A Nutrigenetic approach to investigate the effect of genetic and lifestyle factors on cardiometabolic-disease related traits in ethnically diverse populations.

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DECLARATION OF AUTHORSHIP

I confirm that this is my own work and the use of all materials from other sources has been properly and fully acknowledged.

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ABSTRACT

Cardiometabolic diseases such as cardiovascular diseases (CVD), obesity, hypertension and type 2 diabetes are a major cause of morbidity, mortality, and healthcare spending worldwide, especially in lower-middle-income countries. While cardiometabolic diseases are strongly affected by changes in environmental factors (such as unhealthy diet, sedentary lifestyle, and urbanization), they also have strong genetic determinants. Thus, understandings the role of gene–lifestyle interactions on cardiometabolic diseases and related traits can improve our understanding of disease pathophysiology and contribute to precision nutrition aiming to prevent and treat these diseases. Genome-wide association studies (GWAS) and candidate gene studies have revealed thousands of single nucleotide polymorphisms (SNPs) that have shown to be associated with cardiometabolic traits. However, these studies have been extensively performed in European populations, inadequately representing other ethnic groups. Genetic association studies of cardiometabolic diseases have great potential in terms of informing personalised and prevention medicine. This potential benefit, however, will only be understood by including populations of diverse ancestral backgrounds in these genetic studies. Hence, the main aims of this PhD work were to investigate the individual and joint effect of several SNPs on cardiometabolic disease-related traits in ethnically diverse populations. The interaction of these SNPs with lifestyle factors such as physical activity and dietary macronutrient intake on cardiometabolic disease-related traits was also assessed. This thesis included five different studies: three cross-sectional cohort studies [The Minangkabau Indonesia Study on Nutrition and Genetics (MINANG study; Indonesian women; n=110), The Genetics of Obesity and Nutrition in Ghana (GONG study; Ghanaian adults; n= 302) and The Obesity, Lifestyle and Diabetes in Brazil (BOLD study; Brazilian young adults; n= 200)] and two case-control studies [study in Turkish adults (n= 400) and Chennai Urban Rural Study (CURES; Asian Indian, n=1062)]. Statistical analysis was performed using Statistical Package for the Social Sciences

(SPSS) software (version 24; SPSS Inc., Chicago, IL, USA). We found significant gene-protein interactions on central obesity risk ($P_{\mathbf{interaction}}$=0.044) in the Turkish population, on triglyceride levels and waist circumference (WC) ($P_{\mathbf{interaction}}$=0.003 and 0.002, respectively) in the Indonesian population, and on fasting blood glucose and glycated haemoglobin ($P_{interaction}$=0.01 and 0.007, respectively) in the Indian population. Furthermore, there were GRS-fat intake interactions on WC in the Ghanaian population and on fasting insulin level ($P_{interaction}$=0.017), insulin-glucose ratio ($P_{interaction}$=0.010), homeostasis model assessment estimate of insulin secretion (HOMA-B) ($P_{interaction}$=0.002) and homeostasis model assessment estimate of insulin resistance (HOMA-IR) ($P_{interaction}$=0.051) in the Brazilian population. Also, a significant interaction between the fat mass and obesity-associated (*FTO*) SNP rs9939609 and physical activity on adiponectin concentrations was found in the Turkish population. In summary, the findings from this thesis contribute to the science of nutrigenetics by demonstrating the existence of genetic heterogeneity in gene-diet interactions on cardiometabolic disease-related traits across different ethnic groups. However, these findings need to be replicated using larger cohort and dietary intervention studies before they would be considered for personalised dietary recommendations, which are an innovative and promising approach for the prevention and treatment of cardiometabolic diseases.

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ABBREVIATIONS

MINANG	Minangkabau Indonesia Study on Nutrition and Genetics
CURES	Chennai Urban Rural Epidemiology Study
GONG	Genetics of obesity and nutrition in Ghana
BOLD	Obesity, Lifestyle and Diabetes in Brazil
T2D	Type 2 diabetes
CVD	Cardiovascular diseases
MAF	Minor allele frequency
HWE	Hardy Weinberg Equilibrium
SNP	Single nucleotide polymorphisms
GRS	Genetic risk score
FTO	Fat mass and obesity-associated
MC4R	Melanocortin 4 Receptor
TCF7L2	Transcription factor 7-like 2
ADIPOQ	Adiponectin
KCNQ1	Potassium voltage-gated channel subfamily Q member 1
CDKN2A/2B	Cyclin dependent kinase inhibitor 2A/2B
PPARG	Peroxisome proliferator-activated receptor gamma
CAPN10	Calpain 10
BMI,	Body mass index
WC	Waist circumference
FMI	Fat mass index
HOMA-IR	Homeostasis model assessment estimate of insulin resistance
HOMA-B	Homeostasis model assessment estimate of insulin secretion
HbA1C	Glycated haemoglobin
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol

SBP	Systolic blood pressure
DBP	Diastolic blood pressure
FPG	Fasting plasma glucose
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
MET	Metabolic equivalent of task
dpSNP	Single nucleotide polymorphism database
TEI	Total energy intake
WHO	World health organisation
GPAQ	Global physical activity questionnaire

Chapter 1 Introduction to the thesis

1.1 Introduction

A major epidemic of cardiometabolic diseases including diabetes and cardiovascular diseases (CVD) currently represents a significant public health issue worldwide. In 2016, the World Health Organization (WHO) estimated that approximately 1.6 million individuals died from diabetes and 17.9 million individuals died from CVD (1). Cardiometabolic risk refers to a condition of increased risk of developing CVD and diabetes. There are several risk factors, including insulin resistance (IR), obesity, dyslipidaemia and hypertension, playing major roles in the disease pathophysiology (2).

Evidence has shown strong associations of genetic factors with cardiometabolic diseases. Mapping genes associated with cardiometabolic diseases and related traits has been performed using two main approaches: candidate gene and genome-wide approaches (3). The substantial increase in the prevalence of these diseases in the urban society, characterised by unhealthy diet and sedentary behaviours, also indicates the significant contribution of our lifestyle and environment in disease risk. This highlights the importance of examining interactions between genetic and lifestyle factors (4).

The science of nutrigenetics investigates the effect of genetic factors on an individual's response to dietary interventions, with an ultimate aim of tailoring dietary recommendations based on the individual's genetic profile for preventing or treating cardiometabolic diseases (5).

This chapter will: (i) discuss the effect of genetic and environmental factors on cardiometabolic diseases; (ii) discuss the importance of investigating gene-lifestyle interactions and the implications of personalised nutrition in clinical practice.

1.2 An overview of cardiometabolic diseases

Cardiometabolic diseases including diabetes and CVD have been recognised as a global emergency (6, 7). Cardiometabolic diseases lead to medical impoverishment (8-11) and enforce economic burden especially on low- and middle-income countries (LMICs) (12). While cardiometabolic diseases remain most prevalent among wealthier groups, they are growing at faster rates among groups of poorer socioeconomic status (13). These diseases are strongly clustered amongst individuals of low socioeconomic status in high-income countries (HICs) (10, 11). Furthermore, the highest rates of death from cardiometabolic diseases are found in LMICs (11, 14). It is well-documented that the prevalence of cardiometabolic diseases and their main risk factors vary across populations. Over recent decades, the mortality rate caused by CVD has increased in LMICs (15). Furthermore, the prevalence of diabetes has increased globally but the rate was faster in LMICs (16). Moreover, in LMICs, mortality due to CVD usually occurs at an earlier age (15). The increasing prevalence of cardiometabolic diseases highlights the crucial need for major enhancements in both the timely diagnosis and management of these diseases (17).

1.2.1 Type 2 diabetes

Diabetes is defined as a chronic increase in the blood glucose concentrations caused by insufficient secretion of insulin from the pancreatic beta cells (termed “insulin deficiency”) or limited ability to respond to insulin (termed “IR”). In clinical settings, prediabetes and diabetes are diagnosed by measuring fasting glucose (an overnight fast) and oral glucose tolerance tests (OGTT). For OGTT, individuals are asked for 8-12 hours of fasting before the test. The glucose level is then assessed before and after orally administering a 75g of glucose load for 2 hours. Diabetes is defined by the WHO/IDF as 2h-glucose of ≥11.1 mmol/L or fasting glucose concentration of ≥7.0 mmol/L, impaired glucose tolerance as 2h-glucose of 7.8-11.1 mmol/L and fasting glucose of <7.0mmol/L, and impaired fasting glucose as 2h-glucose of <7.8 mmol/L and fasting plasma glucose of 6.1-6.9 mmol/L (18). It is commonly acknowledged that

the three types of diabetes are type 1 diabetes mellitus (T1D), type 2 diabetes mellitus (T2D) and gestational diabetes (GDM) (17)**.** In T1D, the immune system of the body attacks and destroys the pancreatic beta-cells that produce insulin, wherein T2D, beta-cells cannot produce enough insulin, or the cells of the body do not react to the actions of insulin. In GDM, the levels of blood glucose increase in some women during pregnancy, and their body ability to produce enough insulin is limited (17).

1.2.2 CVDs

CVDs refers to disorders that affect the heart or blood vessels including stroke, peripheral artery disease and coronary heart disease (CHD). The process of atherosclerosis plays a critical role in the development of CVDs (19). In this process, plaque (fatty deposits) accumulates inside the wall of arteries, hardening and narrowing these arteries. As a result, the flow of blood and supply of oxygen to vital organs is restricted, and the risk of developing blood clots which ultimately could block the blood flow to the brain or heart is increased (19). CVDs are the largest contributor to worldwide mortality, accounting for global death of 17.9 million every year (20). Also, CVDs are the main cause of morbidity and mortality in diabetic individuals. The long-term exposure of blood vessels to a high concentration of blood glucose over a long period can be damaging, because of the glycation, release of oxygen free radicals, inactivation of vascular cells proteins, and stimulation of apoptosis of the endothelial cell (21-23).

1.3 Cardiometabolic risk

Cardiometabolic risk refers to risk factors that increase an individual's likelihood of developing a CVD or diabetes. The American Diabetes Association was the first to implement the term "cardiometabolic risk" (24), acknowledging that limiting focus on the clustering of risk factors termed as metabolic syndrome (MetS) was not an ideal approach to determine individual risk for CVD and diabetes (25). Cardiometabolic risk is similar to MetS but with a

much broader meaning. As shown in Figure 1 (26), traditional risk factors for T2D and CVD include obesity (particularly central), IR, hypertension and dyslipidaemia, which can occur as a cluster or in isolation. Obesity can contribute directly to disease risk and indirectly through IR syndrome which can lead to dyslipidaemia, hypertension or hyperglycaemia, however, these abnormalities can also occur independent of IR (26). Lipid abnormalities, including elevated triglycerides, low high-density lipoprotein cholesterol (HDL-C), and increased low-density lipoprotein cholesterol (LDL-C), are also common findings in patients with cardiometabolic risk (2, 26). Family history, advancing age and lifestyle factors including physical inactivity, unhealthy diet and smoking have also shown to increase cardiometabolic risk. Other, newly emerged cardiometabolic risk factors include hypercoagulation and inflammation.

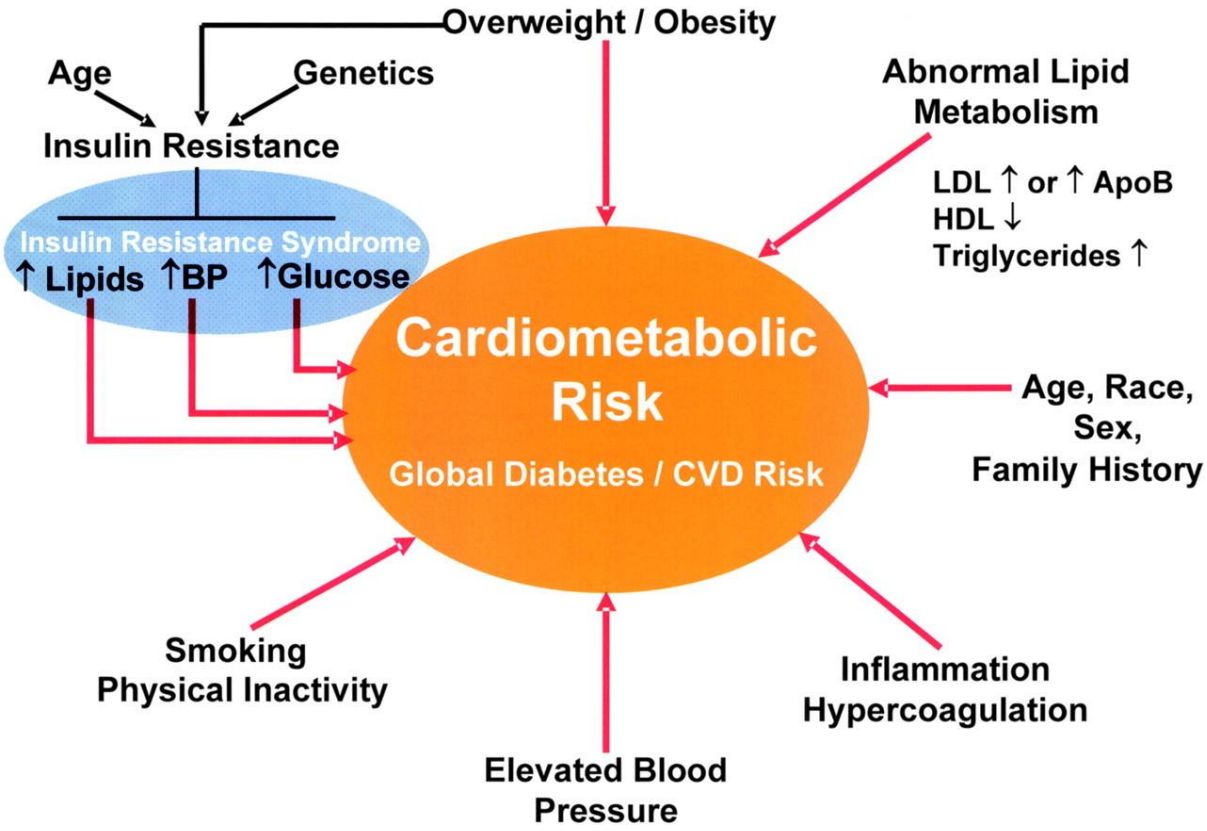

Figure 1: Factors that contribute to cardiometabolic risk of CVD and diabetes.

Abbreviations: BP, blood pressure; LDL, low-density lipoprotein; Apo B, apolipoprotein B; HDL, high-density lipoprotein. Ref. Kahn R. Metabolic syndrome: is it a syndrome? Does it matter? *Circulation. 2007;115(13):1806-10*.

1.3.1 Obesity

Obesity is a medical condition in which excess body fat (adipose tissue) accumulates in the body and may impair health. The management of obesity has been set as the top priority in relation to disease prevention in the WHO public health agenda (27). Fat distribution is more important in risk assessment than total fat where evidence suggests that visceral fat surrounding the intra-abdominal organs is more pathogenic than subcutaneous fat as it releases inflammatory agents, hormones and fatty acids and ultimately leading to higher blood pressure, blood glucose, triglycerides and LDL-C (28). Obesity can be assessed using several measurements including BMI, waist circumference (WC), waist: hip ratio (WHR) and skinfold thickness as well as technology-based methods such as dual-energy X-ray absorptiometry (DXA). BMI is the most frequently used adiposity measure in epidemiological studies and clinical practice as it is easy-to-perform and inexpensive (29). Table 1 shows the WHO classification of BMI for adults (30). The effect of obesogenic environments on disease risk differs across ethnic groups. For instance, diabetes has been shown to develop at a relatively low BMI among Asians (31). The increase of cardiometabolic diseases in LMICs has been closely linked to the increased prevalence of obesity. African data came from 321 population-based surveys observed that BMI rose from 21.9 to 24.9 and from 21.0 to 23.0 in African women and men, respectively, with a positive association being detected between the prevalence of diabetes and BMI (32).

Table 1: The WHO classification of obesity according to BMI.

WHO Classification	BMI	Population description
Underweight	<18.5	Thin
Normal range	18.5-24.9	'Healthy', 'normal', 'acceptable'
Overweight	25.0-29.9	Pre-obese
Obesity class I	30.0-34.9	Moderate obese
Obesity class II	35.0-39.9	Severe obese
Obesity class III	≥ 40.0	Very severe obese

Note: Asians have different criteria. For individuals with the same BMI, sex and age, Asians usually have a higher body fat percentage and a higher risk of developing negative health consequence in comparison with Caucasians (33). The WHO expert committee later recommended new cut-off points for Asians; 23 kg/m2 for overweight and 27.5 kg/m2 for obesity (34).

1.3.2 Central Obesity

Evidence suggests a strong correlation between central obesity, rather than general obesity which is defined by BMI, and the risk of CVD and metabolic diseases (35). A previous study found that centrally obese individuals with normal BMI had the worst long-term survival than their overweight and obese counterparts (36). Furthermore, a study including 42,702 European individuals showed a significant association of central obesity with increased mortality risk even in those of normal weight (35). The most accurate measure for central obesity is computed tomography (CT), quantifying subcutaneous and visceral fat. However, the use of CT is limited due to the increased risk of exposure to radiation and high cost (37). BMI is a common measure of general obesity; however, it might not accurately reflect the degree of abdominal adiposity. Epidemiological and clinical and evidence indicate that WC and WHR are strong indicators of abdominal obesity, providing a better prediction of CVDs risk than BMI (37). Studies have reported that obesity-related outcomes, such as CVDs, can be

predicted using WC (38-40). WC is used as the primary clinical method for assessing central obesity as it is easy to measure and strongly correlated with visceral fat (41). In the Asian population, the prevalence of severe abdominal obesity is higher than in the Caucasian population with identical BMIs, highlighting the need for different diagnostic criteria according to each ethnic group. In 2009, abdominal obesity in Asians was defined as a WC of ≥90 cm and ≥80 cm for men and women, respectively, according to the National Heart, Lung, and Blood Institute and the International Association for the Study of Obesity and the American Heart Association (42).

1.3.3 Insulin resistance

The term IR refers to the impairment in the insulin-mediated disposal of glucose from the bloodstream into body cells. IR is a complex metabolic disorder and several mechanisms have been suggested to cause IR, with inflammation induced by obesity playing a critical role (43). In the early stages of IR, the pancreas compensates for attenuation in insulin effects by secreting more insulin and maintain normal levels of glucose (44). Eventually, however, this compensation reaches a point that, regardless of how much insulin is secreted, glucose in the blood remains consistently above the prediabetic threshold, potentially leading to diabetes development (45). Adipose IR has been identified as a significant factor of cardiometabolic risk, increasing the release of free fatty acids (FFAs) due to increased lipolysis (46, 47). The increased flux of FFAs in the liver leads to high production of triglycerides and glucose as well as the release of very-low-density lipoprotein (VLDL). Associated abnormalities also include increased levels of LDL-C and decreased levels of HDL-C. Furthermore, FFAs decrease muscle insulin sensitivity and contribute to the enhanced pancreatic secretion of insulin, leading to hyperinsulinemia. Studies have shown that hyperinsulinemia (and probably increased levels of FFA) might cause increased activity of the sympathetic nervous system and sodium reabsorption, possibly contributing to hypertension development (46, 47)

Glucose is a major energy source for all body cells. The circulating blood glucose comes from three different sources including intestinal absorption of dietary glucose, glycogenolysis (breakdown of stored glycogen into glucose) and gluconeogenesis (glucose synthesis from precursors of noncarbohydrate) (48). Glucose homeostasis is mainly controlled by the liver and pancreas. The liver regulates several pathways related to utilising and endogenously producing glucose. The pancreatic beta cells secrete insulin in response to increased postprandial blood levels of glucose and amino acid. The disposal of glucose from the bloodstream into peripheral tissues is promoted when insulin binds to its receptors located on the membrane of many cells. Insulin is an anabolic hormone accelerating the synthesis of glycogen in the liver, adipose tissue, and muscle, as well as inhibiting the glucagon actions (48). The hormone glucagon is secreted by the pancreatic alpha cells to stimulate gluconeogenesis and glycogenolysis in the liver, ultimately increasing blood glucose level. Insulin also plays a role in regulating the metabolism of protein and lipids, for instance, insulin decrease lipolysis (fat breakdown) in adipose tissue, raise triglycerides uptake by muscle and adipose tissue and rise the synthesis of VLDL in the liver. The pancreatic beta cells also secrete amylin acting complementary to insulin (48). IR occurs when the concentration of glucose increases in blood. The pancreatic beta cells secrete more insulin and hyperinsulinemia is developed in order to maintain normoglycemia. Over time, cells become less sensitive to insulin action (insulin resistant) and less glucose is absorbed. Eventually, the pancreatic beta cells become unable to maintain the secretion of more insulin to compensate IR, leading to higher levels of blood glucose.

1.3.4 Dyslipidaemia

Plasma lipids include total cholesterol, triglycerides, cholesterol esters and phospholipids. Cholesterol is predominantly transported and stored in the body in the form of cholesteryl esters (i.e.: cholesterol hydroxyl group linked to long-chain fatty acids by

ester bond). Triglycerides and cholesteryl esters bind to a different type of apolipoproteins, forming complex capsules of lipid and protein named lipoproteins that can freely move across blood and tissue fluid. Lipoproteins include very low-density lipoproteins (VLDLs), HDL-C, LDL-C (49). Dyslipidaemia is a disorder of lipoprotein metabolism characterised by raised blood levels of total cholesterol, LDL-C and/or triglycerides, and reduced HDL-C levels, increasing the risk of atherosclerosis (50). Dyslipidaemia plays a critical role in the development of atherosclerosis and it is a significant risk factor for CVD (51). A large body of evidence has shown a strong association between high LDL-C level and atherosclerosis (50), while HDL particles have been consistently established as cardioprotective in epidemiological studies (52). Although the mechanism by which triglycerides affect cardiovascular health is not completely clear, it is commonly agreed that a high blood triglyceride concentration leads to enrichment in HDL and LDL particles via the process of neutral lipid exchange (53). Triglyceride-enriching HDL and LDL particles undergo hydrolysis by hepatic lipase to produce small dense HDL and LDL particles, which are associated with functional loss and higher atherogenic potential, respectively (53).

1.4 Genetic factors

The estimated heritability (proportion of inter-individual variation attributable to genetic factors) for T2D is 30%–70% (54), for obesity is 40–70% (55), for plasma cholesterol and triglycerides are 56%-77% (56), and for blood pressure is 30%-60% (57).

1.4.1 Genetic association studies

Genetic association studies are performed to discover genome regions or candidate genes that contribute to the development of a specific disease by assessing for a correlation between genetic variation and disease status (58). The most common type of genetic variation among individuals is the single nucleotide polymorphism (SNP), which is defined as a variation at a

single position (nucleotide) in a DNA sequence (59). SNPs might locate within genes' coding or non-coding regions, or between genes (in the intergenic regions) (59). SNPs are the most examined markers in association studies. A higher frequency of a genotype or allele in a group of people affected with a specific disease refers to the fact that the examined variant increases the risk of that disease. The simplest and commonly used design in genetic association studies is the case-control study design, in which a group of individuals with the disease of interest (cases) are compared with control individuals free of the disease (58). The significant genetic association might be interpreted in three ways (1) direct association, where the investigated variant is the true causal SNP conferring the susceptibility of a disease of interest; (2) indirect association, where an association is tested with a SNP that is in linkage disequilibrium (LD) with the true causal SNP; or (3) a false-positive finding due to either systematic confounding, including population stratification, or chance (58).

1.4.2 Gene discovery methods

Several gene discovery methods have been used for mapping causal genes for cardiometabolic diseases and related traits including candidate gene and genome-wide approaches (3).

1.4.2.1 The candidate gene approach

A candidate gene study is a hypothesis-driven approach investigating the association between a pre-specified variant within or near a candidate gene and a phenotype of interest. Candidate genes are identified based on previous knowledge suggesting the involvement of these genes in the disease biology and pathophysiology (a biological candidate), or because of its location in a chromosomal region that has been associated with the disease (positional candidate) (60). The first important stage in performing candidate gene studies is the selection of an appropriate candidate gene that might plausibly have a relevant role in the disease or

process under investigation (61). Once a candidate gene is selected, investigators determine the most useful genetic variants for examination based on previous knowledge of existing polymorphisms and decide which of those SNPs lead to proteins functionally altered that may impact the trait of interest (61). Once a candidate gene/ SNP is chosen, the role of the selected gene is commonly tested in a sample of individuals with the disease (i.e., cases) and those without the disease (i.e., controls) (62). The first update of the Human Obesity Gene Map in 2005 listed 127 genes as candidate genes of obesity traits (61). Also, an extensive search of the literature identified 547 candidate genes for obesity-susceptibility (63). Furthermore, more than 60 candidate genes for T2D have been examined in several populations (61, 64). A previous study identified 286 T2D candidate genes associated simultaneously with insulin signaling and mitochondrial genes and thus may possibly act as a molecular bridge between both systems (65). Large-scale candidate gene association studies have reported robust association of genes such as melanocortin 4 receptor (*MC4R*) with obesity and related traits. The most prominent discoveries of candidate association studies are the peroxisome proliferator-activated receptor gamma (*PPARG*) and potassium voltage-gated channel subfamily J member 11 (*KCNJ11),* both of which were associated with T2D (66-69).

1.4.2.2 The genome-wide approaches

Genome-wide scans include genome-wide linkage and genome-wide association studies (GWAS), and both are hypothesis-generating aiming to expand our knowledge in relation to disease pathophysiology. In these studies, the entire genome is scanned to identify associations of novel, unanticipated genetic variants with a disease or trait of interest (70, 71). The linkage studies are based on only populations of related participants, investigating whether certain chromosomal regions co-segregate with disease-related phenotypes across generations (70). The cysteine protease calpain 10 (*CAPN10)* gene was the first type 2 diabetes-associated gene discovered through linkage analysis (72), however, the replication of the observed finding

in subsequent studies was limited. A robust linkage signal of chromosome 10p with T2D was shown in Icelanders and Mexican Americans (73). To find the causal region, data on microsatellite genotype was used to perform analysis of single-marker association and discovered transcription factor7-like 2 (*TCF7L2*) to be significantly associated with T2D (74). The association of the *TCF7L2* rs7903146 with T2D (relative risk of ~1.4) has been consistently replicated in several populations including European, African and Asian (75). Unlike linkage studies, GWAS can be performed using family members, as well as unrelated individuals using larger sample sizes which have been shown to improve statistical power. This approach screens the whole genome at higher resolution levels than genome-wide linkage studies, and hence it is able to narrow down the associated locus more accurately (71).

GWASs have been hugely involved in the detection of tens of thousands of SNPs associated with cardiometabolic diseases and their related traits. The Fat Mass and Obesity-associated gene (*FTO*) was the first and strongest GWAS-identified gene associated with obesity (76, 77). A large GWAS-metanalysis consisting of more than 339,000 European individuals had discovered 97 BMI-associated loci (78). Expression enrichment of genes located near these loci was found in the central nervous system (CNS), suggesting the involvement of hypothalamic control of energy intake in regulating BMI (78). Another large-scale meta-analysis of GWAS of 224,459 individuals of African-American, south and east Asian and European ancestry focusing on body fat distribution identified 49 loci for waist-to-hip ratio adjusted for BMI (79). Genes located near these loci presented expression enrichment in adipose tissue, suggesting that the distribution of fat is mainly controlled in local fat depots (79). A previous review summarised the GWAS-identified loci for obesity-related traits at the genome-wide significance level ($p<5 \times 10^{-8}$) and grouped these loci into seven categories: body fat related (15 loci); BMI (141 loci); birthweight (8 loci); extreme obesity (23 loci); WHR or WC (26 loci); WHR or WC adjusted for BMI (97 loci) and visceral adiposity (2 loci) (80).

The first T2D-GWAS discovered four novel loci associated with T2D in a case-control study in French individuals (n=1,363) (81). Also, three following GWASs observed similar results (82-84). A second wave of GWAS led to the discovery of over 100 loci associated with quantitative glycemic traits, insulin metabolism or diabetes (85-88). To date, the largest GWAS-meta-analysis on T2D (comprising ~74,000 cases and ~824,000 controls from 32 European cohorts) identified 243 loci reaching genome-wide significance (89).

In 2008, the European Network for Genetic and Genomic Epidemiology (ENGAGE) consortium performed the first comprehensive GWAS on lipids (n=17,797 - 22,562 individuals), identifying 22 loci associated with total cholesterol, triglyceride, LDL-C, and HDL-C levels (90). In 2009, a GWAS including 19,840 European individuals and replication in up to European 20,623 individuals identified 30 loci to be associated with lipoprotein levels, 11 of which were novel (91). The Global Lipids Genetics Consortium (GLGC) carried out two large GWASs (n> 100,000 and 188,578 individuals, respectively), reporting 95 loci and 157 loci with independent effect on blood lipids, respectively (92, 93). Recently, a large GWAS based on electronic health records (n= 94,674 individuals) discovered 121 novel lipid-associated SNPs in an ancestrally diverse population (Latino, East and South Asian, Hispanic White and African American) (94).

1.4.3 Cardiometabolic-disease related genes investigated in this thesis

The *TCF7L2* gene is located on chromosome 10q25.2–q25.3, encoding for high mobility group (HMG) box-containing transcription factor which has a major role in the Wnt signaling pathway. The protein has been shown to be involved in the homeostasis of blood glucose (95). The *TCF7L2* expression showed positive associations with insulin gene expression in human pancreatic islets (96). The *TCF7L2* is critical for several processes including development of the pancreas, determination of the beta-cell mass, maintenance of

the beta-cell secretory function, and regulation of the production and processing of insulin (96, 97). Studies have confirmed the associations of *TCF7L2* genetic variants with the risk of T2D among various populations (98-101). A meta-analysis of data from 155 studies with 121,174 participants found significant associations of *TCF7L2* SNPs, rs7903146 [odds ratios (ORs) (95% confidence interval) of 1.39 (1.34–1.45)] and rs12255372 [1.33 (1.27–1.40)] with T2D (102).

The *FTO* gene is located on chromosome 16 and encodes for a 2-oxoglutarate (2-OG) Fe (II)-dependent AlkB family dioxygenase. The association of the *FTO* SNP rs9939609 with increased BMI (effect per allele=0.30-0.39 kg/m^2) has been consistently reported in several genetic association studies (103-106). Studies reported that individuals with the risk allele 'A' of the *FTO* SNP rs9939609 had a high percentage of body fat (107, 108). Although the function and mechanism of the *FTO* gene is not completely known, possible functional alterations caused by the *FTO* gene have been found in the hypothalamic–pituitary–adrenal axis and the reward system in the brain (109, 110)

The *PPARG* is a protein-coding gene located on chromosome 3p25.2, encoding for the PPAR subfamily of nuclear receptors. The *PPARG* gene is recognised as a candidate gene for T2D and CVDs, and evidence shows that the genetic variants of *PPARG* play a key role in controlling the metabolism of glucose and lipid (111-113), with the SNP rs1801282 (also known as Pro12Ala) being extensively examined in epidemiologic studies. The SNP rs1801282 is a missense variant leading to a change of amino acid from Proline (P) to Alanine (A). A study including Finnish and Japanese-American individuals reported that the Pro/Pro genotype was associated with a 4.35-times higher T2D risk compared with Ala/Ala genotype (114). Also, a meta-analysis of 16 studies showed the association of the proline allele of the Pro12Ala SNP with T2D (66). Furthermore, evidence suggested significant associations of the *PPARG* SNPs and CVDs (115, 116).

The *MC4R* gene is a protein-coding gene localised on chromosome 18q22. The protein encoded by this gene is a membrane-bound receptor and member of the melanocortin receptor family. *MC4R* have a major role in regulating energy homeostasis and food intake (117). Genetic variants in the coding region of *MC4R* have been shown to be associated with common and severe types of human obesity (118). Furthermore, polymorphisms in the non-coding region were found to be associated with susceptibility to polygenic obesity. Meta-analyses of GWAS performed in Caucasians (80,957 cases and 220,223 controls) showed a strong association of the *MC4R* SNP rs17782313 (C/T) with higher BMI (119). This variant was also associated with early-onset severe obesity (120, 121), and findings were replicated in various populations including adults, adolescents, and children (119, 122). The SNP rs17782313 also shown to be associated with T2D in numerous studies (123-125).

The potassium voltage-gated channel, KQT-like sub-family, member 1 (*KCNQ1*) gene is located on chromosome 11p15.5 and encodes the pore-forming α-subunit of the voltage-gated K^+ channel, playing a major role in controlling the process of ventricular repolarization (126). Although KCNQ1 is largely expressed in the cardiac cells or tissues, it is also expressed in other organs or tissues such as the pancreas (127). A meta-analysis including 114,140 T2D cases and 167,322 controls with different ethnicities suggested that *KCNQ1* SNPs, rs231362, rs2237892, rs2237897, rs2237895 and rs2283228, were associated with increased risk of T2D (128). Another recent meta-analysis also reported that genetic variants of the *KCNQ1* gene (rs2237892, rs2283228, rs2237895, rs151290, and rs2074196) may be a susceptible factor for T2D, especially in Asians (129).

The cyclin-dependent kinase inhibitor genes 2A/2B (*CDKN2A/B*) are tumour suppressor genes located at chromosome 9p21, and they are highly expressed in the pituitary, pancreas, and adipose tissues (130). *CDKN2A* and *CDKN2B* encode $p16^{INK4a}$ and $p15^{INK4b}$, that act as inhibitors for cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 5

(CDK5), respectively. Both CDK4 and CDK5 have a critical role in beta-cell regeneration and function (130). Previous studies reported the *CDKN2A/B* SNPs, rs10811661 and rs564398, as T2D risk factors (83, 131, 132). SNP rs10811661 also confers risk for T2D in individuals of Framingham Offspring Study (133). A meta-analysis consisting of 24,407 T2D cases and 33,937 controls confirmed the association of the *CDKN2A/B* SNP rs10811661 with the risk of T2D (134).

The adiponectin gene (*ADIPOQ*) is located on chromosome 3q27 and encodes for the protein adiponectin that regulates the metabolism of lipid and glucose as well as insulin sensitivity (135-137). Adiponectin is adipokine that has been associated with obesity and MetS (138). The concentrations of adiponectin have been shown to be influenced by *ADIPOQ* SNPs (139, 140). Several studies have demonstrated the association of genetic variants in *ADIPOQ* with T2D (141) and CVDs (142-144). The three most investigated genetic variants of *ADIPOQ* were rs1501299, rs2241766 and rs266729. Significant association of the *ADIPOQ* SNPs rs2241766 and rs266729 with increased risk of CVD was reported in a meta-analysis including 19,106 cases and 31,629 controls from ethnically diverse populations (145). Furthermore, evidence from the Finnish diabetes prevention study (n=507 individuals) suggested the contribution of the *ADIPOQ* SNPs to variation in serum adiponectin levels and body size (146).

The gene encoding *CAPN10* is a ubiquitously expressed member of the calpain cysteine protease family. *CAPN10* is expressed in several tissues, particularly those involved in the glucose homeostasis regulation, such as liver, pancreatic beta-cell, adipocytes and skeletal muscle (147, 148). *CAPN10* is located on chromosome 2q37.3, and comprises 15 exons spanning 31 kb, and encodes a 672 amino-acid intracellular protease. Significant associations of the *CAPN10* SNPs with IR and T2D have been demonstrated (147, 149-151). These include the extensively studied SNPs: SNP-43 G/A variant, SNP-19 2R (two 32-bp repeats)/3R (three 32-bp repeats), and SNP-63 C/T variant (148, 151).

1.4.4 Single SNP vs. genetic risk score (GRS)

GWASs have discovered thousands of genetic variants associated with cardiometabolic diseases, however, the individual SNPs explain only a small proportion of variation in the complex traits, with limited ability for predicting disease risk (152). Given that cardiometabolic diseases and their related traits are influenced by several genetic variants, with each having a small effect on these traits, combining the effect of several variants as a polygenic score can provide a better understanding of disease risk than single variant approaches (153). The idea of grouping individual SNPs into GRSs has been used to predict and quantify a discrete increment in the overall risk of cardiometabolic diseases, as well as capturing the overall variance in a trait (153). There are several approaches for generating a GRS such as weighted and unweighted methods. Fundamentally, a GRS is constructed by summarising genotype data across multiple genetic variants (154). The most commonly used method is summing the number of alleles that confer risk across all loci (0, 1, or 2) (154). Employing the GRS approach for predicting disease risk has advantages over analysing the effect of individuals SNPs as it decreases the drawback of multiple testing, maximises statistical power, and widens the scope of generalisability of genetic associations (155, 156). Previous studies have emphasised the potential of GRS for predicting the risk of cardiometabolic diseases (157-160). A 28-SNP GRS reported a significant association with high BMI in a Gambia population, whereas no association was detected with the individual SNP analysis (161, 162). A case-control study of 5,148 Indians reported a significant association between an 8-SNP GRS and T2D (159). Another case-control study of 3,357 Indian adults also observed that individuals with a higher 32-SNP GRS were at a higher risk of T2D in comparison to those with lower GRS (160).

1.5 Environmental factors

Epidemiological studies have underlined numerous potential environmental factors associated with the development of cardiometabolic diseases, with the combination of excess calorie intake and physical inactivity being major contributors. However, the effect of several other possible environmental factors has been found, including smoking, alcohol, endocrine disruptors, and sleep deprivation (4, 163-165). It has been shown that obesogenic environments play a significant role in the epidemic of obesity and cardiometabolic diseases in Norwegian individuals (n=118959) who were followed up for 45 years, suggesting that the environment can primarily determine the individuals' metabolic load (166). Evidence from two longitudinal dietary trials (2.8 and 3.2 years, respectively) recruiting ethnically diverse individuals (n= 3234 and 522, respectively) have found that reducing fat consumption is effective in decreasing the incidence of T2D by up to 58% (167, 168). Additionally, the recent epidemiological transition experienced in LMICs has significantly contributed to the increasing burden of cardiometabolic diseases (169). Growing urbanization, economic development, and liberalization of trade in LMICs have led to significant changes in the availability, promotion, composition, affordability and accessibility of foods (170). Importantly, there was evolvement in food prices in LMICs in recent years. The prices for packaged and ready-to-eat food products have been decreased by large-scale retails, making buying foods high in trans and saturated fatty acids, sodium and sugars, such as salty snacks or sugary sodas, cheaper than buying healthy foods such as dairy products, vegetables and fruits, although this association might differ based on the country and its economy (171). As a result, these food products have become available to, and promoted to, the groups of those belonging to lower socioeconomic status. The intake of ultra-processed foods is critically involved in the epidemic of cardiometabolic diseases that impose the biggest economic and health burden in LMICs (172). Thus, elements of the obesogenic environment generated by the epidemiological, urban, and nutritional transitions in LMICs should be targeted for preventing and treating cardiometabolic diseases (173).

1.5.1 Dietary intake

There are a variety of modifiable risk factors for cardiometabolic diseases, including dietary intake and physical activity. Dietary intake can be assessed using several methods including food consumption record, 24-hour dietary recall, dietary record, dietary history and food frequency questionnaire and duplicate diet approach, **(174)**. Diet is a leading risk factor to morbidity and mortality globally and the influence of dietary intake on cardiometabolic traits such as BMI, blood lipids, glycaemia, and glucose-insulin homeostasis is relevant to the management and prevention of cardiometabolic diseases (175). Individual food is composed of a complex matrix of nutrients such as protein, carbohydrate quality, fatty acids and micronutrients that together influence cardiometabolic risk (176).

Although the high intake of protein has been one of the most popular weight-loss strategies for overweight and obesity management (177-179), studies reported inconsistent health effects of high-protein diets on T2D. High animal protein consumption, but not plant protein, was associated with increased risk of T2D in 38,094 European individuals (180), and in 37,309 women from the United States (US) (181). A longitudinal study (n=27,140 Swedish individuals) reported that the consumptions in the highest quintiles of eggs and processed meat were associated with higher T2D risk (182).

The Prospective Urban Rural Epidemiology study (n=135 335 individuals) reported that a higher intake of carbohydrate (>60 % of total energy intake (TEI)) was associated with higher all-cause and cardiovascular mortality (183). However, recent meta-analyses investigating the association between the intake of carbohydrate and cardiovascular health have shown a U-shaped relationship of carbohydrate intake with all-cause mortality, particularly in individuals consuming a diet low in carbohydrate (<40% of TEI) but higher in fat and animal

protein (184, 185). A previous systematic review and meta-analysis of 22 cohort studies observed that the higher intake of dietary fibre was associated with a lower risk of CVDs (186).

The right balance of dietary fat is a major determinant of cardiovascular health. Studies have also long been suggesting that the intake of trans fat and saturated fatty acids (SFA) has harmful effects on cardiovascular health (187, 188). A recent meta-analysis of randomised control trials (RCTs), however, reported that decreasing SFA intake did not significantly affect CVD mortality or total mortality, although it did significantly reduce combined cardiovascular events by 17% (189). Furthermore, greater reductions in CVD events were reported in studies that replaced SFA with polyunsaturated fatty acids (PUFA) when compared to replacement with carbohydrate, monounsaturated fatty acids (MUFA) or protein (189). Furthermore, in a meta-analysis of randomised controlled feeding trials, PUFA intake (in place of SFA, MUFA or carbohydrate) has shown the most consistent favourable effects in relation to improved glycaemia, insulin resistance, and insulin secretion capacity (190). Another meta-analysis including 102 350 participants of European descent reported that the increase of PUFA intake in place of refined starch and sugars is associated with a lower T2D risk, whereas the increase of MUFA intake in place of carbohydrate is associated with a higher T2D risk (191). Therefore, decreasing the intake of SFA and replacing it with unsaturated fat seems to convey the highest cardiovascular benefit and lower the risk of T2D, rather than reducing SFA and replacing it with refined carbohydrates. The sources of SFA (dairy vs animal) can also modify these associations (188). A recent systematic review of RCTs (n=656 individuals) concluded that the intake of SFA from dairy product seems to have a protective impact on some cardiometabolic risk factors (decrease WC and total cholesterol and increase HDL-C) compared to other sources of SFA (188).

1.5.2 Physical activity

Physical inactivity is a key modifiable risk factor for cardiometabolic diseases and related traits. Common clinical approaches of assessing physical activity include questionnaires, heart rate monitoring, pedometers and accelerometers (192). Being physically inactive contributes significantly to obesity, high levels of cholesterol, blood glucose and blood pressure, all of which increase the risk of cardiometabolic diseases (34, 193-195). Studies have demonstrated that physical activity has a wide range of benefits, including decreasing mortality risk, preventing diabetes and CVD, lowering hypertension, improving levels of blood lipids and enhancing functional status (196-199). In both observational and interventional studies, physical activity has been reported to decrease the risk of T2D by 30-60 % (200-202). A systematic review including 310,588 individuals showed that the relative risk of T2D was 31 % lower in individuals with regular physical activity in comparison with those with a sedentary lifestyle (203).

1.6 Nutrigenetic approach

Nutrigenomics and nutrigenetics have a lot of potential for improving dietary guidelines for the general population and individuals. Nutrigenomics explores the effect of certain nutrients on gene expression and, consequently, the proteome (the entire collection of proteins expressed by an organism) and the metabolome (the total number of low molecular weight metabolites) using new high-throughput tools such as "omics" technologies (204). Nutrigenetics is the science that examines the effect of DNA sequence variation in response to diet in relation to individuals' health and disease risk (205). The field of nutrigenetics has undergone rapid progression, generating evidence of gene-nutrient interactions that ultimately can be used to personalise dietary guidelines to prevent or treat disease (205).

1.6.1 Genetic variations and the role of ethnicity in cardiometabolic risk

The human genome is 99.9% identical with only 0.1% variations explaining individual

differences in health and disease states (206). Advances in the field of GWAS have led to the discovery of thousands of genetic variants (such as SNPs located in the *TCF7L2, PPARG* and *FTO* genes) associated with cardiometabolic diseases (3, 63, 207-210). The frequencies of genetic variants vary from one population to another, leading to a difference in the prevalence of diseases across multiple ethnic groups (211). For example, genetic variants in the *TCF7L2* gene, which is one of the strongest T2D associated genes, have shown varied frequency across different ethnicities. While the 'T' allele of the *TCF7L2* SNP rs7903146 has a frequency ranging from 0.180 - 0.462 in Europeans, 0.214 - 0.500 in Africans, and 0.274 - 0.483 in the Middle Eastern population, the allele is almost absent in Native American and Southeast Asian populations (<5%) (212). Therefore, it is important to study the influence of genetic factors on cardiometabolic risk across various ethnicities.

1.6.2 Rationale for investigating gene-diet interactions

The genetic contribution to cardiometabolic diseases has been extensively examined in GWAS. The "common disease/common variant" hypothesis argues that if a heritable disease is common in the population, then the frequency of the genetic contributors in the population will also be high/common (213). So, common genetic components of high frequency have been suggested to explain the heritability of common diseases. However, after discovering thousands of genetic variants that have shown associations with common diseases, the combined effect of these variants only accounted for a small proportion of disease heritability (214). For example, the estimated heritability for BMI was 40–70%. However, the discovered genetic variants only accounted for a small proportion of the actual heritability for BMI, proposing so-described 'missing heritability' (215). Disease risk prediction is complicated by gene-diet interactions which are ubiquitous and might partly explain the missing heritability of diseases (216). Several studies have found that genetic variants can increase the risk of diseases when an individual with a high genetic predisposition is exposed to high-risk lifestyle factors

(217). Thus, identifying the role of gene–lifestyle interactions on cardiometabolic diseases and related traits can improve our understanding of disease pathophysiology and contribute to precision nutrition aiming to prevent and treat these diseases by implementing dietary modification based on an individual's genetic makeup (5).

1.6.3 Importance of investigating gene-diet interactions in ethnically diverse populations

GWASs of cardiometabolic diseases have been extensively performed in European populations, inadequately representing other ethnic groups (218). Given that the prevalence of cardiometabolic diseases varies across worldwide populations, it is necessary to investigate ethnically diverse populations to improve our understanding of the genetic architecture of these diseases (219). In fact, the under-representation of ancestrally diverse individuals in genomic research can hamper the ability of translating genetic research into clinical settings, as well as exacerbating health inequalities in the current policy of public health (219). Genetic studies of cardiometabolic diseases have great potential in terms of informing personalised and prevention medicine. This potential benefit, however, will only be understood by including populations of diverse ancestral backgrounds in these genomic studies (219). Given that the risk of cardiometabolic diseases can also be influenced by lifestyle factors such as dietary intake, it is also necessary to study gene-diet interactions in various populations, so that it will be eventually possible to personalise dietary recommendation based on ethnicity (220, 221). It is important to note that an individual's response to dietary interventions differs across populations (222). Thus, taking into consideration of ethnicity can improve predicting an individual's response to dietary interventions.

1.6.4 Study designs and their role in identifying gene-diet interactions

There are several study designs that potentially can be used for investigating gene-lifestyle interactions including observational study design (such as cross-sectional and case-control studies) and experimental study design (such as RCTs) (223). The cross-sectional design is one of the most used designs, determining the association of exposure with certain outcomes of interest. The case-control design is another illustration of observational studies. In this design individuals who have certain disease or outcome (cases) are compared to matching individuals (controls) who don't have this disease or outcome (58). Observational studies can be performed to generate a hypothesis using large sample sizes as they are relatively inexpensive, easy to use and quick. However, the observational design cannot explain causal relationship as associations are examined at a single point in time (223). Also, the residual effect of confounding factors cannot be eliminated in these studies, highlighting the need for adjusting for confounding in the statistical model (223). However, the cross-sectional design has been commonly used in GWAS, identifying thousands of genetic variants associated with disease risk, and these associations are less likely to be influenced by confounding factors (224). With respect to nutrigenetic research, observational studies can be influenced by inherent bias when investigating interactions between genes and lifestyle factors, as phenotypes can vary significantly over time. For instance, the triglyceride concentrations vary over time, therefore, relying on only fasting triglyceride values taken at a single time point might be a limitation (225). Observational studies have also limitations by difficulties in replicating the initial findings. Dietary intake in observational studies is commonly assessed using a food frequency questionnaire (FFQ) as it is easy to use and can represent individuals' intake over a long period with less burden. However, this method is self-reported and relies on memory, introducing recall bias (226).

Regarding the experimental design (RCTs), individuals are randomly assigned into two groups: the 'intervention group' where they receive a certain treatment and the 'control group'

where they receive a placebo, control or standard treatment, to be assessed for intervention-related changes in certain biomarkers. The main advantage of this design is the possibility that both researcher and participants can be blinded regarding the type of treatment received (223). However, given the high cost of running RCTs, high rate of dropouts and less adherence to received treatments, these trials are usually limited by having a small number of individuals (226). Alternatively, the 'cross over' design is used in experimental studies, where half of the study participants receive the treatment, and the other half receive the placebo for a certain period. Then, both groups undergo a wash-out period to ensure the clearance of the intervention effect from the body. After that, switching of groups between treatment and placebo takes place for another certain period (223). The main advantages of this design are the verification of the study findings among two phases and minimising confounding, which ultimately strengthen these findings. However, the cross-over design has limitations including the small sample size and the requirement for a washout period, which might decrease the power of observing effect sizes for gene-diet interactions and higher participant attrition (224). Another type of experimental studies is the postprandial study design which investigates the effect of a meal or meals given at different time intervals on outcome measures, such as lipaemic response, as well as determining the acute effect of a certain type of fatty acid consumed within the first meal on the postprandial lipaemic response of the second meal (in a two-meal acute study). The majority of the population spends most of the day in a postprandial state and postprandial triglyceride have been identified as an independent risk marker for cardiovascular disease. Changes in lipid profile in the postprandial state emphasise the importance of this design in examining interactions between gene and lifestyle factors (227).

1.6.5 GeNuIne Collaboration

Gene–lifestyle interactions studies have been extensively performed in European populations, suggesting that dietary intake and physical activity could modify the association

of genetic variants with cardiometabolic disease and related traits (228-231). Previous studies reported significant interactions between the risk allele 'T' of the TCF7L2 SNP rs7903146 and fibre intake on T2D risk, where the 'T' allele was associated with a higher risk of T2D and higher HbA1c concentrations among individuals with higher fibre intake (232, 233). However, findings from other nutrigenetic studies were discordant (234-236). Furthermore, high levels of physical activity attenuated the effect of SNPs, such as *FTO* SNPs, on cardiometabolic traits among various populations (229, 237, 238); however, other studies have reported conflicting findings (231, 239-241). This may be due to genetic heterogeneity as well as differences in dietary factors. Genetic make-up differs across ethnicities, emphasising the importance of assessing gene-diet interactions among multiple ethnic groups, which will ultimately allow us to personalise dietary plans based on each population. To address all these challenges, the **Ge**ne–**Nu**trient **In**t**e**ractions (**GeNuIne**) Collaboration (220) has been established to examine the complex interplay between genetic and dietary factors on cardiometabolic disease and related traits using population-based studies in LMICs such as Turkey, India, Indonesia, Brazil, Sri Lanka, Morocco, Thailand and Pakistan.

1.6.6 From Nutrigenetics to Personalised nutrition

Cardiometabolic diseases are major public health problems. Several factors are involved in the pathophysiology of these diseases such as physical inactivity and unhealthy diet. The role of dietary factors is well-known in preventing or treating cardiometabolic disease (4). However, genetic variants also play a significant role in population variation in the risk of cardiometabolic diseases, highlighting the urgent need for the science of nutrigenetics which suggests that elucidating undefined interactions between gene-lifestyle would support the application of personalised nutrition (5). Indeed, personalising dietary recommendations according to genetic makeup would improve an individual's response to nutrition intervention and offers a new dietary strategy for improving health and decreasing disease risk (242).

Over the last two decades, breakthroughs in technologies such as ‘omics’ have been unprecedented, generating mechanistic insights and informing the development of precision/ personalised medicine and nutrition (243). The “omics” technologies include genomics (the study of an organism's genome), transcriptomics (the quantification of the transcriptome), proteomics (the comprehensive analysis of the entire protein produced by a cell type), and metabolomics (the identification and quantification of cellular metabolites) (244). In precision medicine, treatment is individually tailored according to all characteristics of the patient . These characteristics and prognostic indicators are determined using multi-omics data for predicting the toxicity and identifying non-responders and responders (245). As in medicine, the definition of ‘personalisation’ in the nutrition context has been debated (246); and terminology is continuously evolving with the term 'precision' being used more recently in the literature over the previous 5 years. In personalised or precision nutrition, dietary recommendations are tailored according to an individual's biological parameters to improve health (242, 247). For a clinical dietitian or nutritionist, these features include anthropometric, biochemical and clinical measurements, along with assessments of dietary intake, physical activity and lifestyle. However, following the human genome sequencing, came an era of growing research in the fields of nutrigenetics and nutrigenomics, intending to offer personalised nutrition recommendations to avoid diseases associated with dietary intake according to genetic variation and the predicted nutrient responses obtained from genetic profiling (248). Precision nutrition includes strategies for preventing and treating diseases and improving health, considering individual variations in genes, lifestyle, dietary intake, gut microbiome, epigenetic markers and environment by accurately assessing a person's nutritional status (249). Evidence suggests the inclusion of the gut microbiota data in personalised nutrition as it affects the nutritional status and phenotype of the host, playing a role in nutrient absorption and storage regulation (250-252). Advances in the point-of-care diagnostics area

promise to offer a better assessment of nutritional status, as a first step towards the application of precision nutrition and tailoring dietary guidelines at the community and individual levels (249). The precise measurement of nutritional biomarkers would be useful in terms of early prediction of disease risk, identifying individuals who might benefit from a nutrition program, determining the effectiveness and efficacy of a dietary intervention (249).

1.7 Hypothesises, aims and outline of the thesis

Associations of SNPs with cardiometabolic diseases and their related traits have been confirmed in genetic association studies (89, 92, 93, 253-255). Evidence has shown that these associations can be modified by lifestyle factors among various ethnicities (256-262). Thus, given that different ethnicities have different lifestyle factors and genetic makeup, we also hypothesised that the gene-diet interaction will vary across different populations/ ethnic groups.

This thesis aimed to:

1- Investigate the association between selected SNPs, as GRS, and cardiometabolic traits among diverse ethnic groups.

2. Investigate the interactions between these SNPs/ GRS and lifestyle factors (physical activity and dietary intake of fat, protein and carbohydrate) on cardiometabolic traits in multiple ethnic groups. The background and aims of each chapter are outlined below:

Chapter 2: Obesity has been recognised as a serious public health challenge worldwide and it is a multifactorial condition in which environmental and genetic factors are involved (263). The *FTO* gene has been identified as one of the strongest genes associated with obesity (76). Examining gene-lifestyle interactions can offer better approaches for managing obesity. In

Turkey, 64.4% and 28.8% of the population were overweight and obese in 2017, respectively (264). No previous nutrigenetic studies have been conducted in the Turkish population. Therefore, the aims of this study were to examine the effects of the *FTO* SNPs rs9939609 and rs101634090, as well as the effect of both SNPs combined as a GRS, on obesity and related traits. Also, to examine the effect of dietary intake and physical activity on these associations in 400 Turkish adults.

Chapter 3: Non-communicable diseases (NCDs) contributes to 73% of all death rate in Indonesia, with CVD and diabetes accounting for 35% and 6%, respectively (265). The pathophysiology of cardiometabolic diseases involve a complex interplay between genetic variants and lifestyle factors, emphasising the importance of analysing gene-lifestyle interactions (5). Thus, our study aimed to examine the association of a novel GRS constructed from 15 SNPs with cardiometabolic traits and to examine the effect of lifestyle factors including physical activity and dietary intake on these genetic associations among 110 Minangkabau women from Padang, Indonesia.

Chapter 4: The prevalence of obesity is increasing all over the world including LMICs. In Ghana, 43 % of the population are overweight or obese (266). Studies analysing gene–lifestyle interaction have been extensively performed in populations of European, and the replication of these studies in African populations is unknown (267, 268). Thus, for the first time in the African population, we aimed to examine the associations of different GRSs with obesity-related traits and to investigate the effect of physical activity and dietary intake on these associations among 302 healthy Ghanaian adults.

Chapter 5: In Brazil, the prevalence of prediabetes and T2D were 22.0% and 3.3% in the adolescent population, respectively (269). It has been estimated that Brazilian adolescents have a high prevalence of cardiometabolic risk factors including high insulin levels, dyslipidaemia, abdominal obesity, physical inactivity, high blood pressure and unhealthy diet (269-274). Thus, early interventions targeting these risk factors can be an effective method for slowing the progression of T2D and decreasing the risk of CVD (17). T2D is a multifactorial disease caused by complex interactions between genetic and dietary factors. GRS-diet interactions on metabolic traits have not been investigated in young Brazilian adults. Therefore, we aimed to examine the interaction between a 10-SNP metabolic GRS and dietary intake on metabolic traits among 200 healthy Brazilian young adults.

Chapter 6: South Asians have a 50% higher T2D risk than other populations (275, 276). India is a significant contributor to the worldwide elevated prevalence of T2D (17). Between 1990 and 2016, the number of T2D cases in India increased from 26.0 million to 65.0 million (277). Interactions between genetic variants and urbanisation have further worsened the increasing prevalence of T2D in South Asians (278, 279). However, studies assessing interactions of GRS with dietary intake in the Indian population are sparse. Thus, we aimed to investigate the association of a 7-SNP-GRS on T2D and its related traits, as well as the influence of dietary intake in 1,062 urban south Asian Indians on these associations.

Chapter 7: Findings from all 5 studies are discussed in this chapter, in addition to the discussion on the general trends observed across various ethnic groups, strengths, limitations, and future prospects and conclusions of this thesis.

Chapter 2 *FTO* gene–lifestyle interactions on serum adiponectin concentrations and central obesity in a Turkish population

Published (The published version of the paper is attached as an appendix at the end of the thesis)

Isgin-Atici K*, **Alsulami S***, Turan-Demirci B, Surendran S, Sendur SN, Lay I, Karabulut E, Ellahi B, Lovegrove JA, Alikasifoglu M, Erbas T, Vimaleswaran KS and Buyuktuncer Z. *FTO* gene-lifestyle interactions on serum adiponectin concentrations and central obesity in a Turkish population. International journal of food sciences and nutrition**. 2020**;72(3):375-85. https://doi.org/10.1080/09637486.2020.1802580

*** Equal contribution**

Sooad Alsulami's contribution: The cleaning of the dataset was performed before running the statistical analysis. Then, the analysis plan was developed, which was used for running the statistical analysis, using the Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA), and the data was interpretated. The literature search was carried out to write the first draft of the manuscript which was then revised according to the co-authors' comments. The manuscript was formatted based on the journal's guidelines and the reviewer comments were responded.

2.1 Abstract

Aim: 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.

Methods: 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.

Results: 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).

Conclusion: This study demonstrated that the association between *FTO* SNPs and central obesity might be modified by lifestyle factors in this Turkish population.

2.2 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 (280). 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 interactions between genetic and environmental factors is required for the development of a complex disease such as obesity (4). Studies have identified approximately 140 genes to be associated with obesity, and the fat mass and obesity associated (*FTO*) gene has been reported to be the strongest susceptibility gene for human obesity (281).

The *FTO* gene is located on chromosome 16q12.2 and codes for a protein with 2-oxoglutarate dependent nucleic acid demethylase activity which is involved in DNA repair and the accumulation of fat in the body (282). *FTO* is highly expressed in the brain, including the hypothalamus, adipocytes, pancreatic islet cells, and adrenal glands (76). *FTO* gene has been suggested to control energy homeostasis and food intake (283). Previous studies have shown that, of the various obesity susceptibility genes, single-nucleotide polymorphisms (SNPs) located in the first intron of *FTO* gene has provided the strongest evidence for genetic predisposition to obesity (76, 77, 284). The minor allele 'A' of the *FTO* SNP rs9939609 has been consistently associated with higher BMI in various populations (76, 285-288). Furthermore, a meta-analysis reported that the association between the SNP rs9939609 and

BMI was replicated in 13 cohorts with 38,759 participants, where individuals with the 'AA' genotype had 1.67-time higher odds of obesity than those with the 'TT' genotype (76). In the Turkish population, the risk alleles of the *FTO* rs1421085 and rs9939609 polymorphisms were shown to have significant associations with the risk of obesity in women and metabolic syndrome (MetS) in men (289).

Turkish adults are characterized with low levels of total and high-density lipoprotein cholesterol, and high risk of CVD, that distinguish them from Europeans (290). They also have increased susceptibility to impaired glucose tolerance and MetS primarily driven by obesity (291). Among the non-communicable diseases (NCDs) that accounted for 88.0% of deaths in Turkey, CVD has shown to contribute to 47.73% of overall deaths (280). Targeting modifiable risk factors for NCDs including obesity could prevent many deaths. Therefore, several health promotion campaigns such as "Reducing Portion Sizes" and "Move for Health" have been implemented for the prevention of obesity in Turkey (292, 293). However, obesity is a multifactorial disorder, and identifying gene-environment interactions are needed to understand the aetiology and pathophysiology of obesity and also to develop more effective personalised preventative strategies (294). To date, several *FTO*-dietary intake interactions on obesity-related outcomes have been examined in different populations (241, 295-300) however, there are no such studies to date in a Turkish population The investigations of the gene-diet interactions in different ethnic groups are crucial to develop personalised nutrition strategies for each ethnic group due to the genetic heterogeneity (301). The *FTO* SNP rs9939609 has been associated with several dietary components including

dietary protein intake (106, 296, 302) and the SNP rs10163409 in *FTO* was among the top associations in a large genome-wide meta-analysis study (GWAS) for total caloric intake (303). 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.

2.3 Materials and Methods

2.3.1 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 (301). 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 into 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.

2.3.2 Study design

A cross-sectional case-control study design was used, where participants were divided into two groups: obese (BMI ≥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.

2.3.3 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) (304). 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) (305). The waist circumference (WC) was measured by a standard method (306). Increased WC (central obesity) was defined based on cut-points established for Turkish adults (WC ≥ 90 cm for men/ ≥ 80 cm for women) (307). 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) (308). All anthropometrical measurements were taken by the research dieticians.

2.3.4 Biochemical and clinical measures

Serum adiponectin was analysed by enzyme-linked immunosorbent assay (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 (309).

2.3.5 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).

2.3.6 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) (310).

2.3.7 SNPs selection and genotyping

FTO gene was selected based on its consistent and strong associations with obesity traits in large-scale GWASs (76, 77). The SNP rs9939609 is the most commonly studied variant and consistently associated with obesity phenotypes across multiple ethnicities (76, 285-288) and SNP rs10163409 has been shown to be associated with dietary energy intake from macronutrients (303). 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.

2.3.8 Statistical analysis

SPSS software (version 23.0) was used for statistical analysis and the research analysis plan is included as an appendix on Page 236. 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.

2.4 Results

2.4.1 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 2).

2.4.2 Associations between *FTO* variants and obesity-related traits

Genotype distributions and minor allele frequencies (MAFs) for both SNPs are shown in Table 3. 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 4).

Table 2: Anthropometric and biochemical characteristic of the study participants

	Non-obese				Obese				P value**
	Total(N=200)	Men(N=100)	Women(N=100)	P value*	Total(N=200)	Men(N=108)	Women(N=92)	P value *	
Age (year)	33.29±6.83	32.64±6.04	33.93±7.51	0.182	36.37±7	36.09±6.78	36.68±7.27	0.552	<0.001
BMI (kg/m^2)	22.56±1.78	22.83±1.73	22.3±1.79	0.035	29.04±3.38	28.8±3.22	29.33±3.55	0.274	<0.001
WC (cm)	80.68±7.96	85.62±6.52	75.75±5.98	<0.001	95.49±9.84	99.41±8.15	90.89±9.7	<0.001	<0.001
FMI	5.12±1.69	3.97±1.16	6.27±1.31	<0.001	8.72±2.79	7.3±2.16	10.39±2.52	<0.001	<0.001
Adiponectin (ng/ml)	11880.63±6838.36	9095.15±4929.39	14666.11±7350.18	<0.001	9115.13±5766.48	6716.49±3777.58	11930.93±6410.41	<0.001	<0.001
Energy (kcal/day)	2416.44±1064.1	2888.13±1155.72	1944.76±700.65	<0.001	2365.08±1012.1	2728.68±1057.45	1938.23±764.28	<0.001	0.621
Protein (g)	91.34±42.91	106.32±46.98	76.37±32.27	<0.001	87.55±43.81	97.85±50.48	75.45±30.44	<0.001	0.382
Fat (g)	105.31±48	119.48±51.92	91.15±39.13	<0.001	100.8±51.56	113.32±56.41	86.1±40.84	<0.001	0.366
Carbohydrate (g)	270.28±142.83	339.26±157.55	201.29±81.06	<0.001	271.34±128.5	322.74±132.03	211±93.78	<0.001	0.938
Fibre (g)	23.49±10.65	26.71±11.66	20.27±8.43	<0.001	24.03±11.59	26.59±12.84	21.04±9.13	0.001	0.627
SFA (g)	31.13±15.25	33.67±16.56	28.58±13.42	0.018	29.66±16.2	31.87±17.87	27.07±13.64	0.036	0.352
MUFA (g)	35.58±17.78	39.88±19.57	31.27±14.68	0.001	35.12±20.23	39.71±22.78	29.72±15.17	<0.001	0.809
PUFA (g)	28.79±17.35	34.88±19.04	22.7±12.95	<0.001	27.02±16.51	31.57±17.9	21.67±12.88	<0.001	0.296
PAL levels n (%)									
Sedentary	153 (76.5)	78 (78)	75 (75)		165 (82.5)	90 (83.3)	75 (81.5)		
Active	47 (23.5)	22 (22)	25 (25)	0.617	35 (17.5)	18 (16.7)	17 (18.5)	0.737	0.137

P values obtained from independent t-test and chi square tests comparing continuous and categorical variables between men and women within obese

and non-obese categories** P values obtained from independent t-test and chi square tests comparing continuous and categorical variables between total obese and total non-obese. Values are mean ± SD and percentages. BMI, Body Mass Index; WC, Waist Circumference; FMI, Fat Mass Index; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; PAL, Physical activity level.

Table 3: Genotype frequencies of *FTO* SNPs among cases and controls

FTO rs9939609 SNP				
	Non-obese	**Obese**	**Total**	***P value**
Additive n (%)				0.217
TT	77 (38.5)	61 (30.5)	138 (34.5)	
AT	99 (49.5)	115 (57.5)	214 (53.5)	
AA	24 (12)	24 (12)	48 (12)	
Dominant n (%)				0.092
TT	77 (38.5)	61 (30.5)	138 (34.5)	
AT+AA	123 (61.5)	139 (69.5)	262 (65.5)	
HWE	0.36	0.007	0.011	
MAF	0.37	0.41	0.39	
FTO rs10163409 SNP				
Additive n (%)				0.772
AA	85 (42.5)	79 (39.5)	164 (41)	
AT	88 (44)	90 (45)	178 (44.5)	
TT	27 (13.5)	31 (15.5)	58 (14.5)	
Dominant n (%)				0.542
AA	85 (42.5)	79 (39.5)	164 (41)	
TA+TT	115 (57.5)	121 (60.5)	236 (59)	
HWE	0.58	0.525	0.392	
MAF	0.36	0.38	0.37	

*p values obtained from Pearson's chi-squared test comparing genotype frequencies between cases and control. MAF, Minor Allele Frequency; HWE, Hardy Weinberg Equilibrium

Table 4: Associations between *FTO* polymorphisms and anthropometric and biochemical parameters of obesity

	FTO rs9939609			*FTO* rs10163409		
	TT	AT+AA	*p value	AA	TA+TT	*P value
	(n=138)	(n=262)		(n=164)	(n=236)	
BMI (kg/m^2)	24.81±3.65	26.33±4.41	0.001	25.49±4.03	26.02±4.34	0.212
FMI	6.23±2.61	7.29±3.02	0.002	6.72±2.57	7.06±3.15	0.251
WC (cm)	86.08±10.62	89.14±11.99	0.007	87.26±11.47	88.66±11.7	0.234
Adiponectin (ng/ml)	11306.18±7130.97	10072.14±6059.63	0.031	10865.59±6526.36	10242.35±6427.19	0.377

*P values obtained from linear regression analysis adjusted for sex, age, hypertension, cardiovascular diseases and obesity status. Values are mean ± SD. BMI, Body Mass Index; WC, Waist Circumference; FMI, Fat Mass Index

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

2.4.3.1 *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 2).

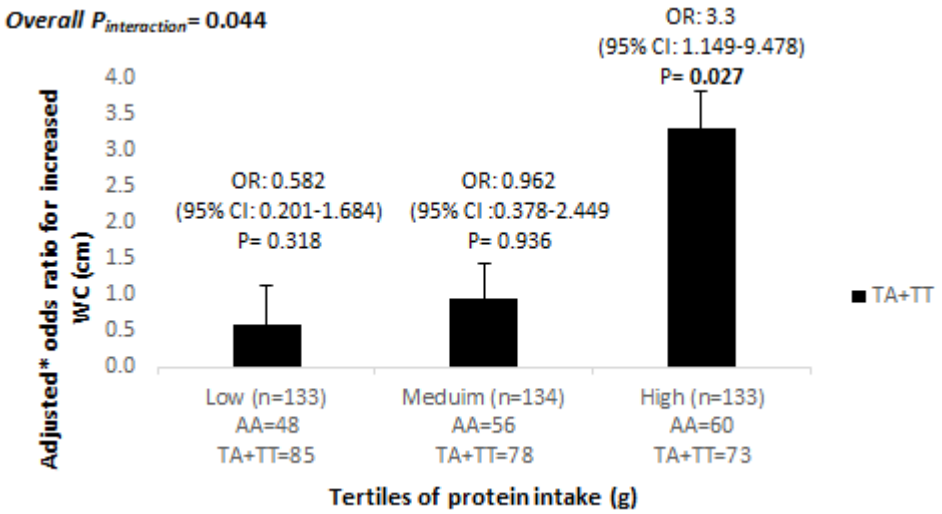

Figure 2: Interactions of the *FTO* rs10163409 with tertiles of protein intake (g) on increased WC.

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. WC: Waist

Circumference. *Odds ratio adjusted for age, gender, hypertension, cardiovascular diseases, total energy intake and obesity status**.**

2.4.3.2 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 3).

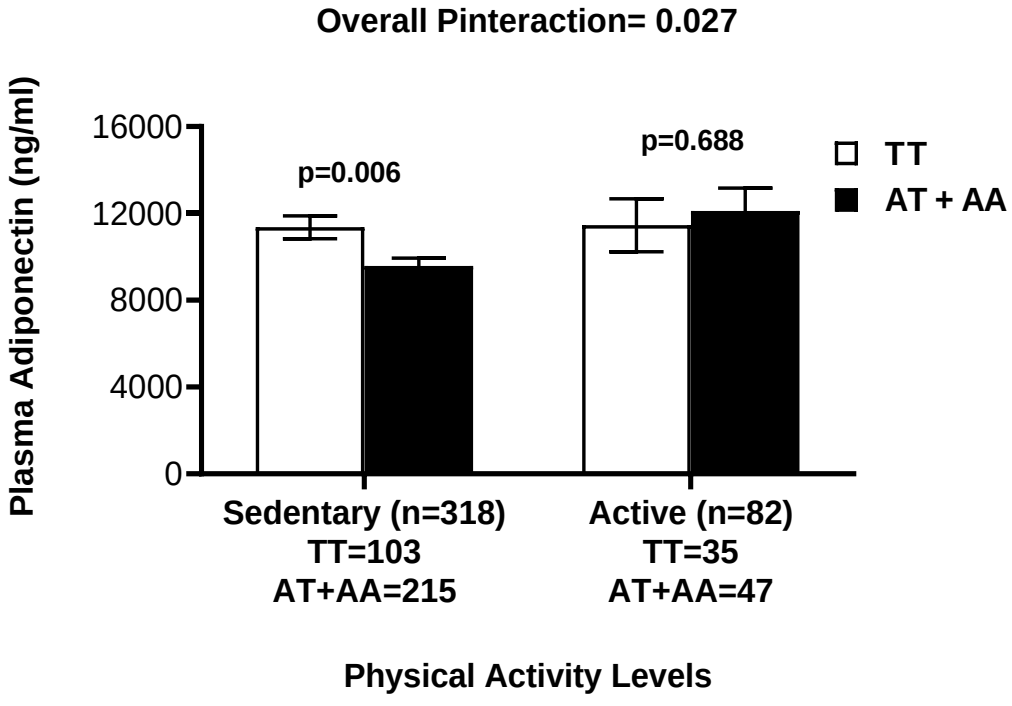

Figure 3: Interactions between *FTO* rs993609 variant and physical activity levels on adiponectin levels.

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).

2.4.4 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 4a, 4b and 4c). However, the interactions between GRS and lifestyle factors on obesity traits were not found statistically significant.

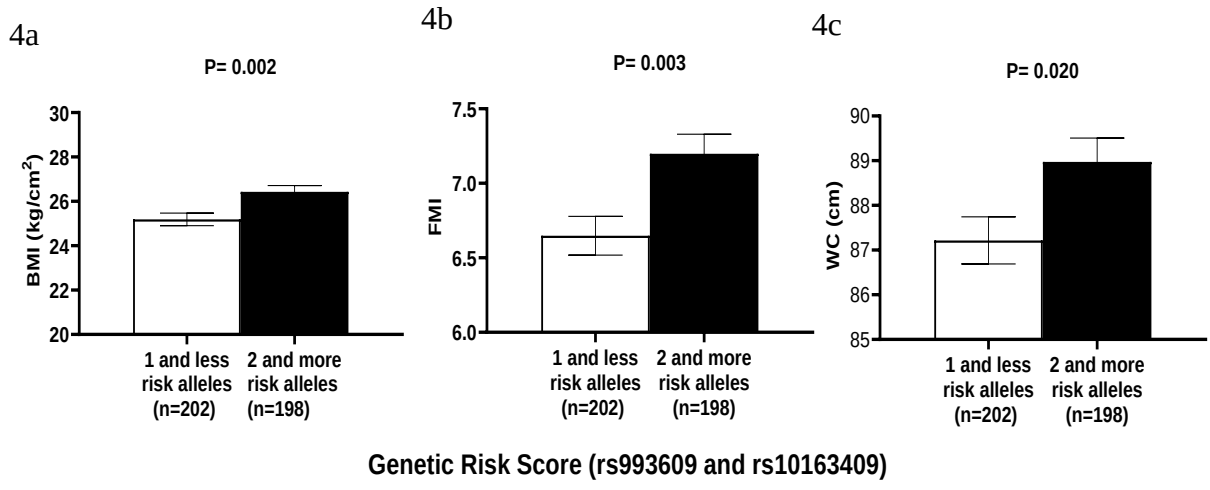

Figure 4: Association between the genetic risk score of the *FTO* rs993609 and rs10163409 and anthropometric measures of obesity.

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 risk alleles. The GRS was significantly associated with BMI (a), FMI (b) and WC (c). (a) Carriers of ≥2 risk alleles of the FTO variants (rs9939609 and rs10163409) had higher BMI (P=0.002) compared to individuals carrying <2 risk alleles. (b) Carriers of _2 risk alleles of the FTO variants (rs9939609 and rs10163409) had higher FMI (P=0.003) compared to individuals carrying <2 risk alleles. (c) Carriers of ≥2 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.

2.5 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 (311). 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 (280), 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 (312).

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 (76, 285-288, 313, 314). 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}) (299). The reported *FTO*-related genetic associations with BMI have also been

confirmed in a study in the Turkish population (289), 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 (300, 315, 316), 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 (303).

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 (302, 317, 318). It has been suggested that following a high protein diet can modulate the genetic effect of *FTO* variants on obesity traits (302, 317, 318). 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 (319). 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 (319). 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 (300). 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 (299). In contrast to our finding, a few large-scale studies demonstrated significant interactions between dietary macronutrient intakes and *FTO* variants in determining BMI (240, 241, 295-298). A cross-sectional study conducted on 4,839 Swedish participants reported an association between the risk allele of the SNP rs9939609 and higher BMI only in individuals with high fat and low carbohydrate consumption (241). A similar interaction between the rs9939609 variant and saturated fatty acids (SFA) intake has been detected in 2,163 individuals from two independent populations of the United States, where individuals homozygous for the risk allele 'AA' had a higher BMI compared to other genotypes, only when the intake of SFA was high (240). Furthermore, the *FTO* SNP rs8050136, in LD with rs9939609, significantly interacted with carbohydrate intake on obesity risk among Asian Indian population (239).

Regarding genetic interactions with physical activity, a previous study conducted among 200 Turkish adults found that BMI was higher in homozygous risk allele 'A' carriers of the SNP rs9939609 than the homozygote the 'T' allele carriers among physically inactive individuals (320). The same interaction but on a biochemical measure of obesity (i.e.: adiponectin level), rather than BMI, was replicated in our study using a larger sample size. We found that, among those with lowest levels of physical activity, the adiponectin concentrations were significantly lower in the carriers of the risk allele 'A' of the *FTO* rs9939609 than 'TT' homozygotes. Adiponectin is a hormone produced and secreted by adipose tissue and commonly known for its antihyperglycemic, anti-inflammatory, antiatherogenic, and

cardioprotective effects (321-323). Studies have reported a strong correlation between the dysregulation of adipokine production and the onset of several metabolic abnormalities including CVD and cancer (324, 325). The positive correlation between adiponectin levels and physical activity has been demonstrated in several studies (326, 327), where higher levels of physical activity have been shown to reduce adiposity which decreases the production of insulin and leptin, and increases adiponectin production (328). 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 (329). An intervention study conducted in 400 obese women showed that a weight reduction program resulted in a significant increase in adiponectin levels (330). 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 (76, 285-288). 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 (76, 77, 284). 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.

2.6 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 (280, 331). 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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Chapter 3 Interaction between the genetic risk score and dietary protein intake on cardiometabolic traits in Southeast Asians.

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Sooad Alsulami's contribution: Before running the statistical analysis, the dataset was cleaned . Then the analysis plan was developed for the study. Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA) was used to conduct the statistical analysis. The data was interpreted and the first draft of the paper was written. The manuscript was revised based on co-authors' comments and suggestions and prepared according to the journal's guidelines. The and reviewer's comments were responded and the galley proof was checked.

3.1 Abstract

Background: Cardiometabolic diseases are complex traits which are influenced by several single nucleotide polymorphisms (SNPs). Thus, analysing the combined effects of multiple gene variants might provide a better understanding of disease risk than using a single gene variant approach. Furthermore, studies have found that the effect of SNPs on cardiometabolic traits can be influenced by lifestyle factors, highlighting the importance of analysing gene-lifestyle interactions.

Aims: In the present study, we investigated the association of 15 gene variants with cardiometabolic traits and examined whether these associations were modified by lifestyle factors such as dietary intake and physical activity.

Methods: The study included 110 Minangkabau women [aged 25-60 years and body mass index (BMI) 25.13±4.2 kg/m2] from Padang, Indonesia. All participants underwent a physical examination followed by anthropometric, biochemical and dietary assessments and genetic tests. A genetic risk score (GRS) was developed based on 15 cardio-metabolic disease-related SNPs. The effect of GRS on cardiometabolic traits was analysed using general linear models. GRS-lifestyle interactions on continuous outcomes were tested by including the interaction term (e.g., lifestyle factor*GRS) in the regression model. Models were adjusted for age, BMI and location (rural or urban), wherever appropriate.

Results: There was a significant association between GRS and BMI, where individuals carrying 6 or more risk alleles had higher BMI compared to those carrying 5 or less risk alleles (P=0.018). Furthermore, there were significant interactions of GRS with protein intake on waist circumference (WC) and triglyceride concentrations ($P_{interaction}$= 0.002 and 0.003, respectively). Amongst women who had a lower protein intake (13.51±1.18% of the total daily energy

intake), carriers of six or more risk alleles had significantly lower WC and triglyceride concentrations compared with carriers of five or less risk alleles (P=0.0118 and 0.002, respectively).

Conclusion: Our study confirmed the association of GRS with higher BMI and further showed a significant effect of the GRS on WC and triglyceride levels through the influence of a low-protein diet. These findings suggest that following a lower protein diet, particularly in genetically predisposed individuals, might be an effective approach for addressing cardiometabolic diseases among South East Asian women.

3.2 Introduction

Cardiometabolic diseases such as cardiovascular diseases (CVD), obesity, hypertension and type 2 diabetes are a major cause of mortality, morbidity and healthcare spending worldwide (332, 333). The prevalence of these diseases has significantly increased and has become a major problem given the significant economic burden that these diseases impose on low- and middle-income countries. Indonesia has the seventh largest number of diabetic patients (7.6 million), despite relatively low prevalence worldwide (4.8%) in 2012 (334). In 2013, it was estimated that there were more than 132.8 million people with diabetes in the Western Pacific (more people than in any other region), and the number is expected to rise to 201.8 million by 2035 (335). Furthermore, obesity is suggested to play a critical role in the development of chronic and non-communicable diseases (NCDs) in the South East (SE) Asia (336). In Indonesia, NCDs are estimated to account for 73% of all deaths (265) of which, CVD contributed to 35% followed by cancers (12%), and diabetes (6%) (265).

Indonesia is the largest island country in the world, consisting of various ethnic groups distributed over 33 provinces (337). Minangkabau community is the world's largest matrilineal society which resides mostly in West Sumatra, where the prevalence of low level of high-density lipoprotein cholesterol (HDL-C), hypertension and central obesity is more than 50% (337). It is reported that the Minangkabau ethnic group had a high risk of dyslipidemia, which is suggested to be driven mainly by the high intake of dietary fat from poor quality sources (338). A study comparing lipid profiles among four ethnic groups reported that the Minangkabau ethnic group has the highest levels of plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C) compared to other larger ethnicities including Sundanese, Javanese and Buginese (339). Furthermore, it has been reported that the prevalence of central obesity is high among Minangkabau women (340). Many environmental exposures contribute

to the increasing prevalence of cardiometabolic diseases, but one key factor is urbanization (341). Countries in SE Asia have undergone rapid epidemiological and nutritional transitions over the past few decades. Furthermore, it has been reported that dietary risks, high blood pressure and tobacco smoking are the three major risk factors contributing to disease burden in Indonesia (342). However, genetic factors also play an important role in the development of cardiometabolic diseases.

Candidate gene studies and genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) relating to cardiometabolic diseases and traits in the Asian populations (343-346). Most cardiometabolic traits are influenced by thousands of SNPs each having a relatively small effect on the trait when present alone. Thus, analysing the combined effects of multiple gene variants might provide a better understanding of traits variability of an individual and improve risk prediction of cardiometabolic diseases than using a single variant approach (347). Furthermore, studies have found that the effect of genetic variants on cardiometabolic traits can be influenced by lifestyle factors (348). It has been confirmed that using genetic risk score (GRS) approaches increase the power to detect gene-lifestyle interactions compared to the common single variant methods (349). Therefore, our study aimed to investigate the association of a novel GRS with cardiometabolic traits and to examine whether lifestyle factors such as dietary intake and physical activity modified these associations in 110 Minangkabau women.

3.3 Methods

3.3.1 Study participants

The study included healthy women who were enrolled in the Minangkabau Indonesia Study on Nutrition And Genetics (MINANG) study, a cross-sectional pilot study conducted in

the city of Padang, West Sumatra, Indonesia, between December 2017 to January 2018. This study is a part of the ongoing GeNuIne (Gene-Nutrient Interactions) Collaboration, which aims to examine the interactions between genetic and dietary factors (nutrigenetics) on cardiometabolic disease and its related traits using population-based studies from several ethnic groups (220). The methodology of the study has been published elsewhere (350). In brief, 133 women were recruited from community health centres in two sub-districts in Padang City including Padang Timur and Kuranji districts to represent both urban and rural areas of Padang population, respectively. The inclusion criteria included: healthy women, aged 25-60 years old and with Minangkabau ethnicity. Of the 133 enrolled women, 10 were excluded from the study according to the following exclusion criteria: being pregnant or lactating (N=0) and taking dietary or vitamin supplements (N=0), have a previous history of hypertension, CVD or type 2 diabetes (N=6), have a body mass index (BMI) of more than 40 kg/m^2 or being classified as morbidly obese by a practitioner (N=0), being blood related to other participants in the study (N=0), have any communicable disease (N=4). Of the remaining 123 participants, we excluded another 5 women who did not undergo blood sampling. Thus, the final sample consisted of 118 participants, of whom seven women did not have complete genetic information about all the investigated SNPs and were excluded from the GRS analysis (N=111). Additionally, one participant with no dietary information available was excluded from the GRS interaction analysis (N=110).

The MINANG study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethical Review Committee of the Medical Faculty, Andalas Univesity (No.311/KEP/FK/2017). All participants gave their written informed consent before participating and had the right to withdraw from the study at will and opt-out from any of the procedures.

3.3.2 Anthropometric Measures

Body weight (to the nearest 100 g) and height (to the nearest mm) were measured using an electronic scale (Seca 803, Seca GmbH. Co. kg, Hamburg, Germany) and a wall-mounted stadiometer (OneMed Medicom stature meter, YF.05.05. V.A.1022, Indonesia), respectively. BMI was calculated as weight (kg)/height (m)2 and categorised according to the Asia-Pacific classification of BMI (351). Waist circumference (WC) was measured in cm using a metal tape (Medline-OneMed Medicom, Jakarta, Indonesia) midway between the 12th rib and the superior border of the iliac crest at the end of normal expiration.

3.3.3 Biochemical and clinical measures

After 12 hours of fasting, blood samples (5ml) were taken to measure the concentrations of glucose, insulin, glycated haemoglobin A1c (HbA1c), total cholesterol, triglycerides, LDL-C and HDL-C. Samples were assayed using the xMark Microplate Spectrophotometer (Bio-Rad Laboratories Inc, Hercules, California, USA). Fasting glucose, insulin and HbA1c were measured using enzyme-linked immunosorbent assay (ELISA) kits from Bioassay Technology Laboratory (Shanghai, China). Blood lipids were analysed using enzymatic colorimetric procedures, namely GPO - PAP for triglycerides, and CHOD-PAP for total cholesterol, LDL and HDL. A sphygmomanometer was used to measure systolic and diastolic blood pressures (SBP and DBP). Measurements were taken twice at 5-minute intervals and the average was recorded.

3.3.4 Assessment of dietary intake and physical activity

Information about dietary intake and physical activity were collected by a well-trained nutritionist in the home or in an integrated health service post. Diet was assessed using a previously validated and published semi-quantitative food frequency questionnaire (SQ-FFQ)

consisting of a list of 223 food items (352). Briefly, participants were asked to report the frequency of consumption (number of times per day, week or month) and portion size of various food items. Participants were provided with portion size images of all relevant foods to enhance reporting accuracy while completing the SQ-FFQ (353). All collected data were double-checked for accuracy and analyzed with the Indonesian Food Database and Nutrisurvey (EBISpro, Germany) to estimate total energy and macronutrient intake. Values of nutrient intake were adjusted for total energy intake using the nutrient (energy-adjusted) residual method, wherever appropriate (354).

"The Global Physical Activity Questionnaire" (GPAQ) was used to calculate an individual's level of physical activity in 3 areas (work, transport and leisure-time) and time spent in sedentary behaviour (355). Total time spent in moderate-to-vigorous physical activity was estimated using to the world health organisation (WHO) STEPwise method and was expressed as metabolic equivalent minutes per day (METmins/day). Participants were defined as "active" if they did ≥600 METmins/week or "inactive" if they accumulated <600 METmins/week.

3.3.5 SNP selection and genotyping

Fifteen genetic variants located at 8 different genes were selected for the present study based on its consistent associations with cardio-metabolic traits in candidate gene studies and GWAS in Asian populations (343-346, 356-365). The selected genetic variants were Calpain 10 (*CAPN10*) rs3792267 and rs5030952, fat mass and obesity-associated (*FTO*)- rs9939609, rs10163409 and rs8050136, melanocortin 4 Receptor (*MC4R*)- rs17782313 and rs2229616, Transcription factor 7-like 2 (*TCF7L2*)- rs12255372 and rs7903146, Potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*)- rs2237895 and rs2237892, Cyclin dependent kinase

inhibitor 2A/2B (*CDKN2A/*2B)- rs10811661, Peroxisome proliferator-activated receptor gamma (*PPARG*)- rs1801282, and adiponectin *(ADIPOQ*)-rs266729 and rs17846866.

Genomic DNA was extracted from peripheral blood leukocytes using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, USA). Furthermore, a NanoDrop spectrophotometer was used to determine DNA concentration. The SNPs were genotyped using the competitive allele-specific PCR-KASP® assay at LGC Genomics (*http://www.lgcgroup.com/services/genotyping).*

3.3.6 Statistical analysis

Statistical analysis was performed using the SPSS software (version 23) and the research analysis plan is included as an appendix on Page 240. Common obesity was defined based on the Asia-Pacific classification of BMI for Asians, where non-obese individuals (BMI<23 kg/m^2) and obese individuals (BMI≥23 kg/m^2) were classed accordingly (366). Central obesity was defined based on WHO classification of WC (WC>80 cm for women) (30). The Hardy-Weinberg equilibrium (HWE) was assessed using the x^2 goodness-of-fit test and the 15 SNPs were in HWE (P>0.05). Normality of distribution of all continuous variables was tested using the Shapiro-Wilk test and those that were not normally distributed were natural log-transformed before the analysis, including glucose, insulin, HbAC1, HDL-C, LDL-C, total cholesterol, triglyceride concentrations and total dietary protein intake (%). Continuous variables are expressed as means and standard deviations (SD) and comparisons between groups were made using the independent t-test. The descriptive statistics for categorical variables, such as physical activity level, were obtained by determining frequency distributions and compared between individuals with and without central obesity using Pearson's chi-squared test. The association between individual SNPs and cardiometabolic traits was analysed using general linear models adjusted for age, residential area (rural or urban) and BMI when

BMI is not an outcome. As the number of individuals with rare homozygous genotypes was low, a dominant model was used, where common homozygous genotypes were compared against combined rare homozygous and heterozygous genotypes.

A GRS was constructed based on 15 SNPs from 8 genes. An additive genetic model was assumed for each gene variant, assigning a score of 0, 1, and 2 to genotypes containing 0, 1, or 2 risk alleles, respectively. The GRS was then calculated for each individual by summing the number of risk alleles in the genetic variants. The count method assumed that each risk allele contributes equally and independently to the development of cardiometabolic traits. The average number of risk alleles per individual for the GRS was 5.12 (SD=2.06), which ranged from 2 to 10. The GRS variable was then categorised into two groups based on the median of risk alleles; "low genetic risk group": individuals with a GRS≤5 risk alleles (N=69) and "high genetic risk group": individuals with GRS>5 risk alleles (N=42). The effects of GRS on cardiometabolic traits were analysed using general linear models. Furthermore, GRS-lifestyle interactions on continuous outcomes were tested using linear regression models by including the interaction terms (e.g., diet*genotype) in these models. Models were adjusted for age, residential area and additionally for BMI when it is not an outcome. Lifestyle factors that were investigated in our study included dietary intake and physical activity. Carbohydrate, protein and fat intakes were expressed as a percentage of total energy intake and fibre intake was expressed in grams. Furthermore, statistically significant interactions were investigated in more depth, where individuals were stratified by the tertiles of dietary intake and the levels of physical activity. A P value of <0.05 was considered statistically significant. Multiple testing correction was not applied given that we had examined only one genetic instrument (i.e., GRS).

3.4 Results

3.4.1 Characteristics of the study participants according to the central obesity status

In the present study, 71 women (64.0%) were centrally obese and 39 (35.1%) were not. The characteristics of the participants are shown in Table 5. In general, centrally obese participants were older, and had higher SBP (P=0.006), fasting plasma glucose (P=0.039), serum triglycerides (P<0.001), serum total cholesterol (P<0.001) and LDL-C (P<0.001) concentrations compared to participants without central obesity. There were no significant differences in fasting HDL-C, serum insulin, HbA1c, DBP, dietary intake and physical activity levels and the distribution of GRS between the two groups (P>0.05).

3.4.2 Associations between GRS and cardiometabolic traits

To explore the combined effect of the 15 SNPs on various cardiometabolic traits, a GRS was calculated. There was a significant association (P=0.018) between the GRS and BMI where individuals carrying 6 or more risk alleles of the SNPs had higher BMI compared with those carrying 5 or less risk alleles (Table 6).

Table 5: Anthropometric and biochemical characteristics of the study participants.

	N	Total (N=111)	N	Non-centrally Obese (WC≤80 cm) (N=39)	N	Centrally Obese (WC>80 cm) (N=71)	P value*
Age (yrs)	111	40.49±10.18	39	37.08±11.68	71	42.58±8.62	0.012
BMI (kgm2)	111	25.13±4.2	39	21.85±3.71	71	26.99±3.24	<0.001
WC (cm)	110	83.85±10.27	39	72.79±6.03	71	89.92±6.26	<0.001
Glucose (mg/dl)	111	92.53±20.67	39	87.21±9.78	71	95.69±24.29	0.039
Insulin (mIU/L)	111	32428.5±25706.13	39	31073.79±28460.35	71	33374.28±24368.83	0.657
HbA1c (ng/ml)	111	655.59±601.59	39	629.22±671.07	71	666.42±568.14	0.759
Triglycerides (mg/dl)	111	98.8±43.47	39	78.26±34.19	71	109.72±44.38	<0.001
Cholesterol (mg/dl)	111	209.31±44.02	39	188.26±30.04	71	221.77±45.74	<0.001
HDL-C (mg/dl)	111	59.12±10.29	39	60.9±10.45	71	58.14±10.22	0.182
LDL-C (mg/dl)	111	128.12±39.85	39	111.49±25.55	71	138.2±42.65	<0.001
SBP (mmHg)	111	113.37±9.07	39	110.14±8.83	71	115.05±8.81	0.006
DBP (mmHg)	111	77.44±6.39	39	76.26±8.35	71	78.06±5.01	0.223
Total energy (kcal/d)	110	1776.24±611.43	39	1789.55±604.31	70	1755.6±613.59	0.781
Carbohydrate intake (%)	110	53.97±9.44	39	52.67±7.86	70	54.91±10.1	0.235
Protein intake (%)	110	16.93±3.32	39	17.13±2.93	70	16.76±3.54	0.579
Fat intake (%)	110	28.95±7.99	39	30.05±6.87	70	28.16±8.45	0.235
Dietary fibre (g)	110	8.78±4.29	39	9.11±4.52	70	8.56±4.19	0.521
SFA (g)	110	20.84±11.22	39	21.77±10.81	70	20.07±11.35	0.447
MUFA (g)	110	8.18±4.6	39	9.00±5.08	70	7.62±4.18	0.129
PUFA (g)	110	6.32±3.5	39	6.67±3.06	70	6.14±3.76	0.541
MET (min/week)	111	1311.89±1877.78	39	1114.87±1625.95	71	1428.45±2016.27	0.407
GRS	110	5.09±2.07	39	4.77±2.01	71	5.31±2.03	0.189
Physical activity levels	44	Sedentary (39.64%)	18	Sedentary (46.15%)	26	Sedentary (36.62%)	0.616
	55	Moderate (49.55%)	17	Moderate (43.59%)	37	Moderate (52.11%)	
	12	Vigorous (10.81%)	4	Vigorous (10.26%)	8	Vigorous (11.27%)	

Data presented as means ± SD for continuous variables and as percentages for categorical variables. *P values for the differences in the means and proportions between non-centrally obese and centrally obese individuals were calculated using the independent t-test and the Chi-

squared test, respectively. Abbreviations: BMI Body mass index; WC Waist circumference; HbA1C glycated haemoglobin A1c; HDL-C High-density lipoprotein cholesterol; LDL-C Low-density lipoprotein cholesterol; SBP Systolic blood pressure; DBP diastolic blood pressure; SFA Saturated fatty acids; MUFA Monounsaturated fatty acids; PUFA Polyunsaturated fatty acids; MET Metabolic equivalent of task; GRS Genetic risk score.

Table 6: Associations between GRS and cardiometabolic traits

	GRS≤5 (N=69)		GRS>5 (N=42)		P value*
	N	Mean±SE	N	Mean±SE	
BMI (kgm2)	69	24.52±0.52	42	26.14±0.6	0.018
WC (cm)	68	84.28±1.22	42	83.16±1.66	0.334
Log Glucose (mg/dl)	69	93.65±2.98	42	90.69±1.72	0.327
Log Insulin (mIU/L)	69	32365.29±3199.95	42	32532.33±3782.96	0.196
Log HbA1C (ng/ml)	69	650.58±71.1	42	663.81±96.65	0.527
Log triglycerides (mg/dl)	69	101.07±5.27	42	95.07±6.67	0.142
Log Cholesterol (mg/dl)	69	212.88±5.59	42	203.43±6.11	0.228
Log HDL-C (mg/dl)	69	58.55±1.26	42	60.05±1.56	0.404
Log LDL-C (mg/dl)	69	131.84±4.97	42	122±5.73	0.197
Log SBP (mmHg)	69	113.12±1.08	42	113.77±1.43	0.679
Log DBP (mmHg)	69	77.59±0.86	42	77.2±0.76	0.535

*P values obtained from linear regression analysis adjusted for age, residential area and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: BMI Body mass index; WC Waist circumference; HbA1C glycated haemoglobin A1c; HDL-C High-density lipoprotein cholesterol; LDL-C Low-density lipoprotein cholesterol; SBP Systolic blood pressure; DBP diastolic blood pressure.

3.4.3 Interactions between GRS and dietary intake on cardiometabolic traits

There were significant interactions between the GRS and protein intake (%) on WC and triglyceride concentrations ($P_{interaction}$=0.002 and 0.003, respectively), Table 7. With low protein intake (13.51±1.18%), carriers of 6 or more risk alleles of SNPs had lower WC and triglyceride concentration compared to carriers of 5 or less risk alleles (P=0.0118 and 0.002, respectively) (Figures 5 and 6). A significant interaction between protein intake and GRS was also detected on cholesterol levels ($P_{interaction}$=0.021). Moreover, there was no other interactions between nutrient intake and GRS on cardio-metabolic traits.

Table 7: Interactions between GRS and lifestyle factors on cardio-metabolic traits.

	Carbohydrate (%)	Protein (%)	Fat (%)	Fibre (g)	Physical activity
BMI (kgm2)	0.961	0.282	0.721	0.876	0.362
WC (cm)	0.224	0.002	0.577	0.614	0.297
Log Glucose (mg/dl)	0.882	0.751	0.732	0.833	0.106
Log Insulin (mIU/L)	0.336	0.341	0.48	0.216	0.909
Log HbA1C (ng/ml)	0.766	0.638	0.935	0.162	0.626
Log Triglycerides (mg/dl)	0.066	0.003	0.355	0.262	0.479
Log Cholesterol (mg/dl)	0.081	0.021	0.261	0.583	0.308
Log HDL-C (mg/dl)	0.978	0.905	0.984	0.323	0.540
Log LDL-C (mg/dl)	0.266	0.337	0.431	0.896	0.721
Log SBP (mmHg)	0.156	0.291	0.208	0.872	0.644
Log DBP (mmHg)	0.966	0.815	0.732	0.292	0.743

Data are p values obtained from linear regression analysis adjusted for age, residential area and BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: BMI Body mass index; WC Waist circumference; HbA1C glycated

haemoglobin A1c; HDL-C High-density lipoprotein cholesterol; LDL-C Low-density lipoprotein cholesterol; SBP Systolic blood pressure; DBP diastolic blood pressure.

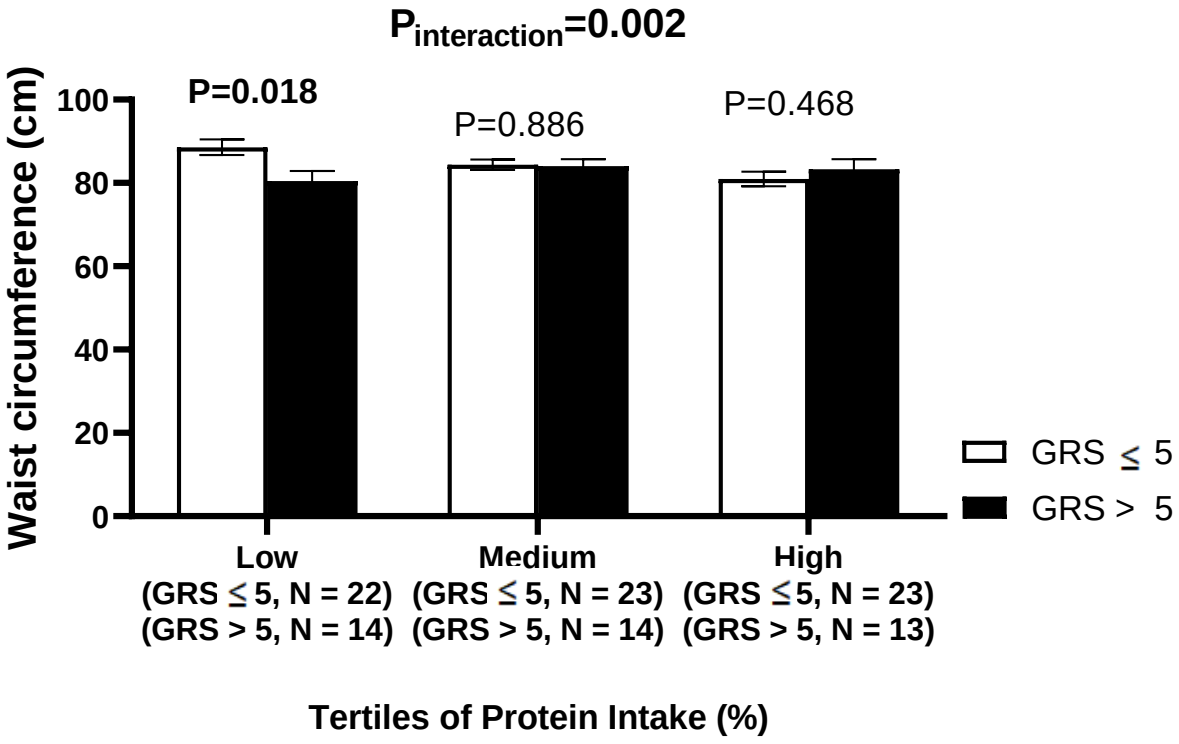

Figure 5: Interaction between genetic risk score (GRS) and log protein intake (%) on waist circumference (WC).

White bars indicate "low genetic risk group": individuals with a GRS≤5 risk alleles; Black bars indicate "high genetic risk group": individuals with GRS>5 risk alleles. Carriers of 6 or more risk alleles had lower WC compared to carriers of 5 or less risk alleles, among individual with lower protein intake (13.51±1.18 %).

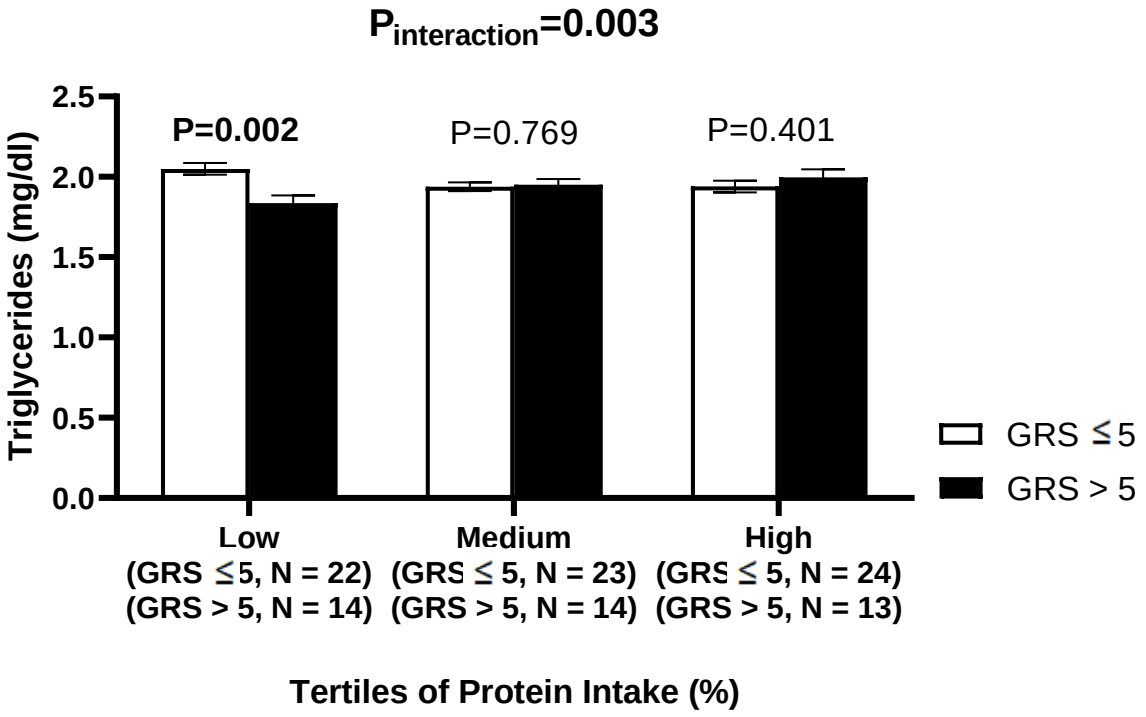

Figure 6: Interaction between genetic risk score (GRS) and log protein intake (%) on log triglyceride levels.

White bars indicate "low genetic risk group": individuals with a GRS≤5 risk alleles; Black bars indicate "high genetic risk group": individuals with GRS>5 risk alleles. Carriers of 6 or more risk alleles had lower triglyceride level compared to carriers of 5 or less risk alleles, among individual with lower protein intake (13.51±1.18 %).

3.4.4 Associations between individual SNPs and cardiometabolic traits

As shown in Supplementary Table 1, we found that the risk alleles of the three *FTO* SNPs rs9939609, rs8050136 and rs10163409 were associated with higher BMI (P=0.006, 0.007 and 0.047, respectively). Furthermore, SNPs rs12255372 (*TCF7L2*), rs2237892 (*KCNQ1*) and rs5030952 (*CAPN10*) were associated with increased fasting serum LDL-C concentrations (P=0.032, 0.039 and 0.04, respectively). A significant association was also found between the

risk allele of the SNP rs17782313 (*MC4R*) and higher insulin level (P=0.036). No significant association was observed between the remaining SNPs and cardiometabolic traits in this population (P>0.05).

3.5 Discussion

The present study aimed to investigate the effects of genetic predisposition and lifestyle factors on cardiometabolic traits in Minangkabau women. In agreement with other studies (3), we have shown that the GRS based on 8 susceptible genes for cardiometabolic diseases is a significant risk factor for higher BMI in our study sample and might be a useful tool in characterising Minangkabau women at high risk for obesity. We found that women carrying 6 or more alleles had significantly higher BMI compared to those carrying 5 or less risk alleles. Furthermore, we found a significant interaction between the GRS and dietary protein intake (%) on WC and triglyceride levels, where, among those who consumed a low protein diet (mean intake ± SD: 13.51±1.18 %) , individuals, despite carrying more than 6 risk alleles, had significantly lower WC and triglyceride levels. Given that Minangkabau women have a high risk of dyslipidemia (339) and the prevalence of common and central obesity is high among this ethnic group (340), it is important to develop effective strategies targeting these conditions to improve public health.

It has been suggested that centrally obese participants defined as normal weight based on BMI had the worst long-term survival even when compared with their overweight and obese counterparts (36). In addition, recent data from 42,702 European participants reported that central obesity is associated with higher mortality risk even in normal-weight individuals (35). This is of concern for Asian populations, where increased levels of visceral adiposity are observed in those with normal BMIs (367-369). Furthermore, the combination of increased WC along with elevated triglyceride levels has been previously defined as the

'hypertriacylglycerolaemic waist' phenotype (370). Studies have shown that individuals with this phenotype have an increased risk of higher visceral adiposity, CVD, insulin resistance and other related outcomes (370). Therefore, targeting this phenotype will have significant public health implications in terms of reducing NCD mortality in Asian populations.

In the present study, the average protein intake was 77±37 g/day, which exceeded the recommended dietary protein daily allowance of 57-59 g/day for Indonesian women (371, 372). Observational studies have shown that higher protein intake was significantly associated with increases in body weight, BMI and fat mass (373-375). These results are in contrast to the finding from intervention studies, which have shown that high protein intake enhances weight loss and provides a better long-term maintenance of reduced intra-abdominal fat stores (366, 376). These inconsistencies might be attributed to the sample size, genetic heterogeneity and gene-lifestyle interactions. Cross-sectional studies have demonstrated the association of several SNPs with obesity-related traits (77, 377-379) and interaction of these SNPs with dietary intake of protein on weight change (382-380). It has been shown that high protein diets can modulate the genetic effect of *FTO* variants on body weight, BMI and WC (302, 317, 318). According to a 2-year weight loss intervention program, carriers of the risk allele 'A' of the *FTO* SNP rs1558902 had a greater reduction in weight and regional fat compared to non-carriers when high protein diets were consumed, whereas an opposite genetic effect was found on changes in fat distribution in response to a low-protein intake (318). However, studies investigating the joint effect of genetic variants have reported conflicting results (383-385), indicating that the influence of genetic predisposition on changes in body weight and WC does not seem to be modulated by protein intake. In contrast, the present study provides evidence for GRS-protein intake interactions on WC and triglyceride concentrations, and these interactions were independent of potential confounding effects. We found that participants with 6 or more risk alleles who consumed a low protein diet (mean intake ± SD: 13.51±1.18%) had significantly

lower WC and triglyceride concentrations compared to those with 5 or less risk alleles. This difference in the findings across the studies might be due to differences in the sample size, methods used to construct GRSs (weighted vs. unweighted), and the number of SNPs included in the GRSs.

The observed interaction between GRS and dietary protein on WC and triglyceride concentrations might be driven by the source of protein consumed, which has not been analysed in our study. Different protein sources have different effects on body weight and fat mass, and the mechanisms behind this are still very speculative and need more investigation. The higher intake of protein from animal sources (protein from red and processed meat and poultry) was found to be associated with an increase in body weight in both genders, with a stronger association in women (374). Diet rich in animal protein might reflect the western pattern diet characterised by high red meat consumption, which has shown to be associated with weight gain (386). In contrast, a study has shown that protein from meat is associated with lower weight gain because it produces a higher 24-h energy expenditure compared to soy protein (387). This hypothesis is, however, based on a mechanistic study and it is still unknown whether this applies in the long run to individuals of the free-living populations. Furthermore, it has been suggested that consuming protein from dairy sources may prevent weight gain and promote abdominal fat loss (388). Here, the suggested mechanism primarily relates to the high content of calcium, which may function synergistically in combination with bioactive compounds, such as angiotensin-converting enzyme inhibitors and the rich concentration of branched-chain amino acids (388). While the above-mentioned studies failed to explore the genetic aspects, our study did not investigate the type of protein that was consumed by the participants; hence, future studies examining the effect of both factors are required.

In agreement with some studies (383, 384), no interactions were detected between GRS and dietary intake of protein, fat and carbohydrate on BMI in the present study. However, a study in the European population (N=48,170 adults) has shown that the joint effect of 93 obesity-related SNPs on BMI might be modulated by the intake of total energy, fat and saturated fat (385). Furthermore, studies have shown that an obesogenic diet and physical inactivity with relatively high intake of sugar-sweetened beverages and prolonged television watching might exaggerate the effect of genetic factors on adiposity (348, 389). Even though several studies have demonstrated that physical activity could attenuate the combined genetic influence of multiple SNPs on BMI and obesity risk (267, 268, 348), no such interactions were detected in the present study.

The strengths of our study include the use of a well-defined population, a validated SQ-FFQ (352) and a genetic risk score generated from the 15 genetic variants associated with cardiometabolic traits. Also, the main exposures investigated in our study were collected by well-trained staff and using validated and standardised operating procedures. However, there are limitations that needs to be acknowledged. Although our analysis was adjusted for several factors, the potential for confounding by unmeasured or unknown factors exist. Even though our study has a small sample size, we were still able to find significant associations and interactions suggesting that our study is well powered. Even though food intake was assessed using validated methods, recall bias and measurement errors in these self-reported FFQs cannot be fully eliminated, which could alter the true underlying interactions between dietary and genetic factors on cardiometabolic traits (390, 391). Finally, our study was restricted to Minangkabau women, and it is unknown whether our findings could be generalised to men or other demographic or ethnic groups.

3.6 Conclusion

In the present study, we have shown a significant effect of the GRS on WC and triglyceride levels through the influence of a low protein intake, where individuals with a high genetic susceptibility can overcome the risk of higher WC and triglyceride levels by consuming a low protein diet. These findings are potentially relevant for public health; however, future trials in both genders with larger sample size and objective measures of protein intake, such as urinary nitrogen, are needed to confirm these findings.

Declarations

Ethics approval and consent to participate*:* The MINANG study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethical Review Committee of the Medical Faculty, Andalas Univesity (No.311/KEP/FK/2017). All participants gave their written informed consent before participating.

Consent for publication: The consent for publication has been obtained from all the participants.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests**:** The authors declare that they have no competing interests.

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Author Contributions**:** SA and ASA performed the statistical analyses and data interpretation; SA and KSV drafted the manuscript; KSV and ASA carried out data collection; KSV designed the nutrigenetics study; NIL, FFY and KSV conceived, supervised and designed the study; UA and SRS contributed to data collection, monitoring and evaluation of participants, and project administration; NT was involved in data collection and dietary data analysis; IRS conducted the laboratory analysis; NIL and JAL critically reviewed the manuscript. All authors read and approved the final manuscript.

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Supplementary Material: The supplementary material is included at the end of this chapter.

Supplementary Table 1. Associations between individual SNPs and cardiometabolic traits.

3.7 Supplementary Material

3.7.1 Supplementary Table 1: Associations between individual SNPs and cardiometabolic traits.

		BMI (kg/m2)	WC (cm)	Log Glucose (mg/dl)	Log Insulin (mIU/L)	Log HbA1C (ng/ml)	Log triglycerides (mg/dl)	Log Cholesterol (mg/dl)	Log HDL-C (mg/dl)	Log LDL-C (mg/dl)	Log SBP (mmHg)	Log DBP (mmHg)
MC4R	rs17782313	0.967	0.552	0.969	0.036	0.135	0.238	0.234	0.565	0.595	0.447	0.928
MC4R	rs2229616	0.81	0.968	0.691	0.301	0.35	0.522	0.846	0.565	0.783	0.52	0.945
FTO	rs9939609	0.006	0.776	0.327	0.553	0.605	0.751	0.961	0.685	0.743	0.982	0.517
FTO	rs8050136	0.007	0.754	0.301	0.558	0.632	0.805	0.867	0.639	0.813	0.882	0.639
FTO	rs10163409	0.047	0.833	0.18	0.985	0.731	0.182	0.411	0.969	0.431	0.749	0.629
TCF7L2	rs7903146	0.645	0.115	0.762	0.439	0.19	0.639	0.58	0.666	0.097	0.762	0.423
TCF7L2	rs12255372	0.722	0.152	0.801	0.316	0.164	0.543	0.276	0.692	0.032	0.538	0.915
ADIPOQ	rs266729	0.837	0.59	0.275	0.581	0.957	0.258	0.682	0.774	0.654	0.693	0.274
ADIPOQ	rs17846866	0.221	0.555	0.21	0.129	0.179	0.797	0.597	0.774	0.845	0.882	0.389
KCNQ1	rs2237895	0.263	0.606	0.847	0.199	0.33	0.782	0.803	0.801	0.746	0.832	0.597
KCNQ1	rs2237892	0.501	0.215	0.937	0.502	0.775	0.868	0.546	0.16	0.039	0.866	0.968
CDKN2A/B	rs10811661	0.392	0.51	0.887	0.617	0.253	0.76	0.815	0.83	0.254	0.497	0.224
PPARG	rs1801282	0.128	0.207	0.988	0.743	0.42	0.4	0.411	0.921	0.101	0.782	0.456
CAPN10	rs3792267	0.977	0.433	0.664	0.427	0.889	0.061	0.374	0.458	0.964	0.341	0.47
CAPN10	rs5030952	0.88	0.852	0.949	0.566	0.849	0.828	0.425	0.373	0.04	0.773	0.912

Data are p values obtained from linear regression analysis adjusted for age, residential area and BMI when BMI is not an outcome. Abbreviations: *FTO* Fat mass and obesity associated gene; *MC4R* Melanocortin 4 Receptor ; *TCF7L2* Transcription factor 7-like 2; *ADIPOQ* Adiponectin*; KCNQ1* Potassium voltage-gated channel subfamily Q member 1; *CDKN2A/2B* Cyclin dependent kinase inhibitor 2A/2B; *PPARG* Peroxisome proliferator-activated receptor gamma and *CAPN10* Calpain 10; BMI Body mass index; WC Waist circumference; HbA1C glycated haemoglobin A1c; HDL-C High-density lipoprotein cholesterol; LDL-C Low-density lipoprotein cholesterol; SBP Systolic blood pressure; DBP diastolic blood pressure.

Chapter 4 Interaction between Metabolic Genetic Risk Score and Dietary Fatty Acid Intake on Central Obesity in a Ghanaian Population.

Published (The published version of the paper is attached as an appendix at the end of the thesis).

Alsulami S.; Nyakotey, D. A.; Dudek, K.; Bawah, A. M.; Lovegrove, J. A.; Annan, R. A.; Ellahi, B.; Vimaleswaran, K. S. Interaction between Metabolic Genetic Risk Score and Dietary Fatty Acid Intake on Central Obesity in a Ghanaian Population. *Nutrients* **2020**, 12(7),1906; https://doi.org/10.3390/nu12071906

Sooad Alsulami's contribution**:** The analysis plan was developed and the dataset was screened before running the statistical analysis. The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA) and the data was interpreted. The literature search was carried out to prepare the first draft of the manuscript which was further revised based on the comments from other co-authors. The manuscript was formatted according to the journal's guidelines and the reviewer comments were responded.

4.1 Abstract

Background: Obesity is a multifactorial condition arising from the interaction between genetic and lifestyle factors. We aimed to assess the impact of lifestyle and genetic factors on obesity-related traits in 302 healthy Ghanaian adults.

Methods: Dietary intake and physical activity were assessed using a 3 day repeated 24 h dietary recall and global physical activity questionnaire, respectively. Twelve single nucleotide polymorphisms (SNPs) were used to construct 4-SNP, 8-SNP and 12-SNP genetic risk scores (GRSs).

Results: The 4-SNP GRS showed significant interactions with dietary fat intakes on waist circumference (WC) (Total fat, $P_{interaction}$ = 0.01; saturated fatty acids (SFA), $P_{interaction}$ = 0.02; polyunsaturated fatty acids (PUFA), $P_{interaction}$ = 0.01 and monounsaturated fatty acids (MUFA), $P_{interaction}$ = 0.01). Among individuals with higher intakes of total fat (>47 g/d), SFA (>14 g/d), PUFA (>16 g/d) and MUFA (>16 g/d), individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles.

Conclusion: This is the first study of its kind in this population, suggesting that a higher consumption of dietary fatty acid may have the potential to increase the genetic susceptibility of becoming centrally obese. These results support the general dietary recommendations to decrease the intakes of total fat and SFA, to reduce the risk of obesity, particularly in individuals with a higher genetic predisposition to central obesity.

4.2 Introduction

Obesity is a known risk factor for several health conditions, including type 2 diabetes, cardiovascular diseases, hypertension and cancer, and hence it is considered as an increasing public health problem worldwide, including in Africa (392, 393). Obesity prevalence varies widely between African countries with a range of 5.3% in Uganda to 30% in Nigeria and 45.7% in South Africa (393). A recent systematic review has reported that nearly 43% of Ghanaian adults are either overweight or obese and that the prevalence of overweight and obesity was higher in women and urban dwellers (266). While obesity is strongly affected by changes in environmental factors (such as dietary intake, sedentary lifestyle, and urbanization), the composition of the gut microbiome, the disruption of circadian rhythms, exposure to endocrine-disrupting chemicals and epigenetic modifications (394-399), it also has strong genetic determinants with a heritability rate from 40 to 70% (80, 400). Genome-wide association studies (GWAS) in European populations have revealed more than 100 loci to be associated with the body mass index (BMI) (78, 284, 401-405). However, these genetic associations have not been consistently replicated in African populations (406-410), which could be attributed to differences in lifestyle and genetic architecture (3).

Given that single nucleotide polymorphisms (SNPs) have relatively small effect sizes on obesity, several studies have aggregated information from multiple-risk variants into a polygenic genetic risk score (GRS) (78, 155, 156, 284, 411-413). Employing a combined risk allele score is an efficient and effective approach in maximising statistical power, decreasing the drawback of multiple testing, and widening the generalisable nature of genetic associations (155, 156). A study among a rural population of Gambia demonstrated a positive association between a GRS of 28 SNPs and BMI and adult weight, whereas no association was found with the single SNP analysis (161, 162). Although genetic research in Africans is

increasing in numbers (409), only a few studies have examined the association of GRS with obesity in Africa (161, 414, 415), which highlights the need for further research in African populations.

Current evidence has shown that heritability estimates for obesity-related traits can be modified by lifestyle factors such as diet and physical activity. Several studies have reported significant GRS–diet interactions on obesity-related traits. Studies in European populations have shown that the genetic association with BMI was stronger with higher intakes of sugar-sweetened beverages (SSBs) and fried foods than among those with lower intakes (416, 417). Studies have also shown that genetic associations with BMI in Europeans can be modified by the levels of physical activity, television watching, and changes in sleep pattern (268, 389). In addition, higher adherence to healthy eating patterns have shown to reduce BMI in Europeans despite having increased genetic susceptibility to obesity (418). Gene–lifestyle interaction studies have largely been conducted in populations of European ancestry, and the replication of these studies in African populations remains unknown (267, 268). Therefore, our study aimed to investigate the association of GRS with obesity-related traits and to examine whether lifestyle factors such as dietary intake and physical activity modified these associations in the Ghanaian population.

4.3 Methods

4.3.1 Study Population

The Genetics of Obesity and Nutrition in Ghana (GONG) study is a cross-sectional study that was conducted in the Oforikrom Municipality in Kumasi, Ashanti region, Ghana. The GONG study was conducted as part of the ongoing GeNuIne (Gene–Nutrient Interactions) Collaboration, the main objective of which is to investigate the effect of gene–

nutrient interactions (nutrigenetics) on metabolic disease outcomes using population-based studies from various ethnic groups (222, 419). The Oforikrom Municipal Assembly is one of the five Municipal Assemblies carved out of the Kumasi Metropolitan Assembly. There are seventeen recognized communities in this Municipal Assembly, with an estimated total population of 360,254. Five communities (Ayeduase, Bomso, Ayigya, Oforikrom and Kotei) were randomly selected from the list of communities in the Oforikrom Municipal Assembly. In each community, a central point was located (a vehicle station, marketplace or other landmarks). A fieldworker entered the first house that faced either North, South, East or West of that central point, and randomly recruited one respondent from each household. Upon exiting a house, the fieldworker entered the next house, and the house-level selection process was repeated.

Three hundred and two free-living and healthy (with no physical complaints or prior diagnosis of cardiometabolic disease) adult volunteers, both men and women, were screened and recruited for the study by trained researchers. The inclusion criteria included the following: healthy individuals aged 25 to 60 years old and being Asante (both parents must be Asante). The exclusion criteria included the following: participants less than 25 years old or older than 60 years, those with existing cardiovascular complications or disease, those with a previous history of hypertension, type 2 diabetes or cardiovascular diseases, participants with any communicable or non-communicable chronic diseases, pregnant women and participants on lipid-lowering drugs, anti-diabetic drugs or anti-hypertensive drugs. A medical screening questionnaire was developed to screen participants for inclusion or exclusion from the study.

This study was approved by the Council for Scientific and Industrial Research (CSIR) Institutional Review Board (IRB) (Ref: RPN 003/CSIR-IRB/2018). In addition, this study

was approved by the Metro Director of Health Services, Kumasi (KMHD/MPHs/13). All participants signed informed consent prior to their participation.

4.3.2 Data Collection

Structured questionnaires were used to elicit information about the participants' demographic characteristics, dietary intakes, physical activity levels, sleep and sunshine exposure patterns and medical history. Fieldworkers were trained before the start of data collection. Survey instruments were also pre-tested on the 10 July 2018 to enhance the field workers' understanding of questionnaires, ensure clearness and avoid ambiguity. Data collection took place from July to September 2018.

4.3.3 Anthropometric Measurements

Height, weight, percentage of body fat and visceral fat, waist circumference (WC) and hip circumference (HC) were measured. The measurements were taken with respondents wearing light clothing. Height was measured with a stadiometer (Seca 213 mobile stadiometer, Hamburg, Germany) to the nearest 0.1 cm with participants standing upright without shoes. Weight was measured using an OMRON Body Composition Analyzer to the nearest 0.1 kg. The same equipment provided values for BMI, percentage of body fat and visceral fat. WC and HC measurements were taken using a non-extensible measuring tape with participants in light clothing. The WC was measured just above the naval to the nearest 0.1 cm whereas the HC was measured at the level of the greater trochanter to the nearest 0.1 cm. The waist-to-hip ratio (WHR) was calculated by dividing WC by HC.

4.3.4 Physical Activity and Dietary Assessments

The health-related physical activity level of participants was measured using the interviewer-administered Global Physical Activity Questionnaire (GPAQ) version 2

developed by the World Health Organization (WHO) for physical activity surveillance (420). This questionnaire contains 16 questions (P1–P16) which gather information on the respondent's engagement in physical activities under three domains or settings (work-related activity, transportation and recreational activities) as well as sedentary behaviours. The total physical activity per week was calculated in Metabolic Equivalents (MET- minutes) and the respondents who had total physical activity ≥ 600 MET- minutes/week were classified as active while those who had < 600 MET- minutes/week were classified as inactive (420).

A three-day repeated (two weekdays and one weekend) 24 h dietary recall method was used to elicit the information concerning the participants' dietary intake. Participants were requested to recollect all the meals taken as well as the times of the meal consumption in the previous day. Common household measures were used to estimate the actual quantities of foods and drinks consumed by the participants. The nutritional composition of the foods eaten was then analysed using the Nutrient Analysis Template (Food Science and Nutrition Department, University of Ghana, Accra, Ghana, 2010).

4.3.5 SNP Selection

Fifteen SNPs near or in 8 obesity-susceptibility loci were chosen for the study based on the previous GWAS for metabolic traits (78, 284, 401-405). These include Transcription factor 7-like 2 (*TCF7L2)* (rs12255372, rs7903146), melanocortin 4 Receptor (*MC4R*) (rs17782313, rs2229616), fat mass and obesity-associated (*FTO*) (rs9939609, rs10163409), adiponectin (*ADIPOQ*) (rs266729, rs17846866), Potassium voltage-gated channel subfamily Q member *1(KCNQ1)* (rs2237892, rs2237895), Cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/2B*) (rs10811661), Calpain 10 (*CAPN10*) (rs3792267, rs5030952, rs2975760) and Peroxisome proliferator-activated receptor gamma (*PPARG*) (rs1801282). Three of these SNPs, *KCNQ1* (rs2237895), *ADIPOQ* (rs17846866) and *CAPN10* (rs2975760), reported

significant deviations from Hardy–Weinberg Equilibrium (HWE) ($p < 0.05$) and were excluded from the current analysis. The detailed information of the 15 SNPs is shown in Table S1.

4.3.6 Genotyping

Blood samples for the measurement of DNA were transported in dry ice to the United Kingdom (UK). Genomic DNA was extracted from a 5 mL whole blood sample from each participant and genotyping was performed at the LGC Genomics (*http://www.lgcgroup.com/services/genotyping*), which employs the competitive allele-specific PCR-KASP® assay.

4.3.7 Construction of the Metabolic GRSs

To evaluate the combined effects of the 12 SNPs on obesity-related traits, an additive model was used to construct the unweighted metabolic GRSs (Figure 7). We did not weigh the risk alleles based on their individual effect sizes, because no previously reported effect sizes were available for these SNPs for the Ghanaian population, and it has been shown that the weighting of risk alleles may only have limited effects (421). The unweighted metabolic GRSs were calculated by the summation of the number of risk alleles across the 12 variants. The risk alleles were defined as alleles previously associated with an increased risk of obesity in the literature. To reduce the bias caused by the missing data, only those participants without any missing data were included in our metabolic GRS analysis. Different metabolic GRSs were constructed including the 12-, 8- and the 4-SNP GRSs. The 12-SNP GRS included the following SNPs: *TCF7L2 (*rs12255372, rs7903146), *MC4R* (rs17782313, rs2229616), *FTO* (rs9939609, rs10163409), *ADIPOQ* (rs266729), *KCNQ1 (*rs2237892), *CDKN2A/2B (*rs10811661), *CAPN10* (rs3792267, rs5030952*)* and *PPARG* (rs1801282), and

the score ranged from 0 to 9 risk alleles. In the 12-SNP GRS analysis, no significant results were identified which might be because four of the SNPs had a minor allele frequency (MAF) of less than 5%. Therefore, we excluded the four SNPs: *MC4R* (rs2229616), *FTO* (rs10163409), *CDKN2B (rs10811661)* and *PPAR* (rs1801282) and created an 8-SNP GRS. No significant findings were observed using the 8-SNP GRS; this might be because four of the eight SNPs (*ADIPOQ (rs266729), KCNQ1 (rs2237892)* and *CAPN10 (rs3792267, rs5030952)*) have not shown consistent associations with obesity-related traits in other populations (362, 422-426). Hence, these four SNPs were removed and a 4-SNP GRS including the SNPs (*TCF7L2* (rs12255372, rs7903146), *MC4R* (rs17782313), *FTO* (rs9939609)) that have shown consistent associations with obesity across several populations was constructed. The 4-SNP GRS ranged from 0 to 6 risk alleles and significant results were observed. Based on the median number of each GRS, the individuals were separated into two groups. Given that there were no previously reported effect sizes available for these SNPs for the Ghanaian population, we were unable to perform sample size calculation.

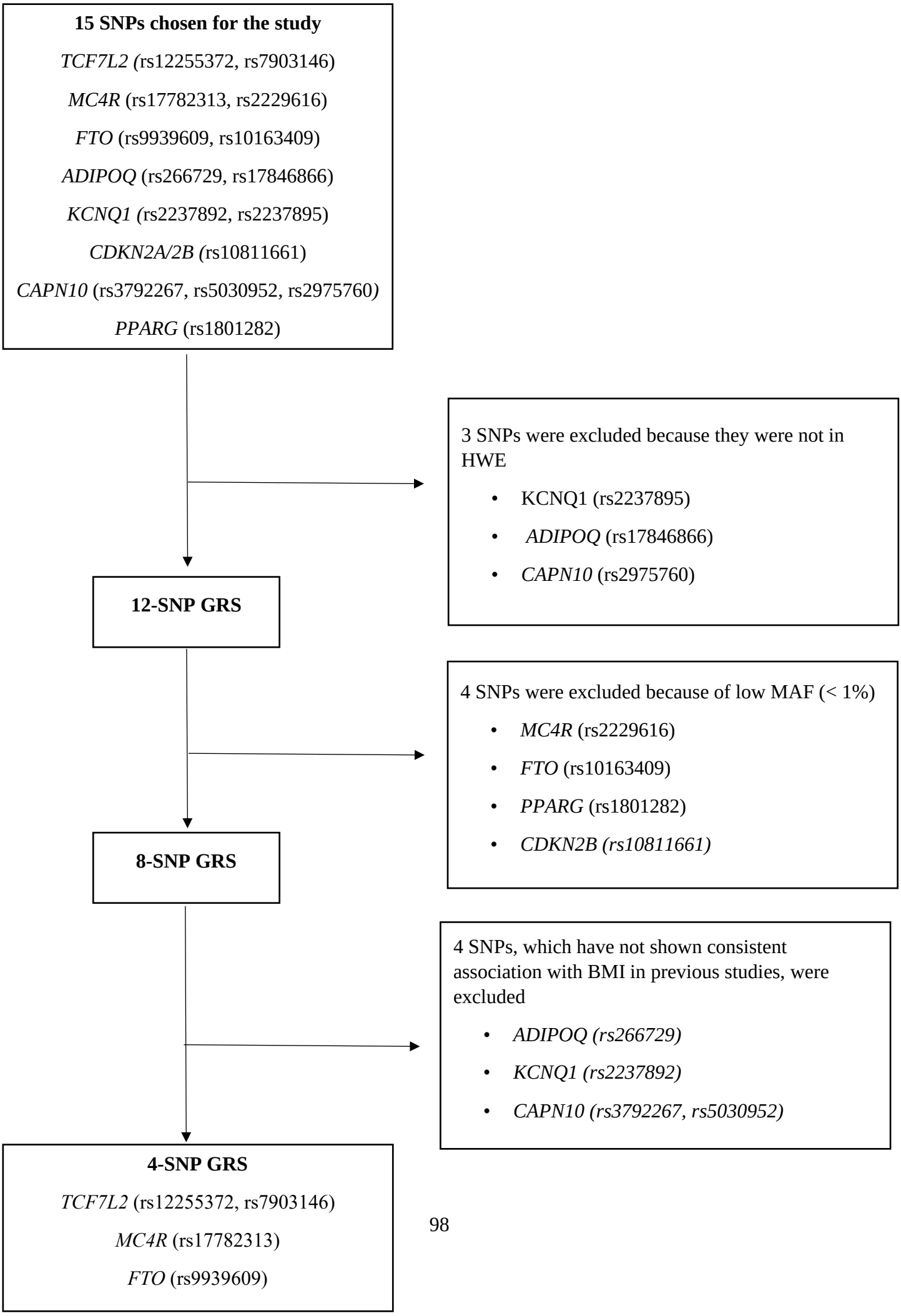
15 SNPs chosen for the study
TCF7L2 (rs12255372, rs7903146)
MC4R (rs17782313, rs2229616)
FTO (rs9939609, rs10163409)
ADIPOQ (rs266729, rs17846866)
KCNQ1 (rs2237892, rs2237895)
CDKN2A/2B (rs10811661)
CAPN10 (rs3792267, rs5030952, rs2975760)
PPARG (rs1801282)
3 SNPs were excluded because they were not in HWE
KCNQ1 (rs2237895)
ADIPOQ (rs17846866)
CAPN10 (rs2975760)
12-SNP GRS
4 SNPs were excluded because of low MAF (< 1%)
MC4R (rs2229616)
FTO (rs10163409)
PPARG (rs1801282)
CDKN2B (rs10811661)
8-SNP GRS
4 SNPs, which have not shown consistent association with BMI in previous studies, were excluded
ADIPOQ (rs266729)
KCNQ1 (rs2237892)
CAPN10 (rs3792267, rs5030952)
4-SNP GRS
TCF7L2 (rs12255372, rs7903146)
MC4R (rs17782313)
FTO (rs9939609)

Figure 7: Steps involved in the construction of the metabolic GRS.

Fifteen SNPs were genotyped in our study; however, the GRS analysis was based only on 12 SNPs as 3 SNPs were not in the HWE. Three different GRSs, including the 12-SNP GRS, 8-SNP GRS and the 4-SNP GRS were constructed. In the 12-SNP GRS analysis, no significant results were identified, which could be because 4 of the SNPs had MAF of less than 5%. Therefore, the 4 SNPs were excluded, and an 8-SNP GRS was created. No significant findings were observed using the 8-SNP GRS; this could be because four of the eight SNPs have not shown a consistent association with obesity-related traits in other populations. Hence, these four SNPs were removed and a 4-SNP GRS including those SNPs that have shown consistent associations with obesity across several populations was constructed. Abbreviations: SNP: single nucleotide polymorphisms; GRS: genetic risk score; HWE: Hardy–Weinberg equilibrium; MAF: minor allele frequency.

Statistical analysis

Data analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA) and the research analysis plan is included as an appendix on Page 244. A natural log transformation was used for the non-normally distributed variables. Unadjusted differences of descriptive characteristics between the overweight/obese and non-obese participants were calculated using an independent samples *t*-test for continuous variables. General linear models were used to examine the association between the metabolic GRSs and obesity traits. GRS–lifestyle interactions were analysed by including the interaction terms in these models. Models were adjusted for covariates including sex, age and BMI (when BMI is not an outcome). Nutrient–GRS interaction analysis was additionally adjusted for total energy intake. All GRS–lifestyle interactions reaching a nominal level of significance ($p < 0.05$) were investigated further using binary

analysis. Based on the median intake of total fat saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), the individuals were separated into two groups: ‘’below the median group’’ and ‘’above the median group”. Within each group, the association between the GRS and the outcome was examined. We also tested for GRS–sex interactions to test if sex influenced the genetic associations with obesity traits. The lifestyle factors investigated in our study included physical activity and the total dietary intake of fat, protein, carbohydrate and fibre. Significant interactions between the GRS and the total fat intake were further investigated to examine the influence of fat subtypes including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Two-tailed value of $p < 0.05$ was considered statistically significant.

4.4 Results

4.4.1 Characteristics of the Study Participants

The anthropometric and dietary characteristics of the study participants are presented in Table 8. The mean age and BMI of the total sample were 38.17 ± 9.64 years and 26.63 ± 4.99 kg/m^2, respectively. Overweight/obese individuals were older than the non-obese ($p < 0.05$). Moreover, the dietary intakes were significantly different between the two groups. Overweight/obese individuals reported significantly lower intakes of total calories, protein, carbohydrate, total fat, fibre, SFA, MUFA and PUFA compared to the non-obese ($p < 0.05$). Women had significantly higher levels of BMI, body fat percentage and WHR compared to men, despite the men consuming significantly higher levels of carbohydrate, protein and fat ($p < 0.05$) (Table S2).

4.4.2 Effect of Metabolic GRSs on Obesity-Related Traits

We first investigated the combined effect of 12 common SNPs on obesity-related traits and no significant associations were observed (Table S3). Similar results were found using an 8-SNP GRS (Table S4) and a 4-SNP GRS (Table 9).

Table 8: Characteristics of the study participants.

	Total (N = 302)	Non-Obese * (N = 126)	Overweight/Obese ** (N = 176)	*p* Value ***
Age (years)	38.17 ± 9.64	35.96 ± 9.55	39.75 ± 9.42	0.001
BMI (kg/m^2)	26.63 ± 4.99	22.01 ± 1.79	29.95 ± 3.75	<0.001
WC (cm)	88.48 ± 12.41	77.99 ± 7.13	96.00 ± 9.61	<0.001
WHR	1.45 ± 6.96	1.55 ± 7.76	1.38 ± 6.34	0.84
Visceral fat (%)	8.02 ± 7.39	6.49 ± 10.97	9.12 ± 2.26	0.01
Body fat (%)	33.12 ± 13.90	22.05 ± 12.47	41.05 ± 8.36	<0.001
Total energy intake (%)	1647.93 ± 685.83	1772.17 ± 723.85	1558.99 ± 644.75	0.008
Protein intake (g/day)	53.24 ± 23.73	57.38 ± 24.52	50.28 ± 22.76	0.01
Total fat intake (g/day)	51.17 ± 26.94	55.00 ± 29.29	48.42 ± 24.85	0.04
Carbohydrates intake (g/day)	239.03 ± 95.84	259.44 ± 104.01	224.42 ± 86.94	0.002
Fibre intake (g/day)	21.31 ± 10.84	23.19 ± 11.44	19.96 ± 10.21	0.01
Total SFA intake (g/day)	16.23 ± 10.36	17.41 ± 11.29	15.39 ± 9.58	0.10
Total MUFA intake (g/day)	18.08 ± 10.49	19.63 ± 11.30	16.96 ± 9.74	0.03
Total PUFA intake (g/day)	9.12 ± 5.03	10.20 ± 5.56	8.35 ± 4.47	0.002

Data presented as the means ± standard deviations. * Non-obese individuals refer to the individuals with a BMI < 25 Kg/m^2, according to the WHO classification of BMI. ** Overweight/obese cases refer to individuals with BMI ≥ 25 Kg/m^2, according to the WHO classification of BMI. *** *p* values for the differences in the means between the two groups were calculated using the independent samples t-test. Abbreviations: BMI: body mass index; WC: waist circumference; WHR: waist–hip ratio; SFA: saturated fatty

acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; WHO: World Health Organisation.

Table 9: Associations of the 4-SNP GRS on obesity-related traits.

	GRS < 3 Risk Alleles (N = 123)	**GRS ≥ 3 Risk Allele (N = 172)**	**** p* Value**
BMI (kg/m^2)	26.13 ± 0.45	26.85 ± 0.37	0.24
WC (cm)	87.13 ± 1.15	89.14 ± 0.92	0.19
WHR	2.27 ± 0.98	0.88 ± 0.01	0.18
Visceral fat (%)	7.89 ± 0.71	8.08 ± 0.55	0.43
Body fat (%)	31.75 ± 1.32	33.87 ± 1.02	0.15

* *p* Values obtained from the linear regression analysis adjusted for age, sex and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: SNP: single nucleotide polymorphism; GRS: genetic risk score; BMI: body mass index; WC: waist circumference; WHR: waist–hip ratio.

4.4.3 GRS–Lifestyle Interactions on Obesity-Related Traits

There was a significant interaction of the 4-SNP GRS with dietary fat intake (g/day) on WC (Total fat, $P_{interaction}$ = 0.01; SFA, $P_{interaction}$ = 0.02; PUFA, $P_{interaction}$ = 0.01 and MUFA, $P_{interaction}$ = 0.01, Table 10). Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with higher intakes of total fat (>47 g/day), SFA (>14 g/day), PUFA (>16 g/day) and MUFA (>16 g/day), (Figure 8a–d). There was also a significant interaction between 4-SNP GRS and dietary fibre intake (g/day) on body fat percentage ($P_{interaction}$ = 0.04). Individuals with <3 risk alleles had a significantly lower body fat percentage compared to those with ≥3 risk alleles (p = 0.02), among

individuals with a higher intake of fibre (>19 g/day). In addition, there was a significant interaction between the 4-SNP GRS and physical activity on WHR ($P_{interaction}$ = 0.002). However, the finding was not significant after stratifying them by physical activity levels. Some significant interactions were observed between the 12- and the 8-SNP GRSs and lifestyle factors on obesity-related traits Table S5 and S6), however, none of these interactions were significant after binary analysis. Given the significant differences in the dietary intakes and obesity-related outcomes between men and women, interactions between the 4-SNP GRS and sex were tested but no significant results were found (Table S7).

Table 10: Interactions between the 4-SNP GRS and the lifestyle factors on obesity-related traits.

	Protein (g/day)	Carbohydrate (g/day)	Fibre (g/day)	Fat (g/day)	SFA (g/day)	MUFA (g/day)	PUFA (g/day)	Physical Activity
BMI (kg/m^2)	0.45	0.22	0.12	0.15	-	-	-	0.76
WC (cm)	0.08	0.21	0.41	0.01	0.02	0.01	0.01	0.24
WHR	0.82	0.88	0.49	0.80	-	-	-	0.002
Visceral fat (%)	0.50	0.35	0.32	0.38	-	-	-	0.93
Body fat (%)	0.46	0.11	0.04	0.75	-	-	-	0.60

Data are *p* values obtained from the linear regression analysis adjusted for age, sex, total energy intake and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: SNP: single nucleotide polymorphism; GRS: genetic risk score; BMI: body mass index; WC: waist circumference; WHR: waist–hip ratio; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

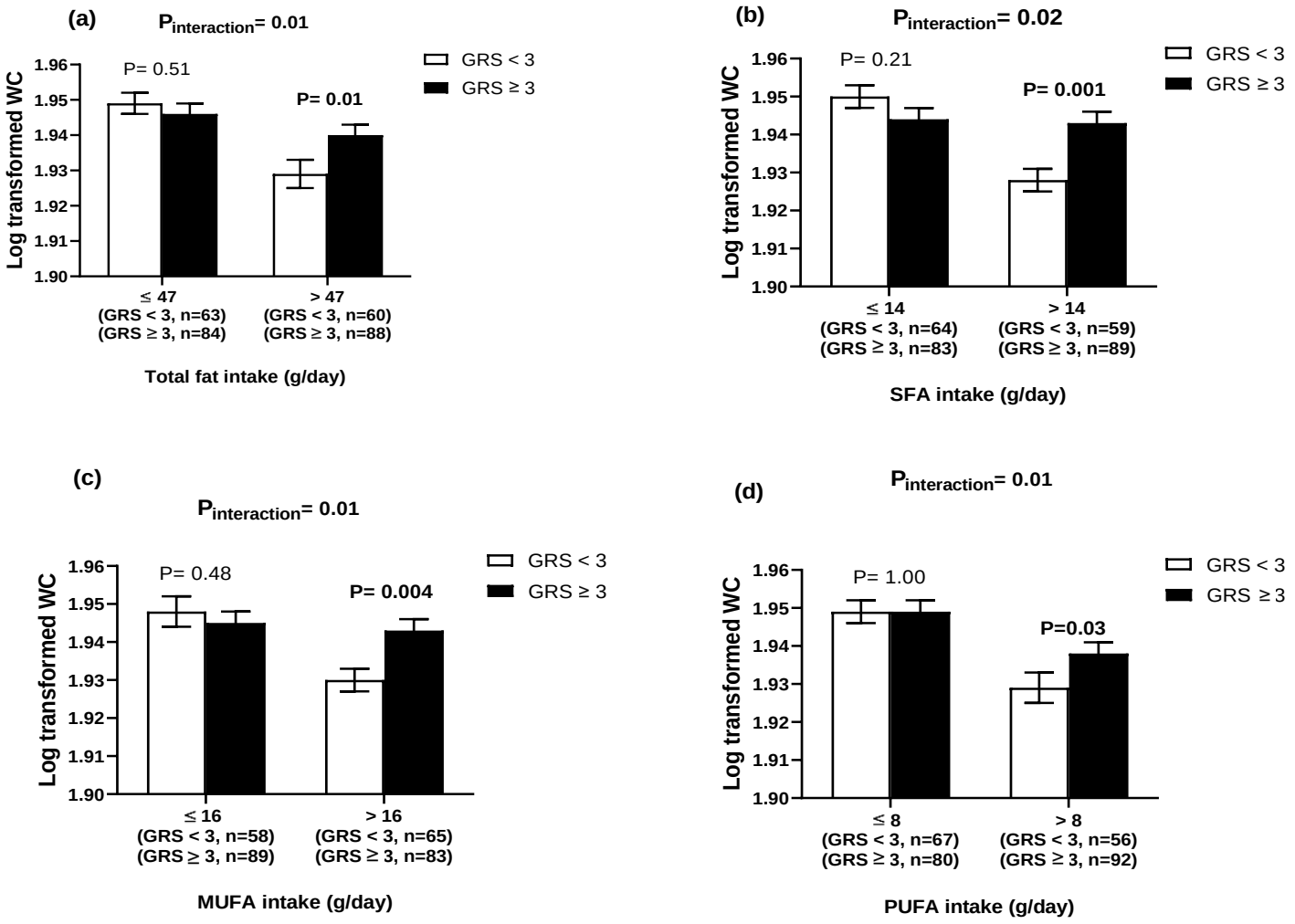

Figure 8: Interaction between the 4-SNP GRS and fat intake (g/day) on the log transformed WC.

(**a**) Interaction between the 4-SNP GRS and the log transformed total fat intake (g/day) on WC**.** White bars indicate individuals with a GRS < 3 risk alleles; the black bars indicate individuals with GRS ≥ 3 risk alleles. Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with a higher total fat intake (above median group > 47 g/day): 71.28 ± 23.68 g/day (34.99 ± 5.54 % TEI); (**b**) the interaction between the 4-SNP GRS and the log transformed SFA intake (g/day) on the log transformed WC**.** White bars indicate individuals with a GRS < 3 risk alleles; the black bars indicate individuals with GRS ≥ 3 risk alleles. Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with a higher SFA intake: 23.50 ± 10.08 g/day (12.19 ± 3.21% TEI); (**c**) the interaction between the 4-SNP GRS and the log transformed MUFA intake (g/day) on the log transformed WC. White bars indicate individuals with a GRS < 3 risk alleles; the black bars indicate individuals with GRS ≥ 3 risk alleles. Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with a higher MUFA intake: 25.72 ± 9.58 g/day (12.79 ± 2.53% TEI); (**d**) the interaction between the 4-SNP GRS and the log transformed PUFA intake (g/day) on the log transformed WC**.** White bars indicate individuals with a GRS < 3 risk alleles; the black bars indicate individuals with GRS ≥ 3 risk alleles. Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with a higher PUFA intake: 12.74 ± 4.7 g/day (6.28 ± 1.08% TEI). Abbreviations: SNP: single nucleotide polymorphisms; GRS: genetic risk score; WC: waist circumference; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TEI: total energy intake. Error bars indicate the standard error of the mean.

4.5 Discussion

To our knowledge, this is the first nutrigenetic study investigating the interaction between metabolic GRSs and lifestyle factors on obesity-related traits in a Ghanaian population. Our study provides evidence for an interaction between the 4-SNP GRS and fat intake on WC, where individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles among those who consumed a diet high in total fat, SFA, MUFA and PUFA. These results are in accordance with the general dietary recommendations, which suggest that the population decrease their intakes of total fat and SFA, to reduce the risk of obesity, and this will be more applicable in individuals with a higher genetic predisposition to obesity. Our findings are of importance to public health, considering the high consumption of foods that are rich in SFA and MUFA in the Ghanaian population (427).

Our study is the first study of its kind, investigating the effect of different metabolic GRSs (the 12-, 8- and the 4-SNP GRS) on obesity-related traits in a Ghanaian population. We found that none of the metabolic GRSs were significantly associated with obesity-related traits in the Ghanaian population, which contradicts the findings of the previous GRS-related studies in European and African populations (155, 156, 161, 284, 411-415). Efforts to replicate previously reported genetic associations of individual SNPs with obesity measures in non-African populations have shown limited success among Africans (162, 410, 428, 429), which is also in line with the findings from the present study. Several factors are likely to be involved in such discrepancies between our findings and genetic association studies in Europeans. First, the metabolic GRS in the present study was constructed based on variants strongly associated with BMI in European populations, which raises the question of the usefulness, applicability and accuracy of using this metabolic GRS in our African population. Analysing the genetic associations of such variants with obesity-related traits in African population may not be ideal

because of differences in risk allele frequency and effect size across populations (430, 431). Second, the 'lead' SNPs identified in Europeans might tag smaller regions in Africans (406, 407, 432) and the 'true' causal polymorphisms might be at different loci (433). A systematic review of genetic research in African samples has reported that more than 300 SNPs in 42 loci analysed in relation to obesity, but only a few positive associations were replicable in Africans (434). Of the 36 variants previously established by GWAS in non-African populations, only the SNPs located at the *FTO* and *MC4R* loci were significantly associated with obesity in Nigerians, Ghanaians and black South Africans (435, 436). Furthermore, in a large-scale GWAS meta-analysis consisting of 71,412 individuals of African ancestry, of the 36 previously identified BMI-associated SNPs in Europeans, only five variants reached a genome-wide significant level in Africans (437). Such inconsistencies in results are likely due, in part, to the variation in the genetic architecture between populations of different ancestry (438). African populations are characterised by greater genetic variation, reduced patterns of linkage disequilibrium (LD) and more haplotype diversity in comparison with populations of another ancestry, which may cause difficulties in replicating previously reported genetic associations (438). Hence, future studies with a larger sample size are needed to investigate the combined effect of a larger number of genetic variants on obesity-related traits in the Ghanaian population.

Our study has identified significant interactions between the 4-SNP GRS and intakes of total fat, SFA, PUFA and MUFA on WC, an indicator of central obesity that has been associated with the increased risk of morbidity and mortality (439, 440). Our findings suggest that dietary fatty acid consumption and composition may have the potential to influence the genetic susceptibility of becoming centrally obese. Evidence is limited concerning the GRS–diet interactions on obesity and its related traits, and most of the research has focused on the influence of a single locus (240, 298, 441), despite the genetic effects on obesity being

polygenic (78). Our results are consistent with previous findings generated from single-locus gene–diet interactions on obesity, in which fat intake is considered as an important lifestyle modulator of genetic associations with obesity-related traits. Two previous studies in 2163 participants from two independent United States (US) populations and in 28,449 individuals living in Malmö have shown significant interactions of the *FTO* SNP rs9939609 with total dietary fat on BMI (240, 241), however, a large-scale meta-analysis of 177,330 individuals (154,439 Whites, 5776 African Americans and 17,115 Asians) failed to identify this interaction (106). In addition, studies in 2163 participants from two US populations, 1754 French individuals and 354 Spanish children and adolescents have demonstrated a significant interaction of *FTO* SNP rs9939609 with SFAs (240, 298, 441) and MUFAs (240) on BMI. Furthermore, a study in 305 obese individuals in Finland reported that the high intake of MUFA was associated with weight loss among carriers of the risk allele (A) *FTO* rs9939609 (442). Additionally, a study in 1680 South Asians has shown a significant interaction of the risk allele 'T' of the *TCF7L2* SNP rs12255372 with fat intake on high-density lipoprotein cholesterol (HDL-C) (261). Studies on GRS–diet interactions on obesity traits have mainly focused on European populations (384, 385, 443). In agreement with our study, data from UK Biobank (385) and two studies from the US (443) have reported significant interactions between the GRS and dietary intakes of total fat and SFA on WC; the GRS was associated with a higher WC among individuals with high intakes of total fat and SFA. However, the interactions on BMI were not identified in the present study, which contradicts the previously reported findings (385, 443). Hence, larger studies are required to replicate our GRS–fat intake interactions on WC in the Ghanaian population.

Several studies have investigated the impact of dietary fat on obesity measures; however, the findings have been inconsistent (444). For instance, prospective studies have examined the relationship between the intake of long-chain omega-3 (LC n−3)-PUFAs and adiposity, but

results have been inconsistent. A study in 124 adults living in the UK found that the plasma levels of n-3 PUFA were negatively associated with anthropometric measures of obesity (445), whereas positive associations were reported in a study of 79,839 women living in the US (446). However, no effect of n-3 LC-PUFA consumption on BMI was found in a 12 year follow-up US cohort of 43,671 men (447). In a randomised controlled trial (RCT) of 27 women, the intake of a 3 g/d of fish oil (1.8 g n−3 PUFAs) for 2 months was associated with adiposity reduction (448). Similar findings were reported in an RCT of 324 men and women from Iceland, Spain and Ireland, in which the intake of either lean fish (3 × 150 g portions of cod/week) or fatty fish (3 × 150 g portions of salmon/week), or fish oil (docosahexaenoic acid/eicosapentaenoic acid capsules) for 8 weeks were associated with weight loss in men (449). However, a 6 week RCT in 195 UK adults found no differences in the anthropometric measures between three intervention diets of specific fatty acid compositions of total energy intake (TEI) (%TEI SFA:%TEI MUFA:%TEI omega-6 PUFA): SFA-rich diet (17:11:4), MUFA-rich diet (9:19:4)or omega-6 PUFA-rich diet (9:13:10) (450). A meta-analysis of 534,906 European individuals revealed that the higher adherence to the Mediterranean diet, which is rich in MUFA, was associated with a beneficial effect on WC (451). However, a recent 4 week intervention found no significant effect of the intake of 50 g/day of olive oil, which is rich in MUFA, on BMI or central obesity in 91 UK adults (452). Conflicting evidence exists regarding the effects of dietary fat on obesity-related traits; this could be because of the genetic heterogeneity and the gene–diet interactions that vary across multiple ethnic groups (453); hence, the influence of both genetic and lifestyle factors should be considered in understanding the pathophysiology of obesity.

In 2018, the WHO recommended that the intake of total fat and SFA should not exceed 30% and 10% of TEI, respectively, to avoid weight gain (454). According to the WHO, the recommended range for PUFA for the general population is 6–11% of TEI (455). It has been

identified that the average consumption of SFA in Africa is between 8.9% and 12.5% TEI (North: 9.1%, Central: 12.2, Eastern: 10.7%, Southern: 8.9% and Western Africa: 12.5%; which is slightly higher than the ≤10% TEI recommended by the WHO). The intake of PUFA is low in many sub-Saharan African countries, suggesting the infrequent use of vegetable oils for cooking or preparing foods (456). The extremely low intake of n-3 long chain PUFA was also identified in Africa, which is explained by the low availability of fish in sub-Saharan Africa countries (456). In the present study, the average consumption of total fat intake was 23.04 ± 9.13% of TEI and the average consumption of SFA, MUFA and PUFA were 8.95 ± 4.10, 9.86 ± 3.65 and 4.99 ± 1.61% of TEI, respectively, which are in accordance with general dietary recommendations. However, nearly one third of the study population had a high consumption of total fat (mean intake: 34.99 ± 5.54 g/day), the group in which the GRS showed a significant association with a higher WC. Hence, our study suggests that following the general dietary recommendations might be an effective way to overcome the genetic susceptibility to central obesity.

The strengths of our study include the analysis of gene–lifestyle interactions in a well characterized Ghanaian population and the use of different metabolic GRSs to maximise statistical power and to reduce multiple testing (155, 156). Nevertheless, some limitations need to be acknowledged. First, our analysis included an only Ghanaian population, which limits the generalisability of our results to other population groups within Africa. Second, our metabolic GRSs were constructed based on BMI-associated loci predominantly identified in Europeans, which might not truly reflect the genetic associations with BMI among Africans. Third, the food intakes were assessed using repeated 24-hour dietary recall method, which is prone to reporting bias and this might have contributed to the discrepancy in the caloric consumption between overweight/obese and non-obese groups (457). Fourth, as with any cross-sectional study design, residual confounding might exist, despite adjustments for several confounding

factors. Fifth, our sample size was small; however, our study had sufficient statistical power to detect significant gene–diet interactions.

4.6 Conclusions

In conclusion, our study has shown that higher intakes of total fat, SFA, MUFA and PUFA can increase the genetic risk on WC in Ghanaian adults. We found that the effect of metabolic risk alleles on WC is stronger among individuals with higher intakes of total fat, SFA, MUFA, PUFA. These results give important insights into the complex interactions between dietary intake and the genetic predisposition to central obesity and highlight the importance of personalising dietary advice according to each ethnic group. Our GRS approach provides insights into cumulative genetic susceptibility; however, studies with a large sample size will be needed to confirm the findings before public health recommendations and personalized nutrition advice can be developed for the Ghanaian population.

Supplementary Materials: The supplementary materials are included at the end of this chapter. Table S1: Genotype distribution of the fifteen SNPs that were included in the metabolic GRS; Table S2: Characteristics of the study participants stratified based on sex; Table S3: Associations of the 12-SNP GRS with obesity-related traits; Table S4: Associations of the 8-SNP GRS with obesity-related traits; Table S5: Interactions between the 12-SNP GRS and lifestyle factors on obesity-related traits' Table S6: Interactions between the 8-SNP GRS and lifestyle factors on obesity-related traits; Table S7: Interactions between the 4-SNP GRS and sex on obesity-related traits.

Author Contributions: Conceptualization, K.S.V.; methodology, K.S.V. and S.A.; software, S.A., D.A.N. and A.-M.B..; validation, D.A.N. and A.-M.B.; formal analysis, S.A. and K.D.; investigation, S.A., R.A.A.; resources, K.S.V., R.A.A. and B.E.; data

curation, K.S.V., S.A. and R.A.A.; writing—original draft preparation, S.A. and K.S.V.; writing—review and editing, K.S.V., S.A., D.A.N., J.A.L, R.A.A., and B.E.; supervision, K.S.V.; project training and administration, K.S.V., R.A.A, and B.E.; funding acquisition, K.S.V. and B.E. All authors have read, edited and approved the published version of the manuscript.

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

4.7 Supplementary materials

4.7.1 Table S1: Genotype distribution of the fifteen SNPs that were included in the metabolic-GRS.

Gene	SNP	Genotype	Genotype Frequency	Nucleotide change	MAF	dbSNP* frequency in Africans	HWE
TCF7L2	rs12255372	GG TG TT	117 144 37	G/T	T= 0.37	G=0.73 T=0.27	0.47
TCF7L2	rs7903146	CC TC TT	162 111 24	C/T	T= 0.27	C=0.74 T=0.26	0.42
MC4R	rs17782313	CC TC TT	19 110 168	C/T	C= 0.25	T=0.66 C=0.34	0.86
MC4R	rs2229616	GA GG	9 290	A/G	A= 0.02	G=0.99 A=0.01	0.79
PPAR	rs1801282	CC GC	298 1	C/G	G=0	C=1 G=NONE	0.98
FTO	rs9939609	AA TA TT	60 163 76	A/T	A=0.47	A=0.52 T=0.48	0.11
FTO	rs10163409	AA TA	296 2	A/T	T=0	A=0.98 T=0.02	0.95
CDKN2B	rs10811661	CT TT	18 280	C/T	C= 0	T=0.98 C=0.02	0.59
KCNQ1	rs2237895	AA CA	230 69	A/C	C=0.12	A=0.85 C=0.15	0.02
KCNQ1	rs2237892	CC TC TT	203 90 4	C/T	T=0.16	C=0.9 T=0.1	0.09
ADIPOQ	rs266729	CC GC	248 49	C/G	G= 0.08	C=0.92 G=0.08	0.12
ADIPOQ	rs17846866	TT	297	G/T	G=0.0	T=1 G=0	-
CAPN10	rs2975760	CC TC TT	1 10 281	C/T	C= 0.02	T=0.7 C=0.3	0.01
CAPN10	rs5030952	CC TC TT	57 145 95	C/T	C=0.44	T=0.53 C=0.47	0.90
CAPN10	rs3792267	AA GA GG	1 67 227	A/G	A= 0.12	G=0.88 A=0.12	0.09

Abbreviations: SNP, Single nucleotide polymorphisms; GRS, Genetic risk score; MAF, Minor allele frequency; HWE, Hardy- Weinberg equilibrium; *TCF7L2,* Transcription factor 7-like 2; *MC4R,* Melanocortin 4 Receptor; *FTO,* Fat mass and obesity-associated; *ADIPOQ,* Adiponectin; *KCNQ1,* Potassium voltage-gated channel subfamily Q member 1; *CDKN2A/2B,* Cyclin dependent kinase inhibitor 2A/2B; *CAPN10,* Calpain 10; *PPARG,* Peroxisome proliferator-activated receptor gamma. * dbSNP database: https://www.ncbi.nlm.nih.gov/snp/

4.7.2 Table S2: Characteristics of the study participants stratified based on sex.

	Total (N=302)	**Men (N=126)**	**Women (N=176)**	**P value ***
Age (years)	38.17 ± 9.64	35.97 ± 9.02	39.74 ± 9.79	<0.001
BMI (kg/m2)	26.63 ± 4.99	23.63 ± 3.12	28.79 ± 4.96	<0.001
WC (cm)	88.48 ± 12.41	81.75 ± 10.05	93.31 ± 11.68	<0.001
WHR	1.45 ± 6.96	0.87 ± 0.09	1.86 ± 9.10	0.15
Visceral fat (%)	8.02 ± 7.39	7.99 ± 10.75	8.04 ± 3.36	0.96
Body fat (%)	33.12 ± 13.90	21.03 ± 11.53	41.78 ± 7.54	<0.001
Total energy intake (%)	1647.93 ± 685.83	1915.18 ± 710.80	1456.61 ± 599.92	<0.001
Protein intake (g/day)	53.24 ± 23.73	64.25 ± 25.10	45.36 ± 19.21	<0.001
Total fat intake (g/day)	51.17 ± 26.94	58.20 ± 29.71	46.13 ± 23.60	0.001
Carbohydrates intake (g/day)	239.03 ± 95.84	279.36 ± 102.02	210.16 ± 79.73	<0.001
Fibre intake (g/day)	21.31 ± 10.84	24.52 ± 11.93	19.00 ± 9.36	<0.001
Total SFA intake (g/day)	16.23 ± 10.36	18.44 ± 11.96	14.66 ± 8.74	0.004
Total MUFA intake (g/day)	18.08 ± 10.49	20.62 ± 11.60	16.25 ± 9.22	0.002
Total PUFA intake (g/day)	9.12 ± 5.03	10.39 ± 5.57	8.21 ± 4.40	0.002

Data presented as means ± standard deviations. *P values for the differences in the means between men and women were calculated using the independent t-test.

Abbreviations: BMI, Body mass index; WC, Waist circumference; WHR, Waist hip ratio, SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

4.7.3 Table S3: Associations of the12-SNP GRS with obesity-related traits.

	GRS ≤ 4 risk alleles (N=149)	**GRS > 4 risk alleles (N=135)**	**P value***
BMI (kg/m2)	26.82 ± 0.43	26.31 ± 0.40	0.74
WC (cm)	89.04 ± 1.05	87.70 ± 1.02	0.29
WHR	2.03 ± 0.81	0.88 ± 0.01	0.28
Visceral fat (%)	8.16 ± 0.63	7.95 ± 0.65	0.65
Body fat (%)	33.35 ± 1.19	32.91 ± 1.14	0.11

Data are means ± standard errors. *P values obtained from linear regression analysis adjusted for age, sex and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: SNP, Single nucleotide polymorphism; GRS, Genetic risk score; BMI, Body mass index; WC, Waist circumference; WHR, Waist hip ratio.

4.7.4 Table S4: Associations of the 8-SNP GRS with obesity-related traits.

	GRS ≤ 4 risk alleles (N=159)	GRS > 4 risk alleles (N=127)	P value*
BMI (kg/m2)	26.76 ± 0.41	26.28 ± 0.41	0.86
WC (cm)	89.02 ± 1.01	87.5 ± 1.06	0.67
WHR	1.96 ± 0.76	0.88 ± 0.01	0.31
Visceral fat (%)	8.1 ± 0.59	7.96 ± 0.69	0.66
Body fat (%)	33.19 ± 1.14	32.84 ± 1.18	0.07

Data are means ± standard errors. *P values obtained from linear regression analysis adjusted for age, sex and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: SNP, Single nucleotide polymorphism; GRS, Genetic risk score; BMI, Body mass index; WC, Waist circumference; WHR, Waist hip ratio.

4.7.5 Table S5: Interactions between the 12-SNP GRS and lifestyle factors on obesity-related traits.

	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Fibre (g/day)	Physical activity
BMI (kg/m2)	0.91	0.46	0.47	0.25	0.87
WC (cm)	0.13	0.98	0.14	0.06	0.43
WHR	0.99	0.77	0.74	0.49	0.02
Visceral fat (%)	0.96	0.62	0.66	0.75	0.54
Body fat (%)	0.22	0.89	0.09	0.11	0.50

Data are P values obtained from linear regression analysis adjusted for age, sex, total energy intake and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: SNP, Single nucleotide polymorphism; GRS, Genetic risk score; BMI, Body mass index; WC, Waist circumference; WHR, Waist hip ratio.

4.7.6 Table S6: Interactions between the 8-SNP GRS and lifestyle factors on obesity-related traits.

	Protein (g/day)	Fat (g/day)	Carbohydrate (g/day)	Fibre (g/day)	Physical Activity
BMI (kg/m2)	0.93	0.47	0.56	0.25	0.48
WC (cm)	0.07	0.82	0.07	0.02	0.83
WHR	0.95	0.76	0.76	0.50	0.04
Visceral fat (%)	0.91	0.64	0.09	0.71	0.46
Body fat (%)	0.14	0.92	0.62	0.11	0.47

Data are P values obtained from linear regression analysis adjusted for age, sex, total energy intake and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: SNP, Single nucleotide polymorphism; GRS, Genetic risk score; BMI, Body mass index; WC, Waist circumference; WHR, Waist hip ratio

4.7.7 Table S7: Interactions between the 4-SNP GRS and sex on obesity-related traits.

Interaction	P value*
4-SNP GRS*Sex interaction on BMI	0.13
4-SNP GRS*Sex interaction on WC	0.29
4-SNP GRS*Sex interaction on WHR	0.25
4-SNP GRS*Sex interaction on Visceral fat (%)	0.42
4-SNP GRS*Sex interaction on Body fat (%)	0.14

*P values obtained from linear regression analysis adjusted for age, sex and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables.

Abbreviations: SNP, Single nucleotide polymorphism; GRS, Genetic risk score; BMI, Body mass index; WC, Waist circumference; WHR, Waist hip ratio.

Chapter 5 Effect of dietary fat intake and genetic risk on glucose and insulin-related traits in Brazilian young adults

The manuscript has been submitted to the Journal of Diabetes & Metabolic Disorders and I am currently responding to the reviewers' comments.

Sooad Alsulami, Nathália Teixeira Cruvinel, Nara Rubia da Silva, Ana Carolina Antoneli, Julie A Lovegrove, Maria Aderuza Horst, and Karani Santhanakrishnan Vimaleswaran. Effect of dietary fat intake and genetic risk on glucose and insulin-related traits in Brazilian young adults. Journal of Diabetes & Metabolic Disorders. 2021. https://doi.org/10.1007/s40200-021-00863-7

Sooad Alsulami's contribution: The dataset was reviewed and cleaned before executing the statistical analysis. Then, an analytical strategy was created and approved by the primary supervisor. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) programme and the data was interpreted. A literature search was carried out for preparing the manuscript which was then revised based on the co-authors' comments. The journal's guidelines were followed for preparing the manuscript for submission.

5.1 Abstract

Purpose: The development of metabolic diseases such as type 2 diabetes (T2D) is closely linked to a complex interplay between genetic and dietary factors. The prevalence of abdominal obesity, hyperinsulinemia, dyslipidaemia, and high blood pressure among Brazilian adolescents is increasing and hence, early lifestyle interventions targeting these factors might be an effective strategy to prevent or slow the progression of T2D.

Methods: We aimed to assess the interaction between dietary and genetic factors on metabolic disease-related traits in 200 healthy Brazilian young adults. Dietary intake was assessed using 3-day food records. Ten metabolic disease-related single nucleotide polymorphisms (SNPs) were used to construct a metabolic-genetic risk score (metabolic-GRS).

Results: We found significant interactions between the metabolic-GRS and total fat intake on fasting insulin level ($P_{interaction}$=0.017), insulin-glucose ratio ($P_{interaction}$=0.010) and HOMA-B ($P_{interaction}$=0.002), respectively, in addition to a borderline GRS-fat intake interaction on HOMA-IR ($P_{interaction}$=0.051). Within the high-fat intake category [37.98 ± 3.39% of total energy intake (TEI)], individuals with ≥5 risk alleles had increased fasting insulin level (P=0.021), insulin-glucose ratio (P=0.010), HOMA-B (P=0.001) and HOMA-IR (P=0.053) than those with <5 risk alleles.

Conclusion: Our study has demonstrated a novel GRS-fat intake interaction in young Brazilian adults, where individuals with higher genetic risk and fat intake had increased glucose and insulin-related traits than those with lower genetic risk. Large intervention and follow-up studies with an objective assessment of dietary factors are needed to confirm our findings.

5.2 Introduction

Metabolic diseases, such as type 2 diabetes (T2D), have been recognised as a significant public health problem worldwide (6, 7), playing a critical role in medical impoverishment (8-11). T2D is a major contributor to morbidity and mortality and individuals with T2D have a five-fold increased risk of developing cardiovascular diseases (CVD) (458)**.** The prevalence of diabetes has increased globally (over 463 million adults) (17) but at a faster rate in low- and middle-income countries (LMICs) (459). In Brazil, the prevalence of T2D has increased by 24% between 2006 and 2019 (460) and an estimate of 65,581 deaths have been shown to be caused by diabetes among adults aged 35–80 years (461). It has been reported that the prevalence of prediabetes and T2D among Brazilian adolescents were 22.0% and 3.3%, respectively (269). Studies have also demonstrated the occurrence of cardiometabolic risk factors including abdominal obesity, high insulin levels, dyslipidaemia, and high blood pressure among Brazilian adolescents (269-271). Hence, early interventions targeting these factors might be an effective strategy to prevent or slow the progression of T2D and decrease the risk of CVD and associated premature mortality (17)**.**

Much of the increase in the prevalence of metabolic diseases in Brazil is attributed to an epidemiological transition characterised by changes in Brazilian age structure, population ageing, reduced rates of infant mortality and fertility and increased low birth weight (462-466). Changes in the cultural and socioeconomic patterns, for instance, increasing urbanisation and economic improvement, have led to negative changes in lifestyle behaviours, including physical inactivity and unhealthy diet, in the Brazilian adolescent/ young adult population (272). A previous study has shown that the intake of saturated fatty acids (SFA) was higher in adolescents than adults in Brazil (273). Animal and human studies have demonstrated an association between increased dietary fat intake and increased insulin resistance (467-469). In addition, the dietary behaviours of Brazilian young adults have been shown to be characterised

by higher intakes of unhealthy foods than middle-aged and older adults, highlighting the need for age-specific public health interventions (274).

The development of metabolic diseases such as T2D is closely linked to a complex interplay between genetic and lifestyle factors, such as diet. Numerous genetic loci have been shown to be associated with T2D (89, 253-255) and related traits (85, 87) and, to date, 243 genetic loci have been identified to be associated with the risk of T2D in multiple ethnic groups (89, 253-255). Single nucleotide polymorphisms (SNPs) have only a modest effect on disease risk, thus, generating a genetic risk score (GRS) combining several SNPs across the genome is necessary for increasing power to identify disease predisposition patterns of an individual (153). Evidence has suggested that the genetic risk of metabolic diseases can be modified by dietary intake (256-260). There are a few gene-diet interaction studies in Brazilians; however, the studies have focused only on cardiovascular disease related traits (470-472). To date, there are no GRS-diet interaction studies on metabolic traits in Brazilians. Hence, we aimed to investigate the interaction of 10 metabolic disease-related SNPs, as a GRS, with dietary intake on metabolic traits in 200 healthy Brazilian young adults.

5.3 Methodology

5.3.1 Study population

Obesity, Lifestyle and Diabetes in Brazil (BOLD) is a cross-sectional study of Brazilian healthy young adults aged 19–24 years recruited at the Federal University of Goiás (UFG) between March and June 2019. This study was conducted as part of the ongoing GeNuIne (gene-nutrient interactions) Collaboration, which aims to investigate the impact of genes and lifestyle factors on chronic diseases using data from multiple ethnic groups (221, 419). All participants completed baseline questionnaires regarding health status, demographics, and socioeconomic status. The study exclusion criteria included those who are 1) using lipid-lowering or

hypoglycemic drugs and mineral or vitamin supplements, 2) undergoing dietary interventions in the last 6 months, 3) having acute clinical conditions such as infection, inflammation, fever or diarrhoea, or confirmed diagnosis of chronic diseases such as diabetes mellitus, moderate/severe hypertension, cancer, rheumatoid arthritis and cardiovascular complications, 4) doing vigorous physical activity. In total, 416 individuals showed interest in participating in the study. However, 207 participants met the inclusion criteria and only 200 completed the study (Figure 9). Out of the 200 participants, only 194 had information on genetic and phenotypic measurements as DNA samples were not available for 6 participants. The study was approved by the Ethics Committee of the Federal University of Goiás (protocol number 3.007.456, 08/11/2018), and performed according to the ethical principles in the Declaration of Helsinki. All participants gave written informed consent for study participation.

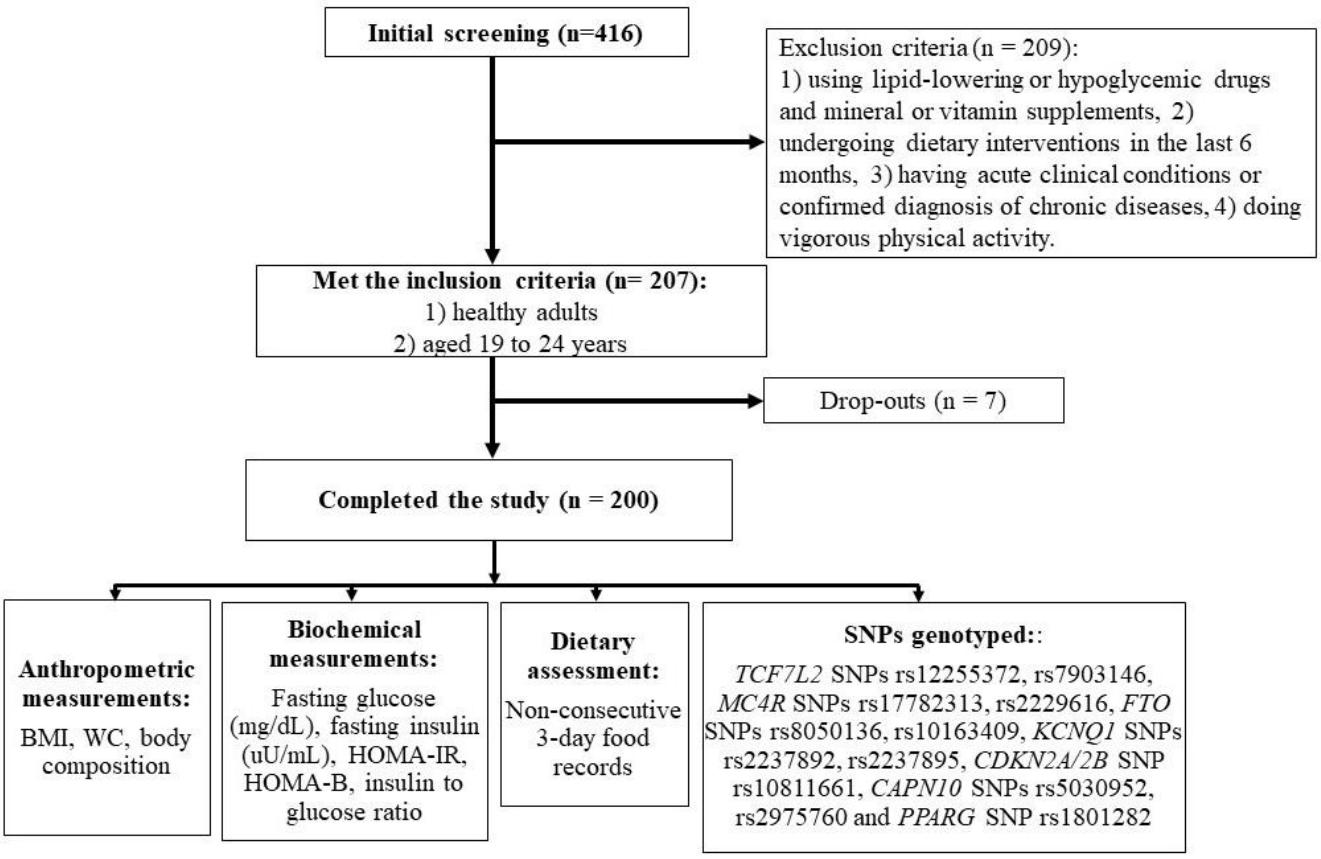


Figure 9: Flow chart showing the participant recruitment process in the BOLD study.

In total, 416 individuals were initially screened. After excluding participants based on the exclusion criteria, 207 were included in the study. However, only 200 completed the study. Abbreviations: BMI: body mass index; WC: waist circumference; HbA1c: glycated haemoglobin A1c; HOMA-IR: homeostasis model assessment estimate of insulin resistance; HOMA-B: homeostasis model assessment estimate of insulin secretion; *TCF7L2* Transcription factor 7-like 2; *MC4R* melanocortin 4 Receptor; *PPARG* Peroxisome proliferator-activated receptor gamma; *FTO* fat mass and obesity-associated; *CDKN2A/2B* Cyclin dependent kinase inhibitor 2A/2B; *KCNQ1* Potassium voltage-gated channel subfamily Q member 1 and *CAPN10* Calpain 10.

5.3.2 Anthropometric and biochemical measurements

Body weight, height and waist circumference (WC) were measured using standardized methods. The weighing was performed on a Tanita® portable electronic scale, with a maximum capacity of 150 Kg. For height, a stadiometer with a movable rod was used. WC was measured using an inelastic measuring tape. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters^2 and WC measurement was taken using a non-extensible measuring tape with partici-pants in light clothing (30). Body composition was performed by Dual Energy Radiological Absorptiometry (DXA), using the Lunar DPX NT model (General Electric Medical Systems Lunar®; Madison, USA).

Blood samples were collected by peripheral venous puncture in the morning after a 12-h fast and the volunteers were advised not to consume alcohol 72 hours before the blood collection. Samples were immediately processed after the collection at the Romulo Rocha Laboratory (Goiânia, Brazil). Fasting serum glucose and insulin were collected in BD Vacutainer® tube and determined by the enzymatic colorimetric method, with an automatic System Vitros

Chemistry 950 XRL (Johnson & Johnson, New Brunswick, NJ, USA). Plasma glycated haemoglobin (HbA1c) was collected in an ethylene-diamine-tetra-acetic acid (EDTA) tubes BD Vacutainer® and measured using high-pressure chromatography (HPLC-Bio-Rad Laboratories, Hercules, CA, USA). Plasma samples were obtained by centrifugation at 3500 rpm for ten minutes at 4°C. The homeostasis model assessment (HOMA) was used to assess the degree of insulin resistance (IR) (HOMA-IR) and β-cell function (HOMA-B). HOMA-IR and HOMA-B were calculated as follows: [fasting insulin levels (mU/l) × fasting glucose levels (mmol/l)/22.5] and [20 × fasting insulin levels)/(fasting glucose levels − 3.5], respectively (473).

5.3.3 Dietary Assessment

Food intake was assessed by trained nutritionists using non-consecutive 3-day food records, including a weekend day (474). Individuals were provided with measuring cups and spoons of different sizes to assist them in estimating portion size for each food. Foods consumed were converted into grams using the Avanutri Online® diet calculation software (Avanutri Informática Ltda, Rio de Janeiro, Brazil).

5.3.4 Genotyping

The blood samples (3ml each) were collected in an EDTA tubes BD Vacutainer® tubes and transported at a controlled temperature (- 80ºC) by the World Courier Company to perform genotyping at LGC Genomics (http://www.lgcgroup.com/services/genotyping), employing the competitive allele-specific PCR-KASP® assay.

5.3.5 SNP selection and GRS calculation

We selected 12 SNPs that have shown associations with metabolic traits in multiple ethnic groups (85, 87, 89, 253-255). The detailed information of these SNPs is shown in Table S1.

Two SNPs were excluded from the current analysis, as the Calpain 10 (*CAPN10*) rs2975760 SNP was not in Hardy-Weinberg equilibrium (HWE) and the melanocortin 4 Receptor (*MC4R*) rs2229616 SNP had a minor allele frequency (MAF) of less than 1%. Unweighted metabolic-GRS was calculated by summing the number of risk alleles present in each individual. The GRS was generated from the following SNPs: rs12255372, rs7903146 of the Transcription factor 7-like 2 (*TCF7L2*) gene, rs17782313 of the *MC4R* gene, rs8050136 and rs10163409 of the fat mass and obesity-associated (*FTO*), rs2237892 and rs2237895 of the Potassium voltage-gated channel subfamily Q member 1(*KCNQ1*) gene, rs10811661 of the Cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/2B*) gene, rs5030952 of the *CAPN10* gene, and rs1801282 of the Peroxisome proliferator-activated receptor gamma (*PPARG*) gene. Genotypes were coded as 0, 1, or 2 according to the number of metabolic-associated risk alleles that are defined based on the literature. These values were then calculated by summing the number of risk alleles for each variant. The GRS was then categorised based on the median risk alleles into two categories: "GRS <5 risk alleles" and "GRS ≥5 risk alleles".

5.3.6 Statistical analysis

Descriptive characteristics of the study population stratified by sex were presented as means and standard deviation (SDs) for continuous variables and compared using an independent samples t-test. Variables were tested for normality using Shapiro-Wilk's W test and non-normally distributed variables were log-transformed including BMI, WC, body fat mass percentage, HbA1c, fasting glucose, fasting insulin, HOMA-IR, HOMA-B, insulin to glucose ratio, total energy intake (TEI), carbohydrate %, protein %, SFA %, and polyunsaturated fatty acids (PUFA) %. We investigated the effects of metabolic-GRS on metabolic traits using general linear models. To test the interactions of the metabolic-GRS with dietary factors on metabolic traits, we included the interaction term (e.g., GRS*fat intake) in the models. The dietary factors investigated in our study included the total dietary intake of fat, protein, and

carbohydrate (percentages of TEI). Significant interactions between the GRS and the total fat intake were analysed in more depth to determine the effect of fat subtypes including SFA, monounsaturated fatty acids (MUFA), and PUFA. The GRS-nutrient interactions that reached statistical significance ($p<0.05$) were tested for the effects of the GRSs on metabolic traits according to tertiles of dietary intakes (low, medium and high intake) using general linear models. All models were adjusted for age, sex and BMI (when BMI is not an outcome). Given that insulin levels are influenced by both the capacity for insulin secretion and IR (475, 476), analysis of HOMA-B was performed with and without adjustment for IR to improve the accuracy of pancreatic β-cell function estimate. All statistical tests were two-sided, and analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA) and the analysis plan is included as an appendix on Page 248.

5.4 Results

5.4.1 Characteristics of the study participants

Table 11 summarises the characteristics of individuals in this study according to sex. Men had higher BMI, WC, fasting glucose, and lower fat mass % compared to women ($P<0.05$ for all). Men also reported higher intakes of total energy and protein than women ($P<0.05$ for all).

5.4.2 Associations between metabolic-GRS and metabolic traits

None of the associations between metabolic-GRS and metabolic-disease related traits was statistically significant except for the association with BMI ($P=0.008$) (Table 12).

Table 11: Characteristics of study participants.

Parameters	Total (n = 200)	Women (n = 147)	Men (n = 53)	*p*-Value
Age (years)	21.35 ± 1.67	21.33 ± 1.70	21.40 ± 1.61	0.815
BMI (kg/m2)	23.35 ± 4.42	22.81 ± 3.97	24.86 ± 5.23	**0.004**
WC (cm)	74.55 ± 13.56	71.10 ± 12.05	84.13 ± 13.01	**0.000**
Body fat mass (%)	33.91 ± 10.72	37.17 ± 8.77	24.84 ± 10.48	**0.000**
HbA1c (%)	4.73 ± 0.25	4.71 ± 0.25	4.78 ± 0.26	0.103
Fasting serum glucose (mg/dL)	87.18 ± 6.84	86.43 ± 6.78	89.26 ± 6.60	**0.009**
Fasting serum insulin (uU/mL)	8.74 ± 3.80	8.69 ± 3.37	8.88 ± 4.82	0.784
HOMA-IR	1.89 ± 0.88	1.86 ± 0.76	1.98 ± 1.15	0.513
HOMA-B	138.32 ± 65.75	142.47 ± 65.65	126.81 ± 65.25	0.137
Insulin to glucose ratio	0.10 ± 0.04	0.10 ± 0.04	0.10 ± 0.05	0.944
Energy (Kcal/day)	1827.81 ± 597.94	1741.52 ± 558.82	2067.15 ± 641.91	**0.001**
Protein (energy %)	17.11 ± 3.63	16.74 ± 3.33	18.14 ± 4.24	**0.016**
Carbohydrate (energy %)	51.09 ± 7.11	51.11 ± 7.01	51.05 ± 7.44	0.961
Fat (energy %)	31.66 ± 5.83	32.12 ± 5.69	30.38 ± 6.08	0.061
SFA (%)	9.43 ± 5.43	9.54 ± 6.030	9.14 ± 3.25	0.652
PUFA (%)	5.13 ± 2.27	5.08 ± 2.38	5.26 ± 1.92	0.628
MUFA (%)	7.72 ± 2.63	7.55 ± 2.55	8.19 ± 2.79	0.129

Data presented as the mean ± SDs. *P* values for the differences in the means between men and women were calculated using the independent samples t-test. Abbreviations: BMI: body mass index; WC: waist circumference; HbA1c: glycated haemoglobin; HOMA-IR: homeostasis model assessment estimate of insulin resistance; HOMA-B: homeostasis model assessment estimate of insulin secretion; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 12: Associations of metabolic-GRS with metabolic traits.

Parameters	GRS<5 (n=93)	GRS≥5 (n=101)	*p*-Value
BMI (kg/m2)	23.90 ± 0.43	22.60 ± 0.43	**0.008**
WC (cm)	75.53 ± 1.27	73.93 ± 1.26	0.967
Body fat mass (%)	35.80 ± 1.05	31.91 ± 1.10	0.663
HbA1c (%)	4.72 ± 0.03	4.73 ± 0.03	0.964
Fasting glucose (mg/dL)	87.54 ± 0.68	86.74 ± 0.72	0.419
Fasting insulin (uU/mL)	8.91 ± 0.43	8.52 ± 0.34	0.542
HOMA-IR	1.93 ± 0.10	1.84 ± 0.08	0.663
HOMA-B	138.76 ± 7.15	138.17 ± 6.32	0.234
HOMA-B adjusted for HOMA-IR	138.76 ± 7.15	138.17 ± 6.32	0.235
Insulin to glucose ratio	0.10 ± 0.00	0.10 ± 0.00	0.477

Data are Mean ± standard error of the mean (SEM). *P* values obtained from the linear regression analysis adjusted for age, sex and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: GRS: genetic risk score; BMI: body mass index; WC: waist circumference; HbAIc: glycated haemoglobin; HOMA-IR: homeostasis model assessment estimate of insulin resistance; HOMA-B: homeostasis model assessment estimate of insulin secretion.

5.4.3 Interactions of metabolic-GRS with dietary factors on metabolic traits

As shown in Table 13, there were statistically significant interactions between the metabolic-GRS and total fat intake (% of TEI) on fasting insulin level ($P_{interaction}$=0.017), insulin-glucose ratio ($P_{interaction}$=0.010) and HOMA-B ($P_{interaction}$=0.002) and a borderline interaction on HOMA-IR ($P_{interaction}$=0.051). Among those in the highest tertile of fat intake (37.98±3.39 % of TEI), individuals with ≥5 risk alleles had higher fasting insulin level (P=0.021), insulin-glucose ratio (P=0.010), HOMA-B (P=0.001) and HOMA-IR (P=0.053), compared to those with <5

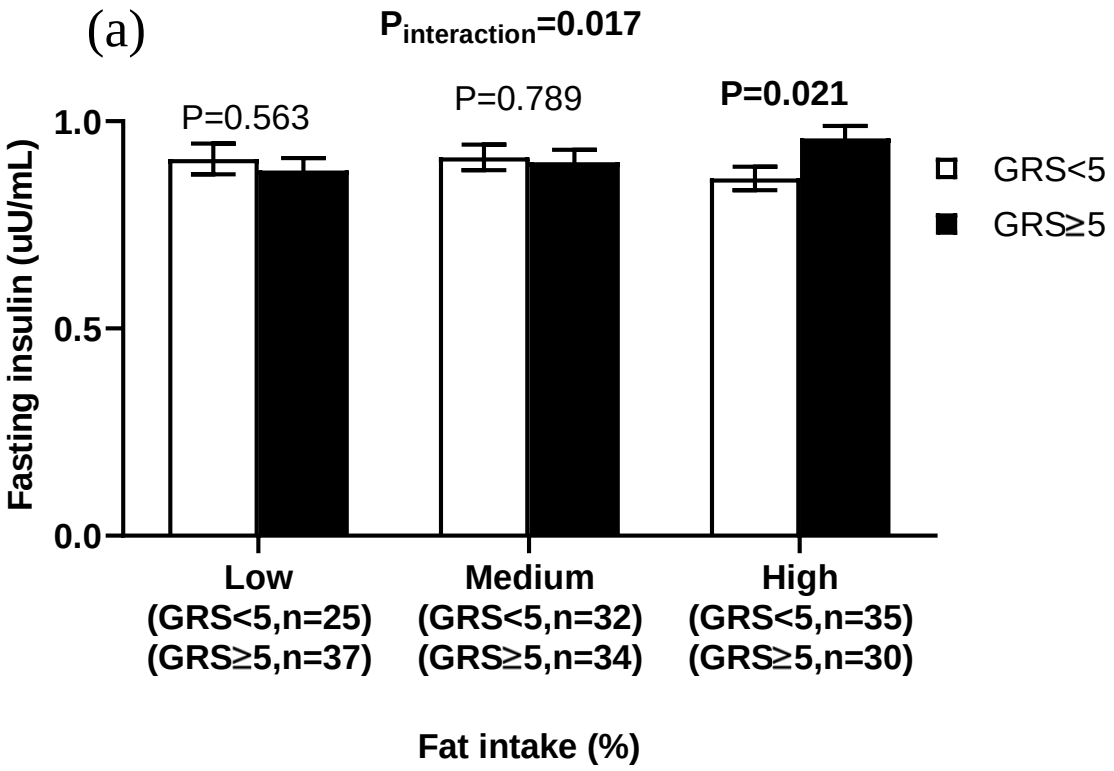
risk alleles (Figures 10 and 11). Interaction on HOMA-B was still significant after adjusting the analysis for HOMA-IR ($P_{interaction}$=0.016), Figure S1. We further examined interactions with fat subtypes on these traits. Significant interactions were detected between the metabolic-GRS and MUFA intake on fasting insulin ($P_{interaction}$=0.021), HOMA-IR ($P_{interaction}$=0.021) and insulin to glucose ratio ($P_{interaction}$=0.031), however, none of these interactions was statistically significant after tertile analysis. Significant interactions were also observed between the metabolic-GRS and intakes of total fat, PUFA and MUFA on percentage of body fat mass ($P_{interaction}$=0.009, 0.033 and 0.038, respectively).

Table 13: Interactions of the metabolic-GRS with dietary factors on metabolic traits.

	Protein (%)	Carbohydrate (%)	Fat (%)	SFA (%)	PUFA (%)	MUFA (%)
BMI (kg/m2)	0.255	0.120	0.922	-	-	-
WC (cm)	0.124	0.303	0.979	-	-	-
Body fat mass (%)	0.451	0.311	**0.009**	0.255	**0.033**	**0.038**
HbA1c (%)	0.955	0.653	0.632	-	-	-
Fasting glucose (mg/dL)	0.764	0.142	0.099	-	-	-
Fasting insulin (uU/mL)	0.898	0.37	**0.017**	0.233	0.809	**0.021**
HOMA-IR	0.944	0.561	**0.051**	0.357	0.837	**0.021**
HOMA-B	0.797	0.089	**0.002**	0.079	0.749	0.123
HOMA-B adjusted for HOMA-IR	0.784	0.084	**0.016**	0.131	0.806	0.952
Insulin to glucose ratio	0.895	0.274	**0.010**	0.154	0.801	**0.031**

Data are P values of interaction which obtained from the linear regression analysis adjusted for age, sex and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: GRS: genetic risk score; BMI: body mass index; WC: waist circumference; HbA1c: glycated haemoglobin; HOMA-IR: homeostasis model assessment estimate of insulin resistance; HOMA-B: homeostasis model assessment estimate

of insulin secretion; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.



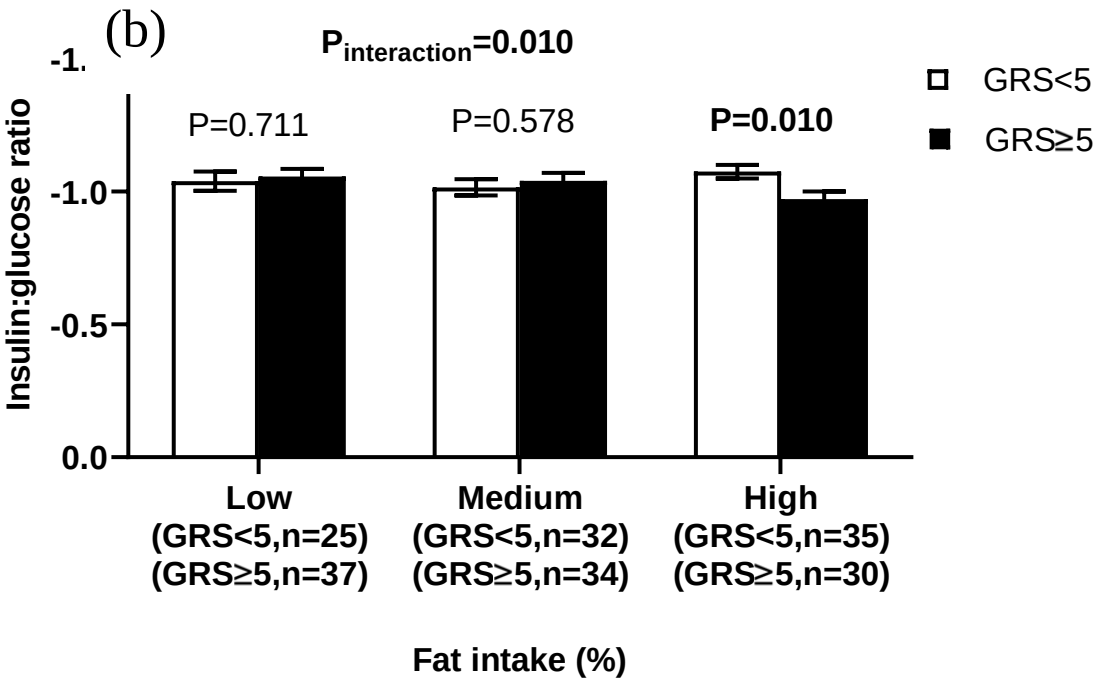


Figure 10: Interaction between the metabolic-GRS and fat intake (%) on fasting insulin levels and insulin: glucose ratio.

White bars indicate individuals with GRS <5 risk alleles; the black bars indicate individuals with GRS ≥5 risk alleles; Error bars indicate the standard error of the mean. Individuals with ≥5 risk alleles had higher fasting insulin (a) and insulin to glucose ratio (b) compared to those with <5 risk alleles, among individuals with a higher total fat intake (37.98±3.39 % of TEI). Abbreviations: GRS: genetic risk score; TEI: total energy intake.

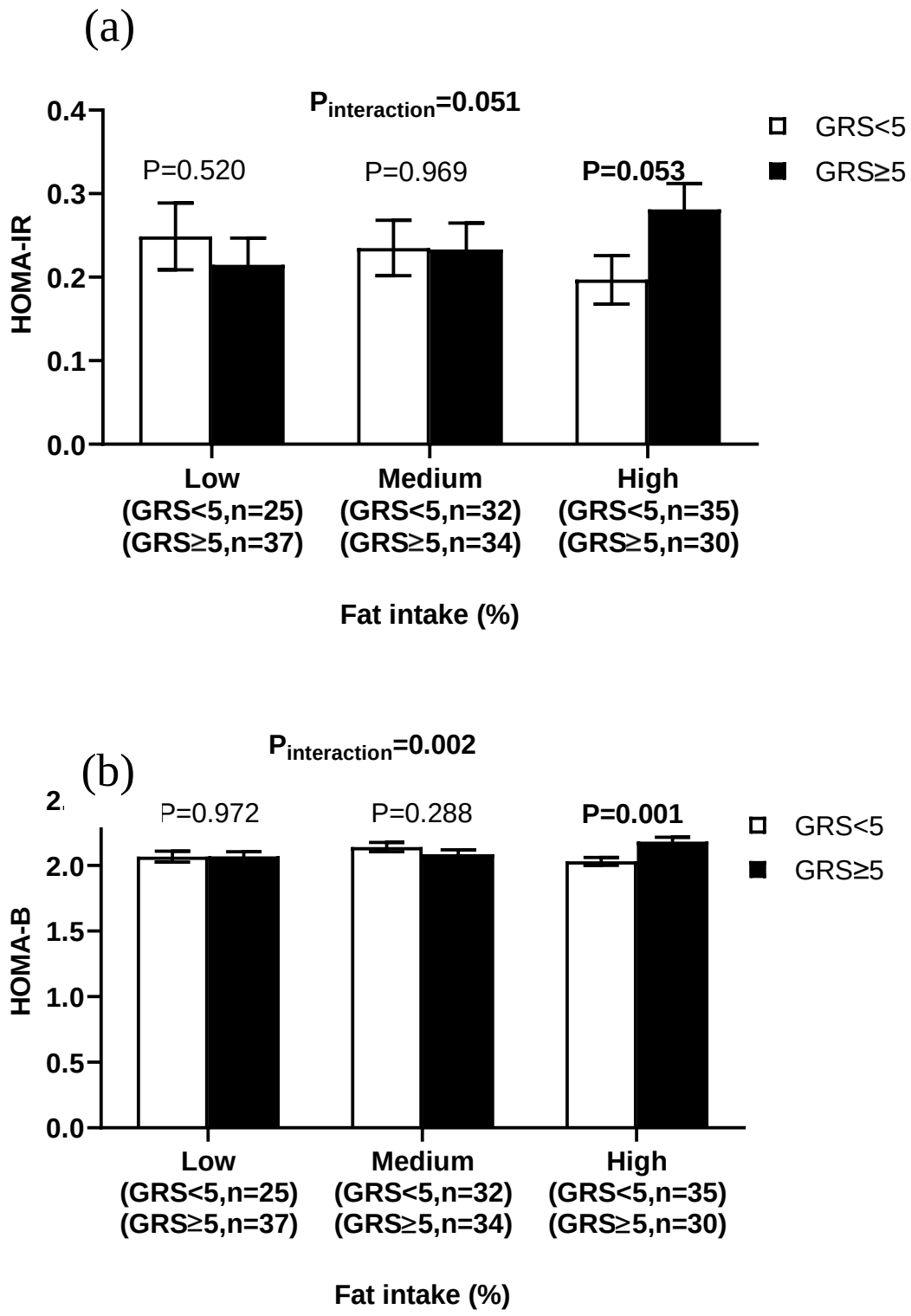


Figure 11: Interaction between the metabolic-GRS and fat intake (%) on HOMA-IR and HOMA-B.

White bars indicate individuals with GRS <5 risk alleles; the black bars indicate individuals with GRS ≥5 risk alleles; Error bars indicate the standard error of the mean. Individuals with

≥5 risk alleles had higher HOMA-IR (a) and HOMA-B (b) compared to those with <5 risk alleles, among individuals with a higher total fat intake (37.98±3.39 % of TEI). Abbreviations: GRS: genetic risk score; TEI: total energy intake; HOMA-IR: homeostasis model assessment estimate of insulin resistance; HOMA-B: homeostasis model assessment estimate of insulin secretion.

5.5 Discussion

The present study investigated the potential interplay between metabolic-GRS and dietary macronutrient intake on metabolic traits in a Brazilian young adult population. Our results provide evidence of significant GRS-fat intake interactions on glucose and insulin-related traits, where individuals with ≥5 risk alleles had higher fasting insulin level, insulin-glucose ratio, HOMA-IR and HOMA-B than those with <5 risk alleles among those in the high fat intake group (37.98±3.39 % of TEI). These findings suggest that individuals with ≥5 risk alleles are sensitive to dietary fat with respect to glucose and insulin-related traits and that these individuals may derive the most benefit from following the Brazilian dietary guidelines which aim at reducing fat intake to less than 30 % of TEI (477). This could have significant implication for public health in terms of providing early intervention to young adults with high genetic risk before the onset of disease, which might help halt the development of T2D.

In the present study, the metabolic-GRS was found to be associated with lower BMI, which contradicts the findings of the previous GRS-related studies in European populations (155, 156, 412, 413). However, the Brazilian population has a mixed genetic ancestry that originates from Europeans, Africans and Native Amerindians, which might explain the discrepancies between our findings and genetic association studies in Europeans (478). Furthermore, a large GWAS of 241,258 European adults showed that the risk allele T of *TCF7L2* rs7903146 was associated with lower BMI compared to the non-risk allele, which may provide a possible explanation of

our findings (479). Metabolic diseases are complex and multifactorial influenced by both environmental and genetic factors including dozens or even hundreds of genetic variants each contributing small effects on these traits (4, 78). Thus, the effect of unmeasured factors on BMI might influence the observed findings.

The present study found that, within the high-fat intake category, individuals with higher metabolic-GRS showed increased fasting insulin level, insulin-glucose ratio, HOMA-IR and HOMA-B, whereas those with lower GRS showed a reduction in these traits. Although direct comparison of our study with previous gene-diet interaction studies is difficult due to differences in the methodology related to the construction of GRSs and measurement of dietary intake, sample size, study design, and ethnicity, our findings are in agreement with some of the previous studies in other populations in which fat intake was found to interact with GRS on metabolic traits (256-258). In a recent study in 302 Ghanaian adults, a GRS of 4 metabolic-related variants was associated with higher WC among individuals with high fat intake (34.99±5.54 % TEI) (480). Data from an intervention study in 733 European adults also reported that higher total fat intake was associated with increased fasting glucose in individuals with higher GRS of 14 fasting glucose-associated SNPs and with decreased fasting glucose among individuals with lower GRS (256). Taken together, these observations suggest that individuals with higher genetic risk might benefit more from reducing fat intake in terms of lowering their metabolic risk.

Dietary guidelines have recommended to limit the dietary intake of total fat (between 15-30 % of TEI) to preserve overall health and reduce the risk of developing metabolic diseases (481). Previous studies have demonstrated that the higher intake of total fat contributes to the development of T2D by inducing IR (469, 482). Lowering total fat intake have been reported to improve glycemic control in a systematic review of clinical trials of diabetic individuals (483). Evidence from two previous intervention studies including individuals from various

ethnic groups (n= 3,234 and 522, respectively) and with long follow-up (2.8 and 3.2 years, respectively) have also shown that decreasing fat intake (from 6.6±0.2 % of TEI and to <30 % of TEI, respectively) is effective in reducing the incidence of T2D by up to 58% (167, 168). In addition, dietary intervention in 48,835 postmenopausal women from the US showed that reducing total fat intake (by ~8% of TEI) and increasing carbohydrate intake (by ~ 8% of TEI) through increasing intake of vegetable/fruit (five servings per day) and grain (six servings per day) were associated with a reduction in glycemia and diabetes progression (484). The dietary intake of Brazilians is characterised by unfavourable fat profile with high intakes of SFA and trans fatty acids and imbalances in the omega-6:omega-3 ratio, being compatible with a high risk of metabolic diseases (273). In our study, the mean fat intake of the total sample (31.66±5.8 % of TEI) and the high fat intake group (37.98±3.39 % of TEI) were above the recommended dietary guidelines for Brazilian adults (< 30% of TEI) (485).

The mechanisms by which dietary fat influences IR and β-cell function are unclear; however, several pathways are biologically plausible. IR is often mediated by increased inflammation that has been shown to be induced mostly by the effect of the fatty acids composition of the diet (486). In particular, SFA and omega-6 have pro-inflammatory effects, and omega-3 fatty acids have anti-inflammatory effects (486). Some of the molecular mechanisms of IR include the lipid-overload hypothesis in which ceramides or diacylglycerides are accumulated leading to the inhibition of insulin signalling and oxidative stress induced by excessive generation of free radicals or endoplasmic reticulum stress induced by excessive calorie intake (487-489). In addition to the insulin-related traits, there was also a significant interaction between GRS and intakes of total fat, PUFA and MUFA on the percentage of body fat mass in our study. Given that adipose tissue is a central metabolic organ that stores excess fat energy in the form of lipid and secretes proinflammatory adipokines that can also influence signalling of insulin, our finding is biologically plausible (490). It is worth observing the intake of SFA, PUFA and

MUFA which were significantly higher in the high fat intake category than low and medium intake groups; this might be one of the reasons for the observed interactions with total dietary fat intake. Evidence suggests that different types of dietary fat have differential effects on IR and insulin secretion. While a cross-sectional study in 538 Spanish individuals suggested that the intake of a MUFA-rich diet was associated with increased HOMA-B (491), a meta-analysis of randomised controlled feeding trials (n=4,220) demonstrated that PUFA intake showed the most consistent favourable effects in relation to improved glycaemia and insulin secretion capacity (190).

Several strengths are worth consideration. This study is the first to examine whether dietary factors interact with metabolic-GRSs on metabolic traits among the Brazilian young adult population. Early prediction of insulin sensitivity in young adults and effective intervention can be a critical factor in terms of delaying or preventing diabetes in normoglycemic individuals who are at risk of diabetes (492). Also, a GRS analysis approach was used, which has the advantage over single-locus approach (153). This approach is especially important for highly polygenic metabolic traits and can identify individuals at risk of metabolic diseases who might benefit from targeted interventions (153). Furthermore, the study outcomes (metabolic traits) were measured using validated methods by trained staff which improve the accuracy of these estimates. Nevertheless, some limitations need to be acknowledged. A major limitation is the small sample size, suggesting that our analysis might be underpowered. However, the use of the GRS approach is suggested to improve the power and significant gene-diet interactions were detected in our study. As with all observational studies, causality between exposure and outcome cannot be inferred and residual confounders might have existed. Given the longitudinal dimension of the development of T2D and the complexity of gene-diet interactions, our cross-sectional study design fails to determine the temporality of the observed findings. Given that dietary intake was assessed using self-reported measures, we cannot

exclude the effect of measurement bias. Another limitation is that the effect of different dietary sources of fat (including meat, dairy and plant) were not considered in the present analysis, which might have provided further explanations to our GRS-fat intake interactions (493). In addition, our GRS was constructed based on 10 SNPs, which account for only a small proportion of the metabolic disease-related genetic variants. As HOMA is a widely validated clinical and epidemiological tool for assessing IR and β-cell function (494), like many other epidemiological studies (256, 258, 482), we also used HOMA-IR and HOMA-B as proxies for IR and insulin secretion, respectively. However, these measures are calculated only using fasting insulin and glucose values which might provide different estimates compared to methods based on dynamic measurements of insulin and glucose responses or those derived from clamp experiments (495). Finally, given that the study was performed with relatively healthy overweight/obese young individuals with normal glucose tolerance who might have a quicker adaptation to changes in fat intake, the findings might not be applicable to those with impaired glucose metabolism or diabetes.

5.6 Conclusion

In conclusion, our study provides evidence of interactions between genetic predisposition and high fat intake on diabetes-related traits among Brazilian young adults. These findings encourage identifying Brazilian young adults with high genetic risk and tailoring dietary recommendations of fat intake according to their metabolic genetic risk profile for the primary prevention of adult-onset T2D. In addition, devising polygenic risk score could be used to provide more insights on understanding the pathophysiology of the genetics of diabetes. However, large interventional and follow up studies with a more comprehensive and objective assessment of environmental factors are needed in Brazilians to confirm our findings and to evaluate the clinical benefit of implementing precision dietary interventions based on an individual's underlying genetic risk of metabolic diseases.

Declarations

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

Availability of data and material: The data that support the findings of this study are available from the corresponding author (KSV) upon reasonable request.

Code availability: Not applicable.

Author Contributions: Conceptualization, K.S.V and M.A.H.; Methodology, K.S.V., M.A.H, and S.A.; Data Collection, N.T.C., N.R.S. and A.C.A.; Software, S.A.; Validation, M.A.H., K.S.V. and S.A.; Formal Analysis, M.A.H. and S.A.; Investigation, K.S.V and M.A.H..; Resources, M.A.H and K.S.V.; Data Curation, K.S.V and M.A.H.; Writing – Original Draft Preparation, S.A. and K.S.V.; Writing – Review & Editing, K.S.V. and S.A.; Supervision, K.S.V., M.A.H. and J.A.L.; Project Administration, K.S.V. and M.A.H.; Funding Acquisition, K.S.V. and M.A.H. All authors have read, edited, and approved the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Federal University of Goiás (protocol number 3.007.456, 08/11/2018).

Consent to participate: All participants gave written informed consent for study participation.

Consent for publication: All participants gave written informed consent for the publication of study findings.

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Supplementary Materials: The supplementary materials are included at the end of this chapter. Table S1: Genotype distribution of the twelve SNPs that were chosen for our study; Figure S1: Interaction between the metabolic-GRS and fat intake (%) on HOMA-B after adjustment of HOMA-IR.

5.7 Supplementary materials

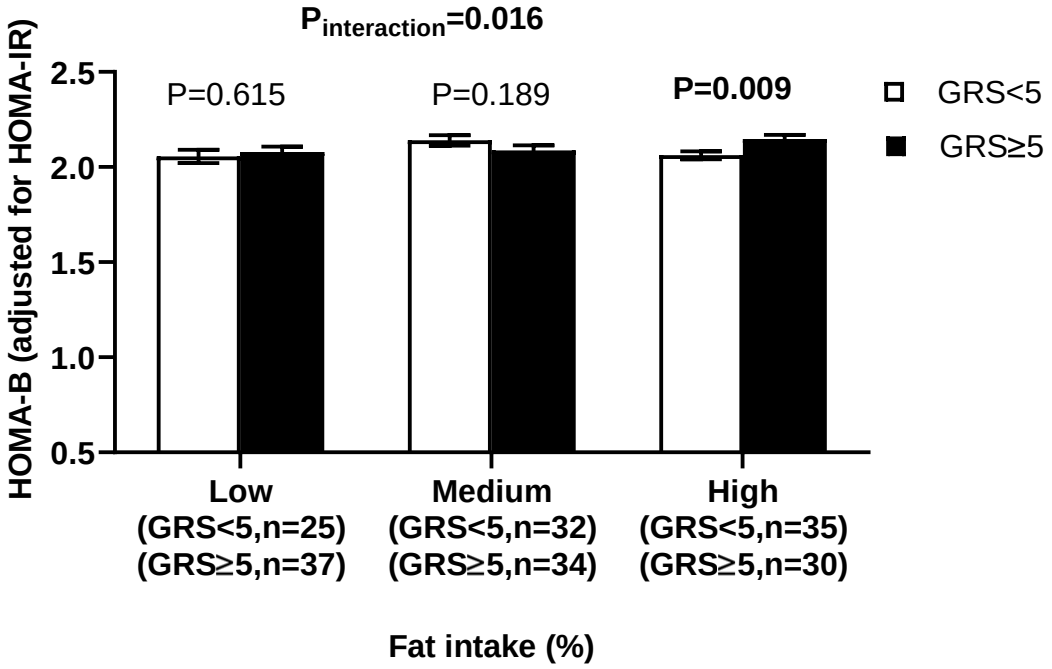
5.7.1 Table S1. Genotype distribution of the twelve SNPs that were chosen for our study.

Gene name	SNP	Location of the SNP	Genotype		Minor Allele	MAF	HWE
Transcription factor 7-like 2 *(TCF7L2)*	rs12255372	Intron Variant	GG	85	T	0.35	0.80
			TG	92			
			TT	23			
Transcription factor 7-like 2 *(TCF7L2)*	rs7903146	Intron Variant	CC	90	T	0.33	0.78
			TC	87			
			TT	23			
Melanocortin 4 Receptor *(MC4R)*	rs17782313	-	CC	6	C	0.17	0.84
			TC	55			
			TT	139			
Melanocortin 4 Receptor *(MC4R)*	rs2229616	Missense Variant	AA	0	A	0.00	0.97
			GA	1			
			GG	199			
Peroxisome proliferator-activated receptor gamma *(PPARG)*	rs1801282		CC	170	G	0.08	0.25

		Missense Variant	GC	30			
			GG	0			
Fat mass and obesity-associated *(FTO)*	rs8050136	Intron Variant	AA	30	A	0.40	0.79
			CA	96			
			CC	71			
Fat mass and obesity-associated *(FTO)*	rs10163409	Intron Variant	AA	117	T	0.25	0.06
			TA	64			
			TT	17			
Cyclin dependent kinase inhibitor 2A/2B *(CDKN2B)*	rs10811661	-	CC	4	C	0.13	0.76
			CT	45			
			TT	151			
Potassium voltage-gated channel subfamily Q member *(KCNQ1)*	rs2237895	Intron Variant	AA	85	C	0.34	0.86
			CA	91			
			CC	23			
Potassium voltage-gated channel subfamily Q member *(KCNQ1)*	rs2237892	Intron Variant	CC	160	T	0.11	0.08
			TC	35			
			TT	5			
Calpain 10 *(CAPN10)*	rs2975760	Intron Variant	CC	15	C	0.15	<0.0001
			TC	31			
			TT	154			

Calpain 10 *(CAPN10)*	rs5030952	-	CC	128	T	0.20	0.47
			TC	66			
			TT	6			

Abbreviations: SNP, single nucleotide polymorphisms; GRS, genetic risk score; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; *TCF7L2*, Transcription factor 7-like 2; *MC4R*, melanocortin 4 Receptor; *PPARG*, Peroxisome proliferator-activated receptor gamma; *FTO*, fat mass and obesity-associated; *CDKN2A/2B*, Cyclin dependent kinase inhibitor 2A/2B; *KCNQ1*, Potassium voltage-gated channel subfamily Q member 1; *CAPN10*, Calpain 10.



5.7.2 Figure S1. Interaction between the metabolic-GRS and fat intake (%) on HOMA-B after adjustment of HOMA-IR.

White bars indicate individuals with GRS <5 risk alleles; the black bars indicate individuals with GRS ≥5 risk alleles; Error bars indicate the standard error of the mean. Individuals with ≥5 risk alleles had higher HOMA-B compared to those with<5 risk alleles, among individuals with a higher total fat intake (37.98±3.39 % of TEI). Abbreviations: GRS: genetic risk score; TEI: total energy intake; HOMA-IR: homeostasis model assessment estimate of insulin resistance; HOMA-B: homeostasis model assessment estimate of insulin secretion.

Chapter 6 Lower dietary intake of plant protein is associated with genetic risk of diabetes-related traits in urban Asian Indian adults.

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Sooad Alsulami's contribution: Before running the statistical analysis, the dataset was cleaned and the analysis plan was developed for the study. Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA) was used to conduct the statistical analysis. The data was interpret and the first draft of the paper was written. The paper was revised according to co-authors' comments and suggestions. The manuscript was formatted based on the journal's guidelines before the paper was submitted to the journal.

6.1 Abstract

Background: The increasing prevalence of type 2 diabetes among South Asians is caused by a complex interplay between environmental and genetic factors.

Ami: We aimed to examine the impact of dietary and genetic factors on metabolic traits in 1,062 Asian Indians.

Methods: Dietary assessment was performed using a validated semi-quantitative food frequency questionnaire. Seven single nucleotide polymorphisms (SNPs) from the Transcription factor 7-like 2 and fat mass and obesity-associated genes were used to construct two metabolic genetic risk scores (GRS): 7-SNP and 3-SNP GRSs.

Results: Both 7-SNP GRS and 3-SNP GRS were associated with a higher risk of T2D (P = 0.0000134 and 0.008, respectively), The 3-SNP GRS was associated with higher waist circumference (P = 0.010), fasting plasma glucose (FPG) (P = 0.002) and glycated haemoglobin (HbA1c) (P = 0.000066). There were significant interactions between 3-SNP GRS and protein intake (% of total energy intake) on FPG ($P_{interaction}$=0.011) and HbA1c ($P_{interaction}$=0.007), where among individuals with lower plant protein intake (<39 g/day) and those with >1 risk allele had higher FPG (P = 0.001) and HbA1c (P = 0.00006) than individuals with ≤1 risk allele.

Conclusion: Our findings suggest that lower plant protein intake may be a contributor to the increased ethnic susceptibility to diabetes described in Asian Indians. Randomized clinical trials with increased plant protein in the diets of this population are needed to see whether the reduction of diabetes risk occurs in individuals with prediabetes.

6.2 Introduction

South Asian populations have a 50% higher risk of type 2 diabetes (T2D) than other populations (275, 276) and this has significant implications, as patients with T2D have a 2–4 times increased risk of cardiovascular diseases (275). The Asian Indian population have a unique phenotype characterised by abdominal and truncal adiposity, as indicated by larger waist to hip ratios and waist circumference (WC) and, higher concentrations of plasma insulin, greater insulin resistance, impaired function of pancreatic β-cell, and a genetic susceptibility to diabetes which ultimately leads to significantly increased diabetes risk (496-498).The burden of T2Dis increasing globally, with India being a major contributor to the worldwide burden (17).The number of diabetic individuals in India rose from 26.0 million in 1990 to 65.0 million in 2016 (277).

The increasing prevalence of T2D among Asian Indians is caused by a complex interplay between environmental and genetic factors including urbanization which plays a large role (261, 262, 499). Urbanization in India is associated with increased consumption of processed foods and dietary fats, decreased level of physical activity and increased mental stress, amplifying the effects of abdominal obesity and insulin resistance (497, 498, 500). Furthermore, the urban areas in India reported higher intake of protein from pulses and animal sources (including meat, fish, eggs and milk) than rural areas (501). Several large longitudinal studies showed that the intake of animal protein was significantly associated with the risk of T2D (180-182, 502, 503). In the context of rapid urbanisation and nutrition transition, interactions between Westernized diet, lifestyle and genetic factors have further escalated T2D prevalence in Asia (504, 505). In South Asians, several single nucleotide polymorphisms (SNPs) have been associated with adiposity (364, 506-508), insulin resistance (509), pancreatic β-cell function (159, 506, 510), and T2D(511 ,510 ,508 ,506 ,364) .The fat mass and obesity-associated *(FTO)* gene has been recognized as one of the strongest obesity-related genes. The

FTO SNPs, rs1588413, rs9939609 and rs8050136, have been shown to increase obesity risk by 1.27, 1.15 and 2.06 times among Indians, respectively(364, 512).Studies have reported strong associations of the Transcription factor 7-like 2 *(TCF7L2)*SNPs, rs7903146 and rs12255372, with T2D risk in Asian Indians(75, 513, 514). To date, evidence has identified 243 genetic loci to be associated with T2D risk in South Asians, East Asians, Europeans, African Americans and Hispanics (89, 253-255). Single genetic variants have only a small to moderate effect on disease risk, thus, combining effects of several SNPs into a genetic risk score (GRS) is required for better detection of individuals with high risk of diabetes (153).

Genome-wide association studies (GWAS) have discovered large number of genetic variants associated with metabolic diseases and related traits; however, these SNPs describe only a small proportion of estimated heritability. Risk prediction of metabolic diseases is complicated by interactions between dietary and genetic factors which may partly explain the missing heritability of diseases (216). Investigating gene-diet interaction is important in understanding pathophysiology of metabolic diseases, which can lead to the development of 'personalised' nutrition focusing on tailoring dietary interventions according to individual genotypic makeup to prevent and treat metabolic diseases (247, 515). The effect of genetic factors on metabolic traits have been shown to be modified by dietary intake in several populations (256-260). However, studies investigating GRS-diet interaction in the Indian population are still sparse. To help fill this gap in knowledge, we assessed the combined effect of 7 genetic variants, as a GRS, on T2D and metabolic traits, and the extent to which dietary intake can influence these genetic associations among 1,062 urban Asian Indians.

6.3 Methods

6.3.1 Study participants

The present study included individuals from the urban area of the Chennai Urban Rural Epidemiology Study (CURES) follow-up study, which is a cross-sectional epidemiological study performed on a representative sample of Chennai city (formerly Madras) in Southern India. The design and procedures of the CURES study have been explained in detail previously (516). In phase 1, a total of 26,001 adult subjects, of which 1,529 'self-reported' or `known diabetic' individuals, were recruited using a method of systematic random sampling. In phase 2, diabetic individuals were invited to the study centre for further investigation, of whom 1,382 responded. In phase 3, every 10th individual of the total sample (n = 26,001 subjects), excluding individuals with self-reported diabetes, were screened using an oral glucose tolerance test (OGTT). Individuals with fasting plasma glucose (FPG)<5.6 mmol/L (100mg/dL) and 2 hr plasma glucose value of 7.8 mmol/L (140mg/dL) were defined as having normal glucose tolerance (NGT) [46]. Those who had 2 hr plasma glucose value of 11.1 mmol/l (200 mg/dl) were categorised as `newly detected diabetic subjects' (n = 222) (Figure S1). The total sample of present study is 1,062 individuals; the NGT individuals were chosen from Phase 3 (n = 496) and T2D individuals were chosen from Phase 2 and Phase 3 of the CURES (n = 566). The study was approved by the Madras Diabetes Research Foundation Institutional Ethics committee and written informed consent was obtained from all study participants.

6.3.2 Anthropometric and biochemical measurements

Anthropometric variables including WC, weight and height were measured using standardised methods. The body mass index (BMI) was calculated with the formula of weight (in kilograms) divided by the square of height (in meters), with obesity being defined as BMI ≥25 according to World Health Organisation Asia Pacific Guidelines for Asians (517).

Biochemical tests were carried out using a Hitachi-912 Auto Analyzer (Hitachi, Mannheim, Germany), with kits provided by Roche Diagnostics (Mannheim). Glycated haemoglobin

(HbA1c) was measured using high-performance liquid chromatography on a Variant machine (Bio-Rad, Hercules, CA, USA). FPG and serum insulin were measured using glucose oxidase-peroxidase and an enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark), respectively.

6.3.3 Dietary assessments

Participants' habitual food intake over the previous year was measured using a validated semi-quantitative food frequency questionnaire (FFQ) administered by an interviewer (518). The FFQ consists of 222 food items and individuals were asked to estimate the usual portion size and frequency (number of times per day, week, month or year/never) of food items listed in the FFQ. Participants were shown common household measures and photographic atlas of different sizes of fruits to help them in estimating portion sizes. The EpiNu® software was used to analyse the recorded data and estimate the intake of energy and macronutrients. The reported intake of various food groups was also estimated. The EpiNu software also provided the source of protein from various food groups. Animal protein intake was summed up using protein intake (g/day from FFQ) from animal food groups such as meat, poultry, fish, egg, and dairy products. Similarly, plant protein intake was estimated from food groups such as cereals, millets, pulses legumes, tubers, nuts, oilseeds, vegetables, and fruits. In addition, dairy protein was estimated separately using the dairy products such as milk products and fermented and unfermented milk.

6.3.4 SNP selection and GRS construction

A total of 7 metabolic disease-associated SNPs which have been extensively studied in various populations including Asian Indians were selected for the study (159, 364, 506-511, 513). The selected SNPsincluded*TCF7L2* SNPs, rs12255372and rs7903146, and *FTO* SNPs, rs8050136, rs918031, rs1588413, rs7193144 and rs1076023. Details regarding these SNPs are summarised

in Tables S1. Each SNP was coded with the expected number of metabolic diseases-associated risk alleles. Consistent with previous studies (257, 519, 520), we used an unweighted method to construct the GRSs by summing the number of risk alleles of each SNP for each participant. The seven SNPs were used to generate a 7-SNP GRS that ranges from 1 to 11 risk alleles. The GRS was divided into 2 categories according to the median number of risk alleles: ‘‘GRS<6 risk alleles’’ and ‘‘GRS≥6 risk alleles’’, indicating individuals with lower and higher risk alleles of the SNPs, respectively. In addition, we constructed a GRS of 3 SNPs (*FTO*SNP rs8050136 and *TCF7L2*SNPs rs12255372 and rs7903146) that have shown consistent associations with metabolic disease-related outcomes across various ethnicities including Asians (77, 102, 521, 522). The 3-SNP GRS ranges from 0 to 6 risk alleles and was divided into 2 categories according to the median number of risk alleles: “GRS ≤1 risk allele” group and “GRS >1 risk allele” group, indicating individuals with lower and higher risk alleles of the SNPs, respectively.

6.3.5 Genotyping

The genotyping methodologies have been previously published (364, 514). The Phenol-chloroform technique was used to extract DNA from whole blood. Genotyping was performed using restriction fragment length polymorphism and confirmed by direct sequencing in which duplicate samples (n = 200; 20%) were genotyped with 100% concordance, suggesting high genotyping accuracy.

6.3.6 Statistical analysis

Descriptive statistics of continuous variables are provided as means with standard deviations (SDs) and compared between T2D and controls using an independent sample t-test. Normality tests were performed and variables with no-normal distribution were log-transformed. For each individual SNP, genotype counts were assessed for Hardy-Weinberg equilibrium (HWE) using

a goodness-of-fit chi-square test. As shown in Table S1, all SNPs were in HWE ($P > 0.092$, for all comparisons). General linear models were utilised to analyse the main associations of the GRS with metabolic traits. Interactions of the GRS with dietary intake were investigated by including the interaction term (GRS*dietary intake) in the models. Furthermore, significant interactions with protein intake were analysed in more depth according to dietary sources of protein (animal and plant protein), where individuals were classified into two groups according to the sample median intake of plant (39g/day) and animal protein (19g/day): below and above median groups. Individuals who consumed below the median were categorised as those who had lower intakes of plant and animal protein, respectively, whereas individuals who consumed above the median were categorised as those who had higher intakes of plant and animal protein, respectively. Dietary intakes as percentage of total energy intake (TEI) included intake of protein, carbohydrate, and fat. Models were adjusted for sex, age, T2D, anti-diabetic medication, and BMI (when BMI is not an outcome). Furthermore, as part of the sensitivity analysis, we further adjusted for duration of diabetes, dairy protein intake, physical activity level, smoking, alcohol consumption, and fibre intake. Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA), with a significance level of 0.05. The research analysis plan is included as an appendix on Page 251.

6.4 Results

6.4.1 Characteristics of study participants

As shown in Table 14, individuals with T2D were significantly older and had higher BMI, WC, HbA1c, FPG, insulin, compared to individuals with NGT ($P<0.05$ for all). Also, diabetic individuals had significantly higher intakes of total protein and carbohydrate, than individuals with NGT ($P<0.05$ for all).

Table 14: Characteristics of study participants

	Total		**NGT controls**		**T2D cases**		**P value**
	n		**n**		**n**		
Sex							
Men (%)	591	56	278	56	313	55	0.807
Women (%)	471	44	218	44	253	45	
Age (years)	1062	45 ± 12	496	38 ± 10	566	51 ± 11	$1.160*10^{-71}$
BMI (kg/m2)	1061	24.6 ± 4.56	496	23.5 ± 4.64	565	25.5 ± 4.30	$1.480*10^{-12}$
WC (cm)	1022	87 ± 12	479	83 ± 12	543	91 ± 10	$5.692*10^{-33}$
HBA1C (%)	1056	7.3 ± 2.4	492	5.6 ± 0.47	564	8.8 ± 2.4	$1.480*10^{-14}$
FPG (mg/dl)	1060	126 ± 64	495	85 ± 8	565	162 ± 69	$1.392*10^{-127}$
Fasting Insulin (μIU/ml)	699	9 ± 7	448	8 ± 6	251	12 ± 7	$6.386*10^{-101}$
Energy (kcal/day)	1062	2536 ± 805	496	2685 ± 708	566	2406 ± 861	8.773*10-9
Protein (%)	1062	11 ± 1	496	11.27 ± 1.17	566	11.45 ± 1.23	0.014
Animal protein (g/day)	1062	22 ± 12	496	25 ± 13	566	19 ± 11	$3.787*10^{-14}$
Plant protein (g/day)	1062	40 ± 14	496	42 ± 15	566	39 ± 13	0.006
Fat (%)	1062	23 ± 5	496	24 ± 5	566	23 ± 5	0.113
Carbohydrate (%)	1062	65 ± 6	496	64 ± 6	566	65 ± 6	0.003
Dietary fibre (g)	1062	32 ± 11	496	32 ± 10	566	31 ± 12	0.150
Total SFA (g)	1062	24 ± 10	496	27 ± 10	566	22 ± 10	$2.295*10^{-12}$
Total MUFA (g)	1062	20 ± 8	496	21 ± 8	566	18 ± 8	$3.943*10^{-9}$
Total PUFA (g)	1062	18 ± 10	496	19 ± 9	566	18 ± 10	0.184

Data presented as Mean±SD.* *P* values are for the mean differences between controls and T2D cases using an independent sample *t*-test. ** *P* values are from the Chi-squared test. Frequency of men and women between controls and cases was compared using a chi-square test. Abbreviations: NGT, normal glucose tolerance, T2D, type 2 diabetes; BMI, body mass index; WC, waist circumference; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

6.4.2 Association between metabolic GRS and metabolic traits.

After adjusting for the potential confounders, there were no significant associations between the 7-SNP GRS and metabolic traits, Table 15.

In the 3-SNP GRS analysis, significant associations were found with WC ($P = 0.010$), FPG ($P = 0.002$) and HbA1c ($P = 0.000066$), where individuals with >1 risk allele had higher WC, FPG and HbA1c compared to individuals with ≤1 risk allele, Table 15. Both 7-SNP GRS and 3-SNP GRS were associated with a higher risk of T2D ($P = 0.0000134$ and 0.008, respectively), Table 16.

6.4.3 Interaction of 7-SNP and 3-SNP GRSs with dietary factors on metabolic traits.

As shown in Table 17, there were significant interactions between the 3-SNP GRS and total protein intake (% of TEI) on FPG ($P_{interaction}=0.011$) and HbA1c ($P_{interaction}=0.007$). Among individuals with lower intake of plant protein (<39 g/day), those with >1 risk allele had higher FPG ($P = 0.001$) and HbA1c ($P = 0.00006$) than individuals with ≤1 risk allele (Figure 12). Furthermore, among individuals with higher intake of animal protein (>19 g/day), those with >1 risk allele had higher FPG ($P = 0.008$) and HbA1c ($P = 0.001$) than individuals with ≤1 risk allele (Figure S2). None of the interactions were significant between the 7-SNP GRS and dietary intakes on metabolic traits except for the interactions between 7-SNP GRS and protein intake on HbA1c ($P_{interaction}=0.032$), and 7-SNP GRS and carbohydrate intake ($P_{interaction}=0.04$) on fasting insulin. However, these interactions were not significant after stratifying based on animal and plant protein.

Table 15: Associations of 7-SNP and 3-SNP GRS and with metabolic traits

	7-SNP GRS					3-SNP GRS			
	n	**GRS <6**	***n***	**GRS ≥6**	**P value**	***n***	**GRS ≤1**	***n***	**GRS >1**	***P* value ***
BMI (kg/m2)	526	24.5 ± 0.2	535	24.7 ± 0.2	0.572	645	24.7 ± 0.2	416	24.5 ± 0.2	0.572
WC (cm)	508	86.7 ± 0.5	514	87.4 ± 0.5	0.668	620	87.0 ± 0.47	402	88.0 ± 0.57	0.010
HBA1C (%)	524	7.1 ± 0.1	532	7.4 ± 0.1	0.935	640	7.0 ± 0.1	416	7.7 ± 0.1	0.000066
FPG (mg/dL)	526	119.9 ± 2.6	534	131.6 ± 2.9	0.181	644	120.0 ± 2.35	416	135.0 ± 3.39	0.002
Fasting insulin (μIU/ml)	373	9.5 ± 0.4	326	9.4 ± 0.3	0.767	419	10.0 ± 0.36	280	9.0 ± 0.33	0.171

Data are Mean ± stander error of the mean. * P values adjusted for sex, age, T2D, anti-diabetic medication and additionally for BMI, when BMI is not an outcome. The analysis was carried out using log-transformed variables. Abbreviations: GRS: genetic risk score; BMI, body mass index; WC, waist circumference; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose.

Table 16: Association of 7-SNP and 3-SNP GRSs with T2D.

GRS	OR	95% CI for OR		*P* Value *
		Lower	Upper	
7-SNP GRS	2.083	1.496	2.898	0.0000134
3-SNP GRS	1.559	1.121	2.170	0.008

* *P* values were obtained from the logistic regression models adjusted for sex, age, anti-diabetic medication, and BMI**.** Abbreviations: GRS: genetic risk score; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; OR, odds ratio; CI, confidence interval; BMI, body mass index

Table 17: Interactions of 7-SNP and 3-SNP GRSs with dietary factors on metabolic traits

	7-SNP GRS			3-SNP GRS		
	Protein (% of TEI)	Fat (% of TEI)	Carbohydrate (% of TEI)	Protein (% of TEI)	Fat (% of TEI)	Carbohydrate (% of TEI)
BMI (kg/m2)	0.176	0.388	0.195	0.36	0.653	0.805
WC (cm)	0.852	0.786	0.892	0.638	0.958	0.914
HBA1C (%)	0.032	0.629	0.618	0.007	0.677	0.756
FPG (mg/dl)	0.249	0.489	0.507	0.011	0.367	0.231
Fasting insulin (µIU/ml)	0.952	0.085	0.04	0.299	0.567	0.999
T2D	0.956	0.214	0.152	0.764	0.508	0.365

Data are $P_{interaction}$ values adjusted for sex, age, T2D, antidiabetic medications and additionally for BMI, when BMI is not an outcome. The analysis was carried out using log-transformed variables. Abbreviations: GRS: genetic risk score; TEI, total energy intake; BMI, body mass index; WC, waist circumference; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose; T2D, type 2 diabetes.

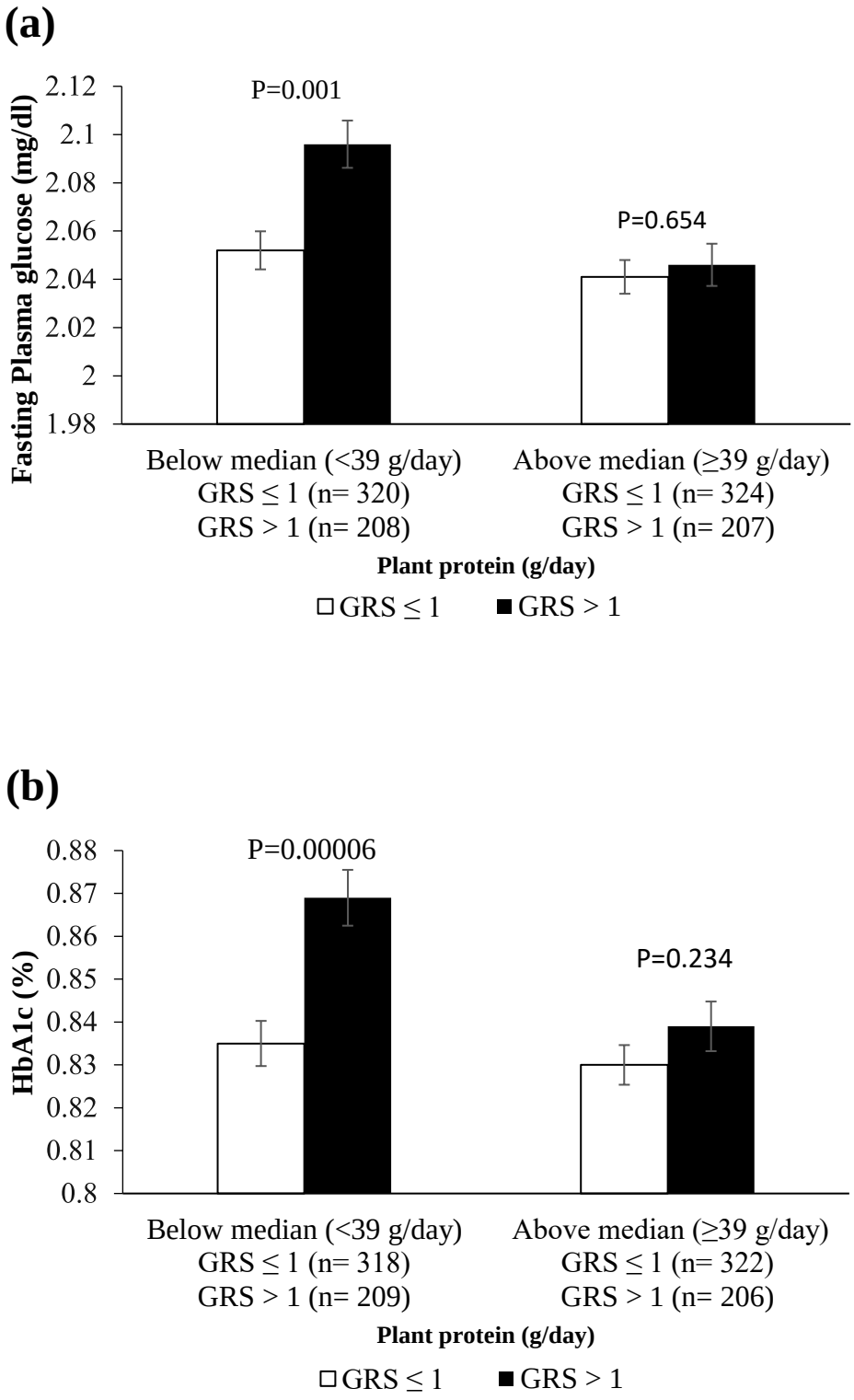

Figure 12: Interaction between 3-SNP GRS and plant protein intake on fasting plasma glucose and glycated haemoglobin.

White bars refer to individuals with GRS≤1 risk allele; the black bars refer to individuals with GRS>1 risk allele. (**a**) Individuals with>1 risk allele had a significantly higher FPG compared to those with ≤1 risk allele, among those with lower intake of plant protein (<39 g/day) (*P* = 0.001). (**b**) Individuals with>1 risk allele had a significantly higher HbA1c compared to those with ≤1 risk allele, among those with lower intake of plant protein (<39 g/day) (*P* = 0.00006). P values were adjusted for age, sex, T2D, BMI, anti-diabetic medication, total fat intake (%) and TEI. Abbreviations: GRS, genetic risk score; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin and TEI, total energy intake.

6.4.4 Sensitivity analyses

We subjected our regression results to a wide range of robustness checks. First, we adjusted for duration of diabetes, and the association of 3-SNP GRS with HbA1c and FPG (*P* = 0.010 and 0.040, respectively) and the interaction of 3-SNP GRS with protein intake (%) ($P_{interaction}$=0.025 and 0.019 for HbA1c and FPG, respectively) were still significant. Second, we excluded individuals with diabetes, and this resulted in a small sample size of 496 NGTs. However, a significant association of 3-SNP GRS with HbA1c (*P* = 0.012) was still observed; but none of the interactions were statistically significant (*P* = 0.126 and 0.405 for HbA1c and FPG, respectively). Third, given the association between dietary fat intake and T2D traits, we adjusted for total dietary fat intake and found that the interaction of 3-SNP GRS with protein intake (%) ($P_{interaction}$= 0.007 and 0.009 for HbA1c and FPG, respectively) was still significant. Fourth, we tested for the interaction between 3-SNP GRS and dairy protein intake to see if the interactions with the animal protein intake were driven by the intake of dairy protein and we found that the interactions between 3-SNP GRS and dairy protein intake were not statistically significant ($P_{interaction}$=0.439 and 0.597 for HbA1c and FPG, respectively) suggesting that dairy

protein intake is unlikely to confound the GRS-animal protein intake interaction on diabetes traits. Fifth, in addition to the aforementioned factors, we adjusted for other possible confounders such as physical activity level, smoking, alcohol consumption, and fibre intake and found that the interactions between the 3-SNP GRS and protein intake on HbA1c and FPG were still significant ($P_{interaction}$= 0.009 and 0.008, on HbA1c and FPG respectively).

6.5 Discussion

The current research provides evidence for the GRS-protein intake interaction on T2D-related traits in an Asian Indians. We found that individuals with >1 risk allele had higher FPG and HbA1c levels than those with ≤1risk allele among individuals with lower intake of plant protein (<39 g/day). Given that the prevalence of obesity, high FPG and T2D has increased in India from 1990 to 2016 (523), our findings are of importance in terms of public health. Our study suggests that increasing the intake of plant protein might be an effective strategy towards better management of blood glucose levels especially in Asian Indians with a higher genetic susceptibility for T2D.

In the present study, the 3-SNP-GRS was associated with higher WC, which is in accordance with the findings in 7,067 individuals from the Indian Migrant Study, where a combined risk score of 8 variants was observed to be nominally associated with higher WC (P=0.02) (524). The 3-SNP GRS was also associated with FPG and HbA1c, where individuals with higher GRS had higher FPG and HbA1c. Similarly, a large GWAS in 159,940 individuals of African, South Asian, East Asian and European ancestries identified 60 genetic variants influencing HbA1c (525), including SNPs located in the *FTO* and *TCF7L2* genes. An association of 8-SNP GRS with T2D was found in a case-control study of 5,148 Indians (including 1,808 individuals withT2D and 1,549 controls) from in and around Pune in western India (159). A case-control study of 3,357 Indian adults (including 2,486 individuals with T2D and 2,678 controls) also

found that individuals with a higher GRS, derived from 32 SNPs, were at a higher T2D risk compared to those with lower GRS (160). The EpiDREAM prospective cohort study (n=15,466 individuals) has shown that South Asians might have a greater genetic load for T2D than Latinos and Europeans (526). If our study findings are confirmed in larger cohorts, our 3-SNP GRS might serve as a diagnostic marker for investigating the cumulative effect of SNPs on diabetes-related traits and identifying Asian Indians with a high genetic risk of T2D.

Increasing evidence has shown that certain dietary factors might interact with genetic susceptibility in relation to the risk of diabetes and related traits (256-258, 260, 527). In our study, individuals with higher 3-SNP GRS had higher fasting glucose and HbA1c concentrations than individuals with lower GRS among individuals with low intake of plant protein. The results of the current analysis are in agreement with a recent study among Southeast Asian women (n=110) showing significant interactions between a 15-SNP GRS and total protein intake. The study found that consuming a low protein diet (13.51±1.18 % of TEI) was associated with lower WC and triacylglycerol concentrations, particularly in individuals with high genetic risk (527). Also, significant interactions of the *FTO* SNPs [rs8044769 (C>T), rs3751812 (G>T) and rs8050136 (A>C)] with protein intake on blood glucose were observed in 819 Polish adults, where high protein intake (>18 % of TEI) was associated with higher blood glucose in individuals with the TT genotype of rs8044769, CC genotype of rs8050136, and GG genotype of rs3751812 (528). However, the effect of protein sources was not analysed in the abovementioned studies, thus, direct comparison between these studies and our findings cannot be performed. In contrast to our study, a large prospective case-cohort study from 8 European countries (n=21,900) found no significant interactions between intake of protein and metabolic GRSs on T2D (529).Similarly, no interaction was found between protein intake and a 10-SNP GRS on T2D risk among 8,842 Korean adults (258). These discrepancies in the findings might be due to differences in ethnicity, dietary assessments, dietary patterns, relative

proportions of different macronutrients, protein sources, sample sizes and GRS construction methods; hence, larger studies in multiple ethnic groups are needed to confirm the GRS-protein intake interactions.

Previous studies have examined the relationship between protein intake and T2D in South Indians. A cross-sectional study of 900 urban South Indians from Chennai demonstrated that individuals with known T2D had significantly higher protein intake (15.9%) than controls (14%) (530). Another study in Asian Indians from different parts of India reported similar findings, where diabetic individuals (n=385) had higher protein intake (14%) than controls (12%) (n=409) (531). A cohort including 146 Asian Indians living in San Francisco found that individuals were at increased T2D risk when the protein intake was high. The same study also reported that the intake of animal protein (32±15 g/day) was more likely to be associated with diabetes risk (P=0.07) in comparison with the intake of vegetable protein (38±8 g/day; P=0.26) (532). Even though consuming diets high in protein have been one of the most popular strategies for losing weight and the management of overweight and obesity (177-179), the health impacts of diets high in protein on T2D are inconsistent. High animal protein intake, but not plant protein, showed significant association with a higher risk of T2D in 38,094 individuals (median intake of animal protein=62 g/day;10 years of a follow-up period) from the European Prospective Investigation into Cancer and Nutrition-Netherlands (EPIC-NL) study (180), and in 37,309 women from the US (median intake of total meat in the highest quintiles=53.5 serving/day; 8.8 years of a follow-up period) from the Women's Health Study (181). Also, a large case-cohort study including 28,557 European individuals reported that high animal protein intake was associated with higher incidence of T2D (per 10 g: 1.05 [1.02–1.08], P_{trend} =0.001) over an average follow-up period of 12 years (502). Furthermore, the high intake of animal protein (5% increase in consumption of protein derived from meat and meat products) was shown to be associated with a 34% increased risk of T2D, whereas the intake of

plant protein was shown to have a considerable protective effect in 1,190 elderly participants from the Mediterranean islands (503). A large study of 92,088 women and 40,722 men from the United States found that substituting 5% of energy intake from animal protein with plant protein was associated with a decrease in T2D risk by 23% (533). Also, a systematic review and meta-analysis of thirteen randomized controlled trials (n=280 middle-aged adults from Iran, Denmark, United States, Germany, Canada and Greece) found significant decreases in HbA1c, fasting insulin and fasting glucose in diets that substituted animal protein with plant protein at a median level of ~ 35% of total protein intake/day (534). Another systematic review and meta-analysis of eleven cohort studies, including individuals from the United States, Europe, Asia, Melbourne and Finland (52,637 cases among 483,174 individuals)**,** showed that the intakes of total protein and animal protein increased T2D risk in both men and women, whereas plant protein intake decreased T2D risk in women (535). Previous cohort studies in the United States (90,239 women and 40,539 men) and in the Netherlands (6798 individuals), found that an association between the higher adherence to a plant-based diet and a lower risk of T2D (536, 537). In contrast, other prospective cohort studies (n=8,370-38,094 individuals) observed no significant associations (180, 538, 539). It is possible that the interactions between genetic factors and protein intake might be one of the reasons for the discrepancies in the effect of dietary protein intake on the risk of T2D and its related traits.

The dietary patterns across different parts of India have been significantly affected by urbanisation. Given that food availability and purchasing power are higher in urban than rural areas, diets of both residents tend to differ significantly (501, 540). Protein intake has been shown to be positively related to individuals' income, where the demand for animal protein increased with the disposable income (501). Higher protein intake has also been reported in urban areas in India, with the overall mean intake of protein being the highest in the high-income group (73.1 g/day) followed by the middle-income group (63.2 g/day), industrial

labourer (59.4 g/day), and low-income group (57.8 g/day) (541, 542). The present study included urban residents and the mean protein intake is 71.6±22.7g/day, which is higher than dietary protein recommendations for Asian Indians (55-60 g/day) (543). However, the mean protein intake is only 11% (percentage calories coming from the protein), which is similar to the previous large studies such as National Family Health and National Nutrition Monitoring Bureau surveys that were conducted in the Indian population (544, 545). A study in 6,907 adults from South India aged > 20 years showed that the consumption of pulses was lower in the rural compared to urban Indian adults (546) and a cross-sectional study including 56,742 men and 99,574 women aged 20-49 years also demonstrated that an inverse association between the daily or weekly legumes and presence of diabetes (547). A recent study in 1,033 Indian adults also showed that a significant decrease in the risk of T2D was observed among those having higher intakes of legumes and pulses (548). In the same population, a study in 2,042 individuals reported that pulses and legumes contributed only to 17.2% of the daily protein suggesting a reduced intake of plant protein (549). Hence, according to the findings from the previous studies and the GRS- plant protein intake interaction from the present study, increasing the intake of plant protein might be an effective strategy to arrest the rising epidemic of T2D among Indian adults.

The strength of this study include is the use of a representative sample of the urban Chennai population. Given that diabetes prevalence continues to be higher in urban residents compared to rural residents in India (276, 550, 551), understanding gene-diet interactions on T2D in urban areas would improve diabetes prevention strategies among urban Indians. Our study used unweighted GRSs to analyse the combined effect of several SNPs, which is an effective approach to study polygenic diseases such as T2D and obesity, providing a better knowledge of disease risk compared to a single-SNP analysis (153). A comprehensive and validated semi-quantitative FFQ was used for analysing dietary intakes (518). Furthermore, anthropometric

outcomes were assessed by qualified staff rather than self-reported to improve the accuracy of anthropometric measurements. However, the study has several limitations. First, the study has a small sample size suggesting that we might have had insufficient power for our analysis. To maximise power, we used a GRS approach, which has an advantage over single-SNP analysis, and significant associations and interactions were found. Second, the observational nature of the study design cannot explain causal relationships or exclude residual confounding; however, sensitivity analyses were carried out where adjustment for additional confounding factors such as diabetes duration, total fat intake, physical activity level, anti-diabetic medication, alcohol consumption, smoking and fibre intake was performed. Third, dietary intake was assessed using self-reported FFQ, which might have introduced recall and measurement bias. Finally, SNPs contributing to our GRSs represent only a small proportion of the increasing number of identified metabolic disease-associated variants in Asian Indians; however, we have chosen SNPs in *TCF7L2* and *FTO* genes that have presented the most consistent and strongest associations with T2D and obesity, respectively in several populations (80, 253).

6.6 Conclusion

In summary, the current study has found a novel GRS-protein intake interaction where individuals with >1 risk allele and lower intake of plant protein (<39 g/day) had higher FPG and HbA1c levels. This suggests that increasing the intake of plant protein may be an effective approach to overcome the genetic risk of diabetes in urban Asian Indians and to prove this hypothesis appropriate randomized clinical trials with diets of higher and lower plant protein intake need to be done. Moreover, there is a need for studies with larger sample sizes to confirm gene-diet interactions. Ultimately there is a need for the assessment of the clinical benefit of targeted interventions based on individuals' underlying genetic risk.

Supplementary Materials: The following are available online at, Table S1: Genotype distribution of the seven SNPs that were chosen for our study (*n* = 1,062). Figure S1: Methodology of the Chennai Urban Rural Epidemiology Study (CURES). Figure S2: Interaction between 3-SNP GRS and animal protein intake (%) on fasting plasma glucose and glycated haemoglobin after adjusting for anti-diabetic medication.

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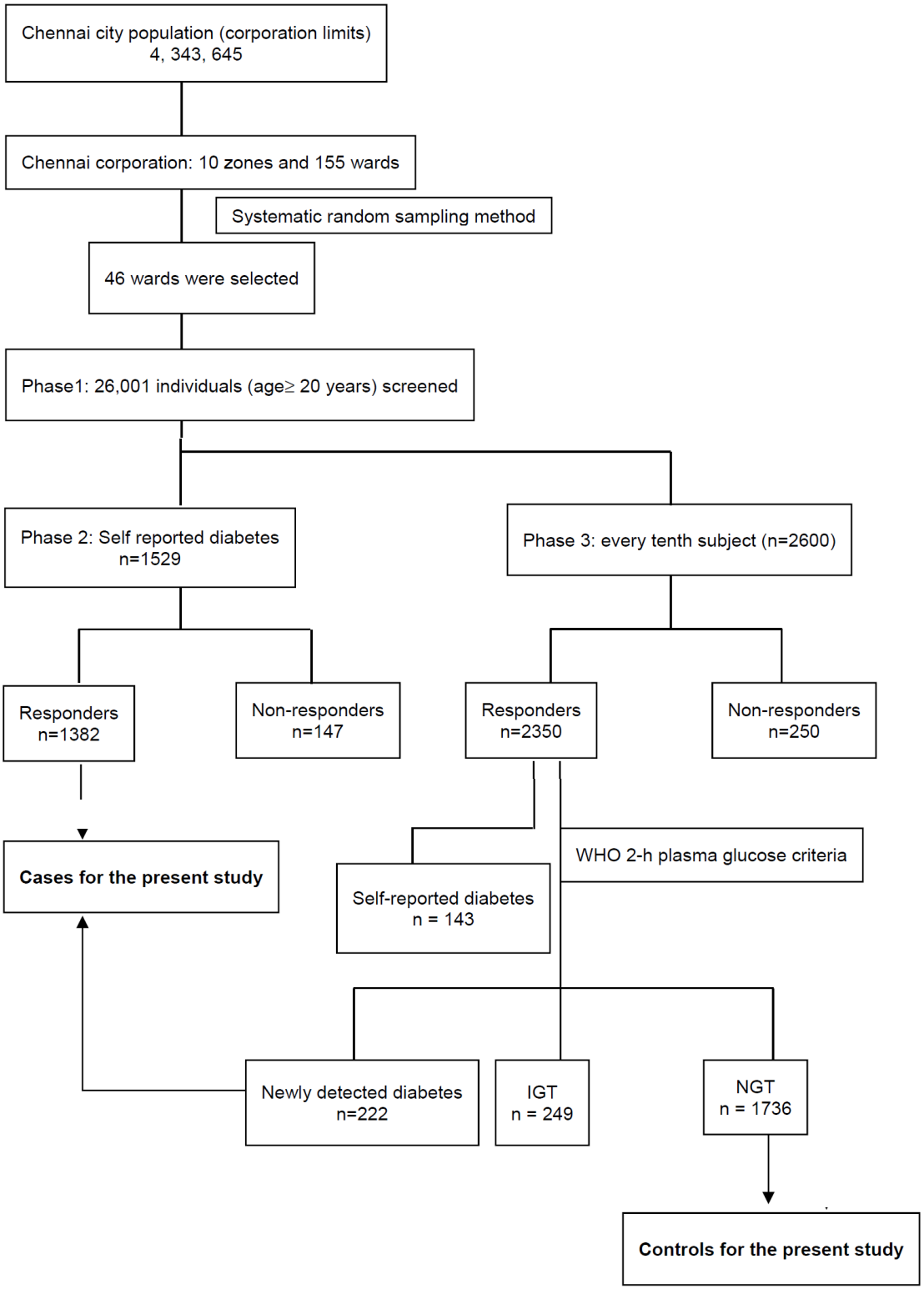
6.7 Supplementary materials

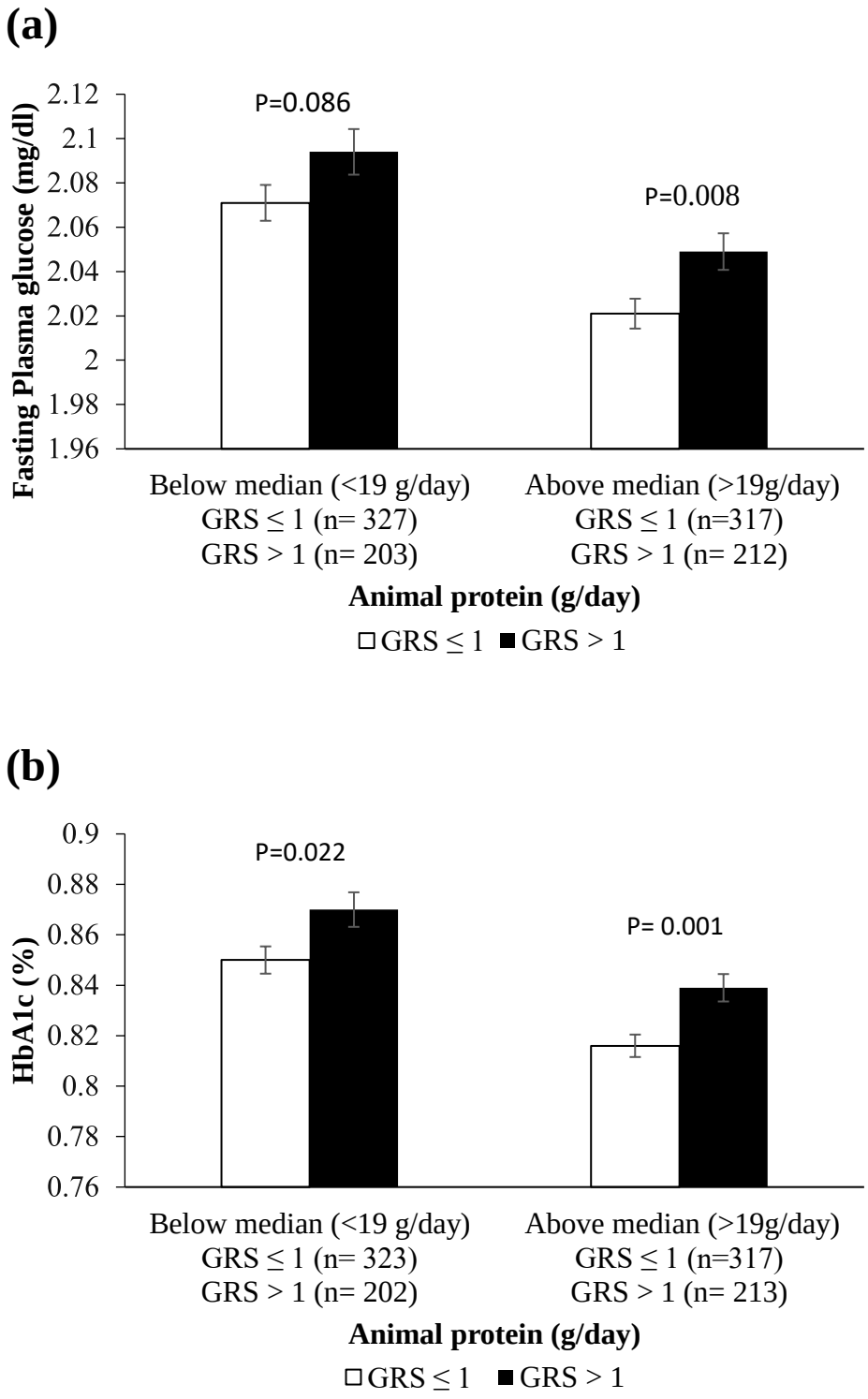
6.7.1 Table S1. Genotype distribution of the seven SNPs that were chosen for our study (n= 1,062)

Gene	SNP	MAF among NGT Controls	MAF among T2D cases	MAF in the present study	dbSNP MAF for SA	HWE
TCF7L2	rs12255372	0.20	0.21	T= 0.21	0.22	0.36
TCF7L2	rs7903146	0.30	0.33	T= 0.31	0.30	0.12
FTO	rs8050136	0.06	0.13	A= 0.10	0.29	0.35
FTO	rs918031	0.49	0.48	C= 0.49	0.48	0.09
FTO	rs1588413	0.25	0.32	T= 0.29	0.26	0.15
FTO	rs11076023	0.49	0.45	T= 0.47	0.36	0.48
FTO	rs7193144	0.13	0.12	T= 0.12	0.29	0.16

Abbreviations: SNP single nucleotide polymorphisms; dbSNP single nucleotide polymorphism database; MAF minor allele frequency; T2D type 2 diabetes; SA South Asians; HWE Hardy-Weinberg equilibrium; *TCF7L2* Transcription factor 7-like 2; *FTO* fat mass and obesity-associated.



6.7.2 Figure S1: Methodology of the Chennai Urban Rural Epidemiology Study (CURES)



6.7.3 Figure S2: Interaction between 3-SNP GRS and animal protein intake (%) on fasting plasma glucose and glycated haemoglobin after adjusting for antidiabetic medications.

White bars indicate individuals with GRS ≤1 risk allele; the black bars indicate individuals with GRS >1 risk allele. (a) Individuals with>1 risk allele had a significantly higher FPG compared to those with ≤1 risk allele, among those with higher intake of animal protein (>19 g/day) (P=0.008). (b) Individuals with>1 risk allele had a significantly higher HbA1c compared to those with ≤1 risk allele, among those with higher intake of animal protein (>19 g/day) (P=0.001). P values were adjusted for age, sex, T2D, BMI, antidiabetic medications, total fat intake (%) and TEI. Abbreviations: GRS, genetic risk score; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin and TEI, total energy intake.

Chapter 7 Discussion and conclusion

7.1 Discussion

The science of nutrigenetics explores gene-diet interactions in relation to individuals' variation in health and disease states, including type 2 diabetes (T2D), obesity and cardiovascular diseases (CVD), to offer personalised dietary advice according to the individual's genetic susceptibility. Personalised nutrition might be a promising approach for preventing or treating cardiometabolic diseases (5). The findings from this thesis contribute to the science of nutrigenetics by demonstrating the occurrence of genetic heterogeneity in gene-diet interactions on cardiometabolic diseases and their related traits across different ethnic groups.

Studies investigating gene-diet interactions on cardiometabolic diseases have reported inconsistent findings. This can be explained by genetic heterogeneity and small sample sizes, limiting the ability of these studies to offer personalised nutrition for each ethnic group (222, 419). Gene-diet interactions have been investigated extensively in developed countries. In contrast, nutrigenetic studies are scarce in developing countries due to limitation in several factors including funding, infrastructure and expertise (222, 419). In this thesis, a genetic approach was used to analyse the association between cardiometabolic disease-related single nucleotide polymorphisms (SNPs) and cardiometabolic traits in various ethnic groups. In addition, a nutrigenetic approach was used to investigate the interaction between these SNPs and lifestyle factors including physical activity and dietary intake of proteins, carbohydrates and fats on cardiometabolic traits in five different ethnic groups.

Given that the genetic make-up differs between populations, this thesis included individuals from different ethnic groups [Turkish adults, Indonesian women, Ghanaian adults, Brazilian young adults and South Asian Indian adults and elderly] to examine gene-lifestyle

interactions, which will allow us to tailor dietary guidelines based on each ethnicity. This project included five studies: three cross-sectional cohort studies [The Minangkabau Indonesia Study on Nutrition and Genetics (MINANG study; Indonesian women; n=110), The Genetics of Obesity and Nutrition in Ghana (GONG study; Ghanaian adults; n= 302) and The Study of Cholesterol, Obesity, Lifestyle and Diabetes (BOLD study; Brazilian young adults; n= 200)] and two case-control studies [study of obesity in Turkish adults (n= 400) and study of diabetes in Chennai Urban Rural Epidemiological Study (CURES; Asian Indian, n=1062)]. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA). Both logistic and general linear models were used for association and interaction analyses. Models were adjusted for age, sex, body mass index (BMI), T2D, residential area, total energy intake (TEI) wherever appropriate. Findings from this thesis are summarised below.

7.2 *FTO* gene–lifestyle interactions on serum adiponectin concentrations and central obesity in a Turkish population

Obesity is a significant global public health problem. In 2017, 64.4% and 28.8% of the Turkish population were overweight and obese, respectively (264). Obesity is caused by complex interactions between lifestyle and genetic factors (263), with the fat mass and obesity-associated (*FTO*) gene being one of the strongest obesity genes **(76)**. Investigating these interactions would provide better approaches for obesity management. There were no previous nutrigenetic studies among the Turkish population. Thus, our study aimed to investigate the associations of two *FTO* SNPs (rs9939609 and rs101634090) with obesity and related traits, including BMI, waist circumference (WC), fat mass index (FMI) and adiponectin level), and to assess whether lifestyle factors modify these association in 400 Turkish adults (aged 24-50 years). A genetic risk score (GRS) was developed using both *FTO* SNPs.

In agreement with previous studies (76, 268, 287, 288, 314, 552, 553), we found that the *FTO* SNP rs9939609 and GRS were both associated with BMI and FMI. Similarly, the *FTO* SNP rs1421085, which is in linkage disequilibrium (LD) with the SNP rs9939609, showed a significant association with higher BMI in a previous study among Turkish adults (289). In the present study, significant interactions were also detected between SNP rs9939609 and physical activity on adiponectin concentrations, and SNP rs10163409 and dietary protein intake on the risk of increased WC. A study including Turkish adults (n=200) showed that homozygous individuals of the risk allele 'A' of the *FTO* SNP rs9939609 had significantly higher BMI than those with the genotype 'TT, among those with low physical activity level (320). Our study (n=400) detected a similar interaction, but on concentrations of adiponectin (which is a biochemical measure of obesity), rather than BMI. We found that individuals with the allele "A" of the SNP rs9939609 had significantly lower adiponectin concentrations than individuals with the "TT" genotype among those with the lowest physical activity level. Adiponectin is a protein hormone generated by adipose tissue and has antiatherogenic, anti-inflammatory, antihyperglycemic and cardioprotective effects (321-323). A strong correlation between dysregulation of adipokine production and the onset of metabolic abnormalities such as CVD and cancer has been reported (324, 325, 554). A positive correlation was also reported between physical activity and adiponectin concentrations (326, 327), where an increased level of physical activity was associated with reduced body adiposity which increases the production of adiponectin and decreases glucose, leptin and insulin production (555). Indeed, adiponectin levels are inversely associated with glucose, BMI, insulin, IR and the levels of triglyceride and visceral adiposity (329). It has been found that levels of adiponectin were significantly increased in response to a weight-loss intervention among 400 obese European women (330).

Furthermore, our study has shown that the higher intake of protein (mean ± SD: 138 ± 38 g/day) was associated with higher central obesity risk in individuals with the "T" allele of

the SNP rs10163409 than individuals with the "AA" genotype. The Turkish population has a sedentary lifestyle (264) and has undergone a nutrition transition in which dietary intake has changed because of changes in food accessibility, globalisation and lifestyle (556). It has been estimated that protein intake per capita has increased by 10.6% (between the period 1961 and 2011) (556). Given these environmental changes along with the increase in obesity prevalence in Turkey, our findings might contribute to the implementation of successful public health policies aimed at preventing and managing central obesity in this population.

7.3 Interaction between the genetic risk score and dietary protein intake on cardiometabolic traits in Southeast Asians

Cardiometabolic diseases are a significant cause of morbidity, mortality and health care spending especially in low-middle income countries (LMICs) **(169)**. In Indonesia, non-communicable diseases (NCDs) contributed to 73% of all death rate, with CVD accounting for 35%, cancers (12%) and diabetes (6%) (265). These diseases are complex traits influenced by several SNPs, as well as lifestyle factors, emphasising the importance of investigating gene-lifestyle interactions (5). Thus, our study aimed to examine the association of a novel GRS constructed from 15 SNPs with cardiometabolic traits and examined whether these associations were modified by lifestyle factors such as dietary intake and physical activity in 110 Minangkabau women (aged 25–60 years) from Padang, Indonesia. The Minangkabau ethnic group is a matrilineal society mostly living in West Sumatra, where the prevalence of obesity, hypertension, and low concentration of high-density lipoprotein cholesterol (HDL-C) is higher than 50% (337). Previous studies have demonstrated that Minangkabau community has an increased risk of dyslipidaemia (338), high prevalence of central obesity (340), as well as the highest concentrations of low-density lipoprotein cholesterol (LDL-C) and total plasma cholesterol than other larger ethnic groups (Buginese, Sundanese and Javanese) (339).

The selected SNPs were rs3792267 and rs5030952 located in Calpain 10 (*CAPN10*) gene; rs9939609, rs10163409 and rs8050136 located in *FTO* gene; rs17782313 and rs2229616 located in melanocortin4 Receptor (*MC4R*) gene; rs12255372 and rs7903146 located in transcription factor 7-like 2 (*TCF7L2*) gene; rs2237895 and rs2237892 located in potassium voltage-gated channel subfamily Qmember 1 (*KCNQ1*) gene; rs10811661 located in cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/2B*) gene; rs1801282 located in peroxisome proliferator-activated receptor gamma (PPARG) gene; and rs266729 and rs17846866 located in adiponectin (*ADIPOQ*) gene.

This study identified an association of the GRS with BMI as well as the *FTO* SNP rs10163409 interaction with protein intake on WC which was also observed in our Turkish study. In the present study, the GRS was significantly associated with BMI, where individuals with > 5 risk alleles had higher BMI than those with ≤ 5 risk alleles, suggesting that having a higher GRS is a strong risk factor for higher BMI. Also, significant interactions were detected between the GRS and protein intake on WC and triglyceride concentrations, where women with > 5 risk alleles had significantly lower WC and triglyceride concentrations than those with ≤ 5 risk alleles among those with a lower intake of protein (13.51 ± 1.18% of TEI). The mean protein intake of our sample was 77± 37 g/day, which is higher than the recommended dietary protein daily allowance of 57–59 g/day for Indonesian women (371, 372). Our study indicates that consuming a diet low in protein (13.51 ± 1.18% of TEI), particularly in individuals with higher genetic risk, may be an effective strategy for preventing cardiometabolic disease in Southeast Asian women. These findings are important in terms of public health, considering the high prevalence of central and common obesity in Minangkabau women (340) A previous study including European individuals (n= 42,702) has reported a significant association between central obesity and a higher risk of mortality even in individuals with normal weight (35). This is of great concern in Asians, where elevated levels of visceral adiposity levels are

present even in individuals with normal BMIs (367-369). The combination of increased triglyceride concentrations along with elevated WC has been termed as the 'hypertriacylglycerolaemic waist' phenotype (370). Evidence has shown that this phenotype is associated with a higher risk of IR, visceral adiposity and CVD (370). Thus, targeting 'hypertriacylglycerolaemic waist' phenotype might have major public health implications in relation to decreasing mortality rate caused by cardiometabolic diseases in Asian populations.

7.4 Interaction between Metabolic Genetic Risk Score and Dietary Fatty Acid Intake on Central Obesity in a Ghanaian Population.

Obesity is a multifactorial condition caused by a complex interplay between genetic and lifestyle factors. It has reported that overweight or obesity account for nearly 43% of Ghanaian adults (266). The majority of gene–lifestyle interaction studies have been performed in European ancestry populations, and the replication of these nutrigenetic studies in Africans is uncertain (267, 268). Thus, we aimed to examine the associations of different GRSs with obesity-related traits and to investigate the effect of physical activity and dietary intake on these associations among 302 healthy Ghanaian adults.

Three metabolic GRSs were calculated including the 12-, 8- and the 4-SNP GRSs. The 12-SNP GRS included the following SNPs: *ADIPOQ* (rs266729), *KCNQ1* (rs2237892), *TCF7L2* (rs12255372, rs7903146), *MC4R* (rs2229616, rs17782313), *CDKN2A/2B* (rs10811661), *CAPN10* (rs5030952, rs3792267), *FTO* (rs10163409, rs9939609) and *PPARG* (rs1801282). No significant associations or interactions were observed with the 12-SNP GRS which could be explained by the fact that four of the SNPs had low minor allele frequency (MAF) of less than 5%; *MC4R* (rs2229616), *FTO* (rs10163409), *CDKN2B* (rs10811661) and *PPARG* (rs1801282). Hence, these four SNPs were excluded, and an 8-SNP GRS was constructed. In the 8-SNP GRS, no significant findings were detected which might be because

the GRS included 4 SNPs [*ADIPOQ* (rs266729), *KCNQ1* (rs2237892) and *CAPN10* (rs3792267, rs5030952)] that have not shown consistent associations with obesity and related traits in several ethnic groups (362, 422-426). Thus, these 4 SNPs were removed, and a 4-SNP GRS was calculated from [*TCF7L2* (rs12255372, rs7903146), *MC4R* (rs17782313) and *FTO* (rs9939609)] that have shown consistent associations with obesity across various populations.

Interestingly, associations between metabolic-GRSs and metabolic traits in other populations examined in this thesis (Turkey, Indonesia and India) were not found and this might be because individuals of African ancestry have decreased patterns of linkage disequilibrium, greater genetic variation and more diverse haplotype than other populations, limiting the replication of previously observed genetic associations **(438)**. Gene-environment and gene-gene interactions as well as differences in sample sizes and GRS construction methods could also explain discrepancies between studies. In this Ghanaian study, the 4-SNP GRS showed significant interactions with dietary fat intakes on waist circumference (WC). Among Ghanaian adults with higher consumptions of total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), individuals with ≤ 3 risk alleles had a significantly higher WC than those with <3 risk alleles. These findings are in agreement with previous studies in the UK (385) and the US (443) populations, reporting significant associations of GRSs with increased WC among individuals with high consumption of total fat and SFA on WC. Our findings are also consistent with previous evidence investigating interactions of a single gene locus with diet on obesity-related traits. For example, data from 2,163 and 28,449 individuals living in the US and Malmö, respectively, showed significant interactions between the *FTO* SNP rs9939609 and total fat consumption on BMI (240, 241). Furthermore, significant interactions of the *FTO* SNP rs9939609 with MUFAs (240) and SFAs (240, 298, 441) on BMI have been reported the US (n= 2,163 individuals), French (n=1,754 individuals) and Spanish (n=354 children and adolescents) populations. In

addition, high MUFA consumption showed an association with lower weight loss among individuals with the risk allele 'A' of *FTO* rs9939609 (557). Also, a significant interaction between the risk allele 'T' of the *TCF7L2* SNP rs12255372 and fat consumption on HDL-C was reported in South Asians (n=1,680) (261).

Our study is the first of its kind in the Ghanaian population, suggesting that a higher intake of dietary fat might have the potential to raise the genetic risk of central obesity. These findings support the current dietary guideline of reducing the intakes of total fat and SFA, to decrease obesity risk, particularly in adults with a higher genetic predisposition to central obesity. Given the high intake of SFA and MUFA- rich foods in the Ghanaian population, our findings are of importance to public health (427).

7.5 Effect of dietary fat intake and genetic risk on glucose and insulin-related traits in Brazilian young adults

T2D prevalence has raised over the world (17), but at a faster rate in LMICs (459). In Brazil, 22.0% and 3.3% of adolescents have prediabetes and T2D, respectively (269). Studies have reported a high prevalence of cardiometabolic risk factors including dyslipidaemia, abdominal obesity and high blood pressure, high insulin levels, physical inactivity, and unhealthy diet among Brazilian adolescents (269-274). Thus, early interventions focusing on these risk factors would be an effective approach for slowing T2D progression and decreasing the risk of CVD (17). T2D pathogeneses involve complex interactions between genetic and dietary factors, however, no previous GRS-diet interaction studies have been performed in young Brazilian adults. Therefore, we aimed to examine the interaction between a metabolic 10-SNP GRS and dietary intake on metabolic traits among 200 healthy Brazilian young adults.

The GRS was calculated from the following SNPs: rs12255372, rs7903146 of the Transcription factor 7-like 2 (*TCF7L2*) gene, rs17782313 of the melanocortin 4 Receptor (*MC4R*) gene,

rs8050136 and rs10163409 of the fat mass and obesity-associated (*FTO*), rs2237892 and rs2237895 of the Potassium voltage-gated channel subfamily Q member 1(*KCNQ1*) gene, rs10811661of the Cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/2B*) gene, rs5030952 of the Calpain 10 (*CAPN10*) gene, and rs1801282 of the Peroxisome proliferator-activated receptor gamma (*PPARG*) gene.

In contrast to the Turkish and Indonesian studies, the GRS was associated with lower BMI. The Brazilian population is characterised by a mixed genetic ancestry including Africans, Europeans and Native Amerindians and this may justify these discrepancies between studies (478). In the present study, significant interactions between the GRS and fat intake observed in the Ghanaian study was also replicated, however, on diabetes-related traits rather than WC. We found that individuals with ≥5 risk alleles had increased fasting insulin level, insulin-glucose ratio, HOMA-B and HOMA-IR than those with <5 risk alleles among the high-fat intake category (37.98 ± 3.39% of total energy intake (TEI)). The GRS-fat interactions observed in this study possibly reflects the dietary pattern of the Brazilian population that is characterised by a high intake of processed foods (273). Although it is difficult to directly compare our findings with previous nutrigenetic studies, because of differences in the study design, ethnicity, sample size, GRS construction and assessment of dietary intake, our study is consistent with previous research, reporting significant interactions between fat intake and GRS on metabolic traits (256-258). An intervention study (n=733 European adults) found that the higher total fat consumption was associated with higher fasting glucose among individuals with a higher 12-SNP GRS and with reduced fasting glucose in those with a lower 12-SNP GRS (256). Similar SNP-fat interactions were observed in studies using single-locus approach analysis (558-560).

Our study proposes that individuals with high genetic risk are sensitive to fat intake with respect to these traits and might obtain the greatest benefit from following the Brazilian dietary

guidelines aiming to reduce the intake of fat to less than 30 % of TEI (477). This might have important public health implications in terms of offering early dietary interventions to young adults in Brazil, particularly those with high genetic predisposition before the development of T2D.

7.6 Low dietary intake of plant protein is associated with genetic risk of diabetes-related traits in urban Asian Indian adults.

South Asian ethnic population have a 50% higher risk of T2D than other ethnic groups (275, 276). India is a major contributor to the global increased prevalence of T2D (17), where the number of T2D cases increased from 26.0 million to 65.0 million between 1990 and 2016 (277). In India, urbanization plays a major role in T2D development as it is associated with a decreased level of physical activity and increased intake of processed foods and dietary fats (497, 498, 500). Furthermore, the consumption of protein from animal sources (including meat, fish, eggs and milk) and pulses in the Indian urban areas is higher in comparison with the rural areas (501). The intake of animal protein has been shown to be associated with increased T2D risk in several large longitudinal studies (180-182, 502, 503), where the intake of plant protein was associated with lower T2D risk in several studies (533-535). Interactions between genetic factors and urbanisation have further escalated the increasing T2D prevalence in South Asians (278, 279). However, studies examining interactions between GRS and nutrient intake among Indians are sparse. Thus, we investigated the joint effect of 7 SNPs on T2D and metabolic traits and interactions with dietary factors in 1,062 urban south Asian Indians.

In the present study, a 7-SNP GRS was created using the following SNPs: *TCF7L2* SNPs (rs7903146 and rs12255372) and *FTO* SNPs (1588413, rs8050136, 7193144, 918031 and 1076023). Additionally, we generated a GRS of only 3 variants (*TCF7L2* rs7903146 and

rs12255372 and *FTO* rs8050136) which have shown consistent associations with metabolic disease and related outcomes in several populations.

Unlike Turkish and Indonesian studies, no significant association between the GRSs and BMI was detected in this Indian study. Similar to the Turkish and Indonesian studies, this study observed significant GRS-protein interactions, however, on diabetes-related traits rather than on central obesity. We found significant associations of the 7-SNP and 3-SNP GRS with higher WC. Also, we detected significant associations between the 3-SNP GRS and higher fasting plasma glucose (FPG) and glycated haemoglobin (HbA1c), and these associations were modified by protein intake. We found that among individuals with low plant protein intake (<39g/day) and high animal protein intake (>19.27g/day), those with >1 risk allele had higher HbA1c (P=0.00005 and P=0.001, respectively) and FPG (P=0.0005 and P=0.008, respectively) than individuals with ≤1 risk allele. In agreement with our findings, a longitudinal study (10 years of a follow-up period) including 38,094 European individuals reported a significant association between the high intake of animal protein, but not plant protein, and increased T2D risk (median animal protein intake =62 g/day) **(180)**. A similar association was reported in the Women's Health Study (n=37,309; 8.8 years of a follow-up period) **(181)**. A large cohort (n=92,088 women and 40,722 men living in the United States (US)) found that replacing 5% of energy intake from animal protein with plant protein was associated with a reduction in T2D risk by 23% (533). Also, a systematic review and meta-analysis of thirteen randomized controlled trials including 280 individuals found significant reductions in HbA1c, fasting insulin and fasting glucose in diets that substituted animal protein with plant protein at a median level of ~ 35% of total protein intake/day (534). Another systematic review and meta-analysis of eleven cohort studies, including individuals from the US, Finland, Asia, Europe and Melbourne (52,637 cases among 483,174 individuals), found that the intakes of total protein and animal protein increased T2D risk in both men and women, whereas plant protein intake

decreased T2D risk in women (535). Furthermore, the high intake of animal protein showed a significant association with higher T2D incidence (per 10 g: 1.05 [1.02–1.08], P_{trend} =0.001) in 28,557 European individuals followed for about 12 years (502). In contrast, no significant associations were found in other large prospective cohort studies (n=8,370-38,094 participants) (180, 538, 539), which could be explained by undetected gene-protein interactions.

Our findings further support the contribution of both *FTO* and *TCF7L2* SNPs in the development of T2D and central obesity and the modification of genetic associations with diabetes-related traits by high intake of protein in urban South Asian Indians. The present study also indicates that higher the intake of plant protein and lower the intake of animal protein are associated with lower diabetes genetic risk in urban Asian Indians. This might have significant public health implication in terms of reducing the high prevalence of obesity, high FPG and T2D in India (523), although requires confirmation in further larger cohorts or intervention studies.

7.7 General trends observed across various ethnic groups.

As shown in Table 18, macronutrient intake is different across the five ethnic groups included in this thesis, highlighting the need for investigating the effect of different dietary factors, as well as gene-diet interactions on cardiometabolic diseases in various populations. The percent of energy from fat was highest in the Turkish population (38.29 ± 8.08%) and lower in Ghanaian adults (22.98 ± 9.11 %) and Indian adults (23.42 ± 4.73 %) than other populations (Indonesian: 28.95 ± 7.99 % and Brazilian young adults: 31.66 ± 5.83 %). The acceptable macronutrient distribution range (AMDR) for fat is 20-35 % of TEI (561). The Turkish population consumed fat above the AMDR, where the fat intake of all other groups was within the AMDR. Protein intake as a percentage of TEI was higher in Brazilian young adults (17.11 ± 3.63 %) and Indonesian women (16.93 ± 3.32 %) and lowest in Indian adults

(11.36 ± 1.21 %) than other populations (Turkish: 15. 58 ± 4.33 % and Ghanaian: 14.11 ± 4.14 %). Protein intake was within the AMDR (10-35%) in all the populations (561). The percent of energy from carbohydrate was higher in the Indian (64.62 ± 6.21 %) and Ghanaian populations (62.48 ± 9.70 %) and lowest in the Turkish population (45.92 ± 9.34 %) than other ethnic groups (Brazilian young adults: 51.09 ± 7.11 % and Indonesian adults: 53.97 ± 9.44 %). The carbohydrate intake of Turkish and Indian populations was very close to the lower and upper limits of the AMDR, respectively, where the intake of other populations was within the AMDR (45-65 %) (561).

Comparison of dietary intake across these five studies might have been affected by sampling methods. The Brazilian population included young adults (19-24 years), whereas the Indian population included adults and elderly participants (25-80 years). Indonesian, Turkish and Ghanaian populations included participants with age ranging between 25-60 years. It is worth noting that adopting new dietary patterns is more likely in younger populations than older populations; hence, to prevent adult onset diseases such as obesity and diabetes, providing dietary advice to the younger population might be an effective strategy. Further research looking at both urban and rural populations and controlling for confounding factors such as socioeconomic status is required (562).

As shown in Table **18**, the highest mean of BMI was observed in the Ghanaian population (26.63±4.99), whereas Brazilians reported the lowest mean (23.35 ± 4.42) and Turkish and Indonesians reported similar means of BMI (25.80 ± 4.22 and 25.13±4.20, respectively). Similar means of percentage of body fat mass were observed in Ghanaian (33.12±13.90) and Brazilian (33.91 ± 10.72) populations. Indians reported the highest means of fasting glucose (125.78 ± 63.95), and HbA1c (7.28 ± 2.4) compared to the other four populations. Given the heterogeneity that exists across these studies, generalizing these findings is difficult.

Table 18: Macronutrient Intakes, Anthropometric and biochemical parameters: A Comparison of the Turkish, MINANG, GONG, BOLD and CURES studies.

Parameters	**Turkish study**	**Indonesian MINANG study**	**Ghanaian GONG study**	**Brazilian BOLD study**	**Indian CURES study**
	(N= 400)	(N=111)	(N=302)	(N= 200)	(N= 1,062)
Total energy (Kcal/day)	2416.44 ± 1064.1	1776.24 ± 611.43	1647.93 ± 685.83	1827.81 ± 597.94	2536.01 ± 804.95
Fat (%)	38.29 ± 8.08	28.95 ± 7.99	22.98 ± 9.11	31.66 ± 5.83	23.42 ± 4.73
Protein (%)	15. 58 ± 4.33	16.93 ± 3.32	14.11 ± 4.14	17.11 ± 3.63	11.36 ± 1.21
Carbohydrate (%)	45.92 ± 9.34	53.97 ± 9.44	62.48 ± 9.70	51.09 ± 7.11	64.62 ± 6.21
BMI (kg/m2)	25.8 ± 4.22	25.13 ± 4.2	26.63 ± 4.99	23.35 ± 4.42	24.59 ± 4.56
WC	88.09 ± 11.61	83.85±10.27	88.48±12.41	74.55 ± 13.56	87.05 ± 11.55
Body fat mass (%)	N/A	N/A	33.12±13.90	33.91 ± 10.72	N/A
Fat Mass Index	6.92 ± 2.93	N/A	N/A	N/A	N/A
WHR	N/A	N/A	1.45±6.96	N/A	N/A
Visceral fat (%)	N/A	N/A	8.02±7.39	N/A	N/A
Plasma adiponectin (ng/ml)	10497.88 ± 6467.17	N/A	N/A	N/A	N/A
FPG (mg/dL)	N/A	92.53±20.67	N/A	87.18 ± 6.84	125.78 ± 63.95
HBA1C (%)	N/A	6.56 ± 6.02	N/A	4.73 ± 0.25	7.28 ± 2.4
Fasting insulin	N/A	32428.5±25706.13	N/A	8.74 ± 3.80	9.45 ± 6.68
(μIU/ml)					
HOMA-IR	N/A	N/A	N/A	1.89 ± 0.88	N/A
HOMA-B	N/A	N/A	N/A	138.32 ± 65.75	N/A
Insulin to glucose ratio	N/A	N/A	N/A	0.10 ± 0.04	N/A
Triglycerides (mg/dl)	N/A	98.8±43.47	N/A	N/A	N/A
Cholesterol (mg/dl)	N/A	209.31±44.02	N/A	N/A	N/A
HDL-C (mg/dl)	N/A	59.12±10.29	N/A	N/A	N/A
LDL-C (mg/dl)	N/A	128.12±39.85	N/A	N/A	N/A
SBP (mmHg)	N/A	113.37±9.07	N/A	N/A	N/A
DBP (mmHg)	N/A	77.44±6.39	N/A	N/A	N/A

Abbreviations: MINANG, Minangkabau Indonesia Study on Nutrition and Genetics; CURES Chennai Urban Rural Epidemiology Study; GONG, Genetics of obesity and nutrition in Ghana; BOLD, Obesity, Lifestyle and Diabetes in Brazil; BMI, body mass index; WC, waist circumference; WHR, waist hip ratio; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; HOMA-IR: homeostasis model assessment estimate of insulin resistance, HOMA-B, homeostasis model assessment estimate of insulin secretion; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Ethnic differences in metabolic traits were observed across the five populations included in this thesis, which could be driven by variations at several genetic loci (Table 19). The *TCF7L2* rs12255372 and rs7903146 SNPs are examples of genetic heterogeneity, where the frequency of minor allele 'T' of the SNP rs12255372 was 9% in the Indonesian population vs 35% in the Brazilian population, whereas Ghanaian individuals reported the highest MAF (37%) compared to other populations. The *FTO* SNP rs9939609 is one of the strongest BMI associated variants in several populations, demonstrating ethnic variations in this project. For example, the MAF of this variant in the Indonesian population was 23% in comparison to the Turkish population (39%). The Ghanaian population has reported the highest MAF (47%) for the SNP rs9939609 which is in line with previously reported values for the African population (*https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?do_not_redirect&rs=rs9939609*). MAFs of *MC4R* and *PPARG* SNPs were extremely low in all populations. Another example is *CDKN2A/B* SNP rs10811661 that was present in 33 % of the Indonesian population and absent in the Ghanaian population.

Table 19: Frequencies of the SNPs: A Comparison of the Turkish, MINANG, GONG, BOLD and CURES studies

Gene	rs number	Major allele	Minor allele	Common Homozygotes n (%)	Heterozygotes n (%)	Rare Homozygotes n (%)	Minor allele frequency	HWE	Ethnicity
TCF7L2	rs12255372	G	T	92 (82.9)	19 (17.1)		0.09	0.32	Indonesian
	rs12255372	G	T	117 (38.7)	144 (47.7)	37 (12.3)	0.37	0.47	Ghanaian
	rs12255372	G	T	85 (42.5)	92 (46)	23 (11.5)	0.35	0.80	Brazilian
	rs12255372	G	T	674 (63.5)	338 (31.8)	50 (4.7)	0.21	0.36	Indian
	rs7903146	C	T	90 (45)	87 (43.5)	23 (11.5)	0.33	0.78	Brazilian
	rs7903146	C	T	512 (48.2)	435 (41)	115 (10.8)	0.31	0.12	Indian
	rs7903146	C	T	162 (53.6)	111 (36.8)	24 (7.9)	0.27	0.42	Ghanaian
	rs7903146	C	T	91 (82)	20 (18)	-	0.09	0.30	Indonesian
MC4R	rs17782313	T	C	168 (55.6)	110 (36.4)	19 (6.3)	0.25	0.86	Ghanaian
	rs17782313	T	C	139 (69.5)	55 (27.5)	6 (3)	0.17	0.84	Brazilian
	rs17782313	T	C	84 (75.7)	26 (23.4)	1 (0.9)	0.13	0.51	Indonesian
	rs2229616	G	A	290 (96)	9 (3)	-	0.02	0.79	Ghanaian
	rs2229616	G	A	199 (99.5)	1 (0.5)	-	0	0.97	Brazilian
	rs2229616	G	A	110 (99.1)	1 (0.9)	-	0	0.96	Indonesian
PPARG	rs1801282	C	G	298 (98.7)	1 (0.3)	-	0	0.98	Ghanaian
	rs1801282	C	G	170 (85)	30 (15)	-	0.08	0.25	Brazilian
	rs1801282	C	G	100 (90.1)	10 (9)	1 (0.9)	0.05	0.21	Indonesian
FTO	rs9939609	T	A	76 (25.2)	163 (54)	60 (19.9)	0.47	0.11	Ghanaian
	rs9939609	T	A	66 (59.5)	38 (34.2)	7 (6.3)	0.23	0.63	Indonesian
	rs9939609	T	A	138 (34.5)	214 (53.5)	48 (12)	0.39	0.01	Turkish
	rs10163409	A	T	296 (98)	2 (0.7)	-	0	0.95	Ghanaian
	rs10163409	A	T	117 (58.5)	64 (32)	17 (8.5)	0.25	0.06	Brazilian
	rs10163409	A	T	100 (90.1)	11 (9.9)	-	0.05	0.58	Indonesian
	rs8050136	C	A	866 (81.5)	183 (17.2)	13 (1.2)	0.1	0.35	Indian
	rs8050136	C	A	66 (59.5)	38 (34.2)	7 (6.3)	0.23	0.63	Indonesian
	rs8050136	C	A	71 (35.5)	96 (48)	30 (15)	0.4	0.79	Brazilian
	918031	T	C	267 (25.1)	558 (52.5)	237 (22.3)	0.49	0.09	Indian

	1588413	C	T	530 (49.9)	454 (42.7)	78 (7.3)	0.29	0.15	Indian
	11076023	T	A	225 (21.2)	540 (50.8)	297 (28)	0.53	0.48	Indian
CDKN2A/B	rs10811661	T	C	280 (92.7)	18 (6)	-	0	0.59	Ghanaian
	rs10811661	T	C	151 (75.5)	45 (22.5)	4 (2)	0.13	0.76	Brazilian
	rs10811661	T	C	47 (42.3)	55 (49.5)	9 (8.1)	0.33	0.20	Indonesian
KCNQ1	rs2237895	A	C	230 (76.2)	69 (22.8)	-	0.12	0.02	Ghanaian
	rs2237895	A	C	85 (42.5)	91 (45.5)	23 (11.5)	0.34	0.86	Brazilian
	rs2237895	A	C	56 (50.5)	45 (40.5)	10 (9)	0.29	0.82	Indonesian
	rs2237892	C	T	203 (67.2)	90 (29.8)	4 (1.3)	0.16	0.09	Ghanaian
	rs2237892	C	T	160 (80)	35 (17.5)	5 (2.5)	0.11	0.08	Brazilian
	rs2237892	C	T	42 (37.8)	50 (45)	19 (17.1)	0.4	0.54	Indonesian
ADIPOQ	rs266729	C	G	248 (48)	49 (42.7)	-	0.08	0.12	Ghanaian
	rs266729	C	G	44 (39.6)	54 (48.6)	13 (11.7)	0.36	0.56	Indonesian
	rs17846866	T	G	297 (98.3)	-	-	0	-	Ghanaian
	rs17846866	T	G	103 (92.8)	8 (7.2)	-	0.04	0.69	Indonesian
CAPN10	rs2975760	T	C	281 (93)	10 (3.3)	1 (0.3)	0.02	0.01	Ghanaian
	rs2975760	T	C	154 (77)	31 (15.5)	15 (7.5)	0.15	<0.0001	Brazilian
	rs5030952	T	C	95 (31.5)	145 (48)	57 (18.9)	0.44	0.90	Ghanaian
	rs5030952	C	T	73 (65.8)	31 (27.9)	7 (6.3)	0.2	0.15	Indonesian
	rs5030952	G	A	227 (75.2)	67 (22.2)	1 (0.3)	0.12	0.09	Ghanaian
	rs3792267	G	A	101 (91)	9 (8.1)	1 (0.9)	0.05	0.14	Indonesian
	rs3792267			813 (76.6)	238 (22.4)	11 (1)	0.12	0.16	Indian

Abbreviations: MINANG, Minangkabau Indonesia Study on Nutrition and Genetics; CURES Chennai Urban Rural Epidemiology Study; GONG, Genetics of obesity and nutrition in Ghana; BOLD, Obesity, Lifestyle and Diabetes in Brazil; SNP single nucleotide polymorphisms; MAF minor allele frequency; HWE Hardy-Weinberg equilibrium; *TCF7L2* Transcription factor 7-like 2; *MC4R* melanocortin 4 Receptor; *PPARG* Peroxisome proliferator-activated receptor gamma; *FTO* fat mass and obesity-associated; *CDKN2A/2B* Cyclin dependent kinase inhibitor 2A/2B; *KCNQ1* Potassium voltage-gated channel subfamily Q member 1; *ADIPOQ*, adiponectin and *CAPN10* Calpain.

7.8 Limitations and strengths

There are some limitations that need to be considered. Some of the studies included in this thesis had relatively small sample sizes; suggesting that we may have had insufficient power for our analysis. However, we used a GRS approach to maximise power and significant associations and interactions were observed. Furthermore, a cross-sectional study design was employed by all 5 studies, limiting our ability to investigate the causal relationship between the GRS-lifestyle interactions on cardiometabolic diseases and related traits. Dietary intake and physical activity were assessed using self-reported measures which might introduce recall bias. Additionally, the effect of food source (plant and animal) on detected GRS-diet interactions was examined only in the CURES study, which might have led to a better understanding of these interactions. The impact of different fats (e.g., omega-6 and omega-3 PUFA), carbohydrates (eg sugars and starch) wasn't determined in any of the studies and these may have differential effects.

The main strengths of this thesis included the use of well-characterised populations and the construction of different GRSs using several genetic variants. This approach is especially important for polygenic traits and has an advantage over single-SNP analysis as it can increase statistical power and provide better identification of an individual's disease risk (153). The CURES and MINANG studies used validated food frequency questioners (FFQs) (352, 518) to measure the long-term macronutrient consumption of the population. Furthermore, study outcomes were measured by trained staff to ensure the accuracy of these measures. GRS-diet interactions on metabolic traits have not been previously analysed in healthy Turkish adults, Ghanaian adults, and Brazilian young adults, thus these studies were the first of their kind in these populations.

7.9 Future prospects

In this thesis, the effects of genetic variants on cardiometabolic traits were found to be influenced by lifestyle factors including physical activity and intakes of protein and fat. Replicating these gene-lifestyle interactions using longitudinal interventional studies with larger sample sizes is important before enforcing any public health recommendations. Mechanistic studies are also required to examine how SNPs interact with lifestyle factors in relation to disease risk. Isolating the macronutrient accountable for any nutrigenetic effects from a complex food matrix is difficult, as these nutrients usually compensate each other (563). Thus, conducting dietary trials is recommended as it can strongly emphasise individuals' compliance to a specific dietary exposure. Future studies should also investigate the modulation effect of micronutrients and food source on genetic associations with cardiometabolic traits; such evidence will have significant implications in terms of applying dietary recommendations in clinical settings. Future research should consider assessing anthropometric parameters using robust measures such as magnetic resonance imaging, dual-energy X-ray absorptiometry, and/or computed tomography scans to assess body composition (29). It is also recommended to examine gene-lifestyle interaction in individuals at high risk of cardiometabolic diseases such as the elderly. Insights from this thesis encourage the use of personalised nutrition in preventing or treating cardiometabolic diseases, whereby genetic information can be used in predicting an individual's risk of cardiometabolic diseases and might be modified by an individual's dietary intake and physical activity.

Although gene-nutrient interactions have been investigated extensively in the population of the West, very few research studies have been performed in the LMICs and, hence, the Gene–Nutrient Interactions (GeNuIne) Collaboration has been established to focus on this missing gap in human nutrition in these countries and significant interactions on cardiometabolic diseases have been detected (220, 221). The field of nutrigenetics has been

remarkably improved, however, little is known regarding cardiometabolic pathways of observed gene-nutrient interactions (564). Hence, there is a need for a science investigating connections between nutrient intake and genetic susceptibility (565). In the broadest context of studying gene-diet interactions, a new field termed "Nutrigenomics" has been identified (204). Both nutrigenetics and nutrigenomics can ultimately help in implementing personalised nutrition and revolutionising our ability in designing optimal nutrition recommendations based on an individual's characteristics for maintaining health and preventing cardiometabolic diseases (243). Currently, dietary recommendations are population-based in which 'one size fits all', showing limited success in terms of preventing or treating diet-related diseases due to several factors such as lack of adherence and motivation to dietary interventions (566). Considering genetic information when designing nutritional interventions has shown to promote better changes in dietary behaviours (564).

The field of 'Foodomics' has become an increasingly important tool, combining 'omics' technologies (i.e., transcriptomics, metabolomics and proteomics) to prevent diet-related diseases (567, 568). Foodomics has helped in understanding interindividual differences in response to dietary interventions, and in identifying interactions of dietary bioactive compounds at the cellular, molecular and biochemical levels (567, 569). For example, numerous studies have examined the effect of dietary polyphenols in controlling intracellular signalling and biochemical mechanisms in relation to cancer prevention (570, 571).

The majority of genetic association studies focusing on disease risk have been performed in European populations (78%), highlighting the need for recruiting more diverse populations (572). The under-representation of diverse ethnic groups in genomic studies in humans can limit our ability of translating these studies into clinical practice. Studying ethnically diverse populations is challenging in many settings, and this might be due to mistrust in the biomedical study originated from previous exploitation experiences (572). Obtaining reliable phenotype

information is important for generating quality genetic associations, emphasising the need for adequate facilities and personnel. In many low middle-income countries that are characterised by great diversity, investment in professional training and infrastructure is primarily required (572).

In summary, improvement in the field of nutrigenetics offers a promising future in terms of implementing personalised nutrition for preventing or treating dietary-related cardiometabolic diseases. However, there is a continuous need for investigating gene-diet interactions in diverse ethnic groups using well-powered interventions with larger sample sizes.

7.10 Conclusion

In conclusion, my research has identified novel interactions between metabolic-GRSs and dietary intakes of protein and fat on central obesity indicators in the Indonesian and Ghanaian populations, respectively. There was also a significant interaction between the *FTO* SNP rs10163409 and protein intake on the risk of central obesity in the Turkish population. Furthermore, metabolic-GRSs showed significant interactions with intakes of protein and fat on T2D-related traits among Indian and Brazilian populations, respectively. High metabolic-GRSs were associated with higher BMI in both Turkish and Indonesian populations, where it was associated with lower BMI in Brazilian young adults. Similarly, having higher GRS was associated with higher WC in Turkish and Indian Individuals. Given that reducing fat intake to less than 30% of TEI and consuming a safe amount of dietary protein are recommended by the WHO for improving health and preventing cardiometabolic diseases (481), the gene-nutrient interaction findings identified in this thesis can have significant public health implications. Interestingly, gene-protein interactions on cardiometabolic traits were similar in the Turkish, Indonesian and Indian populations, where GRS-fat intake interactions on metabolic traits were similar in both Ghanaian and Brazilian individuals. In summary, this thesis contributes to a

better understanding of the complex interplay between genetic and lifestyle factors in the variation of cardiometabolic metabolic traits across multiple ethnic groups **(Figure 13).**

Randomised control trials with larger sample sizes of different ethnicities and more precise and objective measures of lifestyle factors are needed to replicate our findings. Furthermore, prospective genotyping should be considered in future studies to avoid an imbalance in the frequency of genotype between groups, which might confound the findings. This thesis investigated only a limited number of the increasingly identified metabolic-associated SNPs, thus there is a need to utilise a comprehensive panel of genetic variants to construct the GRS.

To conclude, this thesis has demonstrated significant GRS-nutrient interactions on cardiometabolic traits. However, these interactions need to be replicated in larger independent cohorts. Functional studies are also required to understand the molecular aspects of these interactions before applying personalised dietary strategies to prevent or treat cardiometabolic diseases.

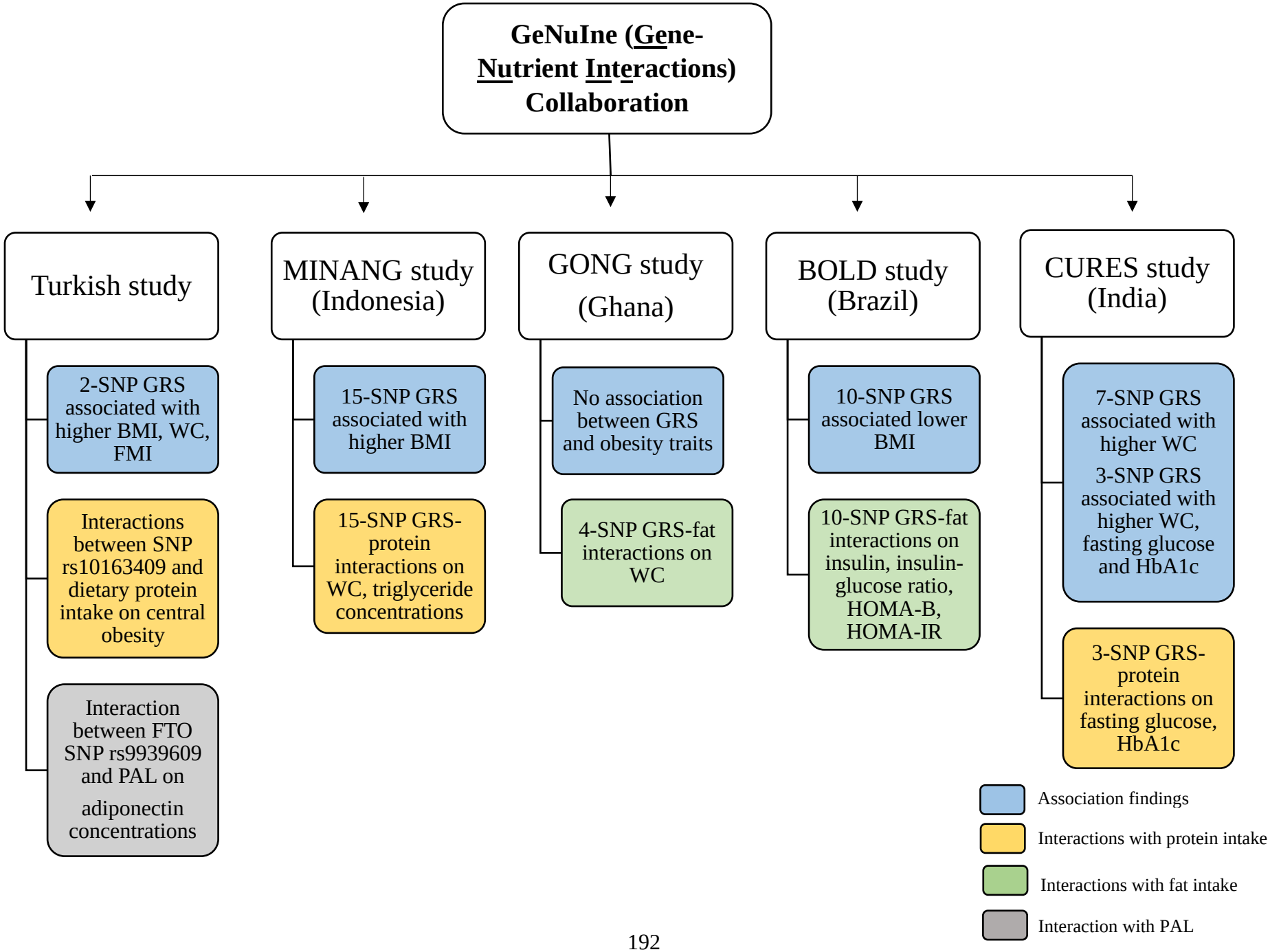

GeNuIne (Gene-Nutrient Interactions) Collaboration
Turkish study
2-SNP GRS associated with higher BMI, WC, FMI
Interactions between SNP rs10163409 and dietary protein intake on central obesity
Interaction between FTO SNP rs9939609 and PAL on adiponectin concentrations
MINANG study (Indonesia)
15-SNP GRS associated with higher BMI
15-SNP GRS-protein interactions on WC, triglyceride concentrations
GONG study (Ghana)
No association between GRS and obesity traits
4-SNP GRS-fat interactions on WC
BOLD study (Brazil)
10-SNP GRS associated lower BMI
10-SNP GRS-fat interactions on insulin, insulin-glucose ratio, HOMA-B, HOMA-IR
CURES study (India)
7-SNP GRS associated with higher WC
3-SNP GRS associated with higher WC, fasting glucose and HbA1c
3-SNP GRS-protein interactions on fasting glucose, HbA1c
Association findings
Interactions with protein intake
Interactions with fat intake
Interaction with PAL

Figure 13: The main findings of the thesis.

Abbreviations**:** GeNuIne, Gene–Nutrient Interactions; MINANG, Minangkabau Indonesia Study on Nutrition and Genetics; CURES Chennai Urban Rural Epidemiology Study; GONG, Genetics of Obesity and Nutrition in Ghana; BOLD, Obesity, Lifestyle and Diabetes in Brazil; SNP, single nucleotide polymorphism; GRS, genetic risk score; BMI, body mass index; WC, waist circumference; FMI, fat mass index; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; HOMA-IR, homeostasis model assessment estimate of insulin resistance and HOMA-B, homeostasis model assessment estimate of insulin secretion.

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Appendix

9.1 Research analysis plan: *FTO* gene–lifestyle interactions on serum adiponectin concentrations and central obesity in a Turkish population.

Main study objective:

The study aimed to investigate the associations of the fat mass and obesity associated *(FTO)* gene single nucleotide polymorphisms (SNPs) with obesity in a Turkish population (n=200) and to examine whether these associations were influenced by lifestyle factors.

Table 1. Previous studies that have examined the association of the selected SNPs with obesity and related traits.

Gene	rs number	Reference
Fat mass and obesity associated (*FTO*)	rs9939609	(76, 285-288)
Fat mass and obesity associated (*FTO*)	rs10163409	(303)

Obesity cut-off values:

1- The body mass index (BMI) classification of the world health organisation (WHO) was used to classify individuals as non-obese (BMI < 25.00 kg/m^2) and obese (BMI ≥ 25.00 kg/m^2) (305).

2- Increased waist circumference (WC) (central obesity) was defined based on cut-points established for Turkish adults (WC ≥ 90 cm for men/ ≥ 80 cm for women) (307)

Table 2. The study objectives.

Objective 1: Determining which genetic model to use for each genetic variant: additive, dominant or recessive.		
Aims:	**Statistical test used:**	**1) Reason for statistical test used** **2) Outcome of statistical test used** **3) covariates** (when appropriate)

1a) Determining genotypes' frequencies to select the appropriate genetic model.	Descriptive statistics: Frequencies	1) Reason for test: Exposure variables (*FTO* SNPs) are categorical variables. 2) Outcome of statistical test used: To identify the frequencies of the common homozygous, heterozygous, and rare homozygous genotypes. Thus, an appropriate genetic model can be selected for each genetic variant: additive, dominant or recessive. Also, the minor allele frequency can be calculated.
Objective 2: Determining whether each genetic variant was in HWE		
2a) Assess whether the observed genotype frequencies are in HWE	Chi-Squared test	1) Reason for test: Comparing the observed genotype frequencies with the expected values under Hardy-Weinberg 2) Outcome of statistical test: Determining whether a population is in HWE at a specific locus (239)
Objective 3: To generate descriptive statistics of the study participants		
3a) To define the descriptive statistics of the study participants	Descriptive statistics: -Descriptive for continuous variables -Frequencies for categorical variables	1. Reason for statistical test used: Determining the demographic, dietary and anthropometric measures of the targeted outcomes in all the study participants (239, 573). 2. Outcome of statistical test used: -Determining the means and standard deviations of the collected demographic, dietary and anthropometric variables: • Age (year) • BMI (kg/m2) • WC (cm) • FMI • Adiponectin (ng/ml) • Energy (kcal/day) • Protein (g) • Fat (g) • Carbohydrate (g) • Fibre (g) • SFA (g) • MUFA (g) • PUFA (g) -Determining the frequency for categorical variables: • Physical activity levels

3b) The descriptive statistics table was then categorised into two groups: non-Obese and Obese.	-Students t-test (Continuous variables) -Chi-square test (Categorical variables)	1. Reason for statistical test used: Comparing the means and standard deviations, as well as the frequencies of the demographic, dietary and anthropometric variables between the two groups (non-Obese and Obese individuals). 2. Outcome of statistical test used: Detecting if the demographic, anthropometric and dietary variables were significantly different between the two groups (241, 441).
Objective 4: To test the associations of the *FTO* SNPs with obesity and related traits; BMI (kg/m2), FMI, WC (cm), Adiponectin (ng/ml).		
4a) To compare the genotype frequencies between obese and non-obese.	Chi-square test	1) Reason for test: The exposure variables (*FTO* SNPs) are categorical variables, and the outcome variable (obesity) is a categorical variable. 2) Outcome of test: Identifying the impact of the *FTO* SNPs on obesity.
4b) To test for the associations of the *FTO* SNPs with obesity-related traits.	Univariate linear regression	1) Reason for test: The exposure variables (*FTO* SNPs) are categorical variables, and the outcome variables (obesity-related traits) are continuous variables. 2) Outcome of test: Identifying the impact of the *FTO* SNPs on obesity-related traits (573, 574). 3) Covariates to be adjusted: Sex, age, hypertension, cardiovascular diseases, and obesity status (258).
Objective 5: Testing the interaction between *FTO* SNPs and lifestyle factors (dietary intake and physical activity) on obesity and related traits.		
5a) Testing the interaction between the *FTO* SNPs and lifestyle factors including dietary intake (carbohydrate, protein, fibre and fat intakes in grams/day) and physical on obesity-related traits	Univariate linear regression	1) Reason for test: The exposure variables (*FTO* SNPs) are categorical variables, and the outcome variables (obesity-related traits) are continuous variables. 2) Outcome of test: Finding the effect of the *FTO* SNPs and lifestyle factors including physical activity and dietary intakes of carbohydrate, protein, fat and fibre in grams on obesity-related traits (261, 575). 3) Covariates to be adjusted: age, gender, hypertension, cardiovascular diseases, total energy intake and obesity status (529).

5b) Testing the interaction between the *FTO* SNPs and lifestyle factors including dietary intake (carbohydrate, protein, fibre and fat intakes in grams/day) and physical on obesity.	Logistic regression	1) Reason for test: The exposure variables (*FTO* SNPs) are categorical variables, and the outcome variable (obesity) is a categorical variable. 2) Outcome of test: Finding the effect of the *FTO* SNPs and lifestyle factors including physical activity and dietary intakes of carbohydrate, protein, fat and fibre (in grams) on obesity (573, 575). 3) Covariates to be adjusted: Age, gender, hypertension, cardiovascular diseases, total energy intake and obesity status. (258)
5c) Statistically significant interactions were investigated in more depth, where individuals were stratified by the tertiles of the lifestyle factors.	-Univariate linear regression -Logistic regression	1) Reason for test: -The exposure variables (*FTO* SNPs) are categorical variables, and the outcome variables (obesity-related traits) are continuous variables. - The exposure variables (*FTO* SNPs) are categorical variables, and the outcome variables (obesity) is a categorical variable. 2) Outcome of test: Determining whether the low, medium, and high intakes of these macronutrients, as well as the levels of physical activity, are causing the observed interactions (261, 575). 3) Covariates to be adjusted: age, gender, hypertension, cardiovascular diseases, total energy intake and obesity status (576).
SPECIAL NOTES:		
When investigating the effect of macronutrients in grams, the analysis should be adjusted for Kcal. However, no adjustment for Kcal is needed when testing interactions with the percentage energy intake of the macronutrients because adjustment has already been performed.	Compute variables	• For carbohydrate interactions: 1g of carbohydrates = 4kcal • For fat interactions: 1g=9 kcal • For protein interactions: 1g= 4kcal (577)

GRS genetic risk score; SNP single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; BMI, body mass index; WC, waist circumference; FMI, fat mass index; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

9.2 Research analysis plan: Interaction between the genetic risk score and dietary protein intake on cardiometabolic traits in Southeast Asians (Indonesian population).

Main study objective:

The study aimed to investigate the association of 15-SNP GRS with cardiometabolic traits and examined whether these associations were modified by lifestyle factors such as dietary intake and physical activity in 110 Minangkabau women.

Table 3. Previous studies that have examined the associations of the selected SNPs with cardiometabolic traits.

Gene	rs number	Reference
Melanocortin 4 Receptor (*MC4R*)	rs17782313	(120, 378, 578)
Melanocortin 4 Receptor (*MC4R*)	rs2229616	(579-581)
Fat mass and obesity associated (*FTO*)	rs9939609	(76, 285-288)
Fat mass and obesity associated (*FTO*)	rs8050136	(582-584)
at mass and obesity associated (*FTO*)	rs10163409	(303)
Transcription factor 7-like 2 (*TCF7L2*)	rs7903146	(585-588)
Transcription factor 7-like 2 (*TCF7L2*)	rs12255372	(589, 590)
Adiponectin *(ADIPOQ*)	rs266729	(145, 591)
Adiponectin *(ADIPOQ*)	rs17846866	(591, 592)
Potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*)	rs2237895	(89, 593)
Potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*)	rs2237892	(594, 595)
Cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/*2B)	rs10811661	(596, 597)
Peroxisome proliferator-activated receptor gamma (*PPARG*)	rs1801282	(598, 599)
Calpain 10 (*CAPN10)*	rs3792267	(600, 601)
Calpain 10 (*CAPN10)*	rs5030952	(600-602)

Obesity cut-off values:

1- Common obesity was defined based on the Asia-Pacific classification of BMI for Asians, where non-obese individuals (BMI<23 kg/m^2) and obese individuals (BMI≥23 kg/m^2) were classed accordingly (366).

2- Central obesity was defined based on WHO classification of WC (WC>80 cm for women) (30).

Table 4. The study objectives.

Objective 1: Determining whether each genetic variant was in HWE		
Aims:	**Statistical test used:**	**1) Reason for statistical test used** **2) Outcome of statistical test used** **3) covariates** (when appropriate)
1a) Assess whether the observed genotype frequencies are in HWE	Chi-Squared test	1) Reason for test: Comparing the observed genotype frequencies with the expected values under Hardy-Weinberg 2) Outcome of statistical test: determining whether a population is in HWE at a specific locus (239)
Objective 2: To generate descriptive statistics of the study participants.		
2a) To define the descriptive statistics of the study participants	Descriptive statistics: -Descriptive for continuous variables - Frequency for categorical variables	1. Reason for statistical test used: Determining the demographic, dietary, biochemical, and anthropometric measures of the targeted outcomes in all the study participants (239, 573). 2. Outcome of statistical test used: -Determining the means and standard deviations of the collected demographic, dietary, biochemical, and anthropometric variables: • Age (yrs) • BMI (kgm2) • WC (cm) • Glucose (mg/dl) • Insulin (mIU/L) • HbA1c (ng/ml) • Triglycerides (mg/dl) • Cholesterol (mg/dl) • HDL-C (mg/dl) • LDL-C (mg/dl) • SB (mmHg) • DBP (mmHg) • Total energy (kcal/d) • Carbohydrate intake (%) • Protein intake (%)

		• Fat intake (%) • Dietary fibre (g) • SFA (g) • MUFA (g) • PUFA (g) • MET (min/week) • GRS - Determining the frequency for categorical variable: • Physical activity levels
2b) The descriptive statistics table was then categorised into two groups: non-centrally obese and centrally obese.	-Students t-test - Chi-squared test	1. Reason for statistical test used: Comparing the mean and standard deviations and the frequencies of demographic, dietary and anthropometric variables between the two groups (non-centrally obese and centrally obese). 2. Outcome of statistical test used: Detecting if the demographic, anthropometric and dietary variables were significantly different between the two groups (241, 441).
Objective 3: To test the associations between cardiometabolic-related genetic variants, as a 15-SNP GRS, and cardiometabolic traits; BMI, WC, glucose, insulin, HbA1C, triglycerides, cholesterol, HDL-C, LDL-C, SB, DBP.		
3a) To test for the association between the GRS and cardiometabolic traits.	Univariate linear regression	1) Reason for test: The exposure variable (GRS) is a categorical variable, and the outcome variables (cardiometabolic traits) are continuous variables. 2) Outcome of test: Identifying the impact of the GRS on cardiometabolic traits (573, 574). 3) Covariates to be adjusted: Age, residential area and additionally for BMI when BMI is not an outcome (258, 603)
Objective 4: Testing the interaction between cardiometabolic-related genetic variants, as a 15-SNP GRS, and lifestyle factors (dietary intake and physical activity) on cardiometabolic traits.		
4a) Testing the interaction between the GRS and lifestyle factors including dietary intakes of protein (% of TEI), fat (% of TEI), carbohydrate (% of TEI) and fibre (grams), and physical activity) on cardiometabolic traits	Univariate linear regression	1) Reason for test: The exposure variable (GRS) is a categorical variable, and the outcome variables (cardiometabolic traits) are continuous variables. 2) Outcome of test: Finding the effect of the GRS and lifestyle factors including physical activity and dietary intakes of carbohydrate, protein, fat and fibre in grams on cardiometabolic-related traits (261, 575). 3) Covariates to be adjusted: Age, residential area and BMI when BMI is not an outcome (258, 529, 603).
4b) Statistically significant interactions were investigated in more depth, where individuals were stratified by the tertiles of the lifestyle factors.	Univariate linear regression	1) Reason for test: The exposure variable (GRS) is a categorical variable, and the outcome variables (cardiometabolic traits) are continuous variables. 2) Outcome of test: Determining whether the low, medium and high intakes of these macronutrients, as well as the levels of physical activity, are causing the observed interactions (261, 575). 3) Covariates to be adjusted: Age, residential area and BMI when BMI is not an outcome (258, 576, 603).

SPECIAL NOTES:		
When investigating the effect of macronutrients in grams, the analysis should be adjusted for Kcal. However, no adjustment for Kcal is needed when testing interactions with the percentage energy intake of the macronutrients because adjustment has already been performed.	Compute variables	• For carbohydrate interactions: 1g of carbohydrates = 4kcal • For fat interactions: 1g=9 kcal • For protein interactions: 1g= 4kcal (577)

GRS genetic risk score; SNP single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; BMI, body mass index; WC, waist circumference; HbA1C, glycated haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; MET, metabolic equivalent of task; TEI, total energy intake

9.3 Research analysis plan: Interaction between Metabolic Genetic Risk Score and Dietary Fatty Acid Intake on Central Obesity in a Ghanaian Population

Main study objective:

The study aimed to investigate the association of selected SNPs, as 12-SNP, 8-SNP and 4-SNP GRS, with obesity-related traits and to examine whether lifestyle factors such as dietary intake and physical activity modified these associations in 302 healthy Ghanaian adults.

Table 5. Previous studies that have examined the association of the selected SNPs with obesity-related traits.

Gene	rs number	Reference
Transcription factor 7-like 2 (*TCF7L2*)	rs12255372	(589, 590)
Transcription factor 7-like 2 (*TCF7L2*)	rs7903146	(589, 590)
Melanocortin 4 Receptor (*MC4R*)	rs17782313	(120, 378, 578)
Melanocortin 4 Receptor (*MC4R*)	rs2229616	(579-581)
Fat mass and obesity associated *(FTO)*	rs9939609	(76, 285-288)
Fat mass and obesity associated *(FTO)*	rs10163409	(303)
Adiponectin *(ADIPOQ*)	rs266729	(145, 591)
Potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*)	rs2237892	(594, 595)
Cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/*2B)	rs10811661	(596, 597)
Calpain 10 (*CAPN10)*	rs3792267	(598, 599)
Calpain 10 (*CAPN10)*	rs5030952	(600-602)
Peroxisome proliferator-activated receptor gamma (*PPARG*)	rs1801282	(598, 599)

Obesity cut off-values:

Non-obese individuals refer to the individuals with a BMI < 25 Kg/m2, according to the WHO classification of BMI. Overweight/obese cases refer to individuals with BMI ≥25 Kg/m2, according to the WHO classification of BMI (305).

Table 6. The study objectives.

Objective 1: Determining whether each genetic variant was in HWE		
Aims:	**Statistical test used:**	**1) Reason for statistical test used** **2) Outcome of statistical test used** **3) covariates** (when appropriate)
1a) Assess whether the observed genotype frequencies are in HWE	Chi-Squared test	1) Reason for test: Comparing the observed genotype frequencies with the expected values under Hardy-Weinberg 2) Outcome of statistical test: Determining whether a population is in HWE at a specific locus (239)
Objective 2: To generate descriptive statistics of the study participants.		
2a) To define the descriptive statistics of the study participants	Descriptive statistics: Descriptive for continuous variables	1. Reason for statistical test used: Determining the demographic, dietary and anthropometric measures of the targeted outcomes in all the study participants (239, 573). 2. Outcome of statistical test used: Determining the means and standard deviations of the collected demographic, dietary and anthropometric variables: • Age (years) • BMI (kg/m2) • WC (cm • WHR • Visceral fat (%) • Body fat (%) • Total energy intake (%) • Protein intake (g/day) • Total fat intake (g/day) • Carbohydrate intake (g/day) • Fibre intake (g/day) • Total SFA intake (g/day) • Total MUFA intake (g/day) • Total PUFA intake (g/day)

2b) The descriptive statistics table was then categorised into two groups: non-Obese and overweight/Obese.	Students t-test	1. Reason for statistical test used: Comparing the means and standard deviations of demographic, dietary and anthropometric variables between the two groups (non-Obese and overweight/Obese individuals). 2. Outcome of statistical test used: Detecting if the demographic, anthropometric and dietary variables were significantly different between the two groups (241, 441).
Objective 3: To test the associations of the 12-SNP, 8-SNP and 4-SNP GRS with obesity-related traits; BMI, percentage of body fat and visceral fat, WC and WHR.		
3a) To test for the association between the 12-SNP, 8-SNP and 4-SNP GRS and obesity-related traits	Univariate linear regression	1) Reason for test: The exposure variables (GRSs) are categorical variables, and the outcome variables (obesity-related traits) are continuous variables. 2) Outcome of test: Identifying the impact of the GRSs on obesity-related traits (573, 574). 3) Covariates to be adjusted: age, sex and additionally for BMI when BMI is not an outcome (258)
Objective 4: Testing the interaction between the 12-SNP, 8-SNP and 4-SNP GRS, and lifestyle factors (dietary intake and physical activity) on obesity-related traits.		
4a) Testing the interaction between lifestyle factors (dietary intake and physical activity) and 12-SNP, 8-SNP and 4-SNP GRS on obesity-related traits	Univariate linear regression	1) Reason for test: The exposure variables (GRSs) are categorical variables, and the outcome variables (obesity-related traits) are continuous variables. 2) Outcome of test: Finding the effect of the GRSs and lifestyle factors including physical activity and dietary intakes of carbohydrate, protein, fat and fibre (in grams) on obesity-related traits (261, 575) 3) Covariates to be adjusted: age, sex, total energy intake and additionally for BMI when BMI is not an outcome (529).
4b) Determining whether the below or above the median intake of these macronutrients are causing the observed interactions	Univariate linear regression	1) Reason for test: The exposure variables (GRSs) are categorical variables, and the outcome variables (obesity-related traits) are continuous variables. 2) Outcome of test: Detecting the effect of GRSs and the intake of various quantities of macronutrients on obesity-related traits. 3) Covariates to be adjusted: Age, sex, total energy intake and additionally for BMI when BMI is not an outcome (576).
SPECIAL NOTES:		
When investigating the effect of macronutrients in grams, the analysis should be adjusted for Kcal. However, no adjustment for Kcal is	Compute variables	• For carbohydrate interactions: 1g of carbohydrates = 4kcal • For fat interactions:

needed when testing interactions with the percentage energy intake of the macronutrients because adjustment has already been performed.		1g=9 kcal • For protein interactions: 1g= 4kcal (577)
Significant GRS*nutrient interactions were analysed in more depth according to the specific type of macronutrient showing the interaction	Univariate linear regression	For fat interactions: Based on the median intake of total fat, SFA, MUFA and PUFA, the individuals were separated into two groups: ‘’below the median group” and ‘’above the median group”. Within each group, the association between the GRS and the outcome was examined (240).

GRS genetic risk score; SNP single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; BMI, body mass index; WC, waist circumference; WHR, waist hip ratio; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

9.4 Effect of dietary fat intake and genetic risk on glucose and insulin-related traits in Brazilian young adults

Main study objective:

The study aimed to investigate the association of selected 10-SNPs, as a metabolic-GRS, with metabolic disease-related traits and to assess the interaction between dietary and genetic factors on these traits in 200 healthy Brazilian young adults.

Table 7. Previous studies that have examined the associations of selected SNPs with metabolic disease-related traits

Gene	rs number	Reference
Melanocortin 4 Receptor (*MC4R*)	rs17782313	(120, 378, 578)
Fat mass and obesity associated (*FTO*)	rs8050136	(582-584)
at mass and obesity associated (*FTO*)	rs10163409	(303)
Transcription factor 7-like 2 (*TCF7L2*)	rs7903146	(585-588)
Transcription factor 7-like 2 (*TCF7L2*)	rs12255372	(589, 590)
Potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*)	rs2237895	(89, 593)
Potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*)	rs2237892	(594, 595)
Cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/*2B)	rs10811661	(596, 597)
Peroxisome proliferator-activated receptor gamma (*PPARG*)	rs1801282	(598, 599)
Calpain 10 (*CAPN10)*	rs5030952	(600-602)

Table 8. The study objectives.

Objective 1: Determining whether each genetic variant was in HWE		
Aims:	**Statistical test used:**	**1) Reason for statistical test used** **2) Outcome of statistical test used** **3) covariates** (when appropriate)
1a) Assess whether the observed genotype frequencies are in HWE	Chi-Squared test	1) Reason for test: Comparing the observed genotype frequencies with the expected values under Hardy-Weinberg 2) Outcome of statistical test: Determining whether a population is in HWE at a specific locus (239)
Objective 2: To generate descriptive statistics of the study participants.		
2a) To define the descriptive statistics of the study participants	Descriptive statistics: -Descriptive for continuous variables	1. Reason for statistical test used: Determining the demographic, dietary, biochemical, and anthropometric measures of the targeted outcomes in all the study participants (239, 573). 2. Outcome of statistical test used: Determining the mean and standard deviation of the collected

		demographic, dietary, biochemical and anthropometric variables: • Age (years) • BMI (kg/m2) • WC (cm) • Body fat mass (%) • HbA1c (%) • Fasting serum glucose (mg/dL) • Fasting serum insulin (uU/mL) • Homeostasis model assessment estimate of insulin resistance (HOMA-IR) • Homeostasis model assessment estimate of insulin secretion (HOMA-B) • Insulin to glucose ratio • Energy (Kcal/day) • Protein (energy %) • Carbohydrate (energy %) • Fat (energy %) • SFA (%) • PUFA (%) • MUFA (%)
2b) The descriptive statistics table was then categorised into two groups: men and women.	Students t-test	1. Reason for statistical test used: Comparing the mean and standard deviations of demographic, dietary and anthropometric variables between the two groups (men and women). 2. Outcome of statistical test used: Detecting if the demographic, anthropometric and dietary variables were significantly different between the two groups (241, 441).
Objective 3: To test the associations of the 10-SNP GRS with metabolic traits; BMI, WC, body fat mass, HbA1c, fasting glucose, fasting insulin, HOMA-IR, HOMA-B, HOMA-B adjusted for HOMA-IR and insulin to glucose ratio		
3a) To test for the association between the metabolic-GRS and metabolic traits.	Univariate linear regression	1) Reason for test: The exposure variable (metabolic-GRS) is a categorical variable, and the outcome variables (metabolic traits) are continuous variables. 2) Outcome of test: Identifying the impact of the metabolic-GRS on metabolic traits (573, 574). 3) Covariates to be adjusted: Age, gender and additionally for BMI when BMI is not an outcome (258)
Objective 4: Testing the interaction between cardiometabolic-related genetic variants, as a GRS, and dietary intake on metabolic traits.		
4a) Testing the interaction between the GRS and lifestyle factors including dietary intakes of protein, fat and carbohydrate (in % of TEI) on metabolic traits.	Univariate linear regression	1) Reason for test: The exposure variable (metabolic-GRS) is a categorical variable, and the outcome variables (metabolic traits) are continuous variables. 2) Outcome of test: Finding the effect of the metabolic-GRS and dietary intakes of carbohydrate, protein and fat on metabolic-related traits (261, 575).

		3) Covariates to be adjusted: Age, gender, and BMI when BMI is not an outcome (529).
4b) Statistically significant interactions were investigated in more depth, where individuals were stratified by the tertiles of dietary intakes.	Univariate linear regression	1) Reason for test: The exposure variable (metabolic-GRS) is a categorical variable, and the outcome variables (metabolic traits) are continuous variables. 2) Outcome of test: Determining whether the low, medium and high intakes of these macronutrients are causing the observed interactions (261, 575). 3) Covariates to be adjusted: Age, gender and BMI when BMI is not an outcome (576).
SPECIAL NOTES:		
When investigating the effect of macronutrients in grams, the analysis should be adjusted for Kcal. However, no adjustment for Kcal is needed when testing interactions with the percentage energy intake of the macronutrients because adjustment has already been performed.	Compute variables	• For carbohydrate interactions: 1g of carbohydrates = 4kcal • For fat interactions: 1g=9 kcal • For protein interactions: 1g= 4kcal (577)
Significant GRS*nutrient interactions were analysed in more depth according to the specific type of macronutrient showing the interaction	Univariate linear regression	For fat interactions: Based on the median intake of total fat, SFA, MUFA and PUFA, the individuals were separated into two groups: 'below the median group" and 'above the median group". Within each group, the association between the GRS and the outcome was examined (240).

GRS genetic risk score; SNP single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; BMI, body mass index; WC, waist circumference; HbA1c: glycated haemoglobin; HOMA-IR: homeostasis model assessment estimate of insulin resistance; HOMA-B: homeostasis model assessment estimate of insulin secretion; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TEI, total energy intake

9.5 Lower Dietary Intake of Plant Protein Is Associated with Genetic Risk of Diabetes-Related Traits in Urban Asian Indian Adults

Main study objective:
The study aimed to examine the impact of dietary and genetic factors, as 7-SNP and 3-SNP GRS, on metabolic traits in 1062 Asian Indians.

Table 9. Previous studies which have examined the association of selected SNPs with metabolic disease-related traits

Gene	rs number	Reference
Transcription factor 7-like 2 (*TCF7L2*)	rs7903146	(585-588)
Transcription factor 7-like 2 (*TCF7L2*)	rs12255372	(589, 590)
Fat mass and obesity associated (*FTO*)	rs918031	(364, 604)
Fat mass and obesity associated (*FTO*)	rs1588413	(364)
Fat mass and obesity associated (*FTO*)	rs7193144	(364, 579, 605)
Fat mass and obesity associated (*FTO*)	rs1076023	(239, 364)
Fat mass and obesity associated (*FTO*)	rs8050136	(582-584)

Disease cut off-values:

1) Subjects with fasting plasma glucose (FPG)<5.6 mmol/l (100mg/dl) and 2 hr plasma glucose value of 7.8 mmol/l (140mg/dl) were defined as having normal glucose tolerance (NGT) according to the WHO (606).
2) Common obesity was defined as BMI ≥25 according to WHO Asia Pacific Guidelines for Asians (517).

Table 10. The study objectives.

Objective 1: Determining whether each genetic variant was in Hardy-Weinberg equilibrium (HWE)		
Aims:	**Statistical test used:**	**1) Reason for statistical test used** **2) Outcome of statistical test used** **3) covariates** (when appropriate)
1a) Assess whether the observed genotype frequencies are in HWE	Chi-Squared test	1) Reason for test: Comparing the observed genotype frequencies with the expected values under Hardy-Weinberg 2) Outcome of statistical test: Determining whether a population is in HWE at a specific locus (239)
Objective 2: To generate descriptive statistics of the study participants.		
2a) To define the descriptive statistics of the study participants	Descriptive statistics: -Descriptive for continuous variables	1. Reason for statistical test used: Determining the demographic, dietary, biochemical and anthropometric measures of the targeted outcomes in all the study participants (239, 573). 2. Outcome of statistical test used:

	-Frequencies for categorical variables	-Determining the means and standard deviations and the frequencies of the collected demographic, dietary, biochemical, and anthropometric variables: • Age (years) • BMI (kg/m2) • WC (cm) • HBA1C (%) • FPG (mg/dl) • Fasting Insulin (μIU/ml) • Energy (kcal/day) • Protein (%) • Animal protein (g/day) • Plant protein (g/day) • Fat (%) • Carbohydrate (%) • Dietary fibre (g) • Total SFA (g) • Total MUFA (g) • Total PUFA (g) - Determining the frequency for the categorical variable: • Sex (%)
2b) The descriptive statistics table was then categorised into two groups: NGT and DM.	-Students t-test for continuous variables -Chi-square for categorical variables	1. Reason for statistical test used: Comparing the means and standard deviations, as well as the frequency of demographic, dietary and anthropometric variables between the two groups (NGT and DM). 2. Outcome of statistical test used: Detecting if the demographic, anthropometric, biochemical and dietary variables were significantly different between the two groups (241, 441).
Objective 3: To test the associations of the 7-SNP and 3-SNP GRS with metabolic traits; BMI, WC, HBA1C, FPG (mg/dl) and fasting insulin.		
3a) To test for the association between the 7-SNP and 3-SNP GRS and metabolic traits	Univariate linear regression	1) Reason for test: The exposure variables (GRSs) are categorical variables, and the outcome variables (metabolic traits) are continuous variables. 2) Outcome of test: Identifying the impact of the GRSs on metabolic traits (573, 574). 3) Covariates to be adjusted: ex, age, T2D, anti-diabetic medication and additionally for BMI, when BMI is not an outcome (258)
Objective 4: To test the associations of the 7-SNP and 3-SNP GRS with DM.		

4a) To test for the association between the 7-SNP and 3-SNP GRS and DM.	Logistic regression	1) Reason for test: The exposure variables (GRSs) are categorical variables, and the outcome variables (DM) is a categorical variable. 2) Outcome of test: identifying the impact of the GRSs on obesity-related traits. 3) Covariates to be adjusted: sex, age, anti-diabetic medication, and BMI (258)
Objective 5: Testing the interaction between 7-SNP and 3-SNP GRS, and dietary intake on metabolic traits.		
5a) Testing the interaction between dietary intake and 7-SNP and 3-SNP GRS on metabolic traits.	Univariate linear regression	1) Reason for test: The exposure variables (GRSs) are categorical variables, and the outcome variables (metabolic traits) are continuous variables. 2) Outcome of test: Finding the effect of the GRSs and dietary intakes of fat, protein and carbohydrate (% of TEI) on metabolic traits (261, 575). 3) Covariates to be adjusted: Sex, age, T2D, antidiabetic medications and additionally for BMI, when BMI is not an outcome (529).
5b) Determining whether the below or above the median intake of these macronutrients are causing the observed interactions	Univariate linear regression	1) Reason for test: The exposure variables (GRSs) are categorical variables, and the outcome variables (metabolic traits) are continuous variables. 2) Outcome of test: Detecting the effect of GRSs and the intake of various quantities of macronutrients on metabolic traits. 3) Covariates to be adjusted: age, sex, total energy intake and additionally for BMI when BMI is not an outcome (576).
SPECIAL NOTES:		
When investigating the effect of macronutrients in grams, the analysis should be adjusted for Kcal. However, no adjustment for Kcal is needed when testing interactions with the percentage energy intake of the macronutrients because adjustment has already been performed.	Compute variables	• For carbohydrate interactions: 1g of carbohydrates = 4kcal • For fat interactions: 1g=9 kcal • For protein interactions: 1g= 4kcal (577)
Significant GRS*nutrient interactions were analysed in more depth according to the specific type of macronutrient showing the interaction	Univariate linear regression	For protein interactions: Significant interactions with protein intake were analysed in more depth according to dietary sources of protein (animal and plant protein), where individuals were classified into two groups according to the sample median intake of plant (39g/day) and animal protein (19g/day): below and above median groups. Individuals who

		consumed below the median were categorised as those who had low intakes of plant and animal protein, respectively, whereas individuals who consumed above the median were categorised as those who had high intakes of plant and animal protein, respectively.

GRS genetic risk score; SNP single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; BMI, body mass index; WC, waist circumference; HbA1c: glycated haemoglobin; FPG, fasting plasma glucose; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; NGT; normal glucose tolerance; DM diabetes mellites; TEI, total energy intake.

nutrients

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Article

Lower Dietary Intake of Plant Protein Is Associated with Genetic Risk of Diabetes-Related Traits in Urban Asian Indian Adults

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Abstract: The increasing prevalence of type 2 diabetes among South Asians is caused by a complex interplay between environmental and genetic factors. We aimed to examine the impact of dietary and genetic factors on metabolic traits in 1062 Asian Indians. Dietary assessment was performed using a validated semi-quantitative food frequency questionnaire. Seven single nucleotide polymorphisms (SNPs) from the Transcription factor 7-like 2 and fat mass and obesity-associated genes were used to construct two metabolic genetic risk scores (GRS): 7-SNP and 3-SNP GRSs. Both 7-SNP GRS and 3-SNP GRS were associated with a higher risk of T2D (p = 0.0000134 and 0.008, respectively). The 3-SNP GRS was associated with higher waist circumference (p = 0.010), fasting plasma glucose (FPG) (p = 0.002) and glycated haemoglobin (HbA1c) (p = 0.000066). There were significant interactions between 3-SNP GRS and protein intake (% of total energy intake) on FPG ($P_{interaction}$ = 0.011) and HbA1c ($P_{interaction}$ = 0.007), where among individuals with lower plant protein intake (<39 g/day) and those with >1 risk allele had higher FPG (p = 0.001) and HbA1c (p = 0.00006) than individuals with ≤1 risk allele. Our findings suggest that lower plant protein intake may be a contributor to the increased ethnic susceptibility to diabetes described in Asian Indians. Randomised clinical trials with increased plant protein in the diets of this population are needed to see whether the reduction of diabetes risk occurs in individuals with prediabetes.

Keywords: genetic risk score; metabolic traits; urban Asian Indian; dietary protein intake; gene–diet interaction; T2D

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1. Introduction

South Asian populations have a 50% higher risk of type 2 diabetes (T2D) than other populations [1,2] and this has significant implications, as patients with T2D have a 2–4 times increased risk of cardiovascular diseases [1]. The Asian Indian population have a unique phenotype characterised by abdominal and truncal adiposity, as indicated by larger waist

to hip ratios and waist circumference (WC), higher concentrations of plasma insulin, greater insulin resistance, impaired function of pancreatic β-cell and a genetic susceptibility to diabetes, which ultimately leads to significantly increased diabetes risk [3–5]. The burden of T2D is increasing globally, with India being a major contributor to the worldwide burden [6]. The number of diabetic individuals in India rose from 26.0 million in 1990 to 65.0 million in 2016 [7].

The increasing prevalence of T2D among Asian Indians is caused by a complex interplay between environmental and genetic factors, including urbanisation, which plays a large role [8–10]. Urbanisation in India is associated with increased consumption of processed foods and dietary fats, decreased level of physical activity and increased mental stress, amplifying the effects of abdominal obesity and insulin resistance [4,5,11]. Furthermore, the urban areas in India reported higher intake of protein from pulses and animal sources (including meat, fish, eggs and milk) than rural areas [12]. Several large longitudinal studies showed that the intake of animal protein was significantly associated with the risk of T2D [13–17]. In the context of rapid urbanisation and nutrition transition, interactions between Westernised diet, lifestyle and genetic factors have further escalated T2D prevalence in Asia [18,19]. In South Asians, several single nucleotide polymorphisms (SNPs) have been associated with adiposity [20–23], insulin resistance [24], pancreatic β-cell function [20,25,26] and T2D [20,22,23,26,27]. The fat mass and obesity-associated *(FTO)* gene has been recognised as one of the strongest obesity-related genes. The *FTO* SNPs, rs1588413, rs9939609 and rs8050136, have been shown to increase obesity risk by 1.27, 1.15 and 2.06 times among Indians, respectively [22,28]. Studies have reported strong associations of the Transcription factor 7-like 2 *(TCF7L2)* SNPs, rs7903146 and rs12255372, with T2D risk in Asian Indians [29–31]. To date, evidence has identified 243 genetic loci to be associated with T2D risk in South Asians, East Asians, Europeans, African Americans and Hispanics [32–35]. Single genetic variants have only a small to moderate effect on disease risk, thus combining effects of several SNPs into a genetic risk score (GRS) is required for better detection of individuals with high risk of diabetes [36].

Genome-wide association studies (GWAS) have discovered a large number of genetic variants associated with metabolic diseases and related traits; however, these SNPs describe only a small proportion of estimated heritability. Risk prediction of metabolic diseases is complicated by interactions between dietary and genetic factors, which may partly explain the missing heritability of diseases [37]. Investigating gene–diet interaction is important in understanding pathophysiology of metabolic diseases, which can lead to the development of 'personalised' nutrition focusing on tailoring dietary interventions according to individual genotypic makeup to prevent and treat metabolic diseases [38,39]. The effect of genetic factors on metabolic traits have been shown to be modified by dietary intake in several populations [40–44]. However, studies investigating GRS–diet interaction in the Indian population are still sparse. To help fill this gap in knowledge, we assessed the combined effect of seven genetic variants, as a GRS, on T2D and metabolic traits, and the extent to which dietary intake can influence these genetic associations among 1062 urban Asian Indians.

2. Methods

2.1. Study Participants

The present study included individuals from the urban area of the Chennai Urban Rural Epidemiology Study (CURES), which is a cross-sectional epidemiological study performed on a representative sample of Chennai city (formerly Madras) in southern India. The design and procedures of the CURES have been explained in detail previously [45]. In phase 1, a total of 26,001 adult subjects, of which 1529 were 'self-reported' or 'known diabetic' individuals, were recruited using a method of systematic random sampling. In phase 2, diabetic individuals were invited to the study centre for further investigation, of whom 1382 responded. In phase 3, every 10th individual of the total sample (n = 26,001 subjects), excluding individuals with self-reported diabetes, were

screened using an oral glucose tolerance test (OGTT). Individuals with fasting plasma glucose (FPG) < 5.6 mmol/L (100 mg/dL) and 2 h plasma glucose value of 7.8 mmol/L (140 mg/dL) were defined as having normal glucose tolerance (NGT) [46]. Those who had 2 h plasma glucose value of 11.1 mmol/L (200 mg/dL) were categorised as 'newly detected diabetic subjects' (n = 222) (Figure S1). The total sample of present study is 1062 individuals; the NGT individuals were chosen from Phase 3 (n = 496) and T2D individuals were chosen from Phase 2 and Phase 3 of the CURES (n = 566). The study was approved by the Madras Diabetes Research Foundation Institutional Ethics Committee and written informed consent was obtained from all study participants.

2.2. Anthropometric and Biochemical Measurements

Anthropometric variables including WC, weight and height were measured using standardised methods. The body mass index (BMI) was calculated with the formula of weight (in kilograms) divided by the square of height (in metres), with obesity being defined as BMI $\geq$ 25 according to World Health Organisation Asia Pacific Guidelines for Asians [47].

Biochemical tests were carried out using a Hitachi-912 Auto Analyzer (Hitachi, Mannheim, Germany), with kits provided by Roche Diagnostics (Mannheim). Glycated haemoglobin (HbA1c) was measured using high-performance liquid chromatography on a Variant machine (Bio-Rad, Hercules, CA, USA). FPG and serum insulin were measured using glucose oxidase-peroxidase and an enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark), respectively.

2.3. Dietary Assessments

Participants' habitual food intake over the previous year was measured using a validated semi-quantitative food frequency questionnaire (FFQ) administered by an interviewer [48]. The FFQ consists of 222 food items and individuals were asked to estimate the usual portion size and frequency (number of times per day, week, month or year/never) of food items listed in the FFQ. Participants were shown common household measures and photographic atlas of different sizes of fruits to help them in estimating portion sizes. The EpiNu® software was used to analyse the recorded data and estimate the intake of energy and macronutrients. The reported intake of various food groups was also estimated. The EpiNu software also provided the source of protein from various food groups. Animal protein intake was summed up using protein intake (g/day from FFQ) from animal food groups such as meat, poultry, fish, egg and dairy products. Similarly, plant protein intake was estimated from food groups such as cereals, millets, pulses and legumes, tubers, nuts, oilseeds, vegetables and fruits. In addition, dairy protein was estimated separately using dairy products such as milk products and fermented and unfermented milk.

2.4. SNP Selection and GRS Construction

A total of 7 metabolic disease-associated SNPs which have been extensively studied in various populations, including Asian Indians, were selected for the study [20–27,29]. The selected SNPs included *TCF7L2* SNPs, rs12255372 and rs7903146, and *FTO* SNPs, rs8050136, rs918031, rs1588413, rs7193144 and rs1076023. Details regarding these SNPs are summarised in Table S1. Each SNP was coded with the expected number of metabolic diseases-associated risk alleles. Consistent with previous studies [41,49,50], we used an unweighted method to construct the GRSs by summing the number of risk alleles of each SNP for each participant. The seven SNPs were used to generate a 7-SNP GRS that ranges from 1 to 11 risk alleles. The GRS was divided into 2 categories according to the median number of risk alleles: "GRS < 6 risk alleles" and "GRS $\geq$ 6 risk alleles", indicating individuals with lower and higher risk alleles of the SNPs, respectively. In addition, we constructed a GRS of 3 SNPs (*FTO*SNP rs8050136 and *TCF7L2*SNPs rs12255372 and rs7903146) that have shown consistent associations with metabolic disease-related outcomes across various ethnicities, including Asians [51–54]. The 3-SNP GRS ranges from

0 to 6 risk alleles and was divided into 2 categories according to the median number of risk alleles: "GRS $\leq$ 1 risk allele" group and "GRS > 1 risk allele" group, indicating individuals with lower and higher risk alleles of the SNPs, respectively.

2.5. Genotyping

The genotyping methodologies have been previously published [22,30]. The phenol-chloroform technique was used to extract DNA from whole blood. Genotyping was performed using restriction fragment length polymorphism and confirmed by direct sequencing in which duplicate samples (n = 200; 20%) were genotyped with 100% concordance, suggesting high genotyping accuracy.

2.6. Statistical Analysis

Descriptive statistics of continuous variables are provided as means with standard deviations (SDs) and compared between T2D and controls using an independent sample t-test. Normality tests were performed and variables with no-normal distribution were log transformed. For each individual SNP, genotype counts were assessed for Hardy–Weinberg equilibrium (HWE) using a goodness-of-fit chi-square test. As shown in Table S1, all SNPs were in HWE (p > 0.092, for all comparisons). General linear models were utilised to analyse the main associations of the GRS with metabolic traits. Interactions of the GRS with dietary intake were investigated by including the interaction term (GRS*dietary intake) in the models. Furthermore, significant interactions with protein intake were analysed in more depth according to dietary sources of protein (animal and plant protein), where individuals were classified into two groups according to the sample median intake of plant (39 g/day) and animal protein (19 g/day): below and above median groups. Individuals who consumed below the median were categorised as those who had lower intakes of plant and animal protein, respectively, whereas individuals who consumed above the median were categorised as those who had higher intakes of plant and animal protein, respectively. Dietary intakes as percentage of total energy intake (TEI) included intake of protein, carbohydrate and fat. Models were adjusted for sex, age, T2D, anti-diabetic medication and BMI (when BMI is not an outcome). Furthermore, as part of the sensitivity analysis, we further adjusted for duration of diabetes, dairy protein intake, physical activity level, smoking, alcohol consumption and fibre intake. Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA), with a significance level of 0.05.

3. Results

3.1. Characteristics of Study Participants

As shown in Table 1, individuals with T2D were significantly older and had higher BMI, WC, HbA1c, FPG and insulin, compared to individuals with NGT (p < 0.05 for all). Moreover, diabetic individuals had significantly higher intakes of total protein and carbohydrate than individuals with NGT (p < 0.05 for all).

Table 1. Characteristics of study participants.

	Total		NGT Controls		T2D Cases		*p* Value
	n		*n*		*n*		
Sex							
Men (%)	591	56	278	56	313	55	0.807 **
Women (%)	471	44	218	44	253	45	
Age (years)	1062	45 ± 12	496	38 ± 10	566	51 ± 11	1.160×10^{-71} *
Diabetes duration	-	-	-	-	566	5.20 ± 5.29	-
Anti-diabetic medication	-	-	-	-	164	15.4%	-
BMI (kg/m^2)	1061	24.6 ± 4.56	496	23.5 ± 4.64	565	25.5 ± 4.30	1.480×10^{-12} *
WC (cm)	1022	87 ± 12	479	83 ± 12	543	91 ± 10	5.692×10^{-33} *
HBA1C (%)	1056	7.3 ± 2.4	492	5.6 ± 0.47	564	8.8 ± 2.4	1.480×10^{-14} *
FPG (mg/dL)	1060	126 ± 64	495	85 ± 8	565	162 ± 69	1.392×10^{-127} *
Fasting Insulin (μIU/mL)	699	9 ± 7	448	8 ± 6	251	12 ± 7	6.386×10^{-101} *
Energy (kcal/day)	1062	2536 ± 805	496	2685 ± 708	566	2406 ± 861	8.773×10^{-9} *
Protein (%)	1062	11 ± 1	496	11.27 ± 1.17	566	11.45 ± 1.23	0.014 *
Animal protein (g/day)	1062	22 ± 12	496	25 ± 13	566	19 ± 11	3.787×10^{-14} *
Plant protein (g/day)	1062	40 ± 14	496	42 ± 15	566	39 ± 13	0.006 *
Fat (%)	1062	23 ± 5	496	24 ± 5	566	23 ± 5	0.113 *
Carbohydrate (%)	1062	65 ± 6	496	64 ± 6	566	65 ± 6	0.003 *
Dietary fibre (g)	1062	32 ± 11	496	32 ± 10	566	31 ± 12	0.150 *
Total SFA (g)	1062	24 ± 10	496	27 ± 10	566	22 ± 10	2.295×10^{-12} *
Total MUFA (g)	1062	20 ± 8	496	21 ± 8	566	18 ± 8	3.943×10^{-9} *
Total PUFA (g)	1062	18 ± 10	496	19 ± 9	566	18 ± 10	0.184 *
Physical activity level							
Sedentary	695	71%	335	73%	360	70%	
Moderate	223	23%	110	24%	113	22%	0.001 **
Vigorously active	58	6%	13	3%	45	8%	
Smoking							
Non-smokers	865	81.5%	396	79.8%	469	82.9%	0.206 **
Smokers	197	18.5%	100	20.2%	97	17.1%	
Alcohol consumption							
Non-alcoholics	793	74.7%	358	72.2%	435	76.9%	0.080 **
Alcoholics	269	25.3%	138	27.8%	131	23.1%	

Data presented as Mean ± SD. * *p* values are for the mean differences between controls and T2D cases using an independent sample *t*-test. ** *p* values are from the Chi-squared test. Frequency of men and women between controls and cases was compared using a chi-square test. Abbreviations: NGT, normal glucose tolerance; T2D, type 2 diabetes; BMI, body mass index; WC, waist circumference; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

3.2. Association between Metabolic GRS and Metabolic Traits

After adjusting for the potential confounders there were no significant associations between the 7-SNP GRS and metabolic traits (Table 2).

Table 2. Associations of 7-SNP and 3-SNP GRS and with metabolic traits.

	7-SNP GRS					3-SNP GRS				
	n	GRS < 6	*n*	GRS ≥ 6	*p* Value	*n*	GRS ≤ 1	*n*	GRS > 1	*p* Value *
BMI (kg/m^2)	526	24.5 ± 0.2	535	24.7 ± 0.2	0.572	645	24.7 ± 0.2	416	24.5 ± 0.2	0.572
WC (cm)	508	86.7 ± 0.5	514	87.4 ± 0.5	0.668	620	87.0 ± 0.47	402	88.0 ± 0.57	0.010
HBA1C (%)	524	7.1 ± 0.1	532	7.4 ± 0.1	0.935	640	7.0 ± 0.1	416	7.7 ± 0.1	0.000066
FPG (mg/dL)	526	119.9 ± 2.6	534	131.6 ± 2.9	0.181	644	120.0 ± 2.35	416	135.0 ± 3.39	0.002
Fasting insulin (μIU/mL)	373	9.5 ± 0.4	326	9.4 ± 0.3	0.767	419	10.0 ± 0.36	280	9.0 ± 0.33	0.171

Data are Mean ± standard error of the mean. * *p* values adjusted for sex, age, T2D, anti-diabetic medication and additionally for BMI, when BMI is not an outcome. The analysis was carried out using log-transformed variables. Abbreviations: GRS, genetic risk score; BMI, body mass index; WC, waist circumference; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose.

In the 3-SNP GRS analysis, significant associations were found with WC ($p = 0.010$), FPG ($p = 0.002$) and HbA1c ($p = 0.000066$), where individuals with >1 risk allele had higher

WC, FPG and HbA1c compared to individuals with ≤1 risk allele (Table 2). Both 7-SNP GRS and 3-SNP GRS were associated with a higher risk of T2D (p = 0.0000134 and 0.008, respectively) (Table 3).

Table 3. Association of 7-SNP and 3-SNP GRSs with T2D.

GRS	OR	95% CI for OR		p Value *
		Lower	Upper	
7-SNP GRS	2.083	1.496	2.898	0.0000134
3-SNP GRS	1.559	1.121	2.170	0.008

* p values were obtained from the logistic regression models adjusted for sex, age, anti-diabetic medication and BMI. Abbreviations: GRS, genetic risk score; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; OR, odds ratio; CI, confidence interval; BMI, body mass index.

3.3. Interaction of 7-SNP and 3-SNP GRSs with Dietary Factors on Metabolic Traits

As shown in Table 4, there were significant interactions between the 3-SNP GRS and total protein intake (% of TEI) on FPG ($P_{interaction}$ = 0.011) and HbA1c ($P_{interaction}$ = 0.007). Among individuals with lower intake of plant protein (<39 g/day), those with >1 risk allele had higher FPG (p = 0.001) and HbA1c (p = 0.00006) than individuals with ≤1 risk allele (Figure 1). Furthermore, among individuals with higher intake of animal protein (>19 g/day), those with >1 risk allele had higher FPG (p = 0.008) and HbA1c (p = 0.001) than individuals with ≤1 risk allele (Figure S2). None of the interactions were significant between the 7-SNP GRS and dietary intakes on metabolic traits except for the interactions between 7-SNP GRS and protein intake on HbA1c ($P_{interaction}$ = 0.032), and 7-SNP GRS and carbohydrate intake ($P_{interaction}$ = 0.04) on fasting insulin. However, these interactions were not significant after stratifying based on animal and plant protein.

Table 4. Interactions of 7-SNP and 3-SNP GRSs with dietary factors on metabolic traits.

	7-SNP GRS			3-SNP GRS		
	Protein	Fat	Carbohydrate	Protein	Fat	Carbohydrate
	(% of TEI)	(% of TEI)	(% of TEI)	(% of TEI)	(% of TEI)	(% of TEI)
BMI (kg/m^2)	0.176	0.388	0.195	0.36	0.653	0.805
WC (cm)	0.852	0.786	0.892	0.638	0.958	0.914
HBA1C (%)	0.032	0.629	0.618	0.007	0.677	0.756
FPG (mg/dL)	0.249	0.489	0.507	0.011	0.367	0.231
Fasting insulin (μIU/mL)	0.952	0.085	0.04	0.299	0.567	0.999
T2D	0.956	0.214	0.152	0.764	0.508	0.365

Data are $P_{interaction}$ values adjusted for sex, age, T2D, antidiabetic medications and additionally for BMI, when BMI is not an outcome. The analysis was carried out using log-transformed variables. Abbreviations: GRS, genetic risk score; TEI, total energy intake; BMI, body mass index; WC, waist circumference; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose; T2D, type 2 diabetes.

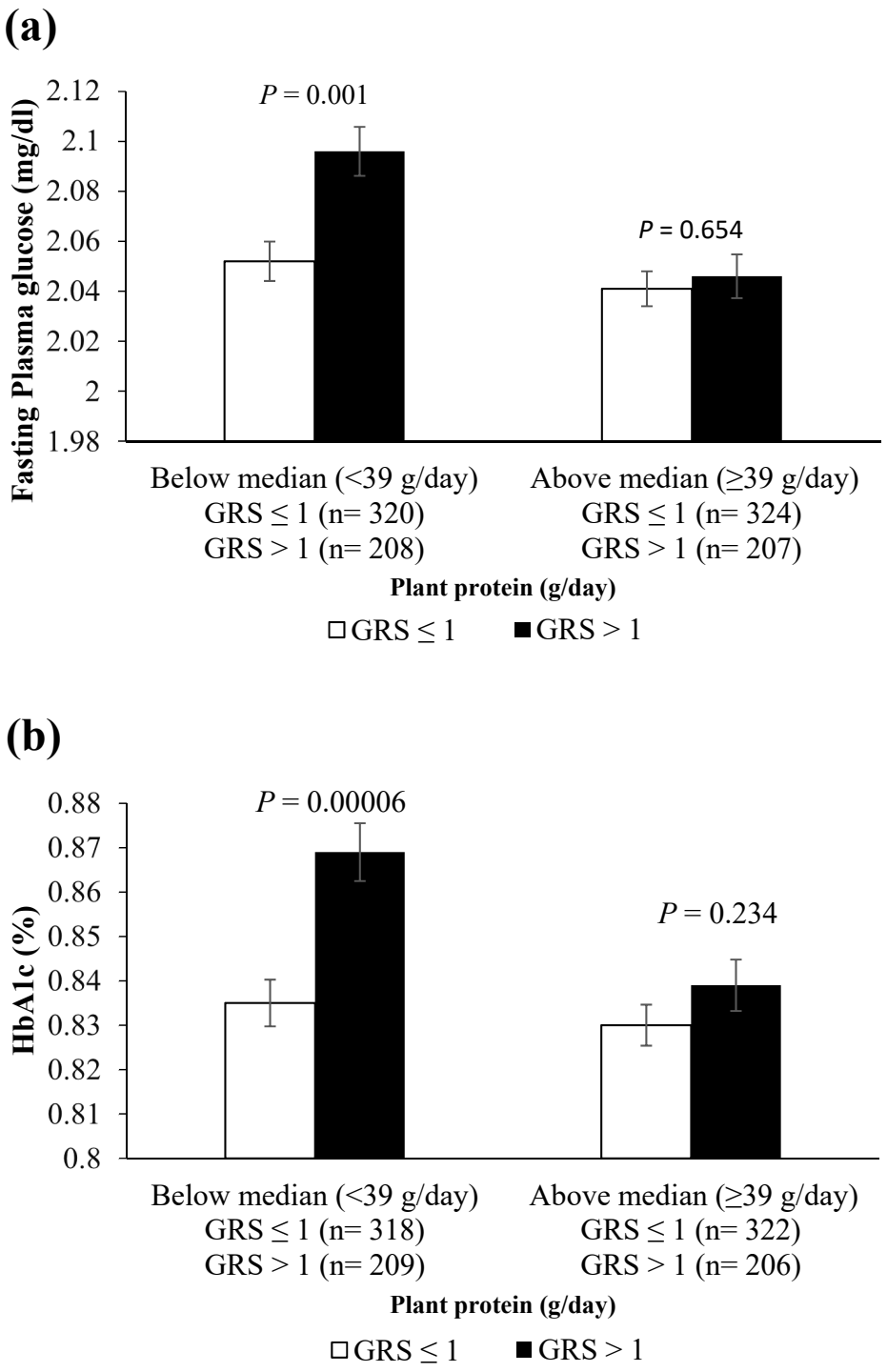

Figure 1. Interaction between 3-SNP GRS and plant protein intake on fasting plasma glucose and glycated haemoglobin. White bars refer to individuals with GRS ≤ 1 risk allele; the black bars refer to individuals with GRS > 1 risk allele. (**a**) Individuals with >1 risk allele had a significantly higher FPG compared to those with ≤1 risk allele, among those with lower intake of plant protein (<39 g/day) (p = 0.001). (**b**) Individuals with >1 risk allele had a significantly higher HbA1c compared to those with ≤1 risk allele, among those with lower intake of plant protein (<39 g/day) (p = 0.00006). p values were adjusted for age, sex, T2D, BMI, anti-diabetic medication, total fat intake (%) and TEI. Abbreviations: GRS, genetic risk score; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; TEI, total energy intake.

3.4. Sensitivity Analyses

We subjected our regression results to a wide range of robustness checks. First, we adjusted for duration of diabetes, and the association of 3-SNP GRS with HbA1c and FPG (p = 0.010 and 0.040, respectively) and the interaction of 3-SNP GRS with protein intake (%) ($P_{interaction}$ = 0.025 and 0.019 for HbA1c and FPG, respectively) were still significant. Second, we excluded individuals with diabetes, and this resulted in a small sample size of 496 NGTs. However, a significant association of 3-SNP GRS with HbA1c (p = 0.012) was still observed, but none of the interactions were statistically significant (p = 0.126 and 0.405 for HbA1c and FPG, respectively). Third, given the association between dietary fat intake and T2D traits, we adjusted for total dietary fat intake and found that the interaction of 3-SNP GRS with protein intake (%) ($P_{interaction}$ = 0.007 and 0.009 for HbA1c and FPG, respectively) was still significant. Fourth, we tested for the interaction between 3-SNP GRS and dairy protein intake to see if the interactions with the animal protein intake were driven by the intake of dairy protein. We found that the interactions between 3-SNP GRS and dairy protein intake were not statistically significant ($P_{interaction}$ = 0.439 and 0.597 for HbA1c and FPG, respectively), suggesting that dairy protein intake is unlikely to confound the GRS–animal protein intake interaction on diabetes traits. Fifth, in addition to the aforementioned factors, we adjusted for other possible confounders such as physical activity level, smoking, alcohol consumption and fibre intake, and found that the interactions between the 3-SNP GRS and protein intake on HbA1c and FPG were still significant ($P_{interaction}$ = 0.009 and 0.008, on HbA1c and FPG, respectively).

4. Discussion

The current research provides evidence for the GRS–protein intake interaction on T2D-related traits in Asian Indians. We found that individuals with >1 risk allele had higher FPG and HbA1c levels than those with ≤1 risk allele among individuals with lower intake of plant protein (<39 g/day). Given that the prevalence of obesity, high FPG and T2D has increased in India from 1990 to 2016 [55], our findings are of importance in terms of public health. Our study suggests that increasing the intake of plant protein might be an effective strategy towards better management of blood glucose levels, especially in Asian Indians with a higher genetic susceptibility for T2D.

In the present study, the 3-SNP GRS was associated with higher WC, which is in accordance with the findings in 7067 individuals from the Indian Migrant Study where a combined risk score of eight variants was observed to be nominally associated with higher WC (p = 0.02) [56]. The 3-SNP GRS was also associated with FPG and HbA1c, where individuals with higher GRS had higher FPG and HbA1c. Similarly, a large GWAS in 159,940 individuals of African, South Asian, East Asian and European ancestries identified 60 genetic variants influencing HbA1c [57], including SNPs located in the *FTO* and *TCF7L2* genes. An association of 8-SNP GRS with T2D was found in a case-control study of 5,148 Indians (including 1808 individuals with T2D and 1549 controls) from in and around Pune in western India [25]. A case-control study of 3357 Indian adults (including 2486 individuals with T2D and 2678 controls) also found that individuals with a higher GRS, derived from 32 SNPs, were at a higher T2D risk compared to those with lower GRS [58]. The EpiDREAM prospective cohort study (n = 15,466 individuals) has shown that South Asians might have a greater genetic load for T2D than Latinos and Europeans [59]. If our study findings are confirmed in larger cohorts, our 3-SNP GRS might serve as a diagnostic marker for investigating the cumulative effect of SNPs on diabetes-related traits and identifying Asian Indians with a high genetic risk of T2D.

Increasing evidence has shown that certain dietary factors might interact with genetic susceptibility in relation to the risk of diabetes and related traits [40–42,44,60]. In our study, individuals with higher 3-SNP GRS had higher fasting glucose and HbA1c concentrations than individuals with lower GRS among individuals with lower intake of plant protein. The results of the current analysis are in agreement with a recent study among Southeast Asian women (n = 110) showing significant interactions between a 15-SNP GRS and total

protein intake. The study found that consuming a low protein diet (13.51 ± 1.18% of TEI) was associated with lower WC and triacylglycerol concentrations, particularly in individuals with high genetic risk [60]. Moreover, significant interactions of the *FTO* SNPs (rs8044769 (C>T), rs3751812 (G>T) and rs8050136 (A>C)) with protein intake on blood glucose were observed in 819 Polish adults, where higher protein intake (>18% of TEI) was associated with higher blood glucose in individuals with the TT genotype of rs8044769, CC genotype of rs8050136 and GG genotype of rs3751812 [61]. However, the effect of protein sources was not analysed in the abovementioned studies, thus a direct comparison between these studies and our findings cannot be performed. In contrast to our study, a large prospective case-cohort study from eight European countries (n = 21,900) found no significant interactions between intake of protein and metabolic GRSs on T2D [62]. Similarly, no interaction was found between protein intake and a 10-SNP GRS on T2D risk among 8842 Korean adults [42]. These discrepancies in the findings might be due to differences in ethnicity, dietary assessments, dietary patterns, relative proportions of different macronutrients, protein sources, sample sizes and GRS construction methods; hence, larger studies in multiple ethnic groups are needed to confirm the GRS–protein intake interactions.

Previous studies have examined the relationship between protein intake and T2D in South Indians. A cross-sectional study of 900 urban South Indians from Chennai demonstrated that individuals with known T2D had significantly higher protein intake (15.9%) than controls (14%) [63]. Another study in Asian Indians from different parts of India reported similar findings, where diabetic individuals (n = 385) had higher protein intake (14%) than controls (12%) (n = 409) [64]. A cohort including 146 Asian Indians living in San Francisco found that individuals were at increased T2D risk when the protein intake was high. The same study also reported that the intake of animal protein (32 ± 15 g/day) was more likely to be associated with diabetes risk (p = 0.07) in comparison with the intake of vegetable protein (38 ± 8 g/day; p = 0.26) [65]. Even though consuming diets high in protein has been one of the most popular strategies for losing weight and the management of overweight and obesity [66–68], the health impacts of diets high in protein on T2D are inconsistent. Higher animal protein intake, but not plant protein, showed significant association with a higher risk of T2D in 38,094 individuals (median intake of animal protein = 62 g/day; 10-year follow-up period) from the European Prospective Investigation into Cancer and Nutrition-Netherlands (EPIC-NL) study [13], and in 37,309 women from the US (median intake of total meat in the highest quintiles = 53.5 serving/day; 8.8 year follow-up period) from the Women's Health Study [14]. Moreover, a large case-cohort study including 28,557 European individuals reported that higher animal protein intake was associated with higher incidence of T2D (per 10 g: 1.05 (1.02–1.08), P_{trend} = 0.001) over an average follow-up period of 12 years [16]. Furthermore, the higher intake of animal protein (5% increase in consumption of protein derived from meat and meat products) was shown to be associated with a 34% increased risk of T2D, whereas the intake of plant protein was shown to have a considerable protective effect in 1190 elderly participants from the Mediterranean islands [17]. A large study of 92,088 women and 40,722 men from the United States found that substituting 5% of energy intake from animal protein with plant protein was associated with a decrease in T2D risk by 23% [69]. Moreover, a systematic review and meta-analysis of thirteen randomised controlled trials (n = 280 middle-aged adults from Iran, Denmark, United States, Germany, Canada and Greece) found significant decreases in HbA1c, fasting insulin and fasting glucose in diets that substituted animal protein with plant protein at a median level of ~35% of total protein intake/day [70]. Another systematic review and meta-analysis of eleven cohort studies, including individuals from the United States, Europe, Asia, Melbourne and Finland (52,637 cases among 483,174 individuals), showed that the intakes of total protein and animal protein increased T2D risk in both men and women, whereas plant protein intake decreased T2D risk in women [71]. Previous cohort studies in the United States (90,239 women and 40,539 men) and in the Netherlands (6798 individuals) found that an association between the higher adherence to a plant-

based diet and a lower risk of T2D [72,73]. In contrast, other prospective cohort studies (n = 8370–38,094 individuals) observed no significant associations [13,74,75]. It is possible that the interactions between genetic factors and protein intake might be one of the reasons for the discrepancies in the effect of dietary protein intake on the risk of T2D and its related traits.

The dietary patterns across different parts of India have been significantly affected by urbanisation. Given that food availability and purchasing power are higher in urban than rural areas, diets of both residents tend to differ significantly [12,76]. Protein intake has been shown to be positively related to an individual's income, where the demand for animal protein increased with the disposable income [12]. Higher protein intake has also been reported in urban areas in India, with the overall mean intake of protein being the highest in the high-income group (73.1 g/day) followed by the middle-income group (63.2 g/day), industrial labourer (59.4 g/day) and low-income group (57.8 g/day) [77,78]. The present study included urban residents and the mean protein intake is 71.6 $\pm$ 22.7 g/day, which is higher than dietary protein recommendations for Asian Indians (55–60 g/day) [79]. However, the mean protein intake is only 11% (percentage calories coming from the protein), which is similar to the previous large studies, such as the National Family Health and National Nutrition Monitoring Bureau surveys that were conducted in the Indian population [80,81]. A study in 6907 adults from South India aged >20 years showed that the consumption of pulses was lower in rural compared to urban Indian adults [82] and a cross-sectional study including 56,742 men and 99,574 women aged 20–49 years also demonstrated that inverse association between daily or weekly legumes and the presence of diabetes [83]. A recent study in 1033 Indian adults also showed that a significant decrease in the risk of T2D was observed among those having higher intakes of legumes and pulses [84]. In the same population, a study in 2042 individuals reported that pulses and legumes contributed to only 17.2% of the daily protein suggesting a reduced intake of plant protein [85]. Hence, according to the findings from the previous studies and the GRS–plant protein intake interaction from the present study, increasing the intake of plant protein might be an effective strategy to arrest the rising epidemic of T2D among Indian adults.

The strength of this study is the use of a representative sample of the urban Chennai population. Given that diabetes prevalence continues to be higher in urban residents compared to rural residents in India [2,86,87], understanding gene–diet interactions on T2D in urban areas would improve diabetes prevention strategies among urban Indians. Our study used unweighted GRSs to analyse the combined effect of several SNPs, which is an effective approach to study polygenic diseases such as T2D and obesity, providing a better knowledge of disease risk compared to a single-SNP analysis [36]. A comprehensive and validated semi-quantitative FFQ was used for analysing dietary intakes [48]. Furthermore, anthropometric outcomes were assessed by qualified staff rather than self-reported to improve the accuracy of anthropometric measurements. However, the study has several limitations. First, the study has a small sample size suggesting that we might have had insufficient power for our analysis. To maximise power, we used a GRS approach, which has an advantage over single-SNP analysis, and significant associations and interactions were found. Second, the observational nature of the study design cannot explain causal relationships or exclude residual confounding; however, sensitivity analyses were carried out where adjustment was performed for additional confounding factors such as diabetes duration, total fat intake, physical activity level, anti-diabetic medication, alcohol consumption, smoking and fibre intake. Third, dietary intake was assessed using self-reported FFQ, which might have introduced recall and measurement bias. Finally, SNPs contributing to our GRSs represent only a small proportion of the increasing number of identified metabolic disease-associated variants in Asian Indians; however, we have chosen SNPs in *TCF7L2* and *FTO* genes that have presented the most consistent and strongest associations with T2D and obesity, respectively, in several populations [32,88].

5. Conclusions

In summary, the current study has found a novel GRS–protein intake interaction where individuals with >1 risk allele and lower intake of plant protein (<39 g/day) had higher FPG and HbA1c levels. This suggests that increasing the intake of plant protein may be an effective approach to overcome the genetic risk of diabetes in urban Asian Indians. To prove this hypothesis, appropriate randomised clinical trials with diets of higher and lower plant protein intake need to be performed. Moreover, there is a need for studies with larger sample sizes to confirm gene–diet interactions. Ultimately, there is a need for the assessment of the clinical benefit of targeted interventions based on an individual's underlying genetic risk.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/nu13093064/s1, Table S1: Genotype distribution of the seven SNPs that were chosen for our study (n = 1062), Figure S1: Methodology of the Chennai Urban Rural Epidemiology Study (CURES), Figure S2: Interaction between 3-SNP GRS and animal protein intake (%) on fasting plasma glucose and glycated haemoglobin after adjusting for anti-diabetic medication.

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RESEARCH ARTICLE

Effect of dietary fat intake and genetic risk on glucose and insulin-related traits in Brazilian young adults

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Abstract

Purpose The development of metabolic diseases such as type 2 diabetes (T2D) is closely linked to a complex interplay between genetic and dietary factors. The prevalence of abdominal obesity, hyperinsulinemia, dyslipidaemia, and high blood pressure among Brazilian adolescents is increasing and hence, early lifestyle interventions targeting these factors might be an effective strategy to prevent or slow the progression of T2D.

Methods We aimed to assess the interaction between dietary and genetic factors on metabolic disease-related traits in 200 healthy Brazilian young adults. Dietary intake was assessed using 3-day food records. Ten metabolic disease-related single nucleotide polymorphisms (SNPs) were used to construct a metabolic-genetic risk score (metabolic-GRS).

Results We found significant interactions between the metabolic-GRS and total fat intake on fasting insulin level ($P_{interaction} = 0.017$), insulin-glucose ratio ($P_{interaction} = 0.010$) and HOMA-B ($P_{interaction} = 0.002$), respectively, in addition to a borderline GRS-fat intake interaction on HOMA-IR ($P_{interaction} = 0.051$). Within the high-fat intake category [37.98 ± 3.39% of total energy intake (TEI)], individuals with ≥ 5 risk alleles had increased fasting insulin level ($P = 0.021$), insulin-glucose ratio ($P = 0.010$), HOMA-B ($P = 0.001$) and HOMA-IR ($P = 0.053$) than those with < 5 risk alleles.

Conclusion Our study has demonstrated a novel GRS-fat intake interaction in young Brazilian adults, where individuals with higher genetic risk and fat intake had increased glucose and insulin-related traits than those with lower genetic risk. Large intervention and follow-up studies with an objective assessment of dietary factors are needed to confirm our findings.

Keywords Genetic risk score · Metabolic traits · Brazil · Fat intake · Gene–diet interaction

Introduction

Metabolic diseases, such as type 2 diabetes (T2D), have been recognised as a significant public health problem worldwide [1, 2], playing a critical role in medical impoverishment [3–6]. T2D is a major contributor to morbidity and mortality and individuals with T2D have a five-fold increased risk of developing cardiovascular diseases (CVD) [7]**.** The prevalence of diabetes has increased globally (over 463 million adults) [8] but at a faster rate in low- and middle-income countries (LMICs) [9]. In Brazil, the prevalence of T2D has increased by 24% between 2006 and 2019 [10] and an estimate of 65,581 deaths have been shown to be caused by diabetes among adults aged 35–80 years [11]. It has been reported that the prevalence of prediabetes and T2D among Brazilian adolescents were 22.0% and 3.3%, respectively [12]. Studies have also demonstrated the occurrence of cardiometabolic risk factors including abdominal obesity, high

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insulin levels, dyslipidaemia, and high blood pressure among Brazilian adolescents [12–14]. Hence, early interventions targeting these factors might be an effective strategy to prevent or slow the progression of T2D and decrease the risk of CVD and associated premature mortality [8].

Much of the increase in the prevalence of metabolic diseases in Brazil is attributed to an epidemiological transition characterised by changes in Brazilian age structure, population ageing, reduced rates of infant mortality and fertility and increased low birth weight [15–19]. Changes in the cultural and socioeconomic patterns, for instance, increasing urbanisation and economic improvement, have led to negative changes in lifestyle behaviours, including physical inactivity and unhealthy diet, in the Brazilian adolescent/young adult population [20]. A previous study has shown that the intake of saturated fatty acids (SFA) was higher in adolescents than adults in Brazil [21]. Animal and human studies have demonstrated an association between increased dietary fat intake and increased insulin resistance [22–24]. In addition, the dietary behaviours of Brazilian young adults have been shown to be characterised by higher intakes of unhealthy foods than middle-aged and older adults, highlighting the need for age-specific public health interventions [25].

The development of metabolic diseases such as T2D is closely linked to a complex interplay between genetic and lifestyle factors, such as diet. Numerous genetic loci have been shown to be associated with T2D [26–29] and related traits [30, 31] and, to date, 243 genetic loci have been identified to be associated with the risk of T2D in multiple ethnic groups [26–29]. Single nucleotide polymorphisms (SNPs) have only a modest effect on disease risk, thus, generating a genetic risk score (GRS) combining several SNPs across the genome is necessary for increasing power to identify disease predisposition patterns of an individual [32]. Evidence has suggested that the genetic risk of metabolic diseases can be modified by dietary intake [33–37]. There are a few gene-diet interaction studies in Brazilians; however, the studies have focused only on cardiovascular disease related traits [38–40]. To date, there are no GRS-diet interaction studies on metabolic traits in Brazilians. Hence, we aimed to investigate the interaction of 10 metabolic disease-related SNPs, as a GRS, with dietary intake on metabolic traits in 200 healthy Brazilian young adults.

Methodology

Study population

Obesity, Lifestyle and Diabetes in Brazil (BOLD) is a cross-sectional study of Brazilian healthy young adults aged 19–24 years recruited at the Federal University of Goiás (UFG) between March and June 2019. This study was conducted as part of the ongoing GeNuIne (gene-nutrient interactions) Collaboration, which aims to investigate the impact of genes and lifestyle factors on chronic diseases using data from multiple ethnic groups [41, 42]. All participants completed baseline questionnaires regarding health status, demographics, and socioeconomic status. The study exclusion criteria included those who are 1) using lipid-lowering or hypoglycemic drugs and mineral or vitamin supplements, 2) undergoing dietary interventions in the last 6 months, 3) having acute clinical conditions such as infection, inflammation, fever or diarrhoea, or confirmed diagnosis of chronic diseases such as diabetes mellitus, moderate/severe hypertension, cancer, rheumatoid arthritis and cardiovascular complications, 4) doing vigorous physical activity. In total, 416 individuals showed interest in participating in the study. However, 207 participants met the inclusion criteria and only 200 completed the study (**Fig. 1**). Out of the 200 participants, only 194 had information on genetic and phenotypic measurements as DNA samples were not available for 6 participants. The study was approved by the Ethics Committee of the Federal University of Goiás (protocol number 3.007.456, 08/11/2018), and performed according to the ethical principles in the Declaration of Helsinki. All participants gave written informed consent for study participation.

Anthropometric and biochemical measurements

Body weight, height and waist circumference (WC) were measured using standardized methods. The weighing was performed on a Tanita® portable electronic scale, with a maximum capacity of 150 kg. For height, a stadiometer with a movable rod was used. WC was measured using an inelastic measuring tape. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters^2 and WC measurement was taken using a non-extensible measuring tape with partici-pants in light clothing [43]. Body composition was performed by Dual Energy Radiological Absorptiometry (DXA), using the Lunar DPX NT model (General Electric Medical Systems Lunar®; Madison, USA).

Blood samples were collected by peripheral venous puncture in the morning after a 12-h fast and the volunteers were advised not to consume alcohol 72 h before the blood collection. Samples were immediately processed after the collection at the Romulo Rocha Laboratory (Goiânia, Brazil). Fasting serum glucose and insulin were collected in BD Vacutainer® tube and determined by the enzymatic colorimetric method, with an automatic System Vitros Chemistry 950 XRL (Johnson & Johnson, New Brunswick, NJ, USA). Plasma glycated haemoglobin (HbA1c) was collected in an ethylene-diamine-tetraacetic acid (EDTA) tubes BD Vacutainer® and measured using high-pressure chromatography (HPLC-Bio-Rad

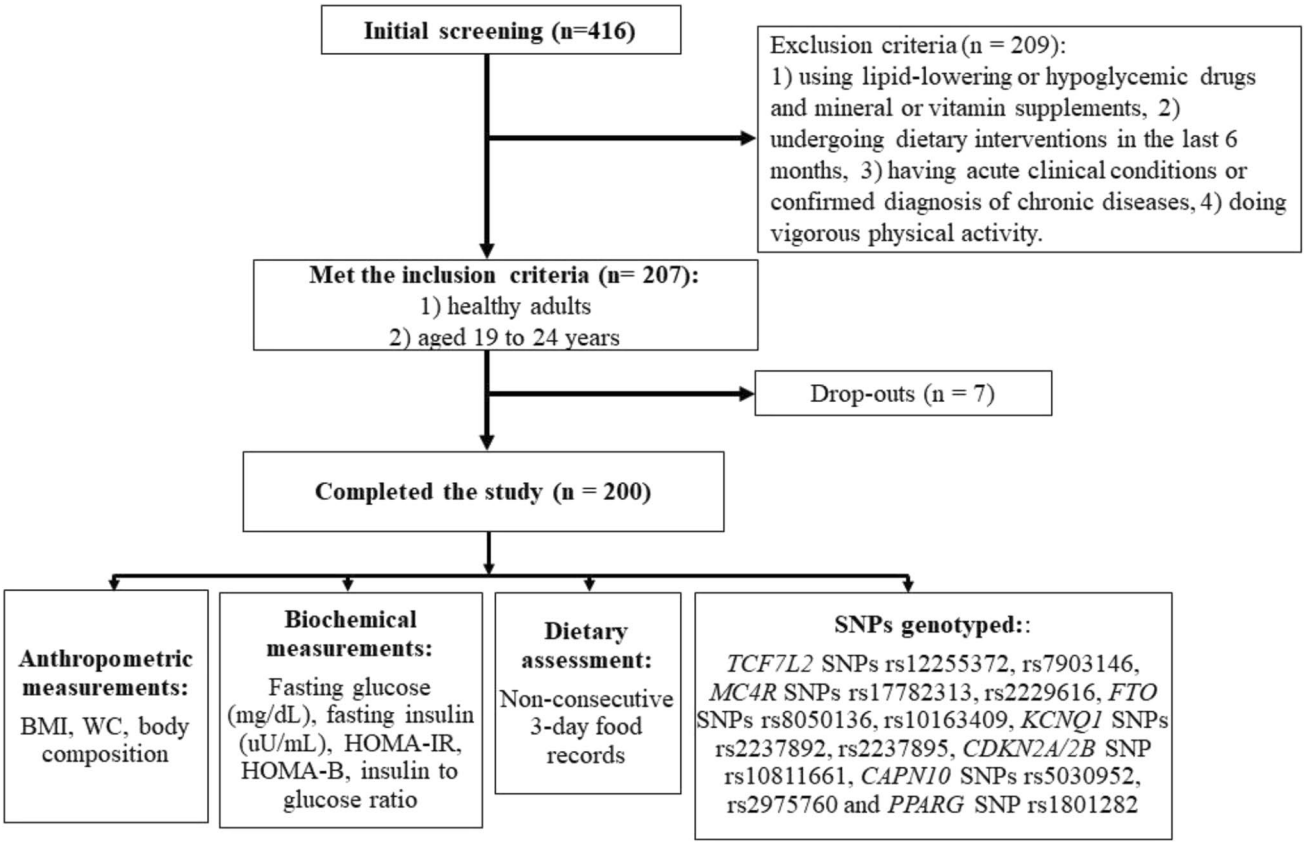

Fig. 1 Flow chart showing the participant recruitment process in the BOLD study. In total, 416 individuals were initially screened. After excluding participants based on the exclusion criteria, 207 were included in the study. However, only 200 completed the study. *BMI* body mass index, *WC* waist circumference, *HbA1c* glycated haemoglobin A1c, *HOMA-IR* homeostasis model assessment estimate of insulin resistance, *HOMA-B* homeostasis model assessment estimate of insulin secretion, *TCF7L2* Transcription factor 7-like 2, *MC4R* melanocortin 4 Receptor, *PPARG* Peroxisome proliferator-activated receptor gamma, *FTO* fat mass and obesity-associated, *CDKN2A/2B* Cyclin dependent kinase inhibitor 2A/2B, *KCNQ1* Potassium voltage-gated channel subfamily Q member 1 and *CAPN10* Calpain 10

Laboratories, Hercules, CA, USA). Plasma samples were obtained by centrifugation at 3500 rpm for ten minutes at 4 °C. The homeostasis model assessment (HOMA) was used to assess the degree of insulin resistance (IR) (HOMA-IR) and β-cell function (HOMA-B). HOMA-IR and HOMA-B were calculated as follows: [fasting insulin levels (mU/l) × fasting glucose levels (mmol/l)/22.5] and [20 × fasting insulin levels)/(fasting glucose levels − 3.5], respectively [44].

Dietary assessment

Food intake was assessed by trained nutritionists using non-consecutive 3-day food records, including a weekend day [45]. Individuals were provided with measuring cups and spoons of different sizes to assist them in estimating portion size for each food. Foods consumed were converted into grams using the Avanutri Online® diet calculation software (Avanutri Informática Ltda, Rio de Janeiro, Brazil).

Genotyping

The blood samples (3 ml each) were collected in an EDTA tubes BD Vacutainer® tubes and transported at a controlled temperature (- 80ºC) by the World Courier Company to perform genotyping at LGC Genomics (http://www.lgcgroup.com/services/genotyping), employing the competitive allele-specific PCR-KASP® assay.

SNP selection and GRS calculation

We selected 12 SNPs that have shown associations with metabolic traits in multiple ethnic groups [26–31]. The detailed information of these SNPs is shown in Table S1. Two SNPs were excluded from the current analysis, as the Calpain 10 (*CAPN10*) rs2975760 SNP was not in Hardy–Weinberg equilibrium (HWE) and the melanocortin 4 Receptor (*MC4R*) rs2229616 SNP had a minor allele frequency (MAF) of less than 1%. Unweighted metabolic-GRS was calculated by summing the number of risk alleles

present in each individual. The GRS was generated from the following SNPs: rs12255372, rs7903146 of the Transcription factor 7-like 2 (*TCF7L2*) gene, rs17782313 of the *MC4R* gene, rs8050136 and rs10163409 of the fat mass and obesity-associated (*FTO*), rs2237892 and rs2237895 of the Potassium voltage-gated channel subfamily Q member 1(*KCNQ1*) gene, rs10811661 of the Cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/2B*) gene, rs5030952 of the *CAPN10* gene, and rs1801282 of the Peroxisome proliferator-activated receptor gamma (*PPARG*) gene. Genotypes were coded as 0, 1, or 2 according to the number of metabolic-associated risk alleles that are defined based on the literature. These values were then calculated by summing the number of risk alleles for each variant. The GRS was then categorised based on the median risk alleles into two categories: "GRS < 5 risk alleles" and "GRS ≥ 5 risk alleles".

Statistical analysis

Descriptive characteristics of the study population stratified by sex were presented as means and standard deviation (SDs) for continuous variables and compared using an independent samples t-test. Variables were tested for normality using Shapiro–Wilk's W test and non-normally distributed variables were log-transformed including BMI, WC, body fat mass percentage, HbA1c, fasting glucose, fasting insulin, HOMA-IR, HOMA-B, insulin to glucose ratio, total energy intake (TEI), carbohydrate %, protein %, SFA %, and polyunsaturated fatty acids (PUFA) %. We investigated the effects of metabolic-GRS on metabolic traits using general linear models. To test the interactions of the metabolic-GRS with dietary factors on metabolic traits, we included the interaction term (e.g., GRS*fat intake) in the models. The dietary factors investigated in our study included the total dietary intake of fat, protein, and carbohydrate (percentages of TEI). Significant interactions between the GRS and the total fat intake were analysed in more depth to determine the effect of fat subtypes including SFA, monounsaturated fatty acids (MUFA), and PUFA. The GRS-nutrient interactions that reached statistical significance ($p < 0.05$) were tested for the effects of the GRSs on metabolic traits according to tertiles of dietary intakes (low, medium and high intake) using general linear models. All models were adjusted for age, sex and BMI (when BMI is not an outcome). Given that insulin levels are influenced by both the capacity for insulin secretion and IR [46, 47], analysis of HOMA-B was performed with and without adjustment for IR to improve the accuracy of pancreatic β-cell function estimate. All statistical tests were two-sided, and analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the study participants

Table 1 summarises the characteristics of individuals in this study according to sex. Men had higher BMI, WC, fasting glucose, and lower fat mass % compared to women ($P < 0.05$ for all). Men also reported higher intakes of total energy and protein than women ($P < 0.05$ for all).

Associations between metabolic-GRS and metabolic traits

None of the associations between metabolic-GRS and metabolic-disease related traits was statistically significant except for the association with BMI ($P = 0.008$) (Table 2).

Interactions of metabolic-GRS with dietary factors on metabolic traits

As shown in Table 3, there were statistically significant interactions between the metabolic-GRS and total fat intake (% of TEI) on fasting insulin level ($P_{interaction} = 0.017$), insulin-glucose ratio ($P_{interaction} = 0.010$) and HOMA-B ($P_{interaction} = 0.002$) and a borderline interaction on HOMA-IR ($P_{interaction} = 0.051$). Among those in the highest tertile of fat intake (37.98 ± 3.39% of TEI), individuals with ≥ 5 risk alleles had higher fasting insulin level ($P = 0.021$), insulin-glucose ratio ($P = 0.010$), HOMA-B ($P = 0.001$) and HOMA-IR ($P = 0.053$), compared to those with < 5 risk alleles (Figs. 2 and 3). Interaction on HOMA-B was still significant after adjusting the analysis for HOMA-IR ($P_{interaction} = 0.016$), Figure S1. We further examined interactions with fat subtypes on these traits. Significant interactions were detected between the metabolic-GRS and MUFA intake on fasting insulin ($P_{interaction} = 0.021$), HOMA-IR ($P_{interaction} = 0.021$) and insulin to glucose ratio ($P_{interaction} = 0.031$), however, none of these interactions was statistically significant after tertile analysis. Significant interactions were also observed between the metabolic-GRS and intakes of total fat, PUFA and MUFA on percentage of body fat mass ($P_{interaction} = 0.009$, 0.033 and 0.038, respectively).

Discussion

The present study investigated the potential interplay between metabolic-GRS and dietary macronutrient intake on metabolic traits in a Brazilian young adult population. Our results provide evidence of significant GRS-fat intake interactions on glucose and insulin-related traits, where

Table 1 Characteristics of study participants

Parameters	Total (n = 200)	Women (n = 147)	Men (n = 53)	*p*-Value
Age (years)	21.35 ± 1.67	21.33 ± 1.70	21.40 ± 1.61	0.815
BMI (kg/m2)	23.35 ± 4.42	22.81 ± 3.97	24.86 ± 5.23	**0.004**
WC (cm)	74.55 ± 13.56	71.10 ± 12.05	84.13 ± 13.01	**0.000**
Body fat mass (%)	33.91 ± 10.72	37.17 ± 8.77	24.84 ± 10.48	**0.000**
HbA1c (%)	4.73 ± 0.25	4.71 ± 0.25	4.78 ± 0.26	0.103
Fasting serum glucose (mg/dL)	87.18 ± 6.84	86.43 ± 6.78	89.26 ± 6.60	**0.009**
Fasting serum insulin (uU/mL)	8.74 ± 3.80	8.69 ± 3.37	8.88 ± 4.82	0.784
HOMA-IR	1.89 ± 0.88	1.86 ± 0.76	1.98 ± 1.15	0.513
HOMA-B	138.32 ± 65.75	142.47 ± 65.65	126.81 ± 65.25	0.137
Insulin to glucose ratio	0.10 ± 0.04	0.10 ± 0.04	0.10 ± 0.05	0.944
Energy (Kcal/day)	1827.81 ± 597.94	1741.52 ± 558.82	2067.15 ± 641.91	**0.001**
Protein (energy %)	17.11 ± 3.63	16.74 ± 3.33	18.14 ± 4.24	**0.016**
Carbohydrate (energy %)	51.09 ± 7.11	51.11 ± 7.01	51.05 ± 7.44	0.961
Fat (energy %)	31.66 ± 5.83	32.12 ± 5.69	30.38 ± 6.08	0.061
SFA (%)	9.43 ± 5.43	9.54 ± 6.030	9.14 ± 3.25	0.652
PUFA (%)	5.13 ± 2.27	5.08 ± 2.38	5.26 ± 1.92	0.628
MUFA (%)	7.72 ± 2.63	7.55 ± 2.55	8.19 ± 2.79	0.129

Data presented as the mean ± SDs. *P* values for the differences in the means between men and women were calculated using the independent samples t-test. *BMI* body mass index, *WC* waist circumference, *HbA1c* glycated haemoglobin, *HOMA-IR* homeostasis model assessment estimate of insulin resistance, *HOMA-B* homeostasis model assessment estimate of insulin secretion, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Table 2 Associations of metabolic-GRS with metabolic traits

Parameters	GRS < 5 (n = 93)	GRS ≥ 5 (n = 101)	*p*-Value
BMI (kg/m2)	23.90 ± 0.43	22.60 ± 0.43	**0.008**
WC (cm)	75.53 ± 1.27	73.93 ± 1.26	0.967
Body fat mass (%)	35.80 ± 1.05	31.91 ± 1.10	0.663
HbA1c (%)	4.72 ± 0.03	4.73 ± 0.03	0.964
Fasting glucose (mg/dL)	87.54 ± 0.68	86.74 ± 0.72	0.419
Fasting insulin (uU/mL)	8.91 ± 0.43	8.52 ± 0.34	0.542
HOMA-IR	1.93 ± 0.10	1.84 ± 0.08	0.663
HOMA-B	138.76 ± 7.15	138.17 ± 6.32	0.234
HOMA-B adjusted for HOMA-IR	138.76 ± 7.15	138.17 ± 6.32	0.235
Insulin to glucose ratio	0.10 ± 0.00	0.10 ± 0.00	0.477

Data are Mean ± standard error of the mean (SEM). *P* values obtained from the linear regression analysis adjusted for age, sex and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. *GRS* genetic risk score, *BMI* body mass index, *WC* waist circumference, *HbAIc* glycated haemoglobin, *HOMA-IR* homeostasis model assessment estimate of insulin resistance, *HOMA-B* homeostasis model assessment estimate of insulin secretion

individuals with ≥ 5 risk alleles had higher fasting insulin level, insulin-glucose ratio, HOMA-IR and HOMA-B than those with < 5 risk alleles among those in the high fat intake group (37.98 ± 3.39% of TEI). These findings suggest that individuals with ≥ 5 risk alleles are sensitive to dietary fat with respect to glucose and insulin-related traits and that these individuals may derive the most benefit from following the Brazilian dietary guidelines which aim at reducing fat intake to less than 30% of TEI [48]. This could have significant implication for public health in terms of providing early intervention to young adults with high genetic risk before the onset of disease, which might help halt the development of T2D.

In the present study, the metabolic-GRS was found to be associated with lower BMI, which contradicts the findings of the previous GRS-related studies in European populations [49–52]. However, the Brazilian population has a mixed genetic ancestry that originates from Europeans, Africans and Native Amerindians, which might explain the discrepancies between our findings and genetic association

Table 3 Interactions of the metabolic-GRS with dietary factors on metabolic traits

	Protein (%)	Carbohydrate (%)	Fat (%)	SFA (%)	PUFA (%)	MUFA (%)
BMI (kg/m2)	0.255	0.120	0.922	-	-	-
WC (cm)	0.124	0.303	0.979	-	-	-
Body fat mass (%)	0.451	0.311	**0.009**	0.255	**0.033**	**0.038**
HbA1c (%)	0.955	0.653	0.632	-	-	-
Fasting glucose (mg/dL)	0.764	0.142	0.099	-	-	-
Fasting insulin (uU/mL)	0.898	0.37	**0.017**	0.233	0.809	**0.021**
HOMA-IR	0.944	0.561	**0.051**	0.357	0.837	**0.021**
HOMA-B	0.797	0.089	**0.002**	0.079	0.749	0.123
HOMA-B adjusted for HOMA-IR	0.784	0.084	**0.016**	0.131	0.806	0.952
Insulin to glucose ratio	0.895	0.274	**0.010**	0.154	0.801	**0.031**

Data are P values of interaction which obtained from the linear regression analysis adjusted for age, sex and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. *GRS* genetic risk score, *BMI* body mass index, *WC* waist circumference, *HbA1c* glycated haemoglobin, *HOMA-IR* homeostasis model assessment estimate of insulin resistance, *HOMA-B* homeostasis model assessment estimate of insulin secretion, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

studies in Europeans [53]. Furthermore, a large GWAS of 241,258 European adults showed that the risk allele T of *TCF7L2* rs7903146 was associated with lower BMI compared to the non-risk allele, which may provide a possible explanation of our findings [54]. Metabolic diseases are complex and multifactorial influenced by both environmental and genetic factors including dozens or even hundreds of genetic variants each contributing small effects on these traits [55, 56]. Thus, the effect of unmeasured factors on BMI might influence the observed findings.

The present study found that, within the high-fat intake category, individuals with higher metabolic-GRS showed increased fasting insulin level, insulin-glucose ratio, HOMA-IR and HOMA-B, whereas those with lower GRS showed a reduction in these traits. Although direct comparison of our study with previous gene-diet interaction studies is difficult due to differences in the methodology related to the construction of GRSs and measurement of dietary intake, sample size, study design, and ethnicity, our findings are in agreement with some of the previous studies in other populations in which fat intake was found to interact with GRS on metabolic traits [33–35]. In a recent study in 302 Ghanaian adults, a GRS of 4 metabolic-related variants was associated with higher WC among individuals with high fat intake (34.99 ± 5.54% TEI) [57]. Data from an intervention study in 733 European adults also reported that higher total fat intake was associated with increased fasting glucose in individuals with higher GRS of 14 fasting glucose-associated SNPs and with decreased fasting glucose among individuals with lower GRS [33]. Taken together, these observations suggest that individuals with higher genetic risk might benefit more from reducing fat intake in terms of lowering their metabolic risk.

Dietary guidelines have recommended to limit the dietary intake of total fat (between 15 and 30% of TEI) to preserve overall health and reduce the risk of developing metabolic diseases [58]. Previous studies have demonstrated that the higher intake of total fat contributes to the development of T2D by inducing IR [24, 59]. Lowering total fat intake have been reported to improve glycemic control in a systematic review of clinical trials of diabetic individuals [60]. Evidence from two previous intervention studies including individuals from various ethnic groups (n = 3,234 and 522, respectively) and with long follow-up (2.8 and 3.2 years, respectively) have also shown that decreasing fat intake (from 6.6 ± 0.2% of TEI and to < 30% of TEI, respectively) is effective in reducing the incidence of T2D by up to 58% [61, 62]. In addition, dietary intervention in 48,835 postmenopausal women from the US showed that reducing total fat intake (by ~ 8% of TEI) and increasing carbohydrate intake (by ~ 8% of TEI) through increasing intake of vegetable/fruit (five servings per day) and grain (six servings per day) were associated with a reduction in glycemia and diabetes progression [63]. The dietary intake of Brazilians is characterised by unfavourable fat profile with high intakes of SFA and trans fatty acids and imbalances in the omega-6:omega-3 ratio, being compatible with a high risk of metabolic diseases [21]. In our study, the mean fat intake of the total sample (31.66 ± 5.8% of TEI) and the high fat intake group (37.98 ± 3.39% of TEI) were above the recommended dietary guidelines for Brazilian adults (< 30% of TEI) [64].

The mechanisms by which dietary fat influences IR and β-cell function are unclear; however, several pathways are

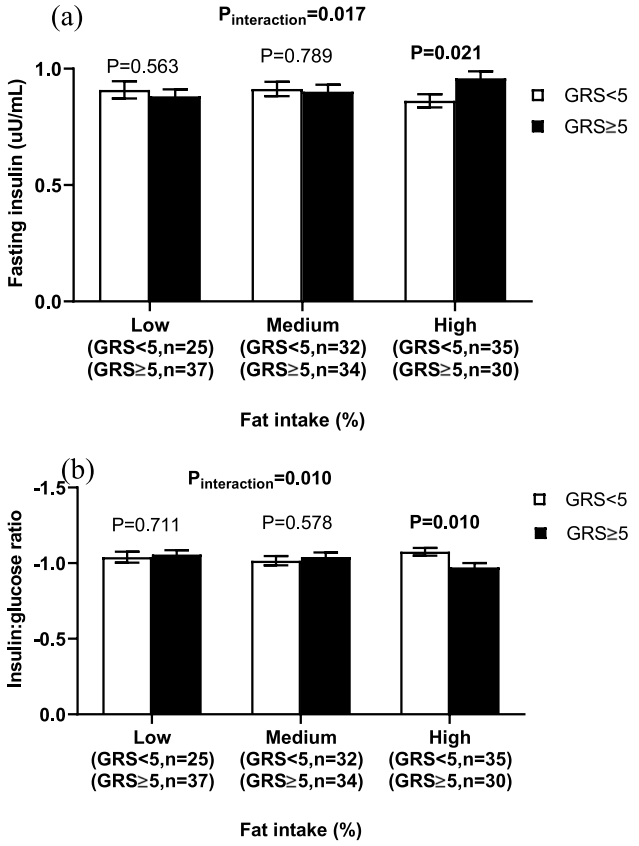

Fig. 2 Interaction between the metabolic-GRS and fat intake (%) on fasting insulin levels and insulin: glucose ratio. White bars indicate individuals with GRS < 5 risk alleles; the black bars indicate individuals with GRS ≥ 5 risk alleles; Error bars indicate the standard error of the mean. Individuals with ≥ 5 risk alleles had higher fasting insulin (**a**) and insulin to glucose ratio (**b**) compared to those with < 5 risk alleles, among individuals with a higher total fat intake (37.98 ± 3.39% of TEI). *GRS* genetic risk score, *TEI* total energy intake

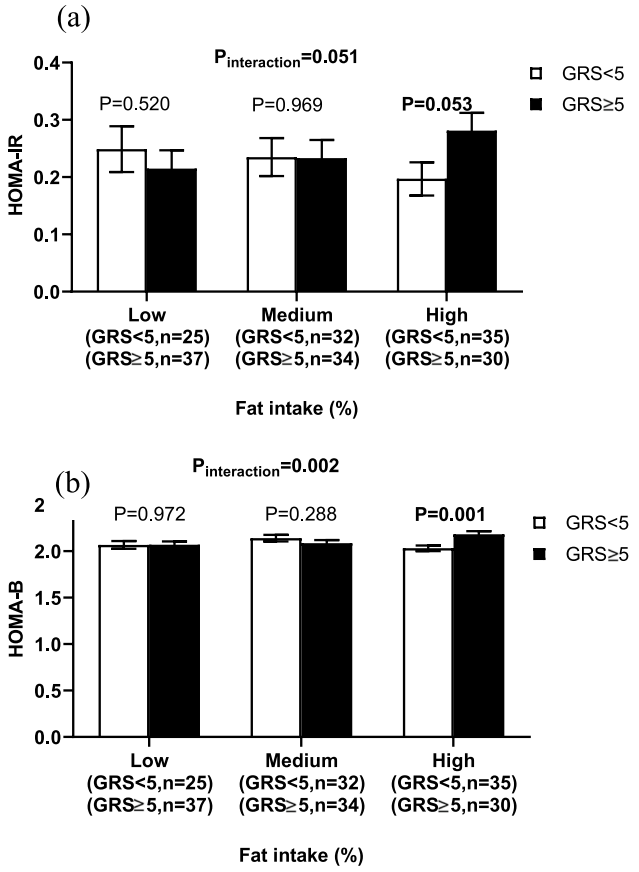

Fig. 3 Interaction between the metabolic-GRS and fat intake (%) on HOMA-IR and HOMA-B. White bars indicate individuals with GRS < 5 risk alleles; the black bars indicate individuals with GRS ≥ 5 risk alleles; Error bars indicate the standard error of the mean. Individuals with ≥ 5 risk alleles had higher HOMA-IR (**a**) and HOMA-B (**b**) compared to those with < 5 risk alleles, among individuals with a higher total fat intake (37.98 ± 3.39% of TEI). *GRS* genetic risk score, *TEI* total energy intake, *HOMA-IR* homeostasis model assessment estimate of insulin resistance, *HOMA-B* homeostasis model assessment estimate of insulin secretion

biologically plausible. IR is often mediated by increased inflammation that has been shown to be induced mostly by the effect of the fatty acids composition of the diet [65]. In particular, SFA and omega-6 have pro-inflammatory effects, and omega-3 fatty acids have anti-inflammatory effects [65]. Some of the molecular mechanisms of IR include the lipid-overload hypothesis in which ceramides or diacylglycerides are accumulated leading to the inhibition of insulin signalling and oxidative stress induced by excessive generation of free radicals or endoplasmic reticulum stress induced by excessive calorie intake [66–68]. In addition to the insulin-related traits, there was also a significant interaction between GRS and intakes of total fat, PUFA and MUFA on the percentage of body fat mass in our study. Given that adipose tissue is a central metabolic organ that stores excess fat energy in the form of lipid and secretes proinflammatory adipokines that can also influence signalling of insulin, our finding is biologically plausible [69]. It is worth observing the intake of SFA, PUFA and MUFA which were significantly higher in the high fat intake category than low and medium intake groups; this might be one of the reasons for the observed interactions with total dietary fat intake. Evidence suggests that different types of dietary fat have differential effects on IR and insulin secretion. While a cross-sectional study in 538 Spanish individuals suggested that the intake of a MUFA-rich diet was associated with increased HOMA-B [70], a meta-analysis of randomised controlled feeding trials (n = 4220) demonstrated that PUFA intake showed the most consistent favourable effects in relation to improved glycaemia and insulin secretion capacity [71].

Several strengths are worth consideration. This study is the first to examine whether dietary factors interact with metabolic-GRSs on metabolic traits among the Brazilian

young adult population. Early prediction of insulin sensitivity in young adults and effective intervention can be a critical factor in terms of delaying or preventing diabetes in normoglycemic individuals who are at risk of diabetes [72]. Also, a GRS analysis approach was used, which has the advantage over single-locus approach [32]. This approach is especially important for highly polygenic metabolic traits and can identify individuals at risk of metabolic diseases who might benefit from targeted interventions [32]. Furthermore, the study outcomes (metabolic traits) were measured using validated methods by trained staff which improve the accuracy of these estimates. Nevertheless, some limitations need to be acknowledged. A major limitation is the small sample size, suggesting that our analysis might be underpowered. However, the use of the GRS approach is suggested to improve the power and significant gene-diet interactions were detected in our study. As with all observational studies, causality between exposure and outcome cannot be inferred and residual confounders might have existed. Given the longitudinal dimension of the development of T2D and the complexity of gene-diet interactions, our cross-sectional study design fails to determine the temporality of the observed findings. Given that dietary intake was assessed using self-reported measures, we cannot exclude the effect of measurement bias. Another limitation is that the effect of different dietary sources of fat (including meat, dairy and plant) were not considered in the present analysis, which might have provided further explanations to our GRS-fat intake interactions [73]. In addition, our GRS was constructed based on 10 SNPs, which account for only a small proportion of the metabolic disease-related genetic variants. As HOMA is a widely validated clinical and epidemiological tool for assessing IR and β-cell function [74], like many other epidemiological studies [33, 35, 59], we also used HOMA-IR and HOMA-B as proxies for IR and insulin secretion, respectively. However, these measures are calculated only using fasting insulin and glucose values which might provide different estimates compared to methods based on dynamic measurements of insulin and glucose responses or those derived from clamp experiments [75]. Finally, given that the study was performed with relatively healthy overweight/obese young individuals with normal glucose tolerance who might have a quicker adaptation to changes in fat intake, the findings might not be applicable to those with impaired glucose metabolism or diabetes.

In conclusion, our study provides evidence of interactions between genetic predisposition and high fat intake on diabetes-related traits among Brazilian young adults. These findings encourage identifying Brazilian young adults with high genetic risk and tailoring dietary recommendations of fat intake according to their metabolic genetic risk profile for the primary prevention of adult-onset T2D. In addition, devising polygenic risk score could be used to provide more insights on understanding the pathophysiology of the genetics of diabetes. However, large interventional and follow up studies with a more comprehensive and objective assessment of environmental factors are needed in Brazilians to confirm our findings and to evaluate the clinical benefit of implementing precision dietary interventions based on an individual's underlying genetic risk of metabolic diseases.

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Data availability The data that support the findings of this study are available from the corresponding author (KSV) upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Federal University of Goiás (protocol number 3.007.456, 08/11/2018).

Consent to participate All participants gave written informed consent for study participation.

Consent for publication All participants gave written informed consent for the publication of study findings.

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Interaction between Metabolic Genetic Risk Score and Dietary Fatty Acid Intake on Central Obesity in a Ghanaian Population

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Abstract: Obesity is a multifactorial condition arising from the interaction between genetic and lifestyle factors. We aimed to assess the impact of lifestyle and genetic factors on obesity-related traits in 302 healthy Ghanaian adults. Dietary intake and physical activity were assessed using a 3 day repeated 24 h dietary recall and global physical activity questionnaire, respectively. Twelve single nucleotide polymorphisms (SNPs) were used to construct 4-SNP, 8-SNP and 12-SNP genetic risk scores (GRSs). The 4-SNP GRS showed significant interactions with dietary fat intakes on waist circumference (WC) (Total fat, $P_{interaction}$ = 0.01; saturated fatty acids (SFA), $P_{interaction}$ = 0.02; polyunsaturated fatty acids (PUFA), $P_{interaction}$ = 0.01 and monounsaturated fatty acids (MUFA), $P_{interaction}$ = 0.01). Among individuals with higher intakes of total fat (>47 g/d), SFA (>14 g/d), PUFA (>16 g/d) and MUFA (>16 g/d), individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles. This is the first study of its kind in this population, suggesting that a higher consumption of dietary fatty acid may have the potential to increase the genetic susceptibility of becoming centrally obese. These results support the general dietary recommendations to decrease the intakes of total fat and SFA, to reduce the risk of obesity, particularly in individuals with a higher genetic predisposition to central obesity.

Keywords: genetic risk score; obesity; Ghana; GONG; fat intake; gene–diet interaction

1. Introduction

Obesity is a known risk factor for several health conditions, including type 2 diabetes, cardiovascular diseases, hypertension and cancer, and hence it is considered as an increasing public health problem worldwide, including in Africa [1,2]. Obesity prevalence varies widely between African countries with a range of 5.3% in Uganda to 30% in Nigeria and 45.7% in South Africa [2]. A recent

systematic review has reported that nearly 43% of Ghanaian adults are either overweight or obese and that the prevalence of overweight and obesity was higher in women and urban dwellers [3]. While obesity is strongly affected by changes in environmental factors (such as dietary intake, sedentary lifestyle, and urbanization), the composition of the gut microbiome, the disruption of circadian rhythms, exposure to endocrine-disrupting chemicals and epigenetic modifications [4–9], it also has strong genetic determinants with a heritability rate from 40 to 70% [10,11]. Genome-wide association studies (GWAS) in European populations have revealed more than 100 loci to be associated with the body mass index (BMI) [12–18]. However, these genetic associations have not been consistently replicated in African populations [19–23], which could be attributed to differences in lifestyle and genetic architecture [24].

Given that single nucleotide polymorphisms (SNPs) have relatively small effect sizes on obesity, several studies have aggregated information from multiple-risk variants into a polygenic genetic risk score (GRS) [13,15,25–29]. Employing a combined risk allele score is an efficient and effective approach in maximising statistical power, decreasing the drawback of multiple testing, and widening the generalisable nature of genetic associations [25,27]. A study among a rural population of Gambia demonstrated a positive association between a GRS of 28 SNPs and BMI and adult weight, whereas no association was found with the single SNP analysis [30,31]. Although genetic research in Africans is increasing in numbers [22], only a few studies have examined the association of GRS with obesity in Africa [30,32,33], which highlights the need for further research in African populations.

Current evidence has shown that heritability estimates for obesity-related traits can be modified by lifestyle factors such as diet and physical activity. Several studies have reported significant GRS–diet interactions on obesity-related traits. Studies in European populations have shown that the genetic association with BMI was stronger with higher intakes of sugar-sweetened beverages (SSBs) and fried foods than among those with lower intakes [34,35]. Studies have also shown that genetic associations with BMI in Europeans can be modified by the levels of physical activity, television watching, and changes in sleep pattern [36,37]. In addition, higher adherence to healthy eating patterns have shown to reduce BMI in Europeans despite having increased genetic susceptibility to obesity [38]. Gene–lifestyle interaction studies have largely been conducted in populations of European ancestry, and the replication of these studies in African populations remains unknown [36,39]. Therefore, our study aimed to investigate the association of GRS with obesity-related traits and to examine whether lifestyle factors such as dietary intake and physical activity modified these associations in the Ghanaian population.

2. Methods

2.1. Study Population

The Genetics of Obesity and Nutrition in Ghana (GONG) study is a cross-sectional study that was conducted in the Oforikrom Municipality in Kumasi, Ashanti region, Ghana. The GONG study was conducted as part of the ongoing GeNuIne (Gene–Nutrient Interactions) Collaboration, the main objective of which is to investigate the effect of gene–nutrient interactions (nutrigenetics) on metabolic disease outcomes using population-based studies from various ethnic groups [40,41]. The Oforikrom Municipal Assembly is one of the five Municipal Assemblies carved out of the Kumasi Metropolitan Assembly. There are seventeen recognized communities in this Municipal Assembly, with an estimated total population of 360,254. Five communities (Ayeduase, Bomso, Ayigya, Oforikrom and Kotei) were randomly selected from the list of communities in the Oforikrom Municipal Assembly. In each community, a central point was located (a vehicle station, marketplace or other landmarks). A fieldworker entered the first house that faced either North, South, East or West of that central point, and randomly recruited one respondent from each household. Upon exiting a house, the fieldworker entered the next house, and the house-level selection process was repeated.

Three hundred and two free-living and healthy (with no physical complaints or prior diagnosis of cardiometabolic disease) adult volunteers, both men and women, were screened and recruited for the study by trained researchers. The inclusion criteria included the following: healthy individuals aged 25 to 60 years old and being Asante (both parents must be Asante). The exclusion criteria included the following: participants less than 25 years old or older than 60 years, those with existing cardiovascular complications or disease, those with a previous history of hypertension, type 2 diabetes or cardiovascular diseases, participants with any communicable or non-communicable chronic diseases, pregnant women and participants on lipid-lowering drugs, anti-diabetic drugs or anti-hypertensive drugs. A medical screening questionnaire was developed to screen participants for inclusion or exclusion from the study.

This study was approved by the Council for Scientific and Industrial Research (CSIR) Institutional Review Board (IRB) (Ref: RPN 003/CSIR-IRB/2018). In addition, this study was approved by the Metro Director of Health Services, Kumasi (KMHD/MPHs/13). All participants signed informed consent prior to their participation.

2.2. Data Collection

Structured questionnaires were used to elicit information about the participants' demographic characteristics, dietary intakes, physical activity levels, sleep and sunshine exposure patterns and medical history. Fieldworkers were trained before the start of data collection. Survey instruments were also pre-tested on the 10 July 2018 to enhance the field workers' understanding of questionnaires, ensure clearness and avoid ambiguity. Data collection took place from July to September 2018.

2.3. Anthropometric Measurements

Height, weight, percentage of body fat and visceral fat, waist circumference (WC) and hip circumference (HC) were measured. The measurements were taken with respondents wearing light clothing. Height was measured with a stadiometer (Seca 213 mobile stadiometer, Hamburg, Germany) to the nearest 0.1 cm with participants standing upright without shoes. Weight was measured using an OMRON Body Composition Analyzer to the nearest 0.1 kg. The same equipment provided values for BMI, percentage of body fat and visceral fat. WC and HC measurements were taken using a non-extensible measuring tape with participants in light clothing. The WC was measured just above the naval to the nearest 0.1 cm whereas the HC was measured at the level of the greater trochanter to the nearest 0.1 cm. The waist-to-hip ratio (WHR) was calculated by dividing WC by HC.

2.4. Physical Activity and Dietary Assessments

The health-related physical activity level of participants was measured using the interviewer-administered Global Physical Activity Questionnaire (GPAQ) version 2 developed by the World Health Organization (WHO) for physical activity surveillance [42]. This questionnaire contains 16 questions (P1–P16) which gather information on the respondent's engagement in physical activities under three domains or settings (work-related activity, transportation and recreational activities) as well as sedentary behaviours. The total physical activity per week was calculated in Metabolic Equivalents (MET- minutes) and the respondents who had total physical activity $\geq$ 600 MET- minutes/week were classified as active while those who had < 600 MET- minutes/week were classified as inactive [42].

A three-day repeated (two weekdays and one weekend) 24 h dietary recall method was used to elicit the information concerning the participants' dietary intake. Participants were requested to recollect all the meals taken as well as the times of the meal consumption in the previous day. Common household measures were used to estimate the actual quantities of foods and drinks consumed by the participants. The nutritional composition of the foods eaten was then analysed using the Nutrient Analysis Template (Food Science and Nutrition Department, University of Ghana, Accra, Ghana, 2010).

2.5. SNP Selection

Fifteen SNPs near or in 8 obesity-susceptibility loci were chosen for the study based on the previous GWAS for metabolic traits [12–18]. These include Transcription factor 7-like 2 (*TCF7L2)* (rs12255372, rs7903146), melanocortin 4 Receptor (*MC4R*) (rs17782313, rs2229616), fat mass and obesity-associated (*FTO*) (rs9939609, rs10163409), adiponectin (*ADIPOQ*) (rs266729, rs17846866), Potassium voltage-gated channel subfamily Q member *1(KCNQ1)* (rs2237892, rs2237895), Cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/2B*) (rs10811661), Calpain 10 (*CAPN10*) (rs3792267, rs5030952, rs2975760) and Peroxisome proliferator-activated receptor gamma (*PPARG*) (rs1801282). Three of these SNPs, *KCNQ1* (rs2237895), *ADIPOQ* (rs17846866) and *CAPN10* (rs2975760), reported significant deviations from Hardy–Weinberg Equilibrium (HWE) ($p < 0.05$) and were excluded from the current analysis. The detailed information of the 15 SNPs is shown in Supplementary Table S1.

2.6. Genotyping

Blood samples for the measurement of DNA were transported in dry ice to the United Kingdom (UK). Genomic DNA was extracted from a 5 mL whole blood sample from each participant and genotyping was performed at the LGC Genomics (http://www.lgcgroup.com/services/genotyping), which employs the competitive allele-specific PCR-KASP® assay.

2.7. Construction of the Metabolic GRSs

To evaluate the combined effects of the 12 SNPs on obesity-related traits, an additive model was used to construct the unweighted metabolic GRSs (Figure 1). We did not weigh the risk alleles based on their individual effect sizes, because no previously reported effect sizes were available for these SNPs for the Ghanaian population, and it has been shown that the weighting of risk alleles may only have limited effects [43]. The unweighted metabolic GRSs were calculated by the summation of the number of risk alleles across the 12 variants. The risk alleles were defined as alleles previously associated with an increased risk of obesity in the literature. To reduce the bias caused by the missing data, only those participants without any missing data were included in our metabolic GRS analysis. Different metabolic GRSs were constructed including the 12-, 8- and the 4-SNP GRSs. The 12-SNP GRS included the following SNPs: *TCF7L2* (rs12255372, rs7903146), *MC4R* (rs17782313, rs2229616), *FTO* (rs9939609, rs10163409), *ADIPOQ* (rs266729), *KCNQ1* (rs2237892), *CDKN2A/2B* (rs10811661), *CAPN10* (rs3792267, rs5030952) and *PPARG* (rs1801282), and the score ranged from 0 to 9 risk alleles. In the 12-SNP GRS analysis, no significant results were identified which might be because four of the SNPs had a minor allele frequency (MAF) of less than 5%. Therefore, we excluded the four SNPs: *MC4R* (rs2229616), *FTO* (rs10163409), *CDKN2B (rs10811661)* and *PPAR* (rs1801282) and created an 8-SNP GRS. No significant findings were observed using the 8-SNP GRS; this might be because four of the eight SNPs (*ADIPOQ (rs266729)*, *KCNQ1 (rs2237892)* and *CAPN10 (rs3792267, rs5030952)*) have not shown consistent associations with obesity-related traits in other populations [44–49]. Hence, these four SNPs were removed and a 4-SNP GRS including the SNPs (*TCF7L2* (rs12255372, rs7903146), *MC4R* (rs17782313), *FTO* (rs9939609)) that have shown consistent associations with obesity across several populations was constructed. The 4-SNP GRS ranged from 0 to 6 risk alleles and significant results were observed. Based on the median number of each GRS, the individuals were separated into two groups.

Given that there were no previously reported effect sizes available for these SNPs for the Ghanaian population, we were unable to perform sample size calculation.

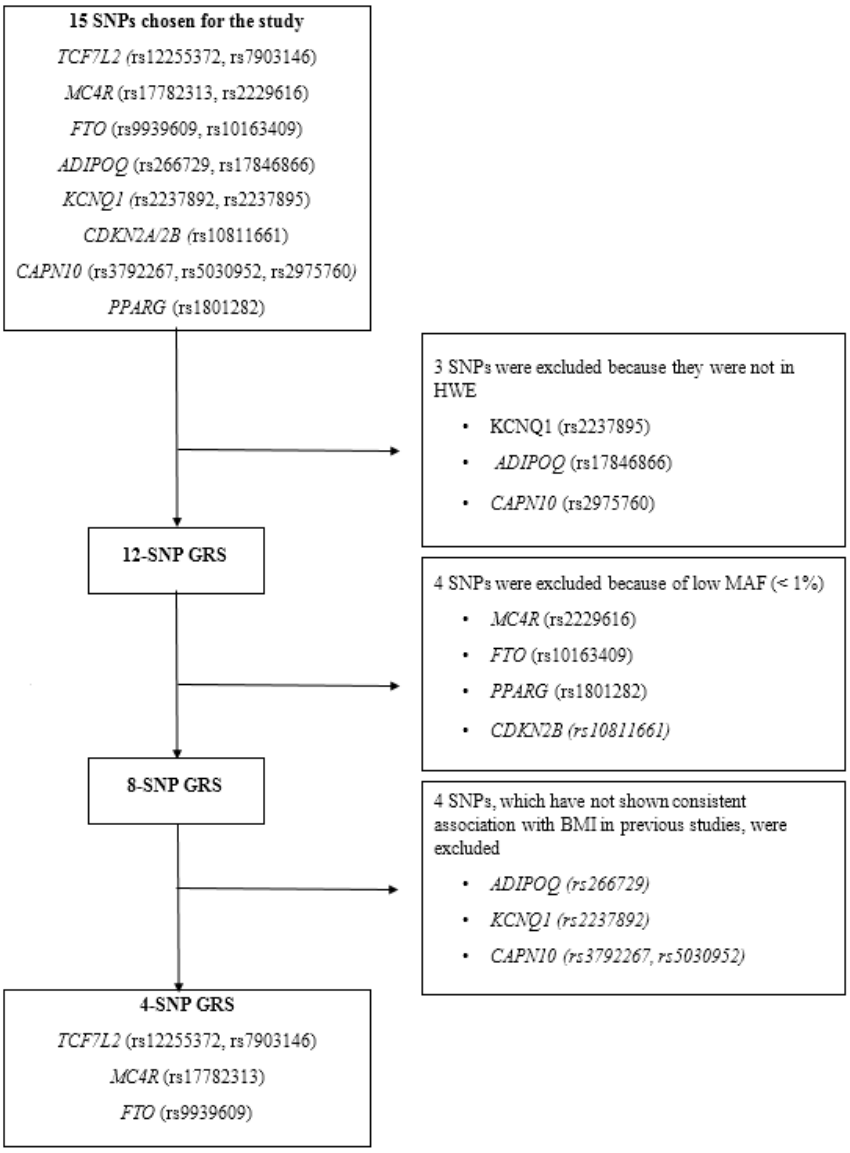

Figure 1. Steps involved in the construction of the metabolic GRS. Fifteen SNPs were genotyped in our study; however, the GRS analysis was based only on 12 SNPs as 3 SNPs were not in the HWE. Three different GRSs, including the 12-SNP GRS, 8-SNP GRS and the 4-SNP GRS were constructed. In the 12-SNP GRS analysis, no significant results were identified, which could be because 4 of the SNPs had MAF of less than 5%. Therefore, the 4 SNPs were excluded, and an 8-SNP GRS was created. No significant findings were observed using the 8-SNP GRS; this could be because four of the eight SNPs have not shown a consistent association with obesity-related traits in other populations. Hence, these four SNPs were removed and a 4-SNP GRS including those SNPs that have shown consistent associations with obesity across several populations was constructed. Abbreviations: SNP: single nucleotide polymorphisms; GRS: genetic risk score; HWE: Hardy–Weinberg equilibrium; MAF: minor allele frequency.

Data analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 24; SPSS Inc., Chicago, IL, USA). A natural log transformation was used for the non-normally distributed variables. Unadjusted differences of descriptive characteristics between the overweight/obese and non-obese participants were calculated using an independent samples *t*-test for continuous variables. General linear models were used to examine the association between the metabolic GRSs and obesity traits. GRS–lifestyle interactions were analysed by including the interaction terms in these models. Models were adjusted for covariates including sex, age and BMI (when BMI is not an outcome). Nutrient–GRS interaction analysis was additionally adjusted for total energy intake. All GRS–lifestyle interactions reaching a nominal level of significance ($p < 0.05$) were investigated further using binary analysis. Based on the median intake of total fat—saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA)—the individuals were separated into two groups: ''below the median group" and ''above the median group". Within each group, the association between the GRS and the outcome was examined. We also tested for GRS–sex interactions to test if sex influenced the genetic associations with obesity traits. The lifestyle factors investigated in our study included physical activity and the total dietary intake of fat, protein, carbohydrate and fibre. Significant interactions between the GRS and the total fat intake were further investigated to examine the influence of fat subtypes including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Two-tailed value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of the Study Participants

The anthropometric and dietary characteristics of the study participants are presented in Table 1. The mean age and BMI of the total sample were 38.17 ± 9.64 years and 26.63 ± 4.99 kg/m^2, respectively. Overweight/obese individuals were older than the non-obese ($p < 0.05$). Moreover, the dietary intakes were significantly different between the two groups. Overweight/obese individuals reported significantly lower intakes of total calories, protein, carbohydrate, total fat, fibre, SFA, MUFA and PUFA compared to the non-obese ($p < 0.05$). Women had significantly higher levels of BMI, body fat percentage and WHR compared to men, despite the men consuming significantly higher levels of carbohydrate, protein and fat ($p < 0.05$) (Supplementary Table S2).

Table 1. Characteristics of the study participants.

	Total (N = 302)	**Non-Obese * (N = 126)**	**Overweight/Obese ** (N = 176)**	***p* Value ***
Age (years)	38.17 ± 9.64	35.96 ± 9.55	39.75 ± 9.42	0.001
BMI (kg/m^2)	26.63 ± 4.99	22.01 ± 1.79	29.95 ± 3.75	<0.001
WC (cm)	88.48 ± 12.41	77.99 ± 7.13	96.00 ± 9.61	<0.001
WHR	1.45 ± 6.96	1.55 ± 7.76	1.38 ± 6.34	0.84
Visceral fat (%)	8.02 ± 7.39	6.49 ± 10.97	9.12 ± 2.26	0.01
Body fat (%)	33.12 ± 13.90	22.05 ± 12.47	41.05 ± 8.36	<0.001
Total energy intake (%)	1647.93 ± 685.83	1772.17 ± 723.85	1558.99 ± 644.75	0.008
Protein intake (g/day)	53.24 ± 23.73	57.38 ± 24.52	50.28 ± 22.76	0.01
Total fat intake (g/day)	51.17 ± 26.94	55.00 ± 29.29	48.42 ± 24.85	0.04
Carbohydrates intake (g/day)	239.03 ± 95.84	259.44 ± 104.01	224.42 ± 86.94	0.002
Fibre intake (g/day)	21.31 ± 10.84	23.19 ± 11.44	19.96 ± 10.21	0.01
Total SFA intake (g/day)	16.23 ± 10.36	17.41 ± 11.29	15.39 ± 9.58	0.10
Total MUFA intake (g/day)	18.08 ± 10.49	19.63 ± 11.30	16.96 ± 9.74	0.03
Total PUFA intake (g/day)	9.12 ± 5.03	10.20 ± 5.56	8.35 ± 4.47	0.002

Data presented as the means ± standard deviations. * Non-obese individuals refer to the individuals with a BMI < 25 Kg/m^2, according to the WHO classification of BMI. ** Overweight/obese cases refer to individuals with BMI ≥ 25 Kg/m^2, according to the WHO classification of BMI. *** *p* values for the differences in the means between the two groups were calculated using the independent samples t-test. Abbreviations: BMI: body mass index; WC: waist circumference; WHR: waist–hip ratio; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; WHO: World Health Organisation.

3.2. Effect of Metabolic GRSs on Obesity-Related Traits

We first investigated the combined effect of 12 common SNPs on obesity-related traits and no significant associations were observed (Supplementary Table S3). Similar results were found using an 8-SNP GRS (Supplementary Table S4) and a 4-SNP GRS (Table 2).

Table 2. Associations of the 4-SNP GRS on obesity-related traits.

	GRS < 3 Risk Alleles (N = 123)	GRS ≥ 3 Risk Allele (N = 172)	* *p* Value
BMI (kg/m^2)	26.13 ± 0.45	26.85 ± 0.37	0.24
WC (cm)	87.13 ± 1.15	89.14 ± 0.92	0.19
WHR	2.27 ± 0.98	0.88 ± 0.01	0.18
Visceral fat (%)	7.89 ± 0.71	8.08 ± 0.55	0.43
Body fat (%)	31.75 ± 1.32	33.87 ± 1.02	0.15

* *p* Values obtained from the linear regression analysis adjusted for age, sex and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: SNP: single nucleotide polymorphism; GRS: genetic risk score; BMI: body mass index; WC: waist circumference; WHR: waist–hip ratio.

3.3. GRS–Lifestyle Interactions on Obesity-Related Traits

There was a significant interaction of the 4-SNP GRS with dietary fat intake (g/day) on WC (Total fat, $P_{interaction}$ = 0.01; SFA, $P_{interaction}$ = 0.02; PUFA, $P_{interaction}$ = 0.01 and MUFA, $P_{interaction}$ = 0.01, Table 3). Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with higher intakes of total fat (>47 g/day), SFA (>14 g/day), PUFA (>16 g/day) and MUFA (>16 g/day), (Figure 2a–d). There was also a significant interaction between 4-SNP GRS and dietary fibre intake (g/day) on body fat percentage ($P_{interaction}$ = 0.04). Individuals with <3 risk alleles had a significantly lower body fat percentage compared to those with ≥3 risk alleles (p = 0.02), among individuals with a higher intake of fibre (>19 g/day). In addition, there was a significant interaction between the 4-SNP GRS and physical activity on WHR ($P_{interaction}$ = 0.002). However, the finding was not significant after stratifying them by physical activity levels. Some significant interactions were observed between the 12- and the 8-SNP GRSs and lifestyle factors on obesity-related traits (Supplementary Tables S5 and S6), however, none of these interactions were significant after binary analysis. Given the significant differences in the dietary intakes and obesity-related outcomes between men and women, interactions between the 4-SNP GRS and sex were tested but no significant results were found (Supplementary Table S7).

Table 3. Interactions between the 4-SNP GRS and the lifestyle factors on obesity-related traits.

	Protein (g/day)	Carbohydrate (g/day)	Fibre (g/day)	Fat (g/day)	SFA (g/day)	MUFA (g/day)	PUFA (g/day)	Physical Activity
BMI (kg/m^2)	0.45	0.22	0.12	0.15	-	-	-	0.76
WC (cm)	0.08	0.21	0.41	0.01	0.02	0.01	0.01	0.24
WHR	0.82	0.88	0.49	0.80	-	-	-	0.002
Visceral fat (%)	0.50	0.35	0.32	0.38	-	-	-	0.93
Body fat (%)	0.46	0.11	0.04	0.75	-	-	-	0.60

Data are *p* values obtained from the linear regression analysis adjusted for age, sex, total energy intake and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables. Abbreviations: SNP: single nucleotide polymorphism; GRS: genetic risk score; BMI: body mass index; WC: waist circumference; WHR: waist–hip ratio; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

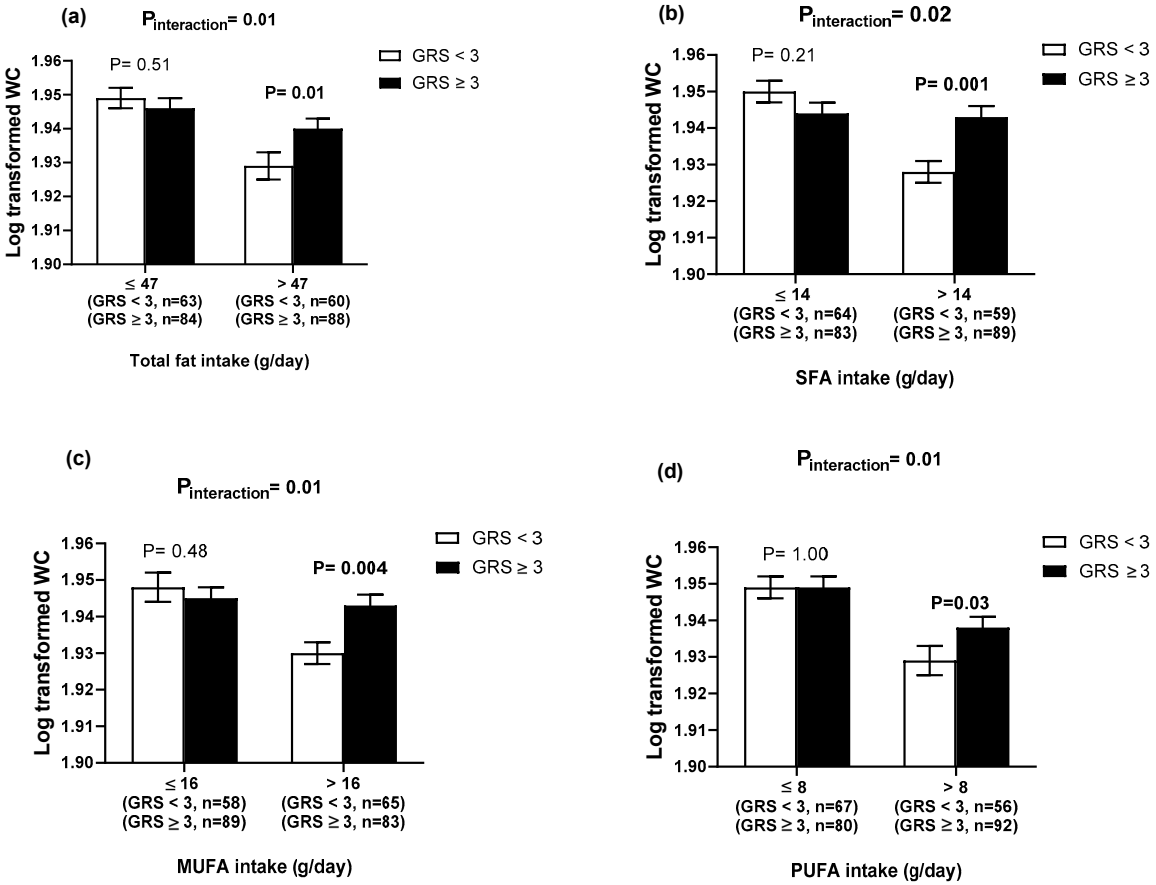

Figure 2. Interaction between the 4-SNP GRS and fat intake (g/day) on the log transformed WC. (**a**) Interaction between the 4-SNP GRS and the log transformed total fat intake (g/day) on WC. White bars indicate individuals with a GRS < 3 risk alleles; the black bars indicate individuals with GRS ≥ 3 risk alleles. Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with a higher total fat intake (above median group > 47 g/day): 71.28 ± 23.68 g/day (34.99 ± 5.54 % TEI); (**b**) the interaction between the 4-SNP GRS and the log transformed SFA intake (g/day) on the log transformed WC. White bars indicate individuals with a GRS < 3 risk alleles; the black bars indicate individuals with GRS ≥ 3 risk alleles. Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with a higher SFA intake: 23.50 ± 10.08 g/day (12.19 ± 3.21% TEI); (**c**) the interaction between the 4-SNP GRS and the log transformed MUFA intake (g/day) on the log transformed WC. White bars indicate individuals with a GRS < 3 risk alleles; the black bars indicate individuals with GRS ≥ 3 risk alleles. Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with a higher MUFA intake: 25.72 ± 9.58 g/day (12.79 ± 2.53% TEI); (**d**) the interaction between the 4-SNP GRS and the log transformed PUFA intake (g/day) on the log transformed WC. White bars indicate individuals with a GRS < 3 risk alleles; the black bars indicate individuals with GRS ≥ 3 risk alleles. Individuals with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles, among individuals with a higher PUFA intake: 12.74 ± 4.7 g/day (6.28 ± 1.08% TEI). Abbreviations: SNP: single nucleotide polymorphisms; GRS: genetic risk score; WC: waist circumference; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TEI: total energy intake. Error bars indicate the standard error of the mean.

4. Discussion

To our knowledge, this is the first nutrigenetic study investigating the interaction between metabolic GRSs and lifestyle factors on obesity-related traits in a Ghanaian population. Our study provides evidence for an interaction between the 4-SNP GRS and fat intake on WC, where individuals

with ≥3 risk alleles had a significantly higher WC compared to those with <3 risk alleles among those who consumed a diet high in total fat, SFA, MUFA and PUFA. These results are in accordance with the general dietary recommendations, which suggest that the population decrease their intakes of total fat and SFA, to reduce the risk of obesity, and this will be more applicable in individuals with a higher genetic predisposition to obesity. Our findings are of importance to public health, considering the high consumption of foods that are rich in SFA and MUFA in the Ghanaian population [50].

Our study is the first study of its kind, investigating the effect of different metabolic GRSs (the 12-, 8- and the 4-SNP GRS) on obesity-related traits in a Ghanaian population. We found that none of the metabolic GRSs were significantly associated with obesity-related traits in the Ghanaian population, which contradicts the findings of the previous GRS-related studies in European and African populations [15,25–30,32,33]. Efforts to replicate previously reported genetic associations of individual SNPs with obesity measures in non-African populations have shown limited success among Africans [23,31,51,52], which is also in line with the findings from the present study. Several factors are likely to be involved in such discrepancies between our findings and genetic association studies in Europeans. First, the metabolic GRS in the present study was constructed based on variants strongly associated with BMI in European populations, which raises the question of the usefulness, applicability and accuracy of using this metabolic GRS in our African population. Analysing the genetic associations of such variants with obesity-related traits in African population may not be ideal because of differences in risk allele frequency and effect size across populations [53,54]. Second, the 'lead' SNPs identified in Europeans might tag smaller regions in Africans [19,20,55] and the 'true' causal polymorphisms might be at different loci [56]. A systematic review of genetic research in African samples has reported that more than 300 SNPs in 42 loci analysed in relation to obesity, but only a few positive associations were replicable in Africans [57]. Of the 36 variants previously established by GWAS in non-African populations, only the SNPs located at the *FTO* and *MC4R* loci were significantly associated with obesity in Nigerians, Ghanaians and black South Africans [58,59]. Furthermore, in a large-scale GWAS meta-analysis consisting of 71,412 individuals of African ancestry, of the 36 previously identified BMI-associated SNPs in Europeans, only five variants reached a genome-wide significant level in Africans [60]. Such inconsistencies in results are likely due, in part, to the variation in the genetic architecture between populations of different ancestry [61]. African populations are characterised by greater genetic variation, reduced patterns of linkage disequilibrium (LD) and more haplotype diversity in comparison with populations of another ancestry, which may cause difficulties in replicating previously reported genetic associations [61]. Hence, future studies with a larger sample size are needed to investigate the combined effect of a larger number of genetic variants on obesity-related traits in the Ghanaian population.

Our study has identified significant interactions between the 4-SNP GRS and intakes of total fat, SFA, PUFA and MUFA on WC, an indicator of central obesity that has been associated with the increased risk of morbidity and mortality [62,63]. Our findings suggest that dietary fatty acid consumption and composition may have the potential to influence the genetic susceptibility of becoming centrally obese. Evidence is limited concerning the GRS–diet interactions on obesity and its related traits, and most of the research has focused on the influence of a single locus [64–66], despite the genetic effects on obesity being polygenic [13]. Our results are consistent with previous findings generated from single-locus gene–diet interactions on obesity, in which fat intake is considered as an important lifestyle modulator of genetic associations with obesity-related traits. Two previous studies in 2163 participants from two independent United States (US) populations and in 28,449 individuals living in Malmö have shown significant interactions of the *FTO* SNP rs9939609 with total dietary fat on BMI [64,67], however, a large-scale meta-analysis of 177,330 individuals (154,439 Whites, 5776 African Americans and 17,115 Asians) failed to identify this interaction [68]. In addition, studies in 2163 participants from two US populations, 1754 French individuals and 354 Spanish children and adolescents have demonstrated a significant interaction of *FTO* SNP rs9939609 with SFAs [64–66] and MUFAs [64] on BMI. Furthermore, a study in 305 obese individuals in Finland reported that the high intake of

MUFA was associated with weight loss among carriers of the risk allele (A) *FTO* rs9939609 [69]. Additionally, a study in 1680 South Asians has shown a significant interaction of the risk allele 'T' of the *TCF7L2* SNP rs12255372 with fat intake on high-density lipoprotein cholesterol (HDL-C) [70]. Studies on GRS–diet interactions on obesity traits have mainly focused on European populations [71–73]. In agreement with our study, data from UK Biobank [72] and two studies from the US [71] have reported significant interactions between the GRS and dietary intakes of total fat and SFA on WC; the GRS was associated with a higher WC among individuals with high intakes of total fat and SFA. However, the interactions on BMI were not identified in the present study, which contradicts the previously reported findings [71,72]. Hence, larger studies are required to replicate our GRS–fat intake interactions on WC in the Ghanaian population.

Several studies have investigated the impact of dietary fat on obesity measures; however, the findings have been inconsistent [74]. For instance, prospective studies have examined the relationship between the intake of long-chain omega-3 (LC n−3)-PUFAs and adiposity, but results have been inconsistent. A study in 124 adults living in the UK found that the plasma levels of n-3 PUFA were negatively associated with anthropometric measures of obesity [75], whereas positive associations were reported in a study of 79,839 women living in the US [76]. However, no effect of n-3 LC-PUFA consumption on BMI was found in a 12 year follow-up US cohort of 43,671 men [77]. In a randomised controlled trial (RCT) of 27 women, the intake of a 3 g/d of fish oil (1.8 g n−3 PUFAs) for 2 months was associated with adiposity reduction [78]. Similar findings were reported in an RCT of 324 men and women from Iceland, Spain and Ireland, in which the intake of either lean fish (3 × 150 g portions of cod/week) or fatty fish (3 × 150 g portions of salmon/week), or fish oil (docosahexaenoic acid/eicosapentaenoic acid capsules) for 8 weeks were associated with weight loss in men [79]. However, a 6 week RCT in 195 UK adults found no differences in the anthropometric measures between three intervention diets of specific fatty acid compositions of total energy intake (TEI) (%TEI SFA:%TEI MUFA:%TEI omega-6 PUFA): SFA-rich diet (17:11:4), MUFA-rich diet (9:19:4) or omega-6 PUFA-rich diet (9:13:10) [80]. A meta-analysis of 534,906 European individuals revealed that the higher adherence to the Mediterranean diet, which is rich in MUFA, was associated with a beneficial effect on WC [81]. However, a recent 4 week intervention found no significant effect of the intake of 50 g/day of olive oil, which is rich in MUFA, on BMI or central obesity in 91 UK adults [82]. Conflicting evidence exists regarding the effects of dietary fat on obesity-related traits; this could be because of the genetic heterogeneity and the gene–diet interactions that vary across multiple ethnic groups [83]; hence, the influence of both genetic and lifestyle factors should be considered in understanding the pathophysiology of obesity.

In 2018, the WHO recommended that the intake of total fat and SFA should not exceed 30% and 10% of TEI, respectively, to avoid weight gain [84]. According to the WHO, the recommended range for PUFA for the general population is 6–11% of TEI [85]. It has been identified that the average consumption of SFA in Africa is between 8.9% and 12.5% TEI (North: 9.1%, Central: 12.2, Eastern: 10.7%, Southern: 8.9% and Western Africa: 12.5%; which is slightly higher than the ≤10% TEI recommended by the WHO). The intake of PUFA is low in many sub-Saharan African countries, suggesting the infrequent use of vegetable oils for cooking or preparing foods [86]. The extremely low intake of n-3 long chain PUFA was also identified in Africa, which is explained by the low availability of fish in sub-Saharan Africa countries [86]. In the present study, the average consumption of total fat intake was 23.04 ± 9.13% of TEI and the average consumption of SFA, MUFA and PUFA were 8.95 ± 4.10, 9.86 ± 3.65 and 4.99 ± 1.61% of TEI, respectively, which are in accordance with general dietary recommendations. However, nearly one third of the study population had a high consumption of total fat (mean intake: 34.99 ± 5.54 g/day), the group in which the GRS showed a significant association with a higher WC. Hence, our study suggests that following the general dietary recommendations might be an effective way to overcome the genetic susceptibility to central obesity.

The strengths of our study include the analysis of gene–lifestyle interactions in a well characterized Ghanaian population and the use of different metabolic GRSs to maximise statistical power and to

reduce multiple testing [25,27]. Nevertheless, some limitations need to be acknowledged. First, our analysis included an only Ghanaian population, which limits the generalisability of our results to other population groups within Africa. Second, our metabolic GRSs were constructed based on BMI-associated loci predominantly identified in Europeans, which might not truly reflect the genetic associations with BMI among Africans. Third, the food intakes were assessed using repeated 24 h dietary recall method, which is prone to reporting bias and this might have contributed to the discrepancy in the caloric consumption between overweight/obese and non-obese groups [87]. Fourth, as with any cross-sectional study design, residual confounding might exist, despite adjustments for several confounding factors. Fifth, our sample size was small; however, our study had sufficient statistical power to detect significant gene–diet interactions.

5. Conclusions

In conclusion, our study has shown that higher intakes of total fat, SFA, MUFA and PUFA can increase the genetic risk on WC in Ghanaian adults. We found that the effect of metabolic risk alleles on WC is stronger among individuals with higher intakes of total fat, SFA, MUFA, PUFA. These results give important insights into the complex interactions between dietary intake and the genetic predisposition to central obesity and highlight the importance of personalising dietary advice according to each ethnic group. Our GRS approach provides insights into cumulative genetic susceptibility; however, studies with a large sample size will be needed to confirm the findings before public health recommendations and personalized nutrition advice can be developed for the Ghanaian population.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/7/1906/s1. Table S1: Genotype distribution of the fifteen SNPs that were included in the metabolic GRS; Table S2: Characteristics of the study participants stratified based on sex; Table S3: Associations of the 12-SNP GRS with obesity-related traits; Table S4: Associations of the 8-SNP GRS with obesity-related traits; Table S5: Interactions between the 12-SNP GRS and lifestyle factors on obesity-related traits' Table S6: Interactions between the 8-SNP GRS and lifestyle factors on obesity-related traits; Table S7: Interactions between the 4-SNP GRS and sex on obesity-related traits.

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Interaction between the genetic risk score and dietary protein intake on cardiometabolic traits in Southeast Asian

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Abstract

Background: Cardiometabolic diseases are complex traits which are influenced by several single nucleotide polymorphisms (SNPs). Thus, analysing the combined effects of multiple gene variants might provide a better understanding of disease risk than using a single gene variant approach. Furthermore, studies have found that the effect of SNPs on cardiometabolic traits can be influenced by lifestyle factors, highlighting the importance of analysing gene-lifestyle interactions.

Aims: In the present study, we investigated the association of 15 gene variants with cardiometabolic traits and examined whether these associations were modified by lifestyle factors such as dietary intake and physical activity.

Methods: The study included 110 Minangkabau women [aged 25–60 years and body mass index (BMI) 25.13 ± 4.2 kg/m^2] from Padang, Indonesia. All participants underwent a physical examination followed by anthropometric, biochemical and dietary assessments and genetic tests. A genetic risk score (GRS) was developed based on 15 cardiometabolic disease-related SNPs. The effect of GRS on cardiometabolic traits was analysed using general linear models. GRS-lifestyle interactions on continuous outcomes were tested by including the interaction term (e.g. lifestyle factor*GRS) in the regression model. Models were adjusted for age, BMI and location (rural or urban), wherever appropriate.

Results: There was a significant association between GRS and BMI, where individuals carrying 6 or more risk alleles had higher BMI compared to those carrying 5 or less risk alleles ($P = 0.018$). Furthermore, there were significant interactions of GRS with protein intake on waist circumference (WC) and triglyceride concentrations ($P_{interaction} = 0.002$ and 0.003, respectively). Among women who had a lower protein intake (13.51 ± 1.18% of the total daily energy intake), carriers of six or more risk alleles had significantly lower WC and triglyceride concentrations compared with carriers of five or less risk alleles ($P = 0.0118$ and 0.002, respectively).

Conclusions: Our study confirmed the association of GRS with higher BMI and further showed a significant effect of the GRS on WC and triglyceride levels through the influence of a low-protein diet. These findings suggest that following a lower protein diet, particularly in genetically predisposed individuals, might be an effective approach for addressing cardiometabolic diseases among Southeast Asian women.

Keywords: GRS, BMI, WC, Triglyceride, Interaction, Indonesian

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Introduction

Cardiometabolic diseases such as cardiovascular diseases (CVD), obesity, hypertension and type 2 diabetes are a major cause of mortality, morbidity and healthcare spending worldwide [1, 2]. The prevalence of these diseases has significantly increased and has become a major problem given the significant economic burden that these diseases impose on low- and middle-income countries. Indonesia has the seventh largest number of diabetic patients (7.6 million), despite relatively low prevalence worldwide (4.8%) in 2012 [3]. In 2013, it was estimated that there were more than 132.8 million people with diabetes in the Western Pacific (more people than in any other region), and the number is expected to rise to 201.8 million by 2035 [4]. Furthermore, obesity is suggested to play a critical role in the development of chronic and non-communicable diseases (NCDs) in the Southeast (SE) Asia [5]. In Indonesia, NCDs are estimated to account for 73% of all deaths [6] of which, CVD contributed to 35% followed by cancers (12%) and diabetes (6%) [6].

Indonesia is the largest island country in the world, consisting of various ethnic groups distributed over 33 provinces [7]. Minangkabau community is the world's largest matrilineal society which resides mostly in West Sumatra, where the prevalence of low level of high-density lipoprotein cholesterol (HDL-C), hypertension and central obesity is more than 50% [7]. It is reported that the Minangkabau ethnic group had a high risk of dyslipidemia, which is suggested to be driven mainly by the high intake of dietary fat from poor quality sources [8]. A study comparing lipid profiles among four ethnic groups reported that the Minangkabau ethnic group has the highest levels of plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C) compared to other larger ethnicities including Sundanese, Javanese and Buginese [9]. Furthermore, it has been reported that the prevalence of central obesity is high among Minangkabau women [10]. Many environmental exposures contribute to the increasing prevalence of cardiometabolic diseases, but one key factor is urbanisation [11]. Countries in SE Asia have undergone rapid epidemiological and nutritional transitions over the past few decades. Furthermore, it has been reported that dietary risks, high blood pressure and tobacco smoking are the three major risk factors contributing to disease burden in Indonesia [12]. However, genetic factors also play an important role in the development of cardiometabolic diseases.

Candidate gene studies and genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) relating to cardiometabolic diseases and traits in the Asian populations [13–16]. Most cardiometabolic traits are influenced by thousands of SNPs each having a relatively small effect on the trait when present alone. Thus, analysing the combined effects of multiple gene variants might provide a better understanding of trait variability of an individual and improve risk prediction of cardiometabolic diseases than using a single variant approach [17]. Furthermore, studies have found that the effect of genetic variants on cardiometabolic traits can be influenced by lifestyle factors [18]. It has been confirmed that using genetic risk score (GRS) approaches increases the power to detect gene-lifestyle interactions compared to the common single variant methods [19]. Therefore, our study aimed to investigate the association of a novel GRS with cardiometabolic traits and to examine whether lifestyle factors such as dietary intake and physical activity modified these associations in 110 Minangkabau women.

Methods

Study participants

The study included healthy women who were enrolled in the Minangkabau Indonesia Study on Nutrition and Genetics (MINANG) study, a cross-sectional pilot study conducted in the city of Padang, West Sumatra, Indonesia, between December 2017 and January 2018. This study is a part of the ongoing GeNuIne (gene-nutrient interactions) Collaboration, which aims to examine the interactions between genetic and dietary factors (nutrigenetics) on cardiometabolic disease and its related traits using population-based studies from several ethnic groups [20]. The methodology of the study has been published elsewhere [21]. In brief, 133 women were recruited from community health centres in two sub-districts in Padang City including Padang Timur and Kuranji districts to represent both urban and rural areas of Padang population, respectively. The inclusion criteria included healthy women, aged 25–60 years old and with Minangkabau ethnicity. Of the 133 enrolled women, 10 were excluded from the study according to the following exclusion criteria: being pregnant or lactating (N = 0) and taking dietary or vitamin supplements (N = 0); have a previous history of hypertension, CVD or type 2 diabetes (N = 6); have a body mass index (BMI) of more than 40 kg/m^2 or being classified as morbidly obese by a practitioner (N = 0); being blood related to other participants in the study (N = 0); have any communicable disease (N = 4). Of the remaining 123 participants, we excluded another 5 women who did not undergo blood sampling. Thus, the final sample consisted of 118 participants, of whom seven women did not have complete genetic information about all the investigated SNPs and were excluded from the GRS analysis (N = 111). Additionally, one participant with no dietary information available was excluded from the GRS interaction analysis (N = 110).

The MINANG study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethical Review Committee of the Medical Faculty, Andalas Univesity (No.311/KEP/FK/2017). All participants gave their written informed consent before participating and had the right to withdraw from the study at will and opt-out from any of the procedures.

Anthropometric measures

Body weight (to the nearest 100 g) and height (to the nearest mm) were measured using an electronic scale (Seca 803, Seca GmbH. Co. kg, Hamburg, Germany) and a wall-mounted stadiometer (OneMed Medicom stature meter, YF.05.05. V.A.1022, Indonesia), respectively. BMI was calculated as weight (kg)/height $(m)^2$ and categorised according to the Asia-Pacific classification of BMI [22]. Waist circumference (WC) was measured in centimetre using a metal tape (Medline-OneMed Medicom, Jakarta, Indonesia) midway between the 12th rib and the superior border of the iliac crest at the end of normal expiration.

Biochemical and clinical measures

After 12 h of fasting, blood samples (5 ml) were taken to measure the concentrations of glucose, insulin, glycated haemoglobin A1c (HbA1c), total cholesterol, triglycerides, LDL-C and HDL-C. Samples were assayed using the xMark Microplate Spectrophotometer (Bio-Rad Laboratories Inc, Hercules, California, USA). Fasting glucose, insulin and HbA1c were measured using enzyme-linked immunosorbent assay (ELISA) kits from Bioassay Technology Laboratory (Shanghai, China). Blood lipids were analysed using enzymatic colorimetric procedures, namely GPO-PAP for triglycerides and CHOD-PAP for total cholesterol, LDL and HDL. A sphygmomanometer was used to measure systolic and diastolic blood pressures (SBP and DBP). Measurements were taken twice at 5-min intervals, and the average was recorded.

Assessment of dietary intake and physical activity

Information about dietary intake and physical activity was collected by a well-trained nutritionist in the home or in an integrated health service post. Diet was assessed using a previously validated and published semi-quantitative food frequency questionnaire (SQ-FFQ) consisting of a list of 223 food items [23]. Briefly, participants were asked to report the frequency of consumption (number of times per day, week or month) and portion size of various food items. Participants were provided with portion size images of all relevant foods to enhance reporting accuracy while completing the SQ-FFQ [24]. All collected data were double-checked for accuracy and analysed with the Indonesian Food Database and Nutrisurvey (EBISpro, Germany) to estimate total energy and macronutrient intake. Values of nutrient intake were adjusted for total energy intake using the nutrient (energy-adjusted) residual method, wherever appropriate [25].

"The Global Physical Activity Questionnaire" (GPAQ) was used to calculate an individual's level of physical activity in 3 areas (work, transport and leisure-time) and time spent in sedentary behaviour [26]. Total time spent in moderate-to-vigorous physical activity was estimated using to the World Health Organization (WHO) STEPwise method and was expressed as metabolic equivalent minutes per day (METmins/day). Participants were defined as "active" if they did ≥ 600 METmins/week or "inactive" if they accumulated < 600 METmins/week.

SNP selection and genotyping

Fifteen genetic variants located at 8 different genes were selected for the present study based on its consistent associations with cardiometabolic traits in candidate gene studies and GWAS in Asian populations [13–16, 27–36]. The selected genetic variants were Calpain 10 (*CAPN10*) rs3792267 and rs5030952; fat mass and obesity-associated (*FTO*)- rs9939609, rs10163409 and rs8050136; melanocortin 4 Receptor (*MC4R*)- rs17782313 and rs2229616; transcription factor 7-like 2 (*TCF7L2*)- rs12255372 and rs7903146; potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*)- rs2237895 and rs2237892; cyclin-dependent kinase inhibitor 2A/2B (*CDKN2A/*2B)- rs10811661; peroxisome proliferator-activated receptor gamma (*PPARG*)- rs1801282; and adiponectin (*ADIPOQ*)- rs266729 and rs17846866.

Genomic DNA was extracted from peripheral blood leukocytes using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, USA). Furthermore, a NanoDrop spectrophotometer was used to determine DNA concentration. The SNPs were genotyped using the competitive allele-specific PCR-KASP® assay at LGC Genomics (http://www.lgcgroup.com/services/genotyping).

Statistical analysis

Statistical analysis was performed using the SPSS software (version 23). Common obesity was defined based on the Asia-Pacific classification of BMI for Asians, where non-obese individuals (BMI < 23 kg/m^2) and obese individuals (BMI ≥ 23 kg/m^2) were classed accordingly [37]. Central obesity was defined based on WHO classification of WC (WC > 80 cm for women) [38]. The Hardy-Weinberg equilibrium (HWE) was assessed using the x^2 goodness-of-fit test, and the 15 SNPs were in HWE ($P > 0.05$). Normality of distribution of all continuous variables was tested using the Shapiro-Wilk test and those that were not normally distributed were natural log-transformed before the analysis, including glucose, insulin, HbAC1, HDL-C, LDL-C, total cholesterol, triglyceride concentrations and total dietary protein

intake (%). Continuous variables are expressed as means and standard deviations (SD), and comparisons between groups were made using the independent *t* test. The descriptive statistics for categorical variables, such as physical activity level, were obtained by determining frequency distributions and compared between individuals with and without central obesity using Pearson's chi-squared test. The association between individual SNPs and cardiometabolic traits was analysed using general linear models adjusted for age, residential area (rural or urban) and BMI when BMI is not an outcome. As the number of individuals with rare homozygous genotypes was low, a dominant model was used, where common homozygous genotypes were compared against combined rare homozygous and heterozygous genotypes.

A GRS was constructed based on 15 SNPs from 8 genes. An additive genetic model was assumed for each gene variant, assigning a score of 0, 1 and 2 to genotypes containing 0, 1 or 2 risk alleles, respectively. The GRS was then calculated for each individual by summing the number of risk alleles in the genetic variants. The count method assumed that each risk allele contributes equally and independently to the development of cardiometabolic traits. The average number of risk alleles per individual for the GRS was 5.12 (SD = 2.06), which ranged from 2 to 10. The GRS variable was then categorised into two groups based on the median of risk alleles: "low genetic risk group"—individuals with a GRS ≤ 5 risk alleles (N = 69) and "high genetic risk group"—individuals with GRS > 5 risk alleles (N = 42). The effects of GRS on cardiometabolic traits were analysed using general linear models. Furthermore, GRS-lifestyle interactions on continuous outcomes were tested using linear regression models by including the interaction terms (e.g. diet*genotype) in these models. Models were adjusted for age, residential area and additionally for BMI when it is not an outcome. Lifestyle factors that were investigated in our study included dietary intake and physical activity. Carbohydrate, protein and fat intakes were expressed as a percentage of total energy intake, and fibre intake was expressed in grammes. Furthermore, statistically significant interactions were investigated in more depth, where individuals were stratified by the tertiles of dietary intake and the levels of physical activity. A P value of < 0.05 was considered statistically significant. Multiple testing correction was not applied given that we had examined only one genetic instrument (i.e. GRS).

Results

Characteristics of the study participants according to the central obesity status

In the present study, 71 women (64.0%) were centrally obese and 39 (35.1%) were not. The characteristics of the participants are shown in Table 1. In general, centrally obese participants were older and had higher SBP (P = 0.006), fasting plasma glucose (P = 0.039), serum triglycerides (P < 0.001), serum total cholesterol (P < 0.001) and LDL-C (P < 0.001) concentrations compared to participants without central obesity. There were no significant differences in fasting HDL-C, serum insulin, HbA1c, DBP, dietary intake and physical activity levels and the distribution of GRS between the two groups (P > 0.05).

Associations between GRS and cardiometabolic traits

To explore the combined effect of the 15 SNPs on various cardiometabolic traits, a GRS was calculated. There was a significant association (P = 0.018) between the GRS and BMI where individuals carrying 6 or more risk alleles of the SNPs had higher BMI compared with those carrying 5 or less risk alleles (Table 2).

Interactions between GRS and dietary intake on cardiometabolic traits

There were significant interactions between the GRS and protein intake (%) on WC and triglyceride concentrations ($P_{interaction}$ = 0.002 and 0.003, respectively) (Table 3). With low protein intake (13.51 ± 1.18%), carriers of 6 or more risk alleles of SNPs had lower WC and triglyceride concentration compared to carriers of 5 or less risk alleles (P = 0.0118 and 0.002, respectively) (Figs. 1 and 2). A significant interaction between protein intake and GRS was also detected on cholesterol levels ($P_{interaction}$ = 0.021). Moreover, there were no other interactions between nutrient intake and GRS on cardio-metabolic traits.

Associations between individual SNPs and cardiometabolic traits

As shown in supplementary Table 1, Additional File 1, we found that the risk alleles of the three *FTO* SNPs rs9939609, rs8050136 and rs10163409 were associated with higher BMI (P = 0.006, 0.007 and 0.047, respectively). Furthermore, SNPs rs12255372 (*TCF7L2*), rs2237892 (*KCNQ1*) and rs5030952 (*CAPN10*) were associated with increased fasting serum LDL-C concentrations (P = 0.032, 0.039 and 0.04, respectively). A significant association was also found between the risk allele of the SNP rs17782313 (*MC4R*) and higher insulin level (P = 0.036). No significant association was observed between the remaining SNPs and cardiometabolic traits in this population (P > 0.05).

Discussion

The present study aimed to investigate the effects of genetic predisposition and lifestyle factors on cardiometabolic traits in Minangkabau women. In agreement with other studies [39], we have shown that the GRS based on 8 susceptible genes for cardiometabolic diseases is a significant risk factor for higher BMI in our study

Table 1 Anthropometric and biochemical characteristics of the study participants

	N	Total (*N* = 111)	*N*	Non-centrally obese (WC ≤ 80 cm) (*N* = 39)	*N*	Centrally obese (WC > 80 cm) (*N* = 71)	*P* value*
Age (years)	111	40.49 ± 10.18	39	37.08 ± 11.68	71	42.58 ± 8.62	0.012
BMI (kg/m^2)	111	25.13 ± 4.2	39	21.85 ± 3.71	71	26.99 ± 3.24	< 0.001
WC (cm)	110	83.85 ± 10.27	39	72.79 ± 6.03	71	89.92 ± 6.26	< 0.001
Glucose (mg/dl)	111	92.53 ± 20.67	39	87.21 ± 9.78	71	95.69 ± 24.29	0.039
Insulin (mIU/L)	111	32,428.5 ± 25, 706.13	39	31,073.79 ± 28,460.35	71	33,374.28 ± 24,368.83	0.657
HbA1c (ng/ml)	111	655.59 ± 601.59	39	629.22 ± 671.07	71	666.42 ± 568.14	0.759
Triglycerides (mg/dl)	111	98.8 ± 43.47	39	78.26 ± 34.19	71	109.72 ± 44.38	< 0.001
Cholesterol (mg/dl)	111	209.31 ± 44.02	39	188.26 ± 30.04	71	221.77 ± 45.74	< 0.001
HDL-C (mg/dl)	111	59.12 ± 10.29	39	60.9 ± 10.45	71	58.14 ± 10.22	0.182
LDL-C (mg/dl)	111	128.12 ± 39.85	39	111.49 ± 25.55	71	138.2 ± 42.65	< 0.001
SBP (mmHg)	111	113.37 ± 9.07	39	110.14 ± 8.83	71	115.05 ± 8.81	0.006
DBP (mmHg)	111	77.44 ± 6.39	39	76.26 ± 8.35	71	78.06 ± 5.01	0.223
Total energy (kcal/day)	110	1776.24 ± 611.43	39	1789.55 ± 604.31	70	1755.6 ± 613.59	0.781
Carbohydrate intake (%)	110	53.97 ± 9.44	39	52.67 ± 7.86	70	54.91 ± 10.1	0.235
Protein intake (%)	110	16.93 ± 3.32	39	17.13 ± 2.93	70	16.76 ± 3.54	0.579
Fat intake (%)	110	28.95 ± 7.99	39	30.05 ± 6.87	70	28.16 ± 8.45	0.235
Dietary fibre (g)	110	8.78 ± 4.29	39	9.11 ± 4.52	70	8.56 ± 4.19	0.521
SFA (g)	110	20.84 ± 11.22	39	21.77 ± 10.81	70	20.07 ± 11.35	0.447
MUFA (g)	110	8.18 ± 4.6	39	9.00 ± 5.08	70	7.62 ± 4.18	0.129
PUFA (g)	110	6.32 ± 3.5	39	6.67 ± 3.06	70	6.14 ± 3.76	0.541
MET (min/week)	111	1311.89 ± 1877.78	39	1114.87 ± 1625.95	71	1428.45 ± 2016.27	0.407
GRS	110	5.09 ± 2.07	39	4.77 ± 2.01	71	5.31 ± 2.03	0.189
Physical activity levels	44	Sedentary (39.64%)	18	Sedentary (46.15%)	26	Sedentary (36.62%)	0.616
	55	Moderate (49.55%)	17	Moderate (43.59%)	37	Moderate (52.11%)	
	12	Vigorous (10.81%)	4	Vigorous (10.26%)	8	Vigorous (11.27%)	

Data presented as means ± SD for continuous variables and as percentages for categorical variables
BMI body mass index, *WC* waist circumference, *HbA1C* glycated haemoglobin A1c, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *MET* metabolic equivalent of task, *GRS* genetic risk score
**P* values for the differences in the means and proportions between non-centrally obese and centrally obese individuals were calculated using the independent *t* test and the Chi-squared test, respectively

sample and might be a useful tool in characterising Minangkabau women at high risk for obesity. We found that women carrying 6 or more alleles had significantly higher BMI compared to those carrying 5 or less risk alleles. Furthermore, we found a significant interaction between the GRS and dietary protein intake (%) on WC and triglyceride levels, where, among those who consumed a low protein diet (mean intake ± SD 13.51 ± 1.18%), individuals, despite carrying more than 6 risk alleles, had significantly lower WC and triglyceride levels. Given that Minangkabau women have a high risk of dyslipidemia [9] and the prevalence of common and central obesity is high among this ethnic group [10], it is important to develop effective strategies targeting these conditions to improve public health.

It has been suggested that centrally obese participants defined as normal weight based on BMI had the worst long-term survival even when compared with their overweight and obese counterparts [40]. In addition, recent data from 42,702 European participants reported that central obesity is associated with higher mortality risk even in normal-weight individuals [41]. This is of concern for Asian populations, where increased levels of visceral adiposity are observed in those with normal BMIs [42–44]. Furthermore, the combination of increased WC along with elevated triglyceride levels has been

Table 2 Associations between GRS and cardiometabolic traits

	GRS ≤ 5 (*N* = 69)		GRS > 5 (*N* = 42)		*P* value*
	N	Mean ± SE	*N*	Mean ± SE	
BMI (kg/m^2)	69	24.52 ± 0.52	42	26.14 ± 0.6	0.018
WC (cm)	68	84.28 ± 1.22	42	83.16 ± 1.66	0.334
Log glucose (mg/dl)	69	93.65 ± 2.98	42	90.69 ± 1.72	0.327
Log insulin (mIU/L)	69	32,365.29 ± 3199.95	42	32,532.33 ± 3782.96	0.196
Log HbA1C (ng/ml)	69	650.58 ± 71.1	42	663.81 ± 96.65	0.527
Log triglycerides (mg/dl)	69	101.07 ± 5.27	42	95.07 ± 6.67	0.142
Log cholesterol (mg/dl)	69	212.88 ± 5.59	42	203.43 ± 6.11	0.228
Log HDL-C (mg/dl)	69	58.55 ± 1.26	42	60.05 ± 1.56	0.404
Log LDL-C (mg/dl)	69	131.84 ± 4.97	42	122 ± 5.73	0.197
Log SBP (mmHg)	69	113.12 ± 1.08	42	113.77 ± 1.43	0.679
Log DBP (mmHg)	69	77.59 ± 0.86	42	77.2 ± 0.76	0.535

BMI body mass index, *WC* waist circumference, *HbA1C* glycated haemoglobin A1c, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure
**P* values obtained from linear regression analysis adjusted for age, residential area and additionally for BMI when BMI is not an outcome. The analysis was performed on log-transformed variables

previously defined as the 'hypertriacylglycerolaemic waist' phenotype [45]. Studies have shown that individuals with this phenotype have an increased risk of higher visceral adiposity, CVD, insulin resistance and other related outcomes [45]. Therefore, targeting this phenotype will have significant public health implications in terms of reducing NCD mortality in Asian populations.

In the present study, the average protein intake was 77 ± 37 g/day, which exceeded the recommended dietary protein daily allowance of 57–59 g/day for Indonesian women [46, 47]. Observational studies have shown that higher protein intake was significantly associated with increases in body weight, BMI and fat mass [48–50]. These results are in contrast to the finding from intervention studies, which have shown that high protein intake enhances weight loss and provides a better long-term maintenance of reduced intra-abdominal fat stores [37, 51]. These inconsistencies might be attributed to the sample size, genetic heterogeneity and gene-lifestyle interactions. Cross-sectional studies have demonstrated the association of several SNPs with obesity-related traits [52–55], and interaction of these SNPs with dietary intake of protein on weight change [56–58]. It has been shown that high protein diets can modulate the genetic effect of *FTO* variants on body weight, BMI and WC [59–61]. According to a 2-year weight loss intervention programme, carriers of the risk allele 'A' of the *FTO* SNP rs1558902 had a greater reduction in weight and

Table 3 Interactions between GRS and lifestyle factors on cardio-metabolic traits

	Carbohydrate (%)	Protein (%)	Fat (%)	Fibre (g)	Physical activity
BMI (kg/m^2)	0.961	0.282	0.721	0.876	0.362
WC (cm)	0.224	**0.002**	0.577	0.614	0.297
Log glucose (mg/dl)	0.882	0.751	0.732	0.833	0.106
Log insulin (mIU/L)	0.336	0.341	0.48	0.216	0.909
Log HbA1C (ng/ml)	0.766	0.638	0.935	0.162	0.626
Log triglycerides (mg/dl)	0.066	**0.003**	0.355	0.262	0.479
Log cholesterol (mg/dl)	0.081	0.021	0.261	0.583	0.308
Log HDL-C (mg/dl)	0.978	0.905	0.984	0.323	0.540
Log LDL-C (mg/dl)	0.266	0.337	0.431	0.896	0.721
Log SBP (mmHg)	0.156	0.291	0.208	0.872	0.644
Log DBP (mmHg)	0.966	0.815	0.732	0.292	0.743

Data are *P* values obtained from linear regression analysis adjusted for age, residential area and BMI when BMI is not an outcome. The analysis was performed on log-transformed variables
BMI body mass index, *WC* waist circumference, *HbA1C* glycated haemoglobin A1c, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

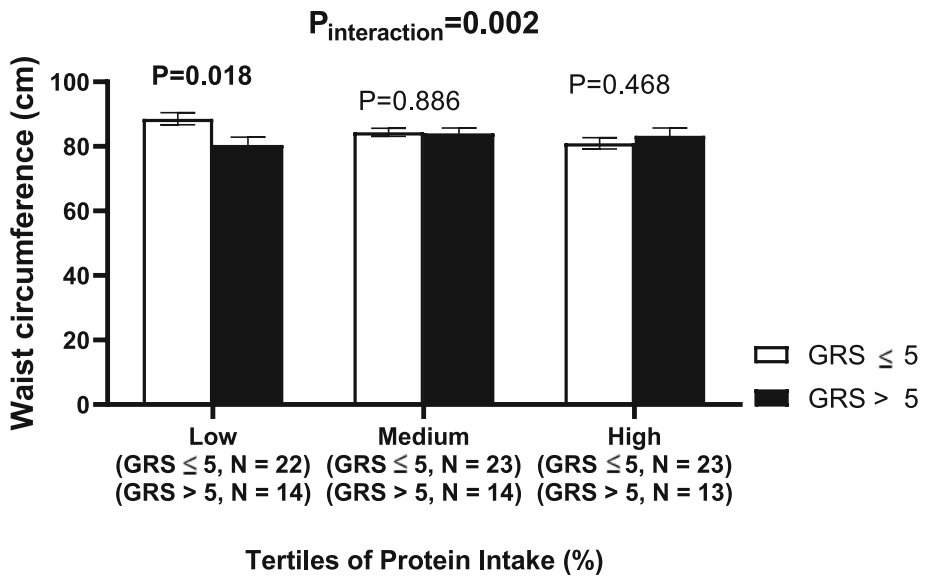

Fig. 1 Interaction between genetic risk score (GRS) and log protein intake (%) on waist circumference (WC). White bars indicate "low genetic risk group": individuals with a GRS ≤ 5 risk alleles; black bars indicate "high genetic risk group": individuals with GRS > 5 risk alleles. Carriers of 6 or more risk alleles had lower WC compared to carriers of 5 or less risk alleles, among individual with lower protein intake (13.51 ± 1.18%)

regional fat compared to non-carriers when high protein diets were consumed, whereas an opposite genetic effect was found on changes in fat distribution in response to a low-protein intake [60]. However, studies investigating the joint effect of genetic variants have reported conflicting results [62–64], indicating that the influence of genetic predisposition on changes in body weight and WC does not seem to be modulated by protein intake. In contrast, the present study provides evidence for GRS-protein intake interactions on WC and triglyceride concentrations, and these interactions were independent of potential confounding effects. We found that participants with 6 or more risk alleles who consumed a low protein diet (mean intake ± SD 13.51 ± 1.18%) had significantly lower WC and triglyceride concentrations compared to those with 5 or less risk alleles. This

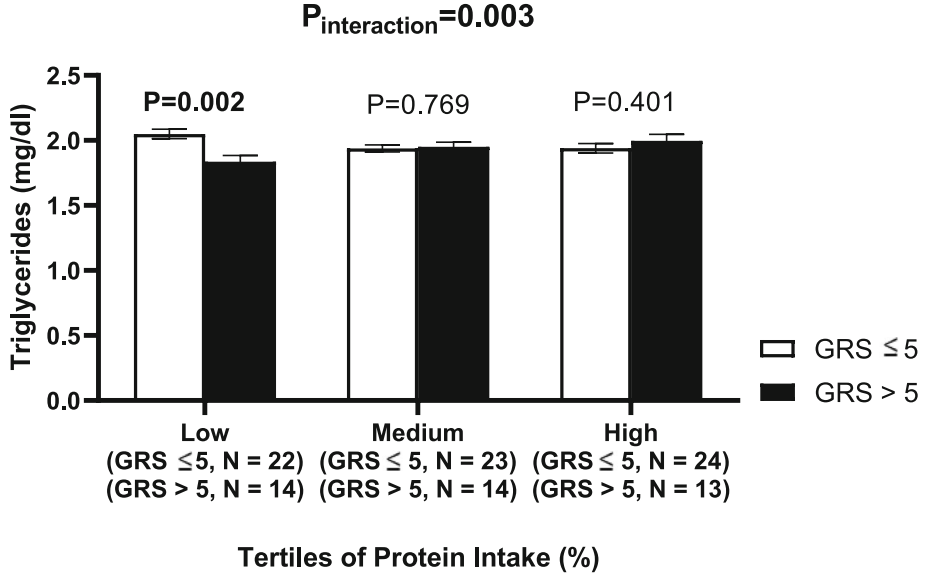

Fig. 2 Interaction between genetic risk score (GRS) and log protein intake (%) on log triglyceride levels. White bars indicate "low genetic risk group": individuals with a GRS ≤ 5 risk alleles; black bars indicate "high genetic risk group": individuals with GRS > 5 risk alleles. Carriers of 6 or more risk alleles had lower triglyceride level compared to carriers of 5 or less risk alleles, among individual with lower protein intake (13.51 ± 1.18%)

difference in the findings across the studies might be due to differences in the sample size, methods used to construct GRSs (weighted vs. unweighted) and the number of SNPs included in the GRSs.

The observed interaction between GRS and dietary protein on WC and triglyceride concentrations might be driven by the source of protein consumed, which has not been analysed in our study. Different protein sources have different effects on body weight and fat mass, and the mechanisms behind this are still very speculative and need more investigation. The higher intake of protein from animal sources (protein from red and processed meat and poultry) was found to be associated with an increase in body weight in both genders, with a stronger association in women [49]. Diet rich in animal protein might reflect the western pattern diet characterised by high red meat consumption, which has shown to be associated with weight gain [65]. In contrast, a study has shown that protein from meat is associated with lower weight gain because it produces a higher 24-h energy expenditure compared to soy protein [66]. This hypothesis is, however, based on a mechanistic study, and it is still unknown whether this applies in the long run to individuals of the free-living populations. Furthermore, it has been suggested that consuming protein from dairy sources may prevent weight gain and promote abdominal fat loss [67]. Here, the suggested mechanism primarily relates to the high content of calcium, which may function synergistically in combination with bioactive compounds, such as angiotensin-converting enzyme inhibitors and the rich concentration of branched-chain amino acids [67]. While the above-mentioned studies failed to explore the genetic aspects, our study did not investigate the type of protein that was consumed by the participants; hence, future studies examining the effect of both factors are required.

In agreement with some studies [62, 63], no interactions were detected between GRS and dietary intake of protein, fat and carbohydrate on BMI in the present study. However, a study in the European population (N = 48,170 adults) has shown that the joint effect of 93 obesity-related SNPs on BMI might be modulated by the intake of total energy, fat and saturated fat [64]. Furthermore, studies have shown that an obesogenic diet and physical inactivity with relatively high intake of sugar-sweetened beverages and prolonged television watching might exaggerate the effect of genetic factors on adiposity [18, 68]. Even though several studies have demonstrated that physical activity could attenuate the combined genetic influence of multiple SNPs on BMI and obesity risk [18, 69, 70], no such interactions were detected in the present study.

The strengths of our study include the use of a well-defined population, a validated SQ-FFQ [23] and a genetic risk score generated from the 15 genetic variants associated with cardiometabolic traits. Also, the main exposures investigated in our study were collected by well-trained staff and using validated and standardised operating procedures. However, there are limitations that need to be acknowledged. Although our analysis was adjusted for several factors, the potential for confounding by unmeasured or unknown factors exist. Even though our study has a small sample size, we were still able to find significant associations and interactions suggesting that our study is well powered. Even though food intake was assessed using validated methods, recall bias and measurement errors in these self-reported FFQs cannot be fully eliminated, which could alter the true underlying interactions between dietary and genetic factors on cardiometabolic traits [71, 72]. Finally, our study was restricted to Minangkabau women, and it is unknown whether our findings could be generalised to men or other demographic or ethnic groups.

Conclusion

In the present study, we have shown a significant effect of the GRS on WC and triglyceride levels through the influence of a low protein intake, where individuals with a high genetic susceptibility can overcome the risk of higher WC and triglyceride levels by consuming a low protein diet. These findings are potentially relevant for public health; however, future trials in both genders with larger sample size and objective measures of protein intake, such as urinary nitrogen, are needed to confirm these findings.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12263-020-00678-w.

Additional file 1:. Supplementary Table 1 Associations between individual SNPs and cardiometabolic traits.

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Authors' contributions

SA and ASA performed the statistical analyses and data interpretation; SA and KSV drafted the manuscript; KSV and ASA carried out data collection; KSV designed the nutrigenetics study; NIL, FFY and KSV conceived, supervised and designed the study; UA and SRS contributed to data collection, monitoring and evaluation of participants, and project administration; NT was involved in data collection and dietary data analysis; IRS conducted the laboratory analysis; NIL and JAL critically reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The MINANG study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethical Review Committee of the Medical Faculty, Andalas Univesity (No.311/KEP/FK/2017). All participants gave their written informed consent before participating.

Consent for publication
The consent for publication has been obtained from all the participants.

Competing interests
The authors declare that they have no competing interests.

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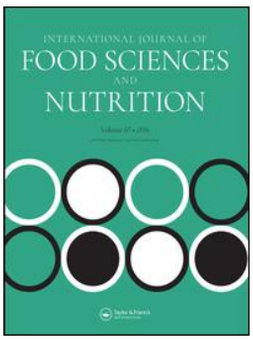

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RESEARCH ARTICLE

FTO gene–lifestyle interactions on serum adiponectin concentrations and central obesity in a Turkish population

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ABSTRACT

The aim of the study was to investigate whether lifestyle factors modify the association between 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). Our study has demonstrated that the association between *FTO* SNPs and central obesity might be modified by lifestyle factors in this Turkish population.

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

The *FTO* gene is located on chromosome 16q12.2 and codes for a protein with 2-oxoglutarate dependent nucleic acid demethylase activity which is involved in DNA repair and the accumulation of fat in the body (Clifton et al. 2006; Chen and Du 2019). *FTO* is highly expressed in the brain, including the hypothalamus, adipocytes, pancreatic islet cells, and adrenal glands (Frayling et al. 2007). *FTO* gene has been suggested to control energy homeostasis and food intake (Abete et al. 2020). Previous studies have shown that, of the various obesity susceptibility genes, single-nucleotide polymorphisms (SNPs) located in the first intron of *FTO* gene has provided the strongest evidence for genetic predisposition to obesity (Frayling et al. 2007; Scuteri et al. 2007; Speliotes et al. 2010;

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Loos and Yeo 2014; Babenko et al. 2019; Fonseca et al. 2020). The minor allele "A" of the *FTO* SNP rs9939609 has been consistently associated with higher BMI in various populations (Frayling et al. 2007; Hertel et al. 2011; Peng et al. 2011; Corella et al. 2012; Li et al. 2012; Qi et al. 2014; Wang et al., 2020; Schlauch et al. 2020). Furthermore, a meta-analysis reported that the association between the SNP rs9939609 and BMI was replicated in 13 cohorts with 38,759 participants, where individuals with the "AA" genotype had 1.67-time higher odds of obesity than those with the "TT" genotype (Frayling et al. 2007). In the Turkish population, the risk alleles of the *FTO* rs1421085 and rs9939609 polymorphisms were shown to have significant associations with the risk of obesity in women and metabolic syndrome (MetS) in men (Guclu-Geyik et al. 2016).

Turkish adults are characterised with low levels of total and high-density lipoprotein cholesterol, and high risk of CVD, that distinguish them from Europeans (Onat 2001). They have also increased susceptibility to impaired glucose tolerance and MetS primarily driven by obesity (Onat and Can 2014). Among the non-communicable diseases (NCDs) that accounted for 88.0% of deaths in Turkey, CVD has shown to contribute to 47.73% of overall deaths (WHO, 2018). Targeting modifiable risk factors for NCDs including obesity could prevent many deaths. Therefore, several health promotion campaigns such as "Reducing Portion Sizes" and "Move for Health" have been implemented for the prevention of obesity in Turkey (WHO 2016; OECD 2017). However, obesity is a multifactorial disorder, and identifying gene-environment interactions are needed to understand the aetiology and pathophysiology of obesity and also to develop more effective personalised preventative strategies (Castillo et al. 2017; Dahlman and Rydén 2020). To date, several *FTO*-dietary intake interactions on obesity-related outcomes have been examined in different populations (Grau et al. 2009; Sonestedt et al. 2009; Lappalainen et al. 2012; Ortega-Azorin et al. 2012; Phillips et al. 2012; Vimaleswaran et al. 2012; Qi et al. 2014; Merritt et al. 2018; Saber-Ayad et al. 2019); however, there are no such studies to date in a Turkish population. The investigations of the gene-diet interactions in different ethnic groups are crucial to develop personalised nutrition strategies for each ethnic group due to the genetic heterogeneity (Vimaleswaran 2017). The *FTO* SNP rs9939609 has been associated with several dietary 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 $\geq 25.00\,kg/m^2$) (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 $\geq$ 90 cm for men / $\geq$ 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 Analyser (USA). Fat mass index (FMI) was calculated based on the fat mass (kg) 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 χ^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 3(a–c)). 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 has 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 a 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 focussing on the prevention

Table 1. Anthropometric and biochemical characteristic of the study participants.

	Non-obese				Obese				
	Total ($N = 200$)	Men ($N = 100$)	Women ($N = 100$)	p value*	Total ($N = 200$)	Men ($N = 108$)	Women ($N = 92$)	p value *	p value**
Age (year)	33.29 ± 6.83	32.64 ± 6.04	33.93 ± 7.51	0.182	36.37 ± 7	36.09 ± 6.78	36.68 ± 7.27	0.552	<0.001
BMI (kg/m^2)	22.56 ± 1.78	22.83 ± 1.73	22.3 ± 1.79	0.035	29.04 ± 3.38	28.8 ± 3.22	29.33 ± 3.55	0.274	<0.001
WC (cm)	80.68 ± 7.96	85.62 ± 6.52	75.75 ± 5.98	<0.001	95.49 ± 9.84	99.41 ± 8.15	90.89 ± 9.7	<0.001	<0.001
FMI	5.12 ± 1.69	3.97 ± 1.16	6.27 ± 1.31	<0.001	8.72 ± 2.79	7.3 ± 2.16	10.39 ± 2.52	<0.001	<0.001
Adiponectin (ng/ml)	11880.63 ± 6838.36	9095.15 ± 4929.39	14666.11 ± 7350.18	<0.001	9115.13 ± 5766.48	6716.49 ± 3777.58	11930.93 ± 6410.41	<0.001	<0.001
Energy (kcal/day)	2416.44 ± 1064.1	2888.13 ± 1155.72	1944.76 ± 700.65	<0.001	2365.08 ± 1012.1	2728.68 ± 1057.45	1938.23 ± 764.28	<0.001	0.621
Protein (g)	91.34 ± 42.91	106.32 ± 46.98	76.37 ± 32.27	<0.001	87.55 ± 43.81	97.85 ± 50.48	75.45 ± 30.44	<0.001	0.382
Fat (g)	105.31 ± 48	119.48 ± 51.92	91.15 ± 39.13	<0.001	100.8 ± 51.56	113.32 ± 56.41	86.1 ± 40.84	<0.001	0.366
Carbohydrate (g)	270.28 ± 142.83	339.26 ± 157.55	201.29 ± 81.06	<0.001	271.34 ± 128.5	322.74 ± 132.03	211 ± 93.78	<0.001	0.938
Fibre (g)	23.49 ± 10.65	26.71 ± 11.66	20.27 ± 8.43	<0.001	24.03 ± 11.59	26.59 ± 12.84	21.04 ± 9.13	0.001	0.627
SFA (g)	31.13 ± 15.25	33.67 ± 16.56	28.58 ± 13.42	0.018	29.66 ± 16.2	31.87 ± 17.87	27.07 ± 13.64	0.036	0.352
MUFA (g)	35.58 ± 17.78	39.88 ± 19.57	31.27 ± 14.68	0.001	35.12 ± 20.23	39.71 ± 22.78	29.72 ± 15.17	<0.001	0.809
PUFA (g)	28.79 ± 17.35	34.88 ± 19.04	22.7 ± 12.95	<0.001	27.02 ± 16.51	31.57 ± 17.9	21.67 ± 12.88	<0.001	0.296
PAL levels n (%)				0.617					
Sedentary	153 (76.5)	78 (78)	75 (75)		165 (82.5)	90 (83.3)	75 (81.5)		
Active	47 (23.5)	22 (22)	25 (25)		35 (17.5)	18 (16.7)	17 (18.5)	0.737	0.137

BMI: Body Mass Index; WC:Waist Circumference; FMI: Fat Mass Index; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; PAL: Physical activity level. Values are presented as mean ± SD and percentages.

*p values obtained from independent t-test and chi square tests comparing continuous and categorical variables between men and women within obese and non-obese categories.

** p values obtained from independent t-test and chi square tests comparing continuous and categorical variables between total obese and total non-obese.

and management of central obesity and CVD in the 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, $P = 3.6 \times 10^{-107}$) (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 was 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 programme, 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 macronutrient intakes and *FTO* variants in determining BMI (Grau et al. 2009; Sonestedt et al. 2009; Corella et al. 2011; Lappalainen et al. 2012; Ortega-Azorin et al. 2012; Phillips et al. 2012). A cross-sectional study conducted on 4839 Swedish participants reported an association between the risk allele of the SNP rs9939609 and higher BMI only in individuals with high fat and low carbohydrate consumption (Sonestedt et al. 2009). A similar interaction between the rs9939609 variant and saturated fatty acids (SFA) intake has been detected in 2163 individuals from two independent populations of the United States, where individuals homozygous for the risk allele "AA" had a higher BMI compared to other genotypes, only when the intake of SFA was high (Corella et al. 2011). Furthermore, the *FTO* SNP rs8050136, in LD with rs9939609, significantly interacted with carbohydrate

Table 2. Genotype frequencies of *FTO* SNPs among cases and controls.

	Non-obese	Obese	Total	*p value
FTO rs9939609 SNP				
Additive *n* (%)				0.217
TT	77 (38.5)	61 (30.5)	138 (34.5)	
AT	99 (49.5)	115 (57.5)	214 (53.5)	
AA	24 (12)	24 (12)	48 (12)	
Dominant *n* (%)				0.092
TT	77 (38.5)	61 (30.5)	138 (34.5)	
AT + AA	123 (61.5)	139 (69.5)	262 (65.5)	
HWE	0.36	0.007	0.011	
MAF	0.37	0.41	0.39	
FTO rs10163409 SNP				
Additive *n* (%)				0.772
AA	85 (42.5)	79 (39.5)	164 (41)	
AT	88 (44)	90 (45)	178 (44.5)	
TT	27 (13.5)	31 (15.5)	58 (14.5)	
Dominant *n* (%)				0.542
AA	85 (42.5)	79 (39.5)	164 (41)	
TA + TT	115 (57.5)	121 (60.5)	236 (59)	
HWE	0.58	0.525	0.392	
MAF	0.36	0.38	0.37	

MAF: Minor Allele Frequency; HWE: Hardy–Weinberg Equilibrium.
*p values obtained from Pearson's chi-squared test comparing genotype frequencies between cases and control.

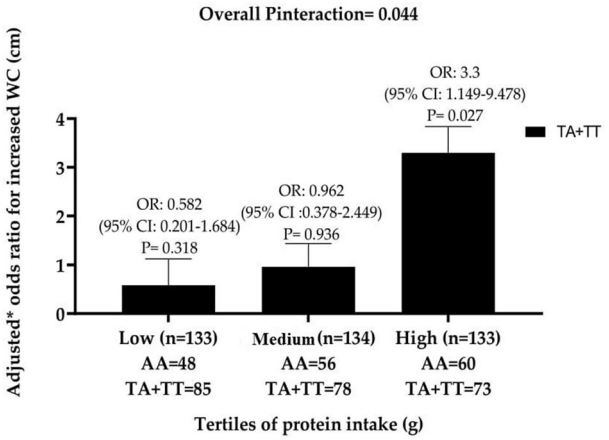

Figure 1. Interactions of the *FTO* rs10163409 with tertiles of protein intake (g) on increased WC. Black bars implicate the "T" allele carriers (TA + TT). *FTO* SNP rs10163409 showed a significant interaction with protein intake (g) on the risk of increased WC ($P_{interaction}$= 0.044). Among those 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. WC: Waist Circumference. *Odds ratio adjusted for age, gender, hypertension, cardiovascular diseases, total energy intake and obesity status.

Table 3. Associations between *FTO* polymorphisms and anthropometric and biochemical parameters of obesity.

	FTO rs9939609			*FTO* rs10163409		
	TT	AT + AA	*p value	AA	TA + TT	*p value
	(*n* = 138)	(*n* = 262)		(*n* = 164)	(*n* = 236)	
BMI (kg/m^2)	24.81 ± 3.65	26.33 ± 4.41	0.001	25.49 ± 4.03	26.02 ± 4.34	0.212
FMI	6.23 ± 2.61	7.29 ± 3.02	0.002	6.72 ± 2.57	7.06 ± 3.15	0.251
WC (cm)	86.08 ± 10.62	89.14 ± 11.99	0.007	87.26 ± 11.47	88.66 ± 11.7	0.234
Adiponectin (ng/ml)	11,306.18 ± 7130.97	10,072.14 ± 6059.63	0.031	10,865.59 ± 6526.36	10,242.35 ± 6427.19	0.377

BMI: Body Mass Index; WC: Waist Circumference; FMI: Fat Mass Index. Values are presented as mean ± SD.
*p values obtained from linear regression analysis adjusted for gender, age, hypertension, cardiovascular diseases and obesity status.

intake on obesity risk among Asian Indian population (Vimaleswaran et al. 2016).

Regarding genetic interactions with physical activity, a previous study conducted among 200 Turkish adults found that BMI was higher in homozygous risk allele "A" carriers of the SNP rs9939609 than the homozygote the "T" allele carriers among physically inactive individuals (Kirac et al. 2016). The same interaction but on a biochemical measure of obesity (i.e., adiponectin level), rather than BMI, was replicated in our study using a larger sample size. We found that, among those with lowest levels of physical activity, the adiponectin concentrations were significantly lower in the carriers of the risk allele "A" of the *FTO* rs9939609 than "TT" homozygotes. Adiponectin is a hormone produced and secreted by adipose tissue and commonly known for its antihyperglycemic, anti-inflammatory, antiatherogenic, and cardioprotective effects (Richard et al. 2020; Esmaili et al. 2020; Lee and Shao 2014). Studies have reported a strong correlation between the dysregulation of adipokine production and the onset of several metabolic abnormalities including CVD and cancer (Avogaro and de Kreutzenberg 2005; De Pergola and Silvestris 2013; Xiang et al. 2019). The positive correlation between adiponectin levels and physical activity has been 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-Bøjsøe et al. 2020). An intervention study conducted in 400 obese women showed that a weight reduction programme 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

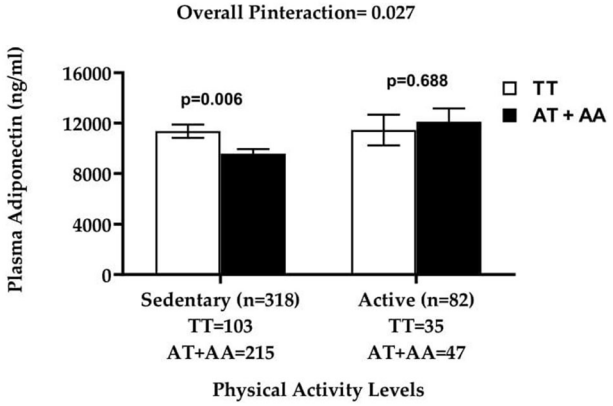

Figure 2. Interactions between *FTO* rs9939609 variant and physical activity on adiponectin levels. White bars indicate carriers of "TT" genotype. Black bars implicate the risk allele, "A", carriers (AT + AA). The regression model was adjusted for age, gender, hypertension, cardiovascular diseases and 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 ($p = 0.006$).

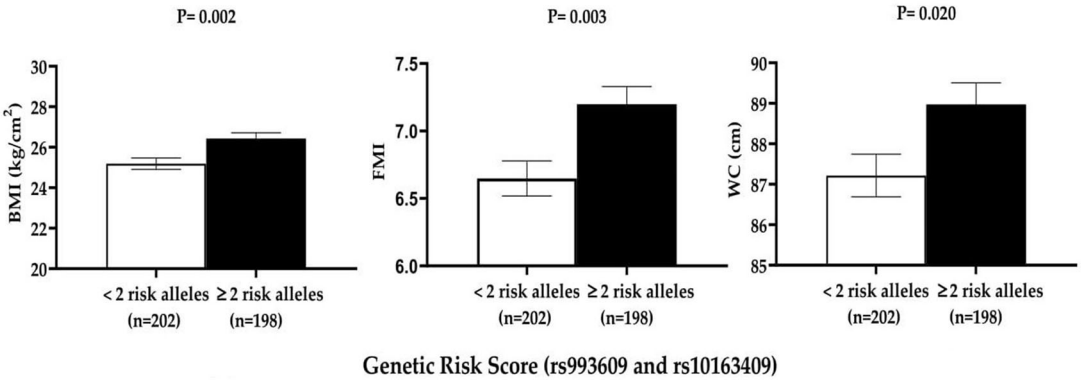

Figure 3. Association between the genetic risk score of the *FTO* SNPs, rs9939609 and rs10163409s and anthropometric measures of obesity. BMI: Body Mass Index; FMI: Fat Mass Index; WC: Waist Circumference. White bars: means of individuals with genetic risk score (GRS) of <2 risk alleles. Black bars: means of individuals with GRS of ≥2 risk alleles. The GRS was significantly associated with BMI (a), FMI (b) and WC (c). (a) Carriers of ≥2 risk alleles of the *FTO* variants (rs9939609 and rs10163409) had higher BMI (P = 0.002) compared to individuals carrying <2 risk alleles. (b) Carriers of ≥2 risk alleles of the *FTO* variants (rs9939609 and rs10163409) had higher FMI (P = 0.003) compared to individuals carrying <2 risk alleles. (c) Carriers of ≥2 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.

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 focussed 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 and 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 of 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. 2018; 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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Disclosure statement

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ORIGINAL ARTICLE

A genetic approach to examine the relationship between vitamin B_{12} status and metabolic traits in a South Asian population

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Abstract

Background Observational studies in South Asian populations have suggested an association between vitamin B_{12} status and metabolic traits; however, the findings have been inconclusive. Hence, the aim of the present study was to use a genetic approach to explore the relationship between metabolic traits and vitamin B_{12} status in a Sri Lankan population and to investigate whether these relationships were modified by dietary intake.

Methods A total of 109 Sinhalese adults (61 men and 48 women aged 25–50 years) from Colombo City underwent anthropometric and biochemical measurements, dietary intake analysis, and genetic tests. Genetic risk scores (GRS) based on 10 metabolic single nucleotide polymorphisms (SNPs) (metabolic-GRS) and 10 vitamin B_{12} SNPs (B12-GRS) were constructed.

Results The B12-GRS was significantly associated with serum vitamin B_{12} ($p = 0.008$) but not with metabolic traits ($p > 0.05$), whereas the metabolic-GRS had no effect on metabolic traits ($p > 0.05$) and vitamin B_{12} concentrations ($p > 0.05$). An interaction was observed between B12-GRS and protein energy intake (%) on waist circumference ($p = 0.002$). Interactions were also seen between the metabolic-GRS and carbohydrate energy intake (%) on waist-to-hip ratio ($p = 0.015$).

Conclusion Our findings suggest that a genetically lowered vitamin B_{12} concentration may have an impact on central obesity in the presence of a dietary influence; however, our study failed to provide evidence for an impact of metabolic-GRS on lowering B_{12} concentrations. Given that our study has a small sample size, further large studies are required to confirm our findings.

Keywords SNP · Body mass index · Obesity · Metabolic traits · Vitamin B_{12} pathway · Sinhalese · Sri Lanka · Nutrigenetics

Abbreviations

SNPs	Single nucleotide polymorphisms
MTHFR	Methylenetetrahydrofolate reductase
CPS1	Carbamoyl-phosphate synthase 1
CUBN	Cubulin
CD320	CD320 molecule

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TCN2	Transcobalamin 2
CLYBL	Citrate lyase beta like
FUT2	Fucosyltransferase 2
TCN1	Transcobalamin 1
FUT6	Fucosyltransferase 6
MUT	Methylmalonyl-CoA mutase
CAP10	Calpain 10
KCNJ11	Potassium voltage-gated channel subfamily J member 11
TCF7L2	Transcription factor 7-like 2
FTO	Fat mass and obesity-associated
MC4R	Melanocortin 4 receptor
BMI	Body mass index
SD	Standard deviations
WC	Waist circumference
WHR	Waist-to-hip ratio

Introduction

In recent years, the incidence of obesity in Sri Lanka has increased markedly [1]. The prevalence of being overweight or obese in Sri Lankan adults is 34.4% (25.2% and 9.2% in 2005 and 2006, respectively), with an upward trend being observed [1, 2]. Obesity increases the risk for certain health conditions, such as insulin resistance, diabetes mellitus, and hypertension [3]. South Asians have been observed to exhibit increased visceral fat and waist circumference (WC), hyperinsulinemia, and insulin resistance; this has been termed the "South Asian phenotype" [4]. Despite a known genetic contribution, the increase in obesity has been largely associated with changes in lifestyle habits [5, 6]. It is imperative that modifiable risk factors for obesity and associated metabolic problems are identified, especially if they can be easily addressed.

Vitamin B_{12} is a micronutrient that has been identified as a modifiable risk factor associated with the progression of metabolic disorders. In humans, vitamin B_{12} acts as an essential coenzyme involved in DNA synthesis and cellular energy production [7]. Subclinical deficiency of vitamin B_{12} has been linked to higher levels of homocysteine; this may have important consequences in the progression of chronic diseases, by inducing oxidative stress and inflammation [8]. Vitamin B_{12} deficiency has also been linked to many other complications including an increased risk of obesity [9–11], diabetes [12–14], and cardiovascular disease [15]. Currently, one study has investigated the effect of genetically instrumented vitamin B_{12} concentrations on body mass index (BMI) in individuals with European ancestry; however, there were no associations between the vitamin B_{12} genetic risk score (GRS) and BMI [16].

Genetic studies have implicated several gene loci in the predisposition to vitamin B_{12} deficiency, but no study has yet been carried out in the Sri Lankan population [17]. The mechanisms by which obesity and its comorbidities are related to vitamin B_{12} deficiency are poorly understood. Hence, we conducted a gene-based approach to explore the relationship between metabolic traits and vitamin B_{12} status in a Sinhalese cohort and investigated whether these relationships were modified by dietary intake in the Genetics Of Obesity and Diabetes (GOOD) study.

Study participants

The GOOD study is a cross-sectional study that was conducted in the city of Colombo, Sri Lanka, between April and August 2017. Healthy adults between the ages of 25 and 50 years were enrolled into the study. Exclusion criteria were having a previous history of type 2 diabetes, cardiovascular disease, or hypertension, having a BMI of more than 40 kg/m^2 or being classed morbidly obese by a physician, being blood related to other participants in the study, having any communicable disease, being pregnant or lactating, taking dietary or vitamin supplements, and taking medications that affect lipid metabolism or hypertension (Fig. 1).

Anthropometric measures

Body weight was measured to the nearest 100 g using an electronic scale (Seca 815, Seca GmbH. Co. kg, Germany) and height was measured to the nearest millimeter using a stadiometer (Seca 217, Seca GmbH. Co. kg, Germany). The BMI calculation was based on the body weight (kg) divided by the square of body height (m). Waist circumference and hip circumference were measured using a metal tape (Lufkin W606PM®, Parsippany, NJ, USA). Body fat percentage was estimated using a handheld bioelectrical impedance analysis technique (Omron Body Fat Monitor BF306, Omron, Milton Keynes, UK).

Biochemical analysis

Blood samples (10 ml) were collected by a trained phlebotomist in the morning, after a 12-h overnight fast. Fasting serum insulin and vitamin B_{12} levels were determined using the chemiluminescent microparticle immunoassay method on an Architect i1000 analyzer (Abbott Laboratories, IL, USA). Fasting plasma glucose concentrations were measured using the glucose hexokinase method using the Beckman Coulter AU5800 analyzer (Beckman Coulter®, California, USA). Glycated hemoglobin (HbA1c) was estimated by high-performance liquid chromatography using the BioRad D10 HPLC analyzer (BioRad, Hercules, CA, USA).

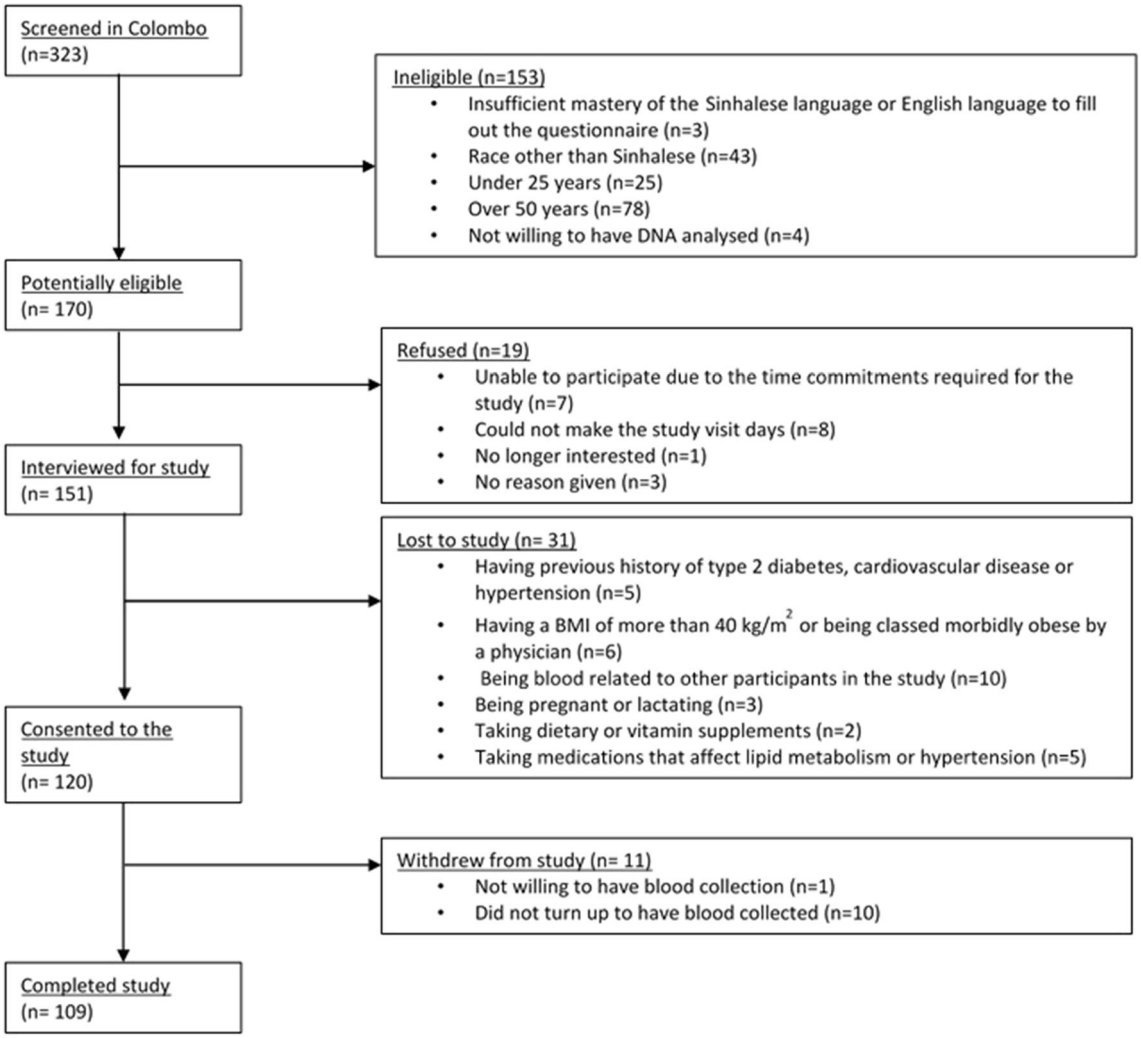

Fig. 1 Flow chart of the subject recruitment process

Dietary intake analysis

Dietary intakes were assessed using a previously validated and published [18] interviewer-administered food frequency questionnaire (FFQ) containing 85 food items. In brief, participants were asked to estimate the usual frequency (number of times per day, week, or month/never) and the portion sizes of various food items. The recorded data was analyzed with the NutriSurvey 2007 database (EBISpro, Germany) to estimate energy as well as macro- and micronutrient consumption [19].

"The Global Physical Activity Questionnaire" (GPAQ), developed by the World Health Organization (WHO), was used to measure physical activity [20]. Individuals were classified as vigorously active, when they both exercised and engaged in demanding work activities, and moderately active, when the participants either exercised or carried out heavy physical work. The remaining study participants were classified into the sedentary group.

SNP selection and genotyping

We selected 10 metabolic disease-related single nucleotide polymorphisms (SNPs) (associated with obesity and diabetes) (fat mass and obesity-associated [*FTO*], rs9939609 and rs8050136; melanocortin 4 receptor [*MC4R*], rs17782313 and rs2229616; transcription factor 7-like 2 [*TCF7L2*], rs12255372 and rs7903146; potassium voltage-gated channel subfamily J member 11 [*KCNJ11*], rs5219; calpain 10 [*CAPN10*], rs3792267, rs2975760, and rs5030952) for our analysis based on previously published candidate gene association and genome-wide association (GWA) studies for metabolic disease-related traits [21–29].

The 10 vitamin B_{12}-related SNPs (methylenetetrahydrofolate reductase [*MTHFR*], rs1801133; carbamoyl-phosphate

synthase 1 [*CPS1*], rs1047891; cubulin [*CUBN*], rs1801222; CD320 molecule [*CD320*], rs2336573; transcobalamin 2 [*TCN2*], rs1131603; citrate lyase beta like [*CLYBL*], rs41281112; fucosyltransferase 2 [*FUT2*], rs602662; transcobalamin 1 [*TCN1*], rs34324219; fucosyltransferase 6 [*FUT6*], rs778805 and methylmalonyl-CoA mutase [*MUT*], rs1141321) were chosen on the basis of the recent review article by Surendran et al. [17].

Blood samples for the measurement of DNA were transported in dry ice to the UK. Genomic DNA was extracted from a 5-ml whole blood sample from each participant and genotyping was performed at LGC Genomics (http://www.lgcgroup.com/services/genotyping), which employs the competitive allele-specific PCR-KASP® assay.

The Hardy-Weinberg equilibrium (HWE) *p* values were computed for the following 20 SNPs. The SNP *FUT2* rs602662 and calpain 10 (*CAP10*) rs3792267 deviated from the HWE; however, these SNPs were not excluded from analysis. The *FUT2* SNP rs602662 previously departed from HWE in a GWA study conducted in India; the authors ruled out that the deviation was not due to a genotyping error and still used this SNP for analysis in their study [30]. In addition, the KASP™ genotyping technology used in our study has been independently assessed to be over 99.8% accurate. Validation of the KASP™ genotyping was conducted at LGC genomics, where the genotyping results were assessed by two project managers separately to confirm that the data was accurate, and this ruled out genotyping artifacts as possible reasons for deviation from HWE. The reasons for deviation from HWE could be due to population or racial grouping substructure (subgrouping), non-random mating, linkage disequilibrium (incomplete mixing of different ancestral population), or chance findings [31].

Statistical analysis

The SPSS statistical package (version 22; SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Allele frequencies were estimated by gene counting (Table 1). The normality of variable distribution was verified by the Shapiro-Wilk test, and data not normally distributed were log transformed prior to analysis. We performed an independent *t* test to compare the means of the quantitative variables between men and women. Comparison of the means between the two groups was analyzed by the chi-square test for categorical outcomes.

A schematic representation of the study design is presented in Fig. 2. The unweighted, risk allele GRS method was calculated for each participant as the sum of risk allele counts across each SNP which predicted vitamin B_{12} status or metabolic disease risk. The B12-GRS was generated from the SNPs in

Table 1 Genotype distribution of vitamin B_{12}-related SNPs and metabolic disease-related SNPs

Gene	rs number	Major allele	Minor allele	Common Homozygotes (%)	Heterozygotes (%)	Rare Homozygotes (%)	Minor allele frequency	HWE *p* value
MTHFR	rs1801133	C	T	89 (81.7)	19 (17.4)	1 (0.9)	0.100	0.990
CPS1	rs1047891	C	A	56 (51.9)	44 (40.7)	8 (7.4)	0.278	0.873
CUBN	rs1801222	C	T	78 (72.2)	29 (26.9)	1 (0.9)	0.144	0.338
CD320	rs2336573	C	T	99 (90.8)	10 (9.2)	0 (0)	0.046	0.616
TCN2	rs1131603	T	C	107 (98.2)	2 (1.8)	0 (0)	0.009	0.923
CLYBL	rs41281112	C	T	105 (96.3)	4 (3.7)	0 (0)	0.018	0.845
FUT2	rs602662	G	A	60 (55.6)	30 (27.8)	18 (16.7)	0.306	**0.000**
TCN1	rs34324219	C	A	107 (98.2)	2 (1.8)	0 (0)	0.009	0.923
FUT6	rs778805	C	T	29 (26.6)	53 (48.6)	27 (24.8)	0.491	0.776
MUT	rs1141321	G	A	28 (25.7)	60 (55.0)	21 (19.3)	0.470	0.271
CAPN10	rs3792267	G	A	79 (72.5)	24 (22.0)	6 (5.5)	0.165	**0.035**
CAPN10	rs2975760	T	C	66 (60.6)	38 (34.9)	5 (4.6)	0.220	0.874
CAPN10	rs5030952	C	T	101 (92.7)	8 (7.3)	0 (0)	0.037	0.691
KCNJ11	rs5219	C	T	49 (45.0)	45 (41.3)	15 (13.8)	0.344	0.373
TCF7L2	rs12255372	G	T	57 (52.3)	45 (41.3)	7 (6.4)	0.271	0.633
TCF7L2	rs7903146	C	T	45 (41.3)	54 (49.5)	10 (9.2)	0.340	0.274
FTO	rs9939609	T	A	48 (44.0)	47 (43.1)	14 (12.8)	0.344	0.641
MCR	rs17782313	T	C	48 (44.0)	50 (45.9)	11 (10.1)	0.330	0.700
FTO	rs8050136	C	A	48 (44.0)	47 (43.1)	14 (12.8)	0.340	0.641
MC4R	rs2229616	G	A	99 (91.7)	9 (8.3)	0 (0)	0.042	0.651

MAF minor allele frequency, *HWE* Hardy-Weinberg equilibrium, χ^2 chi-squared value

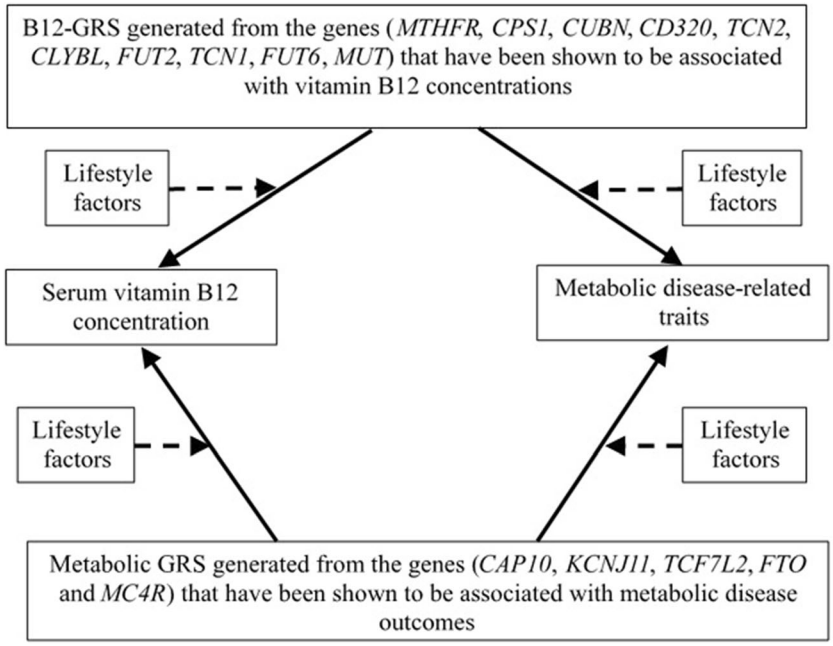

Fig. 2 Diagram representing the study design. The diagram shows four possible associations and four possible interactions. One-sided arrows with unbroken lines represent genetic associations and one-sided arrows with broken lines represent interactions between a lifestyle factor and GRS on serum vitamin B_{12}/metabolic traits. We tested the association between the metabolic-GRS and vitamin B_{12} concentrations and metabolic disease-related traits. We then tested the associations between the B12-GRS and vitamin B_{12} status and metabolic disease-related traits. Lastly, we tested whether these genetic associations were modified by lifestyle factors (macronutrient intake and physical activity levels)

the genes *MTHFR*, *CPS1*, *CUBN*, *CD320*, *TCN2*, *CLYBL*, *FUT2*, *TCN1*, *FUT6*, and *MUT*, which have been shown to be associated with vitamin B_{12} concentrations. Furthermore, another unweighted GRS was created using allele markers previously reported to be associated with metabolic disease traits. The metabolic-GRS was generated from the SNPs in the genes *CAP10*, *KCNJ11*, *TCF7L2*, *FTO*, and *MC4R*. A value of 0.1 or 2 was assigned to each SNP, which denotes the number of risk alleles on that SNP. These values were then calculated by adding the number of risk alleles across each SNP. The average number of risk alleles per person for the B12-GRS was 8.69 (SD = 1.70), which ranged from 5 to 15. The sample was stratified, by the median, into a "low genetic risk group," for those with a GRS ≤ 9 risk alleles ($n = 79$), and into a "high genetic risk group," for those with a GRS ≥ 10 risk alleles ($n = 30$). For the metabolic-GRS, the average number of risk alleles per person was 7.00 (SD = 2.28), which ranged from 1 to 13. The sample was stratified, into a "low genetic risk group," for those with a GRS ≤ 8 risk alleles ($n = 88$), and into a "high genetic risk group," for those with a GRS ≥ 9 risk alleles ($n = 21$). Linear regression was used to examine the association of the two GRS scores with the biochemical and anthropometric outcomes (glucose, insulin, HbAC1, vitamin B_{12}, body fat %, BMI, WC, and waist-to-hip ratio (WHR)). The interaction between the two GRS scores and dietary factors on biochemical and anthropometric outcomes was determined by including interaction terms (GRS × diet) in the regression model. Models were adjusted for age, sex, BMI, and total energy intake, wherever appropriate.

Correction for multiple testing was applied using Bonferroni correction [adjustment p value for association analysis was < 0.00313 [2 GRS × 8 biochemical and anthropometric outcomes (Fasting blood glucose, fasting insulin, glycated hemoglobin, vitamin B_{12}, fat %, BMI, WC, and WHR) = 16 test)] and for interaction < 0.00078 [2 GRS × 8 biochemical and anthropometric × 4 lifestyle factors (dietary carbohydrate energy %, dietary protein energy %, dietary fat energy %, and physical activity levels)) = 64]. Given that there are no studies on GRS and no previously reported effect sizes for the South Asians, we were unable to perform a power calculation.

Results

Characteristics of the participants

In this study, 109 participants (mean age, 38.34 ± 6.92 years; BMI, 24.58 ± 4.12 kg/m^2) were included. Table 2 illustrates the main characteristics of the study participants stratified according to sex. No significant difference between men and women was observed in the levels of fasting glucose, insulin, HbAC1, and plasma vitamin B_{12} ($p > 0.05$).

Table 2 Anthropometric and biochemical characteristics of men and women participants ($n = 109$; men 61, women 48)

	Total (n = 109) Mean ± SD	Men ($n = 61$) Mean ± SD	Women ($n = 48$) Mean ± SD	p value*
Age (years)	38.24 ± 6.92	37.34 ± 6.97	39.38 ± 6.77	0.129
Height (cm)	164.97 ± 9.15	170.95 ± 6.18	157.36 ± 6.16	< 0.0001
Weight (kg)	67.07 ± 13.05	71.76 ± 11.81	61.11 ± 12.17	< 0.0001
BMI (kg/m^2)	24.58 ± 4.12	24.51 ± 3.52	24.68 ± 4.80	0.844
Waist circumference (cm)	83.73 ± 17.97	89.83 ± 14.04	75.99 ± 19.52	< 0.0001
Hip circumference (cm)	91.16 ± 17.78	92.27 ± 13.83	89.75 ± 21.87	0.488
WHR	0.92 ± 0.11	0.98 ± 0.08	0.85 ± 0.11	< 0.0001
Fat (%)	27.25 ± 7.37	23.52 ± 5.12	32.00 ± 7.08	< 0.0001
Obesity cases[a]	40.37%	37.70%	43.75%	0.523
Fasting blood glucose (mg/dL)	85.64 ± 12.64	87.41 ± 15.41	83.40 ± 7.40	0.100
Fasting blood insulin (pmol/L)	68.55 ± 49.97	71.77 ± 59.12	64.46 ± 35.28	0.451
Fasting blood HbA1C (mmol/mol)	35.62 ± 5.91	35.20 ± 5.99	36.16 ± 5.84	0.402
Fasting blood B12 (pmol/L)	380.65 ± 132.83	389.80 ± 135.00	369.02 ± 130.52	0.420
Physical activity levels (low %/moderate%/high%)	72.5/19.3/8.3	70.5/19.7/9.8	75.0/18.8/6.3	0.777
Total energy (kcal/day)	2097.92 ± 456.01	2173.68 ± 427.82	2001.65 ± 476.72	0.050
Protein (energy %)	11.29 ± 2.31	11.25 ± 2.41	11.33 ± 2.20	0.853
Fat (energy %)	21.87 ± 5.31	21.64 ± 5.22	22.16 ± 5.45	0.613
Carbohydrate (energy %)	69.62 ± 8.80	69.89 ± 10.29	69.28 ± 6.52	0.721
Dietary fiber (g)	16.78 ± 8.18	17.24 ± 8.46	16.20 ± 7.85	0.513
Polyunsaturated fatty acids (g)	3.32 ± 1.69	3.36 ± 1.66	3.27 ± 1.75	0.779

Data presented as mean ± SD

BMI body mass index, *SD* standard deviations, *WHR* waist-to-hip ratio

*$p < 0.05$, statistically significant differences in mean values between men/women, unadjusted

[b] Obesity cases refer to the percentage of individuals with a BMI of over 25

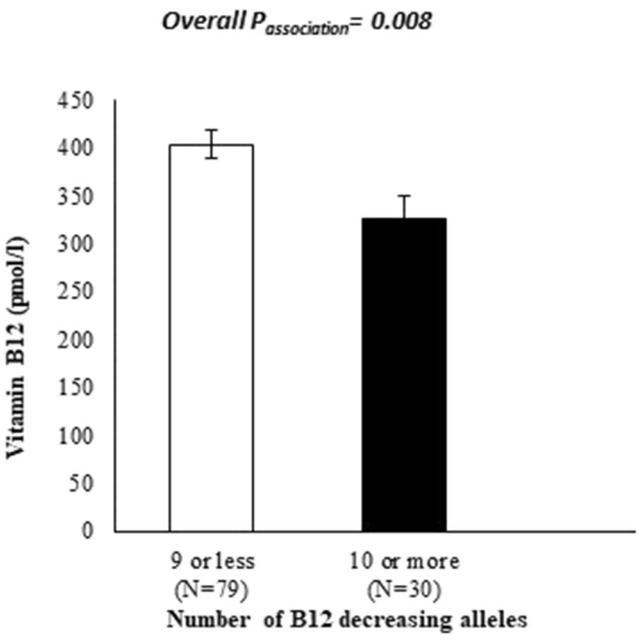

Fig. 3 Association between the B12-GRS and serum vitamin B_{12} levels. Vitamin B_{12} decreasing alleles ranged from 5 to 15. Individuals with ≤ 9 or ≥ 10 alleles were grouped to obtain a reasonable number of individuals in each group

Association between B12-GRS and obesity GRS with biochemical and anthropometric measurements

A significant association between B12-GRS and serum vitamin B_{12} was observed ($p = 0.008$) (Supplementary Table 1 and Fig. 3). However, this finding was not significant after correction for multiple testing. No associations between the B12-GRS and metabolic traits ($p > 0.05$) were observed (Supplementary Table 1). Furthermore, no associations between the metabolic-GRS and vitamin B_{12} or metabolic traits ($p > 0.05$) were observed (Supplementary Table 2).

Interaction between the B12-GRS and dietary factors on biochemical and anthropometric measurements

An interaction was found between the B12-GRS and protein energy (%) on log transformed WC ($p = 0.002$). However, further stratification of participants based on their consumption of low, medium, and high dietary protein (energy %) did not show statistically significant associations between the GRS and the outcome in any of the tertiles, which could account for the small sample size (Supplementary Table 3).

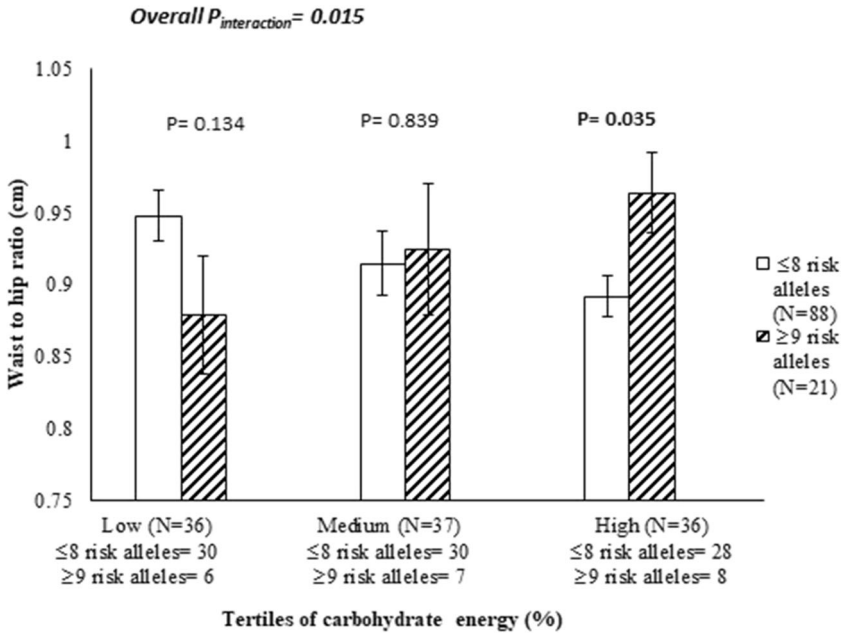

Fig. 4 Interaction between the metabolic-GRS and carbohydrate energy intake (%) on waist-to-hip ratio (cm) ($P_{interaction} = 0.015$). Among those who consumed a high carbohydrate diet, individuals who carried nine or more risk alleles had significantly higher levels of waist-to-hip ratios compared to individuals carrying eight or less risk alleles ($p = 0.035$)

Interaction between the metabolic-GRS and dietary factors on biochemical and anthropometric measurements

We observed a significant interaction between the metabolic-GRS and carbohydrate energy intake (%) on waist-to-hip ratio ($P_{interaction} = 0.015$) (Fig. 4 and Table 3). Individuals who carried eight or less risk alleles for metabolic disease had 7.47% lower WHR measurements (cm) in the highest tertile of carbohydrate energy intake (%) (mean ± SD = 78.00 ± 7.90%) compared to those with nine or more risk alleles ($p = 0.035$) (Table 3).

Interactions were also seen between the metabolic-GRS and carbohydrate energy (%) on log fasting insulin concentrations ($p = 0.011$) and log WC ($p = 0.031$) and the metabolic-GRS and protein energy (%) on log fasting insulin levels ($p = 0.032$) and log WC ($p = 0.011$) (Table 3 and Supplementary Table 3).

Interaction between the B12-GRS and physical activity on biochemical and anthropometric measurements

No statistically significant interactions were observed between the two GRSs (vitamin B_{12} and metabolic) and physical activity on biochemical and anthropometric measurements (Table 3 and Supplementary Table 3). After correction for multiple testing, none of these gene-diet and gene-physical activity interactions remained statistically significant.

Discussion

To our knowledge, this is the first study to use a genetic approach to explore the relationship between metabolic traits and vitamin B_{12} status in a South Asian population. Our study confirmed the strength of the association between B12-GRS and B_{12} concentrations and demonstrated the impact of genetically instrumented B_{12} concentrations on waist circumference, an indicator of central obesity, through the influence of dietary protein intake. Furthermore, our study has also showed a significant effect of metabolic-GRS on waist-to-hip ratio through the influence of high carbohydrate intake. Given that the total daily intake of protein is low and carbohydrate is high in Sri Lankan adults [32], our findings, if replicated in future studies, might carry significant public health implications in terms of revising the food-based dietary guidelines which could prevent central obesity and the associated CVD-related outcomes.

In this study, we constructed a GRS consisting of ten vitamin B_{12} decreasing SNPs in genes involved in vitamin B_{12} metabolism [17]. The B12-GRS was associated with vitamin B_{12} levels, suggesting that it would be an ideal instrument for vitamin B_{12} status. Given the lack of association between the B12-GRS and metabolic disease traits in our study, we were unable to provide evidence for linear decreases in vitamin B_{12} concentrations having substantive effects on metabolic disease traits. However, we found a significant interaction between the B12-GRS and protein energy (%) on log WC. Interestingly, individuals who carried nine or less alleles had lower WC when consuming a high protein diet compared to those consuming a low protein diet. Although no statistically significant differences in WC were observed between the

Table 3 Interaction between the B12-GRS and lifestyle factors on anthropometric measurements

Interaction between the GRS and lifestyle factors on log waist circumference (cm)			
Interaction between B12-GRS and fat energy %	Interaction between B12-GRS and protein energy %	Interaction between B12-GRS and carbohydrate energy %	Interaction between B12-GRS and physical activity levels
0.002 ± 0.004 (0.727)	0.037 ± 0.011 **(0.002)**	− 0.003 ± 0.003 (0.344)	− 0.051 + 0.037 (0.173)
Interaction between metabolic-GRS and fat energy %	Interaction between metabolic-GRS and protein energy %	Interaction between metabolic-GRS and carbohydrate energy %	Interaction between metabolic-GRS and physical activity levels
− 0.007 ± 0.006 (0.212)	− 0.024 ± 0.009 **(0.011)**	0.007 ± 0.003 **(0.031)**	0.020 ± 0.044 (0.654)
Interaction between the GRS and dietary factors on waist-to-hip ratio			
Interaction between B12-GRS and fat energy %	Interaction between B12-GRS and protein energy %	Interaction between B12-GRS and carbohydrate energy %	Interaction between-B12 GRS and physical activity levels
0.002 ± 0.004 (0.660)	0.013 ± 0.010 (0.196)	− 0.003 ± 0.002 (0.241)	0.018 ± 0.032 (0.584)
Interaction between metabolic-GRS and fat energy %	Interaction between metabolic-GRS and protein energy %	Interaction between metabolic-GRS and carbohydrate energy %	Interaction between metabolic-GRS and physical activity levels
− 0.009 ± 0.005 (0.079)	− 0.012 ± 0.008 (0.158)	0.007 ± 0.003 **(0.015)**	0.038 ± 0.039 (0.323)
Interaction between the GRS and lifestyle factors on log BMI			
Interaction between B12-GRS and fat energy %	Interaction between B12-GRS and protein energy %	Interaction between B12-GRS and carbohydrate energy %	Interaction between B12-GRS and physical activity levels
− 0.002 ± 0.003 (0.539[a])	0.009 ± 0.008 (0.259[a])	− 0.001 ± 0.002 (0.762[a])	0.015 ± 0.023 (0.513[a])
Interaction between metabolic-GRS and fat energy %	Interaction between metabolic-GRS and protein energy %	Interaction between metabolic-GRS and carbohydrate energy %	Interaction between metabolic-GRS and physical activity levels
− 0.004 ± 0.004 (0.245[a])	− 0.004 ± 0.006 (0.480[a])	0.002 ± 0.002 (0.322[a])	− 0.005 ± 0.028 (0.851[a])

Values are beta coefficients ± standard errors. *P* values are inserted in brackets. *P* values were obtained by using a general linear model adjusted for age, sex and BMI

Values in bold represent statistical significance ($p < 0.05$)

[a] *P* values were obtained by using a general linear model adjusted for age and sex

alleles of the B12-GRS, the impact of the B12-GRS on WC was observed only under the influence of a high protein diet. Further investigations are required to confirm this finding to determine the clinical significance and potential applications as part of weight management interventions.

At present, carbohydrates constitute the majority of the energy intake among South Asian countries such as Sri Lanka (~71.2%) [32]; in contrast, the consumption of carbohydrates is lower in Western countries (~45%) [33]. Furthermore, high carbohydrate intake has been associated with an increased risk of diabetes in a South Indian population [34] and an increase in WC among premenopausal (20–45 years) Sri Lankan women [35]. In the present study, we found a significant interaction between the metabolic-GRS and carbohydrate energy percentage on waist-to-hip ratio, where the individuals carrying more than nine risk alleles had a higher waist-to-hip ratio among those in the highest tertile of carbohydrate energy percentage. There are no previous reports of the risk variants used in our GRS, but Goni et al. [36] found that carbohydrates (total and complex) interacted with a GRS of 16 obesity/lipid metabolism polymorphisms to modify the effect on body fat mass in 711 individuals of Caucasian ancestry. In our study, we only observed interactions of the metabolic-GRS on WC and waist-to-hip ratio, which suggests that effects are likely to be on central obesity as opposed to common obesity.

South Asians have a higher risk of developing obesity-related non-communicable diseases relative to white Caucasians despite lower BMI levels; this has been termed the "South Asian phenotype." The distinctive features of this phenotype include a higher WC, abdominal adiposity combined with insulin resistance, and a greater predisposition to diabetes [4]. The role of vitamin B_{12} in promoting this adverse phenotype has been suggested by Yajnik et al., who demonstrated that offspring born to mothers with a low vitamin B_{12} and high folate status had a greater risk of developing insulin resistance during childhood [12]. According to Yajnik et al., vitamin B_{12} deficiency prevents the generation of tetrahydrofolate from 5-methyltetrahydrofolate in the one-carbon metabolism cycle; as a result, homocysteine levels accumulate leading to altered lean tissue deposition and reduced protein synthesis [12]. Furthermore, vitamin B_{12} is involved in the conversion of methylmalonyl-CoA to succinyl-CoA by the enzyme methylmalonyl-CoA mutase (adenosyl-B12 as a cofactor). Subsequently, vitamin B_{12} deficiency results in elevated methylmalonyl-CoA, inhibiting the mitochondrial enzyme carnitine palmitoyltransferase, which may promote lipogenesis and insulin resistance [12, 37].

No studies to date have investigated interactions between the two GRSs and physical activity on metabolic traits and B_{12} concentrations in Asian Sri Lankans. Although 60% of Sri Lankan adults are reported to be highly physically active [38], no significant interactions were found between the two GRSs and physical activity on metabolic traits, which could be due to a small sample size and measurement bias associated with self-reported physical activity questionnaire. The strengths of our study include the use of a validated food frequency questionnaire [18] to measure macronutrient intake, the comprehensive measurements of lifestyle factors, and the use of GRSs which increased the statistical power of our study [39]. Nevertheless, some limitations need to be acknowledged. The first limitation concerns the relatively small sample size of the study; however, we were still able to identify significant gene-diet interactions. Furthermore, we used Bonferroni correction to correct for multiple testing and this can often lead to larger power, specifically where studies have a small sample size and a small number of disease-associated markers. This is also true for when studies have a large allele frequency difference due to a small sample size [40]. Secondly, information about the type of oil used for frying, the estimation of different dietary fat components (monounsaturated or saturated fatty acids), and vitamin B_{12} intake was not collected. This could have limited our in-depth analysis of interactions of specific macronutrients and vitamins with the two GRSs. Furthermore, the study was limited to Sinhalese adults in Colombo, and the conclusions may not be applicable to other ethnic groups in Sri Lanka. Finally, none of the genetic associations or gene-lifestyle interactions were statistically significant after correction for multiple testing; however, given that this is the first study using a genetic approach to establish a relationship between vitamin B_{12} status and metabolic disease outcomes in South Asians, we have taken into consideration the significant findings; hence, further large studies are required to replicate our findings.

In summary, our study suggests that a genetically lowered vitamin B_{12} concentration may have an impact on central obesity in the presence of a dietary influence; however, our study failed to show an impact of the metabolic-GRS on lowering B_{12} concentrations through a dietary influence. Our study also showed a significant effect of the metabolic-GRS on waist-to-hip ratio, another indicator of central obesity, through the influence of a high carbohydrate intake. However, after correction for multiple testing, none of these findings were statistically significant. Hence, further replication studies are highly warranted on large samples to confirm or refute our findings.

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Author contributions SS and KSV drafted the manuscript; SS performed the statistical analysis; and SS, VKS, DJA, and RL were responsible for the study conception. KW provided guidance to the research; SS and RL conducted data and sample collection; SS, RJ, and SA were involved in the dietary data analysis; SS and SaS were involved in the physical activity data analysis; KSV designed the gene-diet interaction study; and SS, VKS, JAL, and RJ critically reviewed the manuscript. All authors contributed to and approved the final version of the manuscript.

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Data availability Data from this project will not be shared because additional results from the study are yet to be published.

Compliance with ethical standards

Ethics approval and consent to participate This study was approved by the Ethical Review Committee of the University of Colombo (EC-17-107) and the University of Reading Research Ethics Committee (17/25). All participants signed informed consent prior to their participation.

Consent for publication Not applicable.

Competing interests All authors declare that there is no conflict of interest associated with their contribution to this manuscript.

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