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PRZEMYSŁAW CZAJKOWSKI

Associations between common *FTO* gene polymorphisms and diet, and their impact on obesity as well as its metabolic consequences

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II. Introduction

The interaction of genetic and environmental factors may result in obesity, defined as a body mass index (BMI) greater than 30 kg/m^2 (1). The prevalence of obesity has increased substantially worldwide among children, adolescents, and adults (2). The increasing development of obesity is associated with type 2 diabetes (T2DM) (3), cardiovascular diseases (4) and some forms of cancer (5) prevalence. Hence, obesity is one of the major international public health awareness and economic burden of medical care. The lack of physical activity and over-eating have risen the numbers of overweight people over the years (6, 7). There is a conviction that the identification of obesity genetic factors and other factors that influence on weight gain, can be a target for therapeutic intervention. Moreover, recognition of various environmental factors such as dietary intake may influence on the associations between genetic risk and obesity development. Therefore, an approach to address a balanced diet, that fulfils all nutritional needs, and the genome-customized recommendations, seems to be an efficient strategy to prevent obesity development.

Genome-wide association studies have identified several sequence variants of single nucleotide polymorphisms (SNPs) in the fat mass and obesity-associated (*FTO*) gene, transcription factor-7–like 2 (*TCF7L2*) gene, melanocortin-4 receptor (*MC4R*) gene, and other genes (8). Among the above genes, the *FTO* gene has been described as the gene with a significant correlation with obesity (9). Based on the research concerned on impaired satiety responsiveness conducted among children, it has been shown that the *FTO* gene is widely expressed in many tissues, especially the region involved in appetite regulation, particularly the hypothalamus (10). A newly published population based study, presents the association between large consumption of energy and risk-allele carriers in adults (11). Some of the studies, concerned on the genotype and energy expenditure (12, 13), indicate that the association of obesity and the *FTO* gene is mainly influenced by appetite and satiety regulation. Diet composition and level of physical activity are two main lifestyle factors that may modulate genetic susceptibility. Particularly, several studies indicate that physical activity may interact with a person's genetic predisposition and could reduce the effect of *FTO* SNPs on the risk of obesity in diverse world populations (14-18). Another significant contributor of weight balance is diet composition and food intake. The role of diet in the etiology of obesity remains a controversial matter among the public health debate, due to inconsistent research findings (19, 20) and their relation to specific nutrients or foods. Whereas it should be confirmed which selected components of food intake interact with the *FTO* gene. Therefore, research on the

nutritional area that combines gene-nutrient associations and interactions may have beneficial actions on the health of an individual nowadays and in the future perspective.

III. Aims

Over the last decade, more attention is being focused on the associations of dietary patterns and their relationship with the genetic risk of obesity. Nevertheless, further investigation of the associations between dietary patterns and *FTO* genetic variants is required. Accordingly, advances in this field of science may stand by the development of genome-customized diet recommendations in the prevention of obesity and its metabolic complications.

Therefore, the purpose of this dissertation was to investigate the associations between energy and essential nutrients intake, and some common genetic variants, including the *FTO* gene, obesity and glucose metabolism.

Taking the above into consideration, the following aims of the doctoral dissertation are presented as:

1. Exploration of the potential effect of the dietary factors and *FTO* gene polymorphisms on the obesity-related parameters in the Polish population.
2. Evaluation of whether dietary factors could modify the association between some common genetic variants of the *FTO* gene and obesity and obesity-related parameters.

IV. Materials and Methods

In order to perform the scientific work, a wide research panel was implemented, including standard experimental methods (anthropometric data evaluation and nutritional questionnaires), laboratory analysis, as well as statistical analysis. The schematic protocol of the study is presented at the Fig. 1.

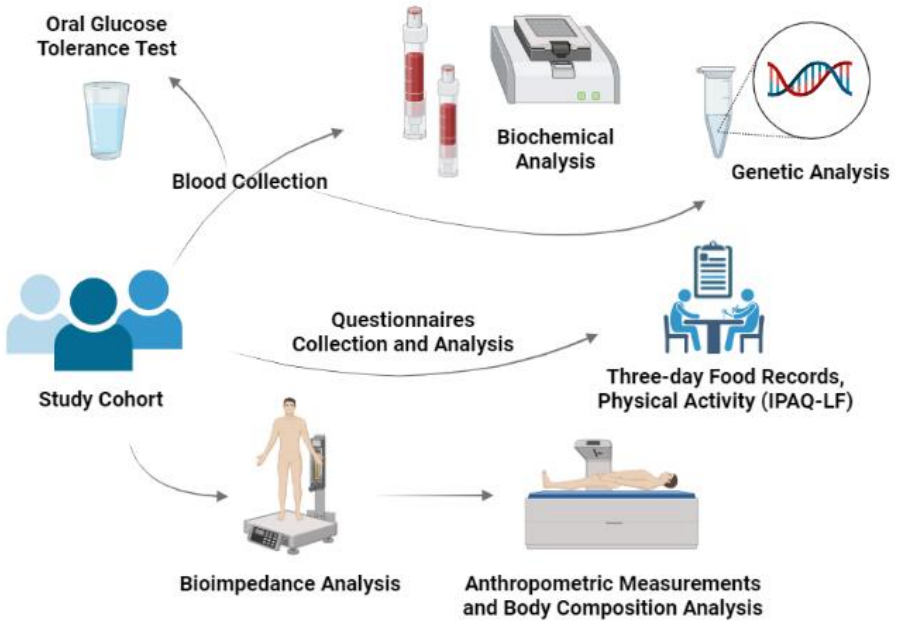

Figure 1. Graphical protocol of the study.

Ethics statement

All of the research methods were carried out in the accordance with ethical experimentation standards on humans and with the Helsinki Declaration, published in 1975, and revised in 1983. The study protocol was approved by the local Ethics Committee (Medical University of Bialystok, Poland, R-I-002/35/2009). Before starting the inclusion procedure, the written informed consent was obtained from all study participants.

Study Population

The study was conducted among 819 subjects (47.5% men and 52.5% women), recruited from 1549 participants of the 1000PLUS Cohort Study of a Polish-origin Caucasian population (registered at www.clinicaltrials.gov as NCT03792685), and described previously (21-25). Individuals who used to take medicines (e.g., anti-diabetic, weight loss, lipid-lowering), diet supplements, and other substances that could influence investigated parameters, were excluded from the analysis. Moreover, subjects who reported any metabolic or other significant diseases, or who had bariatric surgery, which could have an impact on investigated parameters, or followed any special diet or dietary pattern (vegan, vegetarian, Atkins, Dukan, Paleo, etc.), were excluded from the study analysis as well.

Clinical examination - Anthropometry and body composition analysis

The following anthropometric data of each participant were evaluated: body weight, body height, waist circumference and hip circumference, using standardized methods (26). We evaluated body composition (skeletal muscle mass (SMM), fat-free mass (FFM), and fat mass) by the bioelectrical impedance method (InBody 220, Biospace, Korea). Measurements of body fat distribution: visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), VAT/SAT ratio, were carried out by the multi-frequency bioimpedance method (MaltronBioScan 920-2, Maltron International Ltd., Rayleigh, UK). Body Mass Index (BMI) was calculated using the following formula: body weight (kg) divided by height (m) squared. The waist–hip ratio (WHR) was determined by dividing the waist by hip circumference, the similar formula was used for the VAT/SAT ratio calculations.

Blood Collection and Biochemical Analysis

According to the World Health Organization (WHO) recommendations, oral glucose tolerance tests (OGTTs) were performed among participants without a history of diabetes, with a dose of 75 g oral glucose load. The subjects were instructed to fast for 8–12 h prior to the test and to not restrict carbohydrate intake in the 3 days before the test. Blood was collected in time intervals at 0, 30, 60, and 120 min after glucose administration.

Blood samples were obtained and collected to evaluate the concentrations of fasting and during OGTT plasma glucose and insulin, total cholesterol, LDL, HDL, triglyceride (TG), and hemoglobin A1c (HbA1c) level. The samples were prepared for assessment according to the laboratory kit instructions and stored until testing at −20 or −80 °C. Serum insulin concentrations were evaluated by immunoradiometric assay (INS-Irma, DIASource S.A.,

Belgium; Wallac Wizard 1470 Automatic Gamma Counter, PerkinElmer Life Sciences, Turku, Finland). Plasma glucose concentrations were measured using the hexokinase enzymatic method (Cobas c111, Roche Diagnostics Ltd., Switzerland). Lipid concentrations were evaluated by the use of enzymatic colorimetric assay using commercially available kits (Cobas c111, Roche Diagnostic Ltd., Switzerland). By the use of the HPLC (high-performance liquid chromatography; D-10 Hemoglobin Testing System, Bio-Rad Laboratories Inc., Hercules, CA, USA; Bio-Rad, Marnes-la-Coquette, France) HbA1c levels were assessed.

For insulin resistance evaluation the homeostasis model assessment (HOMA-IR) was performed as follows: (fasting plasma glucose concentration (mmol/L)) × (fasting insulin concentration (μU/mL))/22.5. By the use of the following formula: 20 × fasting insulin (μIU/mL)/fasting glucose 100 (mmol/mL) − 3.5, the homeostatic model assessment of β-cell function (HOMA-B) was determined.

Dietary intake and daily physical activity analyses

The International Physical Activity Questionnaire-Long Form (IPAQ-LF) was used to evaluate daily physical activity. Study subjects were stratified as having a low, moderate, or high level of physical activity, expressed by the metabolic equivalent (MET). Values of MET were determined using the following formula: (MET level) × (minutes of activity) × (events per week) (27).

The daily dietary intake among individuals was carried out with the use of 3-day food intake diaries. Participants were instructed on how to estimate portion sizes of foods, based on the provided colour photograph albums of portion sizes, and instructed on how to weigh the food, if possible. We did not include in the analysis diaries that were not fully completed. Total energy, carbohydrate, fat, protein, and fiber intake were estimated using Dieta 6.0 software (National Food and Nutrition Institute, Warsaw, Poland). Dieta software is used to calculate the nutritional value of food and diets based on tables of the nutritional value of local food products and dishes.

To evaluate the associations and interactions between genetic factors and diet, study subjects were divided based on the average daily protein, fat, carbohydrate and fiber intake as follows: lower and higher quantiles of dietary protein intake (≤18% and >18% of total energy intake, respectively), lower and higher quantiles of dietary fat intake (≤30% and >30% of total energy intake, respectively), lower and higher quantiles of dietary carbohydrate intake (≤48% and >48% of total energy intake, respectively), and lower and higher quantiles of dietary fiber intake (≤18g and >18g daily intake, respectively).

Genotyping

Based on the catalogue of genome-wide association studies (28), the 6 *FTO* polymorphisms were selected and genotyped: rs3751812 (G > T), rs9939609 (T > A), rs8050136 (A > C), rs6499640 (G > A), rs7190492 (A > G), and rs8044769 (C > T). The DNA from peripheral blood leukocytes using a classical salting-out method was extracted. Using the TaqMan SNP technology from a ready-to-use human assay library (Applied Biosystems, MA, USA) and with the use of a high-throughput genotyping system, OpenArray (Life Technologies, CA, USA), the SNPs were determined. Moreover, SNP genotyping was performed in duplicate, following the manufacturer's instructions. As a negative control, a sample without a template was used, in the case of possible false positive signals caused by the contamination.

Statistical Analysis

Collected data were summarized with a number of observations (N), standard deviation (SD), and arithmetic mean. The number of observations and frequency (percentage) were presented for categorical data. Study individuals were divided into quantiles based on average daily protein, carbohydrate, fat, and fiber intake, with the thresholds set as the median value of each parameter. Based on the scientific literature and previous findings, risk genotypes of the 6 previously identified *FTO* SNPs were predefined. We did not include in this study comparisons of the allelic and genotypic frequencies, as well as odds ratio calculations, because of the relatively small sample size. Continuous parameters were tested for normality with Shapiro–Wilk's test and by visual inspection. Using Levene's test, the homogeneity of variance across groups was checked. Nonparametric tests were used for response variables that failed the mentioned statistical tests. Differences between studied groups and measured parameters were compared using analysis of variance (ANOVA) or Kruskal–Wallis test for numerical variables, with either Tukey's or Dunn's post hoc test with Holm p-value adjustment, and the chi-squared test for categorical variables. All calculations were prepared in R (version 4.0.3). The statistical significance level was set at <0.05.

In order to study the hypothesis that the relationship between *FTO* genotypes and continuous responses varies in average daily protein, fat, and carbohydrate intake groups, multivariate linear regression models were performed. These models were adjusted for age, sex, BMI (when applicable), and physical activity. The Huber-White robust standard errors (HC1) were calculated. Model fit was estimated using R-squared values plus adjusted R-squared

values. Some of the models were optimized by a stepwise backward elimination based on the Akaike information criterion (AIC).

V. Results

The collected data were evaluated, based on diet-gene associations and interaction, in the group comprised of 819 participants, who met the inclusion criteria. The general clinical characteristics of the studied population and individual characteristics stratified by investigated genotypes were performed and presented in the articles included in the dissertation. Among the investigated *FTO* polymorphisms, some of the loci were in very strong linkage disequilibrium (D' = 1.0 for rs8050136 and rs9939609) (30), therefore only results for rs8050136 were presented.

In general, the population consisted of 52.5% females and 47.5% males. The mean age of the individuals was 42.1 (±14.5) years, and the mean BMI was 28.5 (±6.6) kg/m2. Based on the clinical and anthropometric data, food intake and physical activity, we observed significantly lower hip circumference among studied *FTO* polymorphisms: CC vs AC and AA genotype carriers of rs8050136 (101.2 vs 104.1 and 103.8, respectively, p=0.008), and GG vs GT and TT genotype carriers of rs3751812 (101.3 vs 104.2 and 103.8, respectively p=0.008). Furthermore, TT vs CC and CT genotype carriers of rs8044769 showed the highest total cholesterol concentration (206.7 vs 193.8 and 191.7, respectively, p=0.029). Additionally, CT vs TT and CC genotype carriers of rs8044769 presented the lowest percentage of daily energy intake provided from fat (30.1 vs 31.9 and 32.5, respectively, p=0.005).

Based on the analysis of the interactions between dietary macronutrient intake, genotypes and their effect on investigated parameters, the multivariable linear regression models (dietary macronutrient quantile) × (genotype) with the interaction term were performed. As a result of model implementation, and observation of the association between selected genotypes, patient's glycemic status and variables describing body composition, the hypothesis of the diet and genotypes interaction was confirmed, as presented in the result section of the articles included in the dissertation. With the use of the boxplots in the figures, the differences in median values of the selected responses and the interquartile ranges (IQRs) in different genotypic and dietary strata were shown in the articles included in the dissertation.

The study concerned on macronutrients intake showed that GG genotype carriers of rs3751812 presented lower BMI, hip and waist circumference, and total body fat content (data presented in detail in the article number 1 of this dissertation). Additionally, when energy intake derived from dietary fat did not exceed 30%, the subcutaneous and visceral fat content were the lowest in these participants. Further, if more than 48% of daily energy intake was derived from carbohydrates, the GG genotype carriers of rs3751812 presented significantly

lower obesity-related markers, such as body weight, BMI, fat-free mass levels subcutaneous fat content, and waist and hip circumference, as well as lower fasting blood glucose and higher HDL-cholesterol levels. The CC genotype carriers of rs8050136 presented similar results. Also, a significant impact of diet-gene associations with genotypes of rs8044769 were noted. The TT and CT genotype carriers presented higher body weight and BMI if daily energy intake derived from carbohydrates was equal or less than 48%. In homozygous TT genotype carriers were noted higher blood glucose concentration during the OGTT test, if more than 18% of total energy intake was derived from proteins.

The analysis of fiber intake showed that dietary fiber intake may modify the effect of *FTO* polymorphisms on body composition and lipid profile (data presented in detail in the article number 2 of this dissertation). The results of this analysis showed that, if daily fiber intake was above 18 g per day, the carriers of the CC genotype of rs8050136, GG genotype carriers of rs3751812, and GG genotype carriers of rs6499640, presented lower hip circumference. Surprisingly, higher total cholesterol concentrations were presented in CC genotype carriers of rs8050136 and GG genotype carriers of rs3751812, when subjects were stratified to the group with higher than median fiber intake. Similarly, higher LDL-cholesterol levels were presented in the above genotype variants, particular among CC genotype carriers of rs8050136 and GG genotype carriers of rs3751812, when subjects were stratified to the group with higher than median fiber intake.

VI. Conclusions

The present study findings provide new insights into the role of the associations between diet and *FTO* polymorphisms in the risk of obesity and its metabolic consequences. To the best of our knowledge, this is one of the first studies that present associations between *FTO* SNPs rs3751812, rs8050136, rs9939609, rs6499640, rs7190492 and rs8044769, daily protein, carbohydrate, fat, and fiber intake, and the effect of these relationships on obesity, glucose homeostasis and lipid profile.

Study observations suggest that carriers of the CC genotype of rs8050136 and GG genotype of rs3751812 should follow diets in which not less than 48% of daily energy intake is derived from carbohydrates and not more than 30% from dietary fat. Additionally, study results suggest that carriers of CT and TT genotypes of rs8044769 may benefit from avoiding diets in which carbohydrates provide less than 48% of total energy. Although, carriers of TT genotype of rs8044769 may benefit from avoiding diets in which proteins provide more than 18% of total daily energy. The genome-customized diet recommendations may be important, since we observed that if the mean amounts of macronutrients in the diets are more than 30% for dietary fat, and more than 18% for proteins, and less than 48% of total energy intake for carbohydrates, may have an impact for subjects of the above-mentioned genotypes.

Additionally, another research conclusion of the study is that dietary fiber intake may modify the effect of *FTO* polymorphisms on body composition and lipid profile. Generally it is recommended that the daily fiber intake should be above 18 g per day, to provide health benefits. What was surprising, this analysis suggest that, carriers of CC genotype (rs8050136) and GG genotype (rs3751812) should follow diets in which no more than 18 g of daily energy intake is derived from fiber, to optimize the lipid profile. However, considering all limitations of this study described in the articles, this observation needs further analysis and investigations.

This study may have practical clinical implications in the field of genome-customized diet recommendations in the future, to prevent obesity and its metabolic complications. Nevertheless, further studies are needed to verify these findings, in larger populations and different ethnic groups.

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Summary

Summary in English

The objective of doctoral dissertation in the form of a publication cycle was to investigate the associations between carbohydrate, protein, fat and fiber intake, common genetic variants of the *FTO* gene, obesity, glucose homeostasis and lipid profile. The following two hypotheses were stated and explored: the effect of *FTO* gene polymorphisms on the obesity-related parameters, as well as dietary factors could modify the association between common genetic variants of the *FTO* gene and obesity and obesity-related parameters.

To achieve the aims of the scientific work and to verify the hypotheses, the research was carried out among Caucasian volunteers, selected for gene–diet association and interaction analysis, and genotyped for the *FTO* SNPs (rs3751812, rs8050136, rs9939609, rs6499640, rs8044769, and rs7190492). Measurements of anthropometric parameters, total body fat content and distribution, blood glucose, insulin concentration at fasting and during oral glucose tolerance test (OGTT), and lipid profile were performed. Food intake was analysed based on the three-day food records, and daily physical activity levels were evaluated using the International Physical Activity Questionnaire Long Form (IPAQ-LF). Numerical data were summarized with the number of observations, standard deviation and arithmetic mean. Differences between parameters and dietary groups were compared using the Kruskal-Wallis test for numerical variables, with Dunn's post-hoc test and Holm p-value adjustment, and the chi-squared test for categorical variables. In order to study the hypothesis multivariate linear regression models were used.

Based on the conducted analyses, it was found that associations between *FTO* polymorphisms, daily protein, carbohydrate, fat, and fiber intake, and the effect of these relationships on obesity, glucose homeostasis and lipid profile occur among the study cohort. It was observed that daily macronutrient intake may modulate the impact of *FTO* genetic polymorphisms on investigated parameters. Furthermore, diets based on high-fiber intake, positively influence on the anthropometric parameters, but might be associated with worse lipid profile dependent on the *FTO* genotype. The study findings may have a practical clinical implication in nutritional area, which combine personalized genome-customized diet recommendations.

Keywords: FTO gene; obesity; dietary protein intake; dietary carbohydrates intake; dietary fat intake; dietary fiber intake; macronutrients; gene-diet interaction

Summary in Polish

Celem rozprawy doktorskiej w formie cyklu spójnie tematycznie prac było zbadanie zależności między spożyciem węglowodanów, białek, tłuszczów i błonnika oraz polimorfizmów genu *FTO*, a także otyłością, homeostazą glukozy i profilem lipidowym. Postawiono dwie hipotezy: efekt nosicielstwa polimorfizmów genu *FTO* na parametry związane z otyłością oraz ocena, czy czynniki dietetyczne mogą modyfikować te powiązania.

W celu osiągnięcia założeń pracy naukowej i zweryfikowania postawionych hipotez badawczych, przeprowadzono badania naukowe wśród ochotników rasy kaukaskiej, genotypowanych pod kątem wybranych polimorfizmów genu *FTO* (rs3751812, rs8050136, rs9939609, rs6499640, rs8044769 i rs7190492). U uczestników badania wykonano pomiary antropometryczne, pomiary całkowitej zawartości i dystrybucji tkanki tłuszczowej, stężenia glukozy oraz insuliny na czczo i podczas doustnego testu tolerancji glukozy (OGTT), jak również wykonano badania profilu lipidowego. Spożycie pokarmu analizowano na podstawie trzydniowych dzienniczków żywieniowych, natomiast aktywność fizyczną oceniono za pomocą Międzynarodowego Kwestionariusza Aktywności Fizycznej (IPAQ-LF). Dane liczbowe zestawiono z liczbą obserwacji, odchyleniem standardowym i średnią arytmetyczną. Różnice między parametrami i grupami żywieniowymi porównywano za pomocą testu Kruskala-Wallisa dla zmiennych liczbowych z testem post-hoc Dunna i korektą wartości p Holma oraz testem chi-kwadrat dla zmiennych kategorycznych. W celu zbadania hipotez zastosowano wielowymiarowe modele regresji liniowej.

Na podstawie przeprowadzonych analiz stwierdzono, że w badanej populacji występował związek między nosicielstwem polimorfizmów genu *FTO*, dobowym spożyciem białka, węglowodanów, tłuszczów i błonnika oraz analizowanymi parametrami metabolicznymi. Zaobserwowano, że zależności między polimorfizmami genetycznymi genu *FTO* i badanymi parametrami mogą być zależne również od spożycia badanych makroskładników diety. Ponadto, potwierdzono, że wysoka zawartość błonnika w diecie pozytywnie wpływa na parametry antropometryczne, natomiast może również wiązać się z niekorzystnym profilem lipidowym w przypadku nosicieli wybranych genotypów *FTO*. Wyniki otrzymanych badań mogą mieć praktyczne znaczenie kliniczne w dziedzinie żywienia i w przyszłości będą mogły być podstawą do opracowania spersonalizowanych zaleceń dietetycznych.

Słowa kluczowe: gen FTO; otyłość; zawartość białka w diecie; zawartość węglowodanów w diecie; zawartość tłuszczu w diecie; zawartość błonnika pokarmowego w diecie; makroelementy; interakcja gen-dieta

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The Impact of *FTO* Genetic Variants on Obesity and Its Metabolic Consequences is Dependent on Daily Macronutrient Intake

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Article

The Impact of *FTO* Genetic Variants on Obesity and Its Metabolic Consequences is Dependent on Daily Macronutrient Intake

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Abstract: Numerous studies have identified the various fat mass and obesity-associated (*FTO*) genetic variants associated with obesity and its metabolic consequences; however, the impact of dietary factors on these associations remains unclear. The aim of this study was to evaluate the association between *FTO* single nucleotide polymorphisms (SNPs), daily macronutrient intake, and obesity and its metabolic consequences. From 1549 Caucasian subjects of Polish origin, genotyped for the *FTO* SNPs (rs3751812, rs8044769, rs8050136, and rs9939609), 819 subjects were selected for gene–diet interaction analysis. Anthropometric measurements were performed and total body fat content and distribution, blood glucose and insulin concentration during oral glucose tolerance test (OGTT), and lipid profile were determined. Macronutrient intake was analyzed based on three-day food records, and daily physical activity levels were evaluated using the International Physical Activity Questionnaire Long Form (IPAQ-LF). Our study shows that carriers of the GG genotype of rs3751812 presented lower body weight, body mass index (BMI), total body fat content, and hip and waist circumference and presented lower obesity-related markers if more than 48% of daily energy intake was derived from carbohydrates and lower subcutaneous and visceral fat content when energy intake derived from dietary fat did not exceed 30%. Similar results were observed for rs8050136 CC genotype carriers. We did not notice any significant differences in obesity markers between genotypes of rs8044769, but we did observe a significant impact of diet-gene associations. Body weight and BMI were significantly higher in TT and CT genotype carriers if daily energy intake derived from carbohydrates was less than 48%. Moreover, in TT genotype carriers, we observed higher blood glucose concentration while fasting and during the OGTT test if more than 18% of total energy intake was derived from proteins. In conclusion, our results indicate that daily macronutrient intake may modulate the impact of *FTO* genetic SNPs on obesity and obesity-related metabolic consequences.

Keywords: *FTO* gene; obesity; dietary protein; dietary carbohydrates; dietary fat; macronutrients; gene-diet interaction; glucose homeostasis

1. Introduction

Obesity is a major public health problem worldwide [1] and a leading risk factor for type 2 diabetes mellitus in adolescents [2,3] and children [3,4]. It has already been established that the predictions for diabetes prevalence are not optimistic [5]. Moreover, the increasing prevalence of obesity is associated with lipid metabolism disturbances, such as high concentrations of total cholesterol and low-density lipoprotein (LDL) and low concentrations of high-density lipoprotein (HDL) [6]. Considering the above, obesity also increases the risk of cardiovascular disease [7].

In general, obesity is a result of imbalanced energy homeostasis, but genome-wide association studies have identified many single nucleotide polymorphisms (SNPs) in the fat mass and obesity-associated (*FTO*) gene, melanocortin-4 receptor (*MC4R*) gene, and other genes [8,9] that are associated with the risk of developing obesity. Among these genes, *FTO* has been reported as the gene with the strongest significant correlation with obesity [10]. The *FTO* gene is profoundly expressed in the hypothalamus region, which is involved in appetite regulation [11]. The associations between *FTO* genetic variants, dietary factors, and body weight gain are still under investigation, although it has been postulated that some *FTO* genetic variants may influence the risk of weight gain through larger amounts of consumed food [12] or appetite and satiety regulation [13]. *FTO* rs9939609 SNPs have been associated with increased macronutrient consumption, especially fat and carbohydrates, as well as total energy intake [11,14,15], but these genetic variants do not seem to influence energy expenditure [11,16]. Moreover, environmental factors such as diet may influence the associations between genetic risk and obesity development. Over the last decade, the study of dietary patterns and their relation to genetic risk of obesity has received more attention [17,18]; nevertheless, the associations between *FTO* single nucleotide polymorphisms and dietary patterns need further investigation [19]. Therefore, the aim of our study was to evaluate whether daily macronutrient intake could modify the association between genetic variations of the *FTO* gene and obesity and obesity-related metabolic consequences among the Polish population.

2. Materials and Methods

2.1. Participants

The study was conducted among 1549 Caucasian volunteers of Polish origin (18–79 years old) enrolled in the 1000PLUS Cohort Study (registered at www.clinicaltrials.gov as NCT03792685) from 2007 to 2019, described previously [20–22], which were seeking personalized nutrition for prevention of obesity and treatment of type 2 diabetes mellitus. Individuals who used to take medicines (weight loss, anti-diabetic, lipid-lowering, or any other medication that could have an impact on body weight, body fat content, blood glucose, and other investigated parameters) or diet supplements, which could affect the results, were not enrolled in this study. Subjects who reported endocrine, gastrointestinal, hepatic, renal, metabolic, immunological, or psychiatric disorders or who had bariatric surgery, which could have an impact on investigated parameters, were excluded from the study analysis as well. We excluded all subjects who used to take anti-diabetic (56 subjects, 6.8%) or lipid-lowering medications (47 subjects, 5.7%) and 109 (13.3%) with a previous history of prediabetes or diabetes, as potential cofounders, and others who met the exclusion criteria mentioned above. Moreover, individuals who followed any special diet or dietary pattern (vegetarian, vegan, Atkins, etc.) were not included in the analysis.

2.2. Anthropometric and Body Composition Measurements

The following anthropometric data were collected: body weight, height, and waist and hip circumference. Body mass index (BMI) was calculated using the following formula: body weight (kg) divided by height squared (m). Waist–hip ratio (WHR) was estimated by dividing waist circumference by hip circumference. Total body composition (including fat mass, fat-free mass, and skeletal muscle mass) was evaluated by the bioelectrical impedance method (InBody 220, Biospace, Korea). Body fat distribution analysis, including visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) content, was performed by the multi-frequency bioimpedance method (Maltron BioScan 920-2,

Maltron International Ltd., United Kingdom). The VAT/SAT ratio was calculated by dividing visceral adipose tissue by subcutaneous adipose tissue content.

2.3. Blood Collection, Biochemical Analysis, and Calculations

Oral glucose tolerance tests (OGTTs) were performed in non-diabetic participants according to the World Health Organization (WHO) recommendations with a dose of 75 g oral glucose. The subjects were instructed to fast for 8–12 h prior to the test and to not restrict carbohydrate intake in the 3 days before the test. Blood was collected at 0, 30, 60, and 120 min after glucose administration. Blood samples were obtained and collected to evaluate the concentrations of plasma glucose, insulin, LDL, HDL, total cholesterol and triglyceride (TG), and hemoglobin A1c (HbA1c). The samples were prepared for assessment according to the laboratory kit instructions. Serum insulin concentrations were evaluated by immunoradiometric assay (INS-Irma, DIASource S.A., Belgium; Wallac Wizard 1470 Automatic Gamma Counter, PerkinElmer Life Sciences, Turku, Finland). Plasma glucose concentration was measured using the hexokinase enzymatic method (Cobas c111, Roche Diagnostics Ltd., Switzerland), and lipid profile was evaluated by enzymatic colorimetric assay using commercially available kits (Cobas c111, Roche Diagnostic Ltd., Switzerland). HbA1c levels were measured using high-performance liquid chromatography (HPLC) (D-10 Hemoglobin Testing System, Bio-Rad Laboratories Inc., Hercules, CA, USA; Bio-Rad, Marnes-la-Coquette, France).

The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated following the standard formula: (fasting plasma glucose concentration (mmol/L)) × (fasting insulin concentration (μU/mL))/22.5.

2.4. Daily Physical Activity and Dietary Intake Analyses

To evaluate daily physical activity, the International Physical Activity Questionnaire-Long Form (IPAQ-LF) was used. Metabolic equivalent (MET, min per week) was determined using the following formula: (MET level) × (minutes of activity) × (events per week) [23]. Individuals were stratified as having a low, moderate, or high level of physical activity.

Subjects were asked to record 3-day food intake diaries and were instructed on how to estimate portion sizes of foods based on the provided color photograph albums of portion sizes. Moreover, the subjects were instructed on how to weigh the food, if possible. Daily carbohydrate, protein, fat, and total energy intake were estimated using Dieta 6.0 software (National Food and Nutrition Institute, Warsaw, Poland). Dieta software was developed and is continuously updated by the National Food and Nutrition Institute (Warsaw, Poland), and it is used to calculate the nutritional value of food and diets based on tables of the nutritional value of local food products and dishes. In order to study the interactions between genetic factors and diet, study participants were divided into 2 quantiles based on average daily protein, fat, and carbohydrate intake: lower and higher than median dietary protein intake (≤18% and >18% of total energy intake, respectively), lower and higher than median dietary carbohydrate intake (≤48% and >48% of total energy intake, respectively), and lower and higher than median dietary fat intake (≤30% and >30% of total energy intake, respectively).

2.5. Genetic Analyses

We genotyped 4 common *FTO* SNPs in rs3751812 (G > T), rs8044769 (C > T), rs8050136 (A > C), and rs9939609 (T > A). DNA was extracted from peripheral blood leukocytes using a classical salting-out method. The SNPs were genotyped with TaqMan SNP technology from a ready-to-use human assay library (Applied Biosystems, MA, USA) using a high-throughput genotyping system, OpenArray (Life Technologies, CA, USA). SNP analysis was performed in duplicate, following the manufacturer's instructions. We used a sample without template as a negative control to detect possible false positive signals caused by contamination.

2.6. Ethics Statement

The study methods were carried out in accordance with the ethical standards on human experimentation and with the Helsinki Declaration of 1975 as revised in 1983. Written informed consent was obtained from all participants before inclusion in the study. The study protocol was approved by the local Ethics Committee of the Medical University of Bialystok, Poland (R-I-002/35/2009).

2.7. Statistical Analysis

Numerical data were summarized with number of observations (N), arithmetic mean, and standard deviation (SD). For categorical data, number of observations and frequency (percentage) were presented. Study participants were divided into quantiles based on average daily protein, carbohydrate, and fat intake, with the thresholds set as the median value of each parameter. Risk genotypes of the 4 common *FTO* SNPs were predefined based on the literature and our previous findings. Because of the relatively small sample size, we did not include a comparison of the allelic and genotypic frequencies and odds ratio calculations in this study. Continuous parameters were tested for normality with Shapiro-Wilk's test as well as visual inspection. Homogeneity of variance across groups was studied using Levene's test. Nonparametric tests were used for response variables that failed the mentioned statistical tests. Differences between selected parameters and dietary groups were then compared using analysis of variance (ANOVA) or Kruskal-Wallis test for numerical variables, with either Tukey's or Dunn's post-hoc test with Holm *p*-value adjustment (in case multiple pairwise tests were performed, or when there were multiple grouping variables, as presented in tables and figures), and chi-squared test for categorical variables. In order to study the hypothesis that the relationship between *FTO* genotypes and continuous responses varies in average daily protein, fat, and carbohydrate intake groups, we added (dietary macronutrient quantile) x (genotype) interaction terms to the multivariate linear regression models. These models were adjusted for age, sex, BMI (when applicable), total average energy intake (kcal/day), and physical activity. The Huber-White robust standard errors (HC1) were calculated. Model fit was estimated using R-squared values plus adjusted R-squared values. Some of the models were optimized by a stepwise backward elimination based on the Akaike information criterion (AIC). The statistical significance level was set at <0.05 for all 2-sided tests and multivariate comparisons. All calculations were prepared in R (version 4.0.2) [24].

3. Results

Our analysis identified 411 participants (50.2%) as having prediabetes or diabetes, without any previously known history of glucose homeostasis disturbance.

For the diet-gene interaction analysis, we included data from 819 subjects (Supplementary Figure S1). The general clinical characteristics of the studied population are presented in Table 1, and characteristics stratified by investigated genotypes are presented in Tables 2–4. No significant deviation from the Hardy-Weinberg equilibrium was observed for any of the investigated SNPs ($p > 0.05$). Among the investigated *FTO* SNPs, some of the loci were in very strong linkage disequilibrium ($D' = 1.0$ for rs8050136 and rs9939609) [25], so we present results for rs8050136.

Based on the demographic, anthropometric, behavioral (food intake and physical activity), and laboratory data, we observed that GG genotype carriers of rs3751812 (Table 2) and CC genotype carriers of rs8050136 (Table 3) presented significantly lower hip circumference. We also found that carriers of the TT rs8044769 genotype had the highest total cholesterol levels, and CT carriers presented the lowest percentage of daily energy intake from fat (Table 4).

We did not observe any other significant differences between studied genotypes; however, we noticed a tendency toward higher BMI, total body fat content, and waist circumference in TT genotype carriers of rs3751812 (Table 2) and AA genotype carriers of rs8050136 (Table 3). Between carriers of investigated genetic variants in rs8044769, we noted a tendency for differences in low-density lipoprotein cholesterol (LDL-cholesterol) concentration and daily physical activity level (Table 4).

3.1. Dietary Assessment

The 3-day food diaries were available from 662 subjects from the general cohort group and from 490 subjects who were genotyped for the investigated *FTO* SNPs. We did not find any differences between genotypes and dietary habits, except for the rs8044769 SNP. The heterozygous CT genotype carriers presented the lowest percentage of energy intake provided from dietary fat (Table 4).

We analyzed the interactions between dietary macronutrient intake and individual genotypes and their effect on continuous responses using multivariable linear regression models with the (dietary macronutrient quantile) × (genotype) interaction term. We observed that the association between selected genotypes and variables describing body composition (weight, BMI, and free fat mass) and the patient's glycemic status (fasting glucose levels) varied in different dietary groups, confirming the hypothesis that the effects of diet and genotypes interact. The differences in median values of the selected responses and the interquartile ranges (IQRs) in different genotypic and dietary strata are presented using boxplots in the figures. These results were significant after adjustment for age, sex, BMI (where applicable), and total energy intake.

Table 1. Study group characteristics.

Study Group Characteristics	
N	819
Age	42.1 (14.5)
Female/male (%)	52.5/47.5
BMI (kg/m^2)	28.5 (6.6)
<25.0	273 (33.9%)
25.0–29.9	278 (34.5%)
≥30.0	255 (31.6%)
Total body fat content (kg)	27.1 (13.8)
Total body fat content (%)	31.4 (9.6)
Waist circumference (cm)	96.2 (17.2)
Hip circumference (cm)	103.3 (12.7)
WHR	0.928 (0.088)
Visceral fat (cm^3)	108.4 (80.6)
Visceral fat (%)	37.1 (12.1)
Subcutaneous fat (cm^3)	167.9 (81.7)
Subcutaneous fat (%)	62.8 (12.3)
Visceral/subcutaneous fat ratio	0.669 (0.443)
Total cholesterol	195.4 (46.1)
HDL	59.7 (14.9)
LDL	112.0 (40.0)
TG	118.8 (95.1)
Fasting blood glucose level (mg/dL)	98.8 (23.9)
History of prediabetes or diabetes	
Yes	411 (50.2%)
No	408 (49.8%)
Dietary assessment (n)	490
Daily energy intake (kcal)	1792.5 (697.4)
Daily energy from protein (%)	18.9 (4.8)
Daily energy from fat (%)	31.2 (7.5)
Daily energy from carbohydrates (%)	47.6 (8.6)
Daily physical activity level	
Low	60 (7.3%)
Moderate	173 (21.1%)
High	586 (71.6%)

Data presented as mean and standard deviation (SD). BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; WHR, waist–hip ratio.

3.2. Association of rs3751812 Genetic Variants with Obesity, Anthropometric Measures, Lipid Profile, and Dietary Intake

The comparison between genotypes showed that carriers of the GG genotype presented lower body weight (Figure 1A), BMI (Figure 1B), total body fat content (Figure 1C), and hip (Figure 1D) and waist (Figure 1E) circumference, but higher total cholesterol (Figure 1F) and LDL-cholesterol (Figure 1G) levels, when compared to the GT genotype carriers, and lower body weight (Figure 1A), BMI (Figure 1B), and hip (Figure 1D) and waist (Figure 1E) circumference when compared to the TT genotype carriers.

Table 2. Characteristics of participants stratified by rs3751812 genotypes.

rs3751812	G/G	G/T	T/T	*p*-Value *
N	211	420	181	
Genotype frequency	0.26	0.52	0.22	>0.05
Age	40.5 (14.2)	41.2 (14.7)	39.5 (14.3)	0.33
Female (%)	53.8% (0.49)	53.8% (0.50)	47.8% (0.50)	0.36
BMI (kg/m^2)	27.6 (6.0)	28.7 (6.8)	28.9 (6.8)	0.060
<25.0	81 (38.8%)	136 (32.9%)	53 (29.9%)	
25.0–29.9	69 (33.0%)	141 (34.1%)	66 (37.3%)	0.410
≥30.0	59 (28.2%)	136 (32.9%)	58 (32.8%)	
Total body fat content (kg)	25.3 (12.4)	27.6 (13.8)	28.2 (15.2)	0.080
Total body fat content (%)	30.6 (9.1)	31.8 (9.6)	31.6 (10.3)	0.377
Waist circumference (cm)	94.3 (17.5)	96.7 (17.2)	97.5 (16.7)	0.054
Hip circumference (cm)	101.3 (12.4)	104.2 (13.0)	103.8 (12.5)	0.008
WHR	0.927 (0.091)	0.925 (0.088)	0.937 (0.085)	0.327
Visceral fat (cm^3)	103.0 (81.0)	110.0 (79.9)	112.3 (83.0)	0.379
Visceral fat (%)	36.4 (11.8)	37.5 (12.4)	37.2 (11.7)	0.587
Subcutaneous fat (cm^3)	163.5 (83.1)	167.2 (80.5)	175.0 (82.7)	0.401
Subcutaneous fat (%)	63.7 (11.7)	62.3 (12.9)	62.8 (11.7)	0.557
Visceral/subcutaneous fat ratio	0.642 (0.406)	0.687 (0.475)	0.665 (0.413)	0.554
Total cholesterol	202.7 (56.0)	191.7 (41.3)	194.0 (43.2)	0.070
HDL	60.7 (14.1)	59.8 (15.6)	59.5 (14.5)	0.662
LDL	117.3 (43.3)	109.4 (37.8)	111.2 (41.8)	0.095
TG	123.8 (143.9)	111.9 (69.7)	116.3 (61.9)	0.289
Blood glucose level during OGTT (mg/dL)				
0 min	96.8 (24.1)	95.6 (18.3)	97.1 (20.8)	0.914
30 min	147.0 (44.3)	145.0 (31.6)	150.1 (35.6)	0.312
60 min	132.3 (56.0)	129.5 (46.3)	134.2 (46.3)	0.380
120 min	100.7 (46.1)	99.1 (32.1)	98.8 (31.0)	0.621
History of prediabetes or diabetes				
Yes	103 (48.8%)	209 (49.8%)	95 (52.5%)	0.751
No	108 (51.2%)	211 (50.2%)	86 (47.5%)	
Dietary assessment (n)	126	259	101	
Daily energy intake (kcal)	1807.2 (732.3)	1766.9 (676.0)	1837.4 (713.4)	0.849
Daily energy from protein (%)	18.7 (4.4)	19.0 (4.9)	19.1 (4.9)	0.901
Daily energy from fat (%)	31.2 (7.2)	30.9 (7.5)	31.9 (7.8)	0.568
Daily energy from carbohydrates (%)	47.6 (7.7)	47.8 (9.1)	46.8 (8.5)	0.662
Daily physical activity level				
Low	16 (7.6%)	25 (6.0%)	18 (9.9%)	
Moderate	50 (23.7%)	83 (19.8%)	40 (22.1%)	0.302
High	145 (68.7%)	312 (74.3%)	123 (68.0%)	

Data presented as mean and standard deviation (SD), number of observations, and frequency. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; TG, triglycerides; WHR, waist-hip ratio. * Holm-adjusted Kruskal-Wallis/ANOVA *p*-values.

Based on the analysis of the interactions between rs3751812 genotypes and carbohydrate intake, we observed that GG genotype carriers presented lower body weight (Figure 2A), BMI (Figure 2B),

fat-free mass levels (Figure 2C), subcutaneous fat content (Figure 2D), and waist (Figure 2E) and hip (Figure 2F) circumference, as well as lower fasting blood glucose (Figure 2G) and higher HDL-cholesterol (Figure 2H) levels, when they were stratified to the group with higher than median carbohydrate intake. Moreover, we noted that TT carriers in the group with higher than median carbohydrate intake presented lower fasting insulin levels (Figure 2I) and HOMA-IR values (Figure 2J) compared to participants who were stratified to the group with lower than median carbohydrate intake. The interaction effect of (carbohydrate diet group) × (rs3751812 genotype) on body composition, anthropometric measures, and lipid profile was statistically significant with p-value < 0.05.

Table 3. Characteristics of participants stratified by rs8050136 genotypes.

rs8050136	**C/C**	**A/C**	**A/A**	***p*-Value ***
N	209	424	182	
Genotype frequency	0.26	0.52	0.22	>0.05
Age	40.2 (14.1)	41.3 (14.8)	39.4 (14.3)	0.24
Females (%)	54.3% (0.49)	53.4% (0.49)	47.7% (0.50)	0.36
BMI (kg/m^2)	27.6 (6.1)	28.7 (6.8)	28.9 (6.8)	0.063
<25.0	80 (38.6%)	138 (33.2%)	54 (30.2%)	
25.0–29.9	69 (33.3%)	140 (33.7%)	67 (37.4%)	0.422
≥30.0	58 (28.0%)	138 (33.2%)	58 (32.4%)	
Total body fat content (kg)	25.3 (12.4)	27.5 (13.8)	28.2 (15.2)	0.095
Total body fat content (%)	30.6 (9.1)	31.8 (9.6)	31.6 (10.4)	0.465
Waist circumference (cm)	94.2 (17.6)	96.7 (17.2)	97.4 (16.6)	0.053
Hip circumference (cm)	101.2 (12.5)	104.1 (13.0)	103.8 (12.4)	0.008
WHR	0.927 (0.092)	0.925 (0.088)	0.936 (0.085)	0.382
Visceral fat (cm^3)	103.3 (81.5)	110.2 (80.0)	111.8 (82.5)	0.381
Visceral fat (%)	36.4 (11.7)	37.6 (12.5)	37.1 (11.6)	0.570
Subcutaneous fat (cm^3)	163.7 (83.5)	166.9 (80.8)	175.3 (83.1)	0.405
Subcutaneous fat (%)	63.7 (11.6)	62.2 (13.0)	62.9 (11.6)	0.540
Visceral/subcutaneous fat ratio	0.641 (0.404)	0.690 (0.477)	0.662 (0.410)	0.536
Total cholesterol	201.9 (56.1)	192.1 (41.4)	193.7 (43.1)	0.153
HDL	60.8 (14.0)	59.6 (15.7)	59.7 (14.4)	0.422
LDL	116.3 (43.3)	109.9 (37.9)	111.1 (41.8)	0.189
TG	124.1 (144.3)	113.2 (71.1)	115.1 (61.3)	0.491
Blood glucose level during OGTT (mg/dL)				
0 min	96.8 (24.2)	95.8 (18.5)	96.8 (20.4)	0.922
30 min	146.6 (44.5)	145.2 (31.7)	150.1 (35.4)	0.263
60 min	131.7 (56.4)	129.9 (46.1)	133.9 (46.1)	0.413
120 min	100.2 (46.3)	99.3 (32.2)	98.6 (30.9)	0.493
History of prediabetes or diabetes				
Yes	100 (47.8%)	213 (50.2%)	96 (52.7%)	0.628
No	109 (52.2%)	211 (49.8%)	86 (47.3%)	
Dietary assessment (n)	103	264	123	
Daily energy intake (kcal)	1820.5 (734.9)	1759.6 (673.3)	1853.9 (716.3)	0.645
Daily energy from protein (%)	18.7 (4.5)	19.0 (4.9)	19.0 (4.9)	0.855
Daily energy from fat (%)	31.2 (7.2)	30.9 (7.5)	31.9 (7.8)	0.506
Daily energy from carbohydrates (%)	47.6 (7.7)	47.9 (9.1)	46.8 (8.4)	0.572
Daily physical activity level				
Low	16 (7.7%)	25 (5.9%)	19 (10.4%)	
Moderate	50 (23.9%)	83 (19.6%)	40 (22.0%)	0.179
High	143 (68.4%)	316 (74.5%)	123 (67.6%)	

Data presented as mean and standard deviation (SD), number of observations, and frequency. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; TG, triglycerides; WHR, waist-hip ratio. * Holm-adjusted Kruskal-Wallis/ANOVA p-values.

Table 4. Characteristics of participants stratified by rs8044769 genotypes.

rs8044769	C/C	C/T	T/T	*p*-Value *
N	270	406	138	
Genotype frequency	0.33	0.50	0.17	>0.05
Age	40.4 (14.8)	41.2 (14.6)	39.6 (13.7)	0.54
Females (%)	51.8% (0.50)	51.9% (0.50)	56.2% (0.49)	0.65
BMI (kg/m^2)	28.5 (6.8)	28.7 (6.7)	27.8 (6.1)	0.534
<25.0	88 (33.2%)	131 (32.8%)	51 (37.5%)	
25.0–29.9 (kg/m^2)	92 (34.7%)	143 (35.8%)	43 (31.6%)	0.862
≥30.0 (kg/m^2)	85 (32.1%)	126 (31.5%)	42 (30.9%)	
Total body fat content (kg)	27.7 (15.0)	27.2 (13.6)	25.8 (11.9)	0.676
Total body fat content (%)	31.7 (10.1)	31.4 (9.6)	31.1 (9.0)	0.893
Waist circumference (cm)	96.2 (17.2)	96.8 (17.3)	94.6 (16.8)	0.429
Hip circumference (cm)	103.3 (12.6)	104.0 (13.0)	101.5 (12.2)	0.189
WHR	0.928 (0.089)	0.927 (0.088)	0.929 (0.089)	0.980
Visceral fat (cm^3)	109.2 (78.3)	110.3 (83.7)	99.8 (72.5)	0.648
Visceral fat (%)	37.7 (11.8)	36.9 (12.3)	36.4 (12.0)	0.617
Subcutaneous fat (cm^3)	168.6 (83.3)	169.7 (82.4)	160.9 (74.2)	0.773
Subcutaneous fat (%)	62.3 (11.8)	63.0 (12.8)	63.7 (11.8)	0.598
Visceral/subcutaneous fat ratio	0.689 (0.492)	0.662 (0.421)	0.641 (0.408)	0.590
Total cholesterol	193.8 (40.1)	191.7 (42.7)	206.7 (62.7)	0.029
LDL	111.0 (38.6)	109.6 (38.7)	119.8 (46.5)	0.058
HDL	60.7 (14.7)	59.2 (15.5)	60.4 (13.8)	0.176
TG	110.5 (58.7)	114.6 (73.4)	132.6 (170.6)	0.689
Blood glucose level during OGTT (mg/dL)				
0 min	96.6 (20.0)	96.1 (21.0)	96.0 (20.1)	0.910
30 min	149.6 (35.1)	143.7 (32.0)	148.8 (47.8)	0.229
60 min	133.3 (45.4)	128.7 (47.3)	133.5 (59.3)	0.303
120 min	98.6 (31.8)	99.3 (30.7)	100.6 (53.0)	0.147
History of prediabetes or diabetes				
Yes	143 (53.0%)	201 (49.5%)	64 (46.4%)	0.420
No	127 (47.0%)	205 (50.5%)	74 (53.6%)	
Dietary assessment (n)	157	248	83	
Daily energy intake (kcal)	1775.7 (646.2)	1792.7 (735.8)	1816.0 (686.3)	0.791
% of daily energy from protein	18.9 (4.8)	19.2 (5.0)	18.4 (4.1)	0.608
% of daily energy from fat	32.5 (7.3)	30.1 (7.5)	31.9 (7.4)	0.005
% of daily energy from carbohydrates	46.5 (8.5)	48.3 (8.9)	47.1 (8.1)	0.164
Daily physical activity level				
Low	23 (8.5%)	26 (6.4%)	10 (7.2%)	
Moderate	55 (20.4%)	77 (19.0%)	41 (29.7%)	0.060
High	192 (71.1%)	303 (74.6%)	87 (63.0%)	

Data presented as mean and standard deviation (SD), number of observations, and frequency. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; TG, triglycerides; WHR, waist-hip ratio. * Holm-adjusted Kruskal-Wallis/ANOVA *p*-values.

Our further analysis showed that GG carriers in the group with lower than median protein intake presented lower blood glucose levels at 60 (Figure 3A) and 120 min (Figure 3B) of OGTT, while TT genotype participants in the group with higher than median protein intake presented higher insulin levels at 60 min (Figure 3C). We also observed higher insulin levels at 120 min of OGTT in GG and TT genotype carriers stratified to the group with higher than median protein intake (Figure 3D). The heterozygous GT genotype carriers in the group with lower than median dietary protein intake presented lower body weight (Figure 3E), BMI (Figure 3F) and total body (Figure 3G) and subcutaneous (Figure 3H) fat content. Using linear modeling, we found a significant interaction effect of (protein diet group) × (rs3751812 genotype) on body composition (p-value < 0.05) and blood glucose and insulin levels (p-value < 0.01) at 60 and 120 min of OGTT.

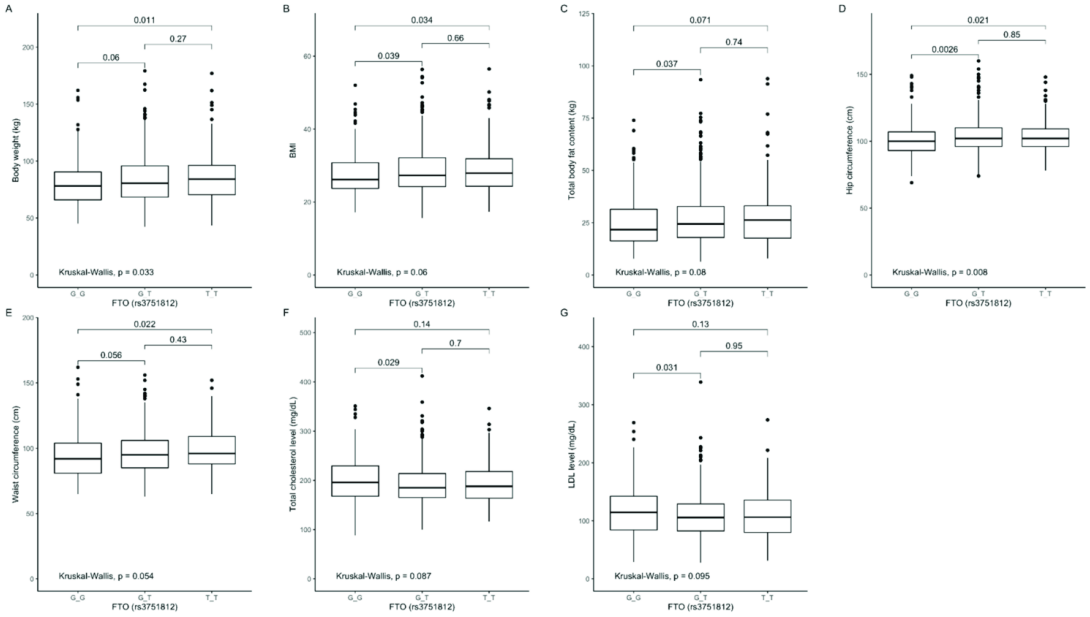

Figure 1. Association of fat mass and obesity-associated (*FTO*) genotype rs3751812 with (**A**) body weight (kg), (**B**) BMI (kg/m^2), (**C**) total body fat content (kg), (**D**) hip circumference (cm^3), (**E**) waist circumference (cm^3), (**F**) total cholesterol level (mg/dL), (**G**) LDL level (mg/dL).

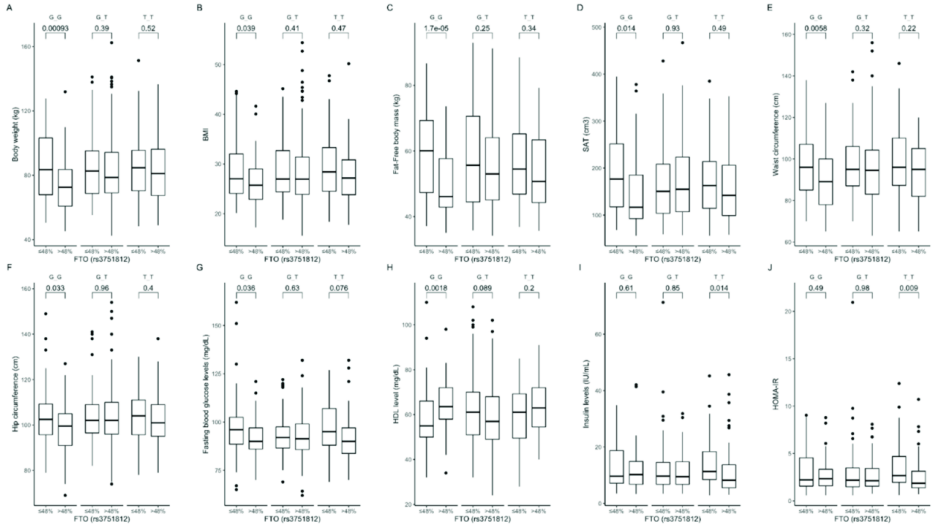

Figure 2. Association of *FTO* genotypes rs3751812 with (**A**) body weight (kg), (**B**) BMI (kg/m^2), (**C**) fat-free body mass (kg), (**D**) subcutaneous adipose tissue (SAT) (cm^3), (**E**) waist circumference (cm^3), (**F**) hip circumference (cm^3), (**G**) fasting blood glucose level (mg/dL), (**H**) HDL level (mg/dL), (**I**) fasting blood insulin level (IU/mL), and (**J**) HOMA-IR by dietary carbohydrate intake strata: ≤48% and >48% of total daily energy intake. HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

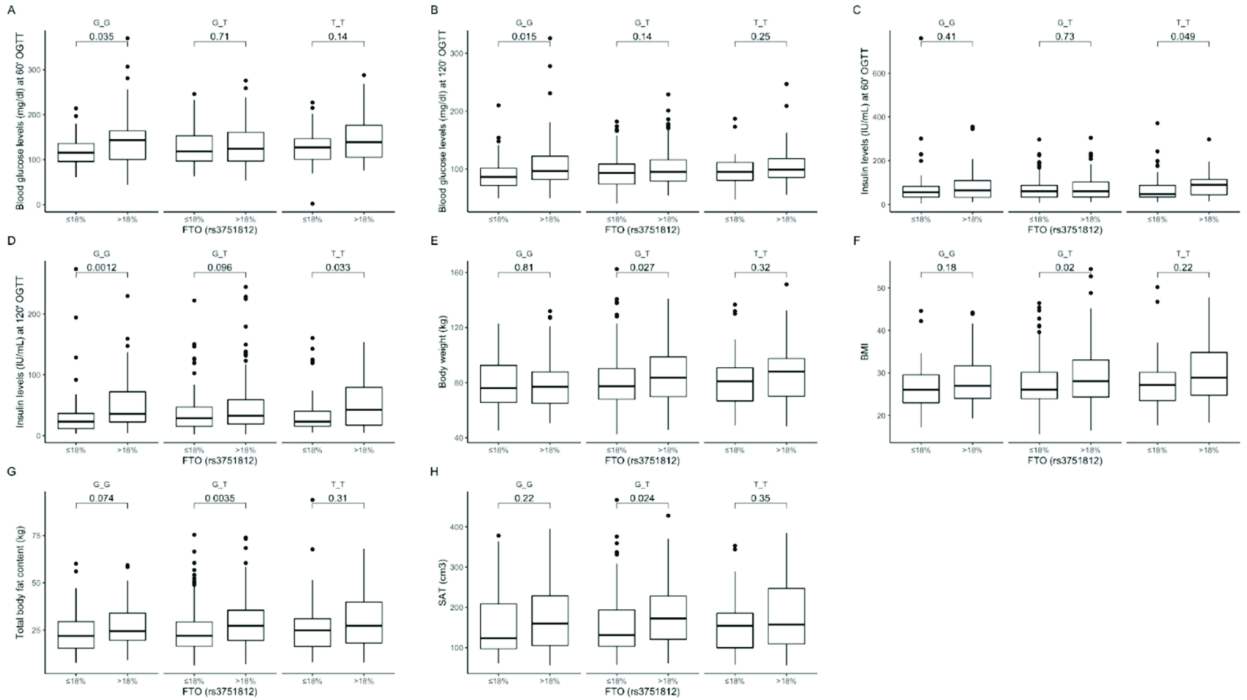

Figure 3. Association of dietary protein intake ≤18% and >18% of total daily energy intake with blood glucose level (mg/dL) at (**A**) 60 min and (**B**) 120 min of OGTT; insulin level (IU/mL) at (**C**) 60 min and (**D**) 120 min of OGTT; (**E**) body weight (kg); (**F**) BMI (kg/m^2); (**G**) total body fat content (kg); and (**H**) SAT (cm^3) in *FTO* rs3751812 genotype carriers.

Analyzing the dietary fat intake, we noted that carriers of the GG genotype stratified to the group with lower than median fat intake presented lower subcutaneous (Figure 4A) and visceral (Figure 4B) fat content. Surprisingly, we observed that GT genotype carriers showed higher HDL levels (Figure 4C) when they were stratified to the group with higher than median fat intake. We did not observe any other association with dietary fat intake. The interaction effect of (fat diet group) × (rs3751812 genotype) on subcutaneous and visceral fat content was statistically significant, with p-value < 0.01, as well as on HDL levels (p-value < 0.002).

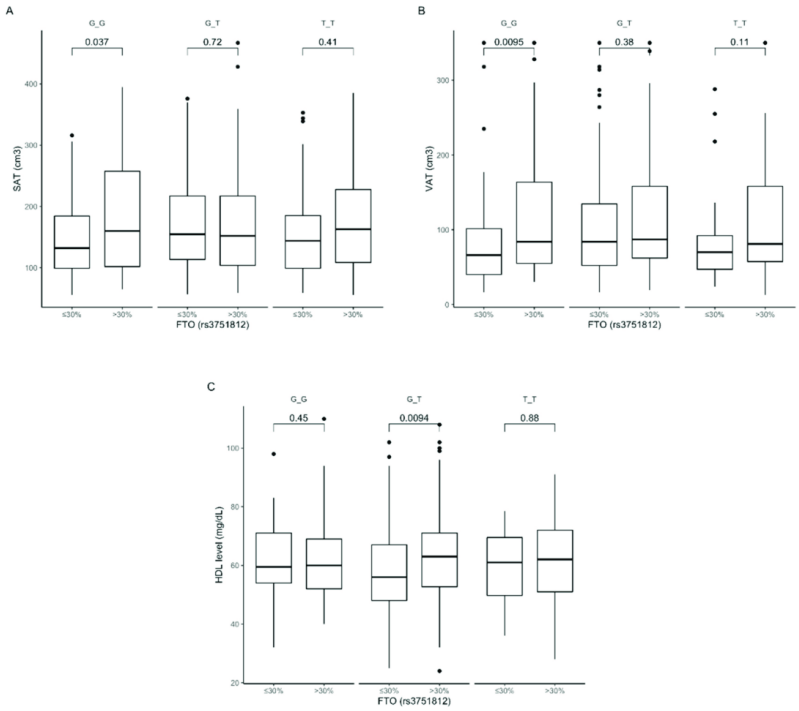

Figure 4. Association of dietary fat intake ≤30% and >30% of total daily energy intake with (**A**) SAT (cm^3), (**B**) VAT (cm^3), and (**C**) HDL level (mg/dL) in *FTO* rs3751812 genotype carriers.

3.3. Association of rs8050136 Genetic Variants with Obesity, Anthropometric Measures, Lipid Profile, and Dietary Intake

Our analysis showed that CC genotype carriers presented significantly lower body weight (Figure 5A), BMI (Figure 5B), and waist (Figure 5D) and hip (Figure 5E) circumference compared to TT, and significantly lower BMI (Figure 5B), total body fat content (Figure 5C), and hip (Figure 5E) circumference when compared to CT genotype carriers.

Based on the analysis of the interactions between rs8050136 genotypes and carbohydrate intake, we observed that CC genotype carriers in the group with higher than median carbohydrate intake presented lower body weight (Figure 6A), fat-free body mass level (Figure 6B), skeletal muscle mass content (Figure 6C), subcutaneous fat content (Figure 6D), and waist circumference (Figure 6E) and higher HDL-cholesterol level (Figure 6F). The interaction effect of (carbohydrate diet group) × (rs8050136 genotypes) on body composition (p-value < 0.05) and HDL (p-value < 0.01) was statistically significant.

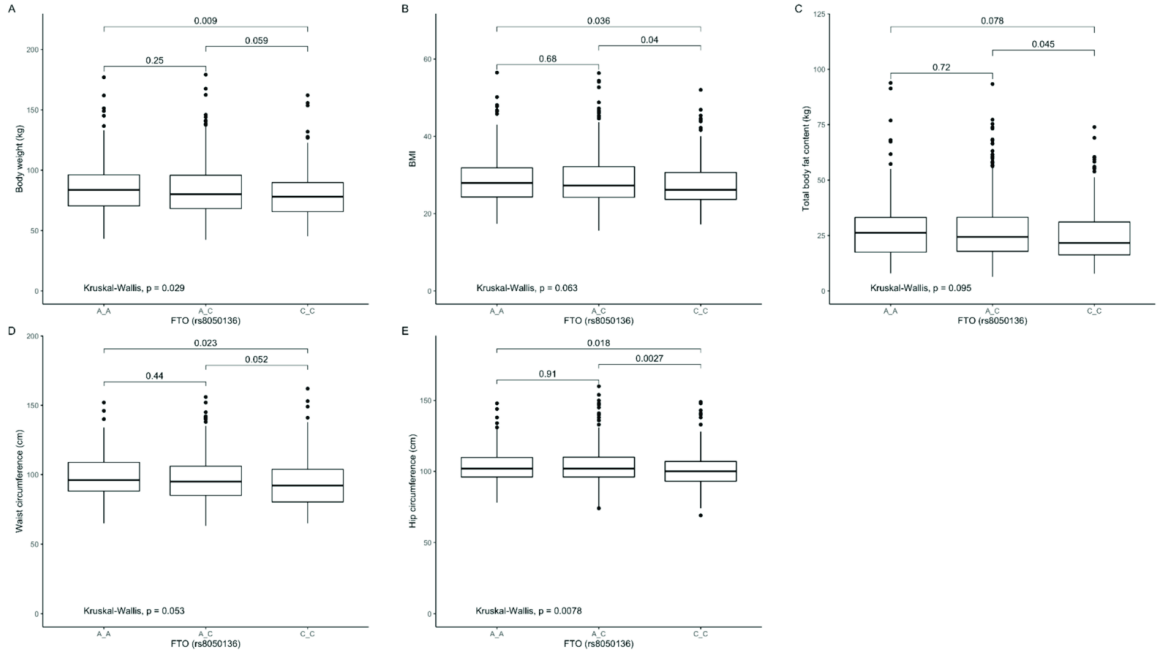

Figure 5. Association of *FTO* genotypes rs8050136 with (**A**) body weight (kg), (**B**) BMI (kg/m^2), (**C**) total body fat content (kg), (**D**) waist circumference (cm^3), (**E**) hip circumference (cm^3).

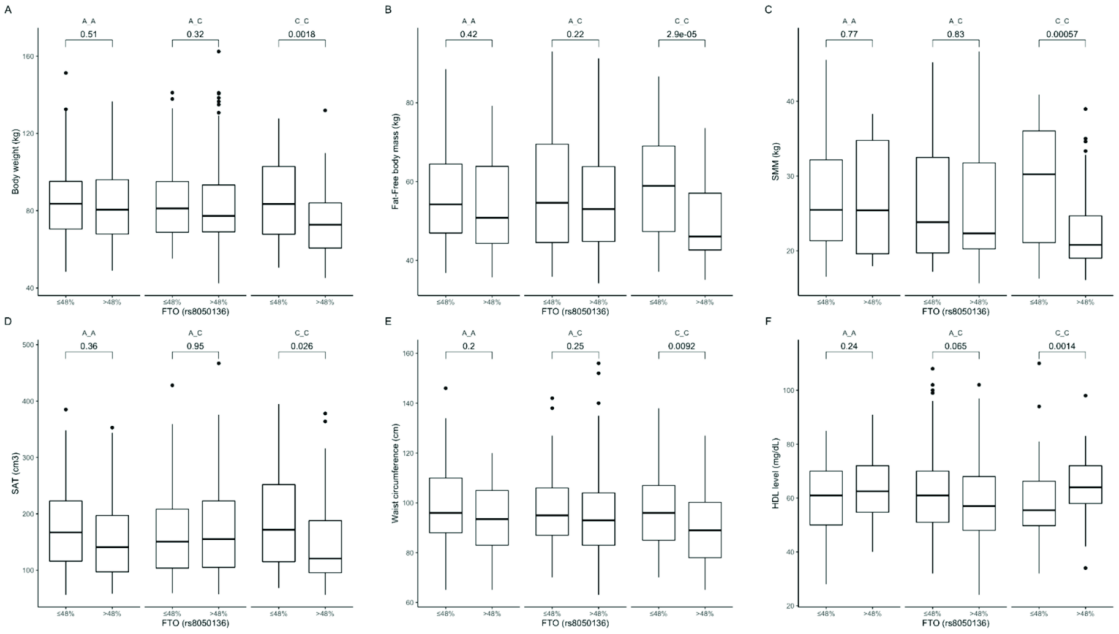

Figure 6. Association of *FTO* genotypes rs8050136 with (**A**) body weight (kg), (**B**) fat-free body mass (kg), (**C**) skeletal muscle mass (SMM) (kg), (**D**) SAT (cm^3), (**E**) waist circumference (cm^3), and (**F**) HDL level (mg/dL) by dietary carbohydrate intake strata: ≤48% and >48% of total daily energy intake.

We observed that AC genotype carriers stratified to the group with lower than median protein intake presented lower body weight (Figure 7A), BMI (Figure 7B), total body fat content (Figure 7C), and subcutaneous fat content (Figure 7D). Additionally, we noticed that CC genotype carriers who were stratified to the group with higher than median protein intake presented higher blood glucose levels at 60 min (Figure 7E) and 120 min (Figure 7F) of OGTT. Higher insulin levels were observed in AA genotype carriers at 60 min (Figure 7G) and 120 min (Figure 7H) of OGTT, and in CC genotype carriers at 120 min (Figure 7H) of OGTT. The interaction effect of (protein diet group) × (rs8050136 genotypes) on body composition, anthropometric measures, and lipid profile was statistically significant with p-value < 0.05.

The analysis of dietary fat intake showed that carriers of the CC genotype stratified to the group with lower than median fat intake presented surprisingly higher subcutaneous fat content (Figure 8A) and lower visceral fat content (Figure 8B), as well as lower VAT/SAT ratio (Figure 8C). In AA genotype carriers, we noticed similar tendencies (Figure 8A,C). In carriers of the AC genotype stratified to the group with higher than median fat intake, we observed higher HDL-cholesterol levels (Figure 8D) compared to those who were stratified to the group with lower than median fat intake. The interaction effect of (fat diet group) × (rs3751812 genotype) on subcutaneous and visceral fat content as well as it ratio was statistically significant with p-value < 0.01, the interaction effect on HDL was the largest, with the p-value of 0.001.

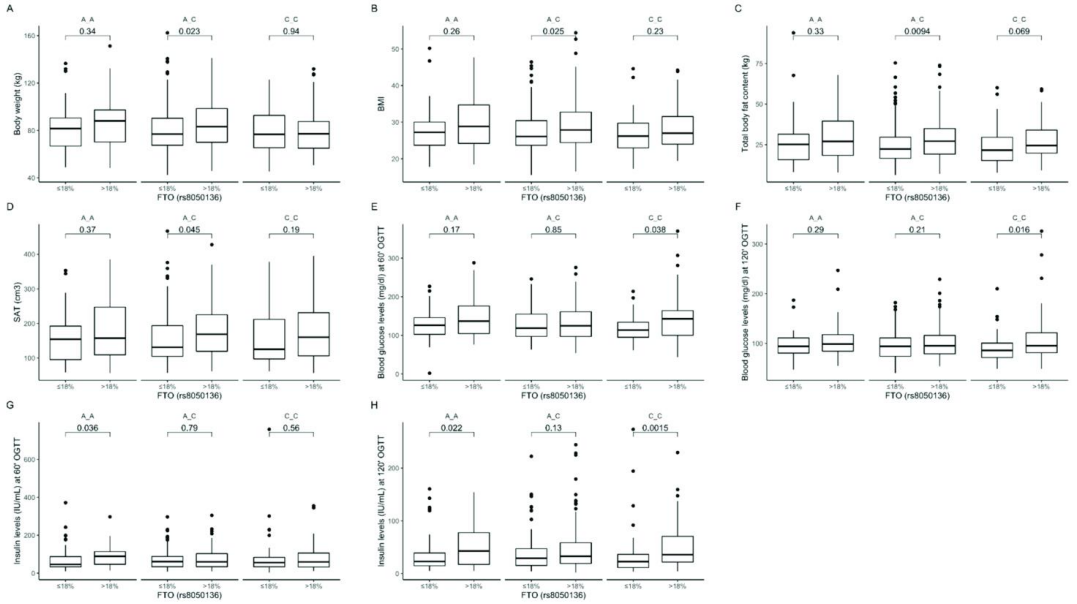

Figure 7. Association of dietary protein intake ≤18% and >18% of total daily energy intake with (**A**) body weight (kg); (**B**) BMI (kg/m^2); (**C**) total body fat content (kg); (**D**) SAT (cm^3); blood glucose level (mg/dL) at (**E**) 60 min and (**F**) 120 min of OGTT; and insulin level (IU/mL) at (**G**) 60 min and (**H**) 120 min of OGTT in *FTO* rs8050136 genotype carriers.

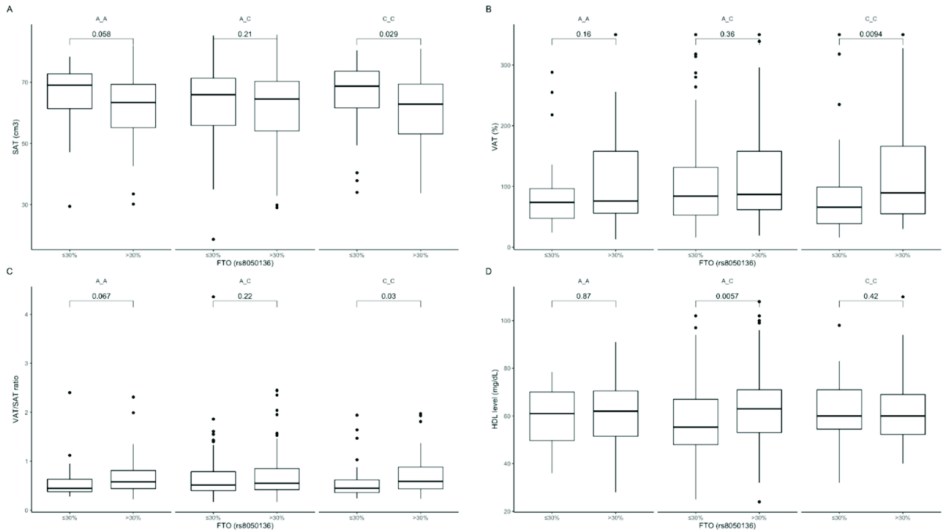

Figure 8. Association of dietary fat intake ≤30% and >30% of total daily energy intake with (**A**) SAT (cm^3), (**B**) VAT (%), (**C**) SAT/VAT ratio, and (**D**) HDL level (mg/dL) in *FTO* rs8050136 genotype carriers.

3.4. Association of rs8044769 Genetic Variants with Obesity, Anthropometric Measures, Lipid Profile, and Dietary Intake

The TT genotype carriers of rs8044769 presented significantly higher total cholesterol (Figure 9A) and LDL-cholesterol (Figure 9B) levels when compared to CT genotype carriers, and similar marginally significant results were noted between TT and CT genotype carriers (Figure 9A,B).

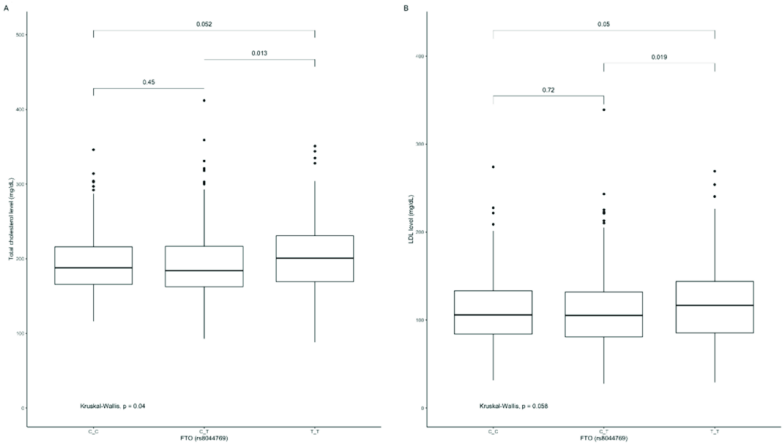

Figure 9. Association of *FTO* genotypes rs8044769 with (**A**) total cholesterol level (mg/dL) and (**B**) LDL level (mg/dL).

The gene-diet interaction analysis showed that TT and CT genotype carriers presented higher body weight (Figure 10A), BMI (Figure 10B), fat-free body mass (Figure 10C), and waist circumference (Figure 10D) when they were stratified to the group with lower than median carbohydrate intake. Homozygous TT carriers in the group with lower than median carbohydrate intake also presented lower HDL-cholesterol levels (Figure 10E) and surprisingly higher skeletal muscle mass content (Figure 10F). We did not notice any association between percentage of daily energy intake provided from carbohydrates and investigated metabolic parameters in CC genotype carriers. The interaction effect of (carbohydrate diet group) × (rs8044769 genotype) on body composition, anthropometric measures, and HDL was statistically significant with p-value < 0.05.

Among individuals in the group with higher than median protein intake, we observed that heterozygous CT carriers showed higher body weight (Figure 11A), BMI (Figure 11B), total body fat content (Figure 11C), subcutaneous fat content (Figure 11D), waist circumference (Figure 11E), and hip circumference (Figure 11F). In addition, we noted that both CC and TT homozygous carriers presented higher insulin concentration at 120 min (Figure 11G); however, higher fasting blood glucose levels (Figure 11H) and blood glucose levels at 30 min (Figure 11I), 60 min (Figure 11J), and 120 min (Figure 11K) of OGTT were noted only in TT genotype carriers stratified to the group with higher than median protein intake. We noted significantly higher values of HOMA-IR in TT genotype carriers, and the same tendency in CC genotype carriers, when dietary protein provided >18% of total energy compared to subjects who were stratified to the group with lower than median protein intake (Figure 11L). The interaction effect of (protein diet group) × (rs8044769 genotype) on body composition, and blood glucose and insulin levels was statistically significant with p-value < 0.05.

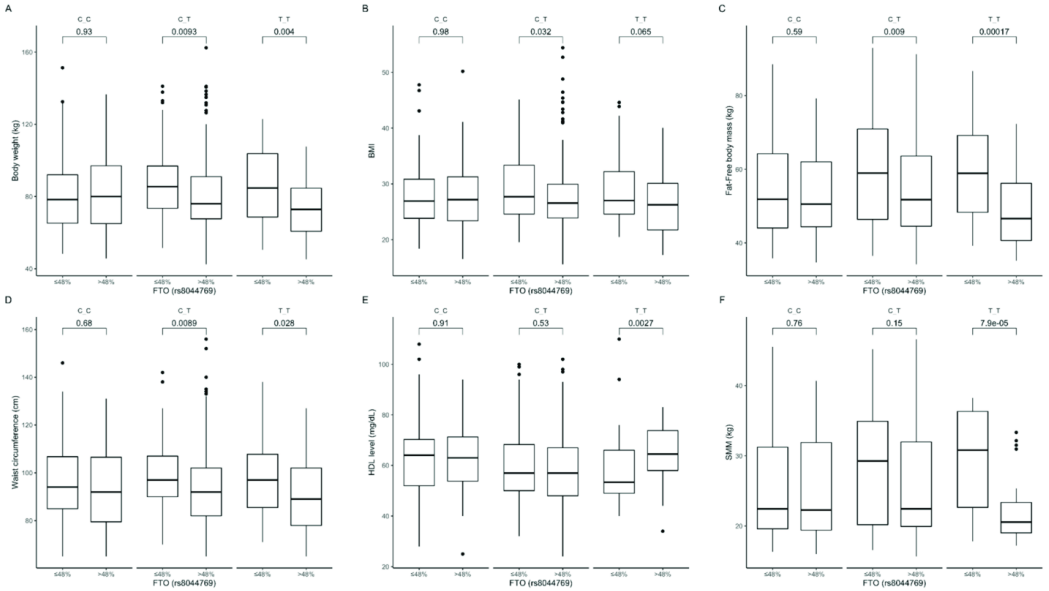

Figure 10. Association of *FTO* genotypes rs8044769 with (**A**) body weight (kg), (**B**) BMI (kg/m^2), (**C**) fat-free body mass (kg), (**D**) waist circumference (cm^3), (**E**) HDL level (mg/dL), and (**F**) SMM (kg) by dietary carbohydrate intake strata: ≤48% and >48% of total daily energy intake.

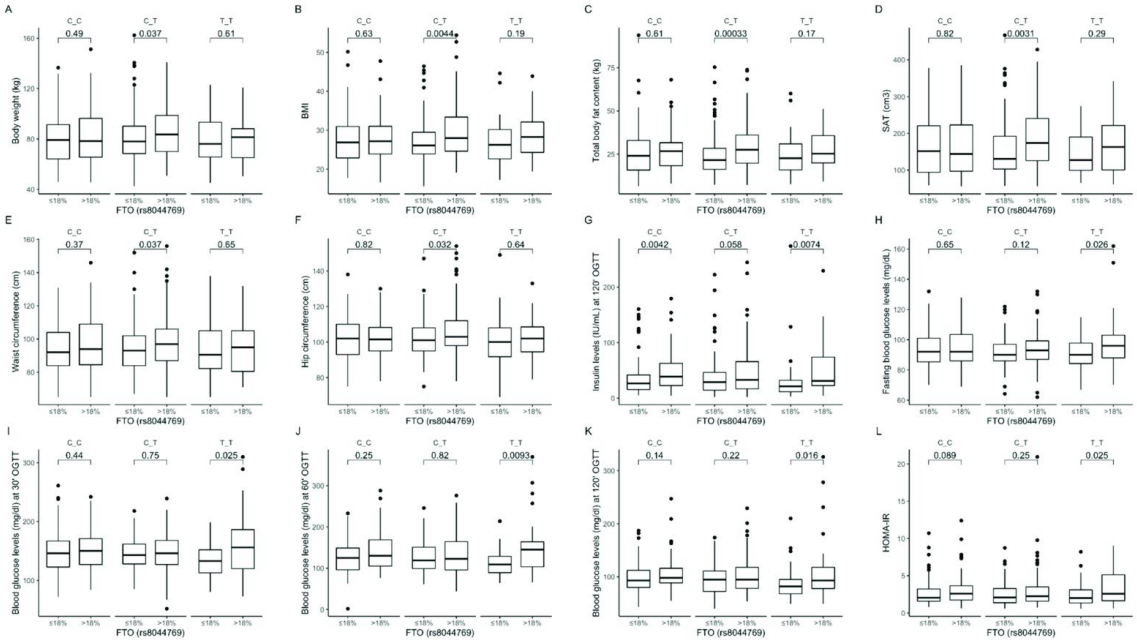

Figure 11. Association of dietary protein intake ≤18% and >18% of total daily energy intake with (**A**) body weight (kg); (**B**) BMI (kg/m^2); (**C**) total body fat content (kg); (**D**) SAT (cm^3); (**E**) waist circumference (cm^3); (**F**) hip circumference (cm^3); (**G**) insulin level (IU/mL) at 120 min of OGTT; (**H**) fasting blood glucose level (mg/dL); blood glucose level (mg/dL) at (**I**) 30 min, (**J**) 60 min, and (**K**) 120 min of OGTT; and (**L**) HOMA-IR in *FTO* rs8044769 genotype carriers.

An association with dietary fat intake was observed in carriers of the heterozygous CT genotype stratified to the group with higher than median fat intake, who presented higher fat-free body mass content (Figure 12A). TT genotype carriers had higher visceral fat content (Figure 12B) and lower subcutaneous fat content (Figure 12C) when they were stratified to the group with higher than median dietary fat intake compared to carriers of the same genotype in the group with lower than median dietary fat intake. The interaction effect of (fat diet group) × (rs8044769 genotype) on subcutaneous and visceral fat content was statistically significant with p-value < 0.05.

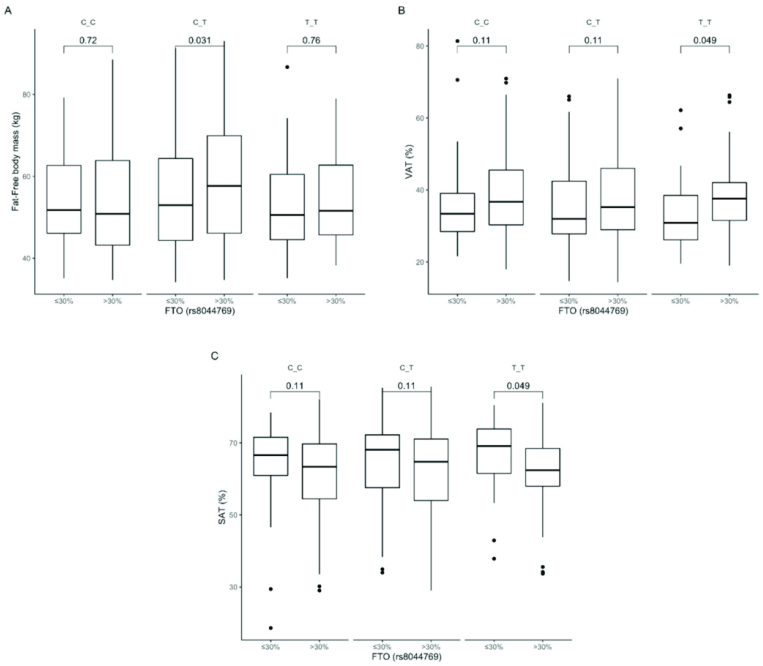

Figure 12. Association of dietary fat intake ≤30% and >30% of total daily energy intake with (**A**) fat-free body mass (kg), (**B**) SAT (cm^3), and (**C**) VAT (%) in *FTO* rs8044769 genotypes carriers.

4. Discussion

Over the past few decades, public awareness in the field of nutrition and physical activity has increased, but obesity and its comorbidities are still serious international health problems [26]. It is widely known that the *FTO* gene is an established genetic susceptibility locus for the risk of obesity development [27]. However, the association between the *FTO* gene and dietary factors is still unclear and there is a scientific need to investigate the associations between environmental and genetic risk factors and their interactions and roles in obesity development and treatment. In our study, we demonstrated an interplay between *FTO* genetic variants and dietary carbohydrate, protein, and fat intake, and the impact of these interactions on body weight, body fat content and distribution, and other anthropometric measures, as well as on glucose homeostasis and lipid profile, in a Polish population of adults. For our study, we chose some of the most common SNPs based on previously published results [25–27].

We observed a protective effect of the GG genotype of rs3751812 against obesity, but GG genotype carriers presented higher total cholesterol and LDL-cholesterol levels. It was shown previously that *FTO* rs3751812 risk allele T is related to increased BMI and body fat distribution compared to the

protective allele G [28]. However, our results indicate that carrying the GG genotype leads to more beneficial effects if more than 48% of total diet energy comes from carbohydrates; then, we could observe significantly lower obesity-related parameters. We did not notice any difference in total body fat content, and lower body weight and BMI could be associated with noted lower fat-free mass. Nevertheless, we did not observe any adverse effects of lower fat-free mass content, since we also noted lower fasting glucose and higher HDL-cholesterol concentration. The impact of the TT genotype, which appears to be a risk genotype for obesity, seems to not be related to carbohydrate intake, except for the associations with fasting insulin concentration and HOMA-IR level.

We also noted associations between dietary protein intake, SNPs, and metabolic parameters for all investigated genetic variants of rs3751812, indicating that we can observe more beneficial results if dietary protein provides no more than 18% of total daily energy intake. These observations are worth underlining, especially in light of the current interest in high-protein diets. Based on the results that we noted for high-risk TT genotype carriers (rs3751812) in the group with higher than median protein intake, including higher post-absorptive insulin levels and higher fasting insulin concentrations and HOMA-IR levels in subjects in the group with lower than median carbohydrate intake, we can hypothesize that these individuals should avoid high-protein, low-carbohydrate diets. It is also worth noting that for TT genotype subjects, we did not observe any differences or adverse effects that would depend on dietary fat intake. Moreover, it has already been found that carriers of minor allele T rs3751812 present a lower risk of obesity when they adhere better to a Mediterranean diet, which consists of higher daily consumption of fats, especially from olive oil and nuts [29]. We noted an association with dietary fat intake only in GG and GT genotypes carriers for body fat distribution and HDL-cholesterol level. In these subjects, if daily energy intake derived from fat was more than 30%, we could observe higher visceral and subcutaneous fat content.

Our study also shows significant associations of *FTO* SNPs in rs8050136 with the investigated markers of obesity. The CC genotype has been shown to play a protective role, and CC genotype carriers presented significantly lower body weight, BMI, total body fat content, and waist and hip circumference. Our observations are in line with results from previous studies [30–33]. Although numerous studies have shown an association of SNPs in rs8050136 with higher values of BMI and waist and hip circumference, the impact of dietary intake on this association is still unclear. In our study, we did not notice any crucial differences in daily dietary macronutrient intake between genotypes. Nevertheless, we observed that CC genotype subjects were more susceptible to the beneficial effects when carbohydrate in their diets provided more than 48% of total daily energy intake and no more than 18% came from dietary protein. Moreover, dietary fat should be limited to less than 30% of total daily energy intake to avoid visceral fat accumulation in these subjects. We also observed that AC genotype carriers of rs8050136 stratified to the group with lower than median dietary fat intake presented lower HDL-cholesterol levels. Bego et al. [34] observed that the risk A allele of rs8050136 was significantly associated with decreased HDL-cholesterol levels in control subjects in type 2 diabetes studies. We did not notice differences between studied genotypes, but surprisingly, we observed lower HDL-cholesterol concentrations in AC genotype carriers only when they followed a diet with less than 30% of energy from dietary fat. In these subjects, when dietary fat provided more than 30% of total energy, then HDL concentrations were significantly higher, without any differences in total cholesterol or LDL-cholesterol levels. We did not evaluate the source of fat, if the diet was rich in saturated or unsaturated fatty acids, or what could explain our observations, because it is well known that various types of fatty acids have different impacts on plasma lipid concentrations [35].

We observed that dietary protein intake might have an impact on obesity-related parameters only in heterozygous CA carriers of rs8050136, while in AA and CC genotype carriers, on glucose homeostasis-related markers, and in all cases, beneficial effects were noted when dietary protein provided no more than 18% of total daily energy intake. It has already been reported by Park et al. that the association of the rs8050136 risk variant may be partially mediated by macronutrient intake [36], but only the association with percentage of energy derived from fat has been detected.

The analysis of differences between genetic variants of rs8044769 showed only that TT genotype carriers presented higher total and LDL-cholesterol levels. We did not notice any other differences between genotypes; however, TT and CT genotype carriers presented lower body weight, BMI, and waist circumference and higher HDL-cholesterol levels when more than 48% of total daily energy was derived from carbohydrates, even if fat-free mass and skeletal muscle mass were also significantly lower. The obesity-related parameters seemed to be associated with dietary protein intake only in heterozygous CT carriers and in TT genotype carriers with glucose homeostasis-related parameters. All of the noted associations were more beneficial if daily energy derived from dietary protein intake did not exceed 18%. Moreover, our study suggests that CT and TT genotype carriers should also not consume dietary fat exceeding 30% of total daily energy intake, to avoid visceral fat accumulation. We did not observe any significant associations for CC genotype carriers that could depend on dietary protein or fat intake, except insulin levels at 120 min of OGTT, which were significantly higher in subjects stratified to the group with higher than median protein intake. There is a very limited number of available studies on *FTO* rs8044769 genetic variants, and some of them present these variants as BMI-associated SNPs [37,38], but in others, the authors did not observe such a relationship [39]. Moreover, considering the fact that all associations can vary with ethnicity, gender, dietary intake, and some other factors, studies in larger and more diverse populations are needed.

The present study has several strengths. As far as we know, this is one of the first studies to present interactions between *FTO* SNPs rs3751812, rs8044769, rs8050136, and rs9939609 and macronutrient intake, and the effect of these relationships on obesity and obesity-related complications. Another strength of our study is that it is based on a relatively large population. It is also worth noting that it has been shown that *FTO* genetic variants may influence dietary factors [14,40–42] or dietary fat intake [36,43,44], while other studies did not confirm these associations [12,15]. In general, we did not observe any significant differences in macronutrient intake between studied genotypes, which can also be interpreted as a strength of our study, because we can exclude the possibility that our results might be affected by the impact of different macronutrient intake on gene expression and the activation of different metabolic pathways. Nevertheless, several limitations of our study also need to be addressed. Some parts of our results are based on self-reported data, such as three-day diaries of food intake, and it has been shown that obese people tend to underreport or misreport their total dietary intake, especially fatty foods and foods rich in carbohydrates [45]. However, dietary questionnaires are the only known implements available for large-scale population investigations so far. Moreover, only Caucasian individuals were recruited for our study; therefore, in order to verify our findings in other ethnic groups, the data should be replicated in other populations.

Our results, if confirmed in larger populations of different ethnic groups, may have also practical clinical implications. Based on our observations, we can recommend that carriers of GG genotype of rs3751812 and CC genotype of rs8050136 follow diets in which no less than 48% of daily energy intake is derived from carbohydrates and no more than 30% from dietary fat. Moreover, carriers of TT and CT genotypes of rs8044769 should avoid diets in which carbohydrates provide less than 48% of total energy, whereas carriers of TT genotype should avoid diets in which proteins provide more than 18% of total daily energy, to prevent glucose homeostasis disturbances. These recommendations seem to be highly important, since we noticed that the mean amounts of macronutrients in the diets of the investigated population were mostly less than 48% of total energy intake for carbohydrates, more than 30% for dietary fat, and more than 18% for proteins, which may have adverse effects for carriers of the above-mentioned genotypes.

5. Conclusions

In conclusion, our findings provide new insights into the role of the interactions between diet and *FTO* SNPs in the risk of obesity and its metabolic consequences. Advances in this field bring us closer to the development of genome-customized diet recommendations to prevent obesity. Detecting *FTO*

risk genotype carriers and modifying dietary intake according to the genetic profile may be a novel, efficient strategy to prevent obesity development.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/11/3255/s1, Figure S1: Study flowchart diagram.

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Dietary Fiber Intake May Influence the Impact of *FTO* Genetic Variants on Obesity Parameters and Lipid Profile - A Cohort Study of a Caucasian Population of Polish Origin

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Dietary Fiber Intake May Influence the Impact of *FTO* Genetic Variants on Obesity Parameters and Lipid Profile—A Cohort Study of a Caucasian Population of Polish Origin

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Abstract: Genetic and environmental factors play a key role in the development of obesity. The aim of this study was to explore the potential effect of fat mass and obesity-associated (*FTO*) rs3751812, rs8050136, rs9939609, rs6499640, rs8044769, and rs7190492 genotypes and dietary fiber intake on the obesity-related parameters and lipid profile in the Polish population. We selected 819 Polish Caucasian adult subjects (52.5% female and 47.5% male) for a final gene–diet interaction analysis, with mean BMI 28.5 (±6.6) kg/m^2. We performed measurements of anthropometric parameters, total body fat content and distribution, and blood glucose, insulin, and lipid concentrations. Daily fiber intake was analyzed based on 3-day food-intake diaries, and daily physical activity was evaluated based on the International Physical Activity Questionnaire—Long Form. Our study shows that carriers of the GG genotype (rs3751812), CC genotype (rs8050136), and GG genotype (rs6499640) presented lower hip circumference if daily fiber intake was above 18 g per day. Additionally, GG genotype (rs3751812) and CC genotype (rs8050136) carriers showed surprisingly higher total cholesterol and LDL-cholesterol levels when they were stratified to the group with higher than median fiber intake. The results of this study highlight that high-fiber diets may positively affect anthropometric parameters but may also worsen lipid profile dependent on the *FTO* genotype.

Keywords: *FTO* gene; dietary fiber; gene–diet interaction; glucose metabolism; macronutrients; obesity

1. Introduction

The prevalence and development of obesity has increased substantially worldwide [1]. It has been established that obesity is a leading factor for diabetes [2] and cardiovascular disease prevalence [3]. In addition, abnormalities in serum lipids and their metabolism are one of the major risk factors of cardiovascular diseases [4]. One of the major strategies that has been attempted to address this is a balanced diet that fulfils all nutritional needs, connected with genome-customized recommendations.

The fat mass and obesity-associated (*FTO*) gene has been reported as the gene with the strongest significant correlation with obesity [5]. The associations between *FTO* genetic variants, dietary factors, and metabolic consequences are still under investigation. However, it has been postulated that associations of some *FTO* variants with obesity can occur due to their influence on dietary intake [6]. Moreover, in our previously published results, we observed that dietary carbohydrate, protein, and fat intake may modulate the impact of

FTO genetic SNPs on obesity and obesity-related metabolic consequences [7]; therefore, we decided to perform additional analyses, including on dietary fiber intake.

Dietary fiber is a non-digestible carbohydrate, derived mainly from fruits and vegetables. Moreover, dietary fiber antioxidant capacity is mainly based on the effect of bioaccessibility and bioavailability of natural antioxidants, including polyphenol compounds, in the diet [8]. Unfortunately, this interaction of dietary fiber and other components of diet could result in retarded absorption of micro- and macronutrients in the small intestine. Nevertheless, it has been shown that dietary fiber intake plays an important role in the improvement of parameters such as glycemic response [9] and plasma lipid profile [10].

The purpose of this study is to evaluate whether daily fiber intake can modify the association between genetic variations of the *FTO* gene and obesity in the Polish Caucasian population. To the best of our knowledge, this study is the first to examine associations between dietary fiber intake and *FTO* genetic variants in the Polish population, based on gene–diet interactions.

2. Materials and Methods

2.1. Participants of the Study Cohort

The study group comprised 819 participants recruited from 1549 Polish-origin Caucasian volunteers (18–79 years old) of the 1000PLUS Cohort Study (registered at www.clinicaltrials.gov as NCT03792685), described previously [7,11–13]. Participants who previously took diet supplements, medicines (weight loss, anti-diabetic, lipid-lowering, etc.), or other chemical and natural-based substances that could affect the results were eliminated from this study. Individuals who reported metabolic, endocrine, hepatic, or gastrointestinal disorders or who had bariatric surgery, which could have an impact on the study, were excluded from the study analysis as well. Subjects who previously took treatment, or who followed any special diet or dietary pattern (vegan, vegetarian, etc.), and others who met the exclusion criteria mentioned above, were not included in the analysis.

2.2. Anthropometric Measurements and Body Composition Analysis

In all subjects, we recorded the body weight and height using a standardized method [14]. Using the bioelectrical impedance method (InBody 220, Biospace, Seoul, Korea), we analyzed total body composition (fat mass, fat-free mass, and skeletal muscle mass). Body fat distribution analysis, including determination of the percentage of total body fat, visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and VAT/SAT ratio, was performed by the multi-frequency bioimpedance method (MaltronBioScan 920-2, Maltron International Ltd., Rayleigh, UK).

2.3. Oral Glucose Tolerance Test (OGTT) Performance

The OGTTs were performed in non-diabetic participants according to the World Health Organization (WHO) recommendations, using a 75 g oral glucose dose. The subjects were instructed to fast for 8–12 h prior to the tests, but not to restrict carbohydrate intake 3 days before the test. Blood was collected at 0, 30, 60, and 120 min after glucose load. Glycemia and insulin levels were measured in all study participants without a history of diabetes.

2.4. Blood Collection and Biochemical Analysis

Blood samples were obtained and collected to evaluate the concentrations of plasma glucose, insulin, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), and hemoglobin A1c (HbA1c). The samples were stored in accordance with the kit instructions until testing at −20 or −80 °C. The samples were prepared for testing in accordance with the instructions provided with the laboratory kit. Concentrations of plasma glucose were measured by the hexokinase enzymatic colorimetric assay (Cobas c111, Roche Diagnostics Ltd., Risch-Rotkreuz, Switzerland). Serum insulin concentrations were evaluated by immunoradiometric assay (INS-Irma, DIASource S.A., Ottignies-Louvain-la-Neuve, Belgium; Wallac Wizard 1470 Automatic Gamma Counter,

PerkinElmer Life Sciences, Turku, Finland). Concentrations of lipids were evaluated by enzymatic colorimetric assay using commercially available kits (Cobas c111, Roche Diagnostic Ltd., Risch-Rotkreuz, Switzerland). HbA1c levels were assessed using HPLC (high performance liquid chromatography; D-10 Hemoglobin Testing System, Bio-Rad Laboratories Inc., Hercules, CA, USA by France, Bio-Rad, Marnes-la-Coquette).

2.5. Calculations

Body mass index (BMI) was calculated using the following formula: body weight (kg) divided by height (m) squared. The waist–hip ratio (WHR) was determined by dividing waist circumference by hip circumference. The VAT/SAT ratio was calculated by dividing visceral adipose tissue content by subcutaneous adipose tissue content. In order to evaluate insulin resistance, we performed the homeostasis model assessment (HOMA-IR), following the standard formula: (fasting plasma glucose concentration (mmol/L)) × (fasting insulin concentration (μU/mL))/22.5. Homeostatic model assessment of β-cell function (HOMA-B) was determined using the following formula: 20 × fasting insulin (μIU/mL)/fasting glucose 100 (mmol/mL) − 3.5. The metabolic equivalent (MET, min per week) was calculated using the following formula: (MET level) × (minutes of activity) × (events per week).

2.6. Daily Physical Activity and Dietary Intake Analyses

Daily physical activity was estimated using the International Physical Activity Questionnaire—Long Form (IPAQ-LF), which is a self-administered questionnaire [15]. The results of the questionnaire were used to assess the level of physical activity, expressed as MET values. Each subject was stratified as having a low, moderate, or high level of physical activity.

We conducted a 3-day food-intake diary analysis. Participants were instructed to weigh food and estimate portion sizes based on provided color photograph albums of portion sizes. Daily carbohydrate, protein, fat, and fiber intake were estimated using Dieta 6.0 software (National Food and Nutrition Institute, Warsaw, Poland). Dieta 6.0 software is used to calculate the nutritional value of food and diets based on the tables of the nutritional value of local food products. In order to study the interactions between genetic factors and diet, study participants were divided into 2 quantiles based on average daily fiber intake: lower and higher than median dietary fiber intake (≤18 g a day and >18 g a day of total fiber intake, respectively).

2.7. Genetic Analysis

We selected and genotyped 6 previously identified *FTO* SNPs in rs3751812 (G > T), rs8050136 (A > C), rs9939609 (T > A), rs6499640 (G > A), rs8044769 (C > T), and rs7190492 (A > G), based on the validated catalog of published genome-wide association studies [16]. DNA was extracted from peripheral blood leukocytes using a classical salting-out method. The SNPs were genotyped with TaqMan SNP technology from a ready-to-use human assay library (Applied Biosystems, Beverly, MA, USA) using a high-throughput genotyping system, OpenArray (Life Technologies, South San Francisco, CA, USA). SNP analysis was performed in duplicate, following the manufacturer's instructions. As a negative control, we used a sample without a template. The negative control was applicable in measuring any false positive signal caused by contamination.

2.8. Ethics Statement

The study methods were carried out in accordance with the ethical standards of the responsible committee on human experimentation (Ethics Committee of the Medical University of Bialystok, Poland, R-I-002/35/2009) and with the Helsinki Declaration of 1975. Written informed consent was obtained from all participants included in the study.

2.9. Statistical Analysis

Numerical data were summarized with number of observations (N), arithmetic mean, and standard deviation (SD). For categorical data, the number of observations and frequency (percentage) are presented. Study participants were divided into quantiles based on average daily fiber intake, with the thresholds set as the median value of each parameter. Risk genotypes of the 6 previously identified *FTO* SNPs were predefined based on the literature and our previous findings. Comparisons of the allelic and genotypic frequencies as well as odds ratio calculations in this study were not included because of the relatively small sample size. Continuous parameters were tested for normality with Shapiro–Wilk's test and by visual inspection. Homogeneity of variance across groups was studied using Levene's test. Nonparametric tests were used for response variables that failed the mentioned statistical tests. Differences between selected parameters and dietary groups were then compared using analysis of variance (ANOVA) or Kruskal–Wallis test for numerical variables, with either Tukey's or Dunn's post hoc test with Holm p-value adjustment (in case multiple pairwise tests were performed, or when there were multiple grouping variables, as presented in tables and figures), and chi-squared test for categorical variables. The statistical significance level was set at <0.05 for all 2-sided tests and multivariate comparisons. All calculations were prepared in R (version 4.0.3).

3. Results

Data from 819 participants (52.5% female and 47.5% male) were included in the gene–diet interaction analysis, as described previously [7]. The mean age of the subjects was 42.1 (±14.5) years, and the mean BMI was 28.5 (±6.6) kg/m^2. Among the individuals, 33.9% had a BMI of <25.00 kg/m^2, 34.5% were overweight with a BMI of ≥25.00 and <30.00 kg/m^2, and 31.6% were obese with a BMI of ≥30.00 kg/m^2. Among the study population, 411 individuals (50.2%) were identified as having prediabetes or diabetes. Of these subjects, 109 participants (13.3%) had a previous history of prediabetes or diabetes, 56 participants (6.8%) previously took anti-diabetic drugs and 47 participants (5.7%) were being treated with lipid-lowering medications. The clinical characteristics, stratified by investigated genotypes, are presented in Tables 1–3. No significant deviation from the Hardy–Weinberg equilibrium was reported for any of the investigated SNPs ($p > 0.05$). Among the investigated FTO SNPs, some of the loci were in strong link disequilibrium (D′ = 1.0 for rs8050136 and rs9939609) [17]; thus, we present the results for one of them: rs8050136. We analyzed the same parameters for all of the investigated SNPs, however, in Figures 1–5 we present only statistically significant results.

Table 1. Characteristics of participants stratified by rs3751812 and rs8050136 genotypes.

	rs3751812				**rs8050136**			
	G/G	**G/T**	**T/T**	***p* Value**	**C/C**	**A/C**	**A/A**	***p* Value**
N	211	420	181		209	424	182	
Genotype frequency	0.26	0.52	0.22	>0.05	0.26	0.52	0.22	>0.05
Age (years)	40.5 (14.2)	41.2 (14.7)	39.5 (14.3)	0.33	40.2 (14.1)	41.3 (14.8)	39.4 (14.3)	0.24
BMI (kg/m^2)	27.6 (6.0)	28.7 (6.8)	28.9 (6.8)	0.060	27.6 (6.1)	28.7 (6.8)	28.9 (6.8)	0.063
○ BMI < 25.0	81 (38.8%)	136 (32.9%)	53 (29.9%)		80 (38.6%)	138 (33.2%)	54 (30.2%)	
○ BMI 25.0–29.9	69 (33.0%)	141 (34.1%)	66 (37.3%)	0.410	69 (33.3%)	140 (33.7%)	67 (37.4%)	0.422
○ BMI ≥ 30.0	59 (28.2%)	136 (32.9%)	58 (32.8%)		58 (28.0%)	138 (33.2%)	58 (32.4%)	
Total body fat content (kg)	25.3 (12.4)	27.6 (13.8)	28.2 (15.2)	0.080	25.3 (12.4)	27.5 (13.8)	28.2 (15.2)	0.095
Total body fat content (%)	30.6 (9.1)	31.8 (9.6)	31.6 (10.3)	0.377	30.6 (9.1)	31.8 (9.6)	31.6 (10.4)	0.465
Waist circumference (cm)	94.3 (17.5)	96.7 (17.2)	97.5 (16.7)	0.054	94.2 (17.6)	96.7 (17.2)	97.4 (16.6)	0.053
Hip circumference (cm)	101.3 (12.4)	104.2 (13.0)	103.8 (12.5)	*0.008*	101.2 (12.5)	104.1 (13.0)	103.8 (12.4)	*0.008*
WHR	0.927 (0.091)	0.925 (0.088)	0.937 (0.085)	0.327	0.927 (0.092)	0.925 (0.088)	0.936 (0.085)	0.382
Visceral fat (cm^3)	103.0 (81.0)	110.0 (79.9)	112.3 (83.0)	0.379	103.3 (81.5)	110.2 (80.0)	111.8 (82.5)	0.381
Visceral fat (%)	36.4 (11.8)	37.5 (12.4)	37.2 (11.7)	0.587	36.4 (11.7)	37.6 (12.5)	37.1 (11.6)	0.570

Table 1. *Cont.*

	rs3751812				rs8050136			
	G/G	**G/T**	**T/T**	***p* Value**	**C/C**	**A/C**	**A/A**	***p* Value**
Subcutaneous fat (cm^3)	163.5 (83.1)	167.2 (80.5)	175.0 (82.7)	0.401	163.7 (83.5)	166.9 (80.8)	175.3 (83.1)	0.405
Subcutaneous fat (%)	63.7 (11.7)	62.3 (12.9)	62.8 (11.7)	0.557	63.7 (11.6)	62.2 (13.0)	62.9 (11.6)	0.540
Visceral/subcutaneous fat ratio	0.642 (0.406)	0.687 (0.475)	0.665 (0.413)	0.554	0.641 (0.404)	0.690 (0.477)	0.662 (0.410)	0.536
Total cholesterol (mg/dL)	202.7 (56.0)	191.7 (41.3)	194.0 (43.2)	0.070	201.9 (56.1)	192.1 (41.4)	193.7 (43.1)	0.153
HDL (mg/dL)	60.7 (14.1)	59.8 (15.6)	59.5 (14.5)	0.662	60.8 (14.0)	59.6 (15.7)	59.7 (14.4)	0.422
LDL (mg/dL)	117.3 (43.3)	109.4 (37.8)	111.2 (41.8)	0.095	116.3 (43.3)	109.9 (37.9)	111.1 (41.8)	0.189
TG (mg/dL)	123.8 (143.9)	111.9 (69.7)	116.3 (61.9)	0.289	124.1 (144.3)	113.2 (71.1)	115.1 (61.3)	0.491
Frequency of prediabetes or diabetes								
○ Yes	103 (48.8%)	209 (49.8%)	95 (52.5%)	0.751	100 (47.8%)	213 (50.2%)	96 (52.7%)	0.628
○ No	108 (51.2%)	211 (50.2%)	86 (47.5%)		109 (52.2%)	211 (49.8%)	86 (47.3%)	
Corrected insulin response level during OGTT ($10 \times mU \times mL \times mg^{-2}$)								
○ 30 min	0.8 (0.7)	0.8 (0.7)	0.9 (1.0)	0.875	0.8 (0.7)	0.8 (0.7)	0.9 (1.0)	0.884
○ 60 min	0.7 (1.1)	0.6 (0.6)	0.7 (0.8)	0.325	0.7 (1.1)	0.6 (0.6)	0.7 (0.8)	0.325
○ 120 min	1.0 (0.7)	1.3 (1.0)	1.5 (1.6)	0.554	1.1 (0.9)	1.2 (1.0)	1.5 (1.6)	0.764
Daily energy intake (kcal)	1807.2 (732.3)	1766.9 (676.0)	1837.4 (713.4)	0.849	1820.5 (734.9)	1759.6 (673.3)	1853.9 (716.3)	0.645
Dietary fiber intake (g)	18.5 (7.2)	18.5 (8.2)	18.3 (7.6)	0.901	18.6 (7.2)	18.5 (8.2)	18.4 (7.7)	0.958
Daily physical activity level								
○ Low	16 (7.6%)	25 (6.0%)	18 (9.9%)	0.302	16 (7.7%)	25 (5.9%)	19 (10.4%)	0.179
○ Moderate	50 (23.7%)	83 (19.8%)	40 (22.1%)		50 (23.9%)	83 (19.6%)	40 (22.0%)	
○ High	145 (68.7%)	312 (74.3%)	123 (68.0%)		143 (68.4%)	316 (74.5%)	123 (67.6%)	

Data presented as mean and standard deviation (SD), number of observations, and frequency. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test. * Holm-adjusted Kruskal–Wallis/ANOVA *p* values.

Table 2. Characteristics of participants stratified by rs6499640 and 8044769 genotypes.

	rs6499640				rs8044769			
	A/A	**A/G**	**G/G**	***p* Value**	**C/C**	**C/T**	**T/T**	***p* Value**
N	307	377	134		270	406	138	
Genotype frequency	0.37	0.46	0.16	>0.05	0.33	0.50	0.17	>0.05
Age (years)	41.0 (14.7)	40.0 (14.2)	41.4 (15.0)	0.97	40.4 (14.8)	41.2 (14.6)	39.6 (13.7)	0.54
BMI (kg/m^2)	28.6 (6.8)	28.6 (6.8)	27.6 (5.8)	0.358	28.5 (6.8)	28.7 (6.7)	27.8 (6.1)	0.534
○ BMI < 25.0	93 (30.8%)	128 (34.5%)	52 (39.4%)		88 (33.2%)	131 (32.8%)	51 (37.5%)	
○ BMI 25.0–29.9	118 (39.1%)	120 (32.3%)	40 (30.3%)	0.230	92 (34.7%)	143 (35.8%)	43 (31.6%)	0.862
○ BMI ≥ 30.0	91 (30.1%)	123 (33.2%)	40 (30.3%)		85 (32.1%)	126 (31.5%)	42 (30.9%)	
Total body fat content (kg)	27.5 (14.5)	27.5 (14.1)	24.9 (10.9)	0.449	27.7 (15.0)	27.2 (13.6)	25.8 (11.9)	0.676
Total body fat content (%)	31.6 (9.8)	31.6 (9.7)	30.6 (8.9)	0.597	31.7 (10.1)	31.4 (9.6)	31.1 (9.0)	0.893
Waist circumference (cm)	96.8 (17.6)	96.7 (17.4)	93.5 (15.5)	0.255	96.2 (17.2)	96.8 (17.3)	94.6 (16.8)	0.429
Hip circumference (cm)	103.4 (12.9)	104.2 (13.3)	100.7 (10.4)	0.080	103.3 (12.6)	104.0 (13.0)	101.5 (12.2)	0.189
WHR	0.933 (0.089)	0.925 (0.085)	0.925 (0.097)	0.485	0.928 (0.089)	0.927 (0.088)	0.929 (0.089)	0.980
Visceral fat (cm^3)	109.6 (84.1)	110.2 (81.3)	101.7 (72.1)	0.822	109.2 (78.3)	110.3 (83.7)	99.8 (72.5)	0.648
Visceral fat (%)	37.3 (11.8)	36.5 (12.0)	38.2 (12.8)	0.483	37.7 (11.8)	36.9 (12.3)	36.4 (12.0)	0.617
Subcutaneous fat (cm^3)	168.9 (85.4)	173.4 (82.6)	151.5 (70.0)	0.124	168.6 (83.3)	169.7 (82.4)	160.9 (74.2)	0.773
Subcutaneous fat (%)	62.7 (11.8)	63.3 (12.6)	61.9 (12.7)	0.503	62.3 (11.8)	63.0 (12.8)	63.7 (11.8)	0.598
Visceral/subcutaneous fat ratio	0.670 (0.410)	0.655 (0.459)	0.706 (0.472)	0.486	0.689 (0.492)	0.662 (0.421)	0.641 (0.408)	0.590
Total cholesterol (mg/dL)	195.3 (51.1)	193.8 (42.3)	196.9 (44.4)	0.646	193.8 (40.1)	191.7 (42.7)	206.7 (62.7)	*0.029*

Table 2. *Cont.*

	rs6499640				rs8044769			
	A/A	**A/G**	**G/G**	***p* Value**	**C/C**	**C/T**	**T/T**	***p* Value**
LDL (mg/dL)	111.0 (39.6)	111.1 (40.3)	114.9 (41.7)	0.487	111.0 (38.6)	109.6 (38.7)	119.8 (46.5)	0.058
HDL (mg/dL)	60.2 (14.6)	60.3 (15.8)	58.3 (13.4)	0.289	60.7 (14.7)	59.2 (15.5)	60.4 (13.8)	0.176
TG (mg/dL)	120.5 (124.2)	111.8 (67.8)	118.6 (71.1)	0.584	110.5 (58.7)	114.6 (73.4)	132.6 (170.6)	0.689
Frequency of prediabetes or diabetes								
○ Yes	152 (49.5%)	187 (49.6%)	71 (53.0%)	0.796	143 (53.0%)	201 (49.5%)	64 (46.4%)	0.420
○ No	155 (50.5%)	190 (50.4%)	63 (47.0%)		127 (47.0%)	205 (50.5%)	74 (53.6%)	
Corrected insulin response level during OGTT (10 × mU × mL × mg^{-2})								
○ 30 min	0.8 (0.9)	0.8 (0.7)	0.9 (0.8)	0.616	0.9 (0.9)	0.8 (0.7)	0.8 (0.7)	0.618
○ 60 min	0.6 (0.7)	0.7 (0.8)	0.7 (0.7)	0.745	0.7 (0.7)	0.7 (0.9)	0.5 (0.5)	0.194
○ 120 min	1.4 (1.4)	1.2 (1.0)	1.2 (1.0)	0.949	1.3 (1.3)	1.4 (1.0)	1.1 (0.8)	0.639
Daily energy intake (kcal)	1786.5 (705.2)	1825.7 (725.8)	1720.7 (594.6)	0.728	1775.7 (646.2)	1792.7 (735.8)	1816.0 (686.3)	0.791
Dietary fiber intake (g)	18.1 (7.1)	19.0 (8.5)	17.9 (7.4)	0.570	18.1 (7.4)	18.8 (8.2)	18.2 (7.4)	0.598
Daily physical activity level								
○ Low	22 (7.2%)	30 (8.0%)	8 (6.0%)	0.736	23 (8.5%)	26 (6.4%)	10 (7.2%)	0.060
○ Moderate	68 (22.1%)	81 (21.5%)	24 (17.9%)		55 (20.4%)	77 (19.0%)	41 (29.7%)	
○ High	217 (70.7%)	266 (70.6%)	102 (76.1%)		192 (71.1%)	303 (74.6%)	87 (63.0%)	

Data presented as mean and standard deviation (SD), number of observations, and frequency. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test. * Holm-adjusted Kruskal–Wallis/ANOVA *p* values.

Table 3. Characteristics of participants stratified by rs7190492 genotype.

	rs7190492			
	G/G	**A/G**	**A/A**	***p* Value**
N	374	358	83	
Genotype frequency	0.46	0.44	0.10	>0.05
Age (years)	40.4 (14.8)	40.8 (14.4)	40.4 (13.6)	1.0
BMI (kg/m^2)	28.7 (6.8)	28.3 (6.5)	28.1 (6.6)	0.605
○ BMI < 25.0	120 (32.5%)	122 (34.8%)	30 (36.1%)	0.461
○ BMI 25.0–29.9	126 (34.1%)	128 (36.5%)	23 (27.7%)	
○ BMI ≥ 30.0	123 (33.3%)	101 (28.8%)	30 (36.1%)	
Total body fat content (kg)	27.8 (14.5)	26.5 (13.4)	26.6 (12.4)	0.533
Total body fat content (%)	31.9 (10.0)	30.8 (9.5)	32.1 (8.8)	0.303
Waist circumference (cm)	96.6 (17.3)	96.2 (17.0)	94.7 (17.6)	0.585
Hip circumference (cm)	103.4 (12.7)	103.6 (12.9)	101.7 (12.5)	0.588
WHR	0.930 (0.090)	0.925 (0.086)	0.927 (0.092)	0.774
Visceral fat (cm^3)	110.7 (83.0)	106.6 (81.2)	103.0 (67.1)	0.777
Visceral fat (%)	37.4 (11.6)	36.6 (12.5)	37.5 (12.5)	0.665
Subcutaneous fat (cm^3)	170.4 (85.7)	166.5 (78.9)	160.7 (72.5)	0.921
Subcutaneous fat (%)	62.4 (12.2)	63.4 (12.5)	62.7 (12.2)	0.653
Visceral/subcutaneous fat ratio	0.674 (0.454)	0.660 (0.434)	0.676 (0.443)	0.652
Total cholesterol (mg/dL)	192.9 (40.4)	194.3 (51.0)	206.0 (47.5)	0.056
LDL (mg/dL)	110.1 (38.2)	111.4 (41.0)	119.6 (45.5)	0.219
HDL (mg/dL)	60.4 (14.6)	59.1 (15.4)	61.0 (14.0)	0.227

Table 3. *Cont.*

	rs7190492			
	G/G	**A/G**	**A/A**	***p* Value**
TG (mg/dL)	111.8 (62.8)	118.6 (119.3)	126.9 (86.2)	0.422
Frequency of prediabetes or diabetes				
○ Yes	194 (51.9%)	176 (49.2%)	38 (45.8%)	0.544
○ No	180 (48.1%)	182 (50.8%)	45 (54.2%)	
Corrected insulin response level during OGTT (10 × mU × mL × mg^{-2})				
○ 30 min	0.8 (0.8)	0.9 (0.7)	0.9 (0.7)	0.421
○ 60 min	0.6 (0.6)	0.8 (1.0)	0.5 (0.4)	0.428
○ 120 min	1.3 (1.3)	1.3 (1.0)	1.2 (0.9)	0.828
Daily energy intake (kcal)	1765.4 (660.6)	1823.5 (728.6)	1771.0 (744.1)	0.609
Dietary fiber intake (g)	18.0 (6.8)	18.8 (8.7)	19.4 (8.4)	0.586
Daily physical activity level				
○ Low	29 (7.8%)	23 (6.4%)	8 (9.6%)	0.626
○ Moderate	75 (20.1%)	75 (20.9%)	21 (25.3%)	
○ High	270 (72.2%)	260 (72.6%)	54 (65.1%)	

Data presented as mean and standard deviation (SD), number of observations, and frequency. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test. * Holm-adjusted Kruskal–Wallis/ANOVA *p* values.

3.1. General Characteristic of Studied Population Stratified by Genotypes

Based on the food intake, physical activity, clinical, anthropometric and demographic data, we observed significant differences only in hip circumference between studied genotypes rs3751812 and rs8050136 (Table 1), and in total cholesterol level between genotypes of rs8044769 (Table 2), which was presented previously [7]. We did not observe any other significant differences between the studied genotypes.

3.2. Assessment of Dietary Intake

The 3-day food diaries were available exclusively from a selected subgroup of 622 participants because not all participants completed diaries properly. We did not find any differences between studied genotypes and dietary fiber intake presented in Tables 1–3. With the use of boxplots in the figures, we showed the contrast between median values of the selected responses and the interquartile ranges (IQRs) in different genotypic and dietary strata.

3.3. Associations between rs3751812 Polymorphism, Anthropometric Measurements, Lipid Profile, and Dietary Fiber Intake

Based on the analysis of the interactions between rs3751812 genotypes and fiber intake, we observed that GG genotype carriers presented lower hip circumferences (Figure 1A), while TT genotype carriers presented lower visceral fat content than heterozygous individuals (Figure 1B) when they were stratified to the group with higher than median fiber intake. Moreover, we noted that GG genotype carriers in the group with higher than median fiber intake presented higher total cholesterol (Figure 1C) and LDL-cholesterol levels (Figure 1D). Additionally, we observed that GG carriers showed lower corrected insulin response levels at 120 min of OGTT (Figure 1E) when they were stratified to the group with lower than median fiber intake.

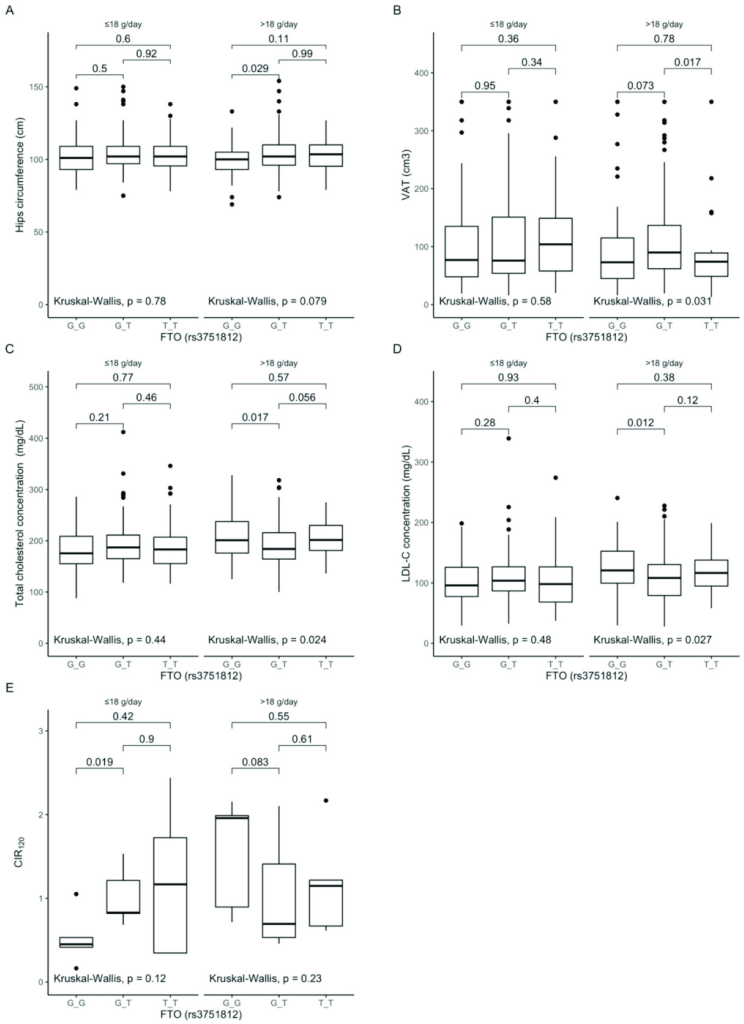

Figure 1. Association of the *FTO* rs3751812 genotypes with (**A**) Hip circumference (cm); (**B**) Visceral adipose tissue (VAT) (cm^3); (**C**) Total cholesterol concentration (mg/dL); (**D**) LDL-C concentration (mg/dL); (**E**) CIR 120 by dietary fiber intake strata: ≤18 g/day and >18 g/day.

3.4. Associations between rs8050136 Polymorphism, Anthropometric Measurements, Lipid Profile, and Dietary Fiber Intake

Analyzing the differences between rs8050136 genotypes dependent on dietary fiber intake, we observed that carriers of the CC genotype stratified to the group with higher than median fiber intake presented lower hip circumferences (Figure 2A), while AA genotype carriers presented lower visceral fat content (Figure 2B). Additionally, we observed that CC genotype carriers showed higher total cholesterol (Figure 2C) and higher LDL-cholesterol levels (Figure 2D), whereas when CC genotype carriers were stratified to the group with

lower than median fiber intake, we noted lower corrected insulin response levels at 120 min of OGTT (Figure 2E).

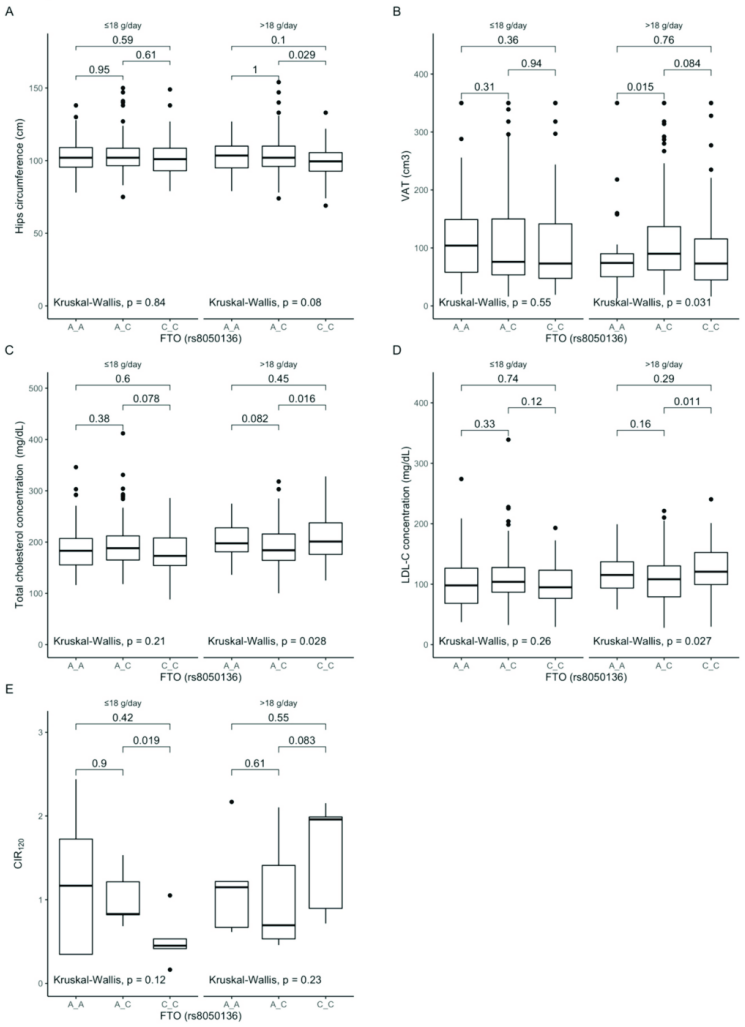

Figure 2. Association of the *FTO* rs8050136 genotypes with (**A**) Hip circumference (cm); (**B**) Visceral adipose tissue (VAT) (cm^3); (**C**) Total cholesterol concentration (mg/dL); (**D**) LDL-C concentration (mg/dL); (**E**) CIR 120 by dietary fiber intake strata: ≤18 g/day and >18 g/day.

3.5. Associations between rs6499640 Polymorphism, Anthropometric Measurements, Lipid Profile, and Dietary Fiber Intake

The gene–diet interaction analysis showed that GG genotype carriers stratified to the group with higher than median fiber intake presented lower hip circumferences (Figure 3A). Additionally, we observed that the AG genotype carriers presented surprisingly lower visceral fat content (Figure 3B), higher subcutaneous fat content (Figure 3C), and lower

VAT/SAT ratio (Figure 3D) compared to the AA genotype carriers. We noted that homozygous AA carriers presented higher HDL-cholesterol levels (Figure 3E), and heterozygous AG carriers presented higher blood glucose levels at 60 min of OGTT (Figure 3F), compared to the heterozygous participants in the group with lower than median fiber intake. The higher levels of triglycerides were noted only in the GG genotype carriers (Figure 3G) when they were stratified to the group with higher than median fiber intake.

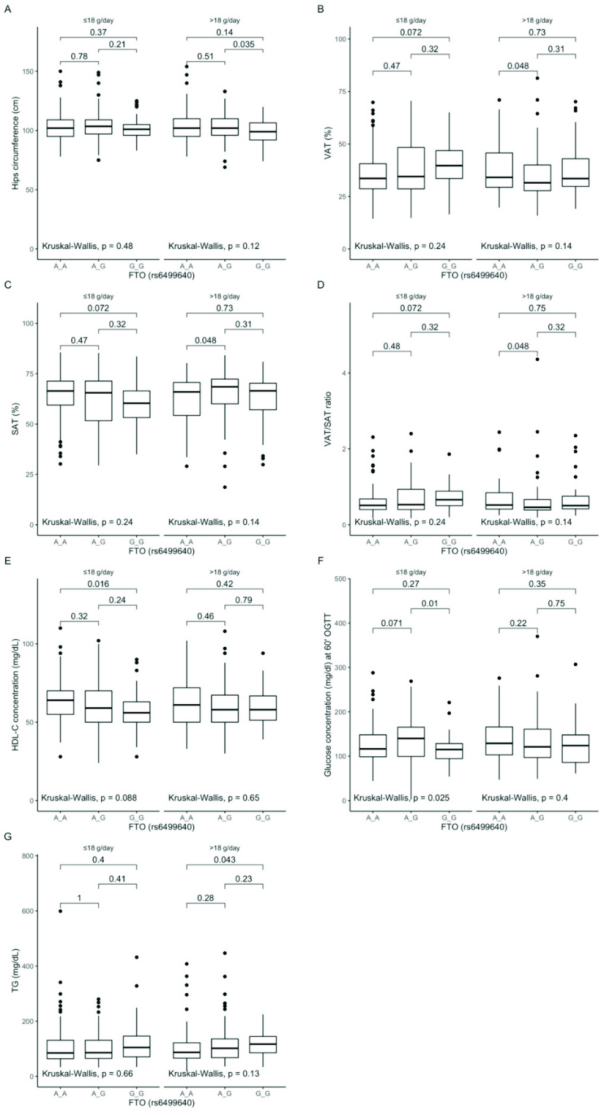

Figure 3. Association of the *FTO* rs6499640 genotypes with (**A**) Hip circumference (cm); (**B**) Visceral adipose tissue (VAT) (cm^3); (**C**) Subcutaneous adipose tissue (SAT) (%); (**D**) VAT/SAT ratio; (**E**) HDL-C concentration (mg/dL); (**F**) Glucose concentration (mg/dL) at 60 min of OGTT; (**G**) Triglycerides (TG) (mg/dL) by dietary fiber intake strata: ≤18 g/day and >18 g/day.

3.6. Associations between rs8044769 Polymorphism, Anthropometric Measurements, Lipid Profile, and Dietary Fiber Intake

Based on the analysis of the interactions between rs8044769 genotypes and fiber intake, we observed that TT genotype carriers, when they were stratified to higher than median fiber intake, presented lower body weight (Figure 4A) and lower fat-free mass content (Figure 4B). Moreover, we noted lower blood glucose levels at 120 min of OGTT in TT genotype carriers (Figure 4C), and lower HDL-cholesterol levels in CT genotype carriers were observed (Figure 4D) when they were stratified to the group with higher than median fiber intake.

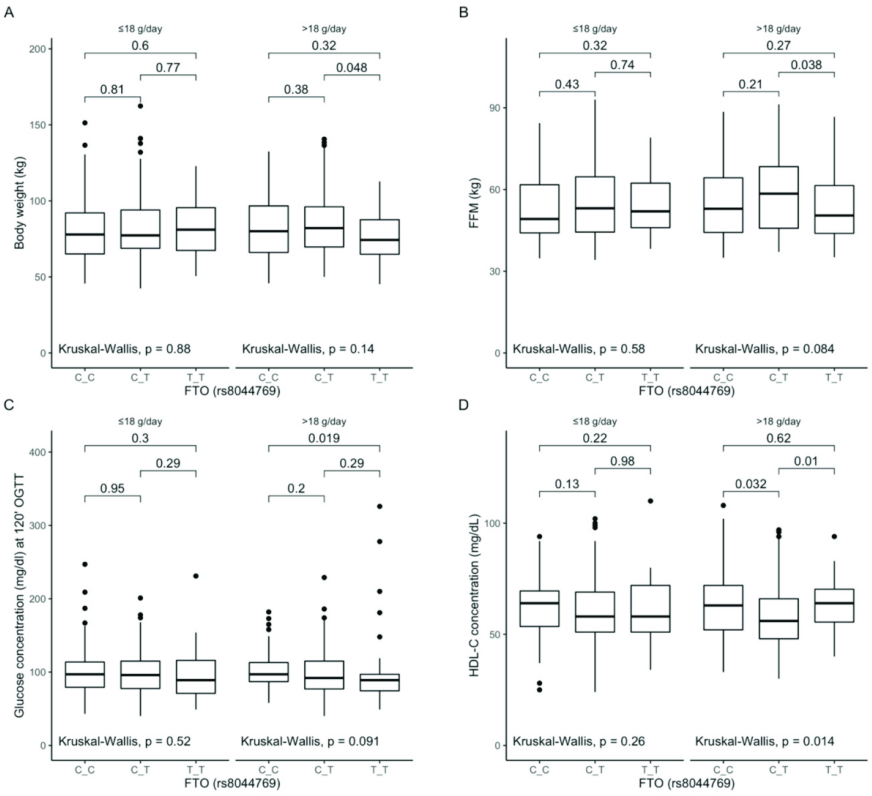

Figure 4. Association of the *FTO* rs8044769 genotypes with (**A**) Body weight (kg); (**B**) Fat-free mass content (FFM) (kg); (**C**) Glucose concentration (mg/dL) at 120 min of OGTT; (**D**) HDL-C concentration (mg/dL) by dietary fiber intake strata: ≤18 g/day and >18 g/day.

3.7. Associations between rs7190492 Polymorphism, Anthropometric Measurements, Lipid Profile, and Dietary Fiber Intake

The analysis of differences between rs7190492 genotypes dependent on dietary fiber intake showed that carriers of the AG genotype stratified to the group with higher than median fiber intake presented higher fat-free mass content (Figure 5A) and higher skeletal muscle mass content (Figure 5B). We did not observe any other differences that were dependent on dietary fiber intake.

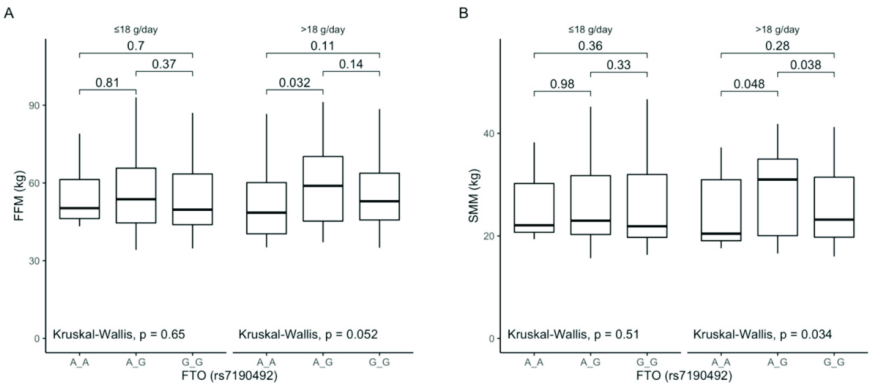

Figure 5. Association of the *FTO* rs7190492 genotypes with (**A**) Fat-free mass content (FFM) (kg); (**B**) Skeletal muscle mass content (SMM) (kg) by dietary fiber intake strata: ≤18 g/day and >18 g/day.

4. Discussion

The role of dietary habits in the prevalence and pathogenesis of obesity is significant. Comprehension of the interactions between genetic variations and dietary intake could be a solution for this dilemma. In our study, we present the association of total diet fiber intake with body composition and the subject's glycemic and lipid profile status among Polish-origin Caucasian participants. The present study reveals that dietary fiber intake may modify the association of the *FTO* SNPs, rs3751812, rs8050136, rs9939609, rs6499640, rs8044769, and rs7190492, especially among those who consume high levels of dietary fiber. Our study demonstrated that associations of the *FTO* SNPs with hip circumference, visceral adipose tissue distribution, total cholesterol and LDL levels, and corrected insulin response levels may be dependent on daily fiber intake. In our previous study, we observed an interplay between *FTO* genetic variants and daily macronutrient intake (carbohydrates, proteins, and fat) with impact on obesity and its metabolic consequences [7]. Nevertheless, the associations between *FTO* genetic variants, dietary fiber intake, and metabolic consequences are still under investigation. Given the high prevalence of obesity in nearly all parts of Europe, and the fact that during the last four decades, the percentage of obese people substantially increased [18,19], our findings presented in this study seem to be significant for public health.

Dietary fiber is defined as a carbohydrate polymer; it is not hydrolyzed by human enzymes and is therefore not digested or absorbed from the digestive tract. Dietary fibers include soluble and insoluble fibers, and are classified based on their solubility in hot water, water holding capacity, and viscosity [20]. It is widely known that high dietary fiber intake, depending on the dietary fiber consumed, has several protective effects against chronic diseases, including diabetes, obesity, metabolic syndrome, inflammatory bowel syndrome, and cardiovascular disease [21–24]. The specific role of dietary fiber in the bioaccessibility and bioavailability of natural antioxidants derived mainly from fruits and vegetables is still widely discussed. It was shown that dietary fiber can reduce the accessibility of macronutrients, especially fat, in the human diet [25–27]. Moreover, dietary fiber is considered as a part of the diet that can interact with antioxidants, including polyphenol compounds. Above interaction with fiber and food components, there could be an effect of prolonged gastric emptying time and retarded absorption of nutrients in the small intestine [8].

We observed significant associations of *FTO* rs3751812 and rs8050136 according to the clinical characteristics of the studied group. Based on the anthropometric data, we noted that homozygous GG genotype carriers of rs3751812 and CC genotype carriers of rs8050136 showed significantly lower only in hip circumference. Our observations are consistent

with the current state of knowledge, based on the associations of *FTO* SNPs and markers of obesity. It has been shown that the CC genotype carriers (rs8050136) present significantly lower BMI, total body fat content, and waist and hip circumferences [28–30]. In this case, the CC genotype carriers (rs8050136) have been shown to play a protective role, which confirms our results. Scuteri et al. [31] showed that rs3751812 was not associated with BMI and hip circumference in the population of African Americans when compared to the Hispanic and European Americans of the GenNetstudy. However, this association may be misleading due to lower minor allele frequencies or smaller effect sizes of variants in the conducted study. The fact that the effect of dietary fiber intake on the association of genotype may differ by race or ethnicity should not be ignored. For example, Villegas et al. [32] found that, in non-Hispanic whites, dietary fiber modified the association between *FTO* rs8050136 and diabetes, whereas no significant interaction was observed among non-Hispanic black participants. Taking the fact that *FTO* rs3751812 risk allele T is related to increased BMI compared to the protective allele G [33], the results that we obtained during our study seem to be appropriate. Currently, numerous studies have shown an association of *FTO* SNPs with anthropometric measurements; nevertheless, the impact of the dietary fiber intake between genotypes is still unclear.

In our study, we did not notice any crucial differences in daily dietary fiber intake between studied genotypes. However, when we stratified the study group dependent on daily fiber intake, we observed that GG genotype (rs3751812), CC genotype (rs8050136), and GG genotype (rs6499640) subjects presented lower hip circumference if daily fiber intake was higher than 18 g a day. These results are consistent with Hosseini-Esfahani et al. [34], who observed the relationship between abdominal obesity, fiber, and *FTO* rs3751812. The findings of Hosseini-Esfahani et al. suggest that individuals with a high number of risk alleles may benefit more from a higher daily dietary fiber intake versus individuals with a low number of risk alleles.

It has been noted that the *FTO* effect on obesity and other comorbidities may be modulated by a healthy dietary pattern. The traditional Mediterranean diet pattern, which may protect against type 2 diabetes [35], is low in saturated fat and includes foods rich in fiber, including vegetables, fruits, legumes, and nuts, which showed interactions with *FTO* rs9939609 [36].

Our study also showed an unexpected observation: that higher fiber intake can have a negative impact on lipid profile. However, soluble fibers have been shown to lower blood cholesterol by several mechanisms [10,37], and we observed that GG genotype (rs3751812) and CC genotype (rs8050136) subjects presented higher levels of total cholesterol and LDL-cholesterol when they were stratified to the high fiber intake quantiles. Moreover, in the same study conditions, but in the low fiber intake quantiles, we noted lower corrected insulin response levels at 120 min of OGTT in the GG genotype (rs3751812) and CC genotype (rs8050136) carriers. These observations might be associated with a source of dietary fiber and its interaction with food components, which we have not analyzed in our study. It is widely known that the source of dietary fiber has an impact on health outcomes [38]. Insoluble fibers present low or no effect on gastric emptying, macronutrient absorption, postprandial glucose responses, or blood lipid levels [39]. In contrast, consumption of soluble fibers influence serum lipids and postprandial glucose responses [40]. Freeland et al. [41] observed that higher consumption of wheat fiber intake results in increased short-chain fatty acid (SCFA) production and glucagon-like peptide-1 (GLP-1) secretion. Since GLP-1 may increase insulin sensitivity and secretion, these effects took over nine months to develop under colonic adaptation to increased wheat fiber intake.

Newly published studies showed that diets might modify the effect of the *FTO* variant on obesity, but these data are conflicting and are limited by a small sample size [42,43]. Some of the intervention studies searching for body fat distribution changes focused on diets with reduced fat or carbohydrates and increased fiber but did not find significant influence of *FTO* polymorphisms (rs8050136 and rs9939609) [44,45]. In our study, in the participants stratified to the higher daily dietary fiber intake, we found that TT genotype

(rs3751812), AA genotype (rs8050136), and AG genotype (rs6499640) subjects presented lower visceral fat distribution (VAT). These results are consistent with studies focused on fiber consumption in healthy adolescent groups [46] or groups with obesity [47], but without the gene–diet analysis impact. We found no more main effects of the FTO variant on body composition during the intervention. As far as we know, there are limited studies on VAT distribution and FTO analysis, taking into consideration fiber intake. Therefore, our analysis presented in this study seems to be significant for public health awareness.

The present study has several strengths. To the best of our knowledge, this is one of the first studies to present associations between *FTO* SNPs rs3751812, rs8050136, rs9939609, rs6499640, rs7190492 and rs8044769, daily fiber intake and obesity, and glucose homeostasis and lipid profile. Moreover, our study has a relatively large sample size with excluded confounders. Another strength of our study is that we did not observe any significant differences in fiber intake between studied genotypes. This can be interpreted as a strength of our study because we can exclude the possibility of interactions between fiber and gene expression, and contribute it to consistency in gene–diet interactions. However, our study has several limitations. Our research is partially based on self-reported data, such as 3-day diaries of food intake, therefore, measurement error due to underreported or misreported data by subjects cannot be completely excluded. Nevertheless, this method is commonly being used for large-scale population investigations thus far. The other limitation is the fact that we analyzed total daily fiber intake without considering fiber source. Finally, although we obtained only Polish Caucasian individuals for our study, the data should be reflected on other populations of different ethnic groups. We included 18–79-year-old volunteers, with a mean age of around 42 years old. We did not perform analyses with an age stratification due to too small of a study group; nevertheless, it is an important factor, since most of the investigated metabolic disturbances are strongly associated with aging. Including only elderly individuals might show more significant associations, which should be considered in future analyses.

5. Conclusions

In summary, we found that dietary fiber intake might modify the effect of *FTO* SNPs on body composition and lipid profile. Our study indicates that daily fiber intake above 18 g per day by carriers of the GG genotype (rs3751812), CC genotype (rs8050136), and GG genotype (rs6499640) may positively affect anthropometric parameters, decreasing hip circumference, but increasing total cholesterol and LDL levels. Advances in this field bring us closer to the development of genome-customized diet recommendations to prevent obesity and its metabolic complications. Further studies are needed to verify our findings and to achieve an efficient strategy to prevent obesity development and metabolic diseases.

Author Contributions: Conceptualization, E.A.-P., M.G. and A.K.; methodology, E.A.-P., M.G. and A.K.; formal analysis, W.B.; investigation, E.A.-P., P.C., W.B., J.F., U.K. and M.M.; writing—original draft preparation, P.C. and E.A.-P.; writing—review and editing, E.A.-P., M.G. and A.K.; visualization, W.B.; supervision, M.G. and A.K.; project administration, E.A.-P.; funding acquisition, E.A.-P., M.G. and A.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study procedures were carried out in accordance with the ethical standards for human experimentation and with the guidelines laid down in the Helsinki Declaration of 1975, revised in 1983. The study protocol was approved by the local Ethics Committee of the Medical University of Bialystok, Poland (R-I-002/35/2009).

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

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Consent from the Bioethics Committee

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Białystok, dn.....

Komisja Bioetyczna Uniwersytetu Medycznego w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP / Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** /na prowadzenie tematu badawczego: „Analiza genetycznych uwarunkowań odpowiedzi metabolicznej na dietę o różnej zawartości węglowodanów, białek i tłuszczy. Poszukiwanie genetycznych markerów do indywidualizacji żywienia pacjentów z otyłością i cukrzycą typu 2" przez prof. dr hab. Marię Górską wraz z zespołem badawczym.

Przewodnicząca Komisji

prof. dr hab. Elżbieta Hassmann-Poznańska

Information about the nature of participation

”The Impact of *FTO* Genetic Variants on Obesity and Its Metabolic Consequences is Dependent on Daily Macronutrient Intake”

authors: Przemysław Czajkowski, Edyta Adamska-Patruno, Witold Bauer, Joanna Fiedorczuk, Urszula Krasowska, Monika Moroz, Maria Górska, Adam Krętowski, published in *Nutrients*, 2020; 12: 3255.

Author’s name and surname	**Nature of participation**	**Contribution in %**
Przemysław Czajkowski	investigation, writing original draft preparation, visualization	51 %
Edyta Adamska-Patruno	conceptualization, methodology, investigation, writing original draft preparation, writing-review, project administration, funding	23 %
Witold Bauer	formal analysis, investigation, visualization	3 %
Joanna Fiedorczuk	investigation	1 %
Urszula Krasowska	investigation	1 %
Monika Moroz	investigation	1 %
Maria Górska	conceptualization, methodology, investigation, writing-review, supervision, funding	10 %
Adam Krętowski	conceptualization, methodology, writing-review, supervision, funding	10 %

"Dietary Fiber Intake May Influence the Impact of FTO Genetic Variants on Obesity Parameters and Lipid Profile—A Cohort Study of a Caucasian Population of Polish Origin"

authors: Przemysław Czajkowski, Edyta Adamska-Patruno, Witold Bauer, Urszula Krasowska, Joanna Fiedorczuk, Monika Moroz, Maria Górska, Adam Krętowski, published in *Antioxidants*, 2021; 10(11): 1793.

Author's name and surname	**Nature of participation**	**Contribution in %**
Przemysław Czajkowski	investigation, writing original draft preparation, visualization, analysis	51 %
Edyta Adamska-Patruno	conceptualization, methodology, investigation, writing original draft preparation, writing review, project administration, funding	23 %
Witold Bauer	formal analysis, investigation, visualization,	3 %
Urszula Krasowska	investigation	1 %
Joanna Fiedorczuk	investigation	1 %
Monika Moroz	investigation	1 %
Maria Górska	conceptualization, methodology, investigation, writing review, supervision, funding	10 %
Adam Krętowski	conceptualization, methodology, writing review, supervision, funding	10 %

I hereby declare that all co-authors agreed to use these articles in the dissertation of Przemysław Czajkowski.

Signature

12.09.2022r., Białystok
date, place

Statement

I agree to use this publication by Przemysław Czajkowski, in the procedure for awarding the doctoral degree in the field of medical sciences and health sciences in the discipline of medical sciences.

I confirm that in the article:

"The Impact of FTO *Genetic Variants on Obesity and Its Metabolic Consequences is Dependent on Daily Macronutrient Intake"*

which is a part of doctoral dissertation of Przemysław Czajkowski, my contribution included:

Author's name and surname	**Nature of participation**	**Contribution in %**	**Signature**
Przemysław Czajkowski	investigation, writing original draft preparation, visualization	51 %	Przemysław Czajkowski
Edyta Adamska-Patruno	conceptualization, methodology, investigation, writing original draft preparation, writing-review, project administration, funding	23 %	Edyta Adamska-Patruno
Witold Bauer	formal analysis, investigation, visualization	3 %	Witold Bauer
Joanna Fiedorczuk	investigation	1 %	Joanna Fiedorczuk
Urszula Krasowska	investigation	1 %	Urszula Krasowska
Monika Moroz	investigation	1 %	Monika Moroz
Maria Górska	conceptualization, methodology, investigation, writing-review, supervision, funding	10 %	
Adam Krętowski	conceptualization, methodology, writing-review, supervision, funding	10 %	

12.09.2022 r., Białystok
date, place

Statement

I agree to use this publication by Przemysław Czajkowski, in the procedure for awarding the doctoral degree in the field of medical sciences and health sciences in the discipline of medical sciences.

I confirm that in the article:

"Dietary Fiber Intake May Influence the Impact of FTO Genetic Variants on Obesity Parameters and Lipid Profile—A Cohort Study of a Caucasian Population of Polish Origin"

which is a part of doctoral dissertation of Przemysław Czajkowski, my contribution included:

Author's name and surname	**Nature of participation**	**Contribution in %**	**Signature**
Przemysław Czajkowski	investigation, writing original draft preparation, visualization, analysis	51 %	Przemysław Czajkowski
Edyta Adamska-Patruno	conceptualization, methodology, investigation, writing original draft preparation, writing review, project administration, funding	23 %	Edyta Adamska-Patruno
Witold Bauer	formal analysis, investigation, visualization,	3 %	Witold Bauer
Urszula Krasowska	investigation	1 %	Urszula Krasowska
Joanna Fiedorczuk	investigation	1 %	Joanna Fiedorczuk
Monika Moroz	investigation	1 %	Monika Moroz
Maria Górska	conceptualization, methodology, investigation, writing review, supervision, funding	10 %	
Adam Krętowski	conceptualization, methodology, writing review, supervision, funding	10 %	

Academic achievements

Article type	Number	Impact Factor	MNiSW points
Articles included in the dissertation	2	13.392	240
Articles not included in the dissertation	11	20.308	486
Conference abstracts	36	-	-
Summary	49	33.70	726

List of articles included in the dissertation

1. ”The Impact of *FTO* Genetic Variants on Obesity and Its Metabolic Consequences is Dependent on Daily Macronutrient Intake”

   authors: Przemysław Czajkowski, Edyta Adamska-Patruno, Witold Bauer, Joanna Fiedorczuk, Urszula Krasowska, Monika Moroz, Maria Górska, Adam Krętowski, published in *Nutrients*, 2020; 12: 3255.

   doi:10.3390/nu12113255

   Impact Factor: 5.719

   MNiSW points: 140

2. “Dietary Fiber Intake May Influence the Impact of FTO Genetic Variants on Obesity Parameters and Lipid Profile—A Cohort Study of a Caucasian Population of Polish Origin”

   authors: Przemysław Czajkowski, Edyta Adamska-Patruno, Witold Bauer, Urszula Krasowska, Joanna Fiedorczuk, Monika Moroz, Maria Górska, Adam Krętowski, published in *Antioxidants*, 2021; 10(11): 1793.

   doi:10.3390/antiox10111793

   Impact Factor: 7.675

   MNiSW points: 100

List of articles not included in the dissertation

1. "An association between diet and MC4R genetic polymorphism, in relation to obesity and metabolic parameters - a cross sectional population-based study"
   authors: Edyta Adamska-Patruno, Witold Bauer, Dorota Bielska, Joanna Fiedorczuk, Monika Moroz, Urszula Krasowska, Przemysław Czajkowski, Marta Wielogórska, Katarzyna Maliszewska, Sylwia Pućkowska, Łukasz Szczerbiński, Danuta Lipińska, Maria Górska, Adam Krętowski,
   published in *International Journal of Molecular Sciences*, 2021; 22: 12044.
   doi: 10.3390/ijms222112044
   Impact Factor: 6.208
   MNiSW points: 140

2. "Dietary macronutrient intake may influence the effects of TCF7L2 rs7901695 genetic variants on glucose homeostasis and obesity-related parameters: a cross-sectional population-based study"
   authors: Witold Bauer, Edyta Adamska-Patruno, Urszula Krasowska, Monika Moroz, Joanna Fiedorczuk, Przemysław Czajkowski, Dorota Bielska, Maria Górska, Adam Krętowski,
   published in *Nutrients*, 2021; 13: 1936.
   doi: 10.3390/nu13061936
   Impact Factor: 6.706
   MNiSW points: 140

3. "The MC4R genetic variants are associated with lower visceral fat accumulation and higher postprandial relative increase in carbohydrate utilization in humans"
   authors: Edyta Adamska-Patruno, Joanna Gościk, Przemysław Czajkowski, Katarzyna Maliszewska, Michał Ciborowski, Anna Golonko, Natalia Wawrusiewicz-Kurylonek, Anna Citko, Magdalena Waszczeniuk, Adam Krętowski, Maria Górska.
   published in *European Journal of Nutrition*, 2019; 58: 2929-2941.
   doi: 10.1007/s00394-019-01955-0
   Impact Factor: 4.664
   MNiSW points: 100

4. “Flow Mediated Skin Fluorescence technique reveals remarkable effect of age on microcirculation and metabolic regulation in type 1 diabetes”

   authors: Joanna Katarzyńska, Anna Borkowska, Przemysław Czajkowski, Agnieszka Łoś, Łukasz Szczerbiński, Agnieszka Milewska-Kranc, Andrzej Marcinek, Adam Krętowski, Katarzyna Cypryk, Jerzy Gębicki.

   published in *Microvascular Research*, 2019; 124: 19-24.

   doi: 10.1016/j.mvr.2019.02.005

   Impact Factor: 2.730

   MNiSW points: 70

5. „Substancje i roślinne surowce lecznicze - wskazania i przeciwskazania do stosowania w czasie trwania ciąży. Substances and healing plant sources - indications and contraindications of their usage during pregnancy.”

   authors: Czajkowski Przemysław, Jastrzębska Katarzyna, Truszkowska Martyna, Nazaruk Jolanta.

   published in *Czasopismo Aptekarskie*, 2016, 8-9 (272-273), s. 28-37.

   MNiSW points: 2

6. „Interakcje leków z pożywieniem w grupie pacjentów geriatrycznych jako problem społeczny i ekonomiczny. Interactions between drugs and food in the group of geriatric patients as the societal and economic problem.”

   authors: Czajkowski Przemysław, Maciejczyk Mateusz, Bogdańska Ewelina, Zaręba Ilona, Rysiak Edyta.

   published in *Polski Przegląd Nauk o Zdrowiu*, 2016, 4 (49), s. 408-410.

   MNiSW points: 7

7. „Schizofrenia - problem społeczny i ekonomiczny. Schizophrenia - social and economic problem.”

   authors: Bogdańska Ewelina, Rysiak Edyta, Czajkowski Przemysław, Zaręba Ilona.

   published in *Polski Przegląd Nauk o Zdrowiu*, 2016 : 4, 49, s. 396-400.

   MNiSW points: 7

8. „Współczesna transplantacja, jako problem ekonomiczny i społeczny. Contemporary transplantation as an economic and social problem”
   authors: Czajkowski Przemysław, Maciejczyk Mateusz, Rysiak Edyta, Zaręba Ilona.
   published in *Zdrowie w XXI wieku: wyzwania, problemy dylematy... T. 2.* Red. Barbara Jankowiak, Beata Kowalewska, Hanna Rolka. Państwowa Wyższa Szkoła Informatyki i Przedsiębiorczości w Łomży, 2016: s. 319-326.
   MNiSW points: 4

9. „Koszty leczenia łuszczycy. Psoriasis treatment costs”
   authors: Zaręba Ilona, Rysiak Edyta, Siemionow Katarzyna, Czajkowski Przemysław, Stelmaszewska Joanna, Zaręba Renata, Cekała Eryk.
   published inW: *Zdrowie w XXI wieku: wyzwania, problemy, dylematy... T. 1.* Red. Barbara Jankowiak, Beata Kowalewska, Hanna Rolka. Państwowa Wyższa Szkoła Informatyki i Przedsiębiorczości w Łomży, 2016: s. 613-620.
   MNiSW: 4

10. „Wpływ działań marketingowych na zakup leków dostępnych bez recepty przez pełnoletnich mieszkańców Białegostoku. The impact of marketing activities for the purchase of OTC drugs by adult residents of Bialystok.”
    authors: Sobolewska Paulina, Czajkowski Przemysław, Zaręba Ilona, Drągowski Paweł, Prokop Izabela, Zadykowicz Rafał, Zaręba Renata, Rysiak Edyta
    published in *Polski Przegląd Nauk o Zdrowiu,* 2015, 3 (44), s. 154-158.
    MNiSW: 7

11. „Rola składników naturalnych w zapobieganiu chorobom neurodegeneracyjnym. The role of natural ingredients in protection from neurodegenerative diseases.”
    authors: Czajkowski Przemysław, Nazaruk Jolanta.
    published in *Geriatria*, 2014 : 8, s. 258-263.
    MNiSW: 5

List of conference abstracts

1. Czajkowski P, Nazaruk J. Rola składników naturalnych w zapobieganiu schorzeniom neurodegeneracyjnym. Dietetyka gerontologiczna - wyzwania i szanse. Poznań, 27.02.2014. (The role of natural ingredients in the prevention of neurodegenerative disorders. Gerontological dietetics - challenges and opportunities)

2. Strawa J, Wyszkowska M, Czajkowski P. Preliminary phytochemical examination of lipophilic fractions of Arctium tomentosum Mill. flower heads. 9th Białystok International Medical Congress for Young Scientists. Białystok, 24-26.04.2014.

3. Nowak K, Czajkowski P, Galicka A. Rola metaloproteinaz (MMPs) i ich inhibitorów tkankowych (TIMPs) w procesie nowotworowym. Ogólnopolska Konferencja Naukowa "Farmakoterapia Kobiet w Ciąży i Elementy Farmakoekonomiki". Białystok, 25.04.2015. (The role of metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in the neoplastic process. Nationwide Scientific Conference "Pharmacotherapy of Pregnant Women and Elements of Pharmacoeconomics")

4. Czajkowski P, Jastrzębska K, Truszkowska M, Rapacz DJ, Nazaruk J. Surowce i substancje pochodzenia naturalnego zalecane kobietom w ciąży. Ogólnopolska Konferencja Naukowa "Farmakoterapia Kobiet w Ciąży i Elementy Farmakoekonomiki". Białystok, 25.04.2015. (Raw materials and natural substances recommended to pregnant women. Nationwide Scientific Conference "Pharmacotherapy of Pregnant Women and Elements of Pharmacoeconomics")

5. Jastrzębska K, Czajkowski P, Truszkowska M, Nazaruk J. Surowce i składniki naturalne przeciwwskazane u kobiet w ciąży. Ogólnopolska Konferencja Naukowa "Farmakoterapia Kobiet w Ciąży i Elementy Farmakoekonomiki". Białystok, 25.04.2015. (Raw materials and natural ingredients contraindicated in pregnant women. Nationwide Scientific Conference "Pharmacotherapy of Pregnant Women and Elements of Pharmacoeconomics")

6. Truszkowska M, Czajkowski P, Jastrzębska K, Nazaruk J. Substancje roślinne stosowane tuż przed i zaraz po porodzie. Ogólnopolska Konferencja Naukowa "Farmakoterapia Kobiet w Ciąży i Elementy Farmakoekonomiki". Białystok, 25.04.2015. (The herbal substances

used just before and immediately after delivery. Nationwide Scientific Conference "Pharmacotherapy of Pregnant Women and Elements of Pharmacoeconomics")

7. Czajkowski P, Andrulewicz E, Bielawska A, Bielawski K, Kałuża Z, Gornowicz A, Galicka A. The effect of new synthetic tylophorine analogs on biological activity in MCF-7 breast cancer cells. 22nd International Student Congress of (BIO)Medical Sciences, Groningen. Netherlands, 2nd - 5th.06.2015.

8. Czajkowski P, Andrulewicz E, Bielawska A, Bielawski K, Kałuża Z, Gornowicz A, Galicka A. Badanie i ocena biologicznej aktywności nowo otrzymanych pochodnych tyloforyny na komórki raka sutka MCF-7. Kongres Polskiego Towarzystwa Farmaceutycznego, FARMACJA 21, "Farmaceuci w ochronie zdrowia". Wrocław, 11-12.09.2015. (Examination and evaluation of the biological activity of newly derived tylophorin derivatives on MCF-7 breast cancer cells. Congress of the Polish Pharmaceutical Society, PHARMACY 21, "Pharmacists in health care")

9. Czajkowski P, Andrulewicz E, Bielawska A, Bielawski K, Kałuża Z, Galicka A. Nowy syntetyczny analog tyloforyny GD41 jako związek o potencjale przeciwnowotworowym. II Ogólnopolska Konferencja "Innowacje w Praktyce". Lublin, 22-23.10.2015. (The new synthetic analog of tylophorine GD41 as a compound with anticancer potential. II National Conference "Innovation in Practice")

10. Czajkowski P, Maciejczyk M, Frątczak J, Surażyński A. Znaczenie porady aptekarskiej w procesie samoleczenia - skutki kliniczne i ekonomiczne. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (The importance of pharmacy advice in the self-healing process - clinical and economic effects. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy)

11. Frątczak J, Czajkowski P, Maciejczyk M, Zieniewska I, Żyłkiewicz M, Kamińska M, Czubaczyński M, Knaś M, Zalewska A. Wpływ antybiotyków na wchłanianie i metabolizm składników odżywczych pożywienia – znaczenie kliniczne. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (Effect of antibiotics on the absorption and metabolism of nutrients of food - clinical significance. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy")

12. Grochowski DM, Jabłonowska MI, Czajkowski P, Tomczyk M. Smaczna terapia - działanie przeciwbakteryjne owoców rodzaju Rubus. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (Tasty therapy - antibacterial effect of Rubus fruit. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy")

13. Czajkowski P, Jastrzębska K, Truszkowska M, Nazaruk J. Olejki eteryczne z rodziny Myrtaceae - substancje o właściwościach przeciwbakteryjnych. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (Essential oils from the Myrtaceae family - substances with antibacterial properties. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy")

14. Osińska M, Sójka A, Zadykowicz R, Krzesicki W, Siemieniuk M, Czajkowski P, Michalski T, Prokop I, Zaręba I, Rysiak E. Antybiogram - szansa na zbilansowanie kosztów leczenia. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (Antibiogram - a chance to balance treatment costs. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy")

15. Maciejczyk M, Marcińczyk N, Czajkowski P, Frątczak J, Rysiak E. Farmakoterapia chorób rzadkich i ultrarzadkich u dzieci - aspekt ekonomiczny. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (Pharmacotherapy for rare and ultra-rare diseases in children - an economic aspect. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy")

16. Bogdańska E, Michalski T, Czajkowski P, Cekała E, Rutkowski M, Zadykowicz R, Laskowski R,Krzesicki W, Siemieniuk M, Osińska M, Maciejczyk M, Kwolek A, Rysiak E. Antybiotykoterapia w dermatologii. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (Antibiotic therapy in dermatology. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy")

17. Cekała E, Czajkowski P, Rutkowski M, Kazberuk A, Zadykowicz R, Raciborska I, Maciejczyk M, Fardorwski B, Kierdylewicz R, Osińska M, Rysiak E, Kwolek A. Alergiczne choroby skóry i reakcje polekowe. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (Allergic skin

diseases and drug reactions. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy")

18. Truszkowska M, Jastrzębska K, Czajkowski P, Nazaruk J. Czosnek - naturalny antybiotyk. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (Garlic - a natural antibiotic. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy")

19. Marcińczyk N, Frątczak J, Czajkowski P, Żyłkiewicz M, Zieniewska I, Kamińska M, Czubaczyński M, Knaś M, Zalewska A. Antybiotykoterapia w czasie ciąży a rozwój dziecka. Ogólnopolskie Sympozjum Naukowe "Problemy Współczesnej Antybiotykoterapii". Białystok, 21.11.2015. (Antibiotic therapy during pregnancy and child development. Nationwide Scientific Symposium "Problems of Contemporary Antibiotic Therapy")

20. Osińska M, Sójka A, Zadykowicz R, Czajkowski P, Rysiak E. Zaburzenia gospodarki wapniowofosforanowej u dzieci - opieka farmaceutyczna. Ogólnopolska Konferencja Młodej Farmacji "Farmaceuta-jeden z filarów ochrony zdrowia". Kraków, 4-6.12.2015. (Disorders of calcium phosphate economy in children - pharmaceutical care. Nationwide Young Pharmacy Conference "Pharmacist - one of the pillars of health protection")

21. Czajkowski P, Radziejewska I. Mucyny żołądkowe i ich rola w aspekcie oddziaływań z Helicobacter Pylori. Ogólnopolska Konferencja Młodej Farmacji "Farmaceuta-jeden z filarów ochrony zdrowia". Kraków, 4-6.12.2015. (Gastric mucins and their role in the aspect of interactions with Helicobacter Pylori. Nationwide Young Pharmacy Conference "Pharmacist - one of the pillars of health protection")

22. Czajkowski P, Osińska M, Maciejczyk M, Sójka A, Bogdańska E, Rysiak E. Opieka farmaceutyczna jako jeden z elementów terapii cukrzycy. Ogólnopolska Konferencja Młodej Farmacji "Farmaceuta jeden z filarów ochrony zdrowia". Kraków, 4-6.12.2015. (Pharmaceutical care as one of the elements of diabetes therapy. Nationwide Conference of Young Pharmacy "Pharmaceuticals with pillars of health protection")

23. Bogdańska E, Śnietka E, Czajkowski P, Kazberuk A, Cekała E, Rutkowski M, Zaręba I, Rysiak E, Prokop I, Osińska M, Kwolek A, Michalski T. Edukacja pacjentów - kluczem do

racjonalizacji farmakoterapii. Ogólnopolska Konferencja Młodej Farmacji "Farmaceuta-jeden z filarów ochrony zdrowia". Kraków, 4-6.12.2015. (Patient education - the key to pharmacotherapy rationalization. Nationwide Young Pharmacy Conference "Pharmacist – one of the pillars of health protection")

24. Czajkowski P, Andrulewicz E, Radziejewska I, Bielawska A, Galicka A. Lectin from Wisteria floribunda influences MUC1 mucin expression on gastric adenocarcinoma cells. 2nd Lublin International Medical Congress for Students and Young Doctors, Lublin, 11th-12th.12.2015.

25. Czajkowski P, Marcińczyk N, Masłowski W, Kramkowski K. Badanie przeciwpłytkowe właściwości CORM-A1 w eksperymentalnym modelu zakrzepicy metodą konfokalnej mikroskopii przyżyciowej. IX Kongres Młodej Farmacji "Farmaceuta w przemyśle", Łódź 29.09.-02.10.2016. (Antiplatelet activity of CORM-A1 in an experimental model of thrombosis by confocal imaging microscopy. IX Congress of Young Pharmacy "Pharmacist in industry")

26. Leszczyńska A, Marcińczyk N, Jarmoc D, Czajkowski P, Kramkowski K. The effect of CORM-A1, NaNO2 and indomethacin on mice breast cancer cells (4T1-IRFP) metastases formation in the liver and lungs. XXII Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego oraz Komitetu Nauk Fizjologicznych i Farmakologicznych Polskiej Akademii Nauk, Gdańsk, 26-28.10.2017.

27. Czajkowski P, Marcińczyk N, Jarmoc D, Leszczyńska A. Antithrombotic properties of carvedilol in real time laser induced thrombosis. 12th BIMC Bialystok International Medical Congress for Young Scientists, Bialystok, Poland, 20-22nd April 2017.

28. Jarmoc D, Leszczyńska A, Marcińczyk N, Czajkowski P, Sikora A, Kramkowski K. Antithrombotic effect of HNO donors. XXII Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego oraz Komitetu Nauk Fizjologicznych i Farmakologicznych Polskiej Akademii Nauk, Gdańsk, 26-28.10.2017.

29. Czajkowski P, Marcinczyk N, Jarmoc D, Leszczyńska A, Kramkowski K. Antiplatelet effect of carvedilol in interavital real time laser-induced thrombosis in mice. XXII Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa

Kardiologicznego oraz Komitetu Nauk Fizjologicznych i Farmakologicznych Polskiej Akademii Nauk, Gdańsk, 26-28.10.2017.

30. Przesław K, Bołtromiuk E, Iwanicka M, Zakrzeska A, Jarmoc D, Leszczyńska A, Czajkowski P, Marcińczyk N, Gołaszewska A, Misztal T, Rusak T, Kramkowski K. Antithrombotic effects of ruthin: a new face of an old drug. XXIII Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego i Komitetu Nauk Fizjologicznych i Farmakologicznych PAN, Łódź, 15-17.11.2018.

31. Przesław K, Bołtromiuk E, Iwanicka M, Zakrzeska A, Jarmoc D, Leszczyńska A, Czajkowski P, Marcińczyk N, Gołaszewska A, Misztal T, Rusak T, Kalvinsh I, Kramkowski K. Antithrombotic effects of disulfide isomerase inhibitors. XXIII Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego i Komitetu Nauk Fizjologicznych i Farmakologicznych PAN, Łódź, 15-17.11.2018.

32. Zakrzeska A, Jarmoc D, Leszczyńska A, Czajkowski P, Marcińczyk N, Przesław K, Bołtromiuk E, Iwanicka M, Gołaszewska M, Misztal T, Rusak T, Kramkowski K. Antimetastatic effects of antiplatelet therapy: in vivo tracking of early fate of cancer cells in the liver and lung. XXIII Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego i Komitetu Nauk Fizjologicznych i Farmakologicznych PAN, Łódź, 15-17.11.2018.

33. Czajkowski P, Jarmoc D, Rusak T, Kramkowski K. Antithrombotic properties of carvedilol shown in two independent intravital real time models of thrombosis. Virtual Congress of the International Society on Thrombosis and Haemostasis, July 12-14, 2020; ISTH 2020.

34. Czajkowski P, Marcińczyk N, Jarmoc D, Rusak T, Kramkowski K. Antithrombotic effect of carvedilol after laser or red-ox endothelium injury. 5ThSfRBM & 3rd MCW Redox Biology Symphosium, Winsconsin. May 13-14, 2021.

35. Krasowska U, Adamska-Patruno E, Bauer W, Czajkowski P, Fiedorczuk J, Moroz M, Górska M, Krętowski A. Assessment of the relationship between protein consumption and development of glucose metabolism disturbances in carries of some common single

nucleotide polymorphisms in gene BDNF (rs10835211). 4th European Summer School on Nutrigenomics, Camerino, Italy, 2021.06.21-25.

36. Czajkowski P, Adamska-Patruno E, Bauer W, Krasowska U, Fiedorczuk J, Moroz M, Górska M, Krętowski A. Dietary fiber intake may influence the associations between FTO genetic variants and obesity-related parameters. 4th European Summer School on Nutrigenomics, Camerino, Italy, 2021.06.21-25.

List of scientific activities

Research Projects:

1. Principal Investigator: "Evaluation of antithrombotic effect of carvedilol in intravital real time laser-induced thrombosis"
   The Leading National Scientific Centre (KNOW), 2016, ID: 213/KNOW/16
2. Principal Investigator: "Assessment of the effect of selected drugs of the renin-angiotensin-aldosterone system in hyperhomocysteinemia, considered as a risk factor for the development of cardiovascular diseases"
   European Union funds, PO WER 2014-2020, grant No.03/IMSD/G/2019.
3. Project participant: "Prostacyclin, nitric oxide and carbon monoxide-based pharmacotherapy of endothelial dysfunction and platelet activation - a novel strategy to inhibit cancer metastasis" program - STRATEGMED, METENDOPHA acronym. STRATEGMED1/233226/11/NCBR/2015, supported by the European Union
4. Project participant: "The assessment of phloroglucinol effectiveness as a compound improving the postprandial glucose and lipid metabolism"
   National Science Centre – MINIATURA-4, 2020
5. Project participant: "Assessment of the impact of selected products of natural origin for postprandial glucose metabolism"
   Polish Diabetology Association, 2019-2023

Distinctions and awards:

1. 1st place - "Gerontological dietetics", 27.02.2014,
   "The role of the natural ingredients in the prevention of neurodegenerative disorders"
2. 3th place - "12th Bialystok International Medical Congress", 20-22.04.2017,
   "Antithrombotic properties of carvedilol in real time laser - induced thrombosis"
3. A distinction - National Congress of the Polish Pharmaceutical Society, 11-12.09.2015,
   "Research and evaluation of biological activity of newly derived derivatives of tiloforine on MCF-7"
4. A distinction - National Conference of Young Pharmacy, 4-6.12.2015,
   "Gastric mucins - the aspect of interactions with Helicobacter pylori"