if not significantly after one year of GFD. The iodine deficiency appears much more evident in school age (about 80% at recruitment, after one year of GFD) as compared to pre-school age children. At diagnosis, the median UIC in our patients appears strikingly lower than the median value of 125 µg/L found in Italian schoolchildren in the same period, even if patients 11–13 years old [19]. Iodine absorption slightly increases during the first year of dietary treatment, even if the increase does not reach statistical significance.

In Italy, since 2005, a law (n 55/2005) introduced a nationwide program of iodine prophylaxis through the use of iodized salt (30 ppm of a gram of salt). Ever since the General Direction of Food Safety and Nutrition at the Italian Ministry of Health and the experts of the Italian National Observatory for Monitoring Iodine Prophylaxis (OSNAMI) intensified the national informative campaigns with the slogan "less salt but iodized"; their efforts successfully led to iodine sufficiency [19].

Iodine plays a central role in the physiology of the thyroid gland, where it exerts its role through two iodine containing-hormones, T3 and T4. The dietary requirement of iodine is determined by T4 production without stressing the thyroid iodide trapping mechanism or TSH levels. We ingest iodine in several chemical forms, mostly reduced to iodide ($I-$) in the gut [20]. Dietary iodide is actively taken up in the small intestine through the $Na+/I-$ symporter (NIS) [12], a glycoprotein located in the basolateral membrane of the thyroid follicular cells and at the apical surface enterocytes of the small intestine [21], which actively accumulates iodine. Iodine enters the circulation as plasma inorganic iodide, which is cleared from circulation by the thyroid, to synthesize the thyroid hormones, and by the kidney, to eliminate the excess. All the steps in thyroid hormones biosynthesis are stimulated by TSH and inhibited by excess iodine [22].

The kidney excretes about 90% of the absorbed iodine with urine within 48 h after intake, and thus the urinary excretion is very reliable to evaluate the iodine intake. In this view, the iodine deficiency was more evident at recruitment in patients 6-12-year-old (median UIC 68 μg/L, suggested minimal iodine intake 120 μg/L) and >12-year-old (median UIC 80 μg/L, recommended minimal iodine intake 150 μg/L). In these patients, UIC was similar after one year of GFD. In patients 0–5 years the median UIC was 72 μg/L at recruitment and 112 μg/L after one year of GFD (suggesting a minimal iodine intake of 90 μg/L), indicating that in this age group the iodine intake is more appropriate than in older patients and that the GFD is beneficial in improving the iodine absorption, even if the increase is not statistically significant as compared to baseline.

Among the methods to monitor the iodine intake [23], the 24-h UIC is the most widely used measurement to give a precise estimation of the iodine intake [24,25]. At the same time, UIC from spot samples is recommended for population assessment and monitoring of iodine interventions globally [16,23]. In this view, we decided to evaluate the iodine absorption by measuring the UIC in 24-h urine samples. To reduce biases as much as possible, an experienced dietician gave proper dietary recommendations to guarantee a well-balanced diet with an adequate amount of iodized salt. At baseline, we performed the urine collection 10 days after using iodized salt (30 ppm of iodine), which is the primary intervention strategy for iodine deficiency control and prevention, to reduce the risk of deficiency in iodine intake and to standardize the iodine intake. It might be argued that the window of dietary intake was too short for replenishing depleted body stores at recruitment, although data from literature [19] show that basically, our school children are iodine sufficient.

Thyroid disease is a frequent finding in CD [9], at least 3-fold higher than in healthy controls [7,26,27]. The most frequent etiology is autoimmune (3–6); however, the incidence of NATD is higher in CD patients than in controls [7]. In our cohort, the prevalence of NATD was around 15–20%, and none of the patients developed overt hypothyroidism. It has been hypothesized that a decreased synthesis of thyroid hormones, due to iodine organification defect or to functional hypothalamic-pituitary impairment secondary to malnutrition, may account for that [7]. We have previously ruled out that pituitary autoimmunity could cause changes in TSH levels [28]. Cassio et al. in a longitudinal study in 135 CD children with at least 3-years of follow-up, found that 13% of patients presented NATD at CD diagnosis, confirming previous data and suggesting the existence of a difference as a yet unknown mechanism [6]. Since the small intestine is the site for iodine absorption, they hypothesized that gliadin induced mucosal enteropathy could impair iodine absorption, thus contributing to the etiology of NATD. Iodine absorption occurs throughout the length of the small intestine. Although we do not have data on the extent of the enteropathy,

it is known from two different studies on celiac adults, using video capsule endoscopy (VCE), that around 60% of CD patients at diagnosis have extensive enteropathy from the duodenum into the jejunum. In contrast, only 30% had villus changes confined to the duodenum [29,30]. Both studies showed conflicting results on the correlation of the extent of the enteropathy with symptoms severity: Murray et al. [29] didn't find any association, while Rodonotti et al. [30] showed that patients with entire small bowel enteropathy presented severer (although not significant) symptoms than those with changes limited to the proximal part. Follow-up VCE showed that a GFD for more than 6 months was able to restore intestinal mucosa starting from the distal part [29].

The hypothesis that impaired iodine absorption might be responsible for altered thyroid hormones metabolism has never been proved. Our data rule out that iodine deficiency in CD at diagnosis is associated with higher TSH levels. Still, our study group can be considered too small to draw a final conclusion. It is likely that different factors, such as iodine deficiency and inflammatory cytokines, may interplay affecting the pituitary-thyroid axis.

We did not find any difference in UIC according to the clinical presentation (typical vs atypical CD). This may be explained by the presence of villous atrophy in all the patients. UIC was not different between patients with partial villous atrophy (B1) as compared to patients with total villous atrophy (B2) (Figure 1), and this might be secondary to the patchy pattern of duodenal enteropathy. We found a progressive, although not significant, increase of UIC after 3 and 12 months of GFD as compared to baseline irrespective of serological tests for CD at one year (Figure 2). It is possible to speculate that a complete anatomical/functional recovery of intestinal mucosa requires a longer period of GFD. This point should be considered for optimal nutritional counselling aiming at an appropriate intake of iodized salt. Celiac patients frequently develop thyroid disorders, and it is well acknowledged that a correct iodine intake in the general population may prevent these disorders. This evidence supports the practical advice to support iodine intake recommendation during nutritional counselling.

We also tested the hypothesis if the nutritional status could affect the pituitary-thyroid axis, but we did not find any difference regarding TSH across the BMI tertiles. We believe that the mucosal damage does not affect thyroid hormones metabolism by reducing iodine absorption considering that the mechanisms of iodine absorption are redundant. Therefore, the impact of gastrointestinal disorders, such as CD, on iodine homeostasis has to be considered negligible, as suggested by the absence of iodine deficiency in patients with short bowel syndrome or previous malabsorptive surgical procedures [31].

Recent data suggest that focusing on selenium metabolism in celiac patients may provide innovative insight. Selenium is absorbed by the duodenum and highly present in the thyroid cells as selenoproteins. A possible reduction of selenium absorption, never evaluated in CD patients, could exert a role in the pathogenesis of NATD. The link between gut microbiota and selenium metabolism is quite strong, as selenium may be actively taken up by the intestinal microbes, causing a reduction of its bioavailability even in the presence of redundant mechanisms of absorption [31]. Imbalances in the intestinal microbiota of patients with CD, mainly characterized by increased *Bacteroides* spp. and decrease of *Bifidobacterium* spp. have been shown [32,33].

This paper has some limitations, which deserve comments. First, the relatively small sample size might not have the power to identify iodine deficit correctly; therefore, our findings warrant confirmatory studies in larger cohorts. Second, we recruited only children and adolescents, and thus it could be debated that our results may not be relevant in adulthood.

On the other hand, we think that our paper has two crucial strengths. First, to the best of our knowledge, this is the first study assaying iodine excretion in celiac patients after proper dietary recommendations. Second, the UIC was assayed on a 24-h sample that, at present, is the gold standard to evaluate iodine absorption although, the collection of daily urinary output is a burden for patients.

5. Conclusions

In conclusion, our data suggest that school age celiac patients present at diagnosis iodine deficiency that partially recovers after one year of a gluten-free diet. This stresses the need for proper dietetic advice on a possible long-term iodine supplementation, especially in these age group patients. Further studies in larger cohorts and hopefully over a more extended study period could be of great help to confirm or deny our findings to better understand the mechanism underlying iodine absorption in celiac patients.

Supplementary Materials: The following are available online at https://www.mdpi.com/2072-6643/13/3/808/s1, Table S1. Characteristics of patients who completed the study vs patients who dropped out. No statistical differences were found between the 2 groups. Data are displayed as median (interquartile range).

Author Contributions: Conceptualization, statistical analysis, writing of the manuscript, M.D. and R.F.; patients' recruitment, data collection, data interpretation, F.B., A.G., F.C., M.D., R.F., M.B., P.G., V.N.D. and S.S.; urinary iodine measurement, data interpretation, writing of the manuscript, R.L. and C.C.; substantial contribution to data interpretation, revision of the paper, language editing, F.C., P.G. and V.N.D. All authors have read and agreed to the published version of the manuscript.

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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 privacy policy.

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

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Health-Related Quality of Life and Experiences of Brazilian Celiac Individuals over the Course of the Sars-Cov-2 Pandemic

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Abstract: Since the end of 2019, the world has been facing an unpredicted COVID-19 pandemic with consequences for the economy, environment, society, and health. The COVID-19 pandemic has increased the risk of death, bringing unbearable psychological pressure upon people worldwide. For celiac patients, the pandemic may represent an additional burden concerning the inherent aspects of celiac disease (CD) that compromise these individuals' quality of life (QoL). Therefore, the objective of this study was to evaluate Brazilian celiac patients' QoL during the course of the COVID-19 pandemic caused by its outbreak and rapid spread and subsequent restrictive measures in addition to the dietary restrictions and other burdens caused by CD. This country-wide cross-sectional study was conducted using a self-administered instrument previously validated in Brazilian–Portuguese to investigate the QoL of individuals with CD. Data collected through the online self-administration of the Brazilian version of the celiac disease quality of life questionnaire (CDQ) comprised 674 CD individuals' responses. Although pandemics have historically posed a challenge for Brazilian population, this period was not associated with a negative impact on Brazilian CD individuals' QoL. During the pandemic, the QoL of Brazilian's with CD was more affected by gastrointestinal aspects than emotions and social aspects and worries. Gender, age, marital status, having (or not) children, occupation, and a positive test for COVID-19 did not affect CD individuals' QoL. However, the study revealed a larger burden and diminished QoL for individuals not following a gluten-free diet and those using antidepressants. Additional research is necessary to verify how the length of the pandemic will affect celiac individuals and then compare those outcomes compare to the COVID-19 period and after.

Keywords: celiac disease; quality of life; COVID-19; pandemic

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1. Introduction

Celiac disease (CD) is a chronic enteropathy started by gluten ingestion in genetically susceptible individuals. CD affects approximately 1% of the world's individuals and presents clinical manifestations including intestinal and extraintestinal symptoms [1]. Despite the symptoms and consequences of gluten ingestion, which are directly related to how these patients perceive their quality of life (QoL) [2], following a strict gluten-free diet (GFD) can also affect QoL positively (reducing symptoms) [3] or negatively (due to the social exclusion, lack of information and ability to handle healthy gluten-free meal production, the high price of food, risk of gluten cross-contamination, fear of social

exclusion, insecurity of being safe, and others) [4]. Therefore, diet is one of the most important aspects of the QoL of CD individuals.

The world is facing an unpredicted COVID-19 pandemic generated by Sars-CoV-2 viruses since the end of 2019. COVID-19 has brought consequences upon the economy, society, environment, and health. Worldwide, people are experiencing an increase in the risk of death and unbearable psychological burden [5]. For celiac patients, the pandemic may represent additional burdens concerning CD's inherent aspects that compromise these individuals' QoL. As a result, celiac individuals may perceive their QoL affected in several ways including physical, emotional, economic, and social [3,6,7]. Most governments around the world took severe mitigation measures due to the COVID-19 pandemic, including community-wide lockdowns, home quarantines, social distancing, and the prohibition of social gatherings, to reduce the spread of Sars-CoV-2 [8]. These measures limited people's access to traditional medical routine appointments and access to food from food services and markets, which may represent an even worse disturbance for individuals who present dietary restrictions.

A recent study conducted in Poland with 1033 adults investigated perceived stress as a predictor of consumers' distress of restricted access to food and predictor of food purchase behaviors throughout the pandemic [9]. The study showed that more than half of the participants perceived a reduction in grocery stores' food supplies. The fear experienced during the pandemic is also influenced by changes in food availability, which highlights the importance of available information and confidence in their sources to reduce psychological burden [9]. Hence, we hypothesized that the Sars-Cov-2 pandemic could affect groups that present chronic diseases whose treatment and well-being depend on restricting diet and special foods, like CD, leading to influences on their food security [10] and, consequently, in their QoL.

Therefore, the investigation of celiac patients' health-related QoL during the Sars-Cov-2 pandemic is essential, since they have special dietary needs. Furthermore, CD interferes in the patient's daily life beyond the gastrointestinal and health aspects of the disease such as their social, economic, and emotional status [11]. While several studies have been carried out on the pandemic's psychological effects on the general public, patients, medical staff, children, and elderly [5,12–14], and several other studies were performed on celiac individuals during the pandemic [15–22], none evaluated the QoL of CD patients during the difficult times of the pandemic. Therefore, the objective of this research was to evaluate Brazilian celiac patients' QoL during the pandemic caused by the outbreak, rapid spread, and subsequent restrictive measures caused by COVID-19, in addition to the dietary restrictions and other burdens caused by CD. We aimed to show what influences Brazilian celiac patients' QoL during the pandemic, helping them recover after the pandemic period.

2. Materials and Methods

2.1. Study Design and Instrument

This country-wide cross-sectional research was performed using a self-administered instrument to evaluate a CD individual's quality of life (CDQ) developed by Häuser et al. [23], which was validated in Brazil by Pratesi et al. [6], to investigate the QoL of Brazilian celiac individuals during the pandemic. The CDQ comprises four domains (i.e., emotions, gastrointestinal symptoms, concerns, and social) with 7 items each, comprising a 28-item questionnaire. Each question was evaluated using a 7 point scale ("1" meaning the worst QoL perception and "7" the best QoL perception). Therefore, the highest possible final score for QoL was 196 points, with higher scores reflecting a better level of QoL.

Sociodemographic characteristics were also investigated in this study (e.g., gender, age, marital status, place of residency, educational level, occupation). The GFD adhesion was self-reported using the Brazilian version of the instrument previously validated [6], and it was not confirmed by serological tests since, during the pandemic, we could not access patients. The researchers also included questions on the Sars-CoV-2 period regarding the presence of a positive test for the disease and/or a family member with infection.

The SurveyMonkey®platform was used for CDQ applications from 7 to 28 August 2020. Volunteers were recruited nationwide by invitation through a link to access the study sent via email, messaging apps, Brazilian Celiac Associations, and social networks.

2.2. Participants and Ethics

A convenience sample composed of CD individuals from the entire country was used in this study. The participants were recruited through a research link invitation that included a consent form to certify their agreement to join the study. The research was approved by the Ethics Committee of the University of Brasília (CAEE 69119317.3.0000.0030) and conducted according to the Declaration of Helsinki guidelines.

The inclusion criteria were: (i) to have celiac disease; (ii) live in Brazil; (iii) $\geq$18 years old. Individuals who agreed to participate in the research were directed to the survey's questions, while those who did not agree to participate were directed to a page thanking them for their time.

2.3. Statistical Analysis

Data from the SurveyMonkey®platform were extracted and analyzed using the IBM SPSS Statistics for Windows (Armonk, NY: IBM Corp, USA). The statistical analysis was conducted by scores (higher score indicating a higher QoL). The corresponding dimensions' median value substituted blank questions. The total score was calculated for each individuals' characteristics. If there was more than 20% of blank questions, the questionnaire was not used in the analysis.

Descriptive statistics were presented as the mean, median, standard deviation, and floor and ceiling effect of CDQ's subscales. One-way repeated measures ANOVA followed by Bonferroni's post-hoc test were used to compare domains' means. The Student's *t*-test and one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests were used to compare the domains with the interesting variables. Comparisons of QoL before and during the Sars-Cov-2 pandemic were performed by one-way analysis of covariance (ANCOVA), considering the sociodemographic and health variables as controlling covariates. All tests considered bilateral hypotheses and a significance level of 5%. The factor validity was verified using a confirmatory factor analysis. The Chi-squared test of minimum discrepancy (chi2), the root mean square error of approximation (RMSEA), and the comparative fit index (CFI) evaluated the factor validity [24]. A RMSEA $\leq$ 0.05 and CFI $\geq$ 0.9 indicate a good model fit [25].

3. Results

3.1. Characterization of the Sample

Data collected through the online self-administration of the CDQ comprised responses from a convenience sample of 674 CD individuals. Most participants were female (n = 631; 93.6%), aged 39 years old or less (n = 405; 60.1%). CD diagnosis occurred before or at 39 years old for most participants (n = 512; 75.9%). Other socioeconomic, demographic, and health-related variables were also investigated, and the results are presented in Supplementary Materials Table S1. During the study period, 59.1% of the participants (n = 397) reported not having a partner compared to 40.9% of those with a partner (n = 275). Individuals were also asked whether they lived with children who were under 18 years of age, and most reported that they did not (57.7%; n = 387). Our sample was mainly composed of participants with a higher level of education, college-level and above. A total of 45.5% reported having a postgraduate degree; 38.3% went to college; and the other 16.2% attended high school, at most. In regard to occupation, 51.2% worked as a self-employed professional or in a private company (n = 335), 23.4% worked in public agencies (n = 153), 12.2% were students (n = 80), 13.1% were retired or unemployed (n = 86), and the remaining participants did not answer this question n = 20).

Self-reported adherence to the gluten-free diet was also investigated. Most participants (88.6%; n = 597) reported that they always followed the diet, while 11.4% (n = 77) reported

not always sticking to the CD's dietary restrictions. Concerning the use of antidepressants, most of the sample (78.4%; n = 526) did not take this type of medication. Regarding the specific questions about COVID-19, most participants (93.8%; n = 631) had nether tested positive for the infection up to the questionnaire's completion date nor had an infected family member (65.9%; n = 444). Among those with family members who tested positive for the disease (34.1%; n = 230), most did not live together (77.8%; n = 179).

3.2. Instrument Reliability Analysis

The questionnaire's and subscales' internal consistencies were assessed through the Cronbach's alpha measure (Table 1). The CDQ questionnaire's four domains displayed satisfactory results for Cronbach's alpha ($\alpha > 0.7$), indicating the instrument's good reliability, both separately by domain and as the complete instrument. Each domain's scores ranged from 7 to 49, and the total from 28 to 196. Higher scores reflected a better level of QoL.

Table 1. Analysis of the precision of the subscales (n = 674) of the CDQ instrument.

	Mean (SD)	Median (IQR)	Range	Floor Effect (%)	Ceiling Effect (%)	Internal Consistency (Cronbach' Alpha)
Emotion	34.81 (8.42)	35 (29–42)	10–49	0%	2.1%	0.820
Social	25.82 (8.87)	26 (19–32)	7–49	0.9%	0.1%	0.916
Worries	34.86 (10.25)	36 (27–44)	8–49	0%	6.1%	0.842
Gastrointestinal	29.77 (10.75)	30 (21–39)	7–49	0.6%	1.5%	0.838
Total Score	125.26 (32.02)	127 (100–151)	43–194	0%	0%	0.936

3.3. Quality of Life

The CDQ sub-scores found in this study were subcategorized by sociodemographic data and are presented in Table 2. In general, during the pandemic, the CDQ total score was affected by the age of CD diagnosis, educational level, GDF adhesion, and use of antidepressants.

There was neither a significant difference for the overall QoL score nor for each domain separately regarding gender. Concerning the variable "age", the only significant difference observed was found for the domain "emotions", which presented a lower score for individuals aged 39 years or less. The CD diagnosis age was reflected on the total score of QoL (p = 0.006) and was higher for those $\geq$40 years of age when they were diagnosed. Individuals diagnosed at $\leq$39 years of age displayed lower QoL overall scores and in the "gastrointestinal" and "emotions" domains.

The absence of a partner led to higher scores for the "gastrointestinal" and "emotions" domains in comparison to the presence of a partner (p = 0.021 and p = 0.001, respectively), but it did not reflect significantly better overall QoL (p = 0.264). Children under 18 years old and living in the same house as the participant resulted in a significantly lower score only for the "worries" domain (p = 0.009).

In general, a greater educational level was associated with higher global QoL and higher scores for the domains separately. Regarding the type of occupation, a significant difference was only found for the "worries" domain. Students displayed higher scores for this domain in comparison to retired/unemployed participants.

Adherence to the gluten-free diet—represented by participants' disclosure of "always following the diet"—resulted both in higher overall QoL and higher scores for each domain separately. Concerning antidepressants, participants who took this type of medication showed lower overall QoL and lower scores for each domain individually.

No significant difference in the quality of life was found between participants who had COVID-19 and those who did not. Moreover, having a relative infected with Sars-CoV-2 also did not significantly differ in the QoL.

Table 2. CDQ sub-scores subcategorized by sociodemographic data ($n = 674$).

	Gastrointestinal		Emotions		Social		Worries		Total	
	Mean (SD)	p	Mean (SD)	p	Mean (SD)	p	Mean (SD)	p	Mean (SD)	p
Gender *										
Women ($n = 631$)	34.87 (8.46) [a]	0.534	25.87 (8.75) [a]	0.663	34.85 (10.28) [a]	0.925	29.70 (10.82) [a]	0.548	125.29 (32.07) [a]	0.940
Men ($n = 43$)	34.05 (7.72) [a]		25.14 (10.59) [a]		35.00 (9.77) [a]		30.72 (9.72) [a]		124.91 (31.63) [a]	
Age *										
≤39 ($n = 405$)	34.34 (8.54) [a]	0.090	24.66 (8.54) [a]	<0.001	34.61 (10.42) [a]	0.463	29.49 (10.83) [a]	0.442	123.11 (32.01) [a]	0.039
≥40 ($n = 264$)	35.47 (8.20) [a]		27.51 (9.11) [b]		35.21 (10.02) [a]		30.14 (10.56) [a]		128.34 (31.75) [a]	
Age at diagnosis *										
≤39 ($n = 512$)	34.46 (8.46) [a]	0.049	25.02 (8.59) [a]	<0.001	34.46 (10.35) [a]	0.071	29.40 (10.91) [a]	0.119	123.34 (32.00) [a]	0.006
≥40 ($n = 162$)	35.94 (8.21) [b]		28.35 (9.29) [b]		36.12 (9.81) [a]		30.91 (10.17) [a]		131.33 (31.43) [b]	
Marital status *										
No partner ($n = 397$)	35.46 (8.46) [a]	0.021	26.77 (8.95) [a]	0.001	34.98 (10.19) [a]	0.803	29.30 (10.73) [a]	0.148	126.52 (32.39) [a]	0.264
With partner ($n = 275$)	33.94 (8.29) [b]		24.47 (8.62) [b]		34.78 (10.29) [a]		30.52 (10.75) [a]		123.72 (31.42) [a]	
Children under 18*										
Yes ($n = 284$)	34.89 (8.53) [a]	0.805	25.75 (8.65) [a]	0.856	34.14 (10.25) [a]	0.122	28.46 (10.60) [a]	0.009	123.25 (31.65) [a]	0.174
No ($n = 387$)	34.73 (8.36) [a]		25.88 (9.07) [a]		35.39 (10.24) [a]		30.66 (10.78) [b]		126.66 (32.31) [a]	
Educational level **										
≤High school ($n = 109$)	32.90 (8.98) [a]		23.96 (9.11) [a]		32.59 (9.99) [a]		27.09 (10.98) [a]		116.54 (32.38) [a]	
College ($n = 258$)	33.36 (8.57) [a]	<0.001	25.20 (8.77) [ab]	0.003	33.61 (10.39) [a]	<0.001	28.71 (10.54) [a]	<0.001	120.87 (31.72) [a]	<0.001
Postgraduate degree ($n = 307$)	36.73 (7.67) [b]		27.00 (8.73) [b]		36.71 (9.92) [b]		31.61 (10.55) [b]		132.05 (30.83) [b]	
Occupation **										
Self-employed/private company ($n = 335$)	35.18 (8.06) [a]		25.95 (8.93) [a]		35.51 (9.95) [a]		30.35 (10.67) [ab]		126.99 (31.21) [a]	
Public agency ($n = 153$)	35.39 (8.54) [a]	0.104	26.86 (8.59) [a]	0.076	34.57 (10.44) [a]	0.114	29.28 (11.14) [ab]	0.044	126.10 (33.01) [a]	0.084
Student ($n = 80$)	33.00 (9.26) [a]		24.56 (8.32) [a]		34.91 (9.17) [a]		30.66 (11.27) [a]		123.14 (31.86) [a]	
Retired/unemployed ($n = 86$)	33.85 (8.69) [a]		24.10 (9.71) [a]		32.53 (11.42) [a]		26.86 (9.63) [b]		117.35 (32.90) [a]	
Gluten-free diet *										
Always ($n = 597$)	35.48 (8.36) [a]	<0.001	26.40 (8.77) [a]	<0.001	35.20 (10.26) [a]	0.015	30.38 (10.66) [a]	<0.001	127.46 (31.78) [a]	<0.001
Not always ($n = 77$)	29.71 (7.03) [b]		21.29 (8.35) [b]		32.18 (9.79) [b]		25.03 (10.32) [b]		108.21 (28.77) [b]	
Antidepressants *										
Yes ($n = 145$)	32.46 (8.37) [a]	<0.001	22.15 (7.61) [a]	<0.001	33.28 (10.05) [a]	0.032	27.70 (10.70) [a]	0.008	115.59 (30.27) [a]	<0.001
No ($n = 526$)	35.51 (8.30) [b]		26.86 (8.94) [b]		35.35 (10.25) [b]		30.36 (10.69) [b]		128.08 (31.94) [b]	
Positive test for COVID-19 *										
Yes ($n = 42$)	34.38 (8.85) [a]	0.723	26.71 (9.51) [a]	0.509	36.98 (9.10) [a]	0.131	31.24 (10.74) [a]	0.367	129.31 (32.19) [a]	0.405
No ($n = 631$)	34.86 (8.40) [a]		25.78 (8.82) [a]		34.73 (10.31) [a]		29.69 (10.74) [a]		125.06 (32.00) [a]	
Infected Family Member **										
Yes: living together ($n = 51$)	34.86 (8.80) [a]		27.80 (9.28) [a]		35.69 (8.88) [a]		31.96 (9.41) [a]		130.31 (29.76) [a]	
Yes: do not live together ($n = 179$)	34.02 (8.50) [a]	0.328	25.08 (8.80) [a]	0.147	34.40 (10.13) [a]	0.695	28.77 (10.80) [a]	0.152	122.26 (32.18) [a]	0.222
No ($n = 444$)	35.14 (8.33) [a]		25.89 (8.83) [a]		34.95 (10.45) [a]		29.92 (10.85) [a]		125.89 (32.16) [a]	

Some variables do not add up to 674, since some individuals did not fill in all fields. * Student's t-test. ** ANOVA with Tukey's post-hoc tests. Groups with the same letters do not differ significantly.

4. Discussion

This is the first study on CD individuals' QoL performed in Brazil during a pandemic. Identifying the factors influencing the CD individuals' QoL during the pandemic could help design strategies to mitigate them. Our results showed that most participants were female (n = 631; 93.6%), similar to the study CD individuals' QoL performed in Brazil before the pandemic in which 94% (n = 425) were female and 82.4% (n = 371) were not using antidepressants [6]. The age group (60.1%, ≤39 y) of the participants was also similar to the participants prior to the pandemic, aged 39 years old or less (57%, ≤39 y [6]). In our study and the previous one [6], 88.6% of participants reported following a strict GFD. Since our sample of CD individuals presented characteristics regarding gender, age, and GFD adherence that were similar, we compared our QoL data to the previous study performed using the same QoL questionnaire in Brazil [6] (Supplementary Materials Table S2). The comparison among the groups of Brazilian celiac individuals before and during the pandemic was possible since, despite it being impossible to identify if they were the same individuals, they were from the same population under study and with similar characteristics. In addition, it was corrected using analysis of covariance (ANCOVA) to adjust for possible heterogeneities in the socio-demographic characteristics of the samples from the two studies.

Surprisingly, the results obtained during the pandemic showed a higher total score for the CDQ than the previous application of the instrument in Brazil before the pandemic (p = 0.011). Therefore, our hypothesis that the pandemic could affect Brazilian CD individuals' QoL was not confirmed. Analyzing each domain separately, a statistically higher score was found for "social" and "worries"($p < 0.001$ in both cases) during the pandemic than before. On the other hand, the "gastrointestinal" domain displayed a lower score during the pandemic ($p < 0.001$). Regarding the domain "emotions", the score was not statistically different from the CDQ's prior application (p = 0.082). In theory, staying at home favors time to "eat well and stay well", following a strict GFD [18]. This could positively impact on the "worries" and "emotions" domains (potentially reduced by the lowered fear of consuming gluten-containing products) but have a worse effect in the "social" domain (as occurred in our study) due to the necessary isolation during the pandemic. The study on Brazilian QoL in general individuals also showed that the "social" domain was burdened by pandemic [26].

During the pandemic, the domains "social" and "worries" presented the best scores among the domains, which differed from the study performed before the pandemic in which the best scores were attributed to the "gastrointestinal" and "social" domains [6].

The "gastrointestinal" domain was worse during the pandemic. Although a similar percentage of respondents informed that they followed a strict GFD in both periods (Supplementary Materials Table S2), data from the "gastrointestinal" domain might indicate the possibility of non-intentional consumption of gluten. Considering that CD individuals that live without a partner presented better scores in the "gastrointestinal" domain (Table 2) than the ones who lived with partners (added to the need to be and eat at home), the presence of more than one adult can, potentially, increase the potential for gluten cross-contamination when one of them does not follow the GFD.

Individuals who reported following the GFD presented better QoL overall (and by domains) than those who reported not adhering to a strict GFD. Similar results were found before the pandemic, except for the "worries" domain, which did not differ among individuals who followed the GFD [6]. Probably, people eating more often at home may reduce the perception of the risk of gluten ingestion for those following a strict GFD, and it could have impacted the "worries" domain.

Different from the study that was performed with the general Brazilian population (n = 1877) during the pandemic in which gender differed for all QoL domains (males with better QoL than females) [26], no significant difference was found for the CDQ during the pandemic regarding gender. Our result was also different from the Brazilian CD individuals' QoL study performed before the pandemic in which males' scores for the CDQ

were higher than females, except for the "gastrointestinal" domain. We did not expect the results in which gender, age, and marital status did not differ among the CD individuals' perception of QoL during the pandemic, since previous studies with non-celiac individuals showed that younger and females individuals were at a greater risk for distress and that individuals with a partner showed better scores than the those with no partner [27–30].

The individuals in the older age group handled the "emotions" domain better in this pandemic than the younger age group. This result is aligned with other studies with non-celiac individuals during the pandemic [31,32]. The authors attributed their results to more unsure working conditions and economic burdens for younger people and larger restrictions for younger than for older individuals [31,32]. However, in our study, the "social" domain did not differ between age groups. It is important to mention that the previous Brazilian CD individuals' QoL study [6] showed that prompt diagnosis was related to a better "social" domain score and general QoL, similar to other studies [33,34]. However, in this study, the time of CD diagnosis did not influence QoL (Pearson correlation = 0.1).

Individuals with the highest educational level presented better overall QoL and higher scores for the domains separately, as found in the study performed in CD individuals' QoL in Brazil before the pandemic [6]. This is probably because a higher educational level contributes to an individual's physical, social, and health aspects. Low education increases some chronic health conditions' adverse outcomes because of the low level of knowledge [35–38]. Education level tends to be associated with higher socioeconomic status [36], and income modulates health-seeking behavior and access to health care [39], both related to higher QoL. In this sense, it is well-known that higher education levels can influence some of the QoL aspects. This characteristic of our convenience sample could be a potential bias of our study. Additional studies should be performed to evaluate CD individuals with lower education levels and their influence on individuals' QoL.

Our results showed that 6% (n = 42) of CD individuals experienced COVID-19 as did 34% (n = 230) of their relatives. These results were higher than those found in a study [20] conducted with 138 CD individuals in Italy in April/May 2020, where no diagnosis of COVID-19 were reported, while 19 participants presented flu-like symptoms (one having a negative nasopharyngeal swab test). Further, 7.9% (n = 11) CD individuals reported a relative presenting respiratory symptoms suggestive of Sard-CoV-2 infection [20]. Our findings were worse compared to the study with the general Brazilian population [26] in which 2.7% of the participants had COVID-19 and 16% (n = 300) of the participants had a relative who presented Sars-Cov-2 infection. Considering that the Brazilian study with the general population ended data collection (14 August 2020) 14 days before our study (28 August 2020) and the number of cases and deaths were continuously increasing, it is not possible to affirm that the incidence of COVID-19 was higher in CD individuals or their relatives than the general population in Brazil. As only 42 patients (6.2%) had SARS-CoV-2 infection when the questionnaire was completed, differences in QoL perception were not likely significant.

A large study performed with 10,737 CD patients from Argentina, Australia, Canada, Italy, Mexico, New Zealand, Spain, Uruguay, and the United States during the pandemic showed that CD patients have similar chances of contracting Sars-CoV-2, and it is unnecessary to take additional care to prevent exposure aside from the recommendations to the general population [22]. However, the comorbidities identified as a significant predictor of morbidity and mortality on COVID-19 were more frequent in CD individuals than control ones [22]. A cohort study conducted in Sweden with 40,963 CD individuals showed that they were neither at increased risk of hospitalization for COVID-19 than the control ones nor at high risk for severe disease outcomes and mortality [21].

Individuals that contracted or did not contract COVID-19 did not differ in QoL. The same was observed among participants whose relatives contracted or did not contract COVID-19, probably because the uncertainties about the disease and its outcomes lead people to continue to fear infection by the virus [40,41], despite having been contaminated themselves or one of their relatives.

A potential study bias is the method of survey dissemination (i.e., internet, email, and social media) and the use of a convenience sample. However, if random sampling was used, it would be impossible to reach a large sample. Also, during the pandemic period and social isolation, use of the internet is the primary way to reach respondents. Despite similar characteristics between the samples of the two studies conducted on CD individuals' QoL in Brazil, it was not possible to affirm that the same individuals participated in both studies. As the vast majority of the respondents to the survey were female celiac subjects, the results did not reflect the perceptions of the male population.

5. Conclusions

The COVID-19 pandemic poses a historic challenge for many worldwide, but this period has not been associated with a negative impact on the Brazilian CD individuals' QoL. In the course of the pandemic, CD individuals' QoL was more affected by gastrointestinal and social aspects than emotional aspects and worries in Brazil. Gender, age, marital status, having (or not) children, occupation, and a positive COVID-19 test did not affect the CD individuals' QoL. However, the study revealed a major QoL burden for individuals who did not follow the GFD and for those using antidepressants. Additional research is necessary to verify how the length of the pandemic will affect celiac individuals and then to compare this period during and after COVID-19.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/nu13051582/s1. Table S1: Socioeconomic, demographic, and health-related results of the study sample; Table S2: Comparison between the application of the CDQoL questionnaire before and during the Sars-Cov-2 pandemic.

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Nutritional Status in Spanish Adults with Celiac Disease Following a Long-Term Gluten-Free Diet Is Similar to Non-Celiac

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Abstract: The only available treatment for celiac disease is life-long gluten exclusion. We conducted a cross-sectional age- and gender-matched study in 64 celiac adults on a long-term (>1 year) gluten-free diet and 74 non-celiac volunteers from Spain, using dietary, anthropometric, and biochemical parameters, as well as assessing bone mineral density and physical activity. Celiac adults had deficient intake (below 2/3 of the recommended intake) for folates, vitamin E, and iodine and low intake of calcium (below 80% of the recommended intake). Iron intake was also below 2/3 of the recommended intake in celiac women. Vitamin D intake was extremely low, and 34% of celiac patients had moderately deficient plasma levels. According to bone mineral density, celiac women may be more prone to osteopenia and osteoporosis. However, we found a perfectly analogous nutritional status scenario in celiac as compared to healthy volunteers, with the dietary deviations found being similar to those of the Spanish population, i.e., both groups followed a high-lipid, high-protein, and low-carbohydrate diet. Values for biochemical parameters were found within the reference ranges. Celiac disease had no influence on body weight, but body fat in celiac patients tended to be higher. According to our results, vitamin D, calcium, folates, vitamin E, iodine, and iron nutritional status should be specifically assessed and monitored in the celiac population.

Keywords: celiac disease; gluten-free diet; nutritional assessment; adults; dietary intake; nutrient intake; anthropometric measures; bone mineral density; physical activity

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1. Introduction

Celiac disease (CD) is an autoimmune systemic disorder triggered by the intake of gluten and its prolamins, which affects genetically susceptible individuals, causing progressive atrophy of the intestinal villi [1]. The only existing treatment is to follow a strict gluten-free diet (GFD) that repairs intestinal damage and restores adequate nutrient absorption [2]. The GFD should meet the recommended nutritional goals for energy and nutrients, just as it is for the general population.

The prevalence of CD in European and American populations of European descent is 1% [3]. In addition, it is estimated that there is a high percentage of undiagnosed cases [4]. So far, there are few studies that assess the overall nutritional status of people with celiac disease following a long-term GFD diet, but most of them agree that energy and nutrient intake are far from recommendations. Several studies agree on a higher intake of fats, proteins, and simple carbohydrates [5–12]. At the same time, some studies describe insufficient intake of fiber and carbohydrates, as well as of certain vitamins and minerals, possibly due to the exclusion of grains naturally rich in fiber, and the incorporation of commercial gluten-free products, whose content in refined flours and fats is higher than in their gluten-containing counterparts [13,14]. The high fat intake described

in celiac population, together with a higher content of saturated fats and hydrogenated fatty acids in gluten-free products and a higher prevalence of overweight and obesity found in celiac population, makes it necessary to manage GFDs nutritionally to prevent health complications, such as the risk of developing metabolic syndrome and cardiovascular disease [15–17]. Interestingly, a higher glycemic index has been described in gluten-free products compared to their gluten-containing counterparts, since they are made with highly refined cereals and the amount of fiber is lower [13,18,19].

Furthermore, insufficient vitamin D and calcium intake are also documented in celiac population. This could be explained by the restriction of dairy products, due to a high prevalence of lactose intolerance in people with CD. In these cases, calcium and vitamin D supplementation, as well as using lactose-free products should be considered [2]. Low intake of calcium and vitamin D, together with the fact that low bone mineral density (BMD) is widely documented in CD patients [20–22], are further reasons why the contribution of diet to the nutritional status of people with CD should be assessed, to avoid the development of long-term bone alterations.

The reason why CD has been associated with low BMD is mainly due to the characteristic malabsorption, with the consequent deficiency in vitamin D and intestinal absorption of calcium. Other deficiencies in fat-soluble vitamins (A, K, and E) and minerals would also affect normal bone metabolism [23]. On the other hand, given the close hormonal interrelationship, calcium and vitamin D deficiencies stimulate the secretion of parathormone (PTH), and hyperparathyroidism would itself be another factor involved, since elevated levels of PTH have been associated with loss of bone mass by activation of bone resorption. Elevated PTH serum levels have been found in patients on a GFD [22].

Nonetheless, the available data on the effect of GFD on BMD provide discordant results, probably due to the different characteristics of the population studied in terms of age and sex, and due to the different amount of time on a GFD. Once the elimination of gluten occurs, the greatest gain in bone mass is established in the first year [24], and the GFD leads to a 5% increase in bone mass after one year of implementation [25]. However, in several studies [26,27], improvement in BMD after undertaking a GFD was only observed in patients who initially had secondary hyperparathyroidism, low serum calcium and vitamin D. Therefore, it may not be easy to normalize BMD in adults with CD [28]. In fact, there is a current debate on whether the GFD is sufficient to normalize bone alterations, or whether calcium and vitamin D supplementation should be recommended for these patients. Calcium fortification of gluten-free products has been suggested as a strategy to improve the calcium content in the diet of celiac patients [29]. Taken together, nutritional status may play a very relevant role in the maintenance and recovery of BMD in patients with CD in the long term, making it necessary to monitor intake of nutrients of vital importance for bone metabolism. Moreover, the same may be applied to physical activity, since people who are regularly active have greater bone mass and a lower number of fractures than those who lead a sedentary life.

The data available in the literature regarding body composition of people with CD following a GFD are not very clarifying of the real situation of this population group. Firstly, due to the variability in the age range of the populations studied, and secondly, due to the difference in the moment at which anthropometric and BMD measurements are made with respect to the beginning of the GFD. Nevertheless, there is some consensus on the favorable response of body composition to a GFD, normalizing situations of thinness, overweight, obesity and low BMD after the recovery of nutrient absorption functionality [30–32]. Classically, it has been described that patients with CD who follow a GFD, with a complete remission of the disease clinic, have lower BMI and body fat as compared to non-celiac [6]. This is also described in a recent review [33], which concluded that BMI is lower in subjects who have not yet begun the GFD compared to those who are on a GFD, and in celiac following a GFD compared to the population without the pathology. In Spain, a study carried out in the Basque Country has found different eating patterns between men and women, and a difference in anthropometric measurements. Thus, celiac women had

lower BMI compared to the general population, low prevalence of overweight (6.5%) and no cases of obesity [9]. In the case of men, they found a higher prevalence of overweight (26.2%) and obesity (11.9%), as compared to celiac women, but still this prevalence was lower than in general population [34]. It should be noted that, in the adult population, the histological recovery of the intestinal mucosa is not as effective as in children.

On these premises, the aim of this study was to perform a thorough analysis of nutritional status in a Spanish CD adult population, including dietary quality, level of exercise, together with the analysis of body composition and biochemical parameters, all of which are necessary to describe how long-term adherence to a GFD impacts on nutritional status.

2. Materials and Methods

2.1. Subjects

This is a cross-sectional age- and gender-matched study in celiac and non-celiac adult volunteers. People with CD were located through a patient's association (Asociación de Celíacos y Sensibles al Gluten) in Madrid (Spain). The criteria for inclusion in the study for celiac were confirmed diagnosis of celiac condition, adherence to a gluten-free diet for more than one year, absence of associated diseases, and not taking nutritional supplements. In the non-celiac group, the inclusion criteria were not being diagnosed with any chronic disease, not having symptoms or signs of digestive disease on a regular basis, and not taking nutritional supplements. All participants had to be between 18 and 59 years old. In both cases, volunteers who did not meet the inclusion criteria were excluded, as well as women who were pregnant or breastfeeding. In the case of the non-celiac, those volunteers who tested positive in the anti-tissue transglutaminase IgA class antibodies (AAtTG) antibody test were also excluded.

Taking into account previous studies in Spain and considering a confidence interval of 95%, an error α of 5%, an error β of 20%, a power of 80%, and a case/control ratio of 1:1, using the EpiInfo v.7 software, a total sample of 110 adults was calculated. Predicting a loss rate of 20%, an initial sample of 75 cases and 75 controls aged 19–59 years was proposed. The final sample included 138 volunteers; 64 had celiac disease and 74 were healthy volunteers. The percentage of lost subjects due to not adequately meeting the participation requirements was 8%. All recruited volunteers completed the study.

The protocol was approved by the Ethics Committee for Human Studies at Universidad San Pablo-CEU (Authorization number 124/16/09). The project was conducted in accordance with legal requirements and guidelines for good clinical practice, as well as the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (revised in October 2008). All volunteers (celiac and non-celiac) were informed and provided their written consent to participate in the study. Anonymity and personal data protection were guaranteed, as established by the Spanish Organic Law on the Protection of Personal Data (3/2018 of December 5th).

2.2. Analysis of Dietary Intake and Eating Habits

A dietitian and trained anthropometrist interviewed the participants. Three 24-hour dietary records were analyzed. The first record was collected during the first face-to-face analysis session, and the two remaining ones were collected by telephone with a one-month interval between each survey. One of them was always carried out on a holiday, following the methodological recommendations of the EFSA (European Food Safety Authority) E-Menu methodology [35]. The interview was conducted with the help of home measurements of food servings, and photographic books to estimate quantities, thus making it easier for participants to describe their dietary intake.

The dietary records were analyzed, by means of the computer software DIAL®, to transform food intake into energy and nutrient consumption. In the case of gluten-free products, nutrient composition was taken from a gluten-free food composition database previously developed in our research group [36]. The results were compared with the

Recommended Intake of Energy and Nutrients for the Spanish Population [37], and with the Nutritional Objectives established by the Spanish Society of Community Nutrition (Sociedad Española de Nutrición Comunitaria, SENC) [38]. Adherence to recommendations was calculated using the following formula: (actual intake/recommended intake) $\times 100$.

For the analysis of eating habits, food consumption frequency questionnaires based on validated questionnaires [39] were applied. The questionnaires provided information on the number of meals per day and the frequency of food consumption by groups (vegetables, fruits, dairy products, cereals, cookies and pastas, nuts., etc.) Subsequently, frequency data were compared with daily recommended servings from each food group, according to the Spanish Society of Community Nutrition (SENC).

2.3. Anthropometric Measures

All anthropometric measurements were taken according to the International Standards for Anthropometric Assessment, issued by the International Society for the Advancement of Kinanthropometry (ISAK) [40]. Measures were taken by a level 1 anthropometrist, on the right side of the volunteers, and a minimum of two times. A third measurement was taken when both measurements differed by more than 5%. The room where the measurements were taken had sufficient privacy and temperature was set to be comfortable for the volunteers. The materials used were the following: SECA® portable stadiometer, accuracy 1 mm; anthropometric box; SECA® digital homologated scale, accuracy 100 g; CESCORF metallic anthropometric tape, accuracy 1 mm; Harpenden® plicometer, accuracy 1 mm; Cescorf® small bone caliper. The following anthropometric measurements were taken according to ISAK methodology, comprising the Complete Restricted Profile [40]: height, weight, subcutaneous folds (triceps, biceps subscapular, iliac crest, supraspinal, abdominal, medial calf, and frontal thigh); girths (relaxed arm, contracted arm, waist, hip, calf); bone breadths (biepicondyle diameter of the humerus and biepicondyle diameter of the femur).

Using these parameters, the following indexes were calculated: Body Mass Index (BMI) (weight (kg)/height^2 (m)) and body fat percentage. Body fat was calculated from the sum of the measured triceps, biceps, subscapular and supraspinal folds, using the Durning and Womersley formula [41].

2.4. Bone Mineral Density

The analysis of bone mineral density (BMD) was performed by a Hologic ultrasonic densitometer model Sahara®, taking the measurement on the calcaneus. The densitometer was calibrated before each measurement according to the manufacturer's instructions. This technique is very simple and non-invasive since it is done by placing a bare foot in the densitometer. The ultrasound measures bone mechanical properties, such as attenuation or BUA (Broadband ultrasound attenuation), and the speed with which the sound passes through the bone or SOS (Speed of Sound), providing information on the elasticity and density of the bone [42]. In addition to these parameters, for the diagnosis of osteoporosis, the T-Score, which takes as a reference the maximum average BMD reached at around 30 years of age, has been evaluated. Reference values consider normality when the BMD is greater than -1 standard deviation from peak bone mass (T score ≥ -1 SD); osteopenia when the BMD is between -1 and -2.5 standard deviations from peak bone mass (T score ≤ -1 and ≥ -2.5 SD), and osteoporosis when the BMD is less than -2.5 standard deviations from peak bone mass (T score ≤ -2.5 SD).

2.5. Blood Parameters

The analytical determinations were carried out by authorized personnel of a certified clinical laboratory in Madrid, Spain (*Megalab S.L.*), extracting a sample of fasting venous blood. The following parameters were determined:

Hematological: Red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MVC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count, median platelets volume

(MPV), leukocyte differential count (lymphocytes, monocytes, neutrophils, eosinophils, basophils).

General biochemistry: iron, basal glucose, homocysteine, total calcium, phosphorus, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol.

Vitamin metabolism: folate, 25-OH Vitamin D.

Malabsorption markers: albumin.

Hormonal alterations: parathormone.

2.6. Physical Activity

For the evaluation of physical activity, the internationally validated IPAQ (International Physical Activity Questionnaire) was used [43]. It includes a sequence of questions about the activities carried out in the last seven days, positively evaluating the intensity and time spent in each type of activity. As described in the methodology of analysis of the questionnaire, according to the intensity of the activity carried out (walking, moderate activity or vigorous activity), the time employed is multiplied by a factor (3.3; 4 or 8 respectively), and by the number of days in which the activity has been carried out, and in this way, we obtain a final result in Metabolic Equivalents of Task (METs), a unit used to estimate the metabolic expenditure for a specific activity. This calculation allowed us to categorize the performed physical activity as insufficient, moderate, or high.

2.7. Data Collection and Statistics

A protocolized collection of data was conducted using templates designed for this purpose.

The statistical analysis was carried out using the SPSS version 24 program. Normal distribution in quantitative variables was checked by means of the Kolmogorov–Smirnov test. To unify data, results are expressed as median (p25–p75). The comparison between the two groups (celiac and non-celiac), both in the global sample, and divided by gender and age groups, was carried out by means of the Mann–Whitney U test and the Student's t-test, as appropriate in the sample, establishing $p \leq 0.05$ as the significance level.

Categorical or qualitative variables (frequencies) were compared through Pearson's chi-squared test, using contingency tables. A significant value of $p \leq 0.05$ was considered. For the analysis of correlations between variables with normal distribution, Pearson's correlation was used, otherwise, Spearman's correlation was used.

3. Results

As shown in Table 1, 67.2% of celiacs were women, and 32.8% were men. In the non-celiac group, 66.2% were women and 33.8% men. The average age in the group of women was 39.17 ± 10.62, and in the case of men, 38.58 ± 9.61.

Table 1. Description of the sample by gender.

	Celiac	Non-Celiac	Total	Age (Years)
Women	43 (67.2%)	49 (66.2%)	92 (66.7%)	39.17 ± 10.62
Men	21 (32.8%)	25 (33.8%)	46 (33.3%)	38.58 ± 9.61
Total	64	74	138	

Results are expressed in total number of participants and corresponding percentage in each case. Age is expressed as mean ± SD.

3.1. Dietary Habits and Nutrient Intake

According to general intake habit/food behavior, subjects in this study consumed four meals a day on average. Most of the celiac volunteers (93.8%) declared to follow a strict gluten-free diet. The results of the food frequency questionnaire are summarized in Table 2. In the group of celiacs, daily consumption of vegetables was higher, as well as weekly consumption of legumes as compared to the non-celiac group. All celiac subjects reported drinking at least one serving of vegetable drinks per week, compared to 76.6% of

non-celiac. The same was observed for pickles, which were eaten at least once a week by 69% of the sample of celiac compared to 40.6% of non-celiac ($p = 0.001$).

However, the consumption of cereals and derivatives is significantly higher in the non-celiac group, as can be expected considering the limitations of the gluten-free diet for this food group.

When considering gender, celiac men had a significantly higher consumption of vegetables and legumes, as well as a higher consumption of meat and eggs, as compared to non-celiac. In the group of women with CD, the consumption of cereals and derivatives was significantly lower.

When comparing with the daily/weekly recommendations of food group servings issued by the Spanish Society of Community Nutrition (SENC) [44], food consumption frequencies in both celiac and non-celiac did not meet recommendations. Number of servings of dairy per day did not reach the three recommended servings, and the same happens with intake of fruits, vegetables, cereals, and legumes, which are insufficient, except in the group of celiac men, who met the legumes weekly intake recommendations. On the contrary, consumption of meat, eggs, fish, and seafood exceeded the recommendations in celiac men, but this was not the case in the rest of the volunteer groups. In the case of pastries and cakes, consumption is higher than recommended, both in women and men, celiac and non-celiac.

No significant differences were found between the number of volunteers who declared they had been breastfed (celiac: 76.6%; non-celiac: 78.4%) and those who had not (celiac: 12.5%; non-celiac: 14.9%), nor in the time of breast-feeding they declared to have received, although the average time was higher in the non-celiac group (5.29 ± 6.86 months) than in the celiac group (3.79 ± 2.60 months).

Recommended macronutrient distribution for the Spanish population is 50–55% of total energy from carbohydrates, 10–15% from proteins and up to 35% from lipids [38]. In the present study (Table 3), percentage contribution of carbohydrates to total energy in celiac was similar to the non-celiac group, and both were below the recommended values. Percentage contribution of proteins to total energy was also similar in all groups but exceeded recommendations. Finally, the contribution of lipids to total energy was higher compared to recommendations in all the groups studied. A slightly lower contribution of lipids was observed in the group of men with CD with respect to the non-celiac group, thus, lipid consumption in celiac men was closer to recommendations. PUFA intake was significantly lower in the celiac group compared to the non-celiac. As for the contribution of simple sugars (mono- and disaccharides) to total energy, no significant differences were found between groups; however, a higher consumption was observed in the groups with CD, both men and women, in both of which the recommendations (<6% of the total energy) were surpassed.

Table 2. Frequency of food consumption in celiac and non-celiac Spanish men and women, expressed as number of servings per day/week.

	Total Sample			Men			Women			SENC [1] *Recommendations*
	Celiac (*n* = 64)	**Non-Celiac (*n* = 74)**	*p*	**Celiac (*n* = 21)**	**Non-Celiac (*n* = 25)**	*p*	**Celiac (*n* = 43)**	**Non-Celiac (*n* = 49)**	*p*	
Dairy (servings/day)	1.6 (0.9–2.2)	1.6 (1.0–2.3)	n.s.	1.6 (0.9–2.3)	1.7 (1.0–2.5)	n.s.	1.6 (0.9–2.3)	1.6 (0.9–2.3)	n.s.	2–3 servings/day
Fruits (servings/day)	1.7 (1.1–2.8)	1.7 (1.0–2.3)	n.s.	1.8 (1.2–3.2)	1.2 (0.8–2.3)	n.s.	1.7 (1.1–2.6)	1.7 (1.0–2.4)	n.s.	3–4 servings/day
Vegetables (servings/day)	1.1 * (1.1–1.5)	1.1 (0.8–1.5)	0.047	1.1 * (0.9–1.3)	0.8 (0.8–1.1)	0.044	1.3 (1.1–1.7)	1.1 (1.1–1.5)	n.s.	2–3 servings/day
Legumes (servings/week)	1.7 * (1.5–3.7)	1.5 (1.5–2.2)	0.009	2.00 * (1.5–4.0)	1.5 (1.5–1.7)	0.042	1.5 (1.5–3.0)	1.5 (1.5–3.0)	n.s.	2–4 servings/day
Meat and eggs (servings/week)	8.5 (4.0–11.0)	8.5 (4.0–11.0)	n.s.	11.0 * (8.2–12.5)	7.0 (6.0–11.0)	0.014	8.0 (6.0–9.5)	8.5 (6.7–11.0)	n.s.	White meats, 3 servings/week Eggs, 3 servings/week
Fish and seafood (servings/week)	4.0 (3.0–6.0)	3.2 (3.0–6.0)	n.s.	5.5 (3.0–6.7)	3.0 (3.0–5.5)	n.s.	4.0 (3.0–5.5)	4.0 (3.0–5.5)	n.s.	3–4 servings/week
Bread/paste/cereals (servings/day)	2.00 * (1.4–2.8)	2.7 (1.7–3.3)	0.005	2.1 (1.4–3.6)	2.9 (2.4–3.3)	n.s.	1.9 * (1.1–2.7)	2.3 (1.6–3.3)	0.022	4–6 servings/day
Pastries/desserts (servings/week)	4.2 (0.4–7.7)	4.0 (0.0–8.0)	n.s.	6.5 (2.2–8.5)	4.5 (1.5–8.0)	n.s.	4.0 (0.0–7.0)	3.0 (0.0–8.5)	n.s.	Optional, occasional, and moderate consumption
Nuts (servings/week)	1.5 (0.0–5.5)	0.0 (0.0–3.0)	n.s.	1.5 (0.0–6.0)	0.0(0.0–1.7)	n.s.	1.5 (0.0–5.5)	0.0 (0.0–4.0)	n.s.	3–7 servings/week
Beer/wine (servings/week)	1.5 (0.0–4.0)	1.5 (0.0–5.1)	n.s.	1.5 (0.2–5.5)	1.5 (0.0–5.5)	n.s.	1.5 (0.0–4.0)	1.5 (0.0–3.0)	n.s.	Optional, occasional, and moderate consumption

Results are expressed as median and range (P25-P75). * Significant differences ($p \leq 0.05$) between celiac and non-celiac. [1] SENC: Spanish Society for Community Nutrition.

Table 3. Energy, macronutrient distribution, expressed as contribution to total energy intake, fiber, and fatty acids in celiac and non-celiac Spanish men and women.

	Total Sample			Men			Women		
	Celiac (n = 64)	Non-Celiac (n = 74)	p	Celiac (n = 21)	Non-Celiac (n = 25)	p	Celiac (n = 43)	Non-Celiac (n = 49)	p
Energy (kcal/d)	1856.5 (1629.8–2134.5)	1799.0 (1570.0–2103.5)	n.s.	2082.0 (1773.5–2365.0)	1932.0 (1739.0–2154.0)	n.s.	1838.0 (1609.0–2031.0)	1691.0 (1433.0–2007.0)	n.s.
Energy (% of RI)	78.9 (67.5–87.5)	71.3 (62.2–86.1)	n.s.	75.3 (59.1–81.7)	64.7 (60.4–76.5)	n.s.	81.1 (73.2–89.7)	73.5 (64.5–88.4)	n.s.
Proteins(g/d)	80.8 (68.0–94.4)	79.4 (69.3–89.1)	n.s.	95.0 (80.1–114.0)	87.9 (79.5–96.0)	n.s.	78.3 (61.3–86.4)	73.1 (66.1–86.3)	n.s.
Proteins (% of RI)	182.2 (160.1–210.6)	174.9 (150.2–195.7)	n.s.	175.9 (148.3–211.1)	162.8 (147.1–177.7)	n.s.	187.1 (161.2–210.7)	178.3 (161.2–210.3)	n.s.
Proteins (% of TE)	16.7 (14.5–20.5)	17.5 (16.0–19.3)	n.s.	17.0 (14.5–21.4)	17.3 (16.2–19.20)	n.s.	16.7 (14.5–19.9)	17.6 (15.7–19.4)	n.s.
Total carbohydrates (% of TE)	38.9 (33.0–43.4)	38.2 (34.3–43.1)	n.s.	39.7 (33.4–44.5)	37.4 (33.9–41.25)	n.s.	38.8 (32.7–43.1)	39.0 (34.5–43.3)	n.s.
Simple sugars (% of TE)	17.1 (13.8–21.6)	16.4 (13.1–20.1)	n.s.	16.9 (12.6–22.0)	14.1 (11.9–18.0)	n.s.	18.1 (14.1–21.6)	17.3 (13.9–20.8)	n.s.
Fiber (g/day)	22.4 (15.6–26.9)	19.9 (15.7–26.3)	n.s.	25.7 (16.2–30.9)	19.4 (16.6–26.4)	n.s.	21.1 (14.9–25.5)	20.3 (14.7–26.3)	n.s.
Total lipids (% of TE)	39.3 (34.8–4.4)	40.3 (36.0–44.4)	n.s.	35.3 (34.1–46.1)	40.6 (38.0–44.3)	n.s.	40.1 (35.5–45.3)	39.8 (35.4–44.5)	n.s.
SFA (% of TE)	12.2 (10.5–14.2)	12.3 (9.9–14.0)	n.s.	11.2* (10.0–14.1)	13.3 (11.6–15.1)	0.046	12.6 (10.0–14.8)	11.4 (9.6–13.6)	n.s.
MUFA (% of TE)	15.6 (12.6–19-6)	17.4 (14.6–19.6)	n.s.	15.7 (12.8–19.2)	17.6 (14.9–19.2)	n.s.	15.5 (12.6–19.7)	16.6 (14.4–20.2)	n.s.
PUFA (% of TE)	4.9 * (3.7–6.4)	5.4 (4.4–6.7)	0.032	4.6 (3.7–6.7)	5.8 (5.2–6.8)	n.s.	4.9 (3.7–6.3)	5.1 (4.4–6.7)	n.s.
Cholesterol (mg/day)	316.0 (231.7–438.0)	312.5 (222.5–414.2)	n.s.	355.0 (251.0–509.5)	364.0 (279.5–440.5)	n.s.	308.0 (226.0–386.0)	292.0 (189.0–390.5)	n.s.
Trans fatty acids (mg/day)	87 (0.0–300)	140 (46–250)	n.s.	90 (46–355)	220 (120–290)	n.s.	84 (0.0–270)	92 (0.0–180)	n.s.
ω6 fatty acids (g/day)	2.0 (1.5–3.0)	2.3 (1.7–4.0)	n.s.	2.3 (1.7–4.5)	3.3 (2.0–5.3)	n.s.	1.9 (1.4–2.5)	1.9 (1.3–3.3)	n.s.
ω3 fatty acids (mg/day)	180 (102–240)	195 (140–292)	n.s.	210 (125–365)	250 (180–350)	n.s.	160 (0.100–0.210)	180 (130–265)	n.s.
EPA (mg/day)	103 (6–367)	145 (38–312)	n.s.	110 (7–435)	140 (39–295)	n.s.	200 (38–370)	200 (38–335)	n.s.
DHA (mg/day)	220 (88–712)	320 (92–600)	n.s.	240 (98–900)	260 (86–580)	n.s.	360 (89–640)	360 (89–625)	n.s.

Results are expressed as median and range (P25-P75). (% of TE): percentage contribution to total energy intake. * Significant differences ($p \leq 0.05$) between celiac and non-celiac. SFA: Saturated Fatty Acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid.

Table 3 also shows the contribution of different lipids to total energy intake, intake of cholesterol and different types of fat. A high contribution of SFA was observed, as compared to recommendations (7–8% of total energy from SFA), both in the groups with CD and in the non-celiac group. In the case of men, percentage contribution of SFA to total energy in celiac was significantly lower as compared to non-celiac, although still above recommendations. In relation to PUFA and MUFA, nutritional objectives for the Spanish population were met more closely (5% and 20% of total energy from PUFA and MUFA, respectively). MUFA percentage contribution to total energy was lower in celiac, but not statistically significant when compared to non-celiac. There are no significant differences in MUFA, cholesterol, trans fatty acids, ω6-fatty acids, ω3-fatty acids, EPA, and DHA intake between the groups with CD and the non-celiac groups, both when comparing the total sample and when dividing it by gender. Cholesterol intake is above the recommendations (<300 mg/day) in all study groups, except for women in the non-celiac group.

In the same way, no significant differences were found between volunteers with CD and non-celiacs in fiber intake. The nutritional objectives for the Spanish population propose a minimum intake of 22 to 25 g/day of fiber in women and a minimum of 25 to 35 g/day for men [38]. Celiac men and women have a higher fiber intake and are, therefore, closer to meeting the recommendations as compared to non-celiac.

Tables 4 and 5 show the adequacy of nutrient intake to reference intake values for the Spanish population, calculated according to the formula %RI = actual intake/recommended intake × 100. It is relevant that, in both celiac and non-celiac, actual intake does not cover 2/3 of the recommended intake of energy, folates, vitamin D, vitamin E, calcium, iodine, zinc, and magnesium. In the case of iron, intake is also low in the total sample and more specifically in the group of women, both celiac and non-celiac. The data concerning vitamin D intake are especially worrying, since their intake does not cover 30% of the recommended intake in any of the study groups. When comparing intake between celiac and non-celiac populations, intake of vitamins C and A in celiac are significantly higher and reach a higher degree of compliance with recommendations. Likewise, vitamin K intake was significantly different, being higher in the group of celiac, especially men. In the men's group, intake of folate and pyridoxine is higher in the celiac population compared to non-celiac; furthermore, folates intake in the non-celiac group fails to reach recommendations, whilst intake is above 2/3 of the recommended intake in celiac men. On the other hand, intake of phosphorus in celiac is significantly lower than in the non-celiac group, although the recommended intake was accomplished in all groups.

Table 4. Percentage contribution of mineral intake to Recommended Intake in celiac and non-celiac Spanish men and women.

	Total Sample			Men			Women		
	Celiac (*n* = 64)	**Non-Celiac (*n* = 74)**	*p*	**Celiac (*n* = 21)**	**Non-Celiac (*n* = 25)**	*p*	**Celiac (*n* = 43)**	**Non-Celiac (*n* = 49)**	*p*
Calcium (% of RI)	71.0 (52.9–93.0)	72.7 (60.0–86.4)	n.s.	75.7 (55.1–107.9)	79.6 (63.7–90.5)	n.s.	68.9 (52.7–92.1)	69.6 (58.5–85.5)	n.s.
Phosphorus (% of RI)	164.8 * (136.1–194.6)	181.1 (156.2–203.4)	0.043	191.6 (165.4–232.6)	195.6 (181.8–214.5)	n.s.	148.7 * (130.9–177.1)	167.4 (141.1–197.8)	0.030
Iron (% of RI)	78.0 (53.3–130.5)	79.4 (67.8–132.0)	n.s.	133.0 (110.0–179.0)	148.0 (129.5–165.0)	n.s.	58.9 (49.4–78.3)	72.2 (61.9–80.8)	n.s.
Zinc (% of RI)	52.0 (46.2–66.5)	57.0 (47.1–65.5)	n.s.	67.3 (52.3–83.3)	60.7 (51.6–75.3)	n.s.	48.0 (42.7–56.7)	54.7 (46.0–63.6)	n.s.
Iodine (% of RI)	68.9 (51.7–89.5)	59.9 (49.7–83.8)	n.s.	64.4 (43.0–79.3)	56.5 (48.1–69.9)	n.s.	70.5 (55.9–90.9)	61.7 (51.7–85.5)	n.s.

Results are expressed as median and range (P25–P75). % of RI: Percentage contribution to recommended intake, calculated as actual intake/recommended intake × 100. * Significant differences ($p \leq 0.05$) between celiac and non-celiac.

Table 5. Percentage contribution of vitamin intake to Recommended Intake in celiac and non-celiac Spanish men and women.

	Total Sample			Men			Women		
	Celiac (n = 64)	Non-Celiac (n = 74)	p	Celiac (n = 21)	Non-Celiac (n = 25)	p	Celiac (n = 43)	Non-Celiac (n = 49)	p
Thiamine (% of RI)	114.6 (98.1–140.6)	126.1 (100.8–146.6)	n.s.	109.1 (89.55–148.10)	127.3 (100.0–145.5)	n.s.	120.0 (98.9–137.5)	122.2 (102.2–158.3)	n.s.
Riboflavin (% of RI)	100.0 (91.8–128.6)	107.1 (85.7–128.6)	n.s.	100.0 (76.50–126.05)	100.0 (76.5–117.1)	n.s.	107.1 (92.3–128.6)	107.7 (92.3–135.7)	n.s.
Pyridoxine (% of RI)	125.0 (100–187.0)	116.7 (100.0–131.3)	n.s.	138.9* (119.45–161.15)	116.7 (97.2–127.8)	0.015	118.8 (93.8–143.8)	112.5 (100.0–134.4)	n.s.
Vitamin B12 (% of RI)	242.5 (181.3–455.0)	237.5 (153.7–355.0)	n.s.	300.0 (195.0–530.0)	275.0 (187.5–480.0)	n.s.	230.0 (160.0–405.0)	215.0 (142.5–282.5)	n.s.
Niacin (% of RI)	103.6 (84.8–125.5)	98.9 (86.5–128.2)	n.s.	111.6 (85.5–125.8)	93.7 (78.7–105.6)	n.s.	102.9 (84.7–125.0)	101.3 (90.0–134.6)	n.s.
Folic Acid (% of RI)	64.7 (53.0–83.5)	61.25 (48.7–72.2)	n.s.	71.50 * (62.1–88.3)	59.5 (48.5–72.1)	0.032	61.3 (49.5–78.8)	62.8 (48.4–72.6)	n.s.
Vitamin C (% of RI)	243.30 * (180.0–304.2)	205.8 (134.3–254.5)	0.029	293.3 * (178.3–332.5)	196.7 (110.2–230.8)	0.012	226.7 (180.0–290.0)	211.7 (142.4–285.0)	n.s.
Vitamin A (% of RI)	122.05 * (89.2–152.9)	91.6 (69.8–117.4)	0.00	120.0 * (85.8–167.1)	79.0 (63.7–98.2)	0.001	123.0 * (92.6–148.3)	101.9 (73.4–137.9)	0.028
Vitamin D (% of RI)	21.35 (11.3–47.0)	22.7 (9.1–36.3)	n.s.	27.3 (12.0–56.3)	22.0 (9.0–35.6)	n.s.	20.0 (9.30–46.0)	22.7 (9.0–37.3)	n.s.
Vitamin E (% of RI)	69.60 (57.7–83.1)	62.1 (49.2–85.6)	n.s.	81.7 (66.7–88.7)	67.5 (54.6–96.6)	n.s.	65.8 (56.7–77.5)	60.0 (46.2–75.0)	n.s.
Vitamin K (% of RI)	138.9 * (103.4–219.8)	109.0 (77.5–165.2)	0.006	149.2 * (118.7–185.0)	100.00 (60.0–155.8)	0.024	135.6 (100.6–242.2)	115.6 (84.2–172.7)	n.s.

Results are expressed as median and range (P25–P75). % of RI: Percentage contribution to recommended intake, calculated as actual intake/recommended intake × 100 * Significant differences ($p \leq 0.05$) between celiac and non-celiac groups.

3.2. Anthropometric Measurements

Celiac men presented lower weight, lower body fat, and lower BMI when compared to their non-celiac counterparts. In the women's group, significant differences are only observed when comparing body fat, which was higher in celiac women (Table 6). There were no significant differences in height. Figure 1 shows the classification of weight status according to body mass index (BMI). In the group of celiac men, a significantly higher proportion of subjects fell in the BMI range classified as normal weight, as well as in the underweight group, as compared to non-celiac. In the non-celiac group, a significantly higher proportion of subjects was found in the ranges of overweight grade I, overweight grade II, and obesity. For women, the proportion of celiac women in the normal-weight range was higher than in the non-celiac group, whilst a higher proportion of non-celiac women were classified in the underweight category (Figure 1).

Table 6. Anthropometric measurements in celiac and non-celiac Spanish men and women.

	Total Sample			Men			Women		
	Celiac (n = 64)	Non-Celiac (n = 74)	p	Celiac (n = 21)	Non-Celiac (n = 25)	p	Celiac (n = 43)	Non-Celiac (n = 49)	p
Weight (kg)	64.0 (55.4–80.6)	64.6 (54.2–73.8)	n.s.	72.3 * (65.4–76.4)	81.4 (72.9–89.3)	0.003	57.4 (51.7–66.5)	56.0 (51.2–64.6)	n.s.
Height (cm)	167.9 (162.0–183.1)	166.9 (160.4–179.9)	n.s.	179.6 (171.7–184.2)	177.5 (174.2–180.2)	n.s.	163.8 (158.0–168.6)	163.1 (158.1–166.9)	n.s.
Body Fat (%)	30.6 (22.1–38.7)	28.7 (23.2–38.7)	n.s.	21.7 * (17.5–26.3)	26.0 (21.6–29.1)	0.019	33.4 * (29.8–36.5)	29.6 (25.0–35.0)	0.027
BMI (kg/m^2)	21.8 (20.2–27.5)	22.7 (20.5–28.2)	n.s.	22.6 * (21.2–24.1)	24.9 (23.3–27.9)	0.000	21.3 (20.0–23.9)	21.7 (18.9–23.2)	n.s.

Results are expressed as median and range (P25–P75). BMI: Body Mass Index. * Significant differences ($p \leq 0.05$) between celiac and non-celiac.

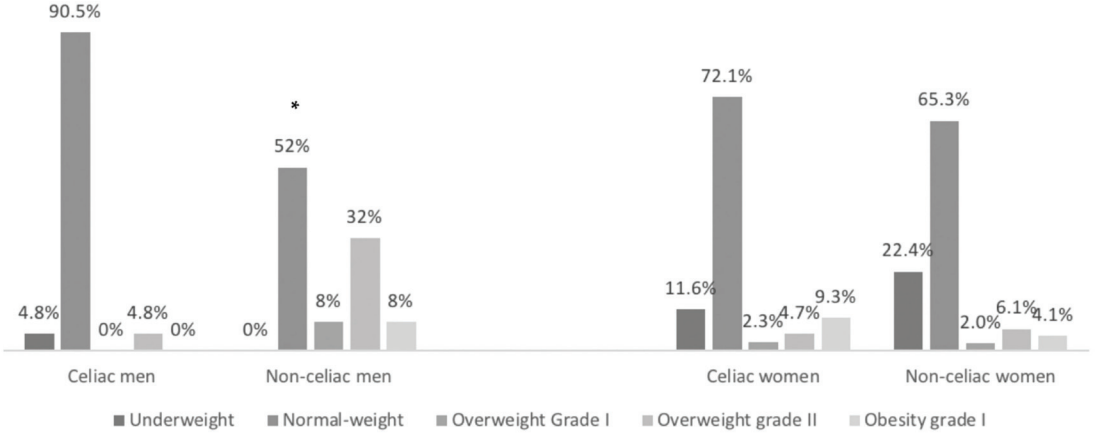

Figure 1. Classification of weight status according to BMI in celiac and non-celiac Spanish men and women. The results are shown as a percentage of subjects classified in each BMI category. Men: Pearson's chi-squared, $p = 0.023$ *.

Because of the influence of age in body fat composition, data on body fat were categorized in two different age groups, young adults (19 to 39) and old adults (40 to 59), and was used to categorize body weight as insufficient, adequate, overweight, or obesity [45]. Data show that in the youngest age group (10–39 years old), women with CD had a higher prevalence of overweight (25%) and obesity (20%) than non-celiac (4.3% and 0%, respectively), and the prevalence of underweight was higher in the non-celiac group (26.1% vs. 0%). Within the healthy category, we found 55% of celiac women versus 69.6% of non-celiac women. In men, it was the non-celiac group who presented the highest

prevalence of overweight and obesity. In the 40–59 years old group, the difference in men with CD with respect to the non-celiac group was relevant and significant, since celiac presented a lower prevalence of overweight (15.4% vs. 33.3%) and obesity (23.1% vs. 53.3%) and a higher percentage of subjects in the healthy category (61.5% vs. 26.7%).

3.3. Bone Mineral Density

Bone mineral density (Table 7) did not differ between celiac and non-celiac, neither in the total sample group nor divided by gender.

Table 7. Bone mineral density in celiac and non-celiac Spanish men and women.

	Total Sample			**Men**			**Women**		
	Celiac (n = 64)	**Non-Celiac (*n* = 74)**	***p***	**Celiac (*n* = 21)**	**Non-Celiac (*n* = 25)**	***p***	**Celiac (*n* = 43)**	**Non-Celiac (*n* = 49)**	***p***
BMD (g/cm^2)	0.520 (0.440–0.610)	0.560 (0.460–0.650)	n.s.	0.540 (0.450–0.650)	0.630 (0.530–0.700)	n.s.	0.510 (0.440–0.600)	0.530 (0.440–0.620)	n.s.

Results are expressed as median and range (P25–P75). BMD: Bone Mineral Density.

The following Figure 2 shows bone mineral density status according to T-score. In men, 29% of celiac and 32% of non-celiac presented values suggestive of osteopenia, and prevalence was similar in both groups. In the case of women, the data obtained showed less favorable results for the group of women with CD, since this group presented a greater and significant risk of osteopenia (44.9%) and osteoporosis (2%) compared to the group of non-celiacs. No relationship was found between these results and blood or intake data for critical nutrients for bone health (calcium, vitamin D, phosphorus) or physical activity.

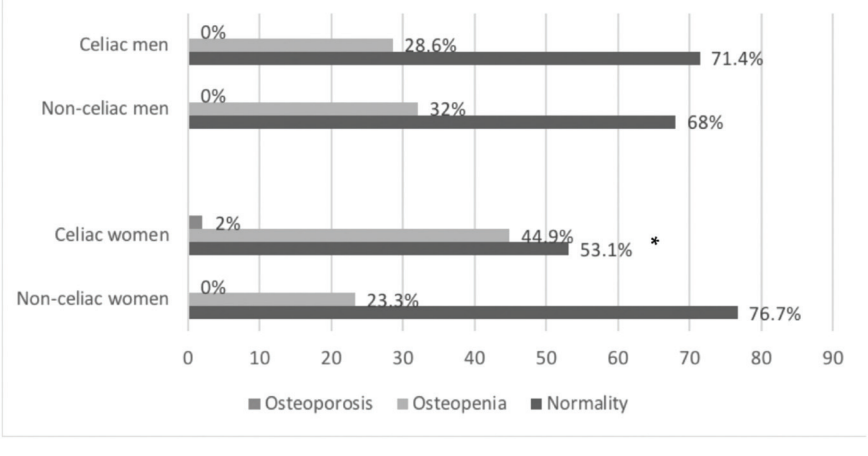

Figure 2. Bone mineral density status according to the T-score in celiac and non-celiac Spanish men and women. The results are shown as a percentage of subjects in each of the T classification categories. Men: Pearson's chi-squared, $p = 0.801$. Women: Pearson's chi-squared, $p = 0.0500$ *.

3.4. Blood Parameters

The data referring to the study of the hemogram and white cell series (Table 8) show that all parameters studied (red blood cell counts, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, and MPV, leukocyte differential count (leucocytes, lymphocytes, monocytes, neutrophils, eosinophils, and basophils)) were within the reference ranges for the population studied. The differences between celiac and control show a significantly lower value in the number of red blood cells in celiac men, and higher values in MCV and MCH. No significant differences were found in the results for women. All the values for the white series were within the reference ranges. Numbers of leukocytes and neutrophils were significantly lower in celiac men as compared to non-celiac.

Table 9 shows the data referring to other biochemical parameters related to nutritional status, and all values were within the reference ranges. Significant differences were found for plasma triglyceride levels, being lower in the group with CD compared to the non-celiac group. On the other hand, a higher LDL/HDL ratio was observed in the group of women with CD compared to the non-celiac group. Finally, plasma albumin levels were significantly higher in the group of men with CD compared to the non-celiac group. In the rest of the blood parameters studied, no significant differences were found between celiac and non-celiac. Plasma levels of folate and homocysteine were similar in celiac and non-celiac, but the prevalence of hyperhomocysteinemia was higher, but not significantly, in the non-celiac group (8.1% vs. 1.6%).

Although we found no significant differences in the plasma values of 25-OH vitamin D, and the median of all the groups was within the reference ranges, we analyzed the distribution of the participants according to reference plasma levels (severe deficit, moderate deficit, recommended value, or excess). The results showed that plasma levels of this vitamin are found to be moderately deficient (10–30 ng/mL) in a greater proportion in the control group, both in the overall sample (41.9% in non-celiac and 34.4% in celiac), and when segregated by gender (men: 44% in non-celiac and 28.6% in celiac; women: 40.8% in non-celiac and 37.2% in celiac). Therefore, the high prevalence of moderate vitamin D deficiency in the general population is of concern, but according to our results, there is no greater deficiency in the group with CD as compared to the control group.

Table 8. Hemogram in celiac and non-celiac Spanish men and women.

	Total Sample			Men			Women			
	Celiac (n = 64)	Non-Celiac (n = 74)	p	Celiac (n = 21)	Non-Celiac (n = 25)	p	Celiac (n = 43)	Non-Celiac (n = 49)	p	Reference Value
Red blood cells/µL ($\times 10^6$)	4.64 (4.4–4.8)	4.73 (4.4–5.1)	n.s.	4.98* (4.7–5.0)	5.17 (4.9–5.4)	0.022	4.5 (4.2–4.7)	4.6 (4.3–4.8)	n.s.	M:4.6–6.20 W:4.20–5.40/µL ($\times 10^6$)
Hemoglobin (g/dL)	14.30 (13.6–15.1)	14.20 (13.5–15.2)	n.s.	15.20 (14.9–15.8)	15.50 (14.8–16.3)	n.s.	13.7 (13.3–14.3)	13.8 (13.0–14.3)	n.s.	M:13.5–18 W:12–16 g/dL
Hematocrit (%)	42.5 (40.0–44.2)	42.6 (39.5–45.0)	n.s.	45.0 (43.9–46.2)	45.7 (43.9–48.0)	n.s.	40.9 (39.6–42.9)	41.1 (38.9–42.9)	n.s.	M:42–52% W:37–47%
MCV (μ^3)	91.1 (88.5–94.5)	90.4 (86.7–93.4)	n.s.	91.7 * (89.8–95.6)	89.8 (86.1–92.4)	0.031	90.4 (88.0–94.0)	90.6 (86.8–94.2)	n.s.	80–96 (μ^3)
MCH (pg)	30.7 (30.1–31.9)	30.4 (29.1–31.6)	n.s.	31.2 * (30.3–32.7)	30.3 (29.3–31.5)	0.018	30.7 (30.0–31.7)	30.5 (28.9–31.7)	n.s.	27–33 pg
MCHC (%)	33.9 (33.2–34.3)	33.6 (33.0–34.2)	n.s.	34.1 (33.3–34.5)	33.8 (33.4–34.5)	n.s.	33.7 (33.0–34.3)	33.3 (32.9–34.0)	n.s.	33–37%
RDW (%)	11.7 (11.5–12.2)	11.9 (11.6–12.5)	n.s.	11.7 (11.4–12.1)	11.9 (11.5–12.2)	n.s.	11.9 (11.5–12.2)	12.1 (11.6–12.7)	n.s.	11–18%
Platelets /µL ($\times 10^3$)	219.0 (181.7–247.0)	214.5 (190.7–256.2)	n.s.	195.0 (182.0–235.5)	206.0 (185.5–226.5)	n.s.	230.0 (181.0–318.6)	229.0 (199.5–299.0)	n.s.	130–450/µL ($\times 10^3$)
MPV (μ^3)	8.5 * (8.0–9.2)	8.8 (8.1–9.3)	0.126	8.6 (8.1–9.1)	9.0 (8.2–9.2)	n.s.	8.4 (7.9–9.2)	8.8 (8.0–9.3)	n.s.	7–13 (μ^3)
Leukocytes /µL ($\times 10^3$)	5.5 * (4.6–6.6)	6.2 (5.0–7.0)	0.014	5.5 * (4.6–6.2)	6.8 (5.4–7.8)	0.003	5.6 (4.6–6.7)	5.8 (5.0–6.8)	n.s.	4.00–11.00/µL ($\times 10^3$)
Lymphocytes /µL	1837.00 (1567.0–2326.0)	2017.5 (1728.7–2393.0)	n.s.	1740.0 * (1571.0–2246.0)	2100.0 (1732.5–2522.0)	0.050	1941.0 (1561.0–2385.0)	1994.0 (1705.0–2361.5)	n.s.	1000–4500/µL
Monocytes /µL	426.0 (362.5–487.2)	456.5 (376.2–534.5)	n.s.	428.0 * (353.0–478.0)	473.0 (411.0–578.0)	0.050	424.0 (362.0–528.0)	446.0 (357.0–525.0)	n.s.	1800–7500/µL
Neutrophils /µL	2926.5 * (2258.5–3567.5)	3361.5 (2645.7–4153.0)	0.024	3038.0 * (2280.0–3458.5)	3452.0 (2914.5–4579.5)	0.028	2854.0 (2230.0–3684.0)	3150.0 (2537.5–3962.5)	n.s.	<800/µL
Eosinophils /µL	148.5 (82.7–225.7)	163.5 (100.2–241.0)	n.s.	167.0.0 (84.5–225.0)	195.0 (107.5–245.0)	n.s.	148.0 (77.0–239.0)	150.0 (99.5–241.5)	n.s.	<800/µL
Basophils /µL	43.0 (23.5–61.5)	51.0 (38.0–63.2)	n.s.	43.0 (22.5–58.5)	51.0 (37.0–74.5)	n.s.	40.0 (25.0–63.0)	52.0 (39.0–62.5)	n.s.	<200/µL

Results are expressed as median and range (P25-P75). * Significant differences ($p \leq 0.05$) between celiac and non-celiac. MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; CMHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Cell Distribution Width. VPM: Medium Platelet Volume.

Table 9. Plasma levels of biochemical parameters in celiac and non-celiac Spanish men and women.

	Total Sample			Men			Women			
	Celiac (n = 64)	Non-Celiac (n = 74)	p	Celiac (n = 21)	Non-Celiac (n = 25)	p	Celiac (n = 43)	Non-Celiac (n = 49)	p	Reference Value
Basal Glucose (mg/dL)	83.0 (77.0–88.0)	81.5 (76.7–87.2)	n.s.	86.0 (78.0–90.0)	84.0 (79.0–91.0)	n.s.	82.0 (77.0–88.0)	80.0 (75.0–86.0)	n.s.	60–110 mg/dL
Albumin (g/dL)	4.5 (4.3–4.7)	4.5 (4.3–4.6)	n.s.	4.7* (4.5–4.9)	4.5 (4.4–4.6)	0.039	4.5 (4.3–4.6)	4.5 (4.3–4.6)	n.s.	3.5–5.2 g/dL
Iron (µg/dL)	100.5 (81.2–121.7)	107.5 (84.7–132.0)	n.s.	106.0 (90.5–133.0)	108.0 (91.0–128.5)	n.s.	97.0 (76.0–113.0)	104.0 (81.0–133.5)	n.s.	37–160 (µg/dL)
Folate (ng/mL)	8.0 (4.3–10.3)	6.3 (4.60–8.62)	n.s.	6.7 (4.0–9.9)	5.5 (4.4–7.3)	n.s.	8.1 (5.7–10.7)	7.0 (4.8–9.2)	n.s.	3–17 ng/mL
Homocysteine (µmol/L)	10.0 (8.4–11.1)	9.6 (8.1–12.1)	n.s.	11.5 (9.7–13.0)	11.7 (9.7–13.4)	n.s.	9.7 (8.4–10.6)	8.8 (7.7–10.4)	n.s.	<15.4 µmol/L
Calcium (mg/dL)	9.4 (9.1–9.6)	9.3 (9.0–9.6)	n.s.	9.4 (9.1–9.5)	9.4 (9.1–9.6)	n.s.	9.4 (9.0–9.6)	9.3 (8.9–9.6)	n.s.	8.2–10.6 mg/dL
Phosphorus (mg/dL)	3.7 (3.4–3.9)	3.6 (3.3–4.0)	n.s.	3.5 (3.0–3.7)	3.4 (3.2–3.6)	n.s.	3.8 (3.7–4.0)	3.8 (3.4–4.1)	n.s.	2.5–5 mg/dL
Cholesterol (mg/dL)	183.5 (148.2–205.5)	177.5 (155.0–206.0)	n.s.	191.0 (145.0–220.0)	186.0 (164.0–210.5)	n.s.	180.0 (152.0–201.0)	174.0 (149.0–204.5)	n.s.	<200 mg/dL
Triglycerides (mg/dL)	59.0 (47.25–74.0)	70.5 (50.5–97.5)	0.044	61.0 (46.5–94.5)	72.0 (60.0–113.5)	*n.s.*	59.0 (48.0–69.0)	64.0 (48.0–95.0)	n.s.	<200 mg/dL
HDL (mg/dL)	63.0 (51.7–73.0)	65.5 (54.2–74.2)	n.s.	56.0 (47.5–68.0)	52.0 (44.0–67.5)	n.s.	66.0 (56.0–74.0)	68.0 (61.0–77.0)	n.s.	>40 mg/dL
LDL (mg/dL)	100.0 (83.2–123.0)	96.0 (76.7–124.0)	n.s.	104.0 (76.0–135.0)	111.0 (96.0–133.5)	n.s.	99.0 (84.0–112.0)	87.0 (69.5–115.0)	n.s.	<130 mg/dL: Primary prevention <100 mg/dL Secondary prevention
Cholesterol/HDL	2.8 (2.4–3.2)	2.7 (2.2–3.4)	n.s.	2.8 (2.5–3.4)	3.4 (2.8–4.2)	n.s.	2.8 (2.4–3.1)	2.5 (2.1–2.9)	n.s.	<4.5
LDL/HDL	1.6 (1.3–1.9)	1.4 (0.97–2.15)	n.s.	1.6 (1.4–2.1)	2.1 (1.6–2.8)	n.s.	1.5 * (1.3–1.9)	1.3 (0.9–1.7)	0.032	M: <3.55 W: <3.22
Vitamin D (ng/mL)	34.7 (24.5–59.9)	33.7 (22.2–66.8)	n.s.	43.7 (26.8–55.3)	31.6 (19.8–63.2)	n.s.	33.8 (22.1–60.6)	38.0 (22.3–68.8)	n.s.	<10 ng/mL: Moderate deficit; 10–30 ng/mL: Severe deficit; 30–96 ng/mL Recommended values; >96 ng/mL excess
Parathormone (pg/mL)	32.85 (24.0–51.3)	34.20 (18.9–48.8)	n.s.	31.30 (24.9–54.9)	36.7 (18.8–46.6)	n.s.	32.9 (23.4–29.9)	34.2 (18.8–51.5)	n.s.	14.5–87.1 pg/mL

Results are expressed as median and range (P25-P75). * Significant differences ($p \leq 0.05$) between celiac and non-celiac.

3.5. Physical Activity

The data obtained from the IPAQ assessing physical activity were used to estimate METs (min/week), and to categorize physical activity as insufficient, moderate, or vigorous according to the scoring protocol of the IPAQ [43]. In none of the cases were significant differences found when comparing physical activity performed by celiac and non-celiac, and most of the studied volunteers fell in the moderate to vigorous physical activity range (Figure 3). According to IPAQ [43], moderate physical activity equals three or more days of vigorous physical activity for at least 20 min per day, or five or more days of moderate physical activity and/or walking for at least 30 thirty minutes per day or five or more days of any combination of walking, moderate or vigorous physical activity achieving at least a total of 600 METs. Vigorous physical activity equals vigorous physical activity at least three days per week achieving a total of at least 1500 METs or seven days of any combination of walking, moderate physical activity, and/or vigorous physical activity, achieving a total of 3000 METs.

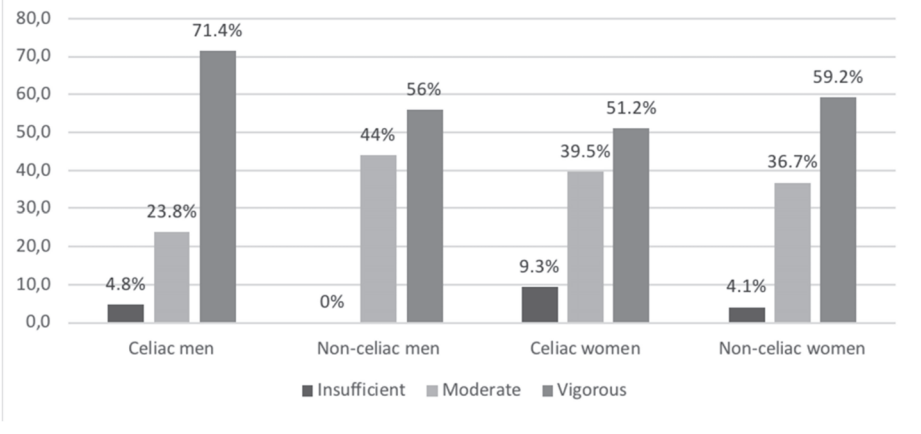

Figure 3. Level of physical activity performed by celiac and non-celiac Spanish men and women. The results are expressed as a percentage of participants classified in each category of physical activity. No significant differences were found between celiac and non-celiac. Men: Pearson's chi-squared: 0.228; women: Pearson's chi-squared: 0.530.

4. Discussion

As far as we know, this is the first study in which a complete evaluation of the nutritional status, through dietary and body composition analysis, as well as biochemical and physical activity measures, has been carried out in Spanish adults diagnosed with celiac disease, once a long-term gluten-free diet has been established at least for a year. In addition, data were compared with non-celiac healthy subjects, according to gender, to detect possible deficiencies and nutritional imbalances in the celiac population.

Patients who follow a GFD necessarily have to exclude many carbohydrate-containing foods with gluten, and it has been postulated that this restriction may lead subjects with CD to make inappropriate choices, and to prefer foods with a high caloric content and a higher proportion of fat and protein [2,16]. In addition, studies show that commercial gluten-free products are often of poorer nutritional quality than their gluten-containing equivalents [36,46,47]. In previous published studies, patients with CD consumed more fat (especially saturated), protein, and simple carbohydrates but less fiber and micronutrients, such as iron, calcium, and vitamin D, than recommended [5,7,16,48,49], as well as compared to healthy subjects [11,15,50]. As reviewed by Penagini et al. [18], the most common nutrient deficiencies found in celiac patients on a GFD included fiber, iron, folate, niacin, vitamin B12, and riboflavin.

In our study, no relevant differences were found in the contribution of macronutrients to total energy when compared to the non-celiac group, however, the same does not apply to vitamin and mineral intake. According to the macronutrient distribution profile, all groups showed high lipid, protein, and simple carbohydrates (mono- and disaccharides) intake, as well as low carbohydrate intake, as compared to the Spanish Nutritional Objectives [38]. Low-carbohydrate plus high-lipid and -protein diets are characteristic of the Spanish population, as shown by the recent national dietary surveys ANIBES and ENIDE [51,52]. According to our results, this same trend is observed in CD patients. Moreover, although celiacs need to avoid some carbohydrate-rich cereals, their carbohydrate intake is close to that of non-celiacs.

When analyzing the quality of lipid intake, none of the groups met recommendations, since the contribution of saturated fatty acids (SFA) to total energy intake was higher, and the contribution of mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) was lower, as compared to guidelines. The intake of PUFA was significantly lower in celiac patients compared to non-celiac subjects, as found in our previous study in children [53]. High intake of SFA and cholesterol (above 300 mg/day), as well as low intake of MUFA and PUFA, is also common in Spanish and European population [51]. PUFA intake is quite relevant since people with CD could benefit from their therapeutical anti-inflammatory effect [54–56].

In the case of fiber, we did find a slightly higher intake in people with CD, especially men, whose fiber intake was closer to meeting the recommendations. These data contrast with those found in previous reviews [13,18], which describe lower fiber intake in people with CD compared to healthy subjects. This is also the conclusion of a study carried out in Italy on 39 celiac adults (21–45 years of age) compared to a control group [10].

When assessing vitamin and mineral intake in celiac adults, we found severely deficient intake of vitamin D, deficient intake (below 2/3 recommended intake) of folates, vitamin E, and iodine, and low intake of calcium. Iron intake was also low (below 2/3 recommended intake) in celiac women. Nutrient intake in celiac patients, especially for vitamin D, folate, and iron, should be assessed, because some studies show a higher risk of bone disease [21,28,29,57,58] and anemia [59] in celiac population. Interestingly, in the present study we did not find lower intake of folate, vitamin D, or iron in celiac as compared to non-celiac. Furthermore, low intake of vitamin D, folate and iron is also commonly described for the general population [51]. When analyzing nutrient intake in celiac, some underestimation of vitamin and mineral intake should be accounted for, since data on gluten-free products' composition are scarce, and almost non-existent for minerals and vitamins [36].

Folate intake in celiac men was significantly higher than that described in non-celiac, in contrast to what we found in our previous study in children and the young population [53], and what previous studies state [8,9,13,14,60]. The main sources of folate in the Spanish diet are vegetables, legumes, fruits, and milk and its derivatives [61], all of which do not need to be excluded in the GFD. Therefore, the higher vitamin intake may be attributable to different eating patterns. In fact, celiac men in our study consumed more vegetables and legumes, thus leading to higher intake of folate, as well as vitamins C, A, and K, which are also present in these food groups. This observation suggests that adults substitute gluten-containing foodstuffs with other food groups and not for commercial gluten-free products, as we found in the case of children and adolescents [53].

Taken all together, we did not find significant differences in nutrient intake in celiac as compared to non-celiac, with the dietary habits found being similar to those of the Spanish population. A similar previous cross-sectional study in Spain found different results, showing significantly different intake between celiac and healthy people for fat, protein, simple carbohydrates, iron, calcium, and vitamin D [11]. Another study in Spain analyzed diets in celiac women [9] as compared to reference values, not to a control group, showing similar deviations. Several reviews also describe nutritional deficiencies in the GFD [13,18].

Some authors attribute these differences to gluten-free product consumption [12]. To explain the difference to other studies, we propose that gluten-free diets in celiac population may have improved because of a closer clinical assessment and follow-up of patients, and availability of more varied gluten-free products. In this sense, the Spanish Ministry of Health, Social Services and Equality published a clinical guide for the early diagnosis of CD [62], focusing on the early detection of CD, but also addressing key issues such as treatment, clinical monitoring of patients, refractoriness, and malignancy, and follow-up and monitoring of CD patients on a gluten-free diet. Patients' associations also contribute to nutritional education for early-diagnosed patients. Finally, we have also demonstrated a slight reformulation in fat composition and salt reduction in gluten-free products [36].

There is a consensus in the fact that a gluten-free diet recovers the intestinal villi and the absorption function in celiac patients. Thus, the values of blood parameters in people with CD who follow a long-term GFD should be similar to those of the general population. In the present study, values for biochemical parameters were found within the reference ranges, both in the celiac group and in the non-celiac group. Celiac men presented a smaller number of red blood cells, but higher mean corpuscular volume and hemoglobin, as compared to controls. Moreover, the number of leukocytes and neutrophils was also smaller in the group with CD, and especially in men. Nonetheless, since all values are within reference ranges, we conclude that there is no indication of abnormality in celiac patients on a long-term gluten-free diet, although this aspect should not be ruled out in some individuals. Conversely, some studies describe common alterations in people with CD, such as vitamin D deficiency [63,64] and anemia [59]. Other studies describe an altered lipid profile, due to diets with a high fat and protein content and a decreased consumption of complex carbohydrates [9,11]. In this context, some authors [13,18,19] describe a higher glycemic index for gluten-free products that could lead to different metabolic alterations. Our results could be again related to an improvement in the adherence to gluten-free diets and improved dietary habits.

Plasma homocysteine levels were within reference ranges, and no significant differences have been found between the prevalence of hyperhomocysteinaemia (>15 μmol/L) in celiac and non-celiac (1.6% in CD vs. 8.1% in non-celiac), although prevalence was higher in the non-celiac group. Again, these results are in contrast with those of another study published in 2002 that describes higher homocysteine levels in 30 individuals with CD compared to the general population [60].

Plasma values of 25-OH-vitamin D were not significantly different between celiac and non-celiac, but we found levels indicative of moderate vitamin D deficiency (values between 10–30 ng/mL) in 41.9% of the non-celiac, and in 34.4% of the celiac. In a more detailed analysis by gender, moderate deficit reaches a prevalence of 44% and 40,8% in healthy men and women, and 28.6% and 37.2% in celiac men and women. Again, our data agree with what is found in the general population, where according to the Kreutz study [65], vitamin D deficiency levels are found in up to 50% of healthy individuals. Vitamin D status proves to be slightly better in celiac than in non-celiac, and this could be due to a higher intake of fish products, a good source of vitamin D, in celiac men.

Studies on anthropometric parameters in people with CD after one year following a GFD provide diverse data. Valletta et al. [66] show an increase in the frequency of overweight, an effect that was also found by Mariani et al. [5] and Norsa et al. [67], who propose that this is explained by the normalization of the intestinal mucosa and the correct absorption of nutrients, accompanied by an unbalanced diet with increased consumption of fats and sugars from GFP. However, other studies show that a good compliance with the GFD had a positive effect on body composition [48–50] with a normalization of BMI, both in underweight and overweight subjects. Thus, Reilly et al. [68] and Bambrilla et al. [30] observed a decreased prevalence of overweight and obesity in patients following a GFD. According to the results of our study, we found a different situation in men and women with CD compared to the non-celiac groups. Thus, celiac men had lower body weight and fat, and lower BMI compared to non-celiac. On the contrary, celiac women presented a higher

percentage of body fat and higher prevalence of overweight and obesity, especially the youngest, although a greater part of them could be classified as normal weight according to BMI. Therefore, we did not find a clear deleterious effect of CD and GFD on weight status, but special attention should be paid to the female gender according to our results.

In people with CD, the risk of suffering lower BMD is well-documented [20,58], mainly due to the characteristic malabsorption of calcium and vitamin D, which results in a decrease in bone mineral content. This means that in many cases, people with untreated CD during the critical periods in which maximum bone mineralization occurs, do not reach an adequate peak of bone mass, causing growth retardation in children and adolescents and increasing the risk of osteopenia and/or osteoporosis in adults [20,69]. In our study, we found no significant differences in BMD between men and women with CD and the non-celiac homologous group, but celiac women have a higher risk of osteopenia and osteoporosis according to the T-Score. Previous studies showed that adherence to the GFD is an important contributor to having a lower degree of bone anomalies [70], and can help to recover a normal BMD [71]. In our study, 93.8% of celiac claim to follow a strict gluten-free diet.

Finally, according to the scores obtained for physical activity, most volunteers engage in moderate to intense physical activity, which has probably contributed to a good nutritional status in our study group, since a physically active lifestyle can compensate for the insufficient intake of calcium and vitamin D found in all volunteers.

Strengths and Limitations

The major strength of this study is that it carries out a complete nutritional assessment, including dietary intake and food habits, blood status, body composition, and physical activity. However, some limitations must be noted. Firstly, it is a cross-sectional study and, consequently, the present results must be interpreted with caution. Secondly, physical activity levels were self-reported (through the IPAQ) instead of objectively measured using accelerometry. Thirdly, there is an important limitation of data on micronutrient composition of commercial gluten-free products. Finally, this is not a randomized study and people participated voluntarily, meaning that bias as a result of more health-conscious volunteers must be considered. The low sample size must also be considered.

5. Conclusions

In conclusion, we found a perfectly equivalent nutritional status scenario in celiac adults as compared to healthy volunteers, with the dietary deviations found being similar to those of Spanish population, i.e., both groups followed a high-lipid, high-protein, and low-carbohydrate diet, ingested too many sugars and low amounts of vitamin D, folates, vitamin E, iodine, calcium, and iron. We did not find hematological signs of anemia, malnutrition, bone alterations, or any pathological situation attributable to CD, except that celiac women may be more prone to osteopenia and osteoporosis. Celiac disease had no clear influence on body weight, but women are also more likely to have more body fat. Nonetheless, vitamin D, calcium, folate, vitamin E, iodine, and iron nutritional status should be assessed and monitored in the celiac population.

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nutrients

MDPI

Review

Iron Deficiency Anemia in Celiac Disease

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Abstract: The iron absorption process developsmainly in the proximal duodenum. This portion of the intestine is typically destroyed in celiac disease (CD), resulting in a reduction in absorption of iron and subsequent iron deficiency anemia (IDA). In fact, the most frequent extra-intestinal manifestation (EIM) of CD is IDA, with a prevalence between 12 and 82% (in relation with the various reports) in patients with new CD diagnosis. The primary treatment of CD is the gluten-free diet (GFD), which is associated with adequate management of IDA, if present. Iron replacement treatment historically has been based on oral products containing ferrous sulphate (FS). However, the absorption of FS is limited in patients with active CD and unpredictable in patients on a GFD. Furthermore, a poor tolerability of this kind of ferrous is particularly frequent in patients with CD or with other inflammatory bowel diseases. Normalization from anemic state typically occurs after at least 6 months of GFD, but the process can take up to 2 years for iron stores to replenish.

Keywords: Iron deficiency Anemia; Celiac disease; iron absorption

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1. Introduction

Iron deficiency anemia (IDA) represents a frequent medical condition encountered in clinical practice by general practitioners, pediatricians and several other specialists. In Western countries, the prevalence of IDA is higher in two phases of the pediatric age: one occurs between the first and third year of life (2.3–15%) and the second occurs in adolescence (3.5–13% in males, 11–33% in females); in adults the prevalence is less than 1% in men <50 years of age, 2–4% in men >50 years of age, 9–20% in menstruating teenagers and young women, and 5 to 7% in post-menopausal women [1–4].

The most common pathogenetic mechanisms of IDA in adults are increased menstrual flow, occult intestinal bleeding or reduced iron absorption, as occurs in celiac disease (CD). In children inadequate intake, increased daily requirement and CD are the leading causes [1–4].

The typical Western diet contains up to 20 mg of daily iron intake, of which 1–2 mg are absorbed; 85–90% of iron is in the non-heme form, mainly as ferric iron (Fe^{3+}), that has to be converted into the ferrous form (Fe^{2+}) in order to be absorbed. This process is regulated by the cytochrome B (DCYTB), a ferrireductase located on the apical membrane of duodenal enterocytes. The absorption of Fe^{2+} primarily occurs in the proximal duodenum, at the brush border of the mucosa cells, through a membrane transport protein called divalent metal transporter (DMT1). Otherwise, heme-iron is absorbed in the same bowel district, but separately from DMT1 and more efficiently than inorganic iron [1–3]. This portion of the duodenum represents the most frequently destroyed region in CD, consequently resulting in a reduction in iron absorption, and therefore IDA, which is another of the most frequent extra-intestinal manifestations (EIM) of CD [5–12]. The prevalence of anemia, different due to the high variability of the studies on this topic, is estimated at between 12 and 82% in patients with new CD diagnosis and about 46% in patients affected by

subclinical CD [5–12]. Furthermore, as noted by Berry, the finding of IDA in patients with CD is less frequent in works from the West (Europe and North America) compared to Asia, Africa and the Middle East; probably, this observation reflects the higher prevalence of IDA, independently from CD, in different regions. Studies focused on prevalence of IDA in celiac patients are summarized in Table 1 [13–26].

Table 1. Studies on prevalence of IDA in celiac patients.

Authors	Country	No. Of Patients	% IDA	Year of the Study
ADULTS				
Koho, et al. [13]	Finland	8	25	1998
Bergamaschi et al. [14]	Italy	132	34	2008
Berry et al. [15]	India	103	81	2018
Binicier et al. [16]	Turkey	195	53	2020
Bottaro et al. [17]	Italy	315	46	1999
Abu Daya et al. [18]	USA	727	21	2013
Sansotta et al. [19]	USA	327	48	2018
De Falco et al. [20]	Italy	505	45	2018
Akbari et al. [21]	Iran	27	52	2006
Kockar et al. [22]	India	434	84	2012
CHILDREN				
Bottaro et al. [17]	Italy	485	35	1999
Sansotta et al. [19]	USA	227	12	2018
Tolone et al. [23]	Italy	385	35	2017
Carroccio et al. [24]	Italy	130	70	1998
Kullogu et al. [25]	Turkey	109	82	2009
Sanseviero et al. [26]	Italy	518	22	2016

IDA often represents the only clinical sign of CD both in children and in adults, especially in patients with subclinical/atypical forms of CD [22–24]. Kocharet al. [22] reported that 39% out of 434 CD patients had anemia as the unique presenting feature. In a multicenter study, including 1026 Italian patients with subclinical/silent CD, the most frequent EIM was IDA (about 39%), found in 46% of adults and 35% of pediatric patients [17].

The counterpart of the relationship between CD and IDA is the high frequency of CD in IDA patients compared to non-anemic ones. However, these studies show a wide variability and diversity in diagnostic methods for CD, and are often performed in selected populations. In a recent systematic meta-analysis, Mahadev identified 18 studies including 2998 patients (adults and children) with IDA, finding CD in 3.2–5.5% of individuals [27]. Shahriari et al. studied the prevalence of IDA in 184 children (92 patients with IDA responsive to iron supplementation, 45 patients with IDA unresponsive to iron supplementation). The authors showed a positive serology of CD in 5.4% and in 28.3% of children with responsive and refractory IDA, respectively [5]. Ertekin et al. reported that among 61 children with IDA, 21.3% had positive serology for CD [28] and Karaman et al. found positive serology for CD in about 8.4% of 250 children with IDA [29]. Kalayciet al. reported a prevalence of CD in about 4.4% of children with IDA [30] and Abd El Dayemet al. in about 44% of 25 children with refractory IDA [31]. Other studies showed different important information: Bansal et al. diagnosed CD in 83 Indian children with IDA unresponsive to conventional therapy [32]. About adults, Dube et al. reported a prevalence of CD between

2.9% and 6% in patients with IDA that increased to 10–15% in patients with IDA and concomitant gastrointestinal symptoms [33]. Hershko, in a cohort of 325 patients, found a 5.2% prevalence of CD [34]. Similar data were obtained by other authors: Corrazza et al. (5.0% out of 200 patients) [35], Carroccio et al. (5.8% out of 85 patients) [24], Annibale (5.6% out of 71patients) [36], Howards (4.7% out of 258 patients) [37], Mandal (1.8% out of 504 patients) [38], Carter et al. (6.0% out of 116 patients) [39] and Lasa (11.11% out of 135 patients) [40].

Paez et al. studied the CD diagnosis delay in 101 adult patients, 52 with gastrointestinal symptoms and 49 without one. Anemia was found in 69.4% of patients without gastrointestinal symptoms, compared with 11.5% in the group with gastrointestinal symptoms. The results showed a mean diagnostic delay of approximately 2.3 months for the group of patients with gastrointestinal symptoms and of 42 months for the group without gastrointestinal manifestations [41].

Given the treatable nature of CD, it is of importance not to delay the diagnosis in order to reduce the effect of the disease on health of the affected subjects. Since patients with IDA, especially in the case of non-responsive forms to treatment, have a higher risk of having CD than the general population, screening with tissue transglutaminase antibodies is strongly recommended in these subjects. Several scientific societies such as the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) [42], the American Gastroenterological Association (AGA) [43], the British Society of Gastroenterology (BSG) [44] and the Italian Association of Hospital Gastroenterologist and Endoscopist (AIGO) and the Italian Society of Pediatric Gastroenterology Hepatology and Nutrition (SIGENP) have drawn up their own guidelines on this topic [45]. However, in clinical practice these recommendations remain often unfollowed. Smukalla found that hematologists rarely screen for celiac disease in the initial workup of IDA patients. According to these authors, this attitude could be a determinant of the underdiagnosis of CD in the United States [46].

After about 24 months of a GFD, an improvement in IDA can be observed [47,48]. De Falco, studying 505 adults with CD, of which 45% had IDA, showed a persistence of anemia after one year of GFD even in the presence of histological normalization of the duodenal mucosa [20].

Saukkonen et al., among 163 adult CD patients, showed greater severity of CD in anemic patients than in non-anemic ones (tTG 65 versus 26.4 U/mL), respectively. The authors showed that the villous height–crypt ratio was lower in anemic patients than in non-anemic ones, with an increased in intraepithelial lymphocytosis, after one year on GFD [49]. Various studies showed similar results; Abu Daya et al. showed a worse villous atrophy in patients with anemia than those presenting with only gastrointestinal symptoms at CD diagnosis [18]. Nurminen et al. demonstrated a greater degree of villous atrophy in patients with CD and IDA, both compared to those with gastrointestinal symptoms at the diagnosis of CD and those whose diagnosis of CD was made following a screening test for serum CD performed for familiarity) [50]. Considering that the presence of extraintestinal manifestations alone may be due to the delayed diagnosis of CD, this is likely to lead to worsening of both the anemia and the degree of villous atrophy at diagnosis.

Two other large series subsequently confirmed the hypothesis of correlation between severity of anemia and severity of intestinal atrophy [15,51].

Similar results have been obtained in children. Repo et al. showed that the median values of hemoglobin, iron, ferritin and transferrin saturation were significantly lower in children with CD and total intestinal villous atrophy compared to those with partial/subtotal atrophy, with potential CD and children belonging to the control group [52].

Schieppattiet al. [53] demonstrated a significant correlation between an increase in hemoglobin concentration and adherence to GFD; Stefanelli et al. [54] and Annibale et al. [36] evidenced a correlation between improving hemoglobin levels and decrease of histological scores of duodenal lesions after starting of a GFD. A normalization of the hemoglobin value was demonstrated after about 6–12 months from the beginning of the

GFD alone, following the restoration of the integrity of the intestinal mucosa. However, data showed that only 50% of patients showed normalization of iron deficiency after 12 months on a GFD [36].

There are several factors that could explain the reason for the persistence of IDA after the adoption of a GFD and oral iron supplementation: non-adherence to a GFD, the presence of ultrastructural and/or molecular alterations of the enterocytes despite the reformation of the integrity of the duodenal mucosa or the presence of specific genetic factors [54].

2. Pathogenesis

Repo et al., evaluating the iron transporter protein expressions in children with CD, found an increased expression of ferroportin and a decreased expression of hephestin in children with histologically confirmed celiac disease compared with the non-celiac controls. There were no other significant differences between the study groups in the expression of iron transporter proteins. In addition, no differences in any of these proteins were detected when it involved anemic and non-anemic children [55].

Several studies have investigated whether it is possible to identify a possible genetic predisposition to IDA in MC. Barisaniet al. showed that the expression of some iron regulatory proteins (DMT1, DCYTB, ferroportin 1, efestin and transferrin receptor) was similar in 25 patients with CD compared to 10 controls [56]. Similarly, Sharma et al. showed greater expression of DMT1 and ferroportin in patients with IDA, regardless of the presence of CD. Other studies have shown a greater expression of ferritin in celiac patients with IDA respect than in those without CD [57].

Toulon et al. showed that the DMT1-IVS4 + 44-AA polymorphism increased the risk of developing anemia, regardless of the degree of atrophy, approximately four times in 387 celiac children compared to 164 control children. In fact, the A allele seems to result in the reduced overexpression of DMT1 necessary in the case of iron deficiency [23].

Other studies investigated if mutations of hemochromatosis genes (HFE), that increase intestinal absorption of iron, could protect CD from IDA. Barisani et al. did not find a protective effect of HFE mutations investigating 203 patients with CD [56]. De Falco et al., on the other hand, demonstrated, with a comparative study of the HFE variants C282Y, H63D and TMPRSS6 in 505 patients with CD at diagnosis and after one year of GFD versus 539 control subjects, how HFE mutations protect celiac patients from the onset of IDA [20]. It was also co-confirmed as TMPRSS6, which modulates the action of epicidin and regulates the oral response of iron [20,55].

In confirmation of these data, Elli et al. showed a higher prevalence and significantly higher TMPRSS6 mutations in celiac patients than in controls, without however demonstrating differences between IDA and non-IDA in patients with CD [58].

Chronic disease anemia (ACD) occurs as a result of an abnormal activation of the immune system following the release of inflammatory cytokines. Among these, it has been shown that, in particular, the interferon-gamma (IFN-y), the interleukin-6 (IL-6) and the tumor necrosis factor (TNF) modulate the synthesis of the iron regulating hormone hepcidin. The latter determines the degradation of ferroportin and inhibits the release of iron by macrophages and enterocytes, thus modifying the reallocation outside the serum [1–3].

Systemic inflammation, subsequent to the increase in blood levels of inflammatory proteins, is a rare event in patients with CD. However, gliadin can favor the activation of mononuclear cells, located in the intestinal lamina propria mucosa, with subsequent local overproduction of proinflammatory cytokines such as IFN-y and IL-6 which favor the onset of ACD [57]. Bergamaschi et al. demonstrated a prevalence of ACD in 17% of CD patients [14]. The data were confirmed by Harper et al., although it was pointed out that patients with CD and ACD do not show signs of systemic inflammation [51]. The percentage instead reported by Berry et al. fluctuates around 3.9% [15].

GFD favors the improvement of intestinal atrophy but also induces a reduction in inflammation with therefore progressive correction of anemia. The mechanism is therefore twofold: increased iron absorption and reduced effects of various inflammatory mediators on iron homeostasis and erythropoiesis.

Therefore, it is evident that the anemia found in subjects with CD has a multifactorial pathogenesis: the most common form is secondary to iron deficiency, especially in patients with more extensive and severe mucosal atrophy, but malabsorption of folate and vitamin B12, a loss of blood or ACD [57].

In the study of Berry, 93% of patients with CD showed anemia, with IDA being the most common cause (81.5%). Other causes included folate deficiency (10.7%), vitamin B12 deficiency (13.6%), mixed nutritional deficiency (16.5%), and ACD (3.9%) [15].

Finally, blood loss due to inflammatory lesions of intestinal mucosa may contribute to IDA in celiac patients, as confirmed in the results provided by Martin Masotet al. [57] and Elli et al. [58].

3. Diagnostic Workup for IDA Diagnosis in CD Patients

We speak of anemia when the blood concentration of hemoglobin (Hb) is less than <2 SD of the normal values, which vary according to age, sex, elevation, smoking habit and physiological conditions such as pregnancy [1–3].

IDA represents a form of hypochromic microcytic anemia. Generally, it is moderate (Hb 9–11 g/dl), rarely severe (<7 g/dl).

The peripheral blood smear shows hypochromic (pale) and microcytic erythrocytes of variable size and shape (aniso-poikilocytosis). A change in red blood cell distribution can be detected early, as assessed by red blood cell distribution width (RDW) and hemoglobin distribution (HDS). Iron deficiency is diagnosed by the presence of low serum iron levels (less than 50 μg/dl) and high serum transferrin levels. Another highly sensitive index for diagnosing IDA is the saturation index of tranferrin, which is less than 10–16%. Low ferritin levels represented an early and highly specific indicator of iron deficiency. However, the international criteria for defining depleted iron deposits vary with age: <12 μg/L for children under 5 years and equal to 15–20 μg/L for those over 5 years and adults.However, it is important to remember that since ferritin is an acute phase reactive protein, its cutoff for the diagnosis of IDA rises to 30–50 μg/L in case of infection or inflammation (correlated with the increase in protein levels C-reactive). More recently, other parameters have been included in the workup of IDA, but they are generally reserved for more complicated diagnosis. Values of Reticulocyte hemoglobin (Chr) less than 27.5 pg are considered very sensitive and specific for IDA (83% and 72% respectively). Other parameters useful for the diagnosis of IDA are an increase in the levels of protoporphyrin IX in red blood cells and zinc protoporphyrin (ZPP). Low levels of serum hepcidin, a new biomarker, can aid in diagnosis, sometimes becoming wearable in the more severe forms of IDA. Frequently, individuals with IDA may experience thrombocytosis (platelet count between 500,000 and 700,000 mm^3) and in severe forms, a low degree of hemolysis can be observed due to the rigidity of the red blood cell membrane [1–3]. Table 2.

Table 2. Parameters for IDA diagnosis.

(a) Red cell parameters values for diagnosis of IDA
➢ Reduction of Hb, RBCs and hematocrit < 2 SD of normal values according to age and gender. For WHO in adult, anemia is defined as hemoglobin < 13 g/dL in men and < 12 g/dL in non-pregnant women. In children, reference values are lower and differ according to age.
➢ Reduction of MCV, MCH and MCHC
➢ Hypochromic cells with a tendency to microcytosis
➢ Increase of RDW > 15%
➢ Reduction of CHr < 27.5 pg

Table 2. *Cont.*

(b) Biochemical parameters values for diagnosis of IDA
➢ Reduction of serum iron < 30mg/dL; increase of total serum transferrin or of TIBC> 350 mg/dL; reduction of IS < 16%; ➢ Reduction of serum ferritin < 10–20 ng/mL if PRC is normal. A ferritin threshold value of <45 ng/mL has a sensitivity for iron deficiency of 85% with a specificity of 92%. In contrast, a ferritin value of < 15 ng/mL has a sensitivity of only 59% and specificity of 99%. A ferritin threshold value of < 45 ng/mL is believed to maximize sensitivity for the diagnosis of IDA with an acceptable number of false-positive diagnoses.
(c) Other parameters evaluable for diagnosis of IDA
➢ Increase of sTfR to a 10–14 mg/L ➢ Reduction of reticulocyte (incostant) ➢ Increase of zincoprotoporftina> 60–80 µmol/mol-heme ➢ Increase of Free Erytrhrocyte Protoporphyrin (FEP) > 10 mg/dL ➢ Increase of platelets count (incostant) between 600.000–1000.000 mmc. ➢ Rarely modest hemolysis

4. Prevention

Patients with CD must follow prevention measures for IDA as recommended also in the non-CD population according to the age, gender, lifestyle and other underlying causes in order to guarantee an adequate iron balance. Furthermore, as CD patients are at high risk for IDA they must be tested for this condition at the diagnosis of CD and during GFD [1–3].

5. Treatment

The key treatment for CD is GFD [59]. GFD alone may improve mild forms of IDA in patients with CD [59]. If, in addition to CD, other underlying causes of IDA exist, they must be addressed when possible, in order to improve iron balance [1–3].

Management of IDA is primary focused on iron stores repletion. To date, discussions remain open on the most suitable modality for iron supplementation, between oral or intravenous therapy [1–3].

The most commonly undertaken therapy for oral iron replacement is with ferrous sulphate (FS), as it is cheaper, easier to administer and presents no risk of life-threatening events. Unfortunately, treatment with FS is limited by gastrointestinal side effects such as abdominal pain, nausea, diarrhea, vomiting and constipation that interest approximately 50% of patients [1–3].

In children, the recommended iron dose is 2–6 mg/kg/day in terms of elemental iron. In adolescents and adults, it is 100–200 mg daily [1–3]. Numerous other forms of iron (bivalent or trivalent) have been commercially available for a few years. Generally, these types of ferrous are better tolerated than FS but are inferior to FS in effectiveness of iron replacement; furthermore, bivalent compounds result as more absorbed than trivalent ones [1–3]. Large variations were observed in mean non-heme iron absorption between studies, which depended on iron status (diet had a greater effect at low serum and plasma ferritin concentrations) and dietary enhancers and inhibitors. So far, it is well known that Vitamin C, hydrochloric acid, sorbitol, ethanol, lactic acid, tartaric acid as well as meat, poultry, fish, affect the increase in absorption while Vitamin E, phytates, (tea, coffee), polyphenols, calcium and dairy products, animal proteins (milk and eggs-albumin), micronutrients (zinc and copper) fiber, casein, legume proteins, calcium, magnesium carbonate and cigarettes contribute to the decrease in absorption. Interactions of iron with manganese, chromium and selenium are still under investigation [1–3].

Absorption of FS is limited in patients with active CD and unpredictable in patients on a GFD [54–57]. Furthermore, a poor tolerability is particularly frequent in patients with

CD or with other inflammatory bowel diseases. A possible solution is the concomitant use of probiotic or prebiotic. Some studies have shown that the association of a probiotic such as *Lactobacillus plantarum 299v* and *Bifidobacteriumlactis HN019* could facilitate a better iron absorption, but the results are controversial [49,60]. Promising results are derived from a pilot clinical trial, which evaluated the synergistic effect of a prebiotic (oligofructose-enriched inulin) on iron homeostasis in children and adolescents with celiac disease treated with a gluten-free diet [61].

In non-celiac patients, the achievement of normal hemoglobin levels can be generally expected after 2–3 months of oral treatment that should be further continued for 2–4 months in order to fill the body stores [1–3,49]. In some cases of CD, it takes longer to improve intestinal lesions with GFD. On average, it is estimated that anemia occurs after about 6 to 12 months with GFD, but sometimes it takes up to 2 years [54–57].

Ferrous Bisglycinate Chelate (FBC) is a new product consisting of a chelated ferrous iron atom bonded to two glycine molecules, with covalent and coordinated bonds. Several studies have demonstrated the efficacy and safety in the treatment of IDA in both adults and children [62–64]. Our study, through the use of an oral iron absorption test (OIAT) with FBC, has an excellent absorption profile of this product in children with newly diagnosed CD and in patients with GFD, without showing side effects [65,66].

A new FBC compound, called Feralgine®, has recently been developed to improve the bioavailability and tolerability profile. It is a patented compound, with a one-to-one ratio between FBC and sodium alginate using spray drying technologies [67].

Feralgine®, as well as FBC, is effective at a dosage of 30–40% compared to FS. Vernero et al. evidenced that the compound is well absorbed and tolerated in patients with inflammatory bowel disease [68]. Two of our recent studies conducted with OIAT in adult celiac patients confirmed the good level of absorption and tolerance in patients with anemia as well as in non-celiac subjects and in those with onset celiac disease [69,70].

The mechanisms by which this improved absorption occurs in CD subjects are not yet clear. Only the most accredited hypotheses are different; the first is that iron chelated with amino acids is better absorbed in the intestine than inorganic iron, perhaps through the use of different absorption processes. In fact, it has been shown that FBC increases the expression of the DMT1 transcript, PepT1 a heme iron transporter, hypothesizing that the latter favors the direct absorption of FBC as heme iron [71,72]. Furthermore, since the intestinal absorption of FBC occurs with the molecule intact, the iron complex is probably absorbed regardless of the presence of DMT1. However, further studies are needed to better clarify the absorption and bioavailability mechanisms of this product.

If oral iron is not tolerated, or not absorbed due to intestinal inflammation, then intravenous iron should be given. Intravenous (IV) administration of iron is the only alternative to oral administration, as intramuscular iron injections are no longer given due to the many side effects: excessive pain, abnormal skin discoloration and potential risk of developing injection site sarcoma (observed in model animals) [1–3,49]. Intravenous therapy is usually initiated when there is a severe form of IDA that requires rapid correction, forms in which a poor response to oral administration is demonstrated, or when there is difficulty in tolerability and adherence itself [1–3,49,73]. However, although there are numerous clinical studies on its efficacy, its application in daily management has been slow, partly due to the fear of possible adverse events, related to historical anaphylactic reactions associated with iron dextran formulations. Studies show that rates of mild reactions are ~1 in 200 and major reactions are ~1 in 200,000 or more [3,49,73].

Numerous various iron formulation for intravenous use have been developed in recent years. Their efficacy in the management of IDA is greater than possible adverse events, which can be easily managed if suitable measures are implemented to ensure early diagnosis and effective management of allergic reactions [3,49,73]. Low molecular weight iron dextran, introduced in the 1990s, although rarely causing serious reactions, is no longer used, due to the current availability of new formulations with an improved safety profile, such as: Fe-gluconate (Ferlixit®), Fe-sucrose (Venofer®) and Fe-carboxymaltose

(Ferrinject®). New preparations such as Fe-isomaltoside (Monofer®) and Ferumoxytol (Ferraheme®) are currently being studied [3,49,73].

Recently, intravenous iron has been recommended in adults and in children with clinically active IBD, in case of intolerance to oral iron with interesting results [74,75]. Atthe moment there are no indications to treat IDA in CD patients with intravenous formulations. However, in case of severe anemia in patients with compromised conditions, it might be taken into considerations in order to rapidly correct the hematological picture as well as recommended in non-celiac patients.

Only when severe anemia is present, with hemoglobin values below 5–6 g/dl, which requires rapid correction such as in patients with cardiac dysfunction, red blood cell transfusions can be performed [76,77].

6. Conclusions

IDA represents the most frequent EIM of CD. Since patients with IDA, especially if a non-responsive form to treatment has a higher risk of having CD than the general population, screening with tissue transglutaminase antibodies is strongly recommended in these subjects.

If the mainstay of treatment for CD remains adherence to a GFD the management of IDA in CD is primary focused on iron stores repletion. For many patients, oral iron replacement with FS has long been the cornerstone treatment. However, treatment with FS can have some side effects, particularly on the gastrointestinal level and more frequent in patients with CD. Numerous other iron-based products (bivalent or trivalent) have been developed for some years, that are well tolerated than FS, but they may be less effective in correcting IDA, especially in people with CD. However, recent studies have shown that some of them, including Feralgine®, could show a good safety and efficacy profile even in celiac patients. Intravenous formulations are suggested only in patients with severe anemia and/or cardiac compromission who need rapid increase of hemoglobin levels.

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Review

Celiac Disease and the Thyroid: Highlighting the Roles of Vitamin D and Iron

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Abstract: Celiac disease (CD) and autoimmune thyroid diseases (AITD) like Hashimoto's thyroiditis (HT) and Graves' disease (GD) frequently coexist, entailing numerous potential impacts on diagnostic and therapeutic approaches. Possible correlations might exist through gut microbiota, regulating the immune system and inflammatory responses, promoting autoimmune diseases, as well as shared cytokines in pathogenesis pathways, cross-reacting antibodies or malabsorption of micronutrients that are essential for the thyroid like iron or vitamin D. Vitamin D deficiency is a common finding in patients with AITD, but might protect from autoimmunity by wielding immunoregulatory and tolerogenic impacts. Additionally, vitamin D is assumed to be involved in the onset and progression of CD, presumably plays a substantial protective role for intestinal mucosa and affects the thyroid via its immunomodulatory effects. Iron is an essential micronutrient for the thyroid gland needed for effective iodine utilization by the iron-dependent enzyme thyroid iodine peroxidase (TPO). Despite being crucial for thyroid hormone synthesis, iron deficiency (ID) is a common finding in patients with hypothyroidism like HT and is frequently found in patients with CD. A literature research was conducted to examine the interplay between CD, AITD, vitamin D and iron deficiency. This narrative review highlights the relevant correlation of the two disease entities CD and AITD, their reciprocal impact and possible therapeutic options that should be further explored by future studies.

Keywords: thyroid; celiac disease; Hashimoto's thyroiditis; Grave's disease; vitamin D; iron

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1. Introduction

A substantial number of patients with autoimmune thyroid diseases (AITDs) shows an increased prevalence of coexisting autoimmune diseases [1,2]. Celiac disease (CD) is an inflammatory disease of the small intestine with autoimmune traits [3] that entails intolerance to dietary gluten and might be associated with other organ autoimmunity [4]. The ingestion of gluten triggers chronic inflammation, which leads to villous atrophy, deprivation of brush-border proteins, as well as enzymes needed for the absorption of micronutrients such as iron [5]. In contrast, iron deficiency (ID) worsens preexisting thyroid dysfunction due to the decreased activity of the heme-dependent thyroid peroxidase (TPO) [6]. Diminished levels of iron, folate, vitamin B12, vitamin D, zinc and magnesium are a frequent finding in untreated CD. Deficiencies of various micronutrients frequently coexist and may compromise physical growth and neurological development, as well as raise the risk of morbidity and mortality [7]. Micronutrient deficiencies are associated with a lower quality of life, given various side effects including fatigue, weakness, headache, dizziness or shortness of breath [8,9]. Although a correlation would be biologically plausible, studies yielded conflicting results so far on the relationship of thyroid hormone balance and trace element levels.

Hashimoto's thyroiditis (HT) and Graves' disease (GD) are the major causes of hypo- and hyperthyroidism, being mediated by different immunological mechanisms [10]. HT is generally the most prevalent autoimmune disease, frequently clustering with other

autoimmune endocrinopathies. The presence of TPO or thyroglobulin antibodies, as well as potentially elevated serum thyroid stimulating hormone (TSH) concentrations can help diagnose the disease. Further, in sonography, a hypoechoic and mostly undersized thyroid gland with inhomogeneous tissue and isolated scarred hyperechoic tissue defines HT. The main feature of GD is circulating TSH receptor stimulating antibodies that bind and stimulate the TSH receptor on thyroid cells, promoting hypertrophy and hyperplasia, eventually resulting in goiter. Patients are predominantly women and may also show high serum concentrations of antibodies against thyroglobulin and TPO [3].

Up to 30% of first-degree relatives of patients with CD and/or AITDs are afflicted by the other disease, respectively. The genes predisposing endocrine autoimmunity, such as diabetes type 1 or AITDs, namely DR2-DQ2 and DR4-DQ8 are substantial genetic parameters of CD, which is an HLA-linked disease as well [11]. CD and endocrine autoimmunity share a similar genetic background. Single nucleotide polymorphisms of several immunoregulatory genes have been found to be overlap susceptibility genes for both CD as well as monoglandular or polyglandular autoimmunity [12]. Genetic overlap between CD and other autoimmune disease may be of clinical relevance, but genetic screening is not yet sensitive nor specific enough to predict the disease onset and progression [13]. Nonetheless, patients with CD should be screened for type 1 diabetes or AITD and vice versa.

Considering the complexity of the mentioned interactions and the partly minor evidence, this review aims to investigate the role of vitamin D and iron, as well as their interplay with the gut microbiota on CD and thyroid function.

2. Methods and Material

A literature research was conducted using PubMed and Google Scholar to assess novel, as well as established knowledge concerning the correlation of CD and AITDs, as well as the influence of iron, vitamin D and the gut microbiota on their onset and progression. Main key words were “Celiac disease”, “Hashimoto’s thyroiditis”, “Grave’s disease”, “Autoimmune thyroid disorders”, “iron”, “vitamin D” and “microbiota”, as well as “microbiome”. Eighty-one manuscripts were reviewed; primarily, randomized controlled trials were included but also systematic reviews and meta-analyses that were considered relevant for the subject.

3. Vitamin D

Vitamin D is the term of a group of hormones responsible for regulating calcium, magnesium, and phosphate levels in the blood. The majority of the daily requirement of vitamin D is covered by endogenous synthesis, triggered by sunlight/UV-B exposure on the skin, and the remaining part by dietary intake. Calcitriol (or 1,25-dihydroxyvitamin D_3) is the biologically most active form. It is formed in the kidneys after hydroxylation of calcidiol (or 25-hydroxyvitamin D_3) by the action of an enzyme called 1-α-hydroxylase [14]. The binding of calcitropic vitamin D to intracellular vitamin D receptors (VDR) in target cells triggers the expression of genes necessary for intestinal absorption of calcium and phosphate, tubular calcium reabsorption in the kidneys, and elevated bone metabolism [15]. Thus, both adequate vitamin D levels in the blood and activity of VDR are crucial for vitamin D signaling and gene expression. In cases of insufficient supply due to lack of sunlight or malabsorption, the use of vitamin D supplements may be required.

3.1. Vitamin D and the Immune System

Besides its crucial role in calcium and phosphate homeostasis, a normal vitamin D status protects against respiratory tract infections [16]. Vitamin D is also thought to have preventive effects against other infections and autoimmune diseases, as VDR are also expressed on immune cells like lymphocytes and antigen presenting macrophages [17,18]. In addition, some antigen presenting cells have proven to be able to synthesize vitamin D metabolites in an autocrine/paracrine way. Vitamin D signaling triggers cellular growth and the development of naïve T-cells [15]. It also has a key role in the terminal differentia-

tion of promyelocytes into monocytes, which in turn differentiate into macrophages as part of the myeloid lineage of the immune system [19]. Furthermore, vitamin D appears to have regulatory effects in the production of immunomodulating cytokines to promote inflammatory reactions. This affects immune cell proliferation and differentiation, promoting self tolerance and protective immunity, and thus may prevent the progression of autoimmune diseases. The need for vitamin D in modulating immune responses is undisputed in the literature. Before the discovery of effective antibiotics, patients with tuberculosis were exposed to sunlight/UV-B, triggering the endogenous synthesis of vitamin D and improving outcomes [20].

3.2. Vitamin D Deficiency

In the current literature, vitamin D deficiency is determined by a calcidiol (or 25-hydroxyvitamin D_3) level below 50 nmol/L or 20 ng/mL in the blood [21]. Low vitamin D levels are associated with AITDs such as Hashimoto's thyroiditis (HT) and Graves' disease (GD) (Figure 1) [18,21]. Both endocrine diseases can be attributed to a genetic predisposition and environmental factors, as well as lymphocytic infiltration and elevated autoantibodies against thyroid tissue [18]. Compared to controls, low vitamin D levels were found more frequently in patients with new-onset autoimmune thyroid diseases [22], as well as inverse relationships of vitamin D and anti-thyroglobulin antibodies (ATAs) [2]. Autoantibodies against TSH receptors permanently stimulate triiodothyronine (T3) and thyroxine (T4) production, leading to enlarged thyroid and hyperthyroidism in GD. Impaired T-cell-suppression due to vitamin D deficiency seems to boost the release of inflammatory cytokines, leading to the destruction of thyroid tissue and hypothyroidism in HT [17]. Oral intake of vitamin D supplements was found to reduce titers of thyroid autoantibodies in levothyroxine-treated women with postpartum thyroiditis. The beneficial effect of vitamin D on autoimmunity may be enhanced by additional selenium supplementation, as a recent study from Krysiak et al. suggested [23].

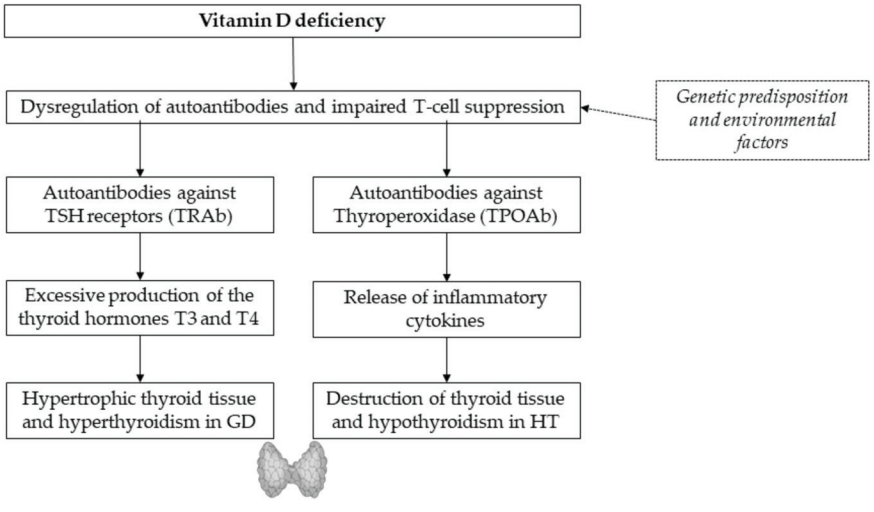

Figure 1. Pathogenic pathway from vitamin D deficiency to AITDs. Further studies are required.

Patients with AITD display a higher prevalence of coexisting celiac disease (CD). The coexistence of autoimmune diseases may be explained by the common immunopathogenetic mechanisms, as findings of Naiyer et al., suggest the same antibodies affecting both thyroid and intestinal tissue [24]. In CD, the protein gliadin, a component of gluten, triggers proinflammatory gene expression and cytokine release in the intestinal submucosa, leading

to chronic inflammation, villous atrophy and impaired absorption of micronutrients such as calcium, vitamin D and iron [25]. Severe iron deficiency worsens preexisting thyroid dysfunction due to the decreased activity of the heme dependent TPO [6], and the lack of calcium and vitamin D has negative effects on bone turnover and bone health.

There is a growing evidence associating vitamin D deficiency with an increased individual susceptibility to other autoimmune diseases [22]. However, the correlations are controversial and require further studies. It is not entirely clear whether low vitamin D levels can be considered a cause or rather a consequence of autoimmune diseases. In GD, effective treatment of the disease did not change vitamin D status, which rather indicates a more causal effect of vitamin D [26].

4. Iron Deficiency

Iron is an essential micronutrient that takes part in numerous and important physiological processes in all cells of the human body including oxygen transport and utilization, oxidative phosphorylation, as well as DNA and ATP biosynthesis [27]. Its concentration is regulated via iron absorption in the proximal small intestine by divalent metal ion transporter 1 (DVT1) for non-heme iron and heme iron transporter 1 (HCP1), which also transports folate and is upregulated by hypoxia and iron deficiency (ID) [28–31]. ID is considered to be the most widespread nutritional deficiency affecting approximately two billion people worldwide [28], being responsible for most anemias. ID anemia (IDA) is the most common extraintestinal finding in patients with CD with a prevalence of about 7% to 81% at the time of diagnosis [5]. In contrast, approximately one in 31 patients with IDA has histologic evidence of CD [32]. Due to permanent inflammation in the small intestines, as well as villous atrophy, patients with CD typically exhibit micronutrient malabsorption and deficiencies [32]. It may even be the only presenting clinical feature of CD in patients without diarrhea or weight loss [33]. Another reason for IDA in CD patients could be systemic inflammation, accordingly high ferritin leading to anemia of chronic disease [34]. Rarely, IDA through blood loss may be present in CD from superimposed small intestinal diseases including neoplasms, ulcerations, gastric or colonic diseases or very scarcely immune-mediated hemolytic anemia with urine blood loss. All these symptoms respond to gluten free diet [33].

Likewise, iron is involved in the maintenance of ideal thyroid function. A considerable amount of animal and human studies indicates that ID—either with or without anemia—compromises thyroid metabolism [35]. Iron is needed for the catalyzation of thyroid hormone synthesis through TPO, which is heme dependent. Additionally, it is required for conversion from T4 to T3, thus if deficient resulting in lower concentrations of circulating T3 and T4 [6]. Many proteins and enzymes involved in the thyroid metabolism are iron and iodine dependent. A systematic review including 57 studies found observational evidence suggesting that iron, selenium and zinc are positively associated with iodine status but data from randomized controlled trials failed to confirm this correlation [6] (urinary iodine does not correlate directly with thyroid function but can be seen as an indicator for the risk of thyroid diseases in a population setting [36]). They also reported no significant effect of iron supplementation on TSH, T3 and T4 [6]. Another systematic review and meta-analysis by Talebi et al., investigating 32 observational studies reported that selenium, as well as zinc, was significantly lower and lead was significantly higher in patients with hypothyroidism compared to healthy controls. Likewise, there was no difference in the concentrations of iron, copper or magnesium between hypothyroid patients and controls [37]. A double-blind controlled clinical trial performed in southern Iran came to the conclusion that treatment with 300 mg ferrous sulfate five times a week significantly increases T4, T3 and triiodothyronine resin uptake, as well as significantly decreases reverse triiodothyronine in comparison to initial values (12%, $p < 0.001$; 3.5%, $p < 0.001$; 16%, $p < 0.05$ and 47%, $p < 0.001$, respectively) [38]. An Indian double-blind randomized intervention study investigated the effect of 190 mg iodine with additional 300 mg ferrous sulphate five times a week with only ferrous sulphate or placebo and concluded that the improvement of iron status is correlated

with an improved thyroid function [39]. However, other studies came to conflicting results: Yavuz et al., investigated the effect of iron status on the thyroid hormone profile in school aged children in an iodine-deficient Turkish area and found no link between iron status and thyroid hormone levels [40].

Developing countries in particular are facing the double burden of co-existing high prevalence of iron and iodine deficiency. Zimmermann et al., provided dual fortification of salt with iodine and ferric pyrophosphate and compared the efficiency of the dual-fortified salt (DFS) with that of iodized salt in a 10-month, randomized, double-blind trial in iodine-deficient 6 to 15-year-old children (n = 158). After 10 months of treatment, hemoglobin in the DFS group increased by 16 g/L, iron status and body stores increased significantly, and the prevalence of IDA decreased from 30% at baseline to 5% significantly. Moreover, thyroid volume and urinary iodine improved significantly [41]. Subclinical hypothyroidism is associated with diverse side effects such as hypercholesterolemia, infertility or poor obstetric outcomes, Specifically, IDA has been reported to be associated with hypothyroidism [42], which is also correlated with low levels of folate or vitamin B12 [43]. The high prevalence of ID in HT patients could as well be a consequence of autoimmune gastritis, a common co-morbidity [2].

Subclinical hypothyroidism should be treated if the patient suffers from IDA concomitantly, according to a randomized controlled trial by Cinemre and colleagues who showed that unresponsiveness of iron replacement therapy could be resolved by thyroid hormone supplementation [42]. A randomized controlled, double-blind trial including 60 patients with hypothyroidism investigated the effect of levothyroxine plus iron salts compared to each treatment alone. The results showed that the increase from baseline levels in hemoglobin and ferritin in the levothyroxine plus iron salt group was significantly higher than in the control group. Additionally, TSH in the study group significantly decreased in the treatment group. These results indicate that treatment with levothyroxine can improve the response to iron salts and that this combination is superior to each component alone [44].

There is a clear link between low levels of certain micronutrients, including iron, vitamin D, selenium, zinc and iodine and thyroid dysfunction. These micronutrient deficiencies might as well contribute to extra-intestinal clinical manifestations of CD, such as neurological symptoms, psychiatric symptoms or bone alterations [45]. These manifold effects implicate the need for a higher awareness of interconnected thyroid and celiac disease and their common micronutrient deficiencies.

Concerning the potential intercorrelations between iron, thyroid disease and CD, the importance of evaluating ID or IDA in CD or AITDs and potential overlaps must be considered. Even though there is conflicting data, ID is a common finding in CD as well as in AITDs. A relevant relationship between ID and these diseases would imply important treatment capabilities.

5. Microbiota and Autoimmunity

Novel research suggests that the composition and diversity of the gut microbiota is involved in the onset of autoimmune disorders, such as AITDs, as well as CD [46]. The microbiota is able to affect and regulate the immune system, functions as a "reservoir" for thyroid drugs and is involved in several micronutrient deficiencies, e.g., via absorption. Taking into account these manifold effects on human health, the microbiota could constitute an important link between CD, AITDs and micronutrient deficiencies. Healthy human intestines are colonized by trillions of microorganisms, which co-evolved symbiotically with us, their host, influenced by both, genetics and evolving environment [47,48]. Those microorganisms are mainly bacteria, predominantly *Bacteroidetes* and *Firmicutes* and to a lower extent *Actinobacteria*, *Proteobacteria*, *Fusobacteria* and *Verrucomicrobia phyla*, as a whole representing even an own vital organ for the provision of nutrients and micronutrients like iodine, selenium and iron, the regulation of epithelial development and developing innate immunity [10,47,49,50]. Bacteria and bacterial antigens have been believed to be

causally involved in inducing autoimmune diseases for a while. Relevant mechanisms behind their involvement in the onset of autoimmunity include molecular mimicry, where antigen-activated T or B cells are cross-reacting to the body's own tissue. Epitope spreading, bystander activation in an inflammatory environment during infection and cryptic antigens are further possible models [51]. Gut bacteria generate vitamins such as vitamin K, enable the digestion of insoluble fiber, and refine nutritive, as well as immunomodulatory compounds such as short-chain fatty acids (SCFA) [52]. Microbiota collaborate with physiological processes in the host, including carbohydrate fermentation and digestion, development of gut-associated lymphoid tissue (GALT) and alignment of specific immune responses, as well as the protection from pathobionts [47]. The delivery mode and nutrition through first years of life not only severely influences gut microbiota composition but additionally the onset of autoimmune diseases [53]. There is a higher incidence of infections and an increased susceptibility to allergic diseases after caesarean delivery and formula use, which stresses the strong impact of microbiota on immunity [53,54]. A randomized controlled trial found that aberrant immunoglobulin A (IgA) susceptibility to the gut microbiota precedes asthma and progression of allergies in infants, pointing towards a deteriorated mucosal barrier function in children with allergies [55]. Breastmilk is an essential source of microbes and maternal IgA antibodies for babies and higher microbial richness lowers the risk of developing an allergy during childhood. Aside from that, probiotic treatment of the mother can influence the breastmilk microbiota composition [56].

As mentioned above, gut microbiota is required for the normal function of the immune system, immune system maturation, as well as GALT development, which constitutes 70% of the entire immune system [57] and plays an important role to develop tolerance to autoantigens in the gut mucosa by controlling its toll-like-receptors (TLRs). Microbiota impacts mucus layer thickness, the number of CD4+ T cells and contributes to a healthy epithelial barrier [47]. A breakdown of this epithelial barrier entails an inflammatory cascade, including the secretion of pro-inflammatory cytokines and the engagement of the adaptive immune system [58,59]. This condition is called "leaky gut", implying an increased permeability of epithelial cells allowing toxins, antigens and bacteria to passage into the blood stream. Growing evidence supports that the microbiota regulate intestinal permeability, which is deteriorated by antibiotics and strengthened by probiotics. Modulation of the microbiota is therefore thought to help alter the course of autoimmunity [60].

5.1. Microbiota and CD

The composition as well as the function of the gut microbiota may be associated with the onset and progression of CD. The sophisticated equilibrium between self-tolerance and immunity is regulated by the intestinal epithelial barrier and its intercellular tight junctions [25,61]. In genetically susceptible individuals, this balance may become impaired and intestinal as well as extraintestinal autoimmune disorders can occur. In CD, tight junctions open up, presumably to zonulin upregulation [62] and the antigenic trigger, gliadin, triggers proinflammatory gene expression and cytokine liberation within the intestinal submucosa [25]. Serum zonulin levels reduce immediately, once gluten is removed from the diet. Without gluten, the intestine retrieves its barrier function, auto antibody titers decrease, the autoimmune mechanisms discontinue and, eventually, the intestinal damage heals completely.

The key to the link between gut microbiota and CD may lie in early childhood. The higher prevalence of CD in children born with C-section could be explained through their changed microbiota compared to children born through vaginal delivery, but this remains controversial [52]. Since oligosaccharides of human milk support the growth of beneficial *Bifidobacteria* and prevent the growth of pathogens like *Clostridium difficile* [63], breast-feeding might be an essential part for the engraftment of a healthy, symbiotic gut microbiota. Furthermore, early gastrointestinal infections may favor the onset of CD [64]. On the other hand, an Italian study showed an increase in the prevalence of CD, even after the initiation of rotavirus vaccination [65].

The active state of CD is accompanied by T regulatory cell (Treg) dysfunction, which are normally involved in immune response to antigens and to preserve self-tolerance. It has been shown that cholecalciferol is able to enhance suppressor function of Tregs in patients suffering from diabetes type 1 in a randomized controlled trial by Treiber et al., [66]—similar effects may be expected in other endocrinopathies, or even CD. The nuclear transcription factor forkhead box protein 3 (FoxP3) has been found to be essential for the regulation of Treg-cells and mutations of FoxP3 have been linked to several autoimmune diseases. An imbalance of FoxP3 isoforms, shifted towards a non-functional isoform, investigated in intestinal biopsies seems to be associated with CD. Serena et al., reported the link between alterations in the intestinal microenvironment and host epigenetic alterations were mechanistically connected with immune surveillance. The proinflammatory intestinal microenvironment of CD active patients is enriched in butyrate producing bacteria and may contribute to this disequilibrium of FoxP3 isoforms [67]. HLA-DQ genotype, which contributes to the susceptibility of developing CD, is able to influence the composition of the gut microbiota. A study compared the intestinal colonization of infants with high genetic risk of CD and low genetic risk of CD. High genetic risk was associated with bigger fractions of certain bacterial strains and total gram-negative bacteria count. Higher numbers of affected celiac relatives, especially if the mother is affected, seems to be linked to higher proportions of bacteria like *E. rectale*, *C. coccoides*, *E. coli*, *C. lituseburense* and *Streptococcus-Lactococcus*, that are related to increased risk for CD in children [68]. Children with a genetic risk for CD more prevalently show a higher incidence of pathogenic bacteria such as *enterotoxigenic E. coli* [69]. Sellitto hypothesized that the gut microbiota as a whole, rather than certain infections influence the change from tolerance to immune responses in genetically susceptible individuals. She and her research group investigated longitudinal changes in the microbial strains of genetically susceptible infants for CD from birth to the age of two years. Late exposure to gluten after the age of 12 months was associated with a lower rate of CD autoimmunity compared to early exposure. Metabolomics analysis revealed potential biomarkers for predicting CD. Microbiota of genetically predisposed infants was generally lacking the phylum *Bacteroidetes* but showed an abundance of *Firmicutes*. Moreover, their microbiota did not resemble the composition of adults even at 2 years of age [70].

5.2. Microbiota and Thyroid

Research suggests a link between the endocrine system and the gut microbiota. Hormonal levels interrelate with the presence of specific gut microbiota, which is able to produce, secrete, regulate and respond to hormones of the host, affecting not only metabolism and immunity, but also behavior, sexual attraction and appetite [71]. Several studies suggest that in patients with thyroid disorders like HT and GD the composition of the gut microbiota is changed and even increases the diseases prevalence (Figure 2). Microbiota have a remarkable metabolism of thyroid hormones, regulating hormone levels by controlling micronutrient absorption, depletion and enterohepatic cycling [10]. Because of the different underlying immunologic mechanisms in HT and GT, respectively, there may be various roles of the microbiota in these diseases as well. Pathogen-free rats showed an increased susceptibility to HT when microbiota were transferred from conventional rats, supposedly caused by cross-reacting antibodies with TPO and thyroglobulin [72]. Besides, in hypothyroid patients, microbiota diversity is even higher than in healthy controls, which may be due to longer gastrointestinal transit time which is prevalently seen in those patients [10]. High microbial diversity, even if often proposed as beneficial for human health, can entail increased protein catabolism, as well as decreased polyphenol conversion, epithelial turnover and mucus secretion [10,73]. HT patients not only show alterations in their gut microbiota but their microbiota composition is also correlated with clinical parameters, indicating that data about individual microbial composition could help with diagnosis and therapy [74]. In GD, the constitution of the gut microbiota, especially the presence of certain strains like *Paludibacter* and *Allobaculum*, *Limibacter*, *Anaerophaga*

and *Ureaplasma* seems to increase susceptibility to disease [75]. Common risk factors for GD like female gender, stress, pregnancy or smoking induce changes in the gut microbiota compared to healthy controls but a definite correlation between dysbiosis and GD has not been found yet [76], even though there are numerous hypothesized mechanisms. Köhling et al., report that remains unclear if bacterial infections can trigger autoimmune thyroid diseases [76].

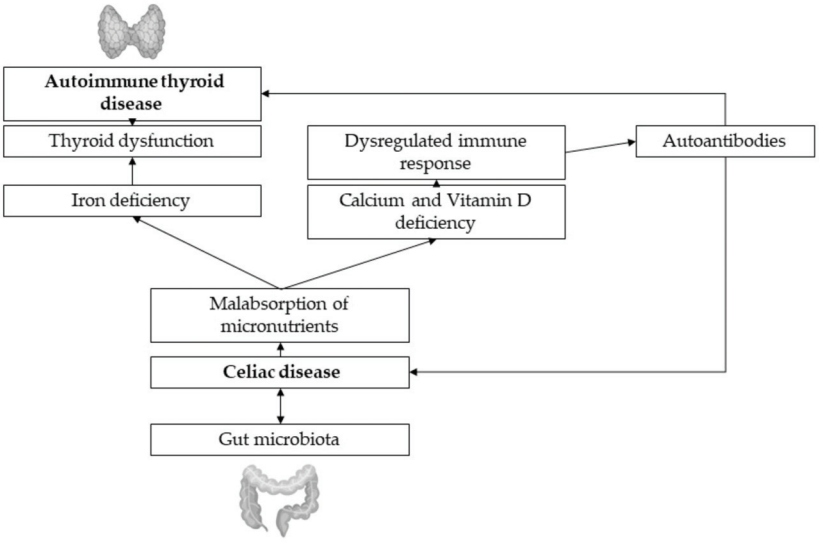

Figure 2. Thyroid-gut-axis.

Numerous organs including the gut are capable of deiodination of T3 or T4. Microbiota seem to have an own deiodinase activity by binding and oxidatively degrading T3 and T4. Through this, they are able to inhibit TSH, and therefore have a direct impact on thyroid hormone levels. Deiodinase activity has been found in intestinal walls of rats [77] and was also identified in the human intestine [78].

L-thyroxine substitution therapy is typically recommended for hypothyroidism. Levothyroxine has a narrow therapeutic index and absorption can be easily disturbed by numerous factors, e.g., if not taken on an empty stomach, or with high fiber or calcium intake. Interestingly, gut microbiota such as microbes like *E. coli* could constitute a reservoir for thyroid hormones by binding it to bacterial thyroid-binding hormone. Taking into account that certain obligate anaerobic bacteria in the gut display glucuronidase activities, gut microbiota research should also focus on thyroxine (T4) metabolism. Through hydrolyzation of conjugated T4 in the intestine, the hormone is able to reenter physiological circulation via the hepatoenteral circulation, joining the iodothyronine pool again [79]. Yao et al., recently showed that the differences in L-thyroxine dose that different patients require to maintain TSH level stability had a relationship with gut microbial composition. Relative abundance of certain strains correlated with thyroxine metabolism were found different between distinct doses of L-thyroxine. Their findings could be due to disparities in the gut´s capacity to metabolize thyroxine. Additionally, they suggested relevant effects of serum cholesterol with L-thyroxine in shaping microbiota. The metabolic resemblance of iodothyronines and bile acid in the intestinal lumen might explain the link between the host´s thyroxine and cholesterol levels [80]. Similarly, Spaggiari et al., showed with his findings that a composite of probiotics containing *Lactobacilli* and *Bifidobacteria* were not directly able to alter thyroid function, but led to less dose adjustments, dose reduction and prevention of serum hormonal fluctuation in the treatment group [81]. Gut microbiota

modification through probiotics intake may increase levothyroxine bioavailability and be able to stabilize thyroid function compensation.

6. Conclusions

Celiac disease (CD) and autoimmune thyroid diseases (AITDs) like Hashimoto's thyroiditis (HT) and Graves' disease (GD) frequently coexist, entailing numerous potential impacts on diagnostic and therapeutic approaches. Accumulating data supports the existence of a significant thyroid-gut-axis, indicating effects of the gut microbiome not only on the immune system and the absorption of micronutrients, but also on thyroid function. Micronutrients such as iron and vitamin D often lack in CD, but also frequently in thyroid diseases, implicating intercorrelating mechanisms. This interconnected synergy can be easily disturbed by numerous events, including environmental factors, early infections, birth mode or eating habits.

There is a higher prevalence of coexisting thyroid and gut related disease, including HT and GD, as well as CD—and dysbiosis frequently co-occurs in this context, either. An altered microbiota is able to change the immune response as well as onset of autoimmune diseases and it is probably able to function as a reservoir for thyroid hormone medication. Supported by a proper composition of the gut microbiota which binds it to bacterial thyroid-binding hormone, patients could reduce hormone fluctuations and reduce their dosage of L-thyroxine. The role of microorganisms and microbiota in the development and progression of AITDs and CD is still controversial and needs to be further elucidated. However, increasing evidence suggests the importance of this thyroid–gut axis, which is thought to modulate autoimmune disorders. Patients often refer to changes in their quality of life and thyroid function in relation to dietary changes. Probiotics could represent a novel additional treatment option for patients with need for thyroid hormone substitution.

There is a clear link between the lack of micronutrients such as iron and vitamin D and CD, as well as AITDS. Iron deficiency is a common finding in CD, presumably as a result of permanent inflammation and villous atrophy. ID occurs often in thyroid diseases as well and deteriorates preexisting thyroid dysfunction, for example, through inhibiting the activity of heme dependent TPO. Studies still yield conflicting results, but a correlation between appropriate iron status and proper thyroid function appears to be clear.

Vitamin D is often lacking in patients with AITDs but might protect from autoimmunity by wielding immunoregulatory and tolerogenic impacts. Vitamin D deficiency is associated with AITDs such as HT and GD, but the correlations are controversial and require further studies. It is not entirely clear whether vitamin D deficiency can be considered a cause or rather a consequence of autoimmune diseases, even though studies point towards a rather causative role of vitamin D.

Future studies should try to examine if and how a gluten-free diet can prevent or delay the development of CD and endocrine autoimmunity of children at risk. The manifold consequences implicate the need for a higher awareness of interconnected thyroid and celiac disease and their common micronutrient deficiencies. There is a close relationship between CD and endocrine autoimmunity, which justifies broader immune genetic and endocrinological screenings of celiac patients.

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Knowledge of Medical Students and Medical Professionals Regarding Nutritional Deficiencies in Patients with Celiac Disease

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Abstract: A gluten-free diet provides relief from symptoms for patients with celiac disease, although there is still a risk of nutritional deficiencies. These patients can potentially consume an excessive amount of fat and insufficient amounts of fiber, iron, vitamin D, and calcium. This study aimed to assess the knowledge of medical students and healthcare professionals in Poland regarding nutritional deficiencies and the prevention of such deficiencies in patients with celiac disease who are on a gluten-free diet. Of the 430 survey participants, 46% did not realize the risk of nutritional deficiencies in patients with celiac disease. The knowledge of the participants was lowest regarding the risk of being overweight or obese. Among the healthcare professionals, an acceptable level of correct answers was provided by only 37% of individuals and was highest for the dietitians' group. Our results demonstrate the need to improve the education of healthcare professionals concerning nutrition in patients with celiac disease.

Keywords: celiac disease; gluten-free diet; nutritional deficiencies

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1. Introduction

Celiac disease (CD) is one of the most common chronic diseases with an increasing prevalence as high as 1–2% [1–4]. CD is a primary intestinal disease and can result in severe damage to the intestinal mucosa, malabsorption, and consequently, nutritional deficiencies [5]. It is well known that untreated CD may lead to growth failure, iron deficiency and/or vitamin B12 anemia, and osteopenia, etc. [6].

A gluten-free diet (GFD) is the sole treatment for patients with CD. Once diagnosed, patients must adhere to a GFD for life [6]. When a GFD is strictly adhered to, it is clinically extremely effective. In recent years, gluten-free products have been perceived as "very healthy," and they are commonly consumed by people who do not have CD [7]. However, recent studies have shown that a GFD may also be associated with nutritional deficiencies [8,9]. A systematic review of 35 studies that assessed nutritional deficiencies in children with CD who followed a GFD demonstrated that they were potentially at risk of consuming an excessive amount of fat and insufficient amounts of fiber, iron, vitamin D, and calcium [8]. Alterations in the intake of folate, magnesium, and zinc were also noted. This may contribute to overweight and obesity, increased cardiovascular risk, and to a lower bone density, which is particularly important in the developmental age [10,11].

The knowledge of CD among healthcare workers has been recently studied and was found to be insufficient [12–14]. However, an assessment of the knowledge of healthcare professionals (HCPs) concerning nutritional deficiencies in patients with CD who follow a GFD has not been performed. Therefore, this study aimed to complete such an assessment using a questionnaire.

2. Materials and Methods

An online survey was designed, using Google Forms platform, to assess the knowledge of medical students and HCPs. The link to it was shared by mail and websites of medical societies. The HCPs group included doctors, dietitians, and nurses. Medical students included senior students of following faculties: medicine, nursing, dietetics, emergency medical services, and physiotherapy. According to their curriculums, they have already been aware of impact of nutrition on CD treatment. The survey contained 12 single or multiple-choice questions that focused on the content of macro- and micronutrients in a GFD and the impact of the diet on the health of patients with CD (Supplementary file). The scope of knowledge and the accuracy of the answers were based on a systematic review of nutritional deficiencies in patients with CD who followed a GFD [8]. The respondents were recruited in Poland, in March and April 2021.

A minimum of 60% (5/8) correct answers for questions regarding nutritional deficiencies was considered an acceptable level of knowledge. In the statistical analyses, the incorrect and inconclusive responses were pooled. Statistical analyses were performed using Statistica version 13.1 software (TIBCO Software Inc., Palo Alto, CA, USA). Multivariate logistic regression model was used to evaluate the associations between acceptable level of knowledge and the following variables: sex, age, experience, providing care for patients with CD, primary medical specialization of doctors, and study field for students. The 95% confidence interval (CI) was used to estimate the precision of the odds ratios (OR) and a p-value of <0.05 was considered to be significant.

3. Results

Of the 430 survey participants, 66% (283/430) were medical students and 34% (147/430) were HCPs. The detailed characteristics of the study participants are presented in Table 1. Medical students included students of following faculties: medicine, nursing, dietetics, emergency medical services, and physiotherapy.

As many as 46% (196/430) of the respondents did not realize the risk of nutritional deficiencies in patients with CD. An awareness of the risk of nutritional deficiencies was highest among dietitians compared to physicians, nurses, and students: 82% (14/17) ($p = 0.03$; OR 4.02; 95% CI 1.13–14.30) vs. 52% (53/101), 52% (15/29), and 54% (152/283), respectively.

Of all the respondents, 80% (344/430) agreed that a GFD is healthy, although 93% (401/430) recommended that a dietitian should be consulted before introducing the diet. Only 10% (43/430) of respondents rated their knowledge of a GFD as sufficient, the largest proportion of these were dieticians and dietetics students (35% [6/17] and 10% [6/58], respectively).

Regarding patients with CD, 71% (306/430) of respondents recommended vitamin D supplementation, 50% (212/430) recommended micronutrient supplementation, and 16% (67/430) claimed that these patients do not require any supplementation.

The most incorrect answers were provided to questions concerning an increased risk of being overweight or obese, higher saturated fatty acid content, and a higher glycemic index in processed gluten-free products (Table 2). 27% (115/430) of respondents believed that patients with CD should follow a high-calorie GFD.

An acceptable level of correct answers was provided by only 37% (55/147) of HCPs (Table 3). The largest proportion of correct answers was observed in the dietitians' group (Figure 1). Professional experience of greater than ten years was the only statistically significant risk factor of insufficient knowledge in the doctors' group ($p = 0.035$). No statistically significant results were obtained regarding the factors influencing the level of knowledge of nurses or dietitians. Among the students surveyed, future dietitians showed a statistically significant higher percentage of correct answers ($p = 0.024$).

Table 1. Basic characteristics of the study participants.

Parameter	Variable	Result, n (%)
Sex	Male	67 (15.58%)
	Female	363 (84.42%)
Age	≤30 years	317 (73.72%)
	>30 years	113 (26.28%)
Occupation	Medical students	283 (65.81%)
	Medical professionals	147 (34.19%)
Medical Students (n = 283)		
Studies	medicine	152 (53.71%)
	nursing	53 (18.73%)
	dietetics	58 (20.49%)
	other medical faculties	20 (7.07%)
Medical professionals (n = 147)		
Occupation	doctors	101 (68.71%)
	nurses	29 (19.73%)
	dietitians	17 (11.56%)
Workplace	hospital	115 (78.23%)
	outpatient healthcare	32 (21.77%)
Experience	≤10 years	88 (59.86%)
	>10 years	59 (40.14%)
Provides care for patients with celiac disease	yes	112 (76.19%)
	no	35 (23.81%)
Primary medical specialization of doctors	pediatric	87/101 (86.14%)
	other	14/101 (13.86%)

Table 2. Distribution of correct answers.

No.	Statements	Total, n = 430 % (n)	Medical Professionals, n = 147 % (n)	Medical Students, n = 283 % (n)
Q1.	Patients with celiac disease should follow a gluten-free diet that contains more calories compared to healthy people.	73.26% (315/430)	80.95% (119/147)	69.26% (196/283)
Q2.	Patients with celiac disease can be exposed to nutritional deficiencies by following a gluten-free diet.	54.42% (234/430)	55.78% (82/147)	53.71% (152/283)
Q3.	Individuals with celiac disease who follow a gluten-free diet can become overweight or obese.	25.12% (108/430)	31.97% (47/147)	21.55% (61/283)
Q4.	A gluten-free diet favors eating fewer complex carbohydrates.	60.70% (261/430)	53.74% (79/147)	64.31% (182/283)
Q5.	Gluten-free processed foods contain more saturated fat than their gluten-containing counterparts.	26.98% (116/430)	26.53% (39/147)	27.21% (77/283)
Q6.	Gluten-free processed foods contain more dietary fiber than their gluten-containing counterparts.	47.67% (205/430)	45.58% (67/147)	48.76% (138/283)
Q7.	The glycemic index of gluten-free processed foods is higher compared with their gluten-containing counterparts.	37.91% (163/430)	42.18% (62/147)	35.69% (101/283)
Q8.	All patients with celiac disease should have regular assessment of vitamin D levels, regardless of their supplementation.	60.23% (259/430)	66.67% (98/147)	56.89% (161/283)

Table 3. Level of knowledge among study participants and odds ratios (OR) with 95% confidence interval (CI) (in parentheses) of having acceptable level of knowledge (≥60% correct answers). Significant differences marked in *italic*.

Parameter	Variable	Acceptable Level of Knowledge	OR (95% CI)	*p* Value
Doctors, *n* = 110				
Sex	Male	19.05% (4/21)	reference	-
	Female	36.25% (29/80)	1.44 (0.40–5.15)	0.576
Age	≤30 years	25.00% (4/16)	reference	-
	>30 years	34.12% (29/85)	2.43 (0.65–9.03)	0.185
Experience	≤10 years	41.67% (25/60)	reference	-
	>10 years	*19.51% (8/41)*	*0.33 (0.12–0.92)*	*0.035*
Provides care for patients with celiac disease	No	14.29% (2/14)	reference	-
	Yes	35.63% (31/87)	2.01 (0.37–10.94)	0.419
Primary medical specialization of doctors	Other	14.29% (2/14)	reference	-
	Pediatric	35.63% (31/87)	1.85 (0.34–10.23)	0.480
Students, *n* = 283				
Sex	Male	33.33% (15/45)	reference	-
	Female	32.77% (78/238)	0.70 (0.34–1.45)	0.335
Studies	Other medical faculties	25.00% (5/20)	reference	-
	Medicine	26.32% (40/152)	0.99 (0.33–2.94)	0.983
	Nursing	30.19% (16/53)	1.30 (0.40–4.20)	0.657
	Dietetics	*55.17% (32/58)*	*3.72 (1.19–11.60)*	*0.024*

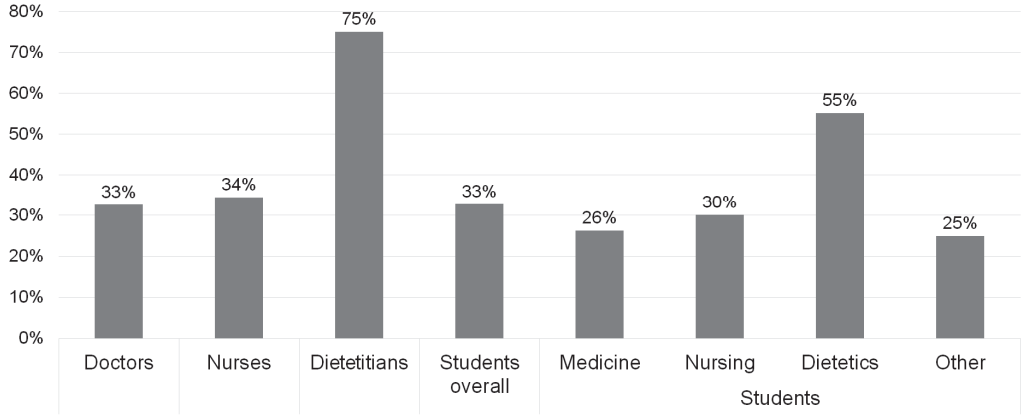

Figure 1. Acceptable level of knowledge (≥60% correct answers) for each occupation.

4. Discussion

The results of this study demonstrate that approximately 46% of participants declared that people with CD who follow a GFD are not at risk of nutritional deficiencies. This corroborates the results of previous studies that assessed the knowledge of HCPs regarding CD. However, none of these studies assessed knowledge strictly regarding nutritional deficiencies associated with a GFD [12,15]. Riznik et al. [12] used a questionnaire that consisted of 22 questions which were divided into three subsections: epidemiology and clinical presentation, diagnostic procedure, and treatment. The authors observed that the knowledge of HCPs (from five European countries: Croatia, Hungary, Germany, Italy, and Slovenia; n = 1381) concerning CD was unsatisfactory. Greater than 50% of the total score was achieved by only 51% of HCPs. Moreover, a study conducted in Romania

demonstrated that only one-third of HCPs performed a total IgA test on patients who were diagnosed with CD [15]. The results of the above-mentioned studies suggest that knowledge of HCPs regarding CD is not satisfactory, regardless of the country in which they live.

An unexpected result obtained in the present study was a low level of knowledge regarding nutritional deficiencies among the HCPs who had a longer period of experience in the field—doctors who worked in the field for greater than ten years had a 70% lower chance of having an acceptable level of knowledge than those who worked in the field for less than ten years. This observation is particularly worrying given the fact that three-quarters of the HCPs surveyed cared for patients with CD. The results of previous studies are dichotomous. Several authors observed that young physicians had improved knowledge of CD compared with older physicians [12,13], however, the authors of other studies did not observe this [16]. Previous studies on the impact of time regarding the medical knowledge of staff (not of CD) demonstrated mixed results. The results of several studies suggested a rapid loss of knowledge, particularly theoretical knowledge [17–19]. Conversely, the results of a separate study showed that the importance of experience gained over time should not be underestimated [20]. The results of the present study indicate the need to intensify the continuous education of HCPs to improve care for patients with CD.

The results of our study demonstrate a significant disproportion concerning the level of knowledge of current and future doctors compared with that of dietitians. This may be due to other educational priorities, as demonstrated in a separate study [21]. However, as nutrition is an essential part of the therapy for many diseases, it should not be neglected in the medical education process [22].

Most of the respondents believed that a GFD is not associated with being overweight or obese. In patients with CD, a GFD heals the intestinal mucosa, decreases intestinal permeability, and improves a patient's condition, including their appetite [23]. If malnutrition is present when CD is diagnosed, a GFD can improve the nutritional status of the patient. In such cases, weight gain is beneficial and can aid in catching up on growth. However, when catch-up growth is achieved, a GFD may lead to a patient becoming overweight or obese. This can be the case for all diets, particularly when they contain additional saturated fatty acids and simple carbohydrates, as most gluten-free processed foods do.

We believe that this result is associated with a separate observation in the present study: greater than 80% of the participants perceived the GFD as healthy. Many individuals believe that, in general, a GFD is a "healthy diet", thus, it cannot be associated with being overweight or obese [24]. On the contrary, "healthy" generally means that weight gain is avoided [25]. In our opinion, this is the most probable explanation for the results we obtained during the present study.

In this study, only 10% of participants believed that their knowledge regarding a GFD was sufficient. This was three times lower than the actual number of HCPs who had sufficient knowledge. In similar studies conducted on nutritional knowledge, approximately half of respondents believed their knowledge was sufficient [26,27]. Our results indicate a lack of confidence in GFD counseling competence among HCPs. This may favor a negative trend of HCPs who provide GFD counseling. Our results demonstrate that there is a requirement to provide HCPs with knowledge concerning nutritional deficiencies in patients with CD who follow a GFD. To our knowledge, this is the first study to assess the knowledge of medical students and HCPs regarding nutritional deficiencies in patients with CD who follow a GFD. The relatively low number of respondents, particularly among nurses and dietitians, may be considered as a limitation of this study. Only Polish students and HCPs participated in the survey and it is questionable to directly extrapolate this data to the global population, however the curricula are similar and GFD is commonly used in the Western countries. There is also a certain overrepresentation of women and pediatricians among the respondents. This may result from the subject of the study itself, CD is most often diagnosed in developmental age, i.e., mainly by pediatricians. On the other hand, the pediatric specialization is statistically more often chosen by women world-

wide [28]. Taking these two facts together women pediatricians are probably the most interested in CD and GFD group among doctors.

5. Conclusions

Healthcare professionals and medical students have insufficient knowledge of the risk of nutritional deficiencies in patients with CD. Medical education should include the diagnostic methods of CD as well as counseling on the appropriate composition of a GFD. Also, longer working doctors should pay more attention to continuous education to improve care for patients with CD.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/nu13061771/s1, Table S1: A survey on a gluten-free diet in patients with celiac disease – part evaluating the knowledge of the respondents.

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Pandemic Effects and Gluten-Free Diet: An Adherence and Mental Health Problem

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Abstract: The COVID-19 pandemic has been present for many months, influencing diets such as the gluten-free diet (GFD), which implies daily challenges even in non-pandemic conditions. Persons following the GFD were invited to answer online ad hoc and validated questionnaires characterizing self-perceptions of the pandemic, current clinical condition, dietary characteristics, adherence to GFD, anxiety, and depression. Of 331 participants, 87% experienced shortage and higher cost of food and 14.8% lost their jobs. Symptoms increased in 29% and 36.6% failed to obtain medical help. Although 52.3% increased food preparation at home and purchased alternative foodstuffs, 53.8% had consumed gluten-containing foods. The Health Eating Index was intermediate/"needs improvement" (mean 65.6 ± 13.3 points); in 49.9% (perception) and 44.4% (questionnaire), adherence was "bad". Anxiety and depression scores were above the cutoff in 28% and 40.4%, respectively. Adherence and mental health were strongly related. The likelihood of poor adherence was 2.3 times higher ($p < 0.004$) in participants declaring that pandemic altered GFD. Those suffering depressive symptoms were 1.3 times more likely to have poor adherence ($p < 0.000$). Depression and faulty GFD (mandatory for treatment) appear, affecting a high proportion of participants, suggesting that support measures aimed at these aspects would help improve the health condition of people that maintain GFD. Comparisons of data currently appearing in the literature available should be cautious because not only cultural aspects but conditions and timing of data collection are most variable.

Keywords: gluten-related disorders; celiac disease; pandemic; adherence; anxiety; depression

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1. Introduction

In December 2019, the first cases of SARS-Cov2 in humans were detected in Wuhan, China [1]. The virus triggered an epidemic that rapidly spread globally as an acute respiratory syndrome [2]; involvement of gastrointestinal and other systems was subsequently reported [3]. The WHO declared a pandemic in March 2020 [4]. Massive worldwide lockdowns and quarantines, and other measures such as physical distancing and self-isolation ensued to protect life and avoid health systems collapse. In Chile, dynamic quarantines were initiated in March and lockdown began in April 2020. Due to the enormous number of COVID-19 cases, ambulatory and follow-up consultations for chronic diseases were usually suspended. All these strategies produced sudden and radical changes in people habits and lifestyles, drastically reducing social interaction, affecting everyday behaviors, and eating habits, among other things.

Today, celiac disease (CD) and other chronic diseases that require treatment with specific restrictive diets raise concern among specialists. The first descriptions of pandemic

effects appeared early; in May 2020, Siniscalchi et al. [5] reported the acute effects identified in 276 adult celiac patients. They did not feel more vulnerable due to their condition, and they did not worry much about the possible shortness of gluten-free food during the epidemic ("not at all" = 48.5%). The most worried were elderly patients, those suffering comorbidities, and females [5]. At the same time, depression and anxiety were reported at 27.9% and 31.6%, respectively, in China [6]; Kontoangelos et al. reviewed the psychological effects of the COVID-19 pandemic, describing that children, older people, and those with underlying health conditions are likely to feel worry, anxiety, and fear, which can be extremely frightening [7].

After experiencing the pandemic effects throughout 2020, we are now learning about the consequences that long isolation measures had on people; indeed, clinical status of diseases, access to food, and mental health are most relevant in patients with gluten-related disorders (GRD) that must keep treatment with gluten-free diet (GFD). Maintaining this diet is always challenging, and patients often feel worried and isolated [8]. GFD is the only known treatment for these conditions [9] and although effective, it may be deficient in macro and/or micronutrients and vitamins, low in fiber, and may have high glycemic index and other shortcomings [7]. Gluten-free foods are typically less available than those forming the regular gluten-containing diet and may be three or more times more expensive than gluten containing equivalents [10]. With the hypothesis that after living for many months in the conditions imposed by the pandemic, the effects on GFD are pronounced and detectable, we invited persons following this diet (due to CD, non-celiac gluten sensitivity, wheat allergy, or GFD as an option of healthy diet) to answer an online questionnaire that characterized four aspects: perceptions about the general pandemic effects, current clinical conditions and dietary characterization, adherence to GFD, and mental health (anxiety and depression).

2. Material and Methods

This population-based, cross-sectional online study was conducted in October–November 2020. An ad hoc questionnaire specially developed for this study asked for sociodemographic characteristics, the perceived effects of the pandemic proper (worrying for risk of infection, shortage of foods, isolation, need to go out of home), dietary data (reasons for maintaining GFD, years on GFD, self-perception of adherence, effects of pandemic on diet, access to gluten-free foods), frequency of home cooking, ingredients/foods the replaced those unavailable, weekly frequency consumption of gluten-containing foods, cost of gluten-free foods, clinical data (diagnosis if any, year of diagnosis, symptoms during last four months, need to consult during last 4–6 months, perceived weight changes), diagnosis of anxiety or depression disorders before or during the pandemic. Validated questionnaires were applied for dietary data, adherence, and mental health: (i) *Healthy eating index* (HEI) [11]; ten variables that define dietary nutritional quality and yield a maximum of 100 points, classifying results into "healthy" (>80), "needs improvement" (51–80), and "not healthy" ($\leq$50). (ii) *Celiac disease adherence test* (CDAT) [12,13]; 7-item scale that classifies responders into "adherent" (patients scoring 7–12 points) and "non-adherents" (scores $\geq$ 13 points) to GFD. (iii) *Generalized anxiety disorder* (GAD-7) [14]; a 7-item scale, where a score of $\geq$10 points define generalized anxiety disorder while cut points of 5, 10, and 15 classify results into mild, moderate, and severe anxiety, and (iv) *Patient health questionnaire* (PHQ-9) [15]; a 9-item scale where the presence of symptoms during the last two weeks classifies responses into major depressive syndrome (5–9 symptoms present in more than half of the days); other depressive syndrome (2, 3, or 4 symptoms present more than half of the days); positive depressive symptoms (presence of at least one or two symptoms not fulfilling the test criteria) and negative depressive symptoms (none of the diagnostic criteria present in at least half of the days). To this study, results of GAD-7 and PHQ-9 were classified into above/below the cutoff.

The protocol was accepted by the IRB of INTA, University of Chile. Participants were invited to answer the questionnaire online, which was released via webpages in

the University of Chile and Coacel (Chilean Celiac Association) websites and WhatsApp, Facebook, Twitter®, and Instagram. This project was approved by the IRB of INTA (INTA 260820). The study was explained in the first page and consenting to participate requested clicking a button. The survey was anonymous, and confidentiality of data was ensured.

Descriptive analyses were conducted, and graphical inspection and the Shapiro–Wilk used to assess variable's distribution. Spearman and/or correlation assessed the association between pandemic, clinical, and mental health variables, as needed. Chi^2 and independent t-test compared categorical and continuous variables, respectively. Univariate analysis by unadjusted logistic regression assessed the association between variables of perception of pandemic effects and the CDAT scores. All those that were statistically significant in the unadjusted regression analysis and those that might convey important information were entered into the multivariate logistic regression model. The adjusted model included adherence to GFD as dependent variables and age, sex, education, geographical macrozone, diagnosis, declared wheat consumption, cost of gluten-free foods, and GAD-7 and PHQ-9 scores as independent variables. Significance level was set at $p < 0.05$. Analyses were performed using STATA 13.1 software (Stata Corp., College Station, TX, USA) and GraphPad Prism 9 (San Diego, CA, USA) and Excel (Microsoft).

3. Results

3.1. Study Population

Of 360 responses obtained, 331 entered analysis. General characteristics of the study group appear in Table 1; 93% were women, 47% were 18–35 years of age, and 16% were older than 50 years; 70% declared university and/or post-graduate studies; 14.8% had lost their jobs; and 45.9% received public health care. Since Chile is a ≈6000 km long strip of land, with clear-cut differences in geographical characteristics and with strong people concentration in the mid macrozone, results were first analyzed by geographical origin of responders (Table 1). No significant differences were detected. Participants reported the following diagnoses: 60.4% CD, 29.3% non-celiac gluten sensitivity (NCGS), 3.2% wheat allergy (WA), and 7.3% followed GFD as a healthy feeding option or fashion, without a diagnosis that justified a restrictive diet; additional diagnoses were reported by 48%, (22.1% of which were autoimmune conditions) (Table 2). No significant differences were detected by diagnosis. Thus, results are presented as one group.

Table 1. General characteristics, diagnoses declared, and mean group scores in dietary and mental health tests by geographic zone of origin of participants.

Variable	Northern Zone (*n* = 26)	Central Zone (*n* = 259)	Southern Zone (*n* = 46)	Total (*n* = 331)	*p* Value
Age (years)	42.1 ± 11.1	37.3 ± 11.9	36.8 ± 11.3		0.13 **
Gender (F), *n* (%)	24 (92.3)	245 (94.6)	41 (89.1)	310 (93.7)	0.93
Urban housing, *n* (%)	24 (92.3)	251 (96.9)	38 (82.6)	313 (94.6)	0.25
Education					
Primary-high school, *n* (%)	4 (15.4)	53 (20.5)	9 (2.0)		0.86 **
University, *n* (%)	17 (65.4)	152 (58.7)	25 (54.4)		
Postgraduate studies	5 (19.2)	54 (20.8)	12 (26.1)		
Public health insurance, *n* (%)	13 (50)	111 (42.9)	26 (56.5)	149 (45.0)	0.27
Diagnoses declared, *n* (%)					
Wheat allergy, *n* (%)	2 (0.8)	8 (3.1)	0	10 (3.2)	
Celiac disease, *n* (%)	15 (57.7)	162 (62.6)	23 (50.0)	200 (60.4)	0.291 **
NCG/WS, *n* (%)	6 (23.1)	72 (27.8)	19 (41.30)	97 (29.3)	
None, *n* (%)	3 (11.5)	17 (6.6)	4 (8.7)	24 (7.3)	
CDAT, score (mean ±SD)	14.6 ± 5.2	14.1 ± 4.7	13.1 ± 4.4		0.30 *
HEI score (mean ±SD)	68.4 ± 9.5	65.2 ± 13.5	66.3 ± 14.2		0.47 **
GAD-7 score (mean-range)	6 (3–11)	7 (4–12)	7(4–13)		0.88 **
PHQ-9 score (mean-range)	9 (5–13)	9 (4–14)	7 (5–13)		0.81 **

Data as mean ± SD. * Kruskal-Wallis Test; ** One-way ANOVA test; Fisher's exact; NCG-WS: non celiac gluten/wheat sensitivity. CDAT: Celiac Dietary Adherence Test; HEI: Healthy Eating Index; GAD-7: Generalized anxiety disorder test; PHQ-9: Patient health questionnaire.

Table 2. CDAT score, non-celiac autoimmune diseases declared, and years on GFD by declared diagnoses.

	CD *	NC/WGS **	WA ***	No Diagnosis Declared ****
n (%)	200 (60%)	97 (29.3%)	10 (3%)	24 (7.2%)
CDAT score < 13 †, *n* (%)	93 (46.5%)	37 (38.1%)	7 (70%)	10 (4.2%)
Additional autoimmune disorders declared	48 (24%)	21 (21.7%)	1 (10%)	3 (12.5%)
Duration of gluten-free diet				
Up to 1 year (*n*, %)	2 (1)	0	0	0
1–5 years (*n*, %)	119 (59.5)	91 (93.8)	5 (50)	23 (95.8)
>5 years (*n*, %)	79 (39.5)	6 (6.2)	5 (50)	1 (4.2)

CD *: celiac disease; NC/WGS ** non-celiac gluten/wheat sensitivity; WA ***: wheat allergy. ****: persons that follow GFD as healthy feeding option or follow a fashion/trend. † CDAT score < 13 points indicate bad adherence to GFD.

3.2. Perceptions of Pandemic General and Clinical Effects

Only 16% declared not to worry because of the pandemic and 37.7% worried very much. Only 14.8% of responders lost their jobs, but 84.3% experienced shortage and higher cost of food, 73% considered that they had no higher risk of infection because of their condition, and 87% declared to be worried because of shortage and higher prices of safe foods. During the last four months, 29% declared their symptoms increased, 15.7% gained weight, and 7.3% lost some, 32.6% felt no need to consult, and 36.6% failed to obtain medical help. Responders declared they increased foods preparation at home (52.3%) and ingredients were changed to some not customarily used but considered safe; 53.8% consumed gluten-containing foods during the pandemic period. Responses of persons on GFD for less than 1 year did not differ from those of participants on the diet for longer periods.

3.3. Health Eating Index (HEI)

The HEI mean score for the study group was 65.6 ± 13.3 points, which classifies in the intermediate level in the HEI scale i.e., "needs improvement" of the feeding patterns. While 15.8% maintained a healthy diet, 71.3% were classified in the intermediate level and in 11.5%, the diet was not healthy.

3.4. Adherence

Given the relevance of GFD in treating GRD, adherence was assessed by both the responder's perception and by CDAT score (Table 3). While 49.9% perceived that their adherence worsened during pandemic, in 44.4% had a CDAT score that classified them in the "bad adherence" category (≥13 points). Positive Spearman correlation consistently found the self-perception of adherence to GFD and CDAT score positively correlated with most dietary and clinical variables measured.

Table 3. Self-perception of COVID-19 pandemic effects on gluten-free diet and celiac disease test adherence (CDAT).

	Adherence Self-Perception of		Adherence CDAT			
Survey Questions	**"Good"**	**"Bad"**	**Poor Adherence**	**Good Adherence**	***p* Value ***	
Has the increase in food prices in general affected the quality of your diet? (yes) *n* (%)	207 (62)	124 (37.4)	129 (38.7)	78 (23.6)	0.0001	0.0001
How many wheat-containing foods do you include in your diet per week? *n* (%)						
1 Four or more times	6 (3.6)	1 (0.5)	7 (3.8)	0 (0)	0.0001	0.0001
2 Two or three times	19 (11.6)	5 (2.9)	22 (12.1)	2 (1.3)		
3 None	115 (70.5)	153 (91)	125 (69)	143 (95.3)		
4 Once	23 (14.1)	9 (5.3)	27 (14.9)	5 (3.3)		
How do you consider your diet? *n* (%)						
1 Excellent	20 (13.2)	55 (32.7)	22 (12.1)	53 (35.3)	0.0001	0.0001
2 Fairly good	79 (48.4)	104 (61.9)	94 (51.9)	89(59.3)		
3 Not so good	58 (35.5)	9 (5.3)	60 (33.1)	7 (4.6)		
4 Badly done	6 (3.6)	0 (0)	5 (2.7)	1 (0.6)		
Does the possibility of shortage of safe gluten-free foods during the pandemic worries you? *n* (%)						
1 Yes	135 (83.8)	80 (47.6)	125 (69)	90 (60)	0.0001	0.012
2 Slightly	22 (13.5)	51 (30.3)	37 (20.4)	36 (24)		
3 No	6 (3.6)	37 (22)	19 (10.5)	24 (16)		
Does maintaining social distancing when going to the supermarket or crowed places worries you? *n* (%)						
1 Yes	112 (68.7)	89 (52.9)	122 (67.4)	79 (52.6)	0.004	0.022
2 A little	34 (20.8)	43 (25.5)	36 (19.8)	41 (27.3)		
3 Not	17 (10.4)	36 (21.4)	23 (12.7)	30 (20)		
Does the COVID-19 pandemic worry you? *n* (%)						
1 Very much concerned	75 (46)	79 (47)	78 (43.1)	76 (50.6)		
2 A lot	74 (45.3)	47(27.9)	79 (43.6)	42 (28)	0.0001	0.012
3 A little	1 (0.6)	1 (0.5)	1 (0.6)	1 (0.6)		
4 No	13 (7.9)	41(24.4)	23 (12.7)	31 (20.6)		

Table 3. *Cont.*

Survey Questions	Adherence Self-Perception of		Adherence CDAT		*p* Value *	
	"Good"	"Bad"	Poor Adherence	Good Adherence		
Do you feel that you have higher risk of infection with COVID-19 due to your diagnosis? *n* (%)						
1 Very much concerned	36 (22)	14 (8.3)	31 (18.1)	19 (12.6)		
2 A lot	30 (18.4)	10(7.2)	29 (16)	11 (7.3)	0.0001	0.03
3 A little	55 (33.7)	94 (55.9)	78 (43.1)	71 (47.3)		
4 No	42 (25.7)	50(29.7)	43 (23.7)	49 (32.6)		
Are you getting the gluten-free foods that you regularly obtained before the pandemic? *n* (%)						
1 I have problems, but I can get them	80 (50)	81(48.2)	82 (45.3)	79 (52.6)		
2 It has been most difficult to get them	66 (40.4)	18(10.7)	58 (32)	26 (17.3)	0.0001	0.002
3 I have not been able to get them	4 (2.4)	0 (0)	4 (2.2)	0 (0)		
4 I have had no problems.	13 (7.9)	69 (41)	37 (20.4)	45 (30)		
Have the prices of gluten-free foods increased? (yes) *n* (%)	141 (42.5)	126 (38.1)	152 (45.9)	115 (34.7)	0.008	0.12
Cooking gluten-free foods at home during the pandemic *n* (%)						
1 Increase	81 (49.6)	92 (54.7)	97 (53.5)	76 (50.6)	0.0001	0.001
2 Decreased	23 (14,1)	1(0.5)	21 (11.6)	3 (2)		
3 Has not changed	59 (36.1)	75 (44.6)	63 (34.8)	71 (47.3)		

* (perception of adherence).

3.5. Mental Health

In total, 28% of the participants obtained scores above 10 (cut off for anxiety) in GAD-7 and 40% obtained above score 10 (cut off for depression) in PHQ-9; 23% were above the cutoff in both tests (Table 1). Chi^2 analyses revealed that positive score in GAD-7 was significantly associated with being worried for the pandemic, risk of infection with COVID-19, and shortage of safe foods (all $p < 0.001$). Spearman and Pearson correlations revealed some diverse but inconsistent correlations with the general and specific variables assessed (Table S1); instead, adherence-related variables showed that the poorer the perception of adherence, the stronger the perception of anxiety or depression or both ($p < 0.0000$, Table 4). Analysis of depression showed that health insurance, perception of dietary quality deterioration, increased prices, wheat consumption, self-perception of adherence, presence of current symptoms, cooking at home, and need to consult were significantly associated with positive PHQ9 scores (all $p < 0.004$, Table 4). Associations of mental health indicators and adherence to GFD were strong, both by perception and by CDAT score (Table 4).

Table 4. Associations of positive anxiety (GAD-7) and depression (PHQ-9) scores with pandemic effects and adherence to diet (by perception and CDAT).

	GAD-7			PHQ-9			Positive Score in Both Tests		
Variable	**Chi^2 (*p*)**	**TE (r)**	**Spear (Pos; *p*) ***	**Chi^2 (*p*)**	**TE (r)**	**Spear (Pos; *p*)**	**Chi^2 (*p*)**	**TE (r)**	**Spear (Pos; *p*)**
Higher cost of food	<0.0001	0.2154	pos < 0.001	<0.0001	0.3469	pos < 0.001	<0.0001	0.1961	pos < 0.001
"Pandemic Affected Adherence"	<0.0001	0.2408	pos < 0.001	<0.0001	0.287	pos < 0.001	<0.0001	0.2143	pos < 0.001
Consumption of Gluten	0.014	0.1747	pos < 0.003	0.0017	0.1978	pos < 0.001	0.0091	0.1653	pos 0.004
Adherence by perception	0.0001	0.203	pos < 0.001	<0.0001	0.2755	pos < 0.001	<0.0001	0.2469	pos < 0.001
CDAT	<0.0001	0.3981	pos < 0.001	<0.0001	0.4682	pos < 0.001	<0.0001	0.3914	pos < 0.001

* Pos; *p* = positive correlation; *p* value.

3.6. Logistic Regression

Given the results of adherence and mental health, logistic regression models using perception of self-adherence and CDAT as dependent variables were calculated. Both models were concordant; the one using adherence (CDAT score) to GFD as a dependent variable and, age, sex, education, geographical macrozone, diagnosis, declared wheat consumption, cost of gluten-free foods, GAD-7 and PHQ-9 scores as independent variables, shows that in participants stating that the pandemic affected their GFD, the likelihood of showing poor adherence by CDAT was 2.3 times higher (OR 2.3, IC 1.3–4.2, $p < 0.004$). Responders that suffered depressive symptoms were 1.3 times more likely to have poor adherence (OR 1.3, IC 1.2–1.4, $p < 0.000$). Older age (OR 0.97, IC 0.94–0.99, $p < 0.038$) and living in the south macrozone (OR 0.5; IC 0.27–0.97, $p < 0.041$) were associated with lower CDAT score, indicating better adherence.

4. Discussion

Results show that after several months of living in COVID-19 pandemic conditions, relevant effects can be indeed detected in persons with CD and other GRD maintaining GFD. Perception of significant deterioration of adherence to diet and mental health are the two most significant findings; unfortunately, because the pandemic situation is unprecedented, it is not possible to evaluate results using a formal before-and-after analysis or against comparison/control groups. Results also suggest the development of strategies to cope with the difficulties faced; it is interesting that those who follow GFD as treatment do not

seem to differentiate from those who follow it as a fashion/trend that consider it a "healthy diet" [16].

4.1. Self-Perceptions of the Pandemic and Clinical Aspects

As previously reported [5], "very much concern" for the pandemic is high (37.7%), and only 16% feel not worried at all. That 73% of responders perceive no higher risk of infection concur with earlier publications and reports [5,17]. Loss of jobs, shortage of food, and higher cost of safe products are the most relevant factors responders identified as determinants of difficulties for adhering to GFD (x2, all $p < 0.0001$); this concurs to an FAO recent statement acknowledging shortage of food as a relevant factor influencing present general dietary quality [18]. It seems reasonable that poor adherence is associated with increasing symptoms. Yet, the exact causes for the increase remain unclear because several factors may be involved, such as the rapid deterioration of adherence to GFD, increased stress inducing unspecific gastrointestinal complaints; mild COVID-19 infection [19], altered permeability [20,21], and modifications to the microbiome that can favor local inflammation [21,22]. It was unexpected that persons declaring CD and NCGS reported the same frequency of autoimmune conditions; however, autoimmune symptoms have been already described in NCGS by other authors [23,24]. Participants developed strategies to meet their dietary needs, the proportion of patients with "unhealthy diets" was low (11.5%), and one-third (32.6%) of responders did not need to seek medical help; but at the same time, symptoms increased during the last four months, and a high proportion of patients did try to find medical help. So, since the participants who were chronic patients were used to challenges when deciding what to eat, we interpret these results as that they are certainly resilient, which agrees with Monsani's report [25], but they definitely suffer adverse pandemic effects, and these are strong and consistent. It is intriguing that the HEI group mean score (65.7 points) observed, which classifies as "needs improvement", does not differ from the mean score described for general population in the previous national survey (2017) [26]. Lack of data in persons on GFD prior to the pandemic impedes further analysis of these results. Anyway, it is interesting that results of HEI and CDAT (validated tests) are concordant with those obtained when asking for perceptions. It was unexpected that being on GFD for a shorter time made no difference when compared with persons on GFD for many years.

4.2. Mental Health

Both GAD-7 and PHQ-9 were positively related with variables representing the pandemic, dietary, and clinical characteristics; however, relations with positive depression markers were more numerous and consistently significant. Correlation analysis against variables related to diet and clinical aspects showed weak associations (Table S1), and instead, association to variables assessing adherence to GFD was strong (Table 4). The several analyses performed to test the strength of the associations (Table 4) confirmed the relationship between mental health scores and adherence, both by perception and CDAT scores, leaving these variables as the main pandemic effects in daily life of people suffering GRD.

Experiences during EBOLA and SARS pandemics support the idea that mental health could be specially affected during the current COVID-19 pandemic [27,28]. Limitations to free moving, uncertainty, and fear facing the advances of viral infections, lack of physical activity, technological capacities required to access food, remote working, and confinement are fundamental factors shaping the mental health deterioration of the general population during the current pandemic [29]. It is difficult to discuss present results because the available data are scarce and incomplete. During the pandemic, the frequency of depression and anxiety in the general population was high in China (48% and 23%, respectively) [30] and in Hong Kong (20% and 14%, respectively) [29]; and in the USA, depression increased three times (prevalence 28%) [31]. As for CD, a recent systematic review and meta-analysis assessing celiac patients in non-pandemic conditions [32] reported that depression (OR 2.17, 95%

CI 2.17–11.15, $p < 0.0001$) and anxiety (OR 6.03, 95% CI 2.22–16.35, $p < 0.0001$) were higher in celiac patients compared with general population. In Chile, the last National Health Survey (2017) [33] showed the prevalence of depression at 6.2% in general population. Our current results are higher than these figures, but they do not clarify to what extent the differences are due to the presence of disease or the pandemic. A limitation to this study is that the group assessed was formed mainly by women, making it difficult to explain whether sex is a relevant variable in the analysis; anyway, depression was twice more frequent among women than in men (10% vs. 2%). Although logistic regression shows a strong relationship between adherence, being worried by the pandemic and mental health, it is unclear how these factors interact between them. Older age and living in smaller, uncrowded cities appear as the only protective factors detected in this study.

Limitations to this study are clear but mostly unavoidable, forcing it to remain descriptive. Since the COVID-19 pandemic is unprecedented and started abruptly, data prior to the pandemic are not available in the study group and comparisons of before and after the pandemic are not possible. The fact that it is still ongoing impedes having control/comparison groups, which is a situation that will change in time, enabling longitudinal analyses. In addition, due to many persons maintaining anonymity, we did not obtain clinical data. Despite this, the results described are relevant because no matter the exact causes of the effects described, and no matter how they compare to situations prior to the COVID-19 pandemic, depression and faulty GFD (mandatory for treatment) appear to be affecting a high proportion of participants, suggesting that support measures aimed at these aspects would help improve the health condition of people that maintain GFD. Finally, it is also important to emphasize that comparisons between this and other studies available should be cautious, because not only cultural aspects but also the conditions and timing of data collection are most variable. Further studies are indeed necessary to better understand the problems derived from the COVID-19 pandemic in persons that must follow restrictive diets such as GFD.

5. Conclusions

We conclude that after nearly a year living under COVID-19 pandemic conditions, the deterioration of dietary treatment and mental health of individuals suffering GRD appear as main effects of the COVID-19 pandemic. Loss of jobs, shortage of food, prices increase, poor access to safe foods, increase of symptoms, and poor availability of medical help are the relevant difficulties identified by individuals on GFD. Although the group assessed developed some strategies to cope with dietary pandemic problems, these fail to guarantee good adherence to treatment (GFD), maintain a healthy diet, and preserve good mental health. Depression represents the most relevant alteration in mental health assessment. Results of this study allow better understanding the health-related pandemic effects in people following restrictive diets, suggesting that improving the capacity to maintain adherence to diet and provide mental health support are two main areas that may help focusing interventions for this group within the population.

Supplementary Materials: The following is available online at https://www.mdpi.com/article/10.3390/nu13061822/s1, Table S1: Associations of anxiety (GAD-7) and depression (PHQ-9) with pandemic effects.

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Expanded Role of a Dietitian in Monitoring a Gluten-Free Diet in Patients with Celiac Disease: Implications for Clinical Practice

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Abstract: Access to a registered dietitian experienced in celiac disease (CD) is still limited, and consultation when available focuses primarily on the elimination of gluten from the diet. Thus, the aim of this study was to evaluate the nutritional value of a gluten-free diet (GFD) in adult CD patients before, and one year after, the standard dietary education. The study included 72 CD patients on a GFD and 30 healthy controls. The dietary intake of both groups was assessed through a 3-day food diary, while adherence to a GFD in celiac subjects was assessed using Standardized Dietician Evaluation (SDE). Subsequently, all CD patients received detailed education on gluten sources, and 48 of them participated in a one-year follow-up. Results: Comparison with the control group showed that consumption of plant protein in CD patients was significantly lower, whereas fat and calories were higher. At baseline, only 62% of CD patients adhered to a GFD, but the standard dietary education successfully improved it. However, the nutritional value of a GFD after one year did not change, except for a reduced sodium intake. The CD subjects still did not consume enough calcium, iron, vitamin D, folic acid or fiber. In conclusion, while the standard dietary education improved GFD adherence, it did not significantly alter its nutritional value. Therefore, it is necessary to increase the role of a dietitian in the treatment of CD.

Keywords: celiac disease; gluten-free diet adherence; dietary assessment; dietary reference intake

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1. Introduction

Celiac disease (CD) is an autoimmune disease that affects the small intestine in genetically predisposed people after consuming gluten [1] and occurs in about 1% of people in most populations [1]. Although new therapeutic strategies (gluten proteolysis, intestinal tissue transglutaminase 2 inhibitors, probiotics, immunotherapeutic methods) have been tested, the primary treatment for CD is still a gluten-free diet (GFD) [2]. It leads to resolution of symptoms, intestinal mucosa recovery and increased absorption of nutrients. The diet of a CD patient has to be based on grains such as maize, buckwheat, millet, rice, amaranth, tapioca or teff; however, gluten-containing whole grains generally have higher amounts of fiber and nutrients such as B vitamins, calcium and iron, so how to balance a GFD must be addressed [3] because nutritional deficiencies may increase the risk of many CD complications: osteoporosis and osteopenia, micro- and macrocytic anemia, chronic weakness or neurological symptoms, such as peripheral neuropathy and numbness [4]. On the other hand, replacement of whole grain barley, rye and wheat products with gluten-free equivalents may be associated with the increased consumption of fats, especially saturated fatty acids (SFA) and trans fats, as well as salt, sucrose and phosphorus. This can lead to

the development of metabolic disorders like obesity, dyslipidemia, gout, diabetes, hypertension and other cardiovascular complications [5]. An extensive fat intake can also reduce the absorption of other nutrients like magnesium [6]. Furthermore, high fat consumption together with low fiber ingestion led some CD patients, who adhered strictly to a GFD, to develop persistent symptoms such as bloating or abnormal bowel movements [7,8]. For all of the aforementioned reasons, it seems warranted that the dietary control of CD should be more detailed. The standard dietary education of patients with celiac disease usually focuses only on the proper recognition and avoidance of gluten sources; consequently, some patients can choose food that is gluten-free, but highly processed with low nutritional value. Unfortunately, there is very little research on the nutritional value of GFDs in adults. It is also unclear if the dietary education focusing on the elimination of gluten sources affects the nutritional value of this diet. Therefore the aim of the present study was to assess the nutritional value of a GFD in adult patients before and one year after standard dietary education.

2. Materials and Methods

2.1. Study Population

The study was conducted from October 2015 to April 2018 and involved 72 adults (63 women and 9 men) with a diagnosis of CD who were outpatients under the care of the Department of Gastroenterology and Hepatology at the Medical University of Gdansk. They were randomly recruited by a gastroenterologist during a routine consultation and had to meet the following inclusion criteria: be on a GFD, be over 18 years of age and have a diagnosis of CD based on serological and histological tests according to the British Society of Gastroenterology guidelines [9]. The exclusion criteria were being under 18 years, not giving consent to take part in the study, being pregnancy and not having a clear diagnosis of CD. Based on the patients' anamneses, the other most common autoimmune disease was hypothyroidism (15% of participants). A positive result for anti-endomysial antibodies (EMA) and anti-tissue transglutaminase (tTG) IgA together with a biopsy result were adopted as indicators of active disease. In the end, the study group consisted of 26 subjects with active CD and 46 with CD in remission.

The control group consisted of 30 gluten-eating healthy adults matched by age and sex with negative results for tTG-IgA antibodies. All controls were randomly recruited based on an online advertisement posted on the university's social network. They followed a traditional diet without any food elimination or therapeutic modifications. Written consent was obtained from all the participants. Pregnant women were excluded. The project was approved by the university bioethics commission (MUG Bioethics Committee approval number is NKBBN/403/201).

2.2. Serologic and Histologic Tests

In all CD patients, blood was drawn to determine serum levels for EMA and anti-deamidated gliadin peptide (DGP), and a duodenal biopsy was performed, whereas tTG antibodies were checked for both the controls and studied subjects. DGP and tTG antibodies were measured using the enzyme-linked immunosorbent assay (Euroimmun, Wrocław, Poland), while EMA antibodies were assessed using an indirect immunofluorescence technique (Euroimmun, Wrocław, Poland) in the hospital laboratory. The titers were considered positive according to the manufacturer's specifications. No CD subjects had IgA deficiency.

2.3. Dietary Assessment and Education

Dietary intake was assessed through a 3-day food diary which consisted of an accurate description of food intake during two weekdays and one weekend day. The dietitian informed all participants (studied and controls) by telephone how to prepare the food diary before the face-to-face consultation. During a personal appointment, a registered dietitian experienced in the dietary management of gastrointestinal disorders validated the

diary by using 24 h diet recall and asking detailed questions about the use of condiments, cooking methods and brands of foods. The amounts of food were assessed by showing the participants a photographic atlas published by National Food and Nutrition Institute (Poland) [10]. To estimate the intake of energy, macro- and micronutrients, the same dietitian used specialized software (Dietetyk 2012 JuMaR, Warsaw, Poland) based mainly on Polish Food Composition databases published by the National Food and Nutrition Institute in Warsaw, Poland, and on the United States Department of Agriculture database [11,12].

The basal metabolic rate (BMR) was calculated using the Harris–Benedict Equation, as follows:

- For men: BMR = 10 × weight (kg) + 6.25 × height (cm) − 5 x age (years) + 5
- For women: BMR = 10 × weight (kg) + 6.25 × height (cm) − 5 x age (years) − 161

A physical activity level (PAL) was determined for each person to estimate total energy expenditure. The percentage of the dietary reference intake (DRI) for energy and micronutrients was calculated based on the following nutrition standards for the Polish population: (Observed amount/reference amount) × 100 [13]. The following vitamin and mineral figures from the recommended daily allowance (RDA) were used: Vitamin A (as retinol activity equivalents), C, B_1, B_2, niacin, B_6, B_{12}, folate (as dietary folate equivalents) and calcium, phosphorus, magnesium, iron and zinc. For nutrients that do not have RDA values (vitamins D and E, sodium, potassium, fiber) adequate intake (AI) was adopted. The intake norms for fats, protein and carbohydrates according to Polish standards are presented as a percentage of the energy derived from them.

In the CD group, GFD adherence was also evaluated by the standardized dietitian evaluation (SDE), which is considered to be the gold standard for testing compliance with a GFD [14]. The usefulness of applying this method in the Polish population with CD was presented in our previous work [15]. The SDE consists of a 3-day diary, food label quiz and a detailed interview conducted by a trained dietitian about reading medicine labels, dietary supplements, eating out and the risk of gluten cross-contamination. The results were presented on a 6-point Likert scale: 1 point—perfect GFD adherence; 2 points—good GFD adherence; 3 points—fair GFD adherence; 4 points—poor GFD adherence; 5 points—very poor GFD adherence, and 6 points—no GFD.

During the consultation, the dietitian also provided an individual one-hour detailed education about the GFD with particular emphasis on hidden sources of gluten. After the meeting, dietary recommendations were sent by e-mail to the patient to summarize the information about gluten sources: a list of manufacturers of gluten-free foods, local stores that specialized in gluten-free products and a list of gluten-free food additives and ingredients. Additionally, two months after the educational consultation, the dietitian called or emailed the CD subjects to remind them of all the dietary recommendations. Each patient could also contact the dietitian for one year if any questions about the GFD diet had arisen. The SDE with a 3-day food diary was repeated one year after the education. The scheme of the study is presented in Figure 1.

2.4. Anthropometric Measurements

Anthropometric measurements were collected by the same dietitian during the first and second face-to-face consultations. Body weight was measured using a body composition analyzer (Jawon Medical X-Contact 350, Daejeon, Korea), while height was measured to the nearest 5 mm using a stadiometer (SECA 213, Hamburg, Germany). The body mass Index (BMI) was calculated from weight and height (kg/m^2) and values were categorized according to World Health Organization criteria.

2.5. Data Analyses

The data are expressed as mean ± SD or median and interquartile range (Q1–Q3). The results of the SDE were additionally divided into two groups: good (Good–Perfect) and bad (Fair–Poor–Very Poor). A Kolmogorov–Smirnov test was used to verify whether the variable distribution was normal. The differences between groups were evaluated

by an independent Student's *t*-test and U Mann–Whitney test (when the distribution of the variable was not normal) or chi-square tests, as appropriate. Data before and after dietary education were compared with the use of the paired Student's *t*-test or Wilcoxon signed-rank test (when the distribution of the variable was not normal). Statistical analysis was performed using STATISTICA version 13.3 (StatSoft, Kraków, Poland), and p values < 0.05 were considered statistically significant.

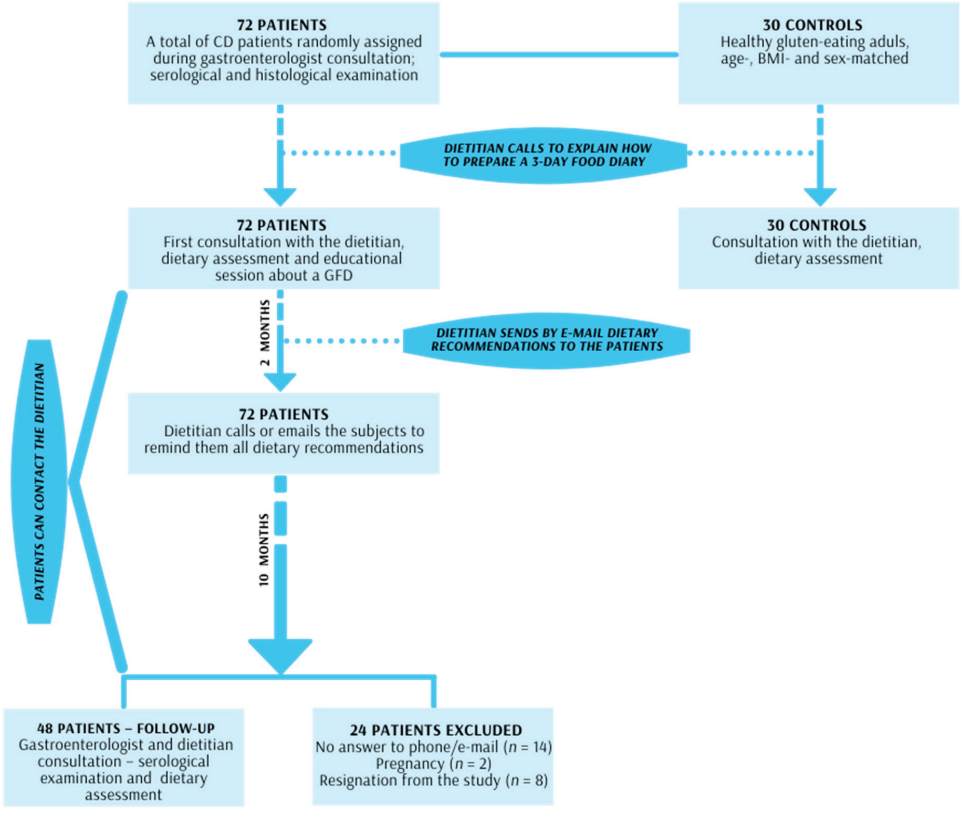

Figure 1. Scheme of the study.

3. Results

3.1. Characteristics of the CD Patients and Control Subjects

The characteristics of the studied population are presented in Table 1. Although the mean time from the diagnosis of CD was 4.8 ± 7.0 years, only 17 of 72 patients (24%) admitted that they had never been consulted by a dietician prior to the study.

3.2. Analysis of Energy and Nutrient Intake in all CD Patients (n = 72) and Control Group (n = 30)

The intake of energy and nutrients assessed through a 3-day food diary in all CD patients and healthy controls is presented in Tables 2 and 3.

Table 1. The characteristics of the study groups.

Characteristic	All CD Patients (*n* = 72)	Follow-Up CD Group (*n* = 48)	Controls (*n* = 30)
Female (%)	87.5	91.7	90
Age (years)	40.3 ± 11.9	42.3 ± 11.2	36.6 ± 12.1
BMI (kg/m^2)	22.2 ± 3.6	21.8 ± 3.3	23.1 ± 3.7
Classic presentation of CD (%)	21	15	-
Disease's duration (years)	4.8 ± 7.0	5.9 ± 8.1	-
Diagnosis over a year ago (%)	68	73	-
Meeting a dietitian before the study (%)	24	29	-
Active CD (%)	36.1	37.5	-
Other autoimmune diseases (%)	36.1	27.1	-

There were no significant differences between the groups. Abbreviations: BMI—-body mass index; CD—-celiac disease.

Table 2. Intake of macronutrients per person, per day in all CD patients (*n* = 72) and controls (*n* = 30).

Observed Component	Recommended	All CD Patients (*n* = 72)		Controls (*n* = 30)	
		Mean ± SD	% of E	Mean ± SD	% of E
Protein (g)	10–20% of E	84 ± 29	16	78 ± 20	18
Plant protein (g)	NA	19 ± 9	-	23 ± 9 *	-
Animal protein (g)	NA	53 ± 26	-	49 ± 21	-
Fat (g)	20–35% of E	93 ± 47	38	75 ± 27 *	37
SFA (g)	as low as possible	31 ± 16	13	29 ± 10	14
MUFA (g)	NA	33 ± 23	13	28 ± 12	13
PUFA (g)	NA	12 ± 9	5	11 ± 5	6
Cholesterol (mg)	NA	324 ± 182	-	333 ± 135	-
Carbohydrates (g)	45–60% of E	247 ± 87	46	222 ± 61	46
Sucrose (g)	NA	56 ± 31	-	45 ± 23	-
Fiber (g)	25 g	23 ± 9	-	22 ± 9	-

* Significant differences (p <0.05) between celiac and control subjects. Abbreviations: CD—-celiac disease; E—-energy; MUFA—-monounsaturated fatty acids; NA—-not available; PUFA—-polyunsaturated fatty acids; SFA—-saturated fatty acids.

Table 3. Intake of energy and micronutrients per person per day in all CD patients (*n* = 72) and controls (*n* = 30).

Observed Component	All CD Patients (*n* = 72)		Controls (*n* = 30)	
	Mean ± SD	% of DRI	Mean ± SD	% of DRI
Energy (kcal)	2138 ± 718	107	1822 ± 451 *	103
Sodium (mg)	2296 ± 1337	161	2317 ± 825	155
Potassium (mg)	3529 ± 1139	101	3255 ± 947	93
Calcium (mg)	929 ± 470	91	834 ± 237	80
Phosphorus (mg)	1221 ± 454	174	1240 ± 325	177
Magnesium (mg)	344 ± 109	105	306 ± 96	94
Iron (mg)	12 ± 5	81	12 ± 4	82
Zinc (mg)	9 ± 4	107	9 ± 3	113
Vitamin A (µg)	1429 ± 1285	198	960 ± 566	135
Vitamin D (µg)	5 ± 6	31	6 ± 10	40
Vitamin E (mg)	11 ± 8	129	12 ± 6	144
Vitamin B_1 (mg)	1.2 ± 0.6	105	1.1 ± 0.4	101
Vitamin B_2 (mg)	1.6 ± 0.6	141	1.5 ± 0.4	130
Niacin (mg)	20 ± 11	141	19 ± 7	133
Vitamin B_6 (mg)	2.2 ± 0.9	163	1.9 ± 0.6	145
Vitamin B_{12} (µg)	4.7 ± 5	197	4.4 ± 3.2	182
Vitamin C (mg)	135 ± 106	177	116 ± 85	153
Folate (µg)	287 ± 136	72	318 ± 118	80

* Significant differences (p < 0.05) between celiac and control subjects. Abbreviations: CD—-celiac disease; DRI—-dietary recommended intake.

As can be seen in Tables 2 and 3, consumption of plant protein in CD patients was significantly lower, whereas consumption of fat and calories was higher than in the control group. There were no other differences between the groups, but the intake of fiber, calcium, iron, vitamin D and folic acid was too low according to the DRI in both CD patients and the control group.

The diet of CD patients who were diagnosed more than one year before the study showed differences in energy consumption from those who had a shorter disease duration. They had, respectively, a higher proportion of energy from fats (39.7% ± 9.1 vs. 34.8% ± 10.4, $p = 0.04$) and MUFAs (14.8% ± 5.8 vs. 10.6% ± 5.0, $p = 0.006$) but lower energy from carbohydrates (44.3% ± 9.7 vs. 50.2% ± 10.2, $p = 0.04$).

3.3. Analysis of Energy and Nutrient Intake in Two Subgroups of CD Patients: In Remission (n = 46) or with Active CD (n = 26) in Comparison to Control Group (n = 30)

No differences were observed among the three groups in age, sex or BMI. There were was also no difference in the macro- and micronutrient composition of the diets of patients in remission or with the active CD.

In a comparison of the nutritional value of the diet of patients with active CD to that of the control subjects, a lower proportion of energy from plant protein was found in the active CD group: (25% ± 14 vs. 30% ± 11, $p = 0.04$) and PUFA (4.5% ±2.0 vs. 5.6% ± 1.9, $p = 0.04$).

It was observed that CD participants in remission consumed more energy than control subjects (2135 ± 764 vs. 1822 kcal ± 451, $p = 0.047$). On the other hand, a lower percentage of energy from plant protein was observed in CD patients in remission than in the control group (24% ± 12 vs. 30% ± 11, $p = 0.007$).

3.4. Assessment of Adherence to a GFD in all CD Patients (n = 72) at Baseline

The results of the SDE are presented in Figure 2, and showed that 62% ($n = 72$) presented perfect or good adherence to a GFD. The median SDE score was 2 and the interquartile range (Q1–Q3) was 1–3. The SDE score did not differ for those who admitted to having met a dietitian prior to the study to those who had never met one. The results of the SDE also did not differ among those who had a disease duration shorter or longer than one year, but the patients in CD remission more often presented perfect or good adherence to a GFD than those with an active disease (74% vs. 42%, $p = 0.008$).

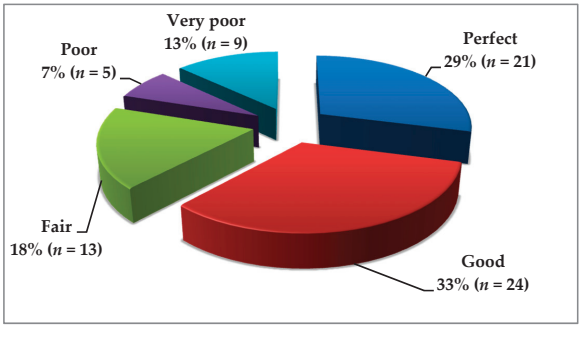

Figure 2. Adherence to a GFD in CD patients ($n = 72$) assessed by the Standardized Dietitian Evaluation (SDE).

3.5. Analysis in Follow-Up CD Group (n = 48)

Forty-eight participants of the CD group (44 women, 4 men) gave their permission to attend follow-up consultations with a gastroenterologist and a dietitian after one year to re-evaluate the serology, dietary intake and nutritional status using the same methods as during the first appointment.

3.5.1. Changes in Nutritional Status, Adherence to a GFD and Autoantibody Levels after One Year of Follow-Up

Patients in the follow-up CD group (n = 48) had a higher BMI one year after dietary education (21.8 kg/m^2 ± 3.3 vs. 22.4 ± 3.3, p = 0.001).

The changes in adherence to a GFD in the whole follow-up group are presented in Figure 3. Based on the SDE results, 60% (29/48) presented better adherence (a lower SDE score) than at baseline, and only 8% (4/48) were given a worse SDE score. In a subgroup of CD patients with a bad SDE score (between 3 and 5, n = 17) at the beginning of the study, as many as 53% (9/17) followed a GFD perfectly or well (SDE score 1 or 2) one year after the education. The median SDE score for the whole follow-up group decreased from 2 points to 1 point (p = 0.0001). The serum titers of EmA IgA, tTG IgA and DPG IgA also significantly decreased in the follow-up group (p values, were, respectively 0.04, 0.02, and 0.0001).

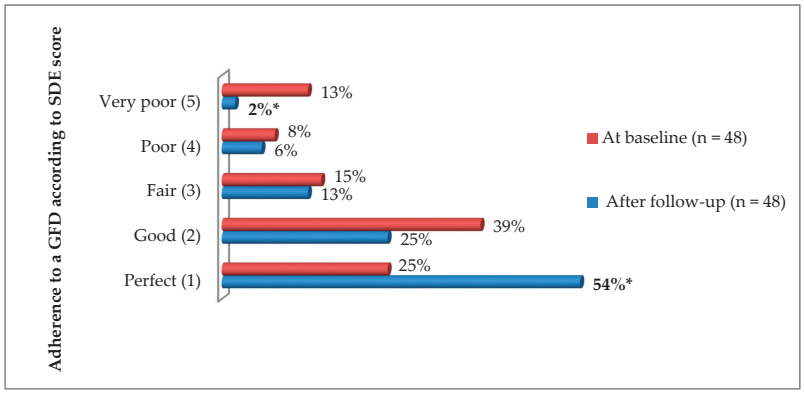

Figure 3. The adherence to a GFD assessed by the Standardized Dietitian Evaluation (SDE) in follow-up CD patients (n = 48) before and one year after education. * Differences were statistically significant ($p < 0.05$).

3.5.2. Energy and Nutrient Intake in Follow-Up CD Group

A comparison of the nutritional value of the GFD in CD patients (n = 48) at the beginning and after one year is presented in Tables 4 and 5. As can be seen, there were no significant differences between the baseline and after follow-up consumption of macro- and micronutrients, except for reduced sodium intake. According to the DRI, the CD subjects still did not consume enough fiber, calcium, iron, vitamin D or folic acid.

Table 4. Intake of macronutrients per person per day in follow-up CD group (n = 48) before and after one year of follow-up.

Nutrients	Recommended Values	At Baseline (n = 48)		After Follow-Up (n = 48)	
		Mean ± SD	% of E	Mean ± SD	% of E
Protein (g)	10–20% of E	81 ± 25	16	78 ± 25	17
Plant protein (g)	NA	18 ± 8	-	17 ± 10	-
Animal protein (g)	NA	51 ± 24	-	49 ± 19	-
Fat (g)	20–35% of E	90 ± 42	39	84 ± 40	37
SFA (g)	as low as possible	31 ± 15	13	30 ± 15	13
MUFA (g)	NA	32 ± 20	13	30 ± 17	13
PUFA (g)	NA	11 ± 7	5	11 ± 7	5
Cholesterol (mg)	NA	297 ± 180	-	299 ± 166	-
Carbohydrates (g)	45–60% of E	235 ± 76	46	238 ± 78	46
Sucrose (g)	NA	50 ± 28	-	51 ± 26	-
Fiber (g)	25 g	22 ± 6	-	22 ± 11	-

There were no significant differences between baseline and after follow-up. Abbreviations: E—Energy; MUFA—monounsaturated fatty acids; NA—not available; PUFA—polyunsaturated fatty acids; SF—saturated fatty acids.

Table 5. Intake of energy and micronutrients per person per day in follow-up CD group (n = 48) before and after one year of follow-up.

Nutrients	At Baseline (n = 48)		After Follow-Up (n = 48)	
	Mean ± SD	% of DRI	Mean ± SD	% of DRI
Energy (kcal)	2047 ± 584	104	1972 ± 649	101
Sodium (mg)	2274 ± 1216	159	2011 ± 1060 *	142
Potassium (mg)	3415 ± 941	98	3508 ± 1188	100
Calcium (mg)	842 ± 390	82	800 ± 313	78
Phosphorus (mg)	1147 ± 385	164	1196 ± 366	171
Magnesium (mg)	344 ± 106	105	353 ± 129	108
Iron (mg)	11 ± 4	76	11 ± 4	74
Zinc (mg)	9 ± 4	104	9 ± 3	104
Vitamin A (µg)	1408 ± 1360	199	1069 ± 898	151
Vitamin D (µg)	5 ± 7	32	6 ± 9	39
Vitamin E (mg)	10 ± 6	119	10 ± 7	121
Vitamin B_1 (mg)	1.2 ± 0.6	105	1.1 ± 0.5	98
Vitamin B_2 (mg)	1.5 ± 0.6	135	1.5 ± 0.5	132
Niacin (mg)	20 ± 10	142	19 ± 9	135
Vitamin B_6 (mg)	2.1 ± 0.8	157	2.0 ± 0.9	176
Vitamin B_{12} (µg)	5.1 ± 6.0	211	4.3 ± 4.3	180
Vitamin C (mg)	125 ± 103	168	124 ± 88	163
Folate (µg)	264 ± 87	66	276 ± 107	69

* Significant differences (p <0.05) between baseline and after follow-up. Abbreviations: DRI—dietary recommended intake.

4. Discussion

It is well known that the main treatment for CD until now has been lifelong compliance with a GFD. Poor dietary adherence can cause serious health problems, such as the risk of T-cell lymphoma and other autoimmune diseases [16]. However, many adults with CD still misidentify gluten sources with adherence rates ranging from 42% to 91% depending on the study method [17]. In the present study, we used the Standardized Dietician Evaluation, which assesses compliance with a GFD very accurately, and found that only 62% of CD patients followed a GFD properly. Hence we confirmed that the lack of adherence is still a problem. Monitoring compliance with a GFD is critical for achieving serological and histological remission of the disease, but it may not be sufficient to improve the long-term prognosis. Some studies even suggested that a GFD may increase the risk of obesity and type 2 diabetes [5,18]. Therefore, in our current study, we compared the nutritional value of the GFD in adult CD patients with the diet of controls. We also wanted to know if the dietary education provided by a registered and highly experienced dietitian who focuses

mainly on identifying and avoiding gluten sources would change the nutritional value of the diet in CD patients. Our results showed that while such education did improve adherence to a GFD, it did not significantly alter the nutritional value of the diet. After a year, CD patients consumed less sodium, but still ate too much fat, especially SFA and not enough fiber. It is difficult to compare these results with other studies because of a lack of follow-up studies to assess the nutritional value of a GFD after education from a dietitian. A small number of studies focused on the nutritional aspects of a GFD that included children, for whom access to dietary consultations is often easier.

Our observation that improved adherence to a GFD after a dietitian's consultation did not always guarantee better nutritional value is quite similar to that of Sepherd et al. [19]. They applied education focused primarily on gluten avoidance in newly diagnosed CD patients, and the nutritional value was checked before starting the GFD and after 12 months. It was found that nutrient intake (except for starch) did not change after the education. The authors also diagnosed many nutritional inadequacies in patients with long-term treated CD concerning, e.g., vitamin A, thiamin, fiber, folate, calcium and iron. Our patients also had inadequate intake of fiber, calcium, iron and folic acid. Based on our results, we agree with others that the extension of standard education to teaching patients about the quality and nutritional value of gluten-free foods is even more important [19,20]. As can be seen from our study, compliance with a GFD is not the only issue for treating CD patients. Those who only follow a GFD but do not eat well-balanced meals might be at risk for metabolic disorders. Our analysis showed that, in general, CD patients consumed more fat and calories and less plant protein compared to the control subjects. This observation was also in line with other studies for probably two reasons [21,22]. First, foods dedicated to CD patients, especially highly processed, typically still contain higher amount of fats and calories than standard gluten-containing foods because substituting gluten often requires the manufacturer to use more ingredients or food additives [23]. Secondly, CD patients often turn to gluten-free snacks rich in fat, calories and sucrose to improve their mood or because they are simply available and readily certified. In our study we observed that the persistent overconsumption of fat and calories in CD patients led to an increase in the BMI. Similarly, in the study of Mahadev et al., nearly half of CD patients gained weight after starting a GFD [24]. On the other hand, the higher BMI value after follow-up may also be the result of improved intestinal absorption due to reduced inflammation of the duodenum.

It should be emphasized that inadequate nutrient intake was also observed in our healthy control group. Both groups consumed on average more than 300 mg of cholesterol per day, which the European Food Safety Authority (EFSA) and Polish Diabetes Association say increases the risk of metabolic disorders such as diabetes, hypertension, atherosclerosis and stroke [25,26]. Similarly, both CD patients and controls consumed more fat than recommended, which may indicate that overconsumption of fat is a problem not only for people with CD, but also for society as a whole [27]. The study conducted by the Polish National Research Institute confirmed that per capita fat intake in Poland increased from 23.6 kg to 33.5 kg from 1990 to 2015 [28]. It has also been observed that a healthy population consumes more products containing plant fats. In our study, we also noticed that the control group consumed more PUFAs than the patients with active CD, but there was no difference in PUFA intake between CD patients in remission and controls. It should be mentioned that patients with the properly treated CD do not need to restrict fat because they are free from gastrointestinal symptoms and fat is gluten free. Additionally, plant fat is very rich in unsaturated fatty acids, which are increasingly popular with people interested in healthy eating, including those on a GFD. This may explain the increased fat intake, especially MUFAs, which was observed in patients with CD lasting more than a year. In contrast, typical carbohydrates are grains, most of them gluten containing, so we found that, over time, CD patients reduced their intake in favor of fats.

Reduced plant protein intake, which was observed in both patients in remission and with active CD, may be due to a decreased consumption of grains for fear of gluten

contamination or to buying products labeled both gluten free and PKU (a low-protein diet for patients with phenylketonuria), such as bread. In healthy people, gluten is one of the main plant proteins consumed every day since it is found in wheat or rye bread, pasta, barley groats and various baked goods. In addition, people with CD often rely on gluten-free products made from white rice or corn, which contain less plant protein than wheat. Importantly, a good alternative to corn or rice might be buckwheat, oat, teff, amaranth and quinoa. Despite the fact that high nutritional value has made pseudocereals a modern trend in the human diet, in some countries such as Poland the availability of teff or quinoa is still limited partly due to the high price [29]. The lack of reimbursement for gluten-free foods in Poland makes them even more difficult to purchase. The situation is much better with buckwheat, amaranth and gluten-free oat products, because they are easily available and relatively cheap. It is worth pointing out that the market for gluten-free products has been growing steadily in recent years, and the situation is already more favorable than it was 10 years ago [30].

We also found that the patients with CD did not consume enough fiber, calcium, iron, vitamin D, or folic acid although it was seen that the same problem occurred in the control group. Fiber deficiency can lead to impaired intestinal peristalsis as well as dysbiosis of intestinal microbiota [31]. As for the subsequent nutrient deficiencies (calcium, iron, vitamin D, folic acid), patients with CD should pay special attention to them due to possible malabsorption. These patients are also at risk of osteoporosis and osteopenia, anemia, and neurological disorders [32,33]. Low calcium intake may be the consequence of lactose intolerance, which is more common in celiac disease patients than the general population. On the other hand, we noted that, like Zingone et al., controls also consumed insufficient amounts of calcium [34]. In CD patients who cannot achieve adequate intake via a GFD or with documented low serum levels, calcium and vitamin D should be supplemented [16].

Some of the nutritional deficiencies in GFD may also be the result of discrepancies in food fortification policy [35]. Gluten-free products are not fortified in the standard way as conventional foods, so they may contain less fiber, iron, or B vitamins. Therefore, the task of a dietitian is also to show particularly malnourished patients what gluten-free products can be fortified with nutrients or whether supplementation is already needed.

The results of our work clearly indicated that the standard education of patients with CD should be expanded so that their food choices improve the nutritional value of the diet. However, implementation may be difficult in many countries because of limited access to a registered dietitian with experience in the dietary treatment of CD. Bebb et al. found that only 38% of patients with CD diagnosed on average 5.4 years earlier remained under the care of a specialist [36]. Herman et al. also indicated that patients with CD are not consistently followed up [37]. They found that one and five years after diagnosis, only 3.3% and 15.8%, respectively, had met a registered dietitian. For comparison, only 24% of our patients with the disease lasting 4.8 ± 7.0 years had the opportunity to consult a dietician before the beginning of the study. On the other hand, we noticed that patients with CD are not always interested in long-term care by a dietician due to the need to come to appointments or because they are ashamed to admit that they do not follow a GFD. Despite the fact that the dietician called and messaged each of our patients several times to arrange a follow-up visit, many did not respond; eventually, 24 patients refused to participate in the follow-up.

It is also worth emphasizing that the previous meeting with a dietitian did not affect the SDE result in our study. This may, of course, be due to the small sample size, but there is no doubt that not every dietitian is equally trained in treating CD. Experts emphasize that a dietitian working with a gastroenterologist should be highly knowledgeable about a GFD [16]. In their opinion, one of the most important elements for increasing adherence to a GFD is regular dietary consultation.

In our study we also demonstrated that the dietary education in CD patients does not always have to be based on in-patient visits. Because of the COVID-19 pandemic, and difficulties in accessing a dietitian, a good solution may be to combine face-to-face

consultations with follow-ups by e-mail or by phone [38,39]. The results of the studies of Sainsbury et al., Jeanes et al., and Muhammad et al. confirm our suggestion [40–42].

The description of difficulties in accessing a dietitian would not be complete if it were not been mentioned that dietary consultations in countries like Poland are not reimbursed, and gastroenterological walk-in clinics are not obligated to employ a dietitian [43]. To summarize, it is important to emphasize that CD dietary care should not be based on just one consultation focusing on gluten sources. Our conclusion is confirmed by the recently published guidelines of the European Society for the Study of Coeliac Disease [16]. Experts emphasize that dietary education is essential not only to learn how to eliminate gluten from the diet, but also to make the patient aware that a GFD, like any diet, must be properly balanced.

There were several limitations to this study. Firstly, we only recruited patients from one medical center, so the sample was relatively small and consisted mostly of women. However, CD is more common in women, and they are more interested in dietary consultation than men. Secondly, although the dietitian thoroughly explained to the study participants how to prepare the 3-day food diary and checked the patient's food intake during the face-to-face consultation by using 24 h recall, the diary method is subject to random error due to the respondent not admitting to eating a certain food or forgetting to write the food in the diary. Thirdly, concomitant autoimmune diseases could result in avoidance or increased consumption of certain foods.

5. Conclusions

In conclusion, we observed that as many as 38% of CD patients did not adhere to a GFD; however, standard dietary consultation by the dietitian experienced in CD treatment, successfully improved this based primarily on the ability to identify gluten sources. Unfortunately, a better GFD compliance did not change the nutritional value of the GFD, except for a lower sodium intake. We observed that patients with CD ate less plant protein (both active CD and in CD remission), but more total fat and energy (especially those in CD remission) than the control group, which may increase the risk of cardiovascular disease or obesity. Moreover, CD subjects did not consume enough calcium, iron, vitamin D, folic acid or fiber, but they ate too much cholesterol. Therefore, the role of a dietitian in the treatment of CD needs to be increased, so that patients not only learn to follow a diet, but also how to balance it.

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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 fact that they contain information that could compromise the privacy of research participants.

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

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TMPRSS6 rs855791 Polymorphism Status in Children with Celiac Disease and Anemia

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Abstract: Celiac disease (CD) is an autoimmune chronic inflammatory disease occurring in genetically predisposed individuals in response to the intake of gluten. Clinical presentation can be heterogeneous. Iron-deficient anemia (IDA) is one of the most common extra-intestinal manifestations of CD. Although IDA usually reverts with a gluten-free diet (GFD), some patients show persistent IDA, the mechanisms of which are poorly understood. Recent studies suggest an association between the rs855791 polymorphism in the *TMPRSS6* gene and persistent IDA in adults with CD. The current study aimed to assess the potential link between rs855791 and persistent IDA in pediatric patients with CD. The study included 106 children diagnosed with CD between 2015 and 2019. Clinical and blood parameters (including blood count, serum iron) were collected at diagnosis and after ≥12 months of GFD, and the rs855791 genotype was assessed for each patient. IDA was present at diagnosis in 25 patients (23.6%); only three (3%) had persistent IDA after GFD. The prevalence of rs855791 genotypes was 9% (n = 10) for TT, 53% (n = 56) for CT, and 38% (n = 40) for CC. There was a tendency toward a higher proportion of the T allele in patients with IDA and lower hemoglobin in the TT genotype but without statistical significance. An association between rs855791 and persistent IDA was not observed. These findings suggest that persistent IDA is uncommon in pediatric patients with CD. The prevalence of rs855791 in children with CD is reported for the first time.

Keywords: celiac disease; anemia; iron metabolism; gluten-free diet; rs855791; *TMPRSS6*

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1. Introduction

Celiac disease (CD) is an autoimmune chronic inflammatory disease that occurs in genetically predisposed individuals in response to gluten intake [1]. The hallmarks of CD include the presence of auto-antibodies directed against tissue transglutaminase 2 (TTG) and small intestinal enteropathy characterized by villous atrophy, crypt hyperplasia and lymphocytic infiltration of the epithelium. The only therapy for CD is a lifelong gluten-free diet (GFD).

The clinical presentation of CD is heterogeneous. The classical form is characterized by chronic diarrhea and failure to thrive. In non-classical CD, diarrhea is absent; instead, gastrointestinal symptoms may include abdominal pain, bloating, nausea, and constipation. Importantly, both classical and non-classical CD can also be accompanied by a wide range of extra-intestinal symptoms. Iron-deficient anemia (IDA), characterized by microcytosis and low serum iron and ferritin levels, is one of the most common extra-intestinal manifestations of CD, irrespective of patients' age and sex [2]. IDA has been reported in 12% to 69% of cases of newly diagnosed CD [2–4]. On the other hand, one in 31 patients with IDA has histologic evidence of CD [5].

Iron deficiency can result from chronic blood loss, inadequate dietary iron intake, impaired iron absorption, and/or chronic inflammation. The latter two factors, in particular, have been incriminated as potential causes of IDA in patients with CD. A gluten-free diet limits intestinal inflammation, normalizes gut epithelium and restores the absorption of nutrients [6]. However, persistent IDA has been reported in 15% to 28% of adult patients with CD and in 16% of children, despite adherence to GFD [7,8]. The mechanisms responsible for the persistence of anemia and iron deficiency in CD have not yet been elucidated [7,8].

Hepcidin, a key player in iron metabolism, can decrease iron levels by inhibiting intestinal iron absorption and iron release by macrophages and hepatocytes [9,10]. The regulation of hepcidin synthesis is a complex process. Transmembrane Serine Protease 6 (TMPRSS6), also known as Matriptase-2, is a liver-specific enzyme that negatively regulates hepcidin production [11]. Interestingly, several studies have linked the rs85579 single nucleotide polymorphism (SNP) in the *TMPRSS6* gene to hemoglobin levels and/or iron status in large cohorts of healthy adults and adults with CD or with IDA [12–14]. Nevertheless, the potential association between *TMPRSS6* polymorphisms and anemia in children with CD has not been addressed to date.

The aim of this study was to investigate the association between the rs855791 *TMPRSS6* polymorphism, anemia and iron status in children with CD at diagnosis and after treatment with GFD, and to explore the potential link between rs855791 and persistent IDA in this population.

2. Materials and Methods

2.1. Study Design and Population

The study was carried out between October 2018 and August 2020 in the Provincial Specialist Children's Hospital in Olsztyn and the Department of Paediatrics, University Hospital No.1 in Bydgoszcz, Poland. Using patient medical records, patients diagnosed with CD were retrospectively identified in both centers between January 2015 and December 2018 and prospectively enrolled patients diagnosed with CD during 2019. Children were diagnosed with CD if they fulfilled the 2012 ESPGHAN criteria: positive IgA TTG or anti-endomysial antibodies (EMA) and biopsy-proven enteropathy according to Marsh criteria [15]. Patients with concomitant inflammatory bowel disease or with Down syndrome were excluded. In all patients, clinical symptoms, anthropometric data, blood parameters, serum iron levels and serum TTGlevels at diagnosis were retrieved retrospectively from medical records. The presence of concomitant-associated conditions, such as type 1 diabetes mellitus or autoimmune thyroid disease, was also recorded. Children with CD were then subdivided into those with IDA and those without anemia at diagnosis, with anemia being defined based on hemoglobin (HGB) levels according to age and gender using the World Health Organization (WHO) criteria [16] (Supplementary Table S1). These two groups were compared in terms of clinical characteristics, histology, hematological and biochemical parameters, adherence and response to GFD.

Patients were followed at their respective gastroenterology outpatient clinics. A follow-up visit was scheduled for each patient as part of this study after at least 12 months of GFD. Hematologic response to GFD was defined as normalization of HGB levels based on WHO criteria. Adherence to GFD was assessed based on parents'/patients' reports and on normalization or a significant decrease in anti-TTG antibodies.

2.2. Ethics

Parents and caregivers of all study participants were informed about the potential benefits and risks and signed a written consent form. A consent form was also signed by patients aged 16 years or above. The experimental design and all procedures were approved by the Bioethics Committee of the Faculty of Medical Sciences of the University of Warmia and Mazury in Olsztyn (permission No. 32/2017 granted on 22 June 2017).

2.3. Sample Collection

At the follow-up visit, blood samples were collected for blood count, C-Reactive Protein (CRP), serum ferritin, iron, hepcidin and Interleukin-6 (IL-6) levels, and for genomic testing for the *TMPRSS6* rs855791 polymorphism. Peripheral blood samples (2 mL) and serum samples (2 mL) were collected from all patients and stored at −80 °C until the biochemical and genetic analyses.

2.4. DNA Analysis

Genomic DNA was extracted from 500 µL of total peripheral blood stabilized with EDTA using an InnuPREP Blood DNA Midi Kit and Smart Blood DNA Midi Kit (cat. No AJ845-KS-1030050, cat. no 845-KS-8100050, Analytik Jena AG, Jena, Germany), according to the manufacturer's instructions. The quality and quantity of extracted DNA were estimated using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The SNP genotyping analysis was performed in a final volume of 10 µL, using 3 µL of DNA, 0.5 µL of specific primers with probes (TaqMan SNP Genotyping Assay ID: C_32899902_10), Context Sequence [VIC/FAM]: GCGTGGCGTCACCTGGTAGCGATAG[A/G]CCTCGCTGCACAGGTCCTGTGGGAT, and TaqPath™ ProAmp™ Master Mix (cat. No A30865, Applied Biosystem, Waltham, MA, USA). Real-time PCR was carried out on a ViiA™ 7 Real-Time PCR System (Life Technologies) under default thermal cycling conditions. Amplification was performed with pre-read step for 30 s at 60 °C; next, an initial denaturation step for 5 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 60 s at 60 °C. The last step was the post-read step for 30 s at 60 °C. Polymerase chain reactions were performed in duplicate and on negative controls prepared using water instead of the DNA template. The results of *TMPRSS6* genotype and allele frequencies were analyzed using GraphPad Prism 8.4 software (GraphPad Software, Inc., San Diego, CA, USA) using Fisher's exact test.

Hereafter, considering that the rs855791 polymorphism site represents the c.2207 position of the *TMPRSS6* coding sequence based on RefSeq NM_153609.3, thymine at this position (resulting in p.Val736) is designated as the "T" allele, and a cytosine (resulting in p.Ala736) is defined as the "C" allele.

2.5. Serum Biochemical Analyses

The concentration of hepcidin in serum samples was measured using a commercial ELISA kit (FineTest, Wuhan, China). The detection range of the test was 15.625–1000 pg/mL, the sensitivity was 9.375 pg/mL, and the coefficient of variation was below 10%.

The serum concentration of IL-6 was measured using Human IL-6 ELISA Kit (FineTest, Wuhan, China). The detection range of the test was 4.688–300 pg/mL, the sensitivity was 2.813 pg/mL, and the coefficient of variation was below 10%.

ELISA kits were used according to manufacturers' protocols. All ELISAs were analyzed using a Biochrom® Asys UVM 340 Microplate Reader (Biochrom Ltd., Cambridge, UK).

2.6. Statistical Analysis

All statistical analyses were performed using the Statistica 12 (StatSoft, Tulsa, OK, USA) and GraphPad Prism version 8.0.0 for Windows (San Diego, CA, USA) software. The normality of quantitative variables was tested with the Shapiro–Wilk W test. Differences in characteristics between the genotypes were tested with the parametric ANOVA test or the non-parametric Kruskal–Wallis test, as appropriate. A comparison between the results at diagnosis and after 12 months of GFD was analyzed using Student's t-test for dependent variables or Wilcoxon signed-rank tests, as appropriate, according to the normality, considering the significance at three levels: (*) = p-value < 0.05; (**) = p-value < 0.01; (***) = p-value < 0.001. Correlations between parameters were analyzed using a Spearman's rank correlation coefficient test.

3. Results

During the study period, 128 children with CD were enrolled and the results of 106 children, including 62 girls (58.5%) and 44 boys (41.5%), were used for the analysis. Twenty-two participants have been removed because of the lack of blood test results in the enrollment or follow-up visit. All patients were treated for at least 12 months with GFD and vitamin D3 supplementation. There was no statistically significant difference in GFD duration between the anemic and non-anemic groups (mean, 29.9 and 30.0 months, respectively). Clinical characteristics and the rs855791 polymorphism status of CD patients with and without IDA at diagnosis are shown in Table 1.

Table 1. Characteristics of CD patients with and without IDA at diagnosis and *TMPRSS6* gene polymorphism status.

Parameter	CD with IDA	CD without IDA
Number	25 23.6%	81 76.4%
Gender		
Girls *n.*	20 80.0%	42 51.9%
Boys *n.*	5 20.0%	39 48.1%
Age [years]	8.06 ± 4.89	8.04 ± 4.12
Height [m]	1.23 ± 0.29	1.28 ± 0.24
Weight [kg]	28.66 ± 19.43	28.10 ± 15.38
BMI [kg/m^2]	16.99 ± 3.97	16.18 ± 2.82
Main complaints		
Gastrointestinal *n.* *	15 60.0%	51 62.9%
Classical	11 73.3%	29 56.9%
Non-Classical	4 26.7%	22 43.1%
Extraintestinal *n.* **	25/3 [1] 100.0%/12.0%	62 76.5%
Diabetes type 1 *n.*	0.0 0.0%	17 20.9%
Polymorphism of rs855791		
TT homozygote *n.*	3 12.0%	7 8.6%
CT heterozygote *n.*	14 56.0%	42 51.9%
CC homozygote *n.*	8 32.0%	32 39.5%
Allele frequency		
Allele T *n.*	20 40.0%	56 34.6%
Allele C *n.*	30 60.0%	106 65.4%

Abbreviations: BMI, body mass index; *n.* number; Values are presented as mean ± standard deviation (SD), *, e.g. abdominal pain, diarrhea, constipation, weight loss, **, e.g. weight and height deficiency, osteoporosis, anemia, [1] Three patients presented anemia as the only symptom.

At the time of diagnosis, sixty-six patients (62%) presented gastrointestinal symptoms (e.g., abdominal pain, diarrhea and/or constipation), and 22 (21%) had weight and/or height deficiency. In 17 cases (16%), CD co-occurred with type 1 diabetes. IDA was present at diagnosis in 25 patients (23.6%). Of those, ten patients had mild anemia, fifteen had moderate anemia, and three had severe anemia, according to WHO classification (Supplementary Table S1).

There was a tendency toward a higher proportion of the T allele in patients with IDA at diagnosis than in those without IDA (40% vs 34.6%, respectively), but this difference did not reach statistical significance (Table 1, Figure 1).

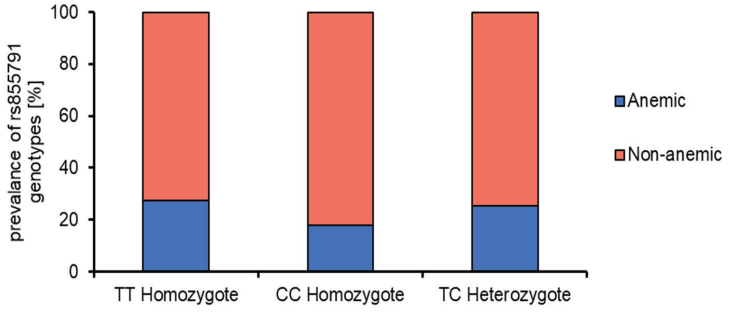


Figure 1. Proportion of the frequency between the polymorphism varieties in anemic and non-anemic children with celiac disease.

Hematological and biochemical parameters at diagnosis in patients stratified according to the rs855791 polymorphism status are presented in Table 2. No statistically significant differences in blood parameters at diagnosis were observed between the three rs855791 genotypes (i.e. homozygous TT, heterozygous CT, and homozygous CC), although there was a tendency toward lower HGB levels in the TT genotype and higher HGB levels in the CC genotype (median HGB = 11.95 g/dL, 12.60 g/dL, and 13.10 g/dL for homozygous TT, heterozygous CT, and homozygous CC, respectively). When each genotype group was further subdivided into children with or without anemia, significant differences between anemic and non-anemic children were observed in terms of blood parameters: HGB, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), as expected, but no significant differences in those parameters were found between the genotypes. However, iron serum levels were significantly lower in anemic children with the CC genotype (median, 23 μg/dL) than in anemic children with the other two genotypes (median, 56 μg/dL for the TT genotype and 36 μg/dL for the CT genotype).

Hematological and biochemical parameters after 12 months of GFD in patients stratified according to the rs855791 polymorphism status are shown in Table 3. A significant reduction in TTG levels as compared to levels at diagnosis supported good dietary adherence in all subgroups. In general, twenty-two of the 25 patients (88%) who initially showed IDA had normalized their HGB levels after 12 months of GFD. In three children (12%), anemia persisted despite 12 months GFD, although its severity decreased. The rs855791genotypes of these three patients included: one patient with genotype TT and two with CT. Finally, two further children who did not have IDA at diagnosis developed anemia during 12 months of GFD (these cases were not considered as "persistent IDA" in the study).

Table 2. Hematological characteristics in CD patients at the time of diagnosis according to the genotype. Data are expressed as a median (P25–P75).

Parameter	TT Homozygote		CC Homozygote		CT Heterozygote	
N	10		40		56	
RBC [$\times 10^6$/mm^3]	4.75 (4.53–4.95) [a 1]		4.70 (4.43–5.08) [a]		4.70 (4.40–4.93) [a]	
HGB [g/dL]	11.95 (10.78–13.05) [a]		13.10 (11.68–13.63) [a]		12.60 (11.30–13.30) [a]	
HCT [%]	37.80 (34.40–40.40) [a]		38.80 (35.05–40.80) [a]		37.30 (34.35–40.10) [a]	
MCV [μm^3]	79.00 (75.00–82.25) [a]		81.00 (77.50–86.00) [a]		82.00 (79.00–86.00) [a]	
MCH [pg]	26.05 (23.13–26.40) [a]		27.40 (25.85–28.70) [a]		27.30 (26.15–28.45) [a]	
MCHC [g/dL]	32.50 (31.90–32.80) [a]		32.90 (32.65–33.80) [a]		33.10 (32.30–33.80) [a]	
RDW [%]	12.60 (12.50–14.30) [a]		13.40 (12.60–15.48) [a]		13.20 (12.60–14.20) [a]	
Fe [μg/dL]	59.00 (56.00–84.00) [a]		41.00 (26.50–107.50) [a]		54.00 (41.25–86.00) [a]	
TTG [U/mL]	289.5 (112.8–398.5) [a]		201.0 (49.40–801.0) [a]		191.0 (41.10–539.0) [a]	
	Anemic	Non-anemic	Anemic	Non-anemic	Anemic	Non-anemic
N	3	7	8	32	14	42
RBC [$\times$ 10^6/mm^3]	4.70 (4.70–4.70) [a]	4.80 (4.45–5.00) [a]	4.80 (4.45–4.95) [a]	4.70 (4.43–5.08) [a]	4.85 (4.45–5.00) [a]	4.65 (4.40–4.90) [a]
HGB [g/dL]	10.10 (9.25–10.35) [a]	12.30 (11.95–13.55) [b]	10.20 (9.28–10.48) [a]	13.30 (12.70–13.75) [b]	10.20 (9.60–10.80) [a]	13.05 (12.30–13.70) [b]
HCT [%]	33.90 (33.80–34.00) [a,b]	38.40 (37.15–40.50) [b]	32.75 (30.43–33.43) [a]	40.00 (37.65–41.00) [b]	32.75 (32.60–33.55) [a]	38.50 (37.00–41.20) [b]
MCV [μm^3]	71.00 (61.00–72.50) [a]	80.00 (79.00–84.00) [b]	67.00 (62.75–72.00) [a]	82.00 (80.00–87.00) [b]	69.50 (66.25–77.00) [a]	83.50 (81.00–86.75) [b]
MCH [pg]	21.80 (18.55–22.10) [a]	26.40 (26.05–27.00) [b]	20.40 (19.68–22.38) [a]	27.80 (26.90–28.80) [b]	21.75 (20.10–23.30) [a]	27.80 (27.10–28.70) [b]
MCHC [g/dL]	30.55 (30.08–31.03) [a]	32.80 (32.25–33.20) [a]	31.30 (30.20–31.65) [a]	33.20 (32.80–33.90) [b]	30.95 (30.10–31.83) [a]	33.30 (32.70–34.00) [b]
RDW [%]	17.95 (17.83–18.08) [b]	12.50 (12.40–12.90) [a,b]	16.60 (15.98–16.85) [b]	13.10 (12.53–13.65) [a,b]	15.30 (13.78–15.80) [b]	13.00 (12.40–13.55) [a]
Fe [μg/dL]	56.00 (40.00–75.50) [b]	71.50 (65.25–77.75) [b]	23.00 (20.50–27.75) [a]	105.00 (52.00–114.00) [b]	36.00 (15.75–75.00) [b]	56.50 (51.75–91.25) [b]
TTG [U/mL]	405.6 (331.8–479.3) [a]	321.0 (135.9–396.0) [a]	301.0 (143.7–801.0) [a]	194.5 (34.50–801.0) [a]	240.5 (104.5–456.5) [a]	161.0 (39.15–733.5) [a]

[1] Comparison between the groups using Kruskal–Wallis analysis of variance. Values with the same letter in each row do not differ significantly ($p < 0.05$). RBC—red blood cells; HGB—hemoglobin; HCT—hematocrit; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; RDW—red cell distribution width; Fe—iron; TTG—auto-antibodies directed against tissue transglutaminase 2.

Table 3. Hematological characteristics in children with CD after 12 months of GFD, according to the genotype. Data are expressed as a median (P25–P75).

Parameter	TT Homozygote		CC Homozygote		CT Heterozygote	
N	10		40		56	
RBC [$\times 10^6$/mm^3]	4.65 (4.60–4.93) $^{a\,1}$		4.70 (4.50–5.00) ^{a}		4.60 (4.35–4.80) ^{a}	
HGB [g/dL]	12.80 (11.90–13.20) ^{a,b}		13.30 (12.63–14.13) ^{b} **		12.60 (12.00–13.45) ^{a} **	
HCT [%]	38.60 (37.20–39.53) ^{a,b}		40.15 (37.88–41.35) ^{b} **		37.80 (36.40–40.55) ^{a}	
MCV [μm^3]	82.00 (75.00–84.00) ^{a} *		84.00 (80.75–87.00) ^{a} **		84.00 (80.75–86.25) ^{a}	
MCH [pg]	27.00 (26.10–27.60) ^{a}		27.85 (27.15–29.38) ^{a} ***		28.30 (26.85–29.07) ^{a} **	
MCHC [g/dL]	33.40 (32.53–34.00) ^{a} *		33.35 (32.60–33.75) ^{a}		33.40 (33.00–33.60) ^{a} **	
RDW [%]	13.20 (12.30–14.80) ^{a}		12.90 (12.38–13.65) ^{a}		12.90 (12.55–13.75) ^{a}	
Fe [μg/dL]	75.00 (57.75–107.8) ^{a}		98.50 (63.00–118.5) ^{a}		69.00 (45.25–96.75) ^{a}	
Ferritin [ng/mL]	16.60 (13.00–33.40) ^{a}		18.00 (8.10–29.25) ^{a}		17.95 (10.23–38.70) ^{a}	
TTG [U/mL]	3.00 (0.75–33.00) ^{a} **		5.60 (1.33–14.70) ^{a} **		4.75 (1.48–18.28) ^{a} ***	
Hepcidin [ng/dL]	219.7 (210.1–275.5) ^{a}		219.6 (208.7–293.5) ^{a}		234.6 (208.4–285.7) ^{a}	
	Anemic ^{2}	Non-anemic ^{2}	Anemic	Non-anemic	Anemic	Non-anemic
N	3	7	8	32	14	42
RBC [$\times 10^6$/mm^3]	4.70 (4.60–4.80) ^{a}	4.60 (4.605–5.00) ^{a}	4.70 (4.58–5.10) ^{a}	4.80 (4.58–5.03) ^{a}	4.50 (4.28–4.80 ^{a}	4.60 (4.40–4.90) ^{a}
HGB [g/dL]	12.40 (11.00–12.70) ^{a,b} **	12.80 (11.90–13.40) ^{a,b}	12.50 (12.08–13.20) ^{a,b} **	13.65 (12.98–14.30) ^{b} **	12.05 (11.78–12.73) ^{a} ***	13.10 (12.45–13.60) ^{a,b}
HCT [%]	39.35 (38.80–39.90) ^{a,b} *	38.40 (36.00–39.40) ^{a,b}	37.95 (37.08–40.13) ^{a,b} ***	41.20 (39.20–42.43) ^{b} *	36.55 (35.63–38.08) ^{a} ***	38.70 (37.00–41.10) ^{a,b}
MCV [μm^3]	83.00 (70.00–84.00) ^{a} *	82.00 (79.00–84.00) ^{a}	79.00 (75.00–83.00) ^{a} *	84.00 (82.00–87.25) ^{a} *	81.50 (78.00–85.00) ^{a} **	85.00 (81.00–87.00) ^{a}
MCH [pg]	25.80 (23.00–27.60) ^{a}	27.20 (26.40–28.30) ^{a}	26.25 (24.88–27.55) ^{a} *	28.00 (27.40–29.63) ^{a} **	26.92 (25.50–28.38) ^{a} **	28.45 (27.48–29.18) ^{a}
MCHC [g/dL]	31.90 (31.10–32.70) ^{a}	33.50 (33.00–34.00) ^{a} *	33.00 (32.38–33.63) ^{a}	33.50 (32.75–34.13) ^{a}	33.20 (32.98–33.35) ^{a} **	33.40 (33.20–33.60) ^{a}
RDW [%]	13.65 (11.90–15.40) ^{a}	13.20 (12.60–14.20) ^{a}	13.30 (12.28–14.83) ^{a} *	12.85 (12.40–13.65) ^{a} *	13.50 (12.68–14.43) ^{a}	12.90 (12.40–13.50) ^{a}
Fe [μg/dL]	68.00 (65.00–71.00) ^{a}	106.0 (36.00–113.0) ^{a}	64.00 (55.50–71.00) ^{a}	105.0 (58.00–123.0) ^{a}	63.00 (36.75–77.50) ^{a}	69.50 (53.00–103.3) ^{a}
Ferritin [ng/mL]	13.60 (10.90–16.60) ^{a}	21.20 (13.00–33.40) ^{a}	17.25 (16.50–18.00) ^{a}	23.75 (7.95–30.18) ^{a}	29.70 (2.60–39.80) ^{a}	17.50 (12.00–37.90) ^{a}
TTG [U/mL]	46.50 (9.30–83.70) ^{a} *	2.90 (0.8–16.10) ^{a} *	11.85 (1.98–14.70) ^{a} *	3.10 (1.0–11.90) ^{a} ***	5.20 (1.05–33.05) ^{a} **	3.60 (1.57–19.15) ^{a} ***

[1] Comparison between the groups using Kruskal–Wallis analysis of variance. Values with the same letter in each row do not differ significantly ($p < 0.05$). [2] Classified at diagnosis. *, **, *** Significant differences comparing the results in the moment of diagnosis and after 12 months analyzed using Student's t-test for dependent variables or Wilcoxon signed-rank tests, as appropriate according to the normality. (*) = p-value < 0.05; (**) = p-value < 0.01; (***) = p-value < 0.001. RBC—red blood cells; HGB—hemoglobin; HCT—hematocrit; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; RDW—red cell distribution width; Fe—iron; TTG—auto-antibodies directed against tissue transglutaminase 2.

Hematological parameters in all children with anemia at diagnosis significantly improved on GFD. In non-anemic children with a homozygous CC genotype, these parameters also increased after GFD, which was not the case for the other two genotypes.

A correlation analyses between (Spearman's rank correlation coefficient test) at diagnosis (Figure 2) and after ≥12 months of GFD were performed to evaluate the association between the parameters (Figure 3). The rs855791 polymorphism status was not significantly correlated with any parameters when all patients were analyzed together (not shown). However, several correlations were observed when the anemic and non-anemic groups were analyzed separately (Figures 2 and 3).

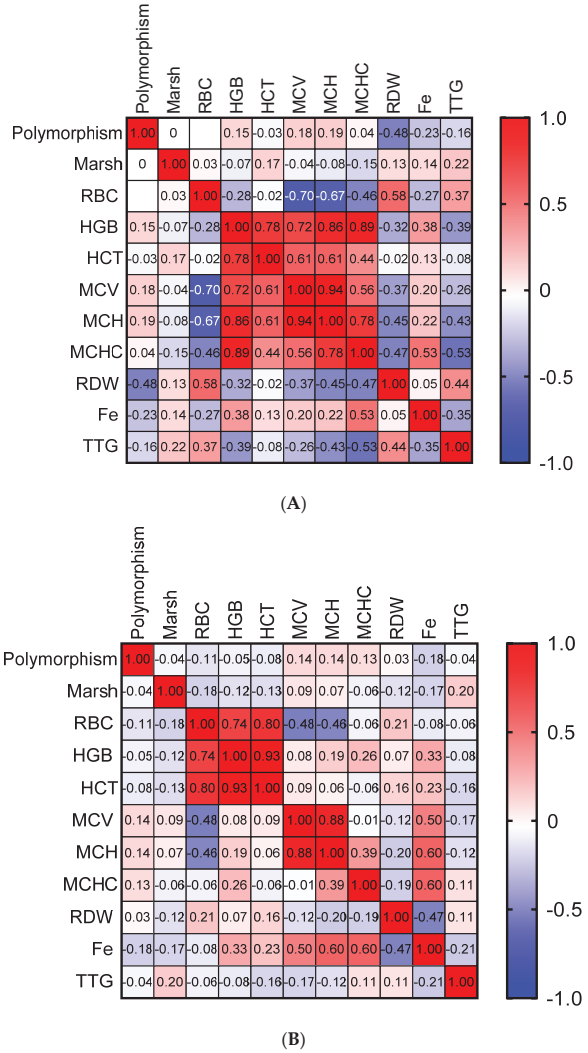
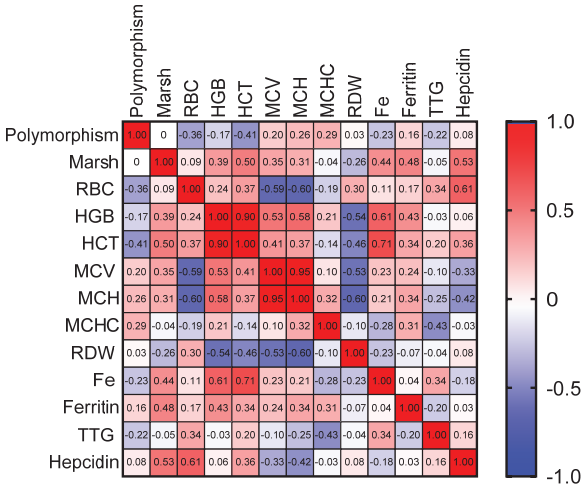
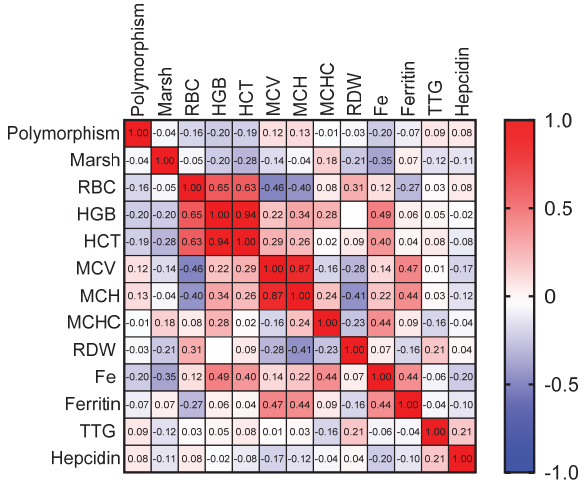


Figure 2. Heatmap representation of the correlations between anemia-related parameters at CD diagnosis in children with (**A**) and without (**B**) anemia. Marsh—Marsh classification; RBC—red blood cells; HGB—hemoglobin; HCT—hematocrit; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; RDW—red cell distribution width; Fe—iron; TTG—auto-antibodies directed against tissue transglutaminase 2.



(**A**)



(**B**)

Figure 3. Heatmap representation of the correlations between anemia-related parameters after 12 months of GFD in children with (**A**) and without (**B**) initial anemia. Marsh—Marsh classification; RBC—red blood cells; HGB—hemoglobin; HCT—hematocrit; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; RDW—red cell distribution width; Fe—iron; TTG—auto-antibodies directed against tissue transglutaminase 2.

At diagnosis, in the anemic group (Figure 2A), a moderate negative correlation between the CT genotype and positive between TT and red cell distribution width (RDW) was observed (r = −0.48, p = 0.018), which was not seen in non-anemic children (Figure 2B). As expected, significant positive correlations were also observed between HGB, HCT, MCV, mean corpuscular hemoglobin concentration (MCHC) and MCH (in particular in the anemic group), and between iron concentration, HGB and MCHC (in both groups). Conversely, a negative correlation between red blood cells (RBC), MCV, and MCH was observed in both groups.

After 12 months of GFD (Figure 3A), in the anemic group, a correlation between the TT genotype and HCT was close to the significance threshold (r = −0.4, $p = 0.06$). A statistically significant negative correlation between hepcidin and MCV and MCH was also noted in this group (MCV: r = 0.53, $p = 0.02$; MCH: r = 0.61, $p = 0.01$). In the non-anemic group, no significant correlations between polymorphism status and other parameters (or between hepcidin levels and other parameters) were observed after 12-months of GFD (Figure 3B). Figure 4 shows a graphical representation of the range of changes in iron metabolism parameters and in TTG levels between diagnosis and the follow-up visit after 12 months of GFD in children with CD and initial anemia, stratified by genotype, calculated as an average change (delta) for the individual patients. The trend of changes for most parameters was similar for all genotypes, except for RBC, in which an increase was noted in the CC genotype, while in homozygous TT and heterozygous CT, a decrease was observed.

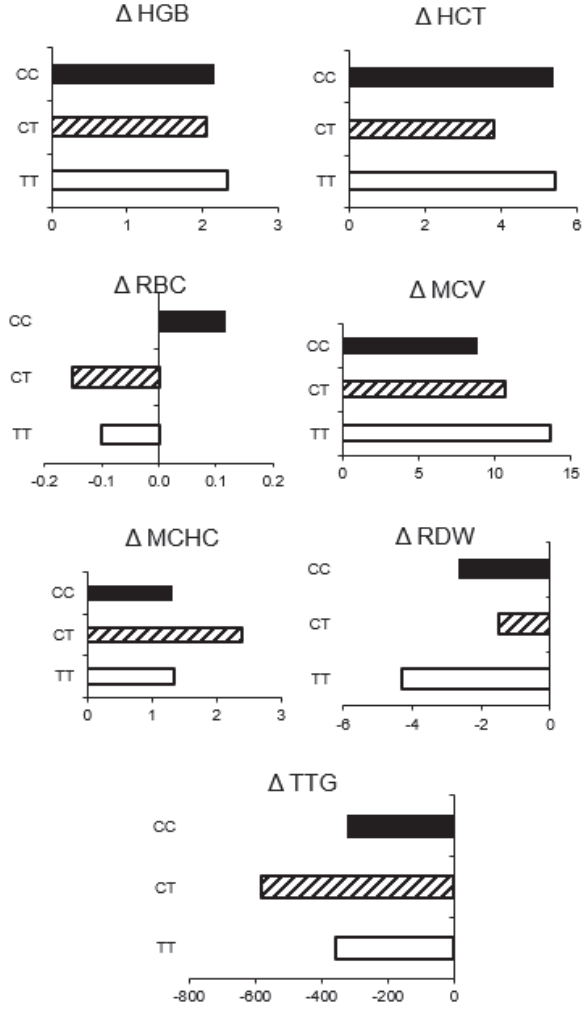


Figure 4. Changes in morphometric parameters and TTG levels between values at diagnosis and after 12 months of GFD in children with CD and initial anemia ($n = 25$), according to the genotype. The results are expressed as the mean of delta values.

4. Discussion

Celiac disease (CD) is a lifelong disorder characterized by a heterogeneous clinical picture with both gastrointestinal and extra-intestinal symptoms and can affect individuals of any age [17]. In recent decades, a shift in terms of frequency of diagnosis from the classical form of CD, characterized by malabsorption, to non-classical forms has been reported. The increasing number of recognized atypical and asymptomatic cases could be explained at least in part by screening at-risk groups and by higher awareness of various clinical presentations of CD [18]. Anemia is one of the most common extra-intestinal symptoms of CD. It can co-occur with other symptoms or even be the only manifestation of the disease [19]. The reported prevalence of iron deficiency and IDA in CD varies between studies, depending on study design, population and patient age [20].

In the current study, IDA at diagnosis was present in 23.6% of cases, which is a prevalence similar to what has been observed in other pediatric studies. In a study in Finland by Nurminen et al. [21], anemia was the second most common extra-intestinal symptom after poor growth in children with CD and it was present in 18% of cases. In another study of preschool children, iron deficiency and growth retardation were the second most common symptom of CD, after abdominal pain [22]. In a recent study of 653 children in Central Europe, the reported prevalence of IDA in participating countries ranged from 4.3% to 24.9% [18]. Nevertheless, some other studies in children reported lower (9%) [23] or higher (84%) [24] frequencies.

Enteropathy is thought to be the main factor responsible for diminished iron uptake in the intestine in CD. The only treatment for CD is a GFD, which results in normalization of the intestinal mucosa and clinical improvement of gastrointestinal and extra-intestinal symptoms [6,25]. However, in a subset of patients, IDA persists despite adherence to GFD [7]. The reported prevalence of persistent IDA in adults with CD ranges from 5% to as high as 45.5% [8,13,26].

Conversely, in the current study, only three of the 25 pediatric patients with CD who had IDA at diagnosis showed persistent anemia after 12 months of GFD, accounting for 2.8% of the entire cohort (n = 106). Overall, the data suggest that persistent IDA is not a common complication of CD in pediatric patients. This is in line with a study by Wessels et al. [23], who reported persistent anemia in only 1% of children with CD, although a higher rate of persistent IDA (16%) was observed by other authors [22,26].

One potential explanation for this difference between pediatric and adult patients is that children are usually diagnosed earlier in the disease. As such, the delay in diagnosis in adults can result in more advanced villous atrophy at diagnosis, more profound iron deficiency and, consequently, more severe anemia [7]. Thus, even prolonged adherence to a GFD without iron supplementation may not be enough to normalize the anemia in adult patients. While IDA in CD has been linked to histopathological changes in the small intestine resulting in iron malabsorption [21], additional genetic factors may contribute to anemia at diagnosis and/or persistent anemia in CD patients. In 2011, two genome-wide association (GWAS) studies identified an association between the rs855791 non-synonymous coding SNP located in the *TMPRSS6* locus and hemoglobin levels and iron status in large populations [12,27]. The *TMPRSS6* protein (Matriptase-2) is a liver-specific type II plasma membrane serine protease, which can negatively regulate hepcidin production by modulating downstream signaling pathways involved in hepcidin gene expression [11]. The expression of hepcidin is induced by high iron stores or inflammation. In turn, hepcidin decreases iron levels by blocking intestinal iron absorption at the basolateral membrane of enterocytes and inhibiting iron release from hepatocytes and macrophages [10]. Thus, the repression of hepcidin by *TMPRSS6* could facilitate iron absorption and potentially promote erythropoiesis. Conversely, *TMPRSS6* loss of function may result in unregulated hepcidin synthesis, reduced iron absorption, and iron-deficiency anemia [28].

The rs85579 non-synonymous coding SNP causes an alanine-to-valine change (p.A736V) in the catalytic domain of the *TMPRSS6* protein (Figure 5). The "T allele" (also referred to as the "A allele" in some studies if using the genomic DNA annotation), results in a valine at the

736aa position, and the "C allele" (also referred to as the "G allele" using the genomic DNA annotation) results in an alanine at the 736aa position [12,27]. The two above-cited GWAS studies found the T allele to be associated with a lower blood HGB concentration per copy of the T allele [12] and lower serum iron levels, transferrin saturation, and MCV [29]. These results were subsequently supported by other studies, whereby the T allele was found to be associated with lower HGB and serum iron levels in Chinese women [30], and the C allele was associated with lower hepcidin and higher iron parameters in the genetically isolated "Val Borbera" population in Italy [31]. *In vitro* experiments provided a functional rationale by showing that the Ala736 form of the *TMPRSS6* protein inhibits hepcidin expression more effectively than the Val736 form. According to available data, the prevalence of rs855791 alleles is heterogenous in different populations. The T allele is the less frequent allele, with a frequency of 0.50 in the white population [29]. In a study by Chambers et al., the TT genotype was presented in 19% of individuals of European ancestry and in 27% of individuals of Indian Asia ancestry [12].

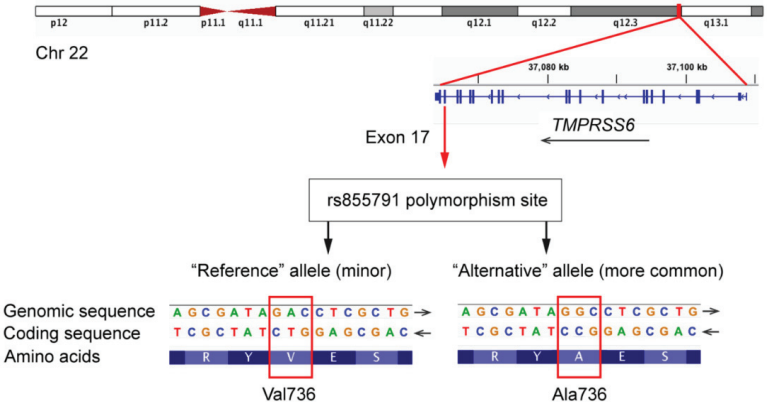


Figure 5. Schematic representation of the rs855791 polymorphism.

The current study is the first to report the prevalence of the rs855791 polymorphism in children with CD. Of note, although the T allele is the "reference" allele in the hg38 and hg19 human reference genomes (Figure 5), it was found to be less frequent than the C allele: the overall allele frequencies across the study cohort (n = 106) were 35.8% for the T allele and 64.2% for the C allele, respectively. This is in agreement with the numbers reported in previous studies [13,14]. Thus, the T allele appears to be a "minor reference allele", which is a known phenomenon in genomic data analysis [32].

The literature contains only two studies addressing the link between rs855791 SNP and anemia in patients with CD. Elli et al. [14] evaluated the association between the rs855791 variant and persistent IDA in 38 adult patients with CD treated by GFD. They found the prevalence of the rs855791 SNP to be significantly higher in adult patients with CD than in the control group of non-CD adults (87% vs. 62%, respectively). Persistent anemia was seen in 16 patients (42%). There was no significant difference in the prevalence of the rs855791 polymorphism between CD adults with persistent IDA and those without IDA. Nevertheless, associations between iron status, hemoglobin and hepcidin levels and the polymorphism status were not evaluated. De Falco et al. [13] also studied the rs855791 polymorphism in adult patients with CD and found that the T allele was significantly more frequent among patients with persistent IDA, and it was associated with a lower response of hematological and iron parameters to oral iron supplementation in patients with persistent IDA.

Contrary to these results, the current study did not find a significant association between the T allele and the persistence of anemia in children with CD who presented IDA

at diagnosis (n = 25). This could be due to the low number of patients with persistent IDA in the current study (3%), as compared to 45.4% of adult anemic patients in the study by De Falco et al. [13]. A tendency was observed toward a higher prevalence of the T allele in CD children with IDA at diagnosis than in those without IDA (40% vs. 34.6%, respectively) and toward lower HGB levels in children with the TT genotype. However, these differences did not reach statistical significance, which was possibly due to the small sample size, and they warrant further investigation in larger studies.

Although genetic factors in anemia in CD have gained much scientific attention, data remain very limited in pediatric patients. One other study found the homozygous intronic IVS4 + 44C > A polymorphism in the DMT1 gene associated with a four-fold risk of developing anemia in children with CD, regardless of the degree of villous atrophy [33]. Additional studies in large patient cohorts are needed to confirm these findings.

As far as it is known, this is the first study to analyze the associations between a *TMPRSS6* polymorphism and anemia in the pediatric population with CD. The main strength of this study is the homogenous nature of the cohort in terms of diagnostic robustness, as the diagnosis was confirmed by histology and immunological markers in all cases. Another important point is the wide range of clinically relevant parameters reported herein, including inflammatory markers and hepcidin levels analyzed in the current study. The principal limitations include the relatively small group of patients, the absence of a control group and the fact that data at diagnosis were collected retrospectively (as a consequence, information on all iron metabolism parameters and symptoms onset to diagnosis was not always available).

5. Conclusions

The current study of 106 children with CD suggests that persistent iron-deficiency anemia (IDA) is not a common finding in pediatric patients with CD. IDA was present at diagnosis in 23.6% of children in the study and normalized on a GFD without iron supplementation in most (88%) patients. Significant differences in the prevalence of the *TMPRSS6* rs855791 polymorphism were not observed between CD children with or without anemia, although a tendency toward a higher proportion of the T allele in patients with IDA was noted. Contrary to what has been reported in adults, the rs855791 polymorphism was not found to be a predicting factor of persistent anemia in children with CD.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/nu13082782/s1, Table S1. Hemoglobin levels to diagnose anemia [g/dL] (WHO recommendation).

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

Data Availability Statement: Data are contained within the article or Supplementary Material.

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nutrients

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Review

Nutritional Imbalances in Adult Celiac Patients Following a Gluten-Free Diet

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Abstract: Celiac disease (CD) is a chronic autoimmune disorder of the small intestine, whose only effective treatment is a gluten-free diet (GFD). It is characterized by the atrophy of the intestinal villi that leads to altered nutrient absorption. This study describes the nutritional imbalances which may be found in adults with CD following a GFD. During the first year of treatment, deficiencies will overcome as the intestinal mucosa recovers. Thus, biochemical data will show this progression, together with the decrease in symptoms. In contrast, in the long term, when a strict GFD is followed and mucosal recovery is achieved, analyzing nutrient intake makes more sense. Macronutrient consumption is characterized by its low complex carbohydrate and fiber intakes, and high fat (especially SFA) and sugar intakes. This profile has been related to the consumption of GFP and their nutritional composition, in addition to unbalanced dietary habits. The most notable deficiencies in micronutrients are usually those of iron, calcium and magnesium and vitamin D, E and some of group B. It is necessary to follow up patients with CD and to promote nutritional education among them, since it could help not only to achieve a gluten free but also a balanced diet.

Keywords: celiac disease; gluten-related disorders; gluten free diet; gluten-free products; nutritional deficiency; nutritional imbalance

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1. Introduction

The incidence of celiac disease (CD) is rising all over the world. This fact is not only due to environmental factors that may decrease tolerance to gluten in diet, but also because there has been an improvement in its diagnosis [1,2]. Prevalence varies with gender, location and age and is more common in women. It has been estimated that in Western countries it is approximately 1% of the population, with a global rate of 0.7–1.4%, detected by biopsy or serologic tests [3,4]. Nevertheless, this disease is sometimes undetected, with a 1/3 to 1/5 ratio between diagnosed and undiagnosed [5]. Although the etiology of CD is not very clear, apart from genetic factors, some environmental factors can be mentioned, such as the consumption of gluten-containing cereals, infections in the first year of life or low economic status along with unsanitary environments [6].

CD is a chronic autoimmune disorder of the small intestine, which is characterized by progressive atrophy of the intestinal villi after the consumption of gluten [2,7]. The main pathological characteristics of the intestinal mucosa in celiac patients are the presence of diverse degrees of atrophy and the existence of intestinal inflammation by lymphocytes infiltrates. The degree of the damage varies between subjects, and thus patients can show a wide range of symptoms [4,7]. Although asymptomatic patients can be found,

the classical form of CD presents gastrointestinal (diarrhea, steatorrhea, weight loss, etc.) and also extraintestinal symptoms like fatigue, osteopenia, iron deficiency, anemia or neurological/psychological disorders [8,9]. The immune response is triggered by dietary gluten, a protein complex present in wheat, rye and barley [4,7]. Prolamin peptides, which arise from an incomplete digestion of gluten, activate the innate and adaptive immune responses [10,11]. As a result, intestinal villi inflammation occurs and nutrients absorption is altered, leading to many deficiencies [4,7,12]. It has been seen that nutritional status of patients with CD depends on the duration of the disease without treatment, the extension and location of the lesions, and the malabsorption degree of several nutrients [13].

Nowadays, following a lifelong gluten-free diet (GFD) is the only effective treatment for this disease. Strict dietary adherence is crucial to improve duodenal mucosa and resolve symptoms [14,15]. The recovery of the intestinal mucosa takes longer in adults than in children, thus it is easier to achieve a complete recovery in children [8]. Nevertheless, even though a strict GFD is followed, the complete avoidance of gluten in the diet seems to be very difficult due to gluten cross-contamination and thus, intestinal atrophy is retained [6,8,16]. In fact, apart from the well-known wheat-based foods such as breads, pasta, pastries and other processed foods as snacks, gluten can be also found as a thickener for sauces or even as a stabilizing or flavoring additive [6]. Consequently, involuntary transgressions of gluten and consequent nutritional deficiencies among people with CD following theoretically a strict GFD is habitual [4,8].

Finally, eating habits of this collective obviously play an important role in their nutritional status. GFD should be not only gluten free but also balanced, covering all the energy and nutrition requirements. Several studies have found unbalanced profile of GFDs, characterized by low cereals, fruits and vegetables intakes and excessive of meat and derivatives [17–19]. Moreover, it has been reported that children and adolescents consume high amounts of specific gluten-free products (GFP). Taking into account that that these products have shown to be poorer nutritionally than their gluten-containing homologues [20,21], observed imbalances in nutrient and energy intakes could be explained, concretely increased fat consumption, which could displace fiber and complex carbohydrate intakes [17,22]. These habits can lead to many micronutrient deficiencies as well [4], less clear in adults, and thus, which need to be more deeply analyzed.

Taking all of the above into account, the need to assess the nutritional status of the celiac population is evident. Their nutritional imbalances should be detailed in depth in order to establish appropriate dietary guidelines for their correction, and so to promote their health and quality of life.

This nutritional unbalance of the GFD was also revised by Vici et al. in 2016 [17]. The present study wants to give an updated vision about this issue with results from studies published after 2015. Moreover, other important differences between these two reviews need to be pointed out. First, whereas Vici et al. described the nutritional deficiencies among celiac children on a GFD, the present review studies in children (as well as those in the general population) have been used for comparison of results obtained in celiac adults. Secondly, the present study shows results related to the dietary adherence of people with celiac disease. Finally, the present review provides the most important dietary guidelines to achieve the nutritional balance with a GFD, so that it could represent a useful tool of nutritional education for dietitians and nutritionists working in the field of celiac disease.

The aim of this review is to describe the nutritional imbalances, deficiencies and excesses, of celiac adults who follow a GFD, at the beginning of treatment and once established, in the long term, and to compare variations of these imbalances between men and women. The dietary profile between celiac patients and the general population is also compared in order to underline the added difficulty of eliminating gluten from the diet and its impact on the observed deficiencies. Taking all into account, some dietary recommendations are suggested.

2. Materials and Methods

PUBMED database was searched for articles published since 2000, and using different combinations of the following terms: celiac disease and gluten-free diet and dietary deficiencies, nutritional deficiencies, nutrient intake, micronutrient, macronutrient, vitamin, mineral, or fiber intake.

Inclusion criteria were as follows: observational studies, case-control studies, cohort studies and systematic reviews were included. Only studies with participants following a GFD and with nutritional assessment in terms of macro- and/or micronutrients, fiber, and/or biochemical data from participants were included. Papers with information on at least one nutrient were included. The selection process of articles regarding the nutritional deficiencies of adult celiac people following a GFD is shown in Supplementary Figure S1.

Nevertheless, along the text some other important studies have been mentioned, in order to clarify, explain or justify some of the observations extracted from selected articles, based on authors' experience (articles concerning deficiencies but in celiac children, articles related to the nutritional composition of GFP, to the dietary adherence, etc.).

3. GFD in CD Treatment

3.1. Newly Diagnosed Patients: Recovery of Previous Nutritional Deficiencies

As previously mentioned, it is well known that untreated celiac patients, as well as those newly diagnosed, present many serious nutritional deficiencies. GFD introduction ameliorates these shortages that are related to the progressive recovery of the intestinal mucosa, which, in turn, depends on the person, the duration of the disease untreated, the severity of mucosal lesions, etc. [23–25]. Table 1 presents data from the selected studies on the nutritional status of people with celiac disease.

Once GFD is established, nutritional deficiencies are not reversed quickly, they improve gradually and sometimes do not even normalize. Regarding iron deficiency, Annibale et al. [24] observed that during the first year of GFD, anemia improved notably in most cases (but not in all) due to the recovery of the intestinal mucosa. Recovery in women appeared to be slower than in men because of menstrual blood loss [26]. Likewise, other authors observed impairment of several nutritional indices, like hemoglobin and folate, after one year of GFD [27]. Thus, in newly diagnosed cases, apart from assessing GFD, it might be interesting to treat deficiencies with supplements, such as iron, especially in women. Afterwards, when normal values are reached and mucosal recovery is supposed to be achieved, a GFD on its own might be enough.

Table 1. Nutritional status of people with celiac disease following the first year GFD: biochemical data and anthropometric parameters.

Author	Sample Size (n) GFD Duration	Type of Study	Country	Biochemical Data	Anthropometric Parameters
Zanchetta et al. (2017) [28]	−n = 26 (women) −1 year GFD	Observational, longitudinal cohort study	Argentina	Low vitamin D Normal Ca, Hb, PTH	Low bone microarchitectural parameters
Annibale et al. (2001) [24]	−n = 20 −GFD: 6, 12 and 24 months		Italy	Low Fe, low Ferr, low Hb Anaemia Normal Glu, TG, proteins, Alb	
Sategna-Guidetti et al. (2000) [27]	−n = 86 −1 year GFD		Italy	Low vitamin D Normal Ca, P, Alb, PA, Hb, Fe, Ferr, Trans, Fol	BMI = 20.85 kg/m^2

GFD: gluten-free diet. Ca: calcium. Hb: hemoglobin. PTH: parathyroid hormone. Fe: iron, Ferr: ferritin. Glu: glucose. TG: triglycerides P: phosphate. Alb: albumin. PA: pre-albumin. Trans: transferrin. Fol: folic acid. BMI: body mass index.

Similar results are found when analyzing vitamin D deficiency and bone structure parameters. Zanchetta et al. [28] and Sategna-Guidetti et al. [27] described that bone microarchitectural parameters and vitamin D levels improved in celiac adults in their first year on a GFD. Nevertheless, these parameters were still low, and also lower than in healthy controls, even after taking supplements. In fact, vitamin D levels did not reach 30 ng/mL, and it is important to note that PTH activity is decreased and not stabilized until this values of vitamin D are achieved [29]. These data agree with those presented in a study from Stenson et al. [30], where CD prevalence was much higher in people with osteoporosis and showing low vitamin D levels. Thus, to face vitamin D deficiencies in celiac patients during the first year on GDF it would be interesting to supplement vitamin D and recommend suitable sun exposure [31].

As mentioned, the recovery of nutritional deficiencies is related to the amelioration of the intestinal mucosa due to the removal of gluten from the diet. However, not all patients show mucosal recovery after one year of GFD [27]. Lanzini et al. (2009) suggested that a complete normalization of duodenal lesions is exceptionally rare in adult celiac patients [32]. Similarly, Tursi et al. (2006) observed that older adult patients (>30 years) show incomplete endoscopic and histological recovery even 24 months after starting a GFD [33]. Nevertheless, it has been proposed that a correct adherence to the GFD leads to an improvement of the intestinal mucosa and symptoms reduction in about 6–12 months [23,24]. These discrepancies could be due to differences in compliance with the GFD. On the one hand, some patients admit to take gluten containing foods from time to time [32]. On the other hand, although it is common to find celiac patients who claim to follow a completely strict GFD, the lack of mucosa recovery could indicate that they may involuntarily consume gluten. For this reason, analyzing dietary adherence together with the dietary habits of this collective is crucial.

3.2. Adherence to the GFD

It has been estimated that gluten transgression in celiac population is very frequent, between 36 and 55% [34]. It is noteworthy that Kurppa et al. claim that adherence to the diet can be achieved; in Finland they obtained 88–90% adherence values in adults due to the high prevalence and good knowledge of CD in the country [35]. Even so, some authors suggest that GFD adherence observed in studies may not be representative of total celiac population, since participants in clinical trials are probably more aware of following a correct GFD, and also because those that take part in studies are very strict [36].

Many factors have been associated with lower diet adherence or occasional gluten intakes, such as: young age at diagnosis, adolescence, local food culture, lower socioeconomic status, travelling and eating in restaurants, the absence of symptoms at present (asymptomatic patients may have higher occasional gluten consumption [6,35]) and low degree of knowledge or motivation of the patient [6].

Another factor that must be taken into account in celiac patients is the quality of life, since it has been seen that they tend to have a lower perception. After diagnosis, this usually improves with GFD treatment because, symptoms are reduced [37,38]. Even so, after about one year of treatment life quality remains lower, which can be explained by the restrictions and limitations of following this diet [6]. In addition, there are some patients who find so difficult to follow this type of restrictive diet that they tend to seek alternative therapies [39]. Serial endoscopies with collection of duodenal biopsies monitor the effectiveness of the GFD. To assess the compliance with the GFD several procedures are employed, alone or in combination, such as periodic visits to nutritionist, clinical follow up, the use of structured questionnaires, serological controls of specific antibodies and the determination of gluten peptides derived from gluten in feces and/or urine [40]. Appropriate detection of dietary transgression could help to predict indirectly that sufficient recovery of the intestinal mucosa is not achieved. Nevertheless, it must be pointed out that the intestinal mucosa structure recovery neither ensures a normal intestinal function at molecular level nor the expression of some genes necessary for the absorption of some micronutrients [41,42].

3.3. Nutritional Composition of the GFD

Studies on nutritional deficiencies in the first year of GFD show some possible deficiencies based on biochemical data, but only a few nutrients are described. Once the mucosa is recovered, it is assumed that a strict diet without gluten has been followed in a long term and thus, measuring nutrient intake in celiac people to assess their nutritional status, with supposedly no absorption problems, makes sense [43].

3.3.1. Macronutrient Intake

Macronutrient intake and distribution in GFD is presented in Table 2. Various imbalanced patterns are repeated across different studies, which have been carried out in people from several countries who followed GFD in different durations.

Table 2. Dietary profile of people with celiac disease following a GFD.

Author	Sample GFD Duration Adherence	Type of Study	Country	Macronutrient Intake	Micronutrient Intake
González et al. (2018) [18]	n = 42 men, 31.5 y ± 11.9 ≥1 year GFD ND	Observational, transversal cohort study	Spain	High fat, specially SFA High protein, Low CHO, Low fiber, High cholesterol.	Low vitamin D and E, folate, iodine, and magnesium.
Churruca et al. (2015) [19]	n = 54 women, 34 y ± 13 Median duration of GFD = 10 years ND	Observational, transversal cohort study	Spain	Low energy intake, Low CHO, Low fiber, High fat.	Low vitamin D and E, Folate, Calcium, Iron, Magnesium, Iodine, Potassium and Selenium.
Bascuñán et al. (2019) [25]	n = 46 (43 women), 41.1 y ± 10.1 ≥1 year GFD Strict adherence 100% of participants	Randomized double bind controlled study	Italy	High fat, low CHO.	Low vitamin D, vitamin E, folate, thiamine (B1), calcium, iron, zinc, sodium and potassium.
Hopman et al. (2006) [36]	n = 132 (87 women), 16.6 y ± 4.4 Median duration of GFD = 9.6 years Strict adherence 75% of participants	Observational, transversal cohort study	Netherlands	High saturated fat, Low fiber.	Low Iron and Calcium.
Hallert et al. (2002) [43]	n = 30 (18 women), 55 y −10 years GFD Strict adherence 100% of participants	Observational, longitudinal cohort study	Sweden	Low folate.	
Wild et al. (2010) [44]	n = 93 62 women, 53 y ± 13; 31 men, 56 y ± 15 ≥6 months GFD (mean duration: 8 y) ND	Observational, longitudinal cohort study	UK	Low fiber, high sugar.	Low vitamin D, folate, calcium, iron, zinc, magnesium and manganese.
Martin et al. (2013) [45]	n = 73 (55 women), 18–80 y Median duration of GFD = 7.5 years ND	Observational, transversal cohort study	Germany	Low CHO, Low fiber.	Low vitamin B1, B2, B6, Folate, magnesium and iron.

Table 2. *Cont.*

Author	Sample GFD Duration Adherence	Type of Study	Country	Macronutrient Intake	Micronutrient Intake
Thompson et al. (2005) [46]	n = 47 (39 women), 51 y ± 11 Median duration of GFD = 5.3 years Strict adherence 100% of participants	Observational, transversal cohort study	USA	Low fiber.	Low iron and calcium.
Jamieson et al. (2020) [47]	n = 35 (29 women), 47 y ± 11.5 Median duration of GFD = 6.7 years ND	Observational, transversal cohort study	Canada	Low CHO, high fat, low fiber.	Low iron, calcium and vitamin C.
Ballestero-Fernández et al. (2021) [48]	n = 64 43 women, 39.17 y ± 10.62; 21 men, 38.58 y ± 9.61 ≥1 year GFD ND	Observational, transversal case-control study	Spain	Low CHO, PUFA and fiber high protein, fat and sugars.	Low folate, vitamin E, vitamin D, iodine, calcium, zinc, magnesium. Low iron in women

GFD: gluten free diet. SFA: saturated fatty acids. CHO: carbohydrates. y: years. PUFA: polyunsaturated fatty acids. ND: not determined.

(a) Fats

All studies agree that fat intake of celiac adults is unbalanced. Some of them only show high fat intakes [19,25,47] but others also observe a high consumption of saturated fatty acids (SFA) or an excessive intake of cholesterol [17,18,36,48]. These results do not differ from those obtained in children, who present high fat intakes, with increased SFA/polyunsaturated (PUFA) ratio [4].

This could be due to the low intake of plant-based foods and high consumption of processed GFP [19,44]. When people with celiac disease follow a GFD, it is common to consume GFP, and these tend to be generally higher in total and SFA than their gluten containing analogues [4,20,21]. Additionally, Wild et al. compiled some records of GFD and described that 47% of the energy intake came from processed products. Therefore, correct classification of GFP is needed, so that celiac patients could be more informed and choose these products appropriately [44].

Unbalanced diets rich in SFA lead to health problems such as increased risk of cardiovascular diseases (CVD), or insulin resistance (IR) in all individuals, general and celiac population [49–51]. In fact, it has been observed that people with CD show higher risk of death from CVD [52]. In addition, the consumption of processed products has been related to higher mortality [4,53]. Another common problem is the chronic low-grade inflammation which has also been related to this context of an unbalanced diet with the aforementioned characteristics [54].

(b) Carbohydrates

When people with CD start on a GFD they have to stop consuming gluten-containing cereal based foods, which are the most commonly consumed cereals. Moreover, cereals are the basis of a balanced diet, and thus, without some suitable guidelines diet can result imbalanced [23,55].

As it can be observed in Table 2, several studies have shown that patients who follow a GFD present low carbohydrate intakes [18,19,25,45,47,48]. This may be due to their fear about consuming gluten that makes them reject cereals. To be more precise, celiac patients do not consume enough complex carbohydrates. Thus, Wild et al. [44] observed a low complex carbohydrate intake; however, total carbohydrate intake seemed to be enough due to the high consumption of simple sugars and processed food. This is consistent with the fact that GFPs tend to have a higher glycemic index than their gluten containing counterparts [50,56]. Thus, it can be assumed that the imbalances observed are not only due to the low or non-existent consumption of gluten-containing cereals, but also to the high intake of processed GFP, and the low consumption of vegetables and legumes [44].

When comparing these data to that of children, similarities can also be observed. Children present a low consumption of foods rich in complex carbohydrates, although the intake of simple carbohydrates is higher due to GFP [4].

This dietary profile with high intakes of simple sugars and high-glycemic index products could also be harmful; they could cause the aforementioned IR, which is associated with the more frequently observed hyperinsulinemia, lower glucose tolerance and increased risk of diabetes [51,57].

(c) Fiber

Foods that are high in carbohydrates are usually also rich in fiber [52], but are unusual in the diets of celiac patients [17,19,44]. Most studies evaluating nutrient intake in GFD show low fiber intake, which is in perfect agreement with the low CHO and high fat intakes mentioned above [18,19,36,44–48]. Regarding the intake of fiber in children, no differences are found either. This low fiber intake also seems to be explained by the low consumption of fiber-rich plant foods and whole-grains [55], and by the high consumption of refined processed foods [4].

Low fiber intake has been linked to a higher prevalence of constipation and increased risk of diverticulitis. Moreover, it has also been related to an increased risk of gastrointestinal symptoms commonly present in CD and even in treated celiac patients [58]. Thus,

even though a direct relation between GFD and constipation or diverticulitis has not been observed, it could be thought that higher fiber intakes among these patients could help to improve the inflammation that is noticeable in the disease [59,60], and reduce symptoms such as abdominal pain [61].

(d) Proteins

Some studies, like those of Martin et al. [45], González et al. [18] and Ballestero-Fernández et al. [48], claim that the protein intake is higher than recommended in celiac patients who follow a GFD, probably due to excessive meat intake. However, other studies do not highlight the same results and neither do the ones obtained in celiac children who follow a GFD. In fact, some studies show contrary data, higher protein consumption among non-celiac children [4,12,62].

3.3.2. Micronutrient Intake

As for macronutrients, the studies listed in Table 2 show that the intake of vitamins and minerals is also impaired. On the one hand, it must be mentioned that most studies do not measure the intake of all micronutrients, so precise and exact conclusion cannot be done [36,43,46]. On the other hand, it is important to take into account that depending on the reference intakes of each country controversial results can be found when defining the deficiencies of each nutrient.

(a) Vitamins

When analyzing vitamin intakes, there are several studies that show deficiencies for the same vitamins, such as that of vitamin D and vitamin E [18,19,25,44,48] followed by low intakes of B group vitamins like folate (B9), thiamine (B1), riboflavin (B2), and pyridoxine (B6) [17–19,25,43–45,55].

Vitamin D deficiency may be of special importance, since a higher prevalence of osteoporosis has been seen in people with CD, and this vitamin is considered of vital importance in bone metabolism [30,31,63]. In addition, as above mentioned, its supplementation has been recommended during the first year of GFD in order to recover the nutritional deficiencies due to low absorption caused by the pathology.

Regarding B group vitamins, the observed deficiencies agree with the biochemical data presented by Hallert et al. who observed low B12 and low folate biochemical levels and declared that homocysteine (tHcy) levels were raised in these patients, higher than in the general population, even when following GFD for a long time [43,64]. It is well known that high tHcy levels are linked to increased risk of CVD [43,65], and, as mentioned before, CD has higher prevalence of this disease [51]. To face this problem, it is important to highlight that the deficiencies mentioned have been associated to a low intake and not to the intestinal malabsorption [43]. Moreover, it has been observed that high tHcy levels can also be normalized thanks to a supplementation of B group vitamins. Thus, dietary treatment and appropriate follow-up are especially important [18,36,44] to mind these aspects too. Di Nardo et al. attached more importance to folate intake, so they recommend consuming pseudo-cereals which are richer in this vitamin, such as quinoa and amaranth [4], apart from its typical consumption through vegetables and pulses.

In addition, low B group vitamin levels have been reported to be associated with a worse sense of quality of life [43], and their supplementation with better general well-being [64].

Deficiencies in B vitamins and vitamin D seem to be common in children with CD too but that of vitamin E is not mentioned [4,43].

(b) Minerals

In general, the most common mineral deficiencies described in the literature are those of iron, calcium and magnesium [17–19,25,36,44–48]. Deficiencies in iodine, potassium and zinc can also be found [18,19,25,44,45]. Finally, there are some studies which present low intakes of selenium, sodium and manganese [19,25,44].

Some of them can be normalized with a suitable GFD-based treatment, such as in the case of zinc deficiency. Nevertheless, GFD may not be enough to overcome other ones, such as magnesium deficiency, due to the fact that cereal-based GFPs have lower mineral content than their gluten-containing analogues [66]. Special attention should be paid to iron deficiency. This is a major problem in non-treated active celiac disease and in patients with incompletely regenerated mucosa who have difficulties in achieving normal iron values [67]. Hallert et al. [43] did not see serum iron deficiencies after 10 years of GFD; since ferritin levels were within normal, but female celiac patients showed lower ferritin levels than male patients [44,46,48]. In addition, iron deficiency has been declared as a common complication of CD, so these low levels should be considered alarming, especially in women [45]. Moreover, it can worsen because of the observed low consumption of legumes and cereals [18,19].

In relation to calcium, controversial results can be found depending on the reference intakes of different countries. Hopman et al. observed a low intake of calcium according to the American recommendations (ARDA) and to Moreiras et al. [55] but adequate results with regard to Dutch recommendations [36]. On the one hand, Thompson et al. reported that 19% of their patients were lactose intolerant and 34% had been intolerant previously. So low calcium intake could also be related to low dairy intake of these patients, or even to the fear of feeling bad or causing harm [46]. Furthermore, although calcium intakes are similar to the general population, it must be taken into account that in recently diagnosed celiac patients too little lactase is produced due to the damaged mucosa and thus they develop a secondary lactose intolerance. Even though this alteration improves with the regeneration of the mucosa, calcium intake together with appropriate levels of vitamin D may benefit newly diagnosed patients, [27,28,45]. Therefore, when meeting patients with lactose intolerance, which seems to be frequent in CD, calcium supplementation could be considered, since dairy consumption will be greatly reduced or avoided [45].

Regarding mineral deficiencies in children, the most described are those of iron, calcium, magnesium and zinc. Even so, and unlike in adults, deficiencies of potassium, iodine, selenium or manganese are not mentioned [4].

Table 3 provides a summary of nutritional deficiencies in adults and children with celiac disease.

Table 3. Summary of nutritional deficiencies in celiac adults and children.

	Celiac Adults	Celiac Children
Fat	High fat and SFA intakes	=
Carbohydrates	Low complex carbohydrate intake, but high simple sugar intake	=
Fiber	Low fiber intake	=
Vitamins	Low intakes of Vitamin D, E and B group vitamins (B1, B2, B6, B9)	Low intakes of Vitamin D and B group vitamins (B1, B2, B6, B9)
Minerals	Low intakes of iron, calcium, magnesium, zinc, iodine, potassium, selenium and manganese.	Low intakes of iron, calcium, magnesium and zinc.

SFA: saturated fatty acids. "=" symbol means that same data were found in children.

It must be pointed out that, according to Larretxi et al., it seems that the influence of GFP on micronutrient deficiencies is limited, since GFPs and their gluten analogues do not contain large amounts of typically lacking micronutrients. Therefore, these deficiencies could be more related to an unhealthy lifestyle: low vegetables, fruits, cereals and nuts intake followed by high meat consumption [67]. Thus, recommendations to correct these mistakes and to promote healthy GFD should be given to amend those habits.

Nevertheless, it is noteworthy that wheat flour and its derivatives are usually fortified with some micronutrients, such as iron or folic acid, but no other alternative flours, like

those used in GFPs [4,22,68,69]. Thus, taking into account observed nutritional deficiencies, the fortification of GFPs could be a matter of interest.

Finally, taking into account that some patients may continue having a suboptimal intestinal absorption throughout their lives, the recommended intakes for the general population may not be valid for all celiac patients, so that it might be more appropriate to establish reference intakes for the celiac population that follows a GFD. Even so, it would be necessary to evaluate individually these patients through biochemical analyses and combine it with the dietary record, in order to give more personalized and effective treatment recommendations [32,45].

3.3.3. Differences between Men and Women

In general terms, unbalanced macronutrient intake and distribution of women and men are quite similar [18,19,44]. Nevertheless, although both genders present a low fiber intake [45], it has been observed that there are fewer women who reach a suitable consumption [18,19,46].

Regarding micronutrient deficiencies, differences among genders have been described. Among vitamins, intake does not vary between genders [18]. Nevertheless, some specific differences can be mentioned, although these are not always observed. Jamieson et al. registered low intake of vitamin C in men, but not in women [47]. Moreover, sometimes contrary results are appreciated, as in the case of B vitamins. One study detected that folate deficiencies were somewhat higher in women [44], while another described more serum folate and pyridoxal 5′-phosphate deficiencies in men [43]. Similarly, Hallert et al. described higher tHcy levels in males than in females, despite being high in both genders. These results have been related to higher vitamin B deficiencies and agree with the fact that celiac men tend to consume 50% less folate-rich foods than celiac women.

In terms of minerals, while women have a better fulfillment of dietary magnesium requirements, men have a better adherence to iron, iodine, potassium and calcium intakes [18,19,43,44,46]. Nevertheless, there are studies that do not find significant differences between men and women either for these or for other minerals that usually present differences, such as magnesium or calcium [45]. Finally, controversial results can also be found, such as for the intake of selenium, which have been shown to be both higher [19] and lower in men [44] than in women in various studies. Table 4 summarizes aforementioned differences between men and women.

Table 4. Differences between celiac men and women.

	Women	Men
Macronutrients	Unbalanced distribution and intake	=
Fiber	Lower fiber intake	Low fiber intake
Vitamins	Various deficiencies	=
Minerals	Lower Fe, Ca, I and K intakes	Lower Mg intake

"=" symbol means that same data were found in men.

Lower intake of micronutrients among women could be because they normally tend to consume lower amounts of food and total energy. Even so, it is important to note that most of the patients participating in the studies are usually female [25,36,44–46,48] as can be seen in the studies included in Table 2. Thus, it can be suggested that the general summary of the GFD present in this review is more representative of the female gender. Therefore, more studies with a greater number of male patients are needed, in order to properly compare the diet quality among genders.

3.4. Comparison with the Healthy General Population

It is of great interest to compare the GFD model with the diet of the general population in order to be able to determine whether the imbalances in the GFD are due exclusively to the difficulty of eliminating gluten from the diet, or if unhealthy habits also influence.

Concerning energy intake, some authors claim that celiac patients tend to have lower energy intake [19,45], while others describe that is higher because of a higher consumption of processed products [44]. Nevertheless, in general terms, differences in energy intake are not usually observed between celiac patients and the healthy general population [18,36].

3.4.1. Macronutrient Comparison

Following on with macronutrient consumption, it has been observed that celiac people who follow a GFD tend to consume too much fat [18,45], but this imbalance is similar in the general population [18,44,48]. Nevertheless, literature comparing these intakes between celiac people and the general population shows that lipid consumption can differ between both groups. Some authors have described that general population presents a higher fat intake than that on a GFD [19]. By contrast, other show a higher fat intake in celiac patients than in the general population [45] and, finally, Hopman et al. found no differences in terms of the total amount of fat, but higher SFA in people on a GFD [36]. These differences could be explained in terms of the magnitude of the consumption of specific GFP products. Taking into account that the composition of these products is higher in fat (used for substituting gluten), it increases fat consumption [4,20,45].

Although different studies have indicated the low carbohydrate intake in the GFD of celiac [18,19,25,45,48] no differences are found with the intakes of the general population [18,19,48]. However, Martin et al. report a slightly lower complex carbohydrate intake in celiac patients than in healthy adults [45]. Also higher intake of simple sugars can be found in celiac patients who follow a GFD [44].

Similarly, previously mentioned low fiber intake is also observed in both groups. Some registered values indicate a slightly higher fiber intake in the general population, although it was still low [18,19,44].

Finally, although a higher protein intake has been reported in the celiac population [18,45], no usual differences with the general population have been seen [18,19,48], which means that both diets are hyperproteic.

3.4.2. Micronutrient Comparison

Regarding the differences in micronutrient intakes, similar deficiencies are usually found in controls subjects [18,19,36,44,48]. Even so, differences have been recorded in some cases. Lower intakes of vitamin E, niacin, iron and magnesium in celiac patients than in the general population have been described, but higher ones of riboflavin, B6, zinc and potassium [18,19]. Controversial results can be found with regard to selenium, folate and B group vitamin intake [19,44,45], observing both higher and lower intakes depending on the study. There are not enough studies that compare micronutrient intake between celiac and general population, thus it is difficult to establish conclusions. Nevertheless, although in some cases higher intakes of these vitamins have been seen in celiac patients, most studies agree that the intakes are usually lower in the patients who follow a GFD [43–45,64].

This is consistent with the lower blood levels of B vitamins and higher tHcy levels that have been found in celiac people, compared to healthy subjects. Moreover, this can be explained, at least in part, by the fact that gluten-free cereal products contain lower amounts of folate [43], as previously mentioned, so that folate intake through bread is higher in the general population than in celiac.

Despite the mentioned results, the problem to highlight is that there is a nutritional deficit of micronutrients in both, celiac and general population. Moreover, some authors claim that celiac do not seem to be at an increased risk of some deficiencies, such as that of B group vitamins, comparing with general population, since no significant differences have been observed in the percentage of participants not reaching reference values [45].

Considering all the above, and according to González et al., the poor macronutrient distribution and the micronutrient deficiencies observed in the GFD could be related, more than to the consequence of eliminating gluten from the diet, to geographical dietary habits: low fruit, vegetables and cereal consumption and high intake of meat. In fact, the same

unbalanced dietary patterns are observed both in celiac and in the general population from the same countries [18,19,55]. Thus, all individuals in the population need dietary advice, not only the persons with celiac disease. It will be more appropriate to give general recommendations to promote the intake of food rich in micronutrients to improve these deficiencies in both, celiac and general population [43]. And in the case of celiac people food that naturally do not contain gluten should be consumed.

Even so, it would be interesting to analyze more biochemical data to compare them with the dietary intakes and thus, to identifying direct interactions between low nutrient intakes and health. It is also important because although micronutrient intakes are enough, the intestinal mucosa is not always recovered, as mentioned above, which could lead to low micronutrient serum levels [45]. Moreover, participants in studies may change their eating habits during the food intake recording period. Therefore, dietary data from those trials may not show the reality, and serum values could help to get closer to the true data [43,46]. It must be also pointed out that most of the studies obtain control intake from national dietary surveys, and it would be desirable to have better age- and gender-matched non-celiac adult controls.

In addition, those studies that measure micronutrient intake of general population show different results, and thus the comparison between them and those of the celiac population can vary. For example, in a study made by Wild et al. celiac females had a higher intake of magnesium and calcium than healthy women, or by contrast lower intakes of magnesium, iron, zinc, manganese, selenium and folate, depending on the control data selected as a reference [44].

Table 5 summarizes the differences between coeliac patients and the general healthy population found in the present review.

Table 5. Differences between celiac patients and healthy general population.

	Healthy Population	Celiac Patients
Energy	=	=
Fat	High fat intake	Higher fat and SFA intake
Proteins	=	=
Carbohydrates	Low carbohydrate intake	Lower complex carbohydrate intake, higher simple sugar intake
Fiber	Low intake	Lower fiber intake
Vitamins	Low folate intake	Lower vitamin E and B group vitamins intakes. Lower folate intake and higher tHcy serum levels.
Minerals		Lower magnesium, selenium, iron and zinc intake

SFA: saturated fatty acids. "=" symbol means that same data were found.

3.5. Dietary Guidelines for a Balanced Diet

Professional nutritional counselling by nutrition adviser is highly desirable from the beginning of the gluten free therapy. It is necessary to follow up patients with CD and to promote their knowledge in relation to the pathology and GFD, especially if symptoms or deficiencies regarding micronutrients persist, since it could help to improve adherence to GFD, and therefore to achieve a gluten free and balanced diet. This practice would lead to a correct recovery of the intestinal mucosa and to a healthy nutritional status. In fact, an adequate and varied GFD, enriched in fruits, vegetables, and fibers, does not necessarily lead to malnutrition. A recent study by Gładyś et al. (2021) evaluated the impact of standard dietary education in adult celiac patients on a GFD, by measuring the nutritional composition of the diet before and after one year of the education. Results showed that although adherence to the diet was improved, nutritional profile of the GFD did not, revealing the necessity to increase the role of dietitians in the treatment of CD [70].

- The most remarkable guideline would be to improve the diet by promoting a greater consumption of plant-based foods, such as fruits, vegetables, legumes, nuts and naturally gluten-free whole grain cereals and pseudocereals followed by reducing GFP consumption.

Regarding macronutrients, these will help to reduce the intake of poor-quality fats and simple sugars, and at the same time help to increase the consumption of complex carbohydrates, fiber, vitamins and minerals [18,19,55].

The consumption of pseudo-cereals, such as quinoa and amaranth, is also an excellent option, since they are considered good sources of some micronutrients such as folate, riboflavin, vitamin C and vitamin E, and they are also not as expensive as GFP [4,55].

- Although we can consider that protein intakes are sufficient, we will have to make sure they understand the importance of protein sources by promoting the intake of high-quality protein rich foods, which will be also related to the intake of high-quality fats, and thus a better dietary lipid profile [55].
- Naturally gluten-free foods rich in micronutrients are proposed before recommending fortified foods or supplements [55]. However, it could be interesting to combine the two options, with the aim of achieving a faster recovery from some vitamin and mineral deficiencies, thus, once suitable levels have been recovered, it could be enough to follow an appropriate GFD.
- Another possible improvement to consider is the one mentioned by González et al. who claim a fortification of the GFP, knowing in advance which are the nutrients that are most needed in the GFD [18]. This, could help to improve micronutrient deficiencies, but on the other hand, the macronutrient content should be corrected (reducing fats for example) and thus total balance could be achieved. In fact, it is noteworthy that the food industry is making great progress in developing healthier GFPs, which is a great challenge that has a direct impact on the health of the patients.
- The deficiencies in iron, calcium and vitamin D are noteworthy, in relation to their involvement in pathologies such as anemia or osteoporosis, which are more prevalent among the celiac population. Thus, it is recommended to take care of their intake through their food sources, such as legumes, cereals and dairy products [55].
- Similarly, to overcome deficiencies observed in B group vitamins, involved, at least in part, in the higher prevalence of CVD in CD, dietary treatment is especially important. Folate is usually given more importance; its consumption should be promoted through vegetables, pulses and pseudocereals. Moreover, this micronutrient is one of those proposed for food fortification and liable to be obtained through supplementation in high risk cases [18,36,44].
- It is common to find nutrient deficiencies in GFD, but this does not mean that it has to be normalized. Thus, Bascuñan et al. claim that although adherence to GFD may seem enough, patients with celiac disease should be continuously supervised to prevent some usual deficiencies, and to ensure that they continue having sufficient adherence to the GFD [25].

Considering what has been described so far, the proposed dietary recommendations for a balanced gluten-free diet and their result are given in Table 6.

Table 6. Dietary guidelines for a balanced GFD.

Guideline for a Secure and Balanced GFD	Result in the Dietary Profile
Promote consumption of plant-based foods, such as fruits, vegetables, legumes, nuts and naturally gluten-free whole grain cereals and pseudocereals (quinoa, amaranth, etc.)	↓ fats, specially saturated ↓ sugars ↑ complex carbohydrates ↑ fiber ↑ vitamins (folate, riboflavin, vitamin C and E, etc.) ↑ minerals
Reduce GFP consumption.	
Fortify naturally gluten-free foods in micronutrients	↑ vitamins ↑ minerals
Fortify GFP in micronutrients and balance their macronutrient content	Helpful in the macronutrient and micronutrient balance achievement
Increase dairy products, as well as legumes and cereals	↑ iron, calcium and vitamin D
Increase vegetables, pulses and pseudocereals	↑ B group vitamins
Continuous supervision of celiac patients going on a GFD	↑ adherence to the diet

GFD: Gluten-free diet. ↓: decrease; ↑: increase.

4. Conclusions

GFD should guarantee the absence of gluten to progressively recover intestinal mucosa at the beginning of the treatment and consequently the nutritional deficiencies caused by the pathology. In the long term, assuming that a strict diet is being followed and mucosa is recovered, the dietary balance should be the goal to achieve, which is possible through a correct and varied GFD. However, GFD is usually unbalanced for macro and micronutrients, in both, celiac women and men. This is mainly due to unhealthy dietary habits, commonly found as well in the general population, but also to the difficulty of eliminating gluten from the diet, that leads to a low cereal intake of cereals and high consumption of processed GFPs.

Taking everything into account, it is vital to carry out a continuous and personalized follow-up of celiac patients from the moment of diagnosis. For this supervision, the presence of a nutritionist is essential, so that these patients could obtain proper nutritional education and thus strictly adhere to the GFD, which will be the key for the long-term balanced diet.

Supplementary Materials: The following is available online at https://www.mdpi.com/article/10.3390/nu13082877/s1, Figure S1: Schema of how articles related to "nutritional deficiencies among adults with celiac disease following a GFD" were selected.

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nutrients

MDPI

Review

Iron Deficiency in Celiac Disease: Prevalence, Health Impact, and Clinical Management

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Abstract: Iron is an essential nutrient to life and is required for erythropoiesis, oxidative, metabolism, and enzymatic activities. It is a cofactor for mitochondrial respiratory chain enzymes, the citric acid cycle, and DNA synthesis, and it promotes the growth of immune system cells. Thus, iron deficiency (ID) leads to deleterious effects on the overall health of individuals, causing significant morbidity. Iron deficiency anemia (IDA) is the most recognized type of anemia in patients with celiac disease (CD) and may be present in over half of patients at the time of diagnosis. Folate and vitamin B12 malabsorption, nutritional deficiencies, inflammation, blood loss, development of refractory CD, and concomitant *Heliobacter pylori* infection are other causes of anemia in such patients. The decision to replenish iron stores and the route of administration (oral or intravenous) are controversial due, in part, to questions surrounding the optimal formulation and route of administration. This paper provides an algorithm based on the severity of symptoms; its impact on the health-related quality of life (HRQL); the tolerance and efficiency of oral iron; and other factors that predict a poor response to oral iron, such as the severity of histological damage, poor adherence to GFD, and blood loss due to mucosal lesions.

Keywords: iron deficiency; iron deficiency anemia; celiac disease; malabsorption; micronutrient deficiencies; gluten-free diet; iron oral; iron intravenous; patient-blood management (PBM)

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1. Introduction

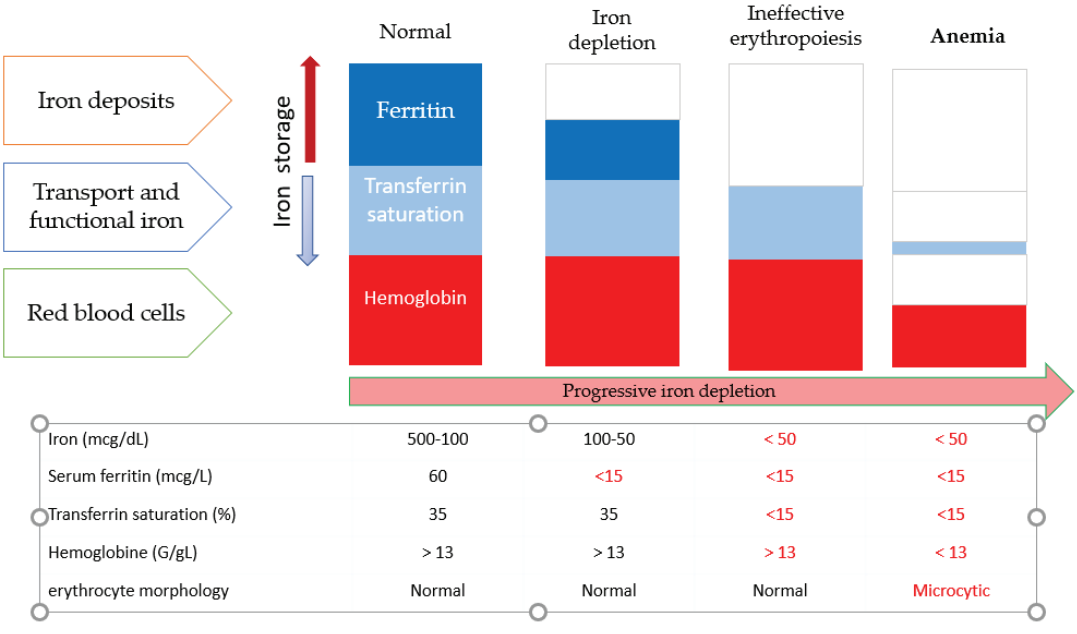
Iron is an essential nutrient to life, and its role in biology is enormous [1–3]. In fact, iron is required for erythropoiesis, oxidative, metabolism, and enzymatic activities, and it is a cofactor for mitochondrial respiratory chain enzymes, the citric acid cycle, and DNA synthesis [4]. It also promotes the growth of immune system cells. Iron deficiency (ID) is the most common deficiency state in the world, affecting more than two billion people globally. Although it is particularly prevalent in less-developed countries, it remains a significant problem in the developed world, where other forms of malnutrition have been almost eliminated [5]. Celiac disease (CD) is a well-recognized cause of IDA, even in asymptomatic patients, and, therefore, it must be considered in the differential diagnosis of IDA [6]. The prevalence of CD among people with anemia (and vice versa), its clinical consequences, and its management in specific contexts is discussed here, providing healthcare professionals with practical guidance and algorithms.

2. Iron Metabolism

Iron is an essential micronutrient with well-established contributions to body functions, such as the formation of red blood cells and hemoglobin, oxygen transport, cell division, energy metabolism, immunity, and cognition [7].

The body and cells need a very precise amount of iron: too much can be toxic and too little is bad for the metabolism [7]. Due to this toxicity, and in the absence of active excretion mechanisms, intestinal iron absorption is extremely limited and regulated tightly (barely 1–2 mg/day) to compensate for natural losses, and it is fundamentally based on recycling and a very precise circular economy. Therefore, internal turnover of iron is essential to satisfy the requirements of erythropoiesis (20–30 mg/d) [8].

ID refers to reduced iron stores and can progress to IDA, which is a more serious condition in which low iron store levels are associated with anemia. It is important to note that ID and anemia are not synonymous; a normal Hb level does not exclude ID (Figure 1). IDA can also be associated with other causes of anemia and other nutritional deficits.



Iron (mcg/dL)	500-100	100-50	< 50	< 50
Serum ferritin (mcg/L)	60	<15	<15	<15
Transferrin saturation (%)	35	35	<15	<15
Hemoglobine (G/gL)	> 13	> 13	> 13	< 13
erythrocyte morphology	Normal	Normal	Normal	Microcytic

Figure 1. Diagnosis of iron deficiency.

The main cause of ID is an inadequate absorption or supply disorder due to an unbalanced diet that does not compensate for the increase in physiological needs at certain times in life (growth, pregnancy, or recovery) or after significant, acute, or chronic blood loss. Gastric and intestinal integrity, both organic and functional, is mandatory.

Dietary non-heme iron primarily exists in an oxidized (Fe^{3+}) form that is not bioavailable and must first be reduced to the Fe^{2+} form by a ferrireductase enzyme, which uses vitamin C as a coenzyme, before being transported across the intestinal epithelium. This is accomplished by a carrier protein called divalent metal transporter 1 (DMT1) [9].

Nearly all absorption of dietary iron occurs in the duodenum. Several steps are involved, including the reduction of iron to a ferrous state, apical uptake, intracellular storage or transcellular trafficking, and basolateral release [8,9].

Once inside the intestinal epithelial cell, most Fe^{2+} is exported by ferroportin 1 across the basolateral membrane of the enterocyte (absorbed iron) and oxidized to Fe^{3+} by hep-

haestin before being bound by plasma transferrin. Ferroportin 1 is also expressed in hepatocytes, reticuloendothelial macrophages, and placental syncytiotrophoblasts [9].

Iron released into the circulation binds to transferrin and is transported to sites of use and storage. About 30–40% of the iron-binding capacity of transferrin is used in normal physiological conditions; thus, there is ∼4 mg of transferrin-bound iron, but this is the most important dynamic iron pool. Transferrin-bound iron enters target cells—mainly erythroid cells, but also immune and hepatic cells—through a highly specific process of receptor-mediated endocytosis [8,9].

In the cell, iron can be stored in two forms: in the cytosol as ferritin, and, after breakdown of ferritin, in the lysosomes as hemosiderin. Iron export from macrophages to transferrin is accomplished primarily by ferroportin 1, the same iron-export protein as expressed in the duodenal enterocyte [8,9]. The liver is the other main storage organ for iron, but RBC mass is the main storage "place".

Iron homeostasis is regulated by two main mechanisms: an intracellular mechanism, which depends on the amount of iron available to the cell, and a systemic mechanism, in which hepcidin plays a crucial role [10].

Hepcidin is the main regulator of systemic iron homeostasis. Hepcidin coordinates the use and storage of iron. It is a mediator in the iron cycle between the liver and the intestine. Hepcidin acts by inhibiting intestinal iron absorption at the level of the basement membrane of the enterocyte, thereby inhibiting iron release by macrophages and enterocytes [10].

The positive regulation of hepcidin by the inflammatory response pathways to stress, its hepatic synthesis through (IL 6) interleukin 6, is an important critical event that triggers withdrawal and systemic iron sequestration due to its negative regulation of iron. Ferroportin causes an increase in iron levels, which, in turn, limit iron transport from the liver and macrophages to plasma; furthermore, it also inhibits the absorption of iron from the diet in the duodenum, which leads to iron restriction. Iron absorption in the presence of increased hepcidin is inhibited.

Anemia and hypoxia, as well as increased erythropoiesis, induce a cascade of changes that, individually or in combination, suppress hepcidin expression [10].

3. Laboratory Tests for the Detection of ID

ID is a progressive process in which iron stores fall from being replete to deplete and, finally, absent, consequently resulting in IDA. Progressive ID can be measured by a variety of biomarkers, as discussed below.

3.1. Full Blood Count, Blood Film and Red Cell Indices

A full blood count is performed routinely in patients with CD and may show low Hb, mean cell volume (MCV) (average volume (size) of the (RBC), mean cell hemoglobin (MCH) (average hemoglobin content in a RBC), and mean cell hemoglobin concentration (MCHC) (average hemoglobin concentration per RBC); a blood film may confirm the presence of microcytic hypochromic red cells. However, for milder cases of ID, the MCV may not have fallen below the normal range. Some analyzers will give a percentage of the hypochromic or microcytes red cells present. Both are fast and most sensitive markers of functional ID.

3.2. Serum Ferritin

Serum ferritin is a stable glycoprotein that accurately reflects iron stores in the absence of inflammatory change. As this glycoprotein is the first to become abnormal as iron stores decrease and since it is not affected by recent iron ingestion, it is examined in laboratory tests. The ferritin test is generally considered the best test to assess ID in patients with malabsorption, although it is an acute phase reactant, and levels will rise when there is active infection or inflammation. A serum ferritin < 30 g/L is a sensitive marker of ID, with <15 g/L being pathognomonic of ID with or without anemia. Ferritin concentrations below 50 or 100 g/L are highly suggestive of ID in the presence of inflammation or "mixed" anemia, whereas in the absence of inflammation, they reflect poor iron "reserves".

3.3. Serum Iron (Fe) and Total Iron Binding Capacity (TIBC)

Serum Fe and TIBC are unreliable indicators of availability of iron to the tissue because of wide fluctuation in levels due to recent ingestion of Fe, diurnal rhythm, and other factors such as infection. However, in the presence of inflammation, transferrin saturation < 20% is a sensitive marker of ID.

3.4. Soluble Transferrin Receptor (sTfR)

Measurement of sTfR is reported to be a sensitive measure of tissue iron supply and is not an acute-phase reactant [11]. The transferrin receptor is a transmembrane protein that transports iron into the cell. Circulating concentrations of sTfR are proportional to cellular expression of the membrane-associated TfR and, therefore, give an accurate estimate of ID. There is little change in the early stages of iron store depletion, but once ID is established, the sTfR concentration increases in direct proportion to total transferrin receptor concentration. However, the higher cost of this test and the lack of standardization restricts its general availability [1,8,10].

3.5. Reticulocyte Hemoglobin Content and Reticulocytes

ID causes a reduction in reticulocyte number and reticulocyte hemoglobin concentration. However, anemia with a decreased (or inappropriately low) reticulocyte count may be due to deficiency of iron, vitamin B12, folate, or copper; medications that suppress the bone marrow; primary bone marrow disorders, including myelodysplastic syndrome (MDS), myelofibrosis, or leukemia, and very recent bleeding (within five to seven days before bone marrow compensation has occurred).

3.6. Red Cell Distribution (RDW)

Red cell distribution width (RDW) is a measure of the variation in RBC size, which is reflected in the degree of anisocytosis on the peripheral blood smear. RDW is calculated as the coefficient of variation (CV) of the red cell volume distribution (RDW = (standard deviation/MCV) $\times$ 100). A high RDW implies a large variation in RBC sizes, and a low RDW implies a more homogeneous population of RBCs. A high RDW can be seen in several anemias, including ID, vitamin B12 or folate deficiency, myelodysplastic syndrome (MDS), and hemoglobinopathies, as well as in patients with anemia who have received transfusions. In fact, it is a parameter for early detection of any erythropoiesis disorder. A review of the peripheral blood smear is often helpful in identifying the cause.

3.7. Bone Marrow Iron

A bone marrow sample stained for iron has been considered the gold standard for assessment of iron stores; however, this test is clearly too invasive and not practical for most patients with CD.

At the time of diagnosis, CD patients have some common nutritional deficiencies. These may include deficiencies of iron, vitamin B12, folate, and copper, which may occur in isolation or simultaneously. Some patients may have ID without anemia and must, therefore, be tested to discard ID before anemia will appear). Iron studies will identify ID (the most likely diagnosis for microcytic anemia). Mild microcytosis with iron studies showing low iron, low TIBC, and high-normal to high ferritin in the appropriate clinical context (e.g., chronic inflammatory condition with normal MCV prior to its development) is consistent with anemia of chronic disease (ACD). Figure 1 shows the evolution of the indicators of ID until the patient develops overt IDA.

Briefly, serum ferritin concentration < 30 g/L, transferrin saturation < 20%, and/or the presence of hypochromic microcytic erythrocytes (mean corpuscular volume < 80 fL, mean corpuscular Hb < 27 pg) are indicative of ID. In the presence of inflammation, transferrin saturation < 20% and ferritin > 100 g/L indicate a functional ID (iron sequestration).

4. Symptoms

Clinical symptoms and signs of IDA are usually nonspecific unless the anemia is severe. Some patients with IDA will be asymptomatic; others will have symptoms that may include the following:

- Symptoms of anemia, which may include weakness, headache, decreased exercise tolerance, fatigue, irritability, or depression. Asthenia, tiredness, and muscle weakness appear even without apparent anemia. IDA may also impair temperature regulation and may make one feel colder than normal.
- Neurodevelopmental delay (children).
- Lack of concentration and lower academic performance (adolescent).
- Worse physical performance (sport competition).
- Pica and pagophagia (ice craving).
- Beeturia (reddish urine after eating beets).
- Restless legs syndrome.

Because storage iron is depleted before a fall in Hb and iron is an essential element in all cells, symptoms of ID may occur even without anemia. These include fatigue, irritability, poor concentration, brittle nails, scratches, depapillation of the tongue, and hair loss.

Of particular interest are the symptoms that can occur in pregnancy [12–21], in children [22–24], and during the productive working age [25–32].

4.1. Pregnancy

ID may contribute to maternal morbidity through effects on immune function with increased susceptibility or severity of infections [13], poor work capacity and performance [14], and disturbances of postpartum cognition and emotions [15]. CD is frequently found in women of childbearing age. There is some evidence for the association between maternal ID and preterm delivery [16], low birth weight [17], possible placental abruption, and increased peripartum blood loss [18]. However, further research on the effect of ID, independent of confounding factors, is necessary to establish a clear causal relationship with pregnancy and fetal outcomes. The fetus is relatively protected from the effects of ID by upregulation of placental iron transport proteins [19], but evidence suggests that maternal iron depletion increases the risk of ID in the first three months of life by a variety of mechanisms [20,21].

4.2. Children

Impaired psychomotor and/or mental development are well described in infants with IDA and may also negatively contribute to infant and social emotional behavior [22]. They also have an association with adult onset diseases, although this is a controversial area [23,24]. Other common nutritional deficiencies in the patient with CD (folate, zinc, cobalamin, niacin, and biotin) contribute to the worsening of these symptoms.

4.3. Productive Working Age

ID and IDA are global health problems leading to deterioration in patients' quality of life and more serious prognosis in patients with chronic diseases [25–32]. People with ID, even without anemia, in the productive working age may present some symptoms, such as loss of concentration and memory, foggy mind, asthenia, fatigue, and depressed mood, which can be very distressing, this being a frequent cause of presenteeism (decreased productivity at the workplace). In fact, there is evidence that HRQL in these patients is severely impaired in many dimensions (physical, social, and emotional). This is a point to bear in mind when considering the route of iron replenishment (oral versus intravenous) (see below).

4.4. Elderly

CD is also possible in the elderly, and, in fact, its incidence has increased in recent decades. A low index of suspicion by a physician may lead to diagnostic delay in recog-

nition or to a distraction to other disorders. The following are three important aspects to consider: first, anemia may be present in up to 80% of cases, even in the presence of no digestive symptoms or in paucisymptomatic patients; second, some elderly patients will be seronegative, but this does not exclude the need to be investigated; and third, elderly patients are vulnerable to the effects of tissue hypoxia, and, in such cases, rapid and effective replenishment of iron stores is imperative when anemia is present (see below).

5. Prevalence of Celiac Disease (CD) in Patients with Anemia

5.1. Global Overview

CD is a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals. Gluten is a complex of water-insoluble proteins from wheat, rye, and barley that are harmful to patients with CD, and, indeed, to confirm a diagnosis of CD (at least for adults), biopsies of the duodenum must be taken when patients are on a gluten-containing diet.

> "Since the Oslo Consensus, published in 2013 [33], the scientific community has cknowledged different patterns of clinical presentation. «Classical» CD presents with signs and symptoms of malabsorption. Examples of classical CD are patients with diarrhea and steatorrhea, but also patients with weight loss and anemia or failure to thrive. In non-classical CD (before 'atypical' CD), the patient does not suffer from malabsorption (e.g., abdominal pain, diarrhea, or constipation, but without any evidence of malabsorption). This consideration is important because some patients with gastrointestinal symptoms with apparent functional criteria (e.g., irritable bowel syndrome) may ultimately be diagnosed with CD if a clinician with a high index of suspicion decides to investigate the cause of an ID (even without anemia) that any other cause cannot explain. Potential celiac disease (PCD) is defined by the presence of positive serum antibodies, HLA-DQ2/DQ8 haplotypes, and a normal small intestinal mucosa (Marsh grade 0–1) [34]. As will be discussed below, these patients may also present with anemia or ID. Subclinical CD has no signs or symptoms sufficient to trigger CD testing in routine practice. However, an unsuspected ID, without anemia, might be discovered in this subgroup if intentionally sought. Finally, refractory CD (RCD) consists of persistent or recurrent malabsorptive symptoms and signs with villous atrophy (VA) despite a strict GFD for more than 12 months. Again, anemia or ID is part of the spectrum of clinical manifestations in this subgroup".

The prevalence of CD among patients with IDA has varied over time and possibly differs according to the geographical area studied [35]. Thus, the pooled global prevalence of CD is 1.4% based on positive results from tests for anti-tissue transglutaminase and/or anti-endomysial antibodies (called seroprevalence). However, the pooled global prevalence of biopsy-confirmed celiac disease is 0.7% (0.4% in South America, 0.5% in Africa and North America, 0.6% in Asia, and 0.8% in Europe and Oceania). The prevalence is higher in female vs male individuals (0.6% vs. 0.4%; $p < 0.001$) and is significantly greater in children than adults (0.9% vs. 0.5%; $p < 0.001$) [35]. Twelve studies assessed the prevalence of CD among patients who were evaluated for anemia [36–48]. In all of these, ID anemia (IDA) was the primary focus of the study or made up the cause of anemia in most of the study patients.

The prevalence of CD among patients suffering from gastro-intestinal symptoms ranged from 10.3% to 15% [36,41,42]. One small study assessed the prevalence of CD in a group of patients who had IDA but no identified gastrointestinal source [40]. In this study, the prevalence of CD by antigliadin antibody (AGA) and confirmed by endomysial antibody (EMA) was 30% [40].

In another study, the investigators assessed the prevalence of CD in premenopausal women with IDA [46]. The overall prevalence of CD in this population was found to be 12.9% by tissue transglutaminase (tTG) and 8.5% after biopsy examination confirmation.

Of interest, CD was found in one of 22 (4.5%) women with hypermenorrhea and 4 of 18 (22%) women with normal menstrual flow.

Finally, four studies assessed the prevalence of CD in asymptomatic IDA patients by serology [38,40,44,45]. The prevalence of CD in this group ranged from 2.3% to 5.0%. Another three studies assessed the prevalence of CD by biopsy examination in asymptomatic IDA patients, finding it to be between 2.9% and 6% [37,42,47].

The data provided by this systematic review were biased by the criteria used for the diagnosis of CD, based in many cases on serological findings without histological confirmation [35]. Mahadev et al. performed a systematic review to determine the prevalence of biopsy verified CD in patients with IDA [48]. This systematic review consisted of examining manuscripts published in PubMed Medline or EMBASE in July 2017 for the term "celiac disease" combined with "anemia or iron-deficiency". The authors identified 18 studies comprising 2998 patients with IDA for inclusion in the analysis. Studies originated from the United Kingdom, United States, Italy, Turkey, Iran, and Israel. Using a weighted pooled analysis, they demonstrated a prevalence of biopsy-confirmed CD of 3.2% (95% CI, 2.6–3.9%) in patients with IDA, although heterogeneity was high (I2 = 67.7%). In the eight studies fulfilling all the quality criteria established by the authors, the pooled prevalence of CD was 5.5% [48].

The CD prevalence in IDA was not influenced by the proportion of females, the average age, or the baseline prevalence of CD in the populations studied. This is notable given that IDA is more common in certain subgroups, such as premenopausal women. These findings suggest that IDA is an important risk factor for CD irrespective of patient demographics, and that endoscopic small bowel biopsy should be a part of the diagnostic workup for the condition, even in patients in which other etiologies may be suspected.

The prevalence of CD has also been investigated in patients with ID without anemia. Abdalla et al. investigated a cohort of 2105 females aged 6 years or older, obtained from the NHANES database, a nationally representative health survey conducted from 2009 to 2010 [49]. ID was defined as serum ferritin level <20 ng/mL and considered positive for CD when subjects were tested positive for both immunoglobulin A (IgA)-tTGA tissue transglutaminase and IgA-EMA. Subjects were divided into two groups (ID and non-ID). Among the sample of 2105 subjects, 569 had ID and 1536 did not have ID. Five people were identified as having CD among the ID group, as were two people in the non-ID group. After adjusting for selected covariates, the prevalence of CD was higher in female subjects with ID with OR of 12.5 (95% CI 1.74–90). These results indicate that CD is more common in patients with ID, which is in line with many different studies conducted in Europe [50,51] and Asia [52–55].

5.2. Children

Some authors have provided data on the prevalence of CD in children suffering from IDA. Narang et al. conducted a cross-sectional analytical study among children aged one to 12 years of age with moderate-to-severe iron IDA and control children without anemia [56]. All children with positive celiac serology underwent upper gastrointestinal endoscopy and duodenal biopsy, and a biopsy finding of Marsh grade 3 was considered positive for CD. Among a total of 152 anemic children and 152 controls with mean (SD) hemoglobin of 7.7 (1.8) and 12.2 (0.74) g/dL, respectively, 16 (10.5%) cases and 3 (2%) control patients had positive serology for CD (OR (95% CI) 5.33 (1.52–18.67), $p = 0.007$). CD was histologically confirmed in 4% of children presenting with moderate-to-severe anemia.

Of great interest is the study conducted by Shahriari et al., in which 184 children, including 92 IDA patients who responded to treatment using iron supplements, 45 non-responding iron deficient patients, and 47 healthy individuals, with the maximum age of 18 years, participated in serologic screening (with anti-TTG antibody and anti-endomysial antibody) for CD. Patients with at least one positive serology test underwent multiple mucosal biopsy from the bulb and duodenum. Interestingly, the frequency of positive serologic tests in the group with IDA resistant to treatment was prominently higher than

that in the other two groups ($p < 0.001$). Among the patients with a positive serologic celiac test who underwent endoscopy and biopsy, no histologic evidence of CD was observed [57]. They were diagnosed as potentially having CD, patients with normal small intestinal mucosa who are at increased risk of developing CD as indicated by positive CD serology [58]. This suggests that even among patients with positive celiac serology and the absence of histological lesions ("potential celiac"), ID or IDA may still be present. This observation is shared by our own clinical practice.

5.3. Index of Suspicion for the Diagnosis of CD in Patients with IDA

Remarkably, despite the information provided in the literature, the index of suspicion for the diagnosis of CD among patients with ID or IDA is surprisingly low. Spencer et al. electronically distributed a survey to primary care physicians (PCPs) who are members of the American College of Physicians. Respondents were asked whether they would test for CD (serologic testing, referral for esophagogastroduodenoscopy (EGD], or referral to GI) in hypothetical patients with new IDA. Testing for CD varied significantly according to patient characteristics but, globally, PCPs are under-testing for CD in patients with IDA, regardless of age, gender, race, or post-menopausal status. In addition, most PCPs surveyed reported that they do not strictly adhere to established guidelines regarding a confirmatory duodenal biopsy in a patient with positive serology for CD [59].

This low awareness has also been reported among hematologists. Smukalla et al. surveyed hematologists to determine rates of CD screening in patients with IDA. The survey was e-mailed to members of the American Society of Hematology. There were 385 complete responses from 4551 e-mails. The percentage of hematologists who would indicate serology in patients with iron IDA was low, ranging from 11 to 18% compared to those who would request colonoscopy and/or gastroscopy for the study of the cause of anemia. Physicians who had recently finished their fellowship and those who saw a high volume of patients with IDA (especially if they were pediatric patients) were more likely to screen for CD [60]. The underdiagnosis of CD has serious health implications for affected individuals, and patients with newly diagnosed IDA present an opportunity for accurate diagnosis that should not be overlooked.

5.4. Prevalence of Celiac Disease among Patients with Anemia of Obscure Origin

Patients with IDA of unknown etiology are frequently referred to a gastroenterologist because, in most cases, the condition has a gastrointestinal origin. On the other hand, it is well known that only a minority of CD patients present with classical malabsorption symptoms of diarrhea and weight loss, whereas most patients have subclinical or silent forms in which IDA can be the sole presentation [13]. Zamani et al. investigated the prevalence of CD in a large group of patients with IDA of obscure origin. [55]. Of the 4120 IDA patients referred to a hematology department, 206 (95 male) patients were found to have IDA of obscure origin after an extensive evaluation of the gastrointestinal tract. Out of a total of 206 patients (14.6%), 30 had gluten-sensitive enteropathy (GSE) based on a positive serological test and abnormal duodenal histology. A gluten-free diet (GFD) was advised for all the GSE patients. Some results of this research deserve to be highlighted:

- The average duration of anemia was 3.6 +/− 1.4 years.
- Most of the GSE patients (73.3%) did not report any gastrointestinal symptoms. Consequently, physicians may fail to consider GSE as a cause of IDA when gastrointestinal symptoms are absent or nonspecific.
- These patients had been treated with oral iron for a mean duration of 1.9 years. Anemia improved in only eight patients (26.8%) treated with oral iron supplementation before GSE diagnosis.
- In GSE patients, the hemoglobin level was inversely correlated with the severity of the histological injury. Patients with Marsh 3 lesions had the most severe anemia, consistent with the role of impaired intestinal absorption in the pathogenesis of IDA. Many authors consider the presence of villous atrophy (e.g., Marsh 3) as one of the

major criteria for diagnosing CD [61,62]. To avoid this controversy in the definition of CD, the authors used the term "gluten sensitive enteropathy" rather than CD to describe patients with any degree of intestinal damage together with positive serologic tests.

- In this study, the authors showed a significant objective improvement in hemoglobin levels with GFD alone in patients with positive serology but no villous atrophy (e.g., Marsh 1 or 2). This would be an important point concerning the route of iron administration (oral versus intravenous) in patients with or without villous atrophy (see below). Furthermore, GFD could improve anemia in IDA patients who have positive tTGA/EMA and mild duodenal lesions without villous atrophy.

Figure 2 shows the reported prevalence of CD in patients investigated for ID anemia in different clinical settings [35–38,40,42,44–47,49,51,56].

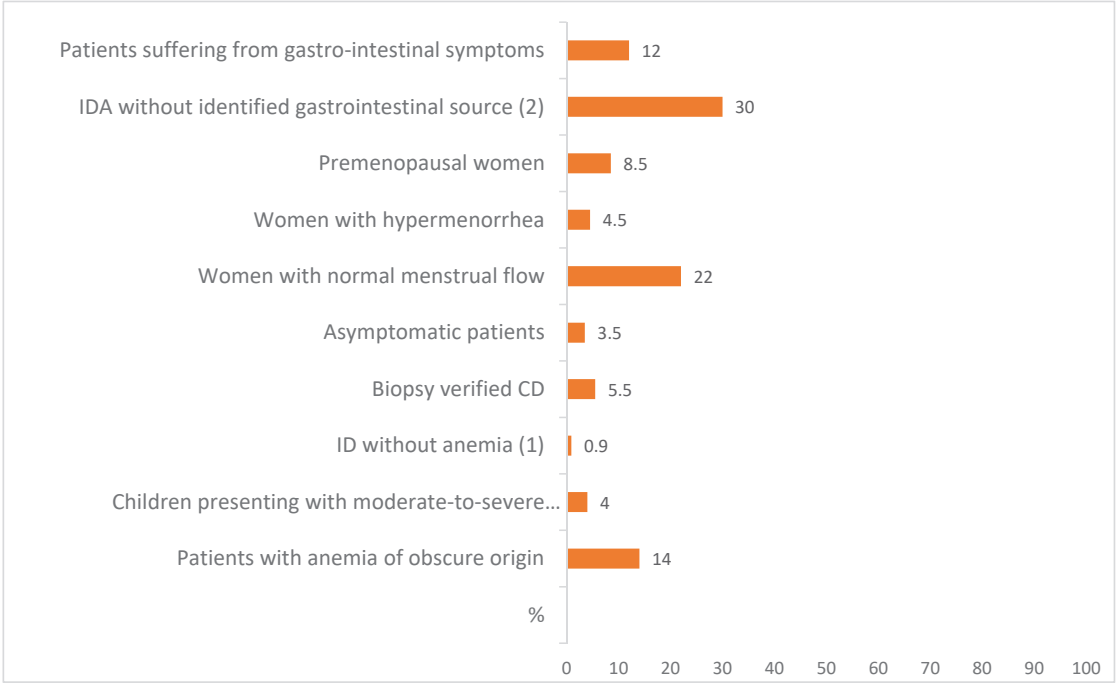


Figure 2. Prevalence of CD in patients with IDA in different setting. (1) OR of 12.5 (95% CI 1.74–90) (compared to the prevalence of CD controls); (2) Diagnosis celiac desease based on serology results without duodenal biopsy.

6. Prevalence of Anemia in CD

6.1. Comprehensive Overview

Indeed, CD is the disease of a thousand faces. Thus, a patient can be severely anemic and not have osteoporosis and vice versa without us truly knowing the reason for this phenomenon. Depending on the study in question, the prevalence of anemia in newly diagnosed patients has varied from 12% to up to 85% [38,63–68].

Saukkonen et al. compared a variety of clinical, serological, and histologic variables between newly diagnosed celiac patients presenting with and without anemia [68]. In this study, 23% of the patients had anemia at CD diagnosis. The anemia group had lower hemoglobin at CD diagnosis (women, 118 vs. 132 g/L, $p < 0.001$: men, 120 vs. 147 g/L, $p = 0.001$). Some findings that should be highlighted in this research are as follows:

- Celiac patients with anemia as a prominent symptom showed signs of more severe disease than those presenting with diarrhea, a finding that has also been reported by other authors [69].
- Anemic celiac patients have a longer duration of symptoms and a more severe serological and histologic presentation at diagnosis, as described by Shing et al. [66].
- Finally, anemic patients also showed a slower histologic response, including a worse recovery in the villus/crypt ratio and a significantly lower decrease in IELs count and in the density of γδ+ IELs.

In conclusion, this study demonstrated that CD patients presenting with anemia at diagnosis have more advanced disease and a slower dietary response than those without anemia. This observation has been supported by numerous studies as reported in an excellent review on the extraintestinal manifestations of CD, highlighting that when anemia is the main reason for presenting with the disease, they have higher anti-transglutaminase levels, lower serum cholesterol, and higher degrees of villous atrophy when compared to those presenting with diarrhea alone [70,71].

6.2. Anemia Outcomes in Celiac Patients after Introduction of a Gluten-Free Diet

Recovery from anemia usually occurs within one year after the commencement of a strict FGD, in most cases even without additional iron supplementation [72]. However, some patients with CD continue to have IDA despite a careful gluten-free diet (GFD). Studies on the effect of GDD on recovery from IDA are scarce [68,72–74]. Annibale et al. evaluated a series of 26 adult patients (24 women, 2 men; 13.7%) with a biopsy-confirmed CD diagnosis. At 12-month control, all but one patient (94.4%) recovered from anemia and 50% from ID. A significant inverse correlation ($r = -0.7141$, $p = 0.0003$) between an increase in Hb concentrations and a decrease in individual histological scores of duodenitis was observed, suggesting that the recovery from anemia occurs in parallel with the normalization of histological alterations of the intestinal mucosa, without iron supply. Note that the recovery of the iron stores occurred in only 50% of cases (mostly women of childbearing age). In another study, a strict gluten-free diet led to an increase in serum iron, resolution of anemia, and restitution of normal mucosal morphology in some celiac patients with severe anemia who had not responded to oral iron replacement [75].

Sansotta et al. conducted a retrospective chart review of patients contained in a registry of children (<18 years of age) and adults (>18 years of age) with CD followed at the University of Chicago between 2002 and 2015 [73]. Out of a total of 554 cases (227 children) with CD, 48% of adults and 8% of children had IDA at the time of diagnosis. All of the patients were instructed to start a strict GFD with the aid of a dietitian following their diagnosis. At the end of the follow-up period (an average of 3.4 years for children and 3.0 years for adults), 85% of adults and almost 100% of children had hemoglobin levels in the normal range.

Of particular interest are the results obtained in the study carried out at Tampere University Hospital and the University of Tampere (Finland) [68]. A total of 163 consecutive adults with confirmed CD were enrolled. The median age of the participants was 49 (range: 16 to 79) years, and 111 (68%) of them were women; 38 (23%) had anemia at CD diagnosis and 10 (6%) still after a one-year diet. Anemia was more common in women, possibly reflecting the generally higher need for iron in premenopausal women, and less common in screen-detected patients. At this point, it could be assumed that active case-finding and screening of at-risk patients has also shortened the diagnostic delay in CD and, thus, further reduced the risk of anemia at diagnosis.

Gamma-delta intraepithelial lymphocyte (γδ + IELs) density in the duodenal mucosa was significantly lower in the anemia group at diagnosis. This finding is of interest as there is evidence that γδ + IELs play an important role in mucosal repair and tumor surveillance, and that their number is reduced in patients with refractory CD [76]. It might thus be hypothesized that the low density of γδ + IELs contributes to the more severe presentation and increased risk of complications in anemic CD patients. In fact, these

patients had more gastrointestinal symptoms, worse indicators of well-being, and higher levels of tTGA. After one year on a gluten-free diet, the mucosal villous height/crypt depth ratio was significantly lower, and all IELs except γδ + IELs were significantly higher in the anemia group, indicating a slower response to the GFD. These results suggest that anemia at diagnosis predisposes one to an inadequate histologic response, warranting careful follow-up for this patient subgroup.

7. Mechanisms that Explain the Presence of Anemia in CD

7.1. Micronutrient Deficiencies

CD leads to an abnormal immune response, which is followed by a chronic inflammation of the small intestinal mucosa with progressive disappearance of intestinal villi leading to a decrease in absorption of many nutrients, including iron, vitamin B12, folate, copper, and zinc [4].

Patients with CD have a predisposition to develop ID, thought to be due to the predominant site of mucosal damage—the duodenum—in CD, which is also the site of maximal iron absorption. In addition, individuals with CD are also predisposed to several other hematologic abnormalities, including vitamin B12 or folate deficiency [77,78].

Harper et al. assessed a variety of hematologic and associated nutritional parameters in a total of 405 celiac patients [64]. Approximately 20% of all patients with CD had anemia at presentation, and ~70% of CD patients had a serum ferritin below the mean for their age- and gender-matched cohort. Macrocytic anemia with concurrent vitamin B12 and/or folate deficiency was rare (<3% of all cases of anemia); however, folate deficiency was present in about 12% of the study population and vitamin B12 deficiency in about 5%. The IDA was present in 13% of the patients with partial villous atrophy and in 34% of those with a subtotal villous atrophy ($p < 0.001$), with no significant difference in the proportion of B12 and folate deficient individuals.

Similar results were reported by Berry et al. in a prospective observational study, where 103 consecutive patients with well-documented CD were included. Overall, ID was seen in 84 (81.5%) patients, followed by vitamin B12 deficiency in 14 (13.6%) and folate deficiency in 11 (10.7%) patients; 17 (16.5%) patients had anemia due to mixed nutritional deficiencies; and four (3.9%) patients had anemia of chronic disease. Again, the mean hemoglobin and median ferritin levels were significantly lower in patients with severe villous atrophy compared to those with mild atrophy [79].

The reason that some, but not all, CD patients develop ID anemia is not well understood but may be related to deficiencies in some regulatory proteins that play a critical role in iron absorption at the level of the enterocyte.

Iron enters the epithelial cell of the duodenal mucosa in ferrous form through the apical or brush border divalent metal transporter DMT1, and the efficiency of iron absorption parallels the level of DMT1 expression [80]. Thus, in the presence of ID, DMT1 increases the spanning of the entire brush border membrane instead of limiting its localization to the villus apical region [81]. Microcytic anemia caused by DMT1 mutations has also been identified in human subjects [82]. Interestingly, the DMT1 iron transporter is known to be upregulated in CD to counteract villous atrophy [83]. Taking advantage of this evidence, Tolone et al. investigated the association between an intronic DMT1 polymorphism, DMT1 IVS4+44C>A, and IDA in a cohort of 387 unrelated celiac children from southern Italy. The authors found that the DMT1 IVS4+44-AA genotype confers a fourfold risk of developing anemia, regardless of atrophy degree. The data from this study suggest, for the first time, that CD may unmask the contribution of the DMT IVS4+44C>A polymorphism to the risk of IDA [84].

In addition to the above mentioned deficiencies (i.e., iron, folic acid, and vitamin B12), at the time of diagnosis, there may be deficiencies in other vitamins and minerals, in particular copper and zinc [85]. Copper deficiency is a rare complication in CD, and its prevalence remains unknown. This deficiency can lead to anemia, thrombocytopenia, neutropenia, and peripheral neuronal involvement [86–89].

Zinc deficiency is also uncommon in celiac patients. In a study, zinc absorption did not appear below usual amounts in subjects with CD, but children with CD have impaired gut function that may affect their zinc nutritional status as shown by a smaller fractional zinc absorption compared with control patients [90]. The mechanism of zinc depletion and its possible implications are unknown [91].

7.2. Infection by Helicobacter pylori

Helicobacter pylori has been proposed to have a role in the homeostasis of iron stores, and many studies have reported that *H. pylori* infection is associated with IDA [92,93]. A systematic review and meta-analysis were conducted by Hudak et al. to examine the prevalence of depleted iron stores among patients infected with *H. pylori.* Compared to uninfected patients, *H. pylori*-infected individuals showed increased likelihood of IDA and ID [94]. Meta-analyses of seven RCTs showed increased ferritin following anti *H. pylori* eradication therapy plus iron therapy as compared with iron therapy alone. Several mechanisms have been postulated to explain this association, including elevated gastric pH due to atrophic gastritis [95,96], elevated serum hepcidin levels [97], and the presence of lymphocytic enteritis, a lesion that is shared by CD itself [98]. In a study conducted by Sapmaz, the serum hepcidin-25, iron, ferritin levels, and total iron-binding capacity were evaluated at baseline and after *H. pylori* eradication to assess whether *H. pylori* eradication plays a role in IDA related to *H. pylori* infection. There was an improvement in hemoglobin, iron, total iron-binding capacity, and ferritin values after *H. pylori* eradication in all subjects. Serum hepcidin-25 levels significantly decreased after *H. pylori* eradication ($p < 0.001$) [97]. This is important, as the presence of *H. pylori* infection upregulates serum hepcidin levels and decreases the response to oral iron therapy in children with iron deficiency anemia [99]. The presence of *H. pylori* infection could have an additive effect that may contribute to the development (or refractoriness) of anemia in CD patients. In fact, some authors have postulated a significant association between *H. pylori* infection in IDA and CD patients [100,101]. There is a low awareness among clinicians of the importance of the concomitance of both diseases (*H. pylori* infection and CD), mainly in children and adolescent groups where iron requirements are increased and eradication of the infection seems mandatory, in addition to a GFD. In conclusion, investigation and eradication of *H. pylori* should be incorporated into the IDA diagnostic workup, especially in populations where the infection is endemic [102,103].

7.3. Anemia of Chronic Disease

Although anemia is still a common presentation of CD, nutritional deficiencies alone do not explain this phenomenon in all cases, and CD has also been associated with other causes of anemia, such as anemia of chronic disease [4,64,104–106]. Systemic inflammation, subsequent to the increase in blood levels of inflammatory proteins, is a rare event in patients with CD. However, gliadin can favor the activation of mononuclear cells, located in the intestinal lamina propria mucosa, with subsequent local overproduction of proinflammatory cytokines (Figure 3). In the study conducted by Harper et al. [64], ferritin was greater than the 50th percentile in 13% of the anemic patients. Ferritin is an acute phase reactant whose serum concentration can increase in response to systemic inflammation. In fact, this subgroup showed an elevated erythrocyte sedimentation rate (ESR). The elevated ESR observed in those with high ferritin values suggests systemic inflammation without significant malabsorption. Thus, the combination of anemia associated with high serum ferritin and evidence of systemic inflammation suggests anemia of chronic disease [64]. Figure 3 illustrates the mechanisms involved in the pathogenesis of anemia of chronic disease [4,64,79,105,106].

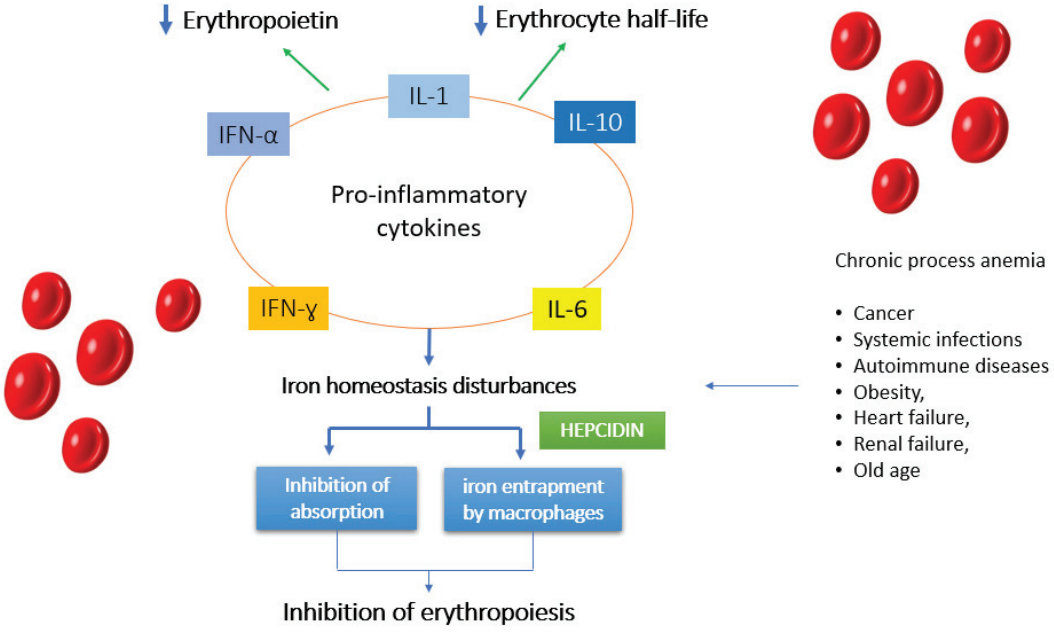


Figure 3. In CD there is a component of systemic inflammation which in some cases may also contribute to the pathogenesis of anaemia. This contribution is less than that due to malabsorption. The figure illustrates some mechanisms involved in the pathogenesis of anemia of chronic disease.

In response to inflammation, cytokines, such as IFN-gamma, TNF-alpha, IL-1, IL-6, and IL-10, are released into circulation. These cytokines act on the liver, causing increased production of hepcidin, an acute phase reactant whose role is to inhibit the duodenal absorption of dietary iron. These cytokines also induce the expression of DMT-1, an iron transporter on macrophages, whose role is to increase iron uptake, and they simultaneously down-regulate the expression of the iron exporting protein ferroportin-1 on macrophages. The net effect is a trapping of circulating iron in the reticuloendothelial system.

7.4. Persistence of Anemia in Patients with CD despite Adopting a GFD

Some patients with CD persist with indicators of IDA (or ID without anemia) refractory to oral iron supplementation after adopting a GFD. Several factors may be involved in this well-documented phenomenon. First, although strict avoidance of gluten typically results in clinical and histological improvement, when a follow-up duodenal biopsy is performed to document mucosal recovery, a significant proportion of patients with CD persist with villous atrophy. This fact has recently been highlighted by Fernández-Bañares et al. in the CADER study, which was designed to evaluate villous atrophy persistence after two years on a GFD in de novo adult patients with CD with strict control of gluten exposure [107]. Seventy-six patients completed the study (36.5 ± 1.6 years, 73% women). The rate of persistent villous atrophy after two years was high (53%) in adult patients with CD on an intentionally strict GFD, despite rigorous prospective dietary monitoring. Two-thirds of participants (69%) had detectable gluten immunogenic peptides in the fecal sample (f-GIPs > 0.08 mg/g) during the study period, without significant differences between patients who achieved recovery and those with persistent villous atrophy. The authors conclude that low-level ongoing inadvertent gluten exposures could be a contributing factor to persistent villous atrophy in highly sensitive patients [107].

Secondly, anemia that is refractory to oral iron supplementation is also seen in CD patients, despite the recovery of duodenal mucosa after adopting a GFD. Several authors have

reported the presence of ultrastructural and/or molecular alterations of enterocytes, such as disrupted and decreased glycocalyx, and irregular or absent microvilli after adopting a GFD [4]. Such findings were only visible when biopsies were investigated by medium- or high-power scanning electron microscopy, whereas low-power scanning electron microscopy did not detect these lesions. Such changes would seem to be involved in the persistence of IDA in this subgroup [108–110]. Ultrastructural and molecular alterations of enterocytes would also appear to be responsible for IDA in patients with non-celiac gluten sensitivity (NCGS), where IDA is present in up to 20% of cases [111,112]. This hypothesis is consistent with the findings observed by the group of Sbarbati et al. The authors revealed alterations of the enterocyte brush border with a significant reduction in the height of microvilli in four out of seven patients with gluten sensitivity, alterations that were not detected by conventional light microscopy [113].

7.5. Blood Loss Due to Inflammatory Lesions

Blood loss due to inflammatory lesions of intestinal mucosa may contribute to IDA in celiac patients [4,106]. Such is the case for patients with concomitant inflammatory bowel disease (IBD) [114], as well as those who have developed refractory celiac disease (RCD) and/or associated complications, including enteropathy associated with T-cell lymphoma (EATL), adenocarcinoma, jejunoileitis, or B-cell lymphoma [115,116]. In this regard, small bowel capsule endoscopy (SBCE) has an established role in the identification and management of IBD and RCD lesions and for the detection of complications [117–121]. The sprue collagen manifests itself in the form of refractoriness, and its occasional association with EATL has also been described [122].

7.6. Aplastic Anemia

Various cases of aplastic anemia associated with CD have been described in the literature, both in pediatric age and in adulthood [123–128]. Pancytopenia is the most common feature, and the clinician should have a high index of clinical suspicion for this entity in the presence of pancytopenia that complicates the evolution of CD. The diagnosis is usually achieved by bone marrow biopsy. Based on the cases reported to date, it seems that the GFD is not enough to improve pancytopenia; therefore, most patients require other treatments.

Figure 4 illustrates the various mechanisms underlying the pathogenesis of anemia in the CD patient.

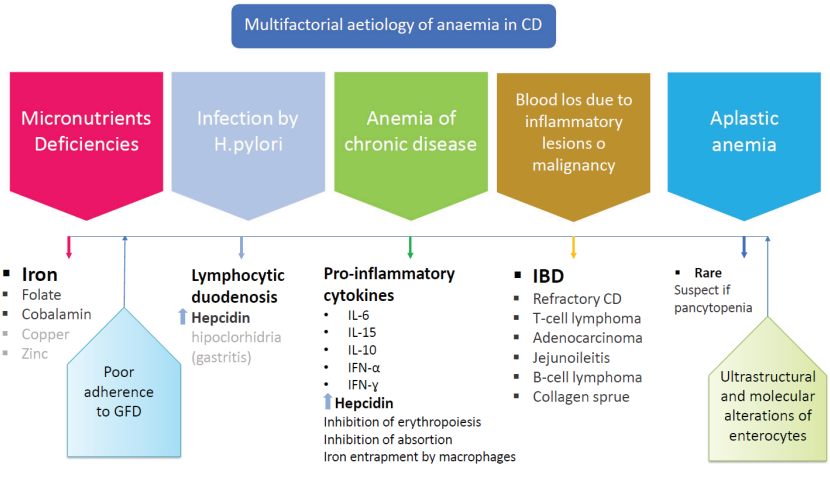


Figure 4. Factors influencing the development of anemia in CD. The main and most frequent cause of anemia in CD is malabsorption. The figure illustrates other components that may contribute to its pathogenesis to a lesser extent. CD "celiac disease"; GFD: "gluten free diet"; IBD: "inflammatory bowel disease".

8. Management of Anemia and Iron Deficiency in Different CD Settings (Algorithms)

8.1. At the Time of CD Disease Diagnosis

A variable proportion of CD patients (children or adults) do not have anemia at diagnosis. However, some of them have indicators of ID and are symptomatic (e.g., asthenia, fatigue, and poor exercise tolerance). Therefore, the clinician should order a battery of laboratory tests to identify any signs suggestive of iron deposition depletion and to act accordingly. ID is diagnosed by the presence of low serum iron levels (less than 50 μg/dL) and high serum transferrin level of IBC > 350 mg/dL. Another highly sensitive index for diagnosing IDA is the saturation index of transferrin, which is less than 10–16%. Low ferritin levels represent an early and highly specific indicator of iron deficiency. However, the international criteria for defining depleted iron deposits vary with age: <12 μg/L for children under five years of age and equal to 15–20 μg/L for those over 5 fiveyears of age and adults [106]. These levels should be adjusted upwards in the presence of inflammation or infection, so that the cut-off for the diagnosis of IDA rises to 30–50 μg/L in this context. Table 1 shows other parameters for IDA diagnosis.

Table 1. Other parameters evaluable for IDA diagnosis [106].

	Parameter	Comment
✓	Reduction in Hb and hematocrit < 2 SD of normal values*.	* These values vary according to age, sex, elevation, smoking habit, and physiological conditions such as pregnancy.
✓	Reduction in MCV, MCH, and MCHC	
✓	Hypochromic cells with a tendency to microcytosis	
✓	Increase in RDW > 15%	
✓	Reduction in CHr < 27.5 pg	
✓	Increase in sTfR to a 10–14 mg/L	
✓	Reduction in reticulocyte (inconstant)	
✓	Increase in free erythrocyte protoporphyrin (FEP) > 10 mg/dL	

* For WHO in adults, anemia is defined as hemoglobin < 13 g/dL in men and <12 g/dL in non-pregnant women. In children, reference values are lower and differ according to age. (MCV: mean corpuscular volume; MCH: mean hemoglobin concentration; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; CHr: reticulocyte hemoglobin concentration; sTfR: soluble transferrin receptor).

8.2. Allogeneic Red Blood Cell Transfusion (When, How, and to Whom?)

Acute post-hemorrhagic anemia is a rare event in the celiac patient, except in cases of complicated CD, especially if patients are receiving anticoagulants [129–132]. The indications for red blood cell (RBC) transfusion in CD patients do not differ from those established for the general population, and all of these patients should benefit from a patient blood management (PBM) program that seeks to minimize blood loss, optimize hematopoiesis (mainly with iron replacement therapy), maximize tolerance of anemia, and avoid unnecessary transfusions [133–135]. Table 2 shows the indications and hemoglobin thresholds for RBC transfusion in the adult with acute or chronic anemia due to gastrointestinal bleeding or malabsorption [136].

Table 2. Indications and hemoglobin thresholds for RBC transfusion in the adult with acute or chronic anemia.

Hemoglobin Thresholds for RBC Transfusion # ‡	
Acute anemia	*Chronic anemia* [1]
Hb < 7 g/dL In those patients without cardiovascular or pulmonary comorbidities [A] or signs of organ dysfunction [B]. Hb < 8 g/dL In those patients with cardiovascular or pulmonary comorbidities [A]. Hb < 9–10 g/dL In those patients with signs of organ dysfunction [B]. [A] Cardiovascular risk factors influencing the decision to transfuse RBC concentrates in patients with acute anemia: ▪ Acute coronary syndrome with active ischemia. ▪ Anginal chest pain ▪ ECG changes suggestive of ischemia ▪ Orthostatic hypotension or tachycardia that does not respond to fluid resuscitation ▪ Severe dyspnea or tachypnea at rest ▪ Coronary artery disease, heart failure, planned surgery this admission, or coronary artery syndrome without active ischemia o Consider transfusion if Hb < 7.5 g/dL* [135].	Hb < 5 g/dL In those patients without cardiovascular or pulmonary comorbidities [A] or signs of organ dysfunction [B]. Hb < 6 g/dL Only in those patients with cardiovascular or pulmonary comorbidities [A]. Hb < 7 g/dL Only in those patients with signs of organ dysfunction [B]. [B] Signs of organ dysfunction They are indicative of severe tissue hypoxia (e.g., in cases of massive hemorrhage, where hemoglobin levels remain "elevated" due to hemoconcentration). ▪ Tachycardia ▪ Hypotension ▪ Dyspnea ▪ Angina ▪ Hypoxia

[1] Iron deficiency anemia due to malabsorption or chronic fecal losses. # Transfusing RBC units one unit at a time, assessing patients after each unit (i.e., "don't give two without review"); ‡ Each unit of RBC provides approximately 200 mg of iron, which is insufficient to replenish iron stores.

RBC transfusion is not usually necessary in CD with chronic IDA, except in cases where a source of chronic gastrointestinal bleeding (e.g., angiodysplasias) is identified, especially in elderly patients, where CD may also occur and coexist with some co-morbidities, such as coronary insufficiency, heart failure, chronic obstructive pulmonary disease (COPD), or renal failure. All of these can exacerbate the effects of tissue hypoxia associated with the loss of red cell mass and may precipitate organ failure. This reflection is important since as the prevalence of CD increases, a greater proportion of new diagnoses are being made in individuals over 60 years of age [137–140]. The atypical (non-classics) patterns of clinical presentation in this age group and the lower sensitivity and specificity of serological tests in the aged population can sometimes cause a delay in diagnosis [140]. It is unknown why the gastrointestinal pattern (GI) of presentation is less common in the elderly than in younger adults; however, in the aged population, deficiency of micronutrients may often represent the only symptom at presentation [141]. Thus, up to 80% of elderly patients with CD in several countries presented with anemia, mainly due to iron deficiency [52,137–139,142]. The clinician must be vigilant since the etiology of anemia in elderly patients includes factors other than ID, such as vitamin B12, folate deficiency and inflammation itself, and some hematological parameters; for example, MCV and ferritin levels may be equivocal [139,141].

8.3. Replenishment of Iron Storage

Iron is an important micronutrient, and CD constitutes one of the groups at the highest risk of ID. ID during the first year of life occurs at a time of rapid neural development and when morphological, biochemical, and bioenergetic alterations may all influence future

functioning [22–24]. The brain is the most vulnerable organ during critical periods of development [19]. Iron is present in the brain from very early in life, when it participates in the neural myelination processes [20], learning, and interacting behaviors, and iron is needed by enzymes involved in the synthesis of serotonin and dopamine neurotransmitters [21]. In adults, IDA results in fatigue and diminished muscular oxygenation, which may affect muscle strength and quality and, subsequently, physical performance. In both populations (children and adults), iron deficiency leads to increased vulnerability to infections, especially of the respiratory tract. Consequently, it is important to replenish iron stores quickly, safely, and effectively in any patient with a gastrointestinal source of ID, either through blood loss or malabsorption.

8.4. How to Proceed with Iron Replenishment: When, How, and to Whom?

GFD favors the improvement of intestinal atrophy but also induces a reduction in inflammation with subsequent progressive correction of anemia. The mechanism is therefore twofold: increased iron absorption and reduced effects of various inflammatory mediators on iron homeostasis and erythropoiesis. The recovery from anemia occurs in parallel with the normalization of histological alterations of the intestinal mucosa, without iron supply, although the recovery of the iron stores occurs in only 50% of cases. At this point, the indications for iron replacement are determined by the impact of ID on symptoms, HRQL, reduced work productivity, and the deleterious effects of anemia on comorbidities, especially in older patients.

8.5. Oral or Intravenous Iron?

The decision to replenish iron stores by oral or intravenous iron administration is controversial [143–145] and depends primarily on the severity of symptoms, the tolerance and efficiency of oral iron, and those factors that predict a poor response to oral iron (e.g., severity of histological lesion; poor adherence to GFD; or blood loss due to mucosal lesions, such as concomitant IBD, jejunoileitis, or malignancy). Figure 5 is an algorithm proposed by the authors for decision making. This proposal should be validated by well-designed studies comparing the efficiency and safety of both replenishment routes in the different settings indicated and their cost-effectiveness.

8.6. Oral Iron Considerations

GFD alone may improve mild forms of IDA in patients with CD [68,72–74]. In fact, GFD is the primary means of preventing anemia in CD patients after diagnosis. However, recovery may be slow [6,83,146,147], and the administration of iron may accelerate the replenishment of iron stores in the body and thus the resolution of ID-dependent symptoms. This strategy can be useful especially in those patients with mild forms of enteropathy (Marsh 1-3a), especially if adherence to the GFD is not good. There are many barriers to following a GFD because gluten is present in many foods, and the cross-contamination is always a cause for concern. Dietary counseling that provides adequate and thorough information to the patients and their families regarding this disease and the need for lifelong adherence to a GFD is necessary. During follow-up, it is important to investigate for a micronutrient deficiency, such as of iron (a complete blood count plus serum ferritin), calcium, folic acid, vitamin B-6, and vitamin B-12 [148]. In contrast, replacement therapy with oral iron formulations is often ineffective and poorly tolerated in patients with more advanced forms of enteropathy (Marsh 3b-3c), because unabsorbed iron impregnates and irritates the duodenal mucosa and is the cause of numerous adverse effects [149]. Table 3 shows some considerations of interest in relation to oral iron replacement [150]. By way of summary:

- Ferrous sulphate (FS) is the most undertaken therapy for oral iron replacement [106,151].
- In children, the recommended iron dose is 2–6 mg/kg/day in terms of elemental iron. In adolescents and adults, it is 100–200 mg daily. Sometimes, these high doses of oral

iron cause a paradoxical decrease in iron absorption due to factors such as elevated plasma hepcidin levels [152,153]. In our practice, formulations that provide 40–80 mg of elemental iron, when administered once (80 mg) or twice (40 mg/12 h) daily, are equally effective and better tolerated.

- Toxicity associated with oral iron is higher in elderly patients, and such patients should be treated with lower doses. In fact, doses of 15, 50, or 150 mg of elemental iron may be equally effective in raising hemoglobin and ferritin levels, while adverse effects are significantly less common with lower doses [154].
- Strategies for reducing side effects and improving tolerability include:
    - Limiting the dose (≤80–100 mg of elemental iron per day).
    - Dividing the total dose and taking it in two daily doses or increasing the time between doses (e.g., every two days) [155].
    - Taking iron after dinner (reduces absorption but improves tolerance).
    - Changing the formulation (e.g., from ferrous sulphate to ferrous gluconate) or presentation used (e.g., from tablets to oral solution, which makes it easier to titrate doses).
    - Some proposed solutions to improve oral iron absorption in CD include the use of probiotics (*Lactobacillus plantarum 299v* and *Bifidobacterium lactis HN019*) [156,157] or prebiotics (oligofructose enriched inulin) [158], as well as the use of ferrous bisglycinate chelate (FBC), or the most recent Feralgine®, a compound of FBC and alginic acid that has recently been developed to improve the bioavailability and tolerability profile. Feralgine® and FBC are effective at a dosage of 30–40% compared to FS. Several studies have demonstrated the efficacy and safety of FBC in the treatment of IDA in both adults and children, without showing side effects [159–162]. In addition, recent studies conducted in adult celiac patients confirmed the good level of absorption and tolerance of Feralgine® in patients with anemia as well as in non-celiac subjects and in those with onset CD [163–165].
    - Another alternative aimed at reducing the risk of adverse effects associated with iron sulphate is sucrosomial iron (SI). SI a preparation of ferric pyrophosphate covered by a phospholipids and sucrester membrane, can be absorbed across intestinal epithelium by an alternative route, non-mediated by the DMT-1 carrier [166], which may contribute to the reduction of side effects and the prevention of iron instability in the gastrointestinal tract. A study evaluated the efficacy and safety of a new SI formulation (30 mg of iron/day) versus iron sulfate (105 mg of iron/day), in patients with CD. After a follow-up of 90 days both groups showed an increase in Hb levels compared to baseline (+10.1% and +16.2% for sucrosomial and sulfate groups, respectively), and a significant improvement in all iron parameters, with no statistical difference between the two groups. However, patients treated with SI reported a lower severity of abdominal symptoms, such as abdominal and epigastric pain, abdominal bloating, and constipation, and a higher increase in general well-being (+33% vs. +21%) compared to the iron sulfate group [167]. Therefore, SI can be effective in providing iron supplementation in difficult-to-treat populations, such as patients with CD, IDA, and known intolerance to iron sulfate.
- Response to oral iron therapy can be considered satisfactory when an increase in hemoglobin levels of at least 2 g/dL is observed within 3–4 weeks, which is also associated with an improvement in physical well-being and anemia-dependent signs and symptoms, including depapillation of the sides of the tongue, which is a good indicator of recovery. For patients with persistent anemia or ID and doubts about correct adherence to the GFD, it may be important to investigate the presence of gluten immunogenic peptides (GIPs) in fecal or urine samples, as these are present in a significant proportion of patients who declare a correct adherence to the diet. This

policy may avoid unnecessary biopsies or limit them to cases where symptoms persist despite good nutritional advice and repeatedly negative GIP results [168].

- If oral iron is not tolerated, or not absorbed due to intestinal inflammation, then intravenous iron should be given.

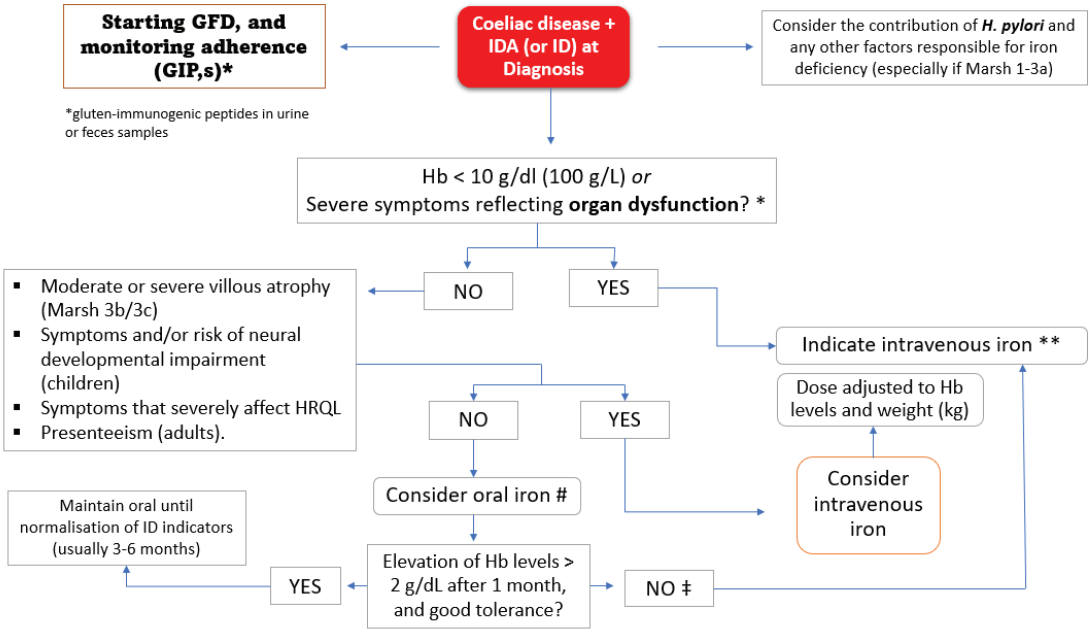


Figure 5. A proposed algorithm for iron replacement in patients with CD and IDA or ID. GFD: gluten-free diet; GIPs: gluten-immunogenic peptides in urine or feces samples; HRQL: health-related quality of life. # In the presence of mild symptoms, consider not giving oral iron and wait for resolution of lesions after GFD. ‡ Some causes of non-response to oral iron replacement include poor adherence to the GFD (consider the presence of GIPs in urine or stool samples as an indicator of poor adherence), slow histological response to the GFD ("slow responders"), refractory celiac, and blood loss due to mucosal lesions (Crohn's disease, jejunoileitis, and malignancy). (*: Consider transfusion of red blood cells.; ** See Tables 5 and 6).

8.7. Intravenous Iron Replacement Therapy

The scenarios in which parenteral iron replacement may be indicated in patients with CD and IDA or ID are diverse and are reflected in Figure 5. These can be grouped into two categories: (1) When there is a clinical need to deliver iron rapidly. Such is the case of patients with severe anemia, often of multifactorial etiology, or poorly tolerated due to the presence of comorbidities (especially in the elderly). In these cases, RBC replenishment is not sufficient to restore the depleted iron stores. (2) When oral iron preparations are not tolerated or not absorbed due to intestinal inflammation, which is highly likely in patients with poorly controlled enteropathy (total or subtotal villous atrophy), poor adherence to the GFD, or concomitant inflammatory status [169]. A third scenario to consider is that of patients with a marked deterioration in HRQL (including fatigue, weakness, poor exercise tolerance, and lack of concentration) or evident risk of neural development disorders (children's) [68,143–145,170,171]. HRQL instruments provide a means of exploring patient perceptions of the effects of IDA/ID on daily living and thus provide additional information that cannot be directly extrapolated from clinical measures. Regarding this point, it is not unusual for the deterioration in HRQL to be recognized only when the iron deposits have been restored in a rapid, safe, and effective manner [172–174].

Table 3. Guidance and considerations in relation to oral iron replacement.

Guidance and Considerations in Relation to Oral Iron Replacement
The dose of oral iron depends on patient age, the estimated iron deficit, how quickly it needs to be corrected, and side effects.
Absorption improves when iron is taken in a moderately acidic medium; therefore, it is recommended that iron be taken with ascorbic acid (250–300 mg) or half a glass of orange juice. Some ferric gluconate formulations contain ascorbic acid with 80 mg of elemental iron.
Some food components, such as phosphates, phytates, and tannates (which are found in coffee, tea, cocoa, and red wine), inhibit iron absorption. Other foodstuffs that impair iron absorption are cereals, dietary fiber, eggs, milk, and generally any foods with a high calcium content. Many of these items regularly form part of patients' breakfasts. The summary of product characteristics for most oral iron products therefore recommend taking oral iron at least 1 h before or 2 h after eating. However, although the administration of oral iron together with food decreases absorption, it improves tolerance and is one of the strategies used by many doctors in the event of side effects (see above).
Iron is best absorbed as the ferrous (Fe++) salt in a mildly acidic medium. Gastric acidity is helpful and medications that reduce gastric acid (e.g., antacids, histamine receptor blockers, proton pump inhibitors) may impair iron absorption. Other medications that impair oral iron absorption are calcium supplements and certain antibiotics (quinolones and tetracyclines), and, therefore, oral iron should be taken at least 2 h before or after these medications. Enteric-coated or sustained-release capsules are less efficient for oral absorption because iron is released too far distally in the intestinal tract (or not at all).
Gastrointestinal symptoms associated with taking oral iron are common and include metallic taste, dyspepsia, nausea, vomiting, flatulence, diarrhea, and constipation. Some patients may also be bothered by the dark green or tarry stools (they should be warned if they are to undergo a colonoscopy). As a result of this, compliance with oral iron administration may be low. The severity and impact of these effects has been demonstrated in various systematic reviews and meta-analyses of randomized studies [149,166], and they are estimated to affect 30–43% of patients, depending on the formulation used. Supplements containing smaller amounts of elemental iron are associated with less gastrointestinal toxicity, especially in elderly patients [154]. Taking iron after dinner reduces absorption but improves tolerance. The reader is referred to the recommended doses in Section 8.6 of the text.

8.8. Iron Formulations for Intravenous Use

Numerous various iron formulations for intravenous use have been developed in recent years. Their efficacy in the management of IDA is greater than the possible adverse events, and, in fact, the rates of mild reactions are ~1 in 200 and those of major reactions are ~1 in 200,000 or more [106]. Table 4 shows the characteristics of the different iron formulations available and Table 5 the advantages and limitations of oral versus intravenous iron. Figure 6 shows a simple algorithm for calculating the dose of iron to be administered based on the patient's weight and hemoglobin level. This pragmatic approach is easier to apply than the classical Ganzoni formula, which, in some contexts, may underestimate the real iron needs.

Ganzoni Equation for Iron Deficiency Anemia

$$\text{Total, iron deficit (mg)} = \text{Body weight (kg)} \times (\text{Target Hb} - \text{Actual Hb})\ [\text{g/dL}] \times 2.4 + \text{iron stores (mg)}$$

Target Hb: 13 g/dL for a body weight of less than 35 kg and 15 g/dL for a body weight of more than 35 kg.

Factor 2.4 = 0.0034 × 0.07 × 10,000, where:

- 0.0034: iron content of hemoglobin (0.34%).
- 0.07: blood volume 70 mL/kg of body weight = 7% of body weight.
- 10,000: conversion factor 1 g/dL = 1000 mg/L.
- iron stores (mg): 500 mg if the body weight is greater than 35 kg or 15 mg/kg if the body weight is less than 35 kg.

Table 4. Characteristics of the different iron formulations available [1].

Brand Name	Venofer®, Feriv® (Iron Sucrose)	Ferlixit®, Ferrlecit® (Fe-Gluconate) [1]	CosmoFer® (Iron Dextran)	Ferinject® (Ferric Carboxymaltose)
Indication	Iron deficiency	Iron deficiency	Iron deficiency	Iron deficiency
Max. iron dose in one infusion	200 mg	125 mg (12.5 mg/mL) [2,3]	20 mg/kg of body weight	1000 mg
Duration of the dose by injection	30 min	1 h	4–6 h	15 min
Max. iron dose by injection	200 mg (3 times/week)	125 mg (12.5 mg/mL) [2,3]	200 mg (3 times/week)	1000 mg (once/week)
No. of hosp. visits for adm. 1000 mg	5	8	1 by infusion 5 by injection	1

[1] New preparations such as Fe-isomaltoside (Monofer®) and ferumoxytol (Ferraheme®) are currently being studied. [2] Product used to treat iron deficiency anemia in adults and children six years and older with chronic kidney disease receiving hemodialysis and supplemental epoetin therapy. [3] Ferrlecit is available in generic form. Data from Ferrlecit postmarketing spontaneous reports indicate that individual doses exceeding 125 mg may be associated with a higher incidence and/or severity of adverse events.

Table 5. Comparative of oral and intravenous iron in the management of IDA.

Oral vs. Intravenous Iron Replacement	Advantages	Limitations
Oral iron	■ Effective in many patients at right dose ■ Low cost ■ Low serious adverse effects	■ Low efficiency in malabsorptive states ■ Poor gastrointestinal tolerance ■ Unsuitable in cases of continuous occult gastrointestinal bleeding (e.g., in the presence of concomitant mucosal lesions) ■ It may take a long time to replenish iron stores
Intravenous iron	■ Effective in most cases ■ Faster correction of anemia ■ High doses administered in a single infusion ■ Adherence is guaranteed ■ No gastrointestinal side effects	■ Intravenous infusion requires monitoring ■ Infusion-related reactions and allergies have been reported ■ Special equipment and trained staff are required to treat potential infusion-related reactions ■ High initial cost

8.9. Intravenous Iron in Children

ID with or without anemia is a common complication of pediatric CD, causing significant morbidity. Despite this, ID remains prevalent and undertreated, related in part to questions surrounding optimal formulation and route of administration. In addition, its application in daily management has been overlooked, partly due to the fear of possible adverse events related to historical anaphylactic reactions associated with iron dextran formulations [101,174]. However, in the case of severe anemia, it might be taken into consideration in order to rapidly correct the hematological picture. Some studies have shown that intravenous iron administration is safe and effective in children with various diseases leading to IDA. Carman et al. conducted a study on a total of 101 pediatric patients 6–18 years of age with IBD and iron deficiency (ID) or iron deficiency anemia (IDA). Patients received ferric carboxymaltose (FCM), a recent formulation of intravenous iron, allowing higher doses and rapid infusion times. Following FCM infusion as a single dose of 15 mg/kg up to 1000 mg over 15–20 min, 64% of patients with IDA had resolution of anemia, with 81% showing resolution for ID without anemia [175]. Similar data have been reported by other authors [176,177].

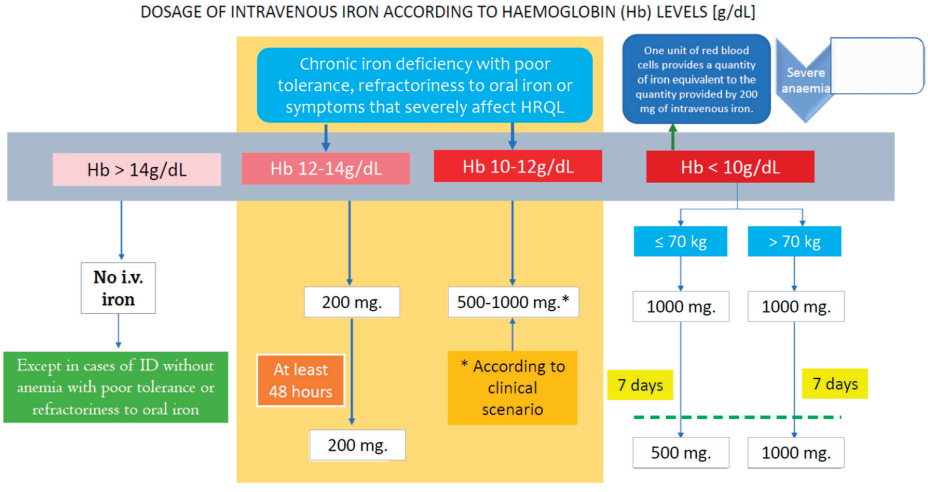


Figure 6. Dosage of intravenous iron according to hemoglobin (Hb) levels (g/dL) (adults). * Dosage may vary depending on the patient's clinical condition and the physician's clinical judgment. Hb: haemoglobin; h: hours; kg: kilograms of patient weight.

CD (and, for that matter, any other disease causing villous atrophy) is a classic scenario where the results of oral iron replacement are poor due impaired absorption inherent to mucosal injury. These children could benefit from a strategy focused on intravenous iron administration under certain conditions (see below). In a retrospective cohort study, a total of 116 IV iron carboxymaltose infusions were administered to 72 patients with IDA refractory to oral iron and were shown to be safe and highly effective in a small yet diverse population of infants, children, and adolescents [178,179].

Among the available intravenous iron preparations, only iron sucrose [180,181], iron carboxymaltose [175,176,178,179,182], and low molecular weight iron dextran [183] have been studied in children, but, to date, none has a pediatric indication. A panel of experts led by Mattiello (SPOG Pediatric Hematology Working Group) has recently provided ID management recommendations based on the best available evidence in response to one of the most common challenges faced by pediatricians [184]. In accordance with these recommendations, IV iron administration can be considered a first-line strategy in patients with chronic IBD or situations with proven malabsorption and a second-line strategy after consultation with a specialist in pediatric iron metabolism (certified pediatric hematologist) (Table 6) under specific conditions:

(1) Failure to achieve correction of IDA after well-conducted oral iron substitution in the setting of good adherence of at least six months of prescribed supplementation and two formulation attempts.
(2) Confirmed malabsorption or chronic oral iron intolerance, including the category of children with severe neurological/neurodevelopmental impairments leading to feeding limitations.

Table 6. Recommendations for the administration of intravenous iron.

Recommendations for the Administration of Intravenous Iron
■ Intravenous iron preparations should only be used at centers that have immediate access to emergency treatments for hypersensitivity reactions.
■ The administration of a test dose is not recommended since cases of allergic reactions have been reported in patients who had previously tolerated the product well. The patient should be monitored for at least 30 min after administration.
■ Intravenous iron preparations are contraindicated in patients who are hypersensitive to any of the components of the medication and should not be used in patients who have suffered severe hypersensitivity reactions to a different preparation.
■ Special care should be taken in patients with known allergies to other medications or with immune or inflammatory diseases, such as patients with a history of asthma or eczema or atopic patients.
■ These preparations should only be used during pregnancy if they are clearly necessary, and their use should be restricted during the second and third trimesters to protect the fetus from potential adverse effects as much as possible.
■ Finally, it is important to remember to report all suspected adverse reactions to the corresponding Autonomous Pharmacovigilance Centre.

8.10. Adverse Effects and Contraindications Related to the Use of Intravenous Iron

Data from studies in adults show that IV iron is contraindicated in the course of infections, in the first trimester of pregnancy, and in patients with a history of iron or of another significant (i.e., anaphylactic) drug allergy. Immediate side effects of an IV iron infusion can be nausea, vomiting, headache, flushing, myalgia, pruritus, arthralgia, and back and chest pain. Hypophosphatemia can be observed but is usually transient and asymptomatic. Possible adverse events can be easily managed if suitable measures are implemented to ensure early diagnosis and effective management of allergic reactions (Tables 6 and 7) [150].

Table 7. Actions to be taken in the event of adverse effects.

Actions to Be Taken in the Event of Adverse Effects
MILD
■ Discontinue the infusion until all symptoms disappear ■ Restart the infusion at a slower rate (slower infusion rate)
MODERATE
■ Discontinue the infusion ■ Administer 1 mg/kg of intravenous methylprednisolone ■ Monitor the patient for 4 h or until all symptoms disappear
SEVERE
■ Administer 1000 mL of saline solution, oxygen (if required), 0.5 mg of intramuscular adrenaline, and 200 mg of intravenous hydrocortisone and admit to hospital if necessary.

9. Summary and Conclusions

ID with or without anemia is a common complication in both children and adults with CD, causing significant morbidity and impairment of HRQL. In some cases, IDA/ID is the main or even the only clinical manifestation of the disease. Importantly, the presence of IDA or ID that does not reverse after oral iron administration should strongly raise the suspicion of CD to ensure that diagnosis is not delayed. Folate and vitamin B12 malabsorption, nutritional deficiencies, inflammation, blood loss, development of refractory CD, and concomitant *H. pylori* infection are other causes of anemia in such patients. Once IDA and ID are detected, the physician must restore iron stores to avoid the deleterious effects of red blood cell mass loss on physical, emotional, and psychological well-being. The decision to replenish iron stores by oral or intravenous iron administration is controversial and depends primarily on the severity of symptoms, its impact on the HRQL, the tolerance and efficiency of oral iron, and those factors that predict a poor response to oral iron

(e.g., the severity of the histological lesion, poor adherence to GFD, and blood loss due to mucosal lesions).

Only when severe anemia is present, with hemoglobin values below 5–6 g/dL (which requires rapid correction such as in patients with cardiac dysfunction) can RBC transfusions be performed following the principles of the PBM strategy.

Finally, the high percentage of subjects with IDA who are celiac reinforces the need for screening CD in patients with IDA or ID.

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