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The aim of the study was to investigate whether lifestyle factors modify the association fat mass and obesity-associated *(FTO)* gene single nucleotide polymorphisms (SNPs) and obesity in a Turkish population. The study included 400 unrelated individuals, aged 24-50 years recruited in a hospital setting. Dietary intake and physical activity were assessed using 24-hour dietary recall and self-report questionnaire, respectively. A genetic risk score (GRS) was developed using *FTO* SNPs, rs9939609 and rs10163409. Body mass index and fat mass index were significantly associated with *FTO* SNP rs9939609 (P=0.001 and P=0.002, respectively) and GRS (P=0.002 and P=0.003, respectively). The interactions between SNP rs9939609 and physical activity on adiponectin concentrations, and SNP rs10163409 and dietary protein intake on increased waist circumference were statistically significant ($P_{interaction}$=0.027 and $P_{interaction}$=0.044, respectively). This study demonstrated that the association between *FTO* SNPs and central obesity might be modified by lifestyle factors in this Turkish population.

Keywords: *FTO* gene variant; obesity; gene-diet interaction; adiponectin; genetic risk score; physical activity

Introduction

Obesity has been recognised as a worldwide public health problem due to its rising prevalence and concomitant health problems. The prevalence of overweight and obesity in Turkey were reported as 64.4% and 28.8%, respectively by WHO (WHO, 2018). Obesity can lead to other chronic diseases including type 2 diabetes (T2D), cardiovascular diseases (CVD), hypertension, cancer and osteoarthritis (Forse et al. 2020). A combination of

67 interactions between genetic and environmental factors is required for the development of a
68 complex disease such as obesity (Franks and McCarthy 2016; Milagro et al. 2020). Studies
69 have identified approximately 140 genes to be associated with obesity, and the fat mass and
70 obesity associated (*FTO*) gene has been reported to be the strongest susceptibility gene for
71 human obesity (Pigeyre et al. 2016).

72 The *FTO* gene is located on chromosome 16q12.2 and codes for a protein with 2-
73 oxoglutarate dependent nucleic acid demethylase activity which is involved in DNA repair
74 and the accumulation of fat in the body (Clifton et al. 2006; Chen and Du 2019). *FTO* is
75 highly expressed in the brain, including the hypothalamus, adipocytes, pancreatic islet cells,
76 and adrenal glands (Frayling et al. 2007). *FTO* gene has been suggested to control energy
77 homeostasis and food intake (Abete et al. 2020). Previous studies have shown that, of the
78 various obesity susceptibility genes, single-nucleotide polymorphisms (SNPs) located in the
79 first intron of *FTO* gene has provided the strongest evidence for genetic predisposition to
80 obesity (Frayling et al. 2007; Scuteri et al. 2007; Speliotes et al. 2010; Loos and Yeo 2014;
81 Babenko et al. 2019; Fonseca et al. 2020). The minor allele 'A' of the *FTO* SNP rs9939609
82 has been consistently associated with higher BMI in various populations (Frayling et al. 2007;
83 Hertel et al. 2011; Peng et al. 2011; Corella et al. 2012; Li et al. 2012; Qi et al. 2014; Wang
84 et al., 2020; Schlauch et al. 2020). Furthermore, a meta-analysis reported that the association
85 between the SNP rs9939609 and BMI was replicated in 13 cohorts with 38,759 participants,
86 where individuals with the 'AA' genotype had 1.67-time higher odds of obesity than those
87 with the 'TT' genotype (Frayling et al. 2007). In the Turkish population, the risk alleles of
88 the *FTO* rs1421085 and rs9939609 polymorphisms were shown to have significant

89 associations with the risk of obesity in women and metabolic syndrome (MetS) in men
90 (Guclu-Geyik et al. 2016).
91 Turkish adults are characterized with low levels of total and high-density lipoprotein
92 cholesterol, and high risk of CVD, that distinguish them from Europeans (Onat 2001). They
93 also have increased susceptibility to impaired glucose tolerance and MetS primarily driven
94 by obesity (Onat and Can 2014). Among the non-communicable diseases (NCDs) that
95 accounted for 88.0% of deaths in Turkey, CVD has shown to contribute to 47.73% of overall
96 deaths (WHO, 2018). Targeting modifiable risk factors for NCDs including obesity could
97 prevent many deaths. Therefore, several health promotion campaigns such as “Reducing
98 Portion Sizes” and “Move for Health” have been implemented for the prevention of obesity
99 in Turkey (WHO, 2016; OECD, 2017). However, obesity is a multifactorial disorder, and
100 identifying gene-environment interactions are needed to understand the aetiology and
101 pathophysiology of obesity and also to develop more effective personalised preventative
102 strategies (Castillo et al. 2017; Dahlman and Ryden 2020). To date, several *FTO*-dietary
103 intake interactions on obesity-related outcomes have been examined in different populations
104 (Grau et al. 2009; Sonestedt et al. 2009; Lappalainen et al. 2012; Ortega-Azorin et al. 2012;
105 Phillips et al. 2012; Vimaleswaran et al. 2012; Qi et al. 2014; Merritt et al. 2018; Saber-Ayad
106 et al. 2019) however, there are no such studies to date in a Turkish population The
107 investigations of the gene-diet interactions in different ethnic groups are crucial to develop
108 personalised nutrition strategies for each ethnic group due to the genetic heterogeneity
109 (Vimaleswaran 2017). The *FTO* SNP rs9939609 has been associated with several dietary
110 components including dietary protein intake (Lappalainen et al. 2012; Qi et al. 2014; Merritt

et al. 2018) and the SNP rs10163409 in *FTO* was among the top associations in a large genome-wide meta-analysis study (GWAS) for total caloric intake (Chu et al. 2013). Therefore, this study aimed to assess whether *FTO* variants, rs9939609 and rs10163409, are associated with obesity in 400 Turkish individuals and to determine whether these SNPs interact with dietary intake and physical activity on obesity outcomes.

Materials and Methods

Study population

A total of 400 unrelated individuals, aged 24-50 years, were recruited from the outpatient clinic of Department of Endocrinology and Metabolism at the Hacettepe University Hospitals, Ankara, Turkey. This study was conducted as part of the GeNuIne Collaboration that investigates the interactions between genetic and dietary factors on metabolic diseases in different ethnic groups (Vimaleswaran 2017). The study participants were screened based on the following inclusion criteria: 1) routine visits to the outpatient clinic, 2) aged 18-50 years, and 3) having a BMI $\geq$ 18.50 kg/m^2. The exclusion criteria were: 1) having specific health problems including, liver and kidney diseases, mental and psychological disorders, history of cancer, and serious endocrine disorders (hypothyroidism, hyperthyroidism or hypopituitarism), 2) history of bariatric surgery, 3) being pregnant or lactating, 4) using drugs that affect body weight. Researchers informed and invited the eligible participants for their participation in to the study. The study was approved by the local ethics committee of Hacettepe University (GO 15/612-11), and all the participants provided the signed written consent.

Study design

A cross-sectional case-control study design was used, where participants were divided into two groups: obese (BMI $\geq$25.00 kg/m^2, n=200) and non-obese (BMI= 18.50-24.99 kg/m^2, n=200). All participants underwent a physical examination by the research endocrinologists,

followed by clinical, biochemical and lifestyle assessments, and genetic analysis of *FTO* SNPs rs9939609 and rs10163409.

Anthropometrical Measurements

Body weight and height were measured by standard methods using a calibrated digital scale (Seca 220 Scale, Germany). BMI calculation was based on the body weight (in kilograms) divided by the square of height (in meter) (WHO, 2020). BMI classification of the WHO was used to classify the individuals as non-obese (BMI < 25.00 kg/m^2) and obese (BMI ≥ 25.00 kg/m^2) (WHO, 2005). The waist circumference (WC) was measured by a standard method (WHO, 2011). Increased WC (central obesity) was defined based on cut-points established for Turkish adults (WC ≥ 90 cm for men/ ≥ 80 cm for women) (Sonmez et al. 2013). Body composition was analysed by bioelectrical impedance using the Tanita MC-980 MA Multi Frequency Segmental Body Composition Analyzer (USA). Fat mass index (FMI) was calculated based on the fat mass (in kilograms) divided by the square of height (in meter) (Peltz et al. 2010). All anthropometrical measurements were taken by the research dieticians.

Biochemical and clinical measures

Serum adiponectin was analysed by ELISA kits (Ebioscience, Austria) at Hacettepe University Hospitals, Clinical Pathology Laboratory. The physical examination included the measurement of systolic (SBP) and diastolic blood pressure (DBP) using a stethoscope and sphygmomanometer in the right arm of the participants after sitting in a comfortable position in a quiet room for at least 15 min. Both blood pressures were measured twice at 5-minute intervals and recorded on average (Frese et al. 2011).

Dietary assessment

Dietary intake was assessed using 24-hour dietary recall method that was carried out by trained research dieticians. A photographic atlas of food portion sizes and common household measures were used to facilitate the quantification of the amount of food consumed. Total energy, macro- and micronutrient intakes of participants were analysed from the records using BeBIS software (BeBIS, Nutrition Information System, Version 8).

Other lifestyle factors

The socio-demographic characteristics, family and medical history, smoking and alcohol consumption were recorded. The physical activity level was assessed using the Turkish version of the International Physical Activity Questionnaire (IPAQ) (Saglam et al. 2010).

SNPs selection and genotyping

FTO gene was selected based on its consistent and strong associations with obesity traits in large-scale GWASs (Frayling et al. 2007). The SNP rs9939609 is the most commonly studied variant and consistently associated with obesity phenotypes across multiple ethnicities (Frayling et al. 2007; Hertel et al. 2011; Peng et al. 2011; Corella et al. 2012; Li et al. 2012; Loos and Yeo 2014; Qi et al. 2014) and SNP rs10163409 has been shown to be associated with dietary energy intake from macronutrients (Chu et al. 2013). Therefore, *FTO* SNPs, rs9939609 and rs10163409, which have been shown to be associated with obesity traits and dietary intake in large GWASs, were genotyped. The genotype frequencies of the *FTO* SNPs, rs9939609 and rs10163409, were in Hardy Weinberg equilibrium ($p>0.05$).

The genomic DNA was extracted from the whole blood in K2EDTA containing tubes by the salting out method. Genotyping of the SNPs, rs9939609 and rs10163409, were performed using KASP assay (a competitive allele-specific polymerase chain reaction that incorporates a fluorescent resonance energy transfer quencher cassette), and the KASP primers were designed using Kraken software system (LGC, https://www.lgcgroup.com). Genotyping assays were carried out according to the manufacturer's instructions with a 7500 Real time PCR System (Applied Biosystems). The following thermal cycling profile were used: 15 min at 94°C; 10 cycles of 20 s at 94°C, 60 s at 61°C with decrement -0.6°C/per cycle and 26 cycles of 20 s at 94°C, 60 s at 55°C; 60 s at 37°C.

Statistical analysis

SPSS software (version 23.0) was used for statistical analysis. The Hardy-Weinberg equilibrium was assessed using the x^2 goodness-of-fit test. Genotype frequencies and distribution in groups were compared using Pearson's chi-squared test. Continuous variables are presented as means and standard deviations (SD), and groups were compared using the independent t-test.

As the number of individuals with rare homozygous genotypes was low, a dominant model was used, where common homozygous genotypes were compared to combined rare homozygous and heterozygous genotypes. A genetic risk score (GRS) was created from both the *FTO* SNPs where the presence of one risk allele of any of the variants was scored as one point. This GRS ranged from 0 (homozygous individuals for non-risk alleles) to 4 points (homozygous individuals for the risk alleles of both *FTO* polymorphisms). The GRS variable

was then categorised into two groups based on the number of points; 1st group: individuals with scores of <2 points; 2nd group: individuals with scores of ≥2 points.

The independent and joint effects of *FTO* SNPs on the risk of obesity were assessed using the odds ratios (ORs) and 95% confidence intervals (CIs) that were calculated by logistic regression models. Also, the associations between *FTO* polymorphisms (separately and joint) and the continuous outcomes were tested using general linear models. Models were adjusted for age, gender, hypertension, CVD and obesity status wherever appropriate. Furthermore, *FTO* gene-environment interactions on continuous and categorical outcomes were tested using linear and logistic regression models, respectively. Interactions were investigated by including the interaction terms (e.g.,carbohydrate*genotype) in the regression models. Environmental factors that were investigated included dietary intake (carbohydrate, protein, fibre and fat intakes in grams/day) and physical activity. Furthermore, statistically significant interactions were investigated in more depth, where individuals were stratified by the tertiles of the lifestyle factor.

Results

Characteristics of the Participants

Obese individuals were older, and had higher BMI, WC and FMI and lower adiponectin levels than the controls (P<0.001, for each). The cases and controls were not statistically different in terms of their food intake and physical activity levels (P>0.05) (Table 1).

Associations between FTO variants and obesity-related traits

Genotype distributions and minor allele frequencies (MAFs) for both SNPs are shown in Table 2. The MAFs of the SNPs, rs10163409 and rs9939609, were T=0.37 and A=0.39,

respectively. The associations between SNP rs9939609 and BMI (P=0.001) and FMI (P=0.002) were found significant where the 'A' (AT/AA) allele carriers had significantly higher BMI and FMI than 'TT' homozygotes (Table 3). Furthermore, 'A' allele carriers had significantly higher WC (P=0.007) and lower adiponectin levels (P=0.031) compared to non-carriers. The *FTO* SNP rs10163409 did not show any significant association with obesity traits (Table 3).

Interactions between FTO variants and dietary intake on obesity-related traits

FTO gene-dietary protein intake interactions

The significant interactions between SNP rs10163409 and protein intake on the risk of increased WC ($P_{interaction}$=0.044) and WC as a continuous variable ($P_{interaction}$=0.007) were observed. Stratification of the dietary protein intake into tertiles showed that, in the highest tertile group with a mean ± SD of 138±38 g/day protein intake, 'T' allele carriers of the SNP rs10163409 had a significantly higher risk of central obesity [OR= 3.3 (95% CI: 1.149-9.478), P=0.027] than those with 'AA' genotype (Figure 1).

Interactions between FTO variants and physical activity on obesity-related traits

The interaction between the SNP rs9939609 and physical activity levels on adiponectin concentrations was statistically significant ($P_{interaction}$= 0.027), where, among those with lowest levels of physical activity, the adiponectin concentrations were significantly lower in the allele 'A' carriers compared to individuals with 'TT' genotype (P=0.006) (Figure 2).

Associations between GRS and obesity-related traits

The GRS was significantly associated with BMI (P=0.002), FMI (P=0.003) and increased WC (P=0.02) (Figures 3a, 3b and 3c). However, the interactions between GRS and lifestyle factors on obesity traits were not found statistically significant.

Discussion

To our knowledge, this is the first study that investigated the interaction between *FTO* SNPs and dietary intake on obesity traits in a Turkish population. This study has identified the

associations of the *FTO* SNP rs9939609 and GRS with obesity traits, and also showed that the physical activity level can modify the effect of the minor allele 'A' of the *FTO* SNP rs9939609 on adiponectin concentrations, a biomarker of metabolic syndrome (Stojanovic et al. 2015). Furthermore, our study has demonstrated that the higher protein intake was associated with higher risk of central obesity among the 'T' allele carriers of the *FTO* SNP rs10163409 compared to non-carriers. Since Turkish adults have a sedentary lifestyle (WHO, 2018), our findings contribute to the development of effective public health strategies focusing on the prevention and management of central obesity and CVD in Turkish population (IHME, 2017).

This study has shown that the risk allele 'A' of the *FTO* SNP rs9939609 was significantly associated with higher BMI and FMI, in agreement with the findings from other populations (Frayling et al. 2007; Do et al. 2008; Hertel et al. 2011; Peng et al. 2011; Corella et al. 2012; Li et al. 2012; Muc et al. 2015; Merra et al. 2020). A meta-analysis performed on 177,330 individuals from multiple ethnicities have demonstrated an association between *FTO* rs9939609 genotype and BMI, suggesting a higher BMI in 'A' allele carriers (effect per allele=0.30 [0.30, 0.35] kg/m^2, $P=3.6*10^{-107}$) (Qi et al. 2014). The reported *FTO*-related genetic associations with BMI have also been confirmed in a study in the Turkish population (Guclu-Geyik et al. 2016), where the *FTO* risk allele, 'C', carriers of the SNP rs1421085, which is in a high linkage disequilibrium (LD) ($D'=0.967$, $r^2=0.85$) with the SNP rs9939609, had significantly increased BMI. Furthermore, parallel to the findings of other studies (Vimaleswaran et al. 2012; De Luis et al. 2016; Saucedo et al. 2017), we have also found that the *FTO* SNP rs9939609 was significantly associated with higher WC and lower adiponectin concentrations. On the contrary, there were no significant association between SNP rs10163409 and obesity. This could be explained by the fact that the SNP rs10163409 is not in LD with other *FTO* variants that have shown significant associations with BMI (Chu et al. 2013).

Our study has provided evidence for gene-diet interaction in the Turkish population. We have demonstrated that, among those in the highest tertile of dietary protein intake, the risk of increased WC/central obesity was higher for the minor allele, 'T', carriers of the *FTO* SNP rs10163409 compared to those with AA genotype. To date, this is the first study analysing gene-diet interactions of the SNP rs10163409, suggesting that high intake of dietary protein might negatively affect WC in genetically susceptible individuals. However, studies investigating other *FTO* SNPs (rs1558902 and rs9939609) have reported conflicting results (Zhang et al. 2012; de Luis et al. 2015; Merritt et al. 2018). It has been suggested that following a high protein diet can modulate the genetic effect of *FTO* variants on obesity traits (Zhang et al. 2012; de Luis et al. 2015; Merritt et al. 2018). According to a 2-year weight loss intervention program, carriers of the risk allele 'A' of the *FTO* rs1558902 had a greater weight loss compared to non-carriers when high protein diets were consumed, whereas a negative genetic effect was found in response to a low-protein intake (Huang et al. 2014). The potential mechanism of *FTO* variants - protein intake interaction is still unclear, however, the regulation of food intake and appetite could be influenced. It has been found that the risk allele 'A' of the SNP rs9939609 was significantly associated with a greater reduction in food cravings and appetite scores among individuals who consumed high-protein diet but not in those in the low-protein diet (Huang et al. 2014). Regarding the SNP rs9939609, there were no significant interactions between the *FTO* variants and any of the dietary components on obesity traits. In agreement with our findings, a study of 11,091 adults from five Europeans countries have found no interactions between the rs9939609 variant and the dietary intake of carbohydrate, glycaemic index, protein or fat on BMI, WC, weight gain and risk of obesity (Vimaleswaran et al. 2012). Furthermore, a meta-analysis of 40 population-based studies reported that the total energy or macronutrient intakes had no effect on the association between the SNP rs9939609 and BMI (Qi et al. 2014). In contrast to our finding, a few large-scale studies demonstrated significant interactions between dietary

298 macronutrient intakes and *FTO* variants in determining BMI (Grau et al. 2009; Sonestedt et
299 al. 2009; Corella et al. 2011; Lappalainen et al. 2012; Ortega-Azorin et al. 2012; Phillips et
300 al. 2012). A cross-sectional study conducted on 4,839 Swedish participants reported an
301 association between the risk allele of the SNP rs9939609 and higher BMI only in individuals
302 with high fat and low carbohydrate consumption (Sonestedt et al. 2009). A similar interaction
303 between the rs9939609 variant and saturated fatty acids (SFA) intake has been detected in
304 2,163 individuals from two independent populations of the United States, where individuals
305 homozygous for the risk allele 'AA' had a higher BMI compared to other genotypes, only
306 when the intake of SFA was high (Corella et al. 2011). Furthermore, the *FTO* SNP
307 rs8050136, in LD with rs9939609, significantly interacted with carbohydrate intake on
308 obesity risk among Asian Indian population (Vimaleswaran et al. 2016).
309 Regarding genetic interactions with physical activity, a previous study conducted among
310 200 Turkish adults found that BMI was higher in homozygous risk allele 'A' carriers of the
311 SNP rs9939609 than the homozygote the 'T' allele carriers among physically inactive
312 individuals (Kirac et al. 2016). The same interaction but on a biochemical measure of obesity
313 (i.e.: adiponectin level), rather than BMI, was replicated in our study using a larger sample
314 size. We found that, among those with lowest levels of physical activity, the adiponectin
315 concentrations were significantly lower in the carriers of the risk allele 'A' of the *FTO*
316 rs9939609 than 'TT' homozygotes. Adiponectin is a hormone produced and secreted by
317 adipose tissue and commonly known for its antihyperglycemic, anti-inflammatory,
318 antiatherogenic, and cardioprotective effects (Richard et al. 2020; Esmaili et al. 2020; Lee
319 and Shao 2014). Studies have reported a strong correlation between the dysregulation of
320 adipokine production and the onset of several metabolic abnormalities including CVD and
321 cancer (Avogaro and de Kreutzenberg 2005; De Pergola and Silvestris 2013; Xiang et al.
322 2020). The positive correlation between adiponectin levels and physical activity has been
323 demonstrated in several studies (St-Pierre et al. 2006; Jurimae et al. 2010; Sirico et al. 2018),

where higher levels of physical activity have been shown to reduce adiposity which decreases the production of insulin and leptin, and increases adiponectin production (Nurnazahiah et al. 2016). Indeed, it has been reported that serum concentrations of adiponectin are inversely related to BMI, visceral body fat and blood concentrations of glucose, insulin, and triglycerides (De Rosa et al. 2013; Frithioff-Bojsoe et al. 2020). An intervention study conducted in 400 obese women showed that a weight reduction program resulted in a significant increase in adiponectin levels (Mavri et al. 2011). Given that this is the first study to report an interaction between *FTO* variant and physical activity on adiponectin concentrations, the findings need to be replicated in a larger Turkish cohort.

The main strengths of this study include the use of a biochemical marker of obesity (i.e., adiponectin) and a well-characterised population. Nevertheless, there are some limitations which include the small sample size and the use of self-reported measurements in the assessment of dietary intake and physical activity. However, this study has still confirmed the associations between *FTO* SNP rs9939609 and obesity traits which were also reported in previous studies (Frayling et al. 2007; Hertel et al. 2011; Peng et al. 2011; Corella et al. 2012; Li et al. 2012; Merra et al. 2020; Schlauch et al. 2020). Given that obesity is a multifactorial condition, several genetic factors and lifestyle behaviours provide a predisposition to obesity; even though we have focused on the two important lifestyle factors, diet and physical activity, only two genetic variants were examined. However, to date, the *FTO* gene has been shown to be the strongest susceptibility gene for common obesity (Frayling et al. 2007; Scuteri et al. 2007; Speliotes et al. 2010; Loos an Yeo 2014). Furthermore, the cross-sectional design of this study limits the proof of causality. Even though our analysis was adjusted for several confounders, we cannot rule out the residual confounding caused by unknown factors. Therefore, the observed interactions needed to be confirmed in further studies with larger sample sizes.

Conclusion

In summary, this study has confirmed the associations between the risk allele 'A' of the *FTO* rs9939609 and GRS, with obesity related traits including BMI and FMI in this Turkish population. Our study suggests that the impact of the *FTO* polymorphisms, rs10163409 and rs9939609, on obesity among Turkish adults might be affected by dietary protein intake and physical activity levels, respectively, suggesting that increased consumption of protein-rich foods and sedentary lifestyle could possibly increase the genetic risk ofc central obesity. Our results provide significant public health implications, given that the rising prevalence of central obesity is a major public health problem in Turkey (Pekcan et al. 2017; WHO, 2018). Further studies with large sample size and objective measures of environmental factors are required to provide a better understanding of how these variants interact with lifestyle factors to develop effective prevention and treatment strategies for obesity.

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References

Abete I, Navas-Carretero S, Zulet MA, Martinez JA. 2020. Genetic Regulation of Energy Homeostasis: Obesity Implications. In: Principles of Nutrigenetics and Nutrigenomics, Ed. Caterina RDE, Martinez JA, Kohlmeier M: Academic Press. p. 175-180.

Avogaro A, de Kreutzenberg SV. 2005. Mechanisms of endothelial dysfunction in obesity. Clin Chim Acta. 360(1-2):9-26.

Babenko V, Babenko R, Gamieldien J, Markel A. 2019. *FTO* haplotyping underlines high obesity risk for European populations. BMC Med Genomics. 13;12(Suppl 2):46.

Castillo JJ, Orlando RA, Garver WS. 2017. Gene-nutrient interactions and susceptibility to human obesity. Genes Nutr. 12:29.

Chu AY, Workalemahu T, Paynter NP, Rose LM, Giulianini F, Tanaka T, Ngwa JS, Qi Q, Curhan GC, Rimm EB et al. 2013. Novel locus including FGF21 is associated with dietary macronutrient intake. Hum Mol Genet. 22(9):1895-1902.

Chen J, Du B. 2019. Novel positioning from obesity to cancer: *FTO*, an m(6)A RNA demethylase, regulates tumour progression. J Cancer Res Clin Oncol.145(1):19-29.

Clifton IJ, McDonough MA, Ehrismann D, Kershaw NJ, Granatino N, Schofield CJ. 2006. Structural studies on 2-oxoglutarate oxygenases and related double-stranded β-helix fold proteins. J Inorg Biochem 100(4):644-669.

Corella D, Arnett DK, Tucker KL, Kabagambe EK, Tsai M, Parnell LD, Lai CQ, Lee YC, Warodomwichit D, Hopkins PN et al. 2011. A high intake of saturated fatty acids strengthens the association between the fat mass and obesity-associated gene and BMI. J Nutr. 141(12):2219-2225.

Corella D, Carrasco P, Sorli JV, Coltell O, Ortega-Azorin C, Guillen M, Gonzalez JI, Saiz C, Estruch R, Ordovas JM. 2012. Education modulates the association of the *FTO*

rs9939609 polymorphism with body mass index and obesity risk in the Mediterranean population. Nutr Metab Cardiovasc Dis. 22(8):651-658.

Dahlman I, Rydén M. 2020. Gene-Environment Interaction and Individual Susceptibility to Metabolic Disorders. In: Beyond Our Genes. Ed.Teperino R, Castillo JJ, Orlando RA, Garver WS.Springer, Switzerland.p.81-94

De Luis DA, Aller R, Izaola O, Primo D, Romero E. 2016. Association of the rs9939609 gene variant in *FTO* with insulin resistance, cardiovascular risk factor and serum adipokine levels in obese patients. Nutr Hosp. 33(5):573.

De Luis DA, Aller R, Izaola O, Primo D, Urdiales S, Romero E. 2015. Effects of a High-Protein/Low-Carbohydrate Diet versus a Standard Hypocaloric Diet on Weight and Cardiovascular Risk Factors: Role of a Genetic Variation in the rs9939609 *FTO* Gene Variant. J Nutrigenet Nutrigenomics. 8(3):128-136.

De Pergola G, Silvestris F. 2013. Obesity as a major risk factor for cancer. J Obes. 2013:291546.

De Rosa A, Monaco ML, Capasso M, Forestieri P, Pilone V, Nardelli C, Buono P, Daniele A. 2013. Adiponectin oligomers as potential indicators of adipose tissue improvement in obese subjects. Eur J Endocrinol. 169(1):37-43.

Do R, Bailey SD, Desbiens K, Belisle A, Montpetit A, Bouchard C, Perusse L, Vohl MC, Engert JC. 2008. Genetic variants of *FTO* influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec Family Study. Diabetes. 57(4):1147-1150.

Esmaili S, Hemmati M, Karamian M. 2020. Physiological role of adiponectin in different tissues: a review. Arc Physiol Biochem. 126(1):67-73.

Fonseca ACPD, Marchesini B, Zembrzuski VM, Voigt DD, Ramos VG, Carneiro JRI, Nogueira Neto JF, Cabello GMK, Cabello PH. 2020. Genetic variants in the fat mass and

obesity-associated (*FTO*) gene confer risk for extreme obesity and modulate adiposity in a Brazilian population. Genet Mol Biol. 6;43(1):e20180264.

Forse RA, Betancourt-Garcia MM, Kissee MC. 2020. Epidemiology and Discrimination in Obesity. In: The ASMBS Textbook of Bariatric Surgery. Ed.Nguyen N, Brethauer S, Morton J, Ponce J, Rosenthal R. Springer, New York, NY.p.3-14.

Franks PW, McCarthy MI. 2016. Exposing the exposures responsible for type 2 diabetes and obesity. Science. 354(6308):69-73.

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW et al. 2007. A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 316(5826):889-894.

Frese EM, Fick A, Sadowsky HS. 2011. Blood pressure measurement guidelines for physical therapists. Cardiopulm Phys Ther J. 22(2):5-12.

Frithioff-Bøjsøe C, Lund MAV, Lausten-Thomsen U, Hedley PL, Pedersen O, Christiansen M, Baker JL, Hansen T, Holm J-C. 2020. Leptin, adiponectin, and their ratio as markers of insulin resistance and cardiometabolic risk in childhood obesity. Pediatr Diabetes. 21(2):194-202.

Grau K, Hansen T, Holst C, Astrup A, Saris WH, Arner P, Rossner S, Macdonald I, Polak J, Oppert JM et al. 2009. Macronutrient-specific effect of *FTO* rs9939609 in response to a 10-week randomized hypo-energetic diet among obese Europeans. Int J Obes (Lond). 33(11):1227-1234.

Guclu-Geyik F, Onat A, Yuzbasiogullari AB, Coban N, Can G, Lehtimaki T, Erginel-Unaltuna N. 2016. Risk of obesity and metabolic syndrome associated with *FTO* gene variants discloses clinically relevant gender difference among Turks. Mol Biol Rep. 43(6):485-494.

Hertel JK, Johansson S, Sonestedt E, Jonsson A, Lie RT, Platou CG, Nilsson PM, Rukh G, Midthjell K, Hveem K et al. 2011. *FTO*, type 2 diabetes, and weight gain throughout adult life: a meta-analysis of 41,504 subjects from the Scandinavian HUNT, MDC, and MPP studies. Diabetes. 60(5):1637-1644.

Huang T, Qi Q, Li Y, Hu FB, Bray GA, Sacks FM, Williamson DA, Qi L. 2014. *FTO* genotype, dietary protein, and change in appetite: the Preventing Overweight Using Novel Dietary Strategies trial. Am J Clin Nutr. 99(5):1126-1130.

IHME: measuring what matters 2017 [Internet]. The Institute for Health Metrics and Evaluation; [cited 2020 July 1]. Available from: http://www.healthdata.org/turkey

Jurimae J, Kums T, Jurimae T. 2010. Plasma adiponectin concentration is associated with the average accelerometer daily steps counts in healthy elderly females. Eur J Appl Physiol. 109(5):823-828.

Kirac D, Kasimay Cakir O, Avcilar T, Deyneli O, Kurtel H, Yazici D, Kaspar EC, Celik N, Guney AI. 2016. Effects of MC4R, *FTO*, and NMB gene variants to obesity, physical activity, and eating behavior phenotypes. IUBMB Life. 68(10):806-816.

Lappalainen T, Lindstrom J, Paananen J, Eriksson JG, Karhunen L, Tuomilehto J, Uusitupa M. 2012. Association of the fat mass and obesity-associated (*FTO*) gene variant (rs9939609) with dietary intake in the Finnish Diabetes Prevention Study. Br J Nutr. 108(10):1859-1865.

Lee B, Shao J. 2014. Adiponectin and energy homeostasis. Rev Endocr Metab Disord. 15(2):149-156.

Li H, Kilpeläinen TO, Liu C, Zhu J, Liu Y, Hu C, Yang Z, Zhang W, Bao W, Cha S et al. 2012. Association of genetic variation in *FTO* with risk of obesity and type 2 diabetes with data from 96,551 East and South Asians. Diabetologia. 55(4):981-995.

Loos RJF, Yeo GSH. 2014. The bigger picture of *FTO*—the first GWAS-identified obesity gene. Nat Rev Endocrinol. 10(1):51-61.

Mavri A, Poredos P, Suran D, Gaborit B, Juhan-Vague I, Poredos P. 2011. Effect of diet-induced weight loss on endothelial dysfunction: early improvement after the first week of dieting. Heart Vessels. 26(1):31-38.

Merra G, Gualtieri P, Cioccoloni G, Falco S, Bigioni G, Tarsitano MG, Capacci A, Piccioni A, Costacurta M, Franceschi F et al. 2020. *FTO* rs9939609 influence on adipose tissue localization in the Italian population. Eur Rev Med Pharmacol Sci. 24(6):3223-3235.

Merritt DC, Jamnik J, El-Sohemy A. 2018. *FTO* genotype, dietary protein intake, and body weight in a multiethnic population of young adults: a cross-sectional study. Genes Nutr. 13:4.

Milagro FI, Moreno-Aliaga MJ, Martínez JA. 2020. Nutrients, Obesity and Gene Expression. In Principles of Nutrigenetics and Nutrigenomics. Ed. Caterina RDE, Martinez JA, Kohlmeier M. Academic Press. p. 431-440.

Muc M, Padez C, Manco LJAJoHB. 2015. Influence of physical activity on the association between the *FTO* variant rs9939609 and adiposity in young adults. 27(5):734-738.

Nurnazahiah A, Lua PL, Shahril MR. 2016. Adiponectin, Leptin and Objectively Measured Physical Activity in Adults: A Narrative Review. Malays J Med Sci. 23(6):7-24.

OECD. Obesity Update- 2017 [Internet]. Organisation for Economic Cooperation and Development; [cited 2020 July 2]. Available from: http://www.oecd.org/health/obesity-update.htm.

Onat A. 2001. Risk factors and cardiovascular disease in Turkey. Atherosclerosis. 156(1):1-10.

Onat A, Can G. 2014. Enhanced proinflammatory state and autoimmune activation: a breakthrough to understanding chronic diseases. Curr Pharm Des. 20(4):575-584.

Ortega-Azorin C, Sorli JV, Asensio EM, Coltell O, Martinez-Gonzalez MA, Salas-Salvado J, Covas MI, Aros F, Lapetra J, Serra-Majem L et al. 2012. Associations of the *FTO* rs9939609 and the MC4R rs17782313 polymorphisms with type 2 diabetes are modulated by diet, being higher when adherence to the Mediterranean diet pattern is low. Cardiovasc Diabetol. 11:137.

Pekcan AG, Samur G, Dikmen D, Kızıl M, Rakıcıoğlu N, Yıldız EA, Özel HG, Mercanlıgil SM, Ersoy G, Karaağaoğlu N. et al. 2018. Population based study of obesity in Turkey: results of the Turkey Nutrition and Health Survey (TNHS)-2010. Prog. Nutr. 19(3):248-56.

Peltz G, Aguirre MT, Sanderson M, Fadden MK. 2010. The role of fat mass index in determining obesity. Am J Hum Biol. 22(5):639-647.

Peng S, Zhu Y, Xu F, Ren X, Li X, Lai M. 2011. *FTO* gene polymorphisms and obesity risk: a meta-analysis. BMC medicine. 9:71-71.

Phillips CM, Kesse-Guyot E, McManus R, Hercberg S, Lairon D, Planells R, Roche HM. 2012. High dietary saturated fat intake accentuates obesity risk associated with the fat mass and obesity-associated gene in adults. J Nutr. 142(5):824-831.

Pigeyre M, Yazdi FT, Kaur Y, Meyre D. 2016. Recent progress in genetics, epigenetics and metagenomics unveils the pathophysiology of human obesity. Clin Sci (Lond). 130(12):943-986.

Qi Q, Kilpeläinen TO, Downer MK, Tanaka T, Smith CE, Sluijs I, Sonestedt E, Chu AY, Renström F, Lin X et al. 2014. *FTO* genetic variants, dietary intake and body mass index: insights from 177,330 individuals. Hum Mol Genet. 23(25):6961-6972.

Richard AJ, White U, Elks CM, Stephens JM. Adipose Tissue: Physiology to Metabolic Dysfunction. 2020. Endotext; [cited 2020 July 2]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK555602/.

Saber-Ayad M, Manzoor S, Radwan H, Hammoudeh S, Wardeh R, Ashraf A, Jabbar H, Hamoudi R. 2019. The *FTO* Genetic Variants Are Associated With Dietary Intake and Body Mass Index Amongst Emirati Population. PLoS One. 17;14(10):e0223808. doi: 10.1371/journal.pone.0223808.

Saglam M, Arikan H, Savci S, Inal-Ince D, Bosnak-Guclu M, Karabulut E, Tokgozoglu L. 2010. International physical activity questionnaire: reliability and validity of the Turkish version. Percept Mot Skills. 111(1):278-284.

Saucedo R, Valencia J, Gutierrez C, Basurto L, Hernandez M, Puello E, Rico G, Vega G, Zarate A. 2017. Gene variants in the *FTO* gene are associated with adiponectin and TNF-alpha levels in gestational diabetes mellitus. Diabetol Metabol Syndr. 9:32-32.

Sirico F, Bianco A, D'Alicandro G, Castaldo C, Montagnani S, Spera R, Di Meglio F, Nurzynska D. 2018. Effects of Physical Exercise on Adiponectin, Leptin, and Inflammatory Markers in Childhood Obesity: Systematic Review and Meta-Analysis. Child Obes. 14(4):207-217.

Schlauch KA, Read RW, Lombardi VC, Elhanan G, Metcalf WJ, Slonim AD, Grzymski JJ. 2020. A Comprehensive Genome-Wide and Phenome-Wide Examination of BMI and Obesity in a Northern Nevadan Cohort. G3: Genes Genom Genet. 10(2):645-664.

Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orru M, Usala G et al. 2007. Genome-wide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. PLoS Genet. 3(7):e115.

Sonestedt E, Roos C, Gullberg B, Ericson U, Wirfalt E, Orho-Melander M. 2009. Fat and carbohydrate intake modify the association between genetic variation in the *FTO* genotype and obesity. Am J Clin Nutr. 90(5):1418-1425.

Sonmez A, Bayram F, Barcin C, Ozsan M, Kaya A, Gedik V. 2013. Waist circumference cutoff points to predict obesity, metabolic syndrome, and cardiovascular risk in Turkish adults. Int J Endocrinol. 2013:767202.

Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, Lango Allen H, Lindgren CM, Luan J, Magi R et al. 2010. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet. 42(11):937-948.

St-Pierre DH, Faraj M, Karelis AD, Conus F, Henry JF, St-Onge M, Tremblay-Lebeau A, Cianflone K, Rabasa-Lhoret R. 2006. Lifestyle behaviours and components of energy balance as independent predictors of ghrelin and adiponectin in young non-obese women. Diabetes Metab. 32(2):131-139.

Stojanovic S, Ilic MD, Ilic S, Petrovic D, Djukic S. 2015. The significance of adiponectin as a biomarker in metabolic syndrome and/or coronary artery disease. Vojnosanit Pregl. 72(9):779-784.

Vimaleswaran KS. 2017. Gene–nutrient interactions on metabolic diseases: Findings from the GeNuIne Collaboration. Nutr Bull. 42(1):80-86.

Vimaleswaran KS, Angquist L, Hansen RD, van der AD, Bouatia-Naji N, Holst C, Tjonneland A, Overvad K, Jakobsen MU, Boeing H et al. 2012. Association between

FTO variant and change in body weight and its interaction with dietary factors: the DiOGenes study. Obesity (Silver Spring). 20(8):1669-1674.

Vimaleswaran KS, Bodhini D, Lakshmipriya N, Ramya K, Anjana RM, Sudha V, Lovegrove JA, Kinra S, Mohan V, Radha V. 2016. Interaction between *FTO* gene variants and lifestyle factors on metabolic traits in an Asian Indian population. Nutr Metab (Lond). 13:39.

Wang D, Wu Z, Zhou J, Zhang X. 2020. Rs9939609 polymorphism of the fat mass and obesity-associated (*FTO*) gene and metabolic syndrome susceptibility in the Chinese population: a meta-analysis. Endocrine. doi: 10.1007/s12020-020-02280-x. Online ahead of print.

WHO. 2005. Preventing Chronic Diseases: a Vital Investment: Geneva, World Health Organization. [Internet]. World Health Organization; [cited 2020 July 1]. Available from: https://www.who.int/chp/chronic_disease_report/en/].

WHO. 2016. Turkish Healthy Nutrition and Active Life Programme 2010–2014 and related initiatives [Internet]. World Health Organisation; [cited 2020 July 2]. Available from: https://www.euro.who.int/en/countries/turkey/publications/turkish-healthy-nutrition-and-active-life-programme-20102014-and-related-initiatives-2016.

WHO. 2020. Body mass index-BMI. [Internet]. World Health Organisation; [cited 2020 July 2]. Available from: http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi].

WHO. 2018. National Household Health Survey – Prevalence of Noncommunicable Disease Risk Factors in Turkey 2017 2018. [Internet]. World Health Organisation; [cited 2020 July 1]. Available from: http://www.euro.who.int/en/countries/turkey/publications/national-household-health-survey-prevalence-of-noncommunicable-disease-risk-factors-in-turkey-2017-2018.

WHO. 2011. Waist Circumference and Waist-Hip Ratio. [Internet]. World Health Organisation Report of a WHO expert consultation, Geneva, 8-11 December 2008;[cited 2020 July 1]. Available from: https://www.who.int/nutrition/publications/obesity/WHO_report_waistcircumference_and_waisthip_ratio/en/.

Xiang Y, Zhou W, Duan X, Fan Z, Wang S, Liu S, Liu L, Wang F, Yu L, Zhou F et al. 2020. Metabolic Syndrome, and Particularly the Hypertriglyceridemic-Waist Phenotype, Increases Breast Cancer Risk, and Adiponectin Is a Potential Mechanism: A Case–Control Study in Chinese Women. Front Endocrinol. 10(905).

Zhang X, Qi Q, Zhang C, Smith SR, Hu FB, Sacks FM, Bray GA, Qi L. 2012. *FTO* genotype and 2-year change in body composition and fat distribution in response to weight-loss diets: the POUNDS LOST Trial. Diabetes. 61(11):3005-3011.

Figure Captions

Figure 1. Interactions of the *FTO* rs10163409 with tertiles of protein intake (g) on increased WC. WC, Waist Circumference. Black bars implicate the 'T' allele carriers (TA+TT). *FTO* SNP rs10163409 showed a significant interaction with protein intake (g) on the risk of increased WC ($P_{interaction}$= 0.044). Among those in the highest tertile of protein intake (mean ± SD: 138±38 g/day), the minor 'T' allele carriers of the SNP rs10163409 had a significantly higher risk of increased WC [OR= 3.3 (95% CI: 1.149-9.478), p = 0.027] than those carrying 'AA' genotype. *Odds ratio adjusted for age, gender, hypertension, cardiovascular diseases, total energy intake and obesity status

Figure 2. Interactions between *FTO* rs9939609 variant and physical activity on adiponectin levels. White bars indicate carriers of 'TT' genotype. Black bars implicate the risk allele, 'A', carriers (AT +AA). The regression model was adjusted for age, gender hypertension, cardiovascular diseases and obesity status. There was a significant interaction between the *FTO* SNP rs9939609 and physical activity on adiponectin levels ($P_{interaction}$= 0.027), where, among those with low physical activity levels, carriers of the 'A' allele had significantly lower adiponectin levels compared to those with 'TT' genotype (p=0.006).

Figure 3. Association between the genetic risk score of the *FTO* SNPs, rs9939609 and rs10163409s and anthropometric measures of obesity.

BMI, Body Mass Index; FMI, Fat Mass Index; WC, Waist Circumference. White bars: means of individuals with genetic risk score (GRS) of <2 risk alleles. Black bars: means of individuals with GRS of ≥2 or more risk alleles. The GRS was significantly associated with BMI (3a), FMI (3b) and WC (3c). Figure 3a; carriers of ≥2 or more risk alleles of the *FTO* variants (rs9939609 and rs10163409) had higher BMI (P=0.002) compared to individuals carrying <2 risk alleles. Figure 3b; carriers of ≥2 or more risk alleles of the *FTO* variants (rs9939609 and rs10163409) had higher FMI (P=0.003) compared to individuals carrying <2 risk alleles. Figure 3c; carriers of ≥2 or more risk alleles of the *FTO* variants (rs9939609 and rs10163409) had higher WC (P=0.020) compared to individuals carrying <2 risk alleles. P values were obtained from linear regression analysis and adjusted for age, gender, hypertension, cardiovascular diseases and obesity status.