

Article

Effect of Acute Consumption of Crackers Enriched with Grape Seed Flour or Barley Flour with Added β -Glucan on Biomarkers of Postprandial Glycemia, Lipidemia, and Oxidative Stress: A Crossover Study

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Citation: Chatziharalambous, D.; Papagianni, O.; Potsaki, P.; Almpounioti, K.; Koutelidakis, A.E. Effect of Acute Consumption of Crackers Enriched with Grape Seed Flour or Barley Flour with Added β -Glucan on Biomarkers of Postprandial Glycemia, Lipidemia, and Oxidative Stress: A Crossover Study. *Appl. Sci.* **2024**, *14*, 4591. <https://doi.org/10.3390/app14114591>

Academic Editors: António José Madeira Nogueira and Andrea Luísa Fernandes Afonso

Received: 17 April 2024

Revised: 22 May 2024

Accepted: 23 May 2024

Published: 27 May 2024



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Abstract: Background: Grape seed polyphenol bioactivity is linked to reduced risk of metabolic syndrome, type 2 diabetes, obesity, and coronary heart disease development. Furthermore, regular consumption of β -glucan is associated with decreased lipidemic and glycemic profiles. The aim was to investigate the acute effect of crackers enriched with either 10% grape seed flour or 40% barley flour with added β -glucan along with a high-fat and high-carbohydrate meal on biomarkers of postprandial glycemia, lipidemia, and oxidative stress. Methods: In a randomized, three-arm crossover design study, 12 healthy subjects were assigned to consume breakfast consisting of bread, butter, and 250 mL water along with crackers containing either (a) wheat flour, (b) 10% grape seed flour, or (c) 40% barley flour enriched with β -glucan. Blood samples were drawn immediately before and 30, 90, and 180 min after the meal. Total antioxidant capacity was measured in plasma with the FRAP method. Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, and uric acid were measured in serum. Results: Tested markers did not differ between the intervention groups at baseline, 30, 90, and 180 min ($p \geq 0.05$) post-prandially. Conclusions: Enriched cracker consumption did not significantly affect the selected markers at the postprandial state, although better serum glucose and lipid levels, similar to baseline values, were maintained.

Keywords: grape seed flour; barley flour; crackers; glycemia; oxidative stress; lipidemia

1. Introduction

Chronic diseases such as obesity, type 2 diabetes mellitus (T2D), metabolic syndrome (MetS), and atherosclerosis, the prevalence of which has increased over the last couple of years, are related to the postprandial state, as postprandial glycemia and lipidemia are critically linked to their onset. Western lifestyle diets lead to the postprandial increase of glucose, triglycerides, and insulin and, therefore, to the induction of postprandial oxidative stress [1]. The consumption of either a high-fat or high-carbohydrate diet or even a combination of both can elicit postprandial inflammation and, hence, the generation of reactive oxygen species (ROS). Oxidative stress-induced damage and inflammation have been reported to play a significant role in the pathogenesis of chronic diseases [2]. Therefore, there is a growing demand for functional food products as the chronic diseases described above can be targeted through a healthy nutrition plan. Functional foods incorporated into a daily nutrition plan could decrease the risk of chronic diseases through the supplementation of bioactive compounds [3]. Over the years, studies have provided evidence for the protective

effects of various polyphenol-rich foods against chronic diseases. Fruits and cereals are rich in phenolic compounds, while grape seeds represent rich sources of polyphenols. Proanthocyanidins, anthocyanins, flavonols, phenolic acids, and stilbenes are the main phenolic compounds found in grapes [4]. The bioavailability of dietary polyphenols, including those present in grapes, has been broadly reported [5,6]. Following ingestion, polyphenols are detected in plasma, interacting with lipoproteins [7]. They represent potent antioxidants as their ability to inhibit risk factors such as hyperlipidemia, hyperglycemia, inflammation, and hypertension, which are involved in chronic diseases such as diabetes and metabolic syndrome, has been broadly reported [8–10].

Grape seed contains the majority of polyphenols, including mainly proanthocyanidins [11], which have been studied for a number of health properties, such as their antioxidant and free-radical scavenging activity, as well as their potential to be used as nutritional supplements for atherosclerosis and cardiovascular disease prevention and in dyslipidemia treatment. There are also many studies indicating gastro-protective, hepato-protective, and anti-obesity effects of grape polyphenols [12–14]. Grape seed flour is a novel exploited flour that possesses a high fiber content and antioxidant capacity due to its rich phenolic content, which includes phenolic acids, flavonoids, and proanthocyanidins [15]. In many scientific studies, grape seed flour has been used for bakery product fortification in, for example, cookies [16,17], butter biscuits [18], bread [19], cereal bars, and pancakes [20]. Therefore, grape seed flour could represent a promising alternative to wheat flour to improve the nutritional properties of bakery products [21]. Epidemiological, *in vitro*, and animal studies have suggested a beneficial effect of polyphenols present in grapes and grape-derived food products in metabolic syndrome biomarkers; however, human clinical trials have demonstrated controversial results [22].

Barley grain represents a great cereal resource of functional nutrients including dietary fiber, phenolic acids, folate, vitamin E, lignans, flavonoids, and phytosterols. Due to its rich composition in bioactive molecules, barley has been reported to possess cardio-protective, anti-diabetic, and antioxidant properties [23]. Dietary fiber is widely applied for the improvement of lipid profiles and its intake has been linked with a reduction in inflammation [24], insulin resistance [25], and cardiovascular disease [26]. Especially viscous fiber β -glucan, abundant in barley grain, has been specifically linked with the improvement of cardiovascular disease-specific biomarkers [27]. The related mechanisms of action involve the reduction of bile acid reabsorption, the modification of colonic microflora metabolism, and the reduction of cholesterol absorption, resulting in a reduction in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL) levels [28,29]. Cereal β -glucan health claims, approved by the European Food Safety Authority (EFSA), involve their implication in the reduction of post-prandial glycemic responses as well as blood cholesterol levels [30,31]. Barley is a cereal grain rich in dietary fiber that contains high levels of β -glucan compared to other cereal grains. It has been incorporated into breads and biscuits [32–34], pasta [35], noodles [36], and chips and tortillas [37,38], as barley consumption on a regular basis may help to minimize blood sugar spikes and prevent insulin resistance due to its low glycemic index. Even though the beneficial action of β -glucan in reducing total and LDL-cholesterol levels is well documented, thus recommending a minimum intake of 3 g/day of soluble fiber from barley or oats [39], results on its effect on glucose levels are inconclusive [40].

Therefore, the aim of this study was to investigate the direct effect of crackers enriched with (i) 10% grape seed flour or (ii) 40% barley flour with added β -glucan along with a high-fat and high-carbohydrate meal on biomarkers of postprandial glycemia, lipidemia, and oxidative stress in a healthy cohort.

2. Materials and Methods

2.1. Study Participants

This study was carried out at the Human Nutrition Unit of the Department of Food Science and Nutrition of the University of the Aegean and the protocol of the study was approved by the University of the Aegean Ethics Committee. This study was also registered

in ClinicalTrials.gov (ID#NCT05977543, Trial Registration: ClinicalTrials.gov NCT05977543) and was conducted in accordance with the principles established in the Declaration of Helsinki and the Principles of Good Clinical Practice. All participants were provided with information where the aims, methods, and outcomes of the study were described in full detail. The members of the lab assured participants of the confidentiality of their data and their voluntary participation. Furthermore, written consent was obtained from all participants before the onset of the study.

A total of 12 ($n = 12$) healthy participants, 4 men and 8 women aged 19–65 years old from Lemnos Island, Greece, were recruited from December 2022 to January 2023 after an initial screening of 24 volunteers. Initially, the screening of volunteers was conducted by the completion of a questionnaire where their demographic characteristics, medical records, frequency of consumption of foods rich in polyphenols, and levels of physical activity were reported. Anthropometric data were also collected by all volunteers in the initial screening. This study excluded subjects over 65 years old; those with a history of chronic illnesses including diabetes, metabolic syndrome, cardiovascular disease or cancer; and heavy smokers and drinkers. Biochemical blood tests were also conducted on volunteers during the initial screening in order to exclude cases with hematological and biochemical medical conditions.

2.2. Study Meals

The control meal consisted of two slices of white bread weighing 80 g (2 slices of 40 g), 3 bars of unsalted butter (Lurpak, Arla Foods, Aarhus, Denmark) weighing 30 g, and wheat flour crackers weighing 55 g (3 pieces). The composition of the functional meals was the same as the control meal, except that they contained 55 gr of the enriched crackers (either 10% grape seed flour crackers or 40% barley flour crackers enriched with β -glucan, 3 pieces). The 10% grape seed flour crackers were formulated according to the recipe mentioned in a previous publication of our research team, whereas the 40% barley flour crackers were formulated using the recipe found in the same publication but with the addition of β -glucan in order to receive 3 gr β -glucan/3 crackers and meet the EFSA health claim [41]. Each meal weighed 165 g. The nutritional composition of the meals is shown in Table 1.

Table 1. Nutritional composition of the meals.

	Control Meal	Functional Meal 1	Functional Meal 2
Energy (kcal)	599.90	597.66	580.77
Fat (total, g)	33.06	33.20	32.48
Carbohydrates (g)	63.55	61.51	60.89
Sugars (total, g)	6.27	6.31	5.73
Dietary fiber (g)	3.18	4.52	5.64
Protein (g)	11.21	11.29	10.62

2.3. Study Design

This was a three-period, cross-over, acute interventional study with a one-week washout period between treatments. The participants were randomly assigned to the C: F1:F2 group (Control: Functional 1: Functional 2), the F1:C:F2 group (Functional 1: Control: Functional 2), or the F2:F1:C group (Functional 2: Functional 1: Control). Participants in the C:F1:F2 group received the control meal on the first visit, functional meal 1 on the second visit, and functional meal 2 on the third visit; those in the F1:C:F2 group received functional meal 1 on the first visit, the control meal on the second visit, and functional meal 2 on the third visit; and those in the F2:F1:C group received functional meal 2 on the first visit, functional meal 1 on the second visit, and the control meal on the third visit. The study design is illustrated in Figure 1.

Participants were recruited by the Human Nutrition Unit on three separate days (control meal, functional meal 1, functional meal 2) at 09:00 a.m., following a 12 h overnight

fast. They filled out a short recall questionnaire referring to the meals consumed over the last 24 h and a fasting blood sample was collected from the forearm vein (referred to as 0 min-baseline). Participants then consumed 165 g of either the control or the functional meals, depending on their group allocation, along with one cup of water (250 mL) within 15 min. Blood samples (10 mL) were drawn at 30, 90, and 180 min following meal consumption.

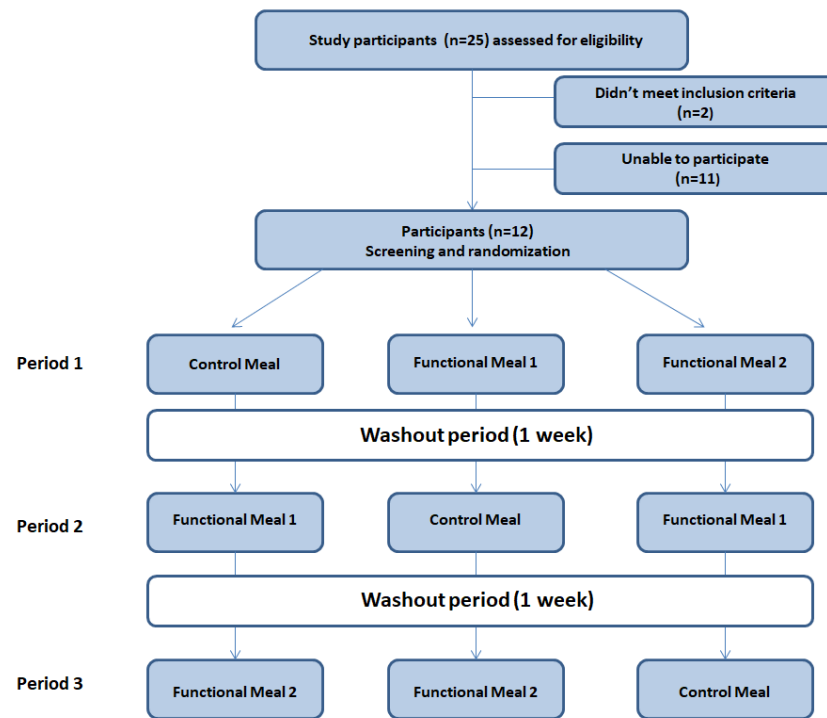


Figure 1. Study design diagram.

Ethylenediaminetetraacetic acid (EDTA)- and citric acid-treated tubes were used for plasma separation and heparinized tubes for serum separation. Plasma and serum samples were separated by centrifugation for 15 and 10 min, respectively, at $3500 \times g$ in a clinical centrifuge after blood sample collection. Aliquots of plasma and serum samples were stored at -40°C until the analysis.

2.4. Blood Samples Analysis

2.4.1. Total Antioxidant Activity of Plasma

The total antioxidant activity of plasma was measured using the FRAP method. A total of 10 μL of plasma was mixed with 300 μL FRAP reagent in a 96-well microplate. The samples were analyzed in triplicates. The 96-well microplate was incubated at room temperature for 5 min and the relative absorbance was measured at 600 nm with a spectrophotometer using Magellan™ Data Analysis Software (version 7.2). The data were expressed as FeSO_4 concentrations (mmol/mL).

2.4.2. Biochemical Biomarkers

Biochemical biomarkers including blood glucose, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides, and uric acid were measured in serum samples with an automated analyzer (COBAS c111, Roche, Switzerland).

2.5. Statistical Analysis

Statistical analysis was performed by IBM SPSS Statistics (Version 17, Chicago, IL, USA). Results were presented as means $\pm$ SD and significance was set at $p \leq 0.05$. Before the statistical analysis, all variables were tested for normal distribution, which was confirmed

through the Kolmogorov–Smirnov test. Repeated ANOVA (analysis of variance) measures were performed on differences between plasma and serum samples at 30 min, 90 min, and 180 min for each intervention and compared to baseline data with respect to venous plasma TAC and serum biochemical biomarkers concentrations. The differences between the two tested groups at every time point and every time point with respect to baseline were also tested by paired *t*-tests. Additionally, the Eta-squared value was calculated for every meal between baseline and 180 min values to determine the size effect on all biomarkers. Cohen’s *d* value was also calculated at 180 min between control and functional meals for glucose, triglycerides, total cholesterol, and LDL concentrations to evaluate the effect of the difference between their means, as lower values were obtained for the functional meals but not at the significance level [42].

The effective sample size of the participants was calculated by GPower statistical software version 3.1.9.2 and a minimum of 12 participants was estimated to observe biologically significant differences between interventions on the tested biomarkers, such as total antioxidant capacity. Considering a probability of 95% that the study would detect a treatment difference at a two-sided 0.05 significance level, the sample of 12 individuals allowed for the detection of a difference of 0.40 mmol TAC/mL between the control group and the two intervention groups.

3. Results

3.1. Baseline Characteristics of the Participants

Twelve participants completed the study, while two volunteers were unable to attend two study visit appointments. The participant characteristics at screening are presented in Table 2. All participants but one were non-smokers.

Table 2. Participant characteristics.

Variable	
General Characteristics	N
Volunteers	12
Men	4
Women	8
Smoking	1
Physical activity	
Low	5
Medium	6
High	1
	Mean ± SD
Age (years)	42.8 ± 17.4
Anthropometry and Body Composition	
Weight (kg)	79.9 ± 17.8
Height (cm)	169.9 ± 10.3
BMI	27.7 ± 6.1
Fat Mass (kg)	23.1 ± 12.6
Muscle Mass (kg)	53.9 ± 14.3
Body water (kg)	36.7 ± 8.3
Bone Mass (kg)	2.8 ± 0.7
Waist/hip circumference ratio	0.8 ± 0.1

3.2. Effect of Control and Functional Meal Consumption on Biochemical Biomarkers

Figures 2–8 show the effect of the acute consumption of the high-carbohydrate and high-fat meal with novel wheat flour crackers enriched with 10% grape seed flour (functional meal 1) or 40% barley flour with added β -glucan (functional meal 2) or the same meal with the non-enriched wheat flour crackers (control meal) on serum and plasma biochemical parameters. Specifically, mean values of serum glucose, cholesterol, triglycerides,

HDL and LDL cholesterol, uric acid, and plasma antioxidant capacity are presented at baseline, 30 min, 90 min, and 180 min after the consumption of the meal.

Regarding glucose levels, no significant overall changes were observed between interventions in all participants. During all interventions, glucose levels increased significantly at 30 min ($p = 0.000$ for control and functional meals) and decreased significantly for all meals at 180 min compared to baseline values ($p = 0.018$ for the control meal, $p = 0.001$ for functional meal 1, and $p = 0.011$ for functional meal 2) (Figure 2). Calculation of the Eta-squared in the paired t -tests conducted between baseline and 180 min revealed a larger effect size of the grape flour and barley flour with added β -glucan crackers on glucose concentration than wheat flour crackers ($\eta^2 = 0.806$ and 0.502 for functional meal 1 and 2, respectively, and $\eta^2 = 0.442$ for the control meal). The consumption of the functional meals did not significantly change the glucose response ($p = 0.153$), and no significance was observed either between time or intervention ($p = 0.691$), although slightly lower post-prandial glucose levels were observed after the functional meals compared to the control meal. Calculation of the Cohen's d value between the control and functional meal 1 at 180 min indicated a small effect difference ($d = 0.38$).

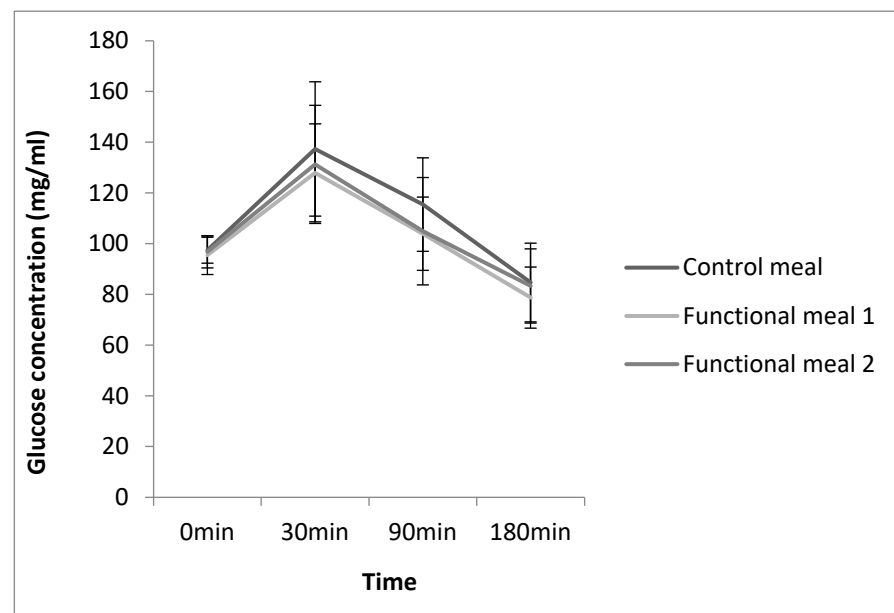


Figure 2. Glucose concentration at 30, 90, and 180 min following meal consumption.

Compared to baseline values, triglyceride levels increased between 30 and 90 min in all interventions for all participants. More specifically, between baseline and 30 min, for the control meal, $p = 0.324$; for functional meal 1, $p = 0.192$; and for functional meal 2, $p = 0.140$. Between baseline and 90 min, for the control meal, $p = 0.010$; for functional meal 1, $p = 0.04$; and for functional meal 2, $p = 0.007$. Additionally, 3 h post-prandially, triglyceride levels were similar to the baseline values for the functional meals ($p = 0.993$ for functional meal 1 and $p = 0.816$ for functional meal 2), whereas, for the control meal, they were slightly higher ($p = 0.174$). Calculation of the Eta-squared in the paired t -test conducted for the control meal between baseline and 180 min revealed a large size effect ($\eta^2 = 0.376$), indicating a higher difference in post-prandial levels compared to baseline. For the functional meals, Eta squared was below 0.01, which was close to baseline post-prandial levels. There was a statistically significant difference between the control and functional meal 2 at 180 min ($p = 0.021$), whereas no statistical difference was observed between the control and functional meal 1 ($p = 0.162$) (Figure 3). Furthermore, Cohen's d value at 180 min between the control and functional meal 1 indicated a low to medium effect difference ($d = 0.43$), whereas, between the control and functional meal 2, there was

a medium to high effect difference ($d = 0.77$). There was no significant difference between interventions ($p = 0.728$) or between time and intervention ($p = 0.739$).

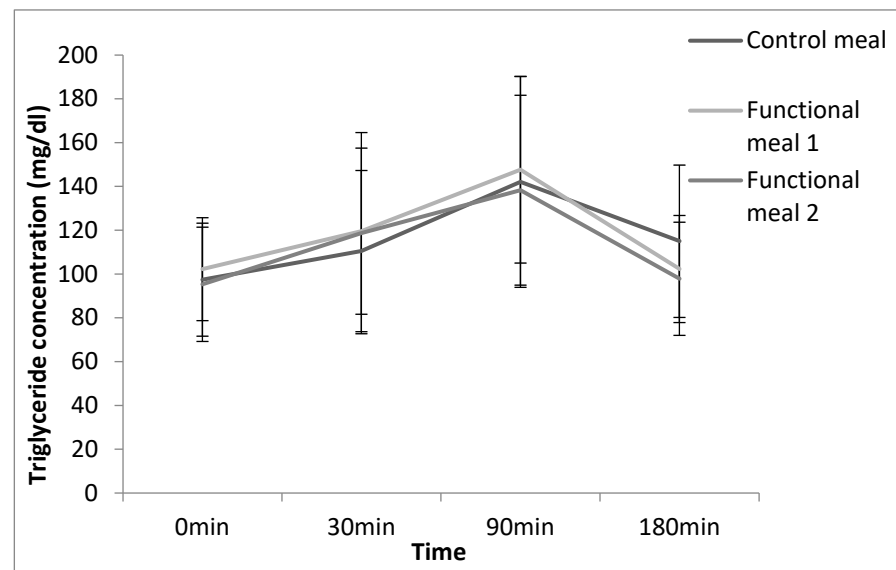


Figure 3. Triglyceride concentration at 30, 90, and 180 min following meal consumption.

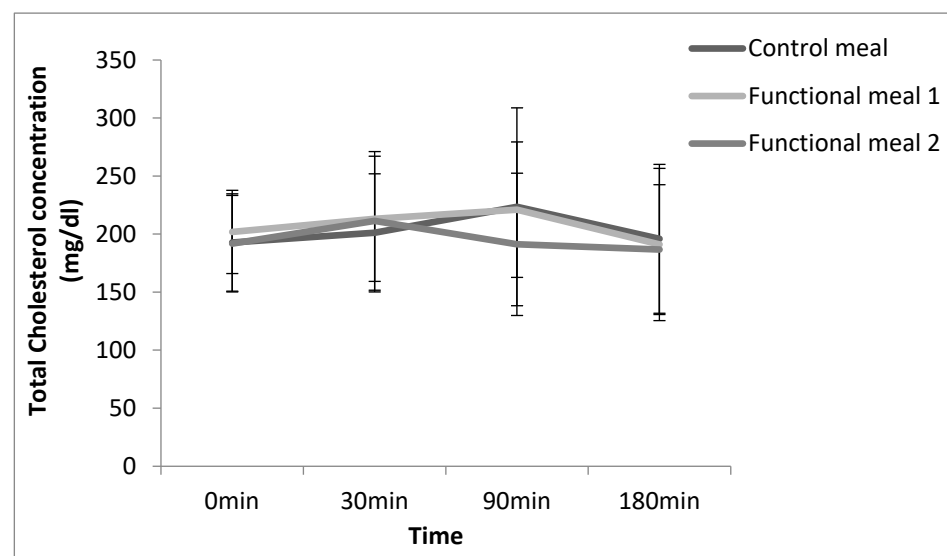


Figure 4. Total cholesterol concentration at 30, 90, and 180 min following meal consumption.

Regarding total cholesterol levels, a non-significant increase was observed for all meals at 30 min ($p = 0.666$ for the control meal, $p = 0.550$ for functional meal 1, and $p = 0.359$ for functional meal 2). The highest values for functional meal 1 and the control meal were observed at 90 min, whereas, for functional meal 2, they were observed at 30 min post-prandially (Figure 4). Values did not differ significantly from baseline for all meals ($p = 0.585$) or between time and intervention ($p = 0.670$). Overall, non-significantly lower concentrations compared to baseline were observed for the functional meals ($p = 0.623$ for functional meal 1 and $p = 0.803$ for functional meal 2), whereas, for the control meal, they were higher ($p = 0.887$). Calculation of Eta-squared in the paired t -tests conducted in all meals between baseline and 180 min revealed a low size effect (below 0.1) of the control and functional meal 2 on the biomarker concentration, whereas a moderate effect ($\eta^2 = 0.07$) was observed for functional meal 1. Cohen's d at 180 min indicated small size

effect differences between the control and functional meals 1 and 2 ($d = 0.08$ and $d = 0.16$, respectively).

HDL cholesterol levels increased non-significantly for all meals at 30 min ($p = 0.932$ for the control meal, $p = 0.398$ for functional meal 1, and $p = 0.485$ for functional meal 2), whereas the highest values for the control and functional meal 1 were observed at 180 min. No statistically significant differences were observed in HDL cholesterol levels between the control and functional meals 1 and 2 ($p = 0.937$ and $p = 0.196$, respectively). For functional meal 2, HDL cholesterol levels decreased at 90 min. At 180 min, they did not differ significantly from baseline ($p = 0.496$) (Figure 5). Calculation of Eta-squared in the paired t -tests conducted for all meals between baseline and 180 min revealed a low size effect of the control and functional meal 2 ($\eta^2 = 0.011$ and 0.045 , respectively) and a moderate effect of functional meal 1 ($\eta^2 = 0.064$). For all interventions, slightly lower levels were observed compared to baseline, with no significant difference between meals ($p = 0.323$) or between time and intervention ($p = 0.217$).

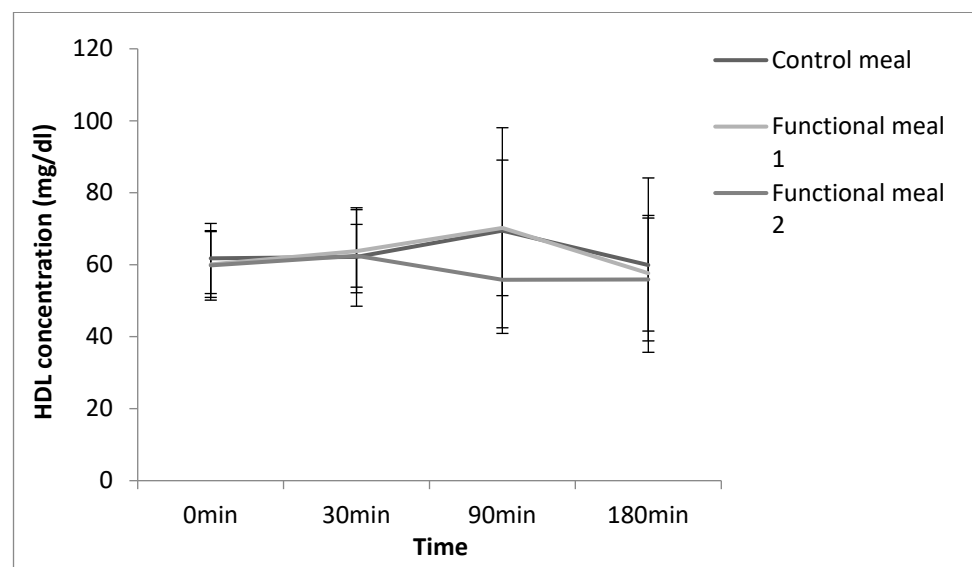


Figure 5. HDL cholesterol concentration at 30, 90, and 180 min following meal consumption.

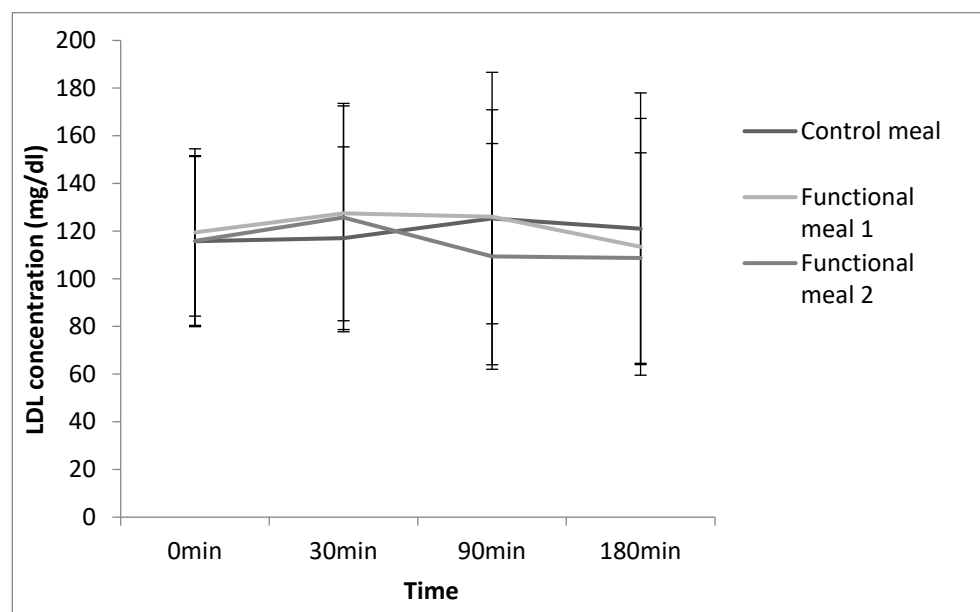


Figure 6. LDL cholesterol concentration at 30, 90, and 180 min following meal consumption.

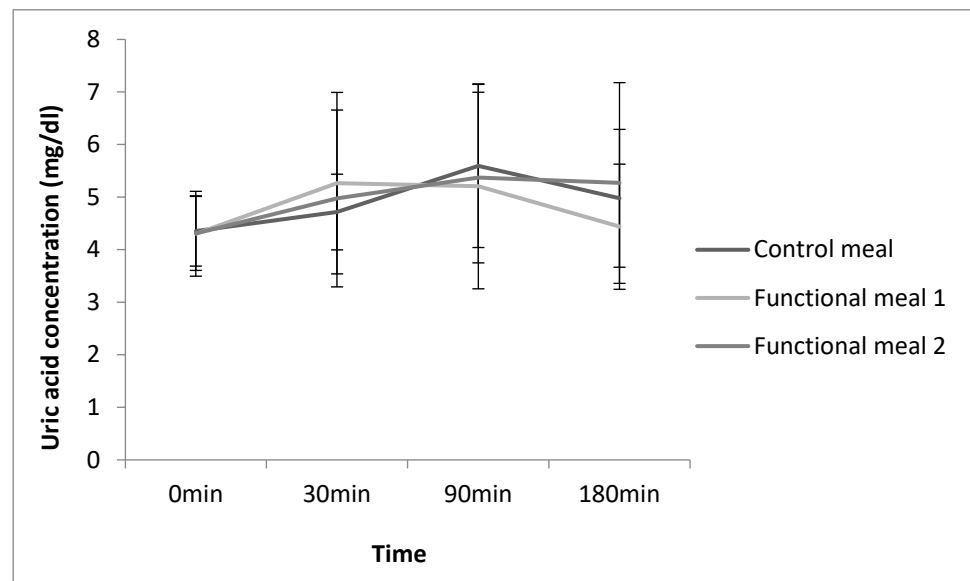


Figure 7. Uric acid concentration at 30, 90, and 180 min following meal consumption.

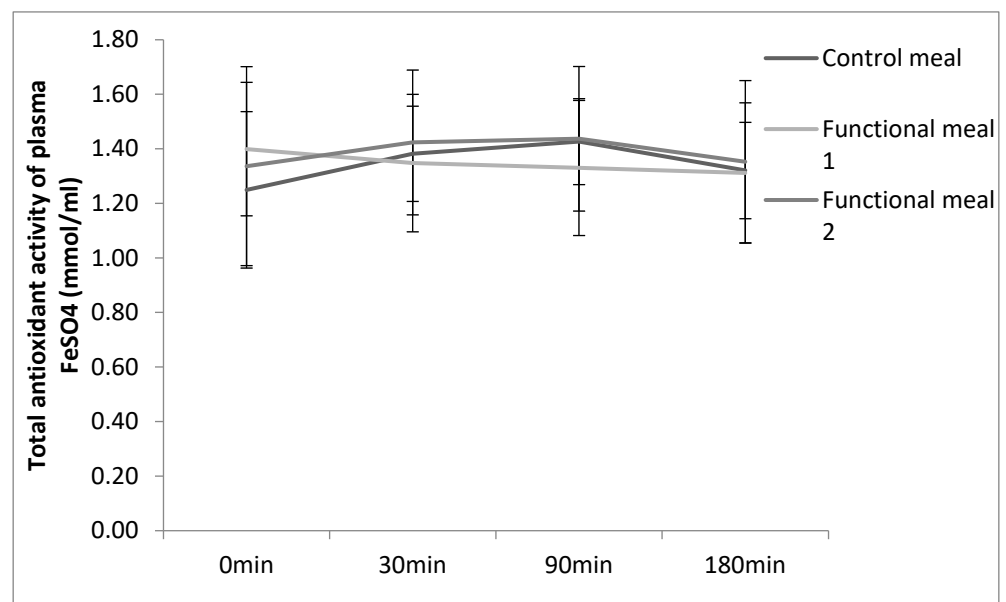


Figure 8. Total antioxidant activity of plasma at 30, 90, and 180 min following meal consumption.

LDL cholesterol levels also increased but not at the significance level for all meals at 30 min ($p = 0.936$ for the control meal, $p = 0.632$ for functional meal 1, and $p = 0.574$ for functional meal 2). The highest concentrations were observed at 90 min for the control and functional meal 1, whereas, for functional meal 2, they were observed at 30 min. At 180 min, LDL levels decreased non-significantly for the functional meals ($p = 0.749$ for functional meal 1 and $p = 0.666$ for functional meal 2), whereas, for the control meal, they were non-significantly increased compared to baseline ($p = 0.791$) (Figure 6). Calculation of Eta-squared in the paired t -tests conducted for all meals between baseline and 180 min showed small size effects of all meals on LDL concentration ($\eta^2 = 0.027$ for the control meal, 0.039 for functional meal 1, and 0.029 for functional meal 2). Cohen's d at 180 min between the control and functional meals indicated small effect differences ($d = 0.30$ for the control and functional meal 1 and $d = 0.15$ for the control and functional meal 2). LDL cholesterol levels did not differ significantly between interventions ($p = 0.372$) or between time and intervention ($p = 0.362$).

Uric acid levels increased non-significantly for all meals at 30 min ($p = 0.220$ for the control meal, $p = 0.100$ for functional meal 1, and $p = 0.225$ for functional meal 2). Uric acid values did not differ significantly between meals ($p = 0.870$) or between time and intervention ($p = 0.070$) and were similar to baseline for the control ($p = 0.163$) and functional meal 1 ($p = 0.751$), but, for functional meal 2, they were non-significantly increased ($p = 0.125$) (Figure 7). Calculation of Eta-squared in the paired t -tests conducted for all meals between baseline and 180 min indicated a high size effect of functional meal 2 and the control ($\eta^2 = 0.27$ and 0.17 , respectively) on biomarkers as the concentrations were elevated compared to baseline levels, whereas, for functional meal 1, a low size effect ($\eta^2 = 0.01$) was detected, as values appeared quite close to baseline.

Regarding the antioxidant capacity of plasma, for the control and functional meal 2, a non-significant increase was observed at 30 min ($p = 0.187$ for the control meal and $p = 0.512$ for functional meal 2) and from 30 to 90 min ($p = 0.516$ for the control meal and $p = 0.472$ for functional meal 2), whereas a gradual slight decrease was observed for functional meal 1 ($p = 0.618$ at 30 min and $p = 0.863$ from 30 to 90 min) (Figure 8). Calculation of Eta-squared in the paired t -tests conducted for all meals between baseline and 180 min indicated a moderate size effect of the control meal ($\eta^2 = 0.03$), as the values appeared slightly higher to baseline; a small size effect of functional meal 2 ($\eta^2 = 0.001$), as values were similar to baseline; and a large size effect of functional meal 1 ($\eta^2 = 0.21$) on the biomarker, as lower values were observed compared to baseline. The antioxidant capacity values were similar to baseline for all meals and there was no significant difference between interventions ($p = 0.153$) or time and intervention ($p = 0.691$).

4. Discussion

A polyphenol-rich diet is associated with several health benefits, including a reduction of the risk of cardiovascular diseases, type 2 diabetes, obesity, and metabolic syndrome. Over the last few years, growing attention has been paid to polyphenols from grapes for their potential to reduce cardiovascular and metabolic disease onset due to their ability to improve associated risk factors [43], reduce oxidative stress [44] and inflammatory status [45], and increase antioxidant capacity [46]. Furthermore, increasing β -glucan intake has been associated with improved glycemic control and a subsequent reduction in diabetes development. Barley β -glucans form a highly viscous solution in the stomach and small intestine, thereby prolonging nutrient transit and absorption through the gut, and have the ability to affect glucose and lipid metabolism. Many studies have demonstrated the LDL cholesterol-lowering effect of products enriched with β -glucan, whereas a number of other studies have not.

Regarding the study of the acute consumption of grape seed flour-enriched bakery products, no similar clinical trials exist in order to directly compare the results of the present study. Limited data concerning grape seed extract intervention studies in healthy cohorts exist in the literature; therefore, comparisons between those findings and the ones demonstrated by our study were performed. Furthermore, concerning barley flour and β -glucan addition in crackers, we are the first to report their acute effect in a healthy cohort as the literature findings retrieved indicated their effect on other bakery products. Therefore, we were the first to assess the effects of the administration of crackers enriched either with 10% grape seed flour or with 40% barley flour with added β -glucan along with a high-fat and high-carbohydrate meal on the metabolic profiles and antioxidant status among a cohort of 12 healthy volunteers.

In the literature, human intervention studies have studied the acute and long-term effects of daily grape seed or pomace supplementation in the form of extracts or powders. Some researchers observed beneficial effects in glycemic and lipid profiles, although others recorded no impact of their action. In regard to lowering lipid profiles, it was reported that the acute administration of 300 mg/kg of a procyanidin-rich grape seed extract along with a high-polyunsaturated-fat meal to eight healthy individuals could reduce postprandial oxidative stress by increasing plasma antioxidant status. However,

no statistically significant differences were observed in LDL cholesterol and triglyceride levels [47]. The consumption of a high-carbohydrate meal along with 100 or 300 mg grape seed extract by eight healthy adults significantly reduced postprandial glucose levels up to 2 h after treatment [48]. In a similar manner, the intake of grape pomace extract along with a high-fat meal in 18 normal and overweight healthy women managed to reduce uric acid in the normal-weight subjects. Extract consumption did not change the glucose response in all participants, although there was a tendency for a time effect. Triglyceride levels were reduced but not at the significance level, demonstrating a significant time effect, whereas total, HDL-, and LDL-cholesterol post-prandial levels were similar to baseline [49].

Only one human intervention study concerning the enrichment of products with grape pomace was retrieved. Twelve healthy participants (men aged 20–40 years old) followed a 3-day low polyphenol diet prior to the beginning of the study, and they consumed, on two different days separated by a one-week washout period, after an overnight fast, either a polyphenol-rich drink (1.562 g gallic acid equivalents (GAE)) or a control drink, followed after 3 h by the consumption of a standard meal. The standard meal contained 18% protein, 30% fat, and 52% carbohydrate (960 kcal). Blood samples were taken at fasting, 3 h after the drink, over 5 h after the standard meal, and at fasting the day after to measure plasma concentrations of glucose and triglyceride levels. The acute consumption of the drink rich in polyphenols from red grape pomace followed after 3 h by a standard meal elicited similar glycemic and triglyceride post-meal responses compared to a control drink in these healthy individuals [50]. This finding is in accordance with our intervention outcome, as the consumption of the novel grape seed flour crackers led to similar post-prandial glucose levels and maintained serum lipid concentrations similar to (regarding triglyceride and HDL concentrations) or lower than (regarding total and LDL cholesterol concentrations) baseline values. The energy intake of functional meal 1, which comprised the grape seed flour crackers, was lower than that of the standard meal offered in the previous study, and the polyphenol-rich drink was administered 3 h before the consumption of the meal, whereas, in our study, the enriched crackers were part of the meal administered. Furthermore, the time points of blood sampling differed from those of our study as the final time point was 2 h after meal consumption, whereas, in the previous study, it was the next day after overnight fasting. Even with the different parameters between our study and the one retrieved, grape polyphenols and their metabolites when consumed daily in a meal in the usual diet could potentially improve glucose homeostasis and lipid metabolism long-term. In our study, even if non-significant differences were observed, the post-prandial levels of lipids and glucose after enriched cracker consumption were retained close or lower to baseline levels, suggesting a possible protective role in lipid and glucose metabolism after a high-fat and high-carbohydrate meal. Furthermore, as our study and the study retrieved employed healthy individuals, a statistically significant difference in lipidemic and glycemic profiles would require long-term supplementation in high doses.

The acute consumption of 40% barley flour crackers enriched with β -glucan elicited a slightly lower glycemic response and retained post-prandial lipid levels similar to the baseline values, as observed in the case of triglyceride and total and HDL cholesterol concentrations, whereas, in respect to LDL cholesterol, lower levels compared to baseline were detected. These findings suggest a possible role of barley flour-enriched products in the regulation of postprandial hyperglycemia and hyperlipidemia after a high-fat and high-carbohydrate meal. Furthermore, plasma levels of FRAP as a measure of total antioxidant capacity did not change. Studies in the literature have investigated the effect of barley flour and β -glucan on post-prandial metabolic markers following the acute consumption of a variety of bakery products; some of them elicited lower glycemic and lipid responses, whereas others observed higher levels or no effect of the intervention, especially in the case of antioxidant measures.

The acute consumption of 17.5% barley flour bread containing 2.5 g β -glucan along with two subsequent standard meals by 23 healthy participants aged 20–30 years resulted in a significantly lower glucose concentration 60 and 90 min post-prandially compared

to refined wheat flour bread. Compared to our study, the participants the day before the trial consumed an energy-adjusted meal between 19:00 and 20:00. After 20:00, their access to food and drink, apart from water, was restricted, similarly to our study. Furthermore, the meals were adjusted to contain 50 g of carbohydrates without dietary fiber and were consumed with 180 mL of lactose-free milk; this is in contrast to our study, where dietary fiber was present and only water was allowed. Participants were instructed to consume each meal during a 10–15 min period, similar to our study instructions, and to drink milk after swallowing. Blood glucose concentrations were measured using the glucose meter just before the consumption of the first meal (0 min) and 15, 30, 45, 60, 90, and 120 min afterwards. The standard meal, which contained three seaweed rice balls and had a higher energy yield than our functional meals (770 kcal, 7% protein, 5% fat, 88% carbohydrate), was then consumed as the second meal. Blood glucose concentration was also measured just before the second meal (0 min) and 15, 30, 45, 60, 90, and 120 min afterwards. The authors pointed out that barley has a tendency to have a first-meal effect in the form of kernels, rather than when present in processed form, such as in porridge or in foods supplemented with dietary fiber extracted from barley. This could be the reason for not observing statistically significant differences in our study [51]. Wheat flour bread containing barley fiber concentrated with 3% β -glucan elicited a similar response in 14 healthy individuals aged 20–29 years old. The β -glucan and control bread had higher fiber and carbohydrate contents and lower fat contents and energy intake compared to the meals provided in our study. Furthermore, the bread was administered with 5 g apricot jam and 125 mL semi-skimmed milk, which differed from our meal composition. Blood samples were taken at baseline (before breakfast) and at 30, 60, 120, and 180 min after breakfast. Even though the enriched bread contained 3 g of β -glucan, similar to the crackers of our study, the type of food used, the subjects of the study, and the viscosity of the β -glucan could explain the contradictory results compared to our study [52]. However, the intake of wheat-based bread enriched with 40% barley flour elicited a lower glycemic response, but not at the significance level, compared to control wheat bread in 14 healthy individuals aged 18–65 years old. The participants had at least a 12 h overnight fast, similar to our study, and were instructed to avoid strenuous exercise, smoking, and alcohol consumption and consume a similar carbohydrate-based meal the evening before the visit; those restrictions were not included in our instructions. There was at least a 48 h washout period between the visits. The portion size of the meal was decreased to retain 25 g of available carbohydrates in the bread. Subjects had to consume the test products within 15 min with 250 mL of water, similar to our study, and blood samples were drawn at baseline and 15, 30, 45, 60, 90, 120, and 180 min after meal consumption. Those results are in accordance with our results regarding glucose levels. Fat and protein content can influence the glucose response, although their contents were low both in the trial and in our study as well. Furthermore, the authors mentioned that the EFSA criteria for the health claim of the lowering of the post-prandial glycemic response require 4 g of β -glucan per 30 g of available carbohydrates, a very high dose that is difficult to achieve in bread. This also applied to our product formulation, as the use of special milling fractions with elevated levels of β -glucan were required instead of regular barley flour. This could be a possible explanation as well for our study outcomes [53].

In another study, chapattis formulated with 15% barley flour resulted in non-significantly lower levels of glucose, total and LDL-cholesterol, and triglycerides compared to wheat flour chapattis in 18 healthy individuals aged 25–40 years old. The participants were instructed to consume two chapattis per day for 30 days and blood samples were drawn at baseline, 15 days, and 30 days. The results highlighted that the inclusion of composite flours with a high fiber content should be encouraged for long-term nutritional supplementation in order to achieve hypoglycemic and hypolipidemic effects in healthy subjects, as also indicated in our study [54]. In a cohort of 10 healthy participants, the glycemic responses elicited by the consumption of barley flour pasta did not differ significantly from those elicited by the consumption of durum semolina pasta, which served as a

control. The portions provided 50 g of carbohydrates, the participants were instructed to consume the meals within 15 min, and blood samples were obtained at 15, 30, 45, 60, 90, and 120 min after the start of the meal. Participants were allowed to drink with the test meal a cup of water, tea, or coffee with 30 mL 2% milk and/or artificial sweetener [55]. The study meal composition differed, although similar outcomes were observed. Furthermore, the consumption of high- β -glucan tortillas elicited a lower glucose response compared to the consumption of low- β -glucan tortillas in 12 healthy participants aged 19–35 years. The washout period was 1 week and, before each visit, participants underwent an overnight fast, similar to our study. Blood was collected at fasting and at 15, 30, 45, 60, 120, and 180 min after the start of the meal. Compared to our study, the tortillas had higher β -glucan and protein contents, which could therefore have contributed to a better control of blood glucose metabolism [56]. A sample of 40% β -glucan-enriched barley flour pasta resulted in a lower glycemic response compared to a control durum wheat pasta sample in a cohort of five healthy, fasted subjects. The barley and durum wheat blend pasta contained 100 g of carbohydrate, 30 g of total dietary fiber, and 12 g of β -glucan, and the durum wheat pasta contained the same amount of carbohydrate, 5 g of total dietary fiber, and negligible amounts of β -glucan. The pasta was supplemented with 300 mL of distilled water, a small amount (5 g) of butter, and a variety of dry seasonings, such as salt, pepper, herbs, and spices. The subjects were given the test meal and consumed it within 15 min; then, blood samples were drawn at 30, 60, 120, and 180 min after the start of the meal. The meal composition differed from our study and provided a higher energetic yield as well as a higher fiber content; also, the time points at which blood samples were drawn differed. Furthermore, cooking methods such as the boiling of pasta have been shown to increase the amount of soluble β -glucan, but baking decreases it [57,58]. The consumption of muffins containing high levels of β -glucan managed to reduce glucose responses in a cohort of 10 control and 10 overweight men (25 to 56 years). All subjects were fed an equilibration diet containing 30% fat, 55% carbohydrate, and 15% protein for 2 days before the visits to ensure that all of the subjects were eating a moderately high carbohydrate diet before the acute meal tests, which was not applied in our study. Each treatment contained 75 g of total carbohydrates compared to our study and blood samples were collected before the treatment and at 0.5, 1, 2, 3, and 4 h after the treatment, a different time range from our study [59]. In a single study concerning barley flour cracker acute consumption, the intake of barley flour-formulated crackers by a cohort of 10 healthy subjects triggered a lower glycemic response than whole-wheat crackers, whereas post-prandial triacylglycerol levels did not differ among test meals. The participants consumed the breakfast after an overnight fast on separate mornings, with a washout period of 2 weeks. Test meals (95 gr) were served with 500 mL of unsweetened tea, contained 40 g of available carbohydrates, and provided a higher energy yield than our functional meal. The meals were consumed in 10 min and blood samples were drawn at the administration of the meal as well as 15, 30, 45, 60, 90, 120, and 180 min and then at hourly intervals for the following 5 h after the meals. Furthermore, a standard light lunch, composed of white bread (50 g), roast beef (70 g), and apple (200 g) (containing 27 g protein, 2.3 g lipid, and 59.2 g carbohydrate; 1481 KJ), was served 4 h after the meals. Participants were also asked to abstain from cigarette smoking and had access to water. The study meal design and the time intervals differed from our study, irrespective of the similar results obtained [60]. In a single study concerning the plasma antioxidant capacity of 13 healthy volunteers aged 40–70 years after oat or barley muffin consumption, no changes were observed after the interventions. The subjects received 48 g of whole grain in two small muffins. The flour used was either whole oat flour, whole barley flour, or refined wheat flour. There was a 1-week washout period between interventions. During each intervention, an oral glucose tolerance test was performed on each participant for 3 h following the consumption of a glucose reference (75 g in 300 mL water) along with the muffins. Each muffin provided 48.7 g flour, 8.2 g fat, 7.8 g protein, and 52.4 g carbohydrates (310 kcal). Blood samples were collected at baseline after 12 h fasting and at 0.5, 1, 1.5, 2, 3,

4, 6, 8, 10, and 24 h after meal administration. The study design and intervention meals differed from ours, although similar results in TAC were obtained [61].

Our results are in accordance with the general glycemic, lipid, and antioxidant capacity responses observed in a number of the previously mentioned studies, although the contradictory findings generally observed in the literature could be explained by the different solubility and molecular weights that characterize β -glucans, which has a direct effect on intestinal viscosity. Greater intestinal viscosity could potentially lower bile acid absorption and, thus, serum cholesterol levels [62,63]. Additionally, intestinal viscosity has been proposed to be affected by methods of processing and the food matrices of β -glucan-enriched products [64]. Also, it has been reported that β -glucan could be degraded by endogenous enzymes present in barley and wheat flour during baking [58], which could be the case for the no-added effect we observed during our study. In fact, Aman et al. demonstrated that baking can result in the enzymatic degradation of β -glucan [65]. Taking this into account, it could be challenging to predict the effect of β -glucan-enriched products on cholesterol levels. Furthermore, Kerckhoffs et al. reported a non-significant reduction in serum LDL cholesterol levels per gram of β -glucan incorporated into bread and cookies [64]; although, when β -glucan was incorporated either into fruit juice, fruit drink, or oat milk, the decrease was significantly greater. The authors suggested that β -glucan potential increases when incorporated into liquid products, which was also supported by further studies [63,64,66]. It was also reported that during the baking of bread or cookies, β -glucan becomes less viscous than raw β -glucan. This reduced viscosity was linked to a reduction in the ability of β -glucan to modify glucose and cholesterol absorption in the gastrointestinal tract [64]. In terms of antioxidant capacity status, relatively small phenolic compound concentrations found in bakery products and their interactions with dietary fibers such as β -glucan may be insufficient to increase antioxidant status and attenuate glucose-induced metabolic stress. Other authors have suggested that there may be only a minor or negligible effect on biomarkers of oxidative stress or antioxidant activity in response to whole grain cereal consumption [61,67]. Therefore, the processing of whole grains and bakery products could alter the fibers' and phytochemicals' natural structure and content in a way that makes them less effective in attenuating cardiometabolic risk factors than originally expected [64]. Taking into account the previously mentioned parameters, the processing and baking of the β -glucan-enriched crackers might therefore provide possible explanations for our study outcomes, where no significant differences were observed between the control meal and functional meal 2 in the healthy participants due to the reduction of the β -glucan molecular weight and action; although, a possible protective effect was demonstrated in the lipid profiles after the high-carbohydrate and high-fat meal.

Another important aspect to consider in the outcomes of the intervention studies is the frequency of administration and the dose of barley β -glucan. In order to affect bile acid secretion and exert its protective effect on glycemic and lipid profiles, the consumption of barley β -glucan-enriched foods is recommended several times daily [68,69]. Furthermore, several postprandial studies have shown that a 50% decrease in the glycemic response can be estimated after the ingestion of 35 g of carbohydrates together with 5 g of β -glucans [69,70]. The amount of β -glucan provided by the barley flour crackers enriched with β -glucan was 3 g in order to meet the daily dietary intake, i.e., the daily amount stated in the FDA health claim for blood lipid effects; therefore, because of the possibly small difference in soluble fiber between the intervention meals and the acute nature of our study, no significant changes were observed in the tested biochemical biomarkers. However, our results showed that barley β -glucan maintained the post-prandial lipid and glucose levels lower or close to the baseline values; therefore, a higher administered dose could possibly achieve a more substantial decrease.

Therefore, in this study, the effect of the consumption of enriched wheat flour crackers on glucose, lipid, and antioxidant profiles may have been mainly due to the daily consumption of at least one healthy meal of the study cohort because of their healthy status; this fact may have overridden the effect of the grape seed polyphenols and barley β -glucan

of the interventions. In conclusion, this study showed that wheat flour crackers enriched with either 10% grape seed flour or 40% barley flour with added β -glucan lowered glucose levels and retained lipid profiles close to baseline levels post-prandially, following the consumption of a high-carbohydrate and high-fat meal in healthy subjects. Even though the reductions were not significantly different from those of the group that consumed the wheat flour control crackers, enriched crackers could represent a healthy alternative, with a positive long-term effect on serum glucose and lipids, due to the higher fiber and polyphenol contents. Healthy and continuous changes in food habits could have a positive effect on glucose, lipid, and antioxidant profiles.

One limitation of our study is the acute nature and the small sample size of the participants. Nevertheless, we were able to detect lower postprandial glucose, triglyceride, and LDL levels after the functional meals compared to the control meal. Thus, it is conceivable that the beneficial effect of the barley crackers enriched with β -glucan consumption could be even greater and more evident with a larger sample size. Although further studies with more volunteers are needed, our study demonstrated the benefits of the acute consumption of crackers containing grape seed or barley flour with added β -glucan and the possible role of these flours on glucose and lipid metabolism, which would contribute to decreasing the risk of type 2 diabetes. The discrepancies between our findings and those of previous reports concerning the effects of enriched cracker supplementation might be explained by the dosage of the grape seed and barley flours used, the acute nature of the intervention, and the study participants. Furthermore, the effect of enriched cracker consumption on inflammatory cytokines, such as C-reactive protein (CRP), interleukin 1 (IL-1), IL-6, and tumor necrosis factor alpha (TNF- α); other biomarkers of oxidative stress, such as oxidized LDL (ox-LDL), superoxide dismutase (SOD), and Gamma-glutamyl transferase (g-GT); and the glutamic transaminase enzymes serum glutamic-oxalacetic (GOT) and serum glutamic-pyruvic (GPT), could not be examined. The final limitation of our study is the lack of further statistical analysis including a calculation of the minimum detectable effect post-hoc through methods such as MDE, MDD, or CIs.

In conclusion, our findings show that the consumption of crackers enriched with alternative flours demonstrated no significant effect on markers of postprandial glycemia and lipidemia or oxidative stress. Higher doses of grape seed polyphenols and β -glucan at a daily dose should be administered in the case of bakery products in order to elicit a positive health effect in the glycemic and lipid profiles of healthy individuals, as the outcomes of our acute study maintained post-prandial levels close to baseline values. Furthermore, higher and daily doses could depict a clearer role of those substances in a greater sample size.

Author Contributions: Conceptualization, D.C. and A.E.K.; Data curation, D.C.; Investigation, D.C., O.P., P.P. and K.A.; Methodology, D.C., O.P., P.P., K.A. and A.E.K.; Project administration, A.E.K.; Resources, D.C., O.P., P.P. and K.A.; Software, D.C., O.P., P.P. and K.A.; Supervision, A.E.K.; Validation, A.E.K.; Visualization, A.E.K.; Writing—original draft, D.C.; Writing—review and editing, A.E.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was co-funded by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH—CREATE—INNOVATE, grant number T2EDK-02137.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of the Aegean (protocol code 7505 and date of approval 20 October 2019).

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

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: We would like to thank all the volunteers who participated in this study.

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

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