A Nutrigenetic approach to investigate the relationship between vitamin D status and metabolic traits in multiethnic 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

Low vitamin D concentration has been shown to be a risk factor for metabolic traits in several observational studies; however, the findings have been inconsistent. Certain genotypes could potentially contribute jointly to obesity and vitamin D deficiency, and these may be modulated by lifestyle factors (dietary factors and physical activity levels) across different ethnic groups. The implementation of a genetic approach to establish the relationship between vitamin D and metabolic diseases could be a more desirable option over observational studies, as results are less prone to confounding factors. Hence, the main aims of this thesis were to examine the association of common vitamin D-related single nucleotide polymorphisms (SNPs) and metabolic SNPs, as genetic risk scores (GRSs), with vitamin D concentrations and metabolic outcomes in multiple ethnic groups. In addition, the interaction between these GRSs and dietary factors (protein, fat, carbohydrate, and fibre) on vitamin D concentrations and metabolic traits was investigated. A total of five different studies were used. These studies included two case-control studies [the Chennai Urban Rural Study (CURES; Asian Indian, n = 545) and a study in Turkish adults (n = 396)] and three cross-sectional cohort studies [the Minangkabau Indonesia Study on Nutrition and Genetics (MINANG study; Indonesian women; n = 110), the Obesity, Lifestyle and Diabetes in Brazil (BOLD study; Brazilian young adults; n = 187), and the Genetics of Obesity and Nutrition in Ghana (GONG study; Ghanaian adults; n = 302)]. Gene-diet interactions were observed in the Indonesian and Ghanaian populations between vitamin D related-GRS and dietary intake on obesity markers, carbohydrate intake (g) on body fat percentage ($p_{interaction} = 0.049$) and fibre intake (g) on body mass index ($p_{interaction} = 0.020$), respectively. Additionally, in the Ghanaian population, an interaction was found between vitamin D-related GRS and dietary fat intake (g) on glycated haemoglobin levels ($p_{interaction} = 0.029$), a marker of long-term glycaemic status. In the Brazilian population, the vitamin D related-GRS showed a significant interaction with protein intake (g)

on 25(OH)D levels ($p_{interaction}$ = 0.006). Furthermore, we observed novel interactions in metabolic disease related-GRSs on vitamin D concentrations with carbohydrate energy intake (%) in the Indian population ($p_{interaction}$ = 0.047), and with fat energy intake (%) in the Turkish populations ($p_{interaction}$ = 0.040). An additional interaction was observed in the Indian population with the metabolic disease related-GRS and fat energy intake (%) on low-density-lipoprotein cholesterol ($p_{interaction}$ = 0.032), a marker of cardiovascular disease. In summary, these studies in multiple ethnic groups show that the relationship between vitamin D status and metabolic outcomes may be influenced by dietary factors such as protein, fat, carbohydrate, and fibre intake. Replication of these findings in larger cohorts and in dietary intervention studies is warranted before these findings could be considered for personalised dietary recommendations.

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LIST OF PUBLICATIONS FROM MY PHD WORK

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ABBREVIATIONS

$1,25(OH)_2D$	1,25 Dihydroxyvitamin D
1958BC	1958 British Birth Cohort
$24,25(OH)_2D$	24,25-Dihydroxyvitamin D
25(OH)D	25 Hydroxyvitamin D
AH	Abdominal Height
BFP	Body Fat Percentage
BG	Blood Glucose
BM	Body Mass
BMI	Body Mass Index
BOLD	Obesity, Lifestyle, and Diabetes in Brazil
CAPN10	Calpain 10
CASR	Calcium Sensing Receptor
CDKN2A/B	Cyclin Dependent Kinase Inhibitor 2A/B
CHOD-PAP	Cholesterol Oxidase Phenol 4-Aminoantipyrine Peroxidase
CURES	Chennai Urban Rural Epidemiological Study
CYP24A1	Cytochrome P450 Family 24 Subfamily A Member 1
CYP27B1	Cytochrome P450 Family 27 Subfamily B Member 1
CYP2R1	Cytochrome P450 Family 2 Subfamily R Member 1
CYP3A4	Cytochrome P450 Family 3 Subfamily A Member 4
DAD	Diode Array Detection
DBP	Diastolic Blood Pressure
DBP	Vitamin D Binding Protein
dbSNP	Single Nucleotide Polymorphism Database
DEQAS	Vitamin D External Quality Assessment Scheme
DHCR7	7-Dehydrocholesterol Reductase
DHSC	Department of Health and Social Care
DXA	Dual Energy Radiological Absorptiometry Scan
ECLIA	Electrochemiluminescence Immunoassay
EDTA	EthyleneDiamine TetraAcetic Acid
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
FFQ	Food Frequency Questionnaire
FM	Fat Mass
FMI	Fat Mass Index
FPG	Fasting Plasma Glucose
FSG	Fasting Serum Glucose
FTO	Fat Mass and Obesity-Associated Gene
GC	Group-Specific Component
GeNuIne	Gene–Nutrient Interactions
GIANT	Genetic Investigation of Anthropometric Traits
GLM	General Linear Model
GONG	Genetics of Obesity and Nutrition In Ghana
GPAQ	Global Physical Activity Questionnaire
GPO-PAP	Glycerine Phosphate Oxidase Peroxidase
GRS	Genetic Risk Score
GWAS	Genome Wide Association Studies
HbA1c	Glycated Haemoglobin
HDL-c	High Density Lipoprotein Cholesterol

HES	Household Expenditure Surveys
HGP	Human Genome Project
HPLC	High Pressure Liquid Chromatography
HWE	Hardy-Weinberg Equilibrium
ID-LC	Isotope Dilution-Liquid Chromatography
IGF-I	Insulin-Like Growth Factor-I
IOM	Institute of Medicine
IPAQ	International Physical Activity Questionnaire
ISI	Insulin Secretion Index
IU	International Unite
KCNQ1	Potassium Voltage-Gated Channel Subfamily Q Member 1
LD	Linkage Disequilibrium
LDL-c	Low Density Lipoprotein Cholesterol
LMICs	Low and Middle Income Countries
MAF	Minor Allele Frequency
MC4R	Melanocortin 4 Receptor Gene
MET	Metabolic Equivalents of Task
MINANG	Minangkabau Indonesia Study on Nutrition and Genetics
MR	Mendelian Randomization
MS	Mass Spectrometry
MUFA	Monounsaturated Fatty Acid
mVDR	Membrane Vitamin D Receptor
NCD	Non-Communicable Diseases
NGT	Normal Glucose Tolerance
NIST	National Institute of Standards and Technology
nVDR	Nuclear Vitamin D Receptor
PBF	Percent Body Fat
PFM	Percentage Fat Mass
PPARG	Peroxisome Proliferator Activated Receptor Gamma
PTF	Percent Total Fat
PTH	Parathyroid Hormone
PUFA	Polyunsaturated Fatty Acid
PVD	Percent Visceral Deposit
RCT	Randomised Control Trials
RDA	Recommended Dietary Allowance
RNI	Recommended Nutrient Intake
RR	Rapid Response
RXR	Retinoid X Receptor
SACN	Scientific Advisory Committee on Nutrition
SBP	Systolic Blood Pressure
SD	Standard Deviation
SFA	Saturated Fatty Acid
SNP	Single Nucleotide Polymorphism
SPF	Sun Protection Factor
SPSS	Statistical Package for the Social Sciences
SSA	Sub-Saharan African
SSFT	Sum of Skin Fold Thickness
SZA	Solar Zenith Angle
T2D	Type 2 Diabetes
TBF	Total Body Fat

TCF7L2	Transcription Factor 7-Like 2 Gene
TSFT	Triceps Skinfold Thickness
US ES	US Endocrine Society
UV	Ultraviolet
UVB	Ultraviolet B
VDD	Vitamin D Deficiency
VDDR1A	Vitamin D Dependent Rickets Type 1A
VDDR1B	Vitamin D Dependent Rickets Type 1B
VDDR2A	Vitamin D-Dependent Rickets Type 2A
VDDR2B	Vitamin D-Dependent Rickets Type 2B
VDDR3	Vitamin D Dependent Rickets Type 3
VDR	Vitamin D Receptor
VDRE	Vitamin D Receptor Elements
VDSP	Vitamin D Standardization Program
VF	Visceral Fat
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist Hip Ratio

Chapter 1: Introduction to the Thesis

1.1 Introduction

Vitamin D is a fat-soluble secosteroid prohormone and an essential micronutrient [1]. It is a unique vitamin because in addition to being obtained from the diet, it can be synthesised in the skin during exposure to sunlight and its active metabolite functions as a hormone within the body [2]. There are two main forms of vitamin D, D_3 and D_2, which get activated in the same metabolic pathway to perform the hormonal functions of this vitamin [3]. 1α,25-dihydroxyvitamin D ($1,25(OH)_2D$) which is the active form of vitamin D, plays an important role in bone mineralization by promoting absorption of calcium and phosphorus and by performing an endocrinological role to control and maintain calcium homeostasis [4]. Additionally, vitamin D has been shown to be involved in various nonskeletal functions [5]. Symptoms of rickets, the severe vitamin D deficiency disease in children, was first described in the late 19th century and later recognised to be associated with lack of sunshine exposure in the early 20th century [6]. In adults, vitamin D deficiency can cause osteomalacia and osteoporosis [7]. Vitamin D deficiency is widespread worldwide and a number of biological and lifestyle factors can influence vitamin D status in humans [8].

Chronic metabolic diseases are prevalent world-wide, and constitute a burden on the health care system of most countries [9, 10]. Numerous epidemiological studies have linked low vitamin D status with a wide range of metabolic diseases such as obesity and type 2 diabetes (T2D) [11-14]. The metabolic diseases are multifactorial diseases with several factors contributing to their development [15]. Obesity and T2D are mostly the result of an interplay between lifestyle factors (diet and physical activity) and genetic factors [16]. The effects of interactions between genetic risk and lifestyle factors have differing effects across diverse populations [17].

The nutrigenetics approach is a novel way to investigate the effects of lifestyle factors and genetic susceptibility on certain diseases [18]. Examining the interactive effects between genetic factors and dietary consumption may lead to personalised dietary recommendations to prevent metabolic diseases in specific ethnic groups based on vitamin D genetic susceptibility.

This chapter will (i) give an overview of vitamin D (ii) explain the nutritional facets of vitamin D (iii) describe aspects of vitamin D deficiency (iv) explain the need for a nutrigenetics approach to study the role of genes and diet on vitamin D deficiency and metabolic traits.

1.2 Synthesis and Metabolism of Vitamin D

The human skin can synthesise the required amount of vitamin D for normal body functions through sufficient exposure to sunlight and endogenously synthesise cholecalciferol (vitamin D_3). Natural food source also provides a small amount of vitamin D as vitamin D_3 or ergocalciferol (vitamin D_2) [3]. Structures of the different vitamin D forms and their precursors have been illustrated in **Figure 1.1**.

In the skin, a precursor for vitamin D_3, 7-dehydrocholesterol (7DHC) absorbs UVB radiation from sunlight, at 290-320 nanometres wavelength, and the absorbed energy causes the cleavage of the 9,10 carbon-carbon bond at the B ring of 7DHC, resulting in the formation of previtamin D_3 in the skin which subsequently undergoes a thermally induced isomerization to form vitamin D_3 [19]. Serum concentrations of vitamin D_3 peak at 24 to 48 hours after UVB irradiation and then their levels decline with a serum half-life ranging from 36 to 78 hours. As a fat-soluble molecule, vitamin D_3 can be taken up by adipocytes and stored in adipose tissue which prolongs its total-body half-life to approximately two months [20]. In situations of prolonged sunlight exposure, previtamin D_3 and vitamin D_3 continue to absorb UV radiation at different wavelengths and become degraded into several inactive photoproducts, thus reaching a steady state, and preventing vitamin D intoxication [21, 22].

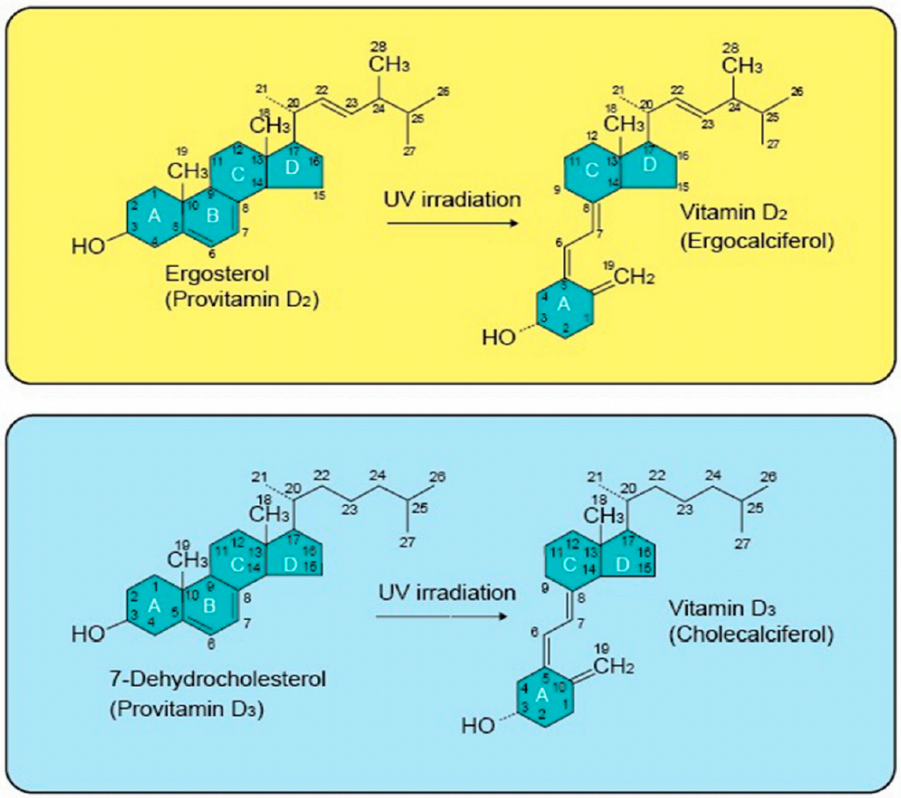


Figure 1. 1: The structure of Vitamin D forms and their precursors.

Adapted From: Sirajudeen et al, 2019 [23].

In circulation, both forms of vitamin D (D_3 and D_2) are transported by serum glycoproteins secreted by the liver, known as vitamin D binding protein (DBP) or group-specific component (GC) [24], and subsequently vitamin D undergoes a two-step hydroxylation process to get activated. The first step occurs in the liver where vitamin D is hydroxylated, by addition of a hydroxyl group on carbon 25, through the action of the enzyme 25-hydroxalase, and converted into 25-hydroxyvitamin D (25(OH)D), also known as calcidiol. The metabolite 25(OH)D is the main circulating form of vitamin D in the serum and is the form that is clinically measured to determine vitamin D status in the body, yet it is still a biologically inactive metabolite and has very minimal activity [5, 20].

The second step of the hydroxylation process takes place in the kidney, which acts as an endocrine organ and uptakes 25(OH)D into the renal proximal tubular cells. Subsequently, through the action of the enzyme 1α-hydroxylase, serum 25(OH)D is catalysed into $1,25(OH)_2D$, also known as calcitriol, which is the biologically active hormonal form of vitamin D. Similar to other hormones, the levels of calcitriol in the circulation is tightly controlled by a number of factors, such as the serum phosphorus levels and parathyroid hormone (PTH) concentrations [20]. The activity of 1α-hydroxylase is also found in extrarenal tissues, suggesting other sites with local activation of vitamin D and implying possible local modulation of vitamin D in other body tissues [25].

The role of DBP is largely to transport vitamin D and about 85% of vitamin D metabolites exist in the circulation primarily bound to DBP, including 25(OH)D and $1,25(OH)_2D$. However, some small amounts do exist unbound in the circulation [5, 26]. In the cell, activated $1,25(OH)_2D$ binds to the nuclear vitamin D receptor (VDR) in the nucleus or in cellular brush border. The ligand $1,25(OH)_2D$ bound to VDR binds to vitamin D response elements (VDREs) that can stimulate or inhibit the effects of vitamin D in tissues. This interaction is enhanced by heterodimerizing with retinoic X receptor (RXR) and serves as a nuclear transcription factor that modulates gene expression (**Figure 1.2**) [25].

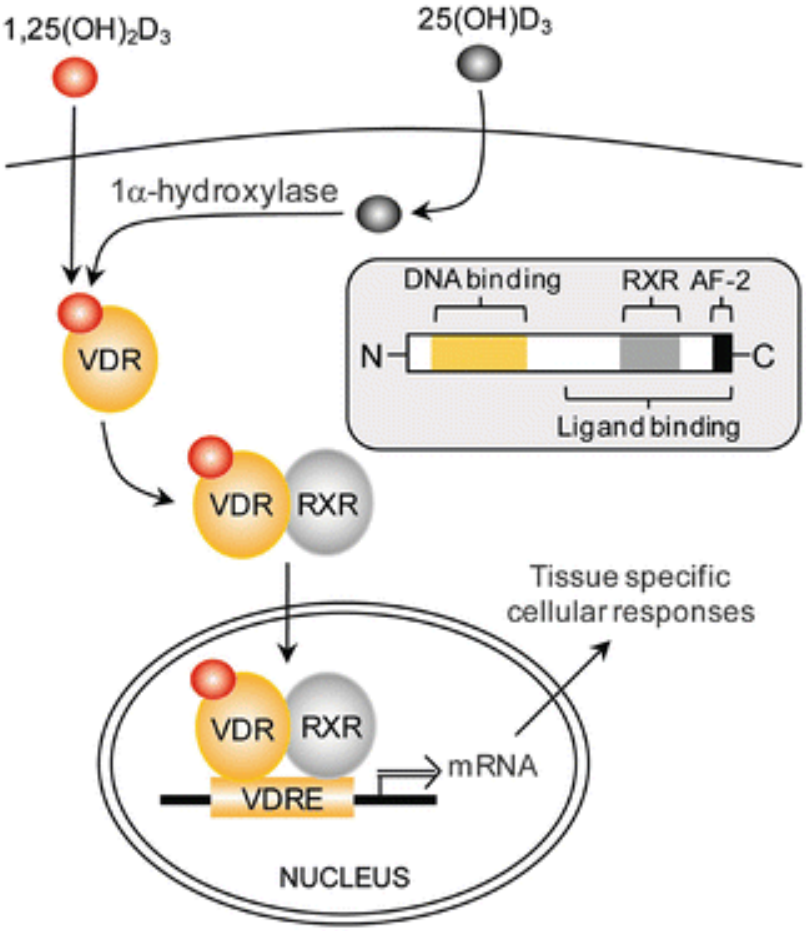


Figure 1. 2: **Ligand binding to Vitamin D receptor.**

Abbreviations: $1,25(OH)_2D_3$, 1,25 dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; VDR, Vitamin D Receptor; AF-2, Activation Function domain; RXR, Retinoid X Receptor; VDRE, Vitamin D Response Elements; mRNA, messenger RNA.

Adapted From: Von Essen et al, 2018 [27].

The active $1,25(OH)_2D$ decreases its own synthesis through a negative feedback mechanism by decreasing the synthesis and secretion of parathyroid hormone secreted by the parathyroid glands and by initiating the catabolism of 25(OH)D and $1,25(OH)_2D$. In the kidney, the enzyme 24-hdroxylase breaks down vitamin D to 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) and calcitroic acid, a water-soluble and biologically inactive substrate, which is excreted in the bile [5, 7].

1.3 Actions of Vitamin D

The biological activities of $1,25(OH)_2D$ are exerted in various cells through genomic responses and non-genomic rapid responses (RR). The genomic action is mediated by the nuclear vitamin D receptor (nVDR), a member of the nuclear receptor superfamily for steroid hormones [23]. The nVDR acts as a ligand-activated transcription factor. The main steps in the activation of gene transcription by nVDR are; 1) $1,25(OH)_2D$ ligand binding to nVDR; 2) heterodimerization with retinoid X receptor (RXR); 3) binding of the heterodimer to vitamin D response elements (VDREs) in the promoter of $1,25(OH)_2D$ target genes; and 4) using protein coactivators and transcription integrators to regulate the rate of gene transcription by the nVDR [28, 29]. It has been estimated that the nVDR can regulate the expression of as many as 300-800 genes in the human genome [5].

Given the complexity of the processes associated with nVDR signalling, vitamin D genomic activity requires time to take effect, from a few hours up to several days, and is regulated by inhibitors of transcription and translation [23]. Nevertheless, it is recognised that vitamin D can exert rapid actions at the cellular level, independent of gene expression [30]. The mechanisms of the non-genomic activity of vitamin D are not clearly defined. However, other steroids have also been shown to have non-genomic activity like testosterone, estrogen, and cortisol [30]. The non-genomic RR of vitamin D is mediated by the binding of the $1,25(OH)_2D$ ligand to specific binding sites localised in the caveolae of plasma membrane referred to as membrane VDR (mVDR) [23, 31]. The mVDR rapidly activates a variety of signal transduction pathways at or near the plasma membrane which are achieved through second messenger systems [32]. The onset of the RR action is a system dependent matter and can occur in seconds or take up to sixty minutes [33]. Interestingly, one shape of $1,25(OH)_2D$ is used for genomic responses (6-s-trans form), and a different shape is used to initiate RR (6-s-cis form) [19]. The majority of recognised actions of $1,25(OH)_2D$ are attributed to genomic

responses with only few known actions attributed to RR, which include rapid absorption of calcium from the intestine, secretion of insulin from pancreatic beta cells, modulation of osteoblast ion channel, and rapid migration of endothelial cells [33].

The genomic responses of $1,25(OH)_2D$ result from its specific binding with the nVDR stimulating bone mineralization and calcium homeostasis. The $1,25(OH)_2D$ produced in the kidney stimulates intestinal calcium and phosphorus absorption; induces bone development and remodelling by osteoblasts and osteoclasts; suppresses parathyroid function by reducing production of PTH and parathyroid cell growth; and induce renal calcium reabsorption to maintain calcium in the extracellular fluid within the appropriate limits essential for normal cell function and skeletal integrity. Other non-classic actions of $1,25(OH)_2D$ include suppression of cell growth, regulation of cell apoptosis, stimulation of cell differentiation, activation of the renin-angiotensin system, influencing insulin secretion, muscle function, nervous system, and stimulating immune system [34].

1.4 Nutritional Aspects of Vitamin D

1.4.1 Sources of Vitamin D

The main source of vitamin D is cutaneous synthesis of the form vitamin D_3 after sunlight UVB irradiation [7]. Sensible sun exposure should be adequate to attain about 90% of vitamin D requirements [35]. Typically, this would be about 5-30 minutes per day between 10 a.m. to 3 p.m. of sunlight exposure [7]. However, several factors can affect the time required and the amount of vitamin D_3 produced in the skin.

Dietary sources of vitamin D_3 are animal products such as, fish liver oils, fatty fish (such as mackerel, salmon, sardines, trout, swordfish, and tuna), egg yolk, beef liver and also from fortified foods such as milk, yogurt, margarine, orange juice, and breakfast cereals [7]. The form vitamin D_2, ergocalciferol, is obtained from plant dietary sources and is available in

minimal amounts. It is mostly obtained from edible fungus like mushrooms [24]. A list of dietary sources of vitamin D is presented in **Table 1.1**. Vitamin D supplements are available in both vitamin D_2 and vitamin D_3 forms, however, although both forms go through the same metabolic pathway and both can increase serum 25(OH)D concentrations, evidences suggest that vitamin D_3 has superior bioavailability than vitamin D_2 and that it can increase serum 25(OH)D levels to a greater extent and maintain higher levels for a longer duration than vitamin D_2 [7, 19, 36].

Table 1. 1: Vitamin D content in selected dietary sources.

Food Type	**Food and serving size**	**Microgram (µg)** *	**International Units (IU)**
Natural sources			
Fish	Cod liver oil (1 teaspoon)	11	450
	Mackerel, raw (3 ounces)	13.7	546
	Trout, raw (3 ounces)	13.5	540
	Swordfish, raw (3 ounces)	11.9	474
	Salmon, raw (3 ounces)	9	375
	Tuna, canned (1 cup)	9.8	393
	Sardines, canned (1 cup)	7.2	288
Mushrooms	Morel (1 cup)	3.4	136
	Chantelle (1 cup)	2.9	114
	Oyster (1 cup)	1	43
	Portabella (1 cup)	0.2	9
Eggs	Whole egg (1 large / 50g)	1	41
Meat	Beef liver (3 ounce)	1	42

Fortified foods			
Milk and airy	Milk, whole 3.25% (1 cup)	3.1	124
	Milk, 2% (1 cup)	3	120
	Milk, 1% (1 cup)	2.9	117
	Milk, non-fat (1 cup)	2.9	115
	Yoghurt (3/4 cup)	2	80
	Cheese (1cup)	2.9-3.4	115-134
Milk Alternatives	Soy (1 cup)	2.9	114
	Almond (1cup)	2.8	110
Juice	Orange juice	2.5	100
Cereals	Ready-to-eat cereals (1 cup)	0.8-2.5	32-100
Fat spreads	Margarin (1 tablespoon)	1.5	60

The data has been extracted from The U.S. Department of Agriculture (USDA) [37].

* Values were rounded up, 1 microgram (μg) = 40 international units (IU).

Variables that can affect synthesis of vitamin D_3 in the skin include: 1) skin pigmentation, as melanin absorbs UVB rays and reduces the efficiency of vitamin D_3 production; 2) time of day, solar zenith angle (SZA) is the angle between the sun's rays and the vertical direction of an area, smaller SZAs occur when the sun is at its highest point in the day, and result in more intense UVB radiation. Moreover, UVB rays is only available in the atmosphere when the sun elevation is approximately at a 35 degree angle and hence we can only produce vitamin D_3 in the midday; 3) latitude/season, the SZA also plays a role in the UVB rays reaching different areas on earth, while locations with 35 degree latitude or less from the equator can get vitamin D_3 every day of the year, areas that are farther away from the equator can only get vitamin D_3 for part of the year; 4) altitude, the atmosphere in higher

altitudes is thinner and sunlight is less blocked resulting in more UVB rays getting absorbed by the skin; 5) weather conditions, the presence of clouds and fog can block UVB rays; 6) clothing reduces the skin exposed to sunlight and prevents absorption of UVB rays ; 7) use of sunscreen, which absorbs about 92-95% of UVB rays on the surface of the skin and prevents the synthesis of vitamin D_3; 8) as glass absorbs most of the UVB rays, exposure to sunshine indoors through a window does not produce vitamin D_3; 9) obesity, gaining weight reduces the bioavailability of vitamin D_3 as it is sequestered in adipose tissues; and, 10) age, the ageing skin has reduced concentrations of 7DHC and thus a reduced capacity to synthesise vitamin D_3, therefore longer periods of sun exposure are required to produce sufficient vitamin D_3 as we get older [6, 7, 20, 38].

1.4.2 Methods for the Analysis of Vitamin D in Food

The method for analysing vitamin D content in food requires a digestion process to break down the food matrix, followed by an extraction procedure to separate it from other fat-soluble components in the food matrix, then a clean-up of the extract to separate the vitamin D from other food components and finally a concentration step before detection. Instruments used for analysing vitamin D in foods include separation by high pressure liquid chromatography (HPLC) and detection by ultraviolet absorption (UV), diode array detection (DAD) or mass spectrometry. The methods vary in the choice of internal standards, extraction solvent, the method of clean-up, the choice of analytical column, and the wavelength for UV detection [39, 40].

1.4.3 Recommended Dietary Intake of Vitamin D

The recommended dietary intake of vitamin D varies between countries. The United States recommends a daily intake of 15 μg per day for ages 1-70, and 20μg per day for

individuals more than 70 years of age [41], while the United Kingdom recommends a daily intake of 10 µg of vitamin D per day, throughout the year, for everyone aged 4 years and older (**Table 1.2**) [42]. For European population, the European Food Safety Authority (EFSA), adequate intake (AI) recommendations are 15 µg/day for all population groups one year and older and 10 µg/day for infants aged 7-11 months [43]. It is common for dietary guidelines to indicate intakes in terms of microgram (µg) per day, whereas supplemental doses are often indicated in terms of international unit (IU) doses; therefore, both units are used throughout this chapter.

Table 1. 2: Vitamin D - Recommended Dietary Allowance (RDA) and Recommended Nutrient Intake (RNI).

Age or condition	US (RDA) [41]		UK (RNI) [42]	
	µg/day	IU/day	µg/day	IU/day
0-1 year			8.5-10**	340-400**
1-4 years	10*	400*	10**	400**
5-70 years	15	600	10	400
> 70 years	15	600	10	400
Pregnant or lactating	20	800	10	400

* This value represents Adequate Intake (AI).

** This value represents Safe Intake (SI).

Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press, 2010. https://nap.nationalacademies.org/read/13050/chapter/1

Scientific Advisory Committee on Nutrition SACN Vitamin D and Health report, 2016. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/537616/SACN_Vitamin_D_and_Health_report.pdf

1.5 Assessment of Vitamin D Status

Total serum 25(OH)D is the biomarker clinically measured to determine vitamin D status. It is the major circulating form of vitamin D, and is considered the best indicator because 25(OH) has a long half-life (2-3 weeks) and represents total vitamin D levels from cutaneous production and dietary intake. The active form of vitamin D, $1,25(OH)_2D$, is not used to determine vitamin D status because it is regulated by the vitamin D endocrine system, and has a short half-life (4-6 hours); thus serum levels of $1,25(OH)_2D$ are often normal for individuals with vitamin D deficiency [44-46].

Several biochemical assays are available for the assessment of serum 25(OH)D vitamin D deficiency. The two most common ones involve immunoassays and chromatography such as high-performance liquid chromatography with UV detection (HPLC-UV) and isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC/tandem MS) [5]. There is considerable variability between available assays for 25(OH)D measurements and between different laboratories, thus, some results can be falsely low or falsely high, depending on the assay used and the laboratory.

There are efforts in progress by the US National Institute of Health in establishing the Vitamin D Standardization Program (VDSP), to standardize 25(OH)D measurement procedures. They aim to provide reliable and consistent evaluation of serum 25(OH)D levels across different laboratories [45, 47, 48]. In the UK, the Vitamin D External Quality Assessment Scheme (DEQAS) is a proficiency testing scheme which provides samples with

reference values for 25(OH)D concentrations using the reference measurement procedures assigned by the U.S. National Institutes of Standards and Technologies (NIST) [49].

1.6 Vitamin D Deficiency

1.6.1 Cut-off Levels of Vitamin D Deficiency

Determining vitamin D cut-off levels is challenging as different scientific authorities in different countries have different recommendations. The most widely used cut-off level in the literature is the earliest defined values assigned by the Institute of Medicine (IOM) in 2011 [41] (**Table 1.3**). A serum level of 25(OH)D less than 12 ng/mL indicates deficiency with adverse effects on bone health and is associated with the disease rickets in children and osteomalacia in adults and a level between 12 to less than 20 ng/mL indicates an insufficient level which is adequate for bone health but is not considered as an optimal level for other health functions of vitamin D in the body. A sufficient serum 25(OH)D level is equal to or more than 20 ng/mL, the level that is considered optimal for bone and overall heath in healthy individuals, and an excessive level of more than 50 ng/mL is associated with adverse effects due to vitamin D toxicity, particularly at levels higher than 60 ng/mL [41].

Table 1. 3: Vitamin D status for adults as defined by the Institute of Medicine Recommendations [41].

Status	Nanograms/mL (ng/mL)	Nanomoles/L (nmol/L)
Deficient	< 12 ng/mL	< 30 nmol/L
Insufficient	12 to < 20 ng/mL	30 to < 50 nmol/L
Sufficient	≥ 20 ng/mL	≥ 50 nmol/L

Excessive	> 50 ng/mL	> 125 nmol/L

1 nmol/L = 0.4 ng/mL

Vitamin D. Washington, DC: National Academy Press, 2010. https://nap.nationalacademies.org/read/13050/chapter/1.

The US Endocrine Society (US ES) recommendations for vitamin D were also published in 2011, which indicated the cut-off for serum 25(OH)D deficiency levels at less than 20 ng/mL, insufficient between 21 to 29 ng/mL, and sufficient between 30 to 100 ng/mL [50]. In 2016, the UK's Scientific Advisory Committee on Nutrition (SACN), set the levels for serum 25(OH)D deficiency as less than 10 ng/mL, insufficient between 10 to 20 ng/mL, and sufficient as greater than 20 ng/mL [42]. Additionally, in 2016, the EFSA defined vitamin D deficiency levels as below 20 ng/mL and sufficient levels as above 20 ng/mL [51]. The differences in vitamin D deficiency cut-off levels arise because of the different viewpoints of the organizations tasked with developing these recommendations and depending on the strength and interpretation of evidence linking vitamin D status to certain health outcomes.

1.6.2 Symptoms of Vitamin D Deficiency

Definition of serum levels of vitamin D deficiency is still a controversial topic; however, most agree that a level of serum 25(OH)D concentration lower than 20 ng/mL is an indication of vitamin D deficiency or insufficiency [4]. The small intestine, kidneys, parathyroid gland, and bones are the primary organs responsive to vitamin D that are involved in calcium and phosphorus absorption and homeostasis that affects skeletal health. In children, chronic severe vitamin D deficiency can cause growth retardation and the bone deformity disease rickets, characterized by bowed legs, bulging of the anterior ribcage ends, bone pain, dental deformities, decreased muscle strength, and impaired growth. In adults, severe vitamin

D deficiency can cause osteomalacia, a disease characterized with soft and week bones that can bend and break more easily than normal. Vitamin D insufficiency can lead to stunted growth and prevent children and adolescents from attaining optimal peak growth and in adults it can lead to and exacerbate osteoporosis and increase the risk of bone fragility fractures [4, 5, 7].

Non-skeletal implications of vitamin D deficiency has been linked to a various array of conditions and diseases including cancer (breast, colorectal, lung, pancreatic, and prostate), cardiovascular disease, stroke, hypertension, depression, autoimmune diseases like multiple sclerosis, type 1 diabetes, T2D, rheumatoid arthritis, inflammatory bowel disease, asthma, sarcopenia, obesity/weight loss, infectious diseases, and most recently COVID-19 [52-65]. Nonetheless, the evidence is inconsistent, and some vitamin D supplementation trials report no significant effects on these diseases [58, 66-75]. So far, causation has not been proven in those diseases and it remains unclear whether vitamin D deficiency is the cause or merely the consequence of numerous chronic diseases [13, 76, 77].

1.6.3 At-risk Groups of Vitamin D Deficiency

Obtaining adequate vitamin D from natural (nonfortified) food sources alone is challenging for most people, however, some groups are at increased risk of vitamin D deficiency due to either reduced dietary intake or sun exposure, reduced skin synthesis, increased requirement, or conditions that reduce bioavailability of vitamin D [41]. Firstly, increased risk of vitamin D deficiency can be caused by low dietary intake and/or inadequate sun exposure in some individuals including breastfed infants due to poor vitamin D content in human milk, vegans or vegetarians due to exclusion of fish from their diets, and individuals with limited sun exposure due to either being homebound/institutionalised, or wearing clothes that cover the whole body for cultural or religious reasons, or having occupations that limit sun

exposure [50, 78-80]. Secondly, reduced vitamin D synthesis occurs in older adults and patients with skin grafts for burns as both groups have decreased 7DHC in the skin; additionally, people with dark skin also have reduced synthesis because melanin, the substance responsible for the dark pigment in the skin, absorbs UV radiation reducing the production of vitamin D [81-83]. Thirdly, increased requirement in pregnant and lactating mothers, infants, and the obese (BMI ≥ 30), can account for the increased risk of vitamin D deficiency in those groups. Physiological needs are increased during pregnancy for the development of the fetus, during lactation for milk production, and during infancy for the rapid growth and bone development. On the other hand, risk of deficiency increases in the obese because of the sequestering of vitamin D in the more abundantly available adipose tissues. This reduces the availably of 25(OH)D in circulation, and thus more amounts of vitamin D is required to normalize serum 25(OH)D. Furthermore, obese individuals who undergo gastric bypass surgery are also at risk of vitamin D deficiency as the procedure bypasses the upper part of the small intestine where vitamin D is absorbed [84-86]. Finally, there is decreased bioavailability in people suffering from liver or kidney diseases, caused by decreased synthesis of vitamin D metabolites in those organs, and in people suffering from malabsorption conditions that affect the absorption of dietary vitamin D from the gut such as, Crohn's disease, celiac disease, cystic fibrosis, and ulcerative colitis [42, 87]. These groups are most likely to have inadequate vitamin D status and thus might require supplementation to meet their vitamin D requirements [80, 88].

1.6.4 Drug-Nutrient Interactions

Some medications can interact with vitamin D leading to low serum levels of vitamin D. These include weight loss medications like orlistat, which can affect absorption of vitamin D from diet and supplements [89]. Anti-inflammatory corticosteroids such as prednisone can decrease calcium absorption and impair vitamin D metabolism [91]. On the other hand, thiazide

diuretic agents that are used to treat hypertension, can decrease urinary calcium excretion. Hence, the combination of vitamin D supplements that enhances calcium absorption with thiazide diuretics should be avoided as it can lead to hypercalcemia particularly in older adults and people suffering from hyperparathyroidism and renal ailments [92].

1.7 Prevalence of Vitamin D Deficiency

Vitamin D deficiency is a globally prevalent health problem and constitutes an unrecognised epidemic in many ethnic populations. It is estimated that more than one billion people have low vitamin D concentrations worldwide [7, 93]. Although multiple review studies have indicated widespread vitamin D prevalence around the world [94-97], there is a lack of population representative prevalence of vitamin D deficiency data in many countries, particularly in Low and Middle Income Countries (LMICs) [93, 98].

Recent review papers have reported vitamin D prevalence in whole population representative samples, from studies using the VDSP standardised serum 25(OH)D measurements, with vitamin D deficiency defined as serum 25(OH)D < 12 ng/mL, where the deficiency was at 5.9% in the US (n = 15,652), 7.4% in Canada (n = 11,336), and 13% in Europe (n = 55,844). Estimates of the prevalence of vitamin D insufficiency (serum 25(OH)D levels < 20 ng/mL), have been reported as 24% in the US, 37% in Canada, and 40% in Europe [8, 98]. In the UK, a recent cross-sectional study using data from the UK biobank (n = 449,942) of adults aged 40-69 years using the SACN cut-off values reported vitamin D deficiency (< 10 ng/mL) at 53.7%, 34.9%, 27.1% and 12% in Asian, black, Chinese, and white participants respectively. The study also reported vitamin D insufficiency (< 20 ng/mL) at 37.3%, 49.7%, 56% and 41.7% in Asian, black, Chinese, and white participants respectively [99].

Several studies reported vitamin D deficiencies and insufficiencies around the world with different subcategories of age and gender [93, 95, 96]. Vitamin D deficiency with a serum

25(OH)D level of less than 10 or 12 ng/mL is common in more than 20% of the population in India, Tunisia, Pakistan, and Afghanistan [49]. However, estimating accurate figures for direct comparison from available studies for the prevalence of vitamin D deficiencies around the world is complicated by the season of sampling, the different measurement methods used which can vary widely in precision, and by the cut-off levels used in the reported data. Nevertheless, a cut-off level of less than 10 or 12 ng ng/mL is usually indicative of severe vitamin D deficiency with increased risk of nutritional rickets and osteomalacia [49, 95].

1.8 Prevention and Treatment of Vitamin D Deficiency

1.8.1 Supplements

Though nutritional supplements can be helpful in fulfilling dietary requirements and preventing deficiencies, they need to be considered along with dietary intakes to ensure that the recommended tolerable upper levels are not exceeded to prevent toxicity. Vitamin D supplements are available in both forms vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_2 supplement is approximately 30% as effective as vitamin D_3 in maintaining serum 25(OH)D [32]. The Global Consensus Recommendation on Prevention and Management of Nutritional Rickets, recommends that all infants from birth to one year are given a 10 μg/day supplementation [88]. Breastmilk is not an adequate source of vitamin D and formula fed infants would need to consume approximately one litre of milk to achieve their requirement, so the recommendation is for all infants regardless of their feeding mode [100]. Pregnant women are recommended 15 μg/day to prevent osteomalacia and congenital rickets [88]. In the absence of food fortification, breastfeeding women, children, older adults, and all at-risk groups, require supplementation [88].

In the UK, The Department of Health and Social Care (DHSC) recommends that babies from birth to 1 year old should be given a vitamin D supplement containing 8.5 to 10 μg/day

throughout the year if they are breastfed or if they consume less than 500 ml of infant formula. Also, those children aged 1-4 years should have a vitamin D supplement containing 10 μg/day throughout the year. For children over 4 years and adults, the recommendation is a daily supplement containing 10 μg of vitamin D during autumn and winter (October to March); alternatively, for the at-risk group, the recommendation is to continue supplementation throughout the year [101].

1.8.2 Fortification

Vitamin D fortification is a public health strategy for the prevention of nutrition dependent vitamin D rickets and improve vitamin D levels in the population. Public health officials in the US and UK have been recommending vitamin D fortification since the 1930s [8]. Milk and dairy products are the most commonly used foods for vitamin D fortification, as they are rich in calcium and optimal for bone health, and both vitamin D_2 and D_3 can be used in fortification. Other products that are used as vehicles for vitamin D fortifications include flour, margarine, breakfast cereals, cooking oil, orange juice and soy-based foods [49].

Infant formula fortification is mandatory in the US, Canada, and Europe. In Europe and the US, food fortification of vitamin D is voluntary in milk, breakfast cereals, yogurt, cheese, juices, and margarine. In the UK, vitamin D fortification of margarine was mandatory since 1940 but it was removed in 2013. Canada currently has mandatory fortification of vitamin D in milk and margarine [42, 98]. There is substantial evidence that vitamin D food fortification can significantly improve vitamin D intake and status and prevent vitamin D deficiency safely and effectively [102-105]. Hence, implementation of vitamin D fortification programs in widely used and culturally appropriate foods around the world would be a helpful and cost-effective public health intervention to closing the gap between current recommendations and population intakes [49, 88, 98].

1.8.3 Treatment

Treatment of vitamin D deficiency and insufficiency is usually done by an oral loading regimen that provides a total of 7,500 μg (300,000 IU) of vitamin D_2 or 3,750 μg (150,000 IU) of D_3, given either by weekly or daily doses over 6 to 10 weeks and for maintenance, a daily dose of 10-20 μg (400-800 IU) is given. For daily treatment, both vitamin D_2 and D_3 are effective, however, for weekly treatment with higher doses, the use of vitamin D_3 is preferable because the synthesised serum 25(OH)D_3 has a longer half-life than 25(OH)D_2. Intermuscular injection of vitamin D can also be used when needed but it has unpredictable bioavailability and a slower repletion onset, hence oral treatments are preferable. Exposure to sunlight or artificial UV-B such as a tanning bed is also helpful during treatment and maintenance especially for patients prone to vitamin D deficiency. It is possible that a new steady state for serum 25(OH)D is reached six months after treatment, therefore, it is prudent to check serum levels 3-6 months after treatment to determine vitamin D status [106].

Nutritional vitamin D deficiency rickets can be treated effectively with the combination of oral vitamin D and calcium for at least 90 days or until healing is confirmed radiographically. Common dosing is 50 μg/day (2000 IU/day) of vitamin D and 500 mg/day of calcium, although this may vary on the basis of age and response to treatment (**Table 1.4**) [49].

Table 1. 4: Nutritional Vitamin D deficiency treatment for rickets.

Age	Daily dose for 3 months	Single Dose	Maintenance daily dose
Birth – 3 months	50 μg (2,000 IU)	N/A	10 μg (400 IU)
3 – 12 months	50 μg	1,250 μg	10 μg

	(2,000 IU)	(50,000 IU)	(400 IU)
> 12 months to 12 years	75 – 150 µg (3,000 – 6,000 IU)	3,750 µg (150,000 IU)	15 µg (600 IU)
> 12 years	150 µg (6,000 IU)	7,500 µg (300,000 IU)	15 µg (600 IU)

Data from Global Consensus Recommendations on Prevention and Management of Nutritional Rickets [88].

1.9 Vitamin D Toxicity

Vitamin D toxicity can occur with high doses of vitamin D intake. The clinical definition of vitamin D intoxication is hypercalcemia and serum 25(OH)D more than 100 ng/mL (250 nmol/L), with hypercalciuria and suppressed PTH [88]. Toxicity can result in deposition of calcium in soft tissues, bone demineralisation, renal and cardiovascular damage [42]. Excessive sunlight exposure does not lead to vitamin D intoxication because excess previtamin D_3 and vitamin D_3 are photolyzed to biologically inactive compounds [4]. Causes of toxicity can be high doses of supplementation and in rare cases it could be caused by excessive food fortification [49]. Reported cases of vitamin D toxicity in infants and young children have been due to exposure to high doses of vitamin D (6,000 – 112,500 µg /240,000 to 4,500,000 IU) [107-111]. Additionally, some case studies have shown that vitamin D supplementation of 1,250 – 7,700 µg/day (50,000–300,000 IU/day) can cause hypercalcemia within a few weeks [112-114]. Therefore, supplementation of vitamin D should be carefully considered with total vitamin D intake from all sources, natural and fortified.

The tolerable intake levels (UL) are the maximum intake levels that can be consumed every day without risk to health set for the population. The IOM has established UL for vitamin D intake to be 100 µg/day (4000 IU/day) in adults and in children from 9 years old [115] (**Table**

1.5). Similarly, SACN and EFSA set the UL at 100 μg/day (4000 IU/day) in adults and in children from 11 years and older [42, 43] (**Table 1.6**). Moreover, the IOM has designated a threshold of 25(OH)D above which there were concerns of adverse effects at 50 ng/mL (125 nmol/L). However, the Global Consensus Recommendation on Prevention and Management of Nutritional Rickets considered the IOM threshold of concern to be too low as a published review on vitamin D safety concluded that serum 25(OH)D levels less than 88 ng/mL (220 nmol/L) posed no known adverse effects [49].

Table 1. 5: The US-based Vitamin D tolerable upper intake level.

Age	RDA – UL
0-6 months	25 μg (1000 IU)
6-12 months	38 μg (1500 IU)
1-3 years	63 μg (2500 IU)
4-8 years	75 μg (3000 IU)
9-50 years	100 μg (4000 IU)
Pregnant	100 μg (4000 IU)
Lactating	100 μg (4000 IU)

Abbreviations: RDA, Recommended Dietary Allowance; UL, tolerable upper intake level.

Table 1. 6: Europe-based Vitamin D tolerable upper intake level.

Age	SACN / EFSA – UL
0-12 months	25 μg (1000 IU)
1-10 years	50 μg (2000 IU)
11-50 years	100 μg (4000 IU)
> 50 years	100 μg (4000 IU)

Pregnant	100 µg (4000 IU)
Lactating	100 µg (4000 IU)

Abbreviations: SACN, Scientific Advisory Committee on Nutrition; EFSA, European Food Safety Authority; UL, tolerable upper intake level.

1.10 Genetic Epidemiology of Vitamin D Status

The human genome consists of around three billion base pairs residing in the 23 pairs of chromosomes [116]. Human beings share at least 99.5% of identical base pairs of the genome [117]. A genetic variant is a single base position change in the DNA sequence. The DNA sequence variation can be defined as either a mutation or a single nucleotide polymorphism (SNP) depending on the frequency of their occurrence [118]. A rare genetic variant detectable in less than 1% of the population which usually, but not always, causes a disease is called a mutation, while a genetic variation that occurs in the population with a frequency equal to or more than 1% in known as a SNP [118]. Researchers study if and how SNPs in a genome influence health, disease, drug response and other traits to optimise health and longevity of an individual and to minimise risk of diseases [119].

Genetic factors can play an important role in determining serum 25(OH)D status [120, 121]. Twin and family-based genetic heritability studies have previously showed that heritable factors play a role in vitamin D deficiency with varying estimates from 20-85% [121]. Furthermore, gene discovery methods have been successful in identifying and replicating genetic associations with vitamin D status [122]. The candidate gene approach has revealed associations of SNPs with serum 25(OH)D concentration from genes that are involved in vitamin D metabolic pathway such as *DHCR7*, *CYP2R1*, *CYP27B1*, *CYP24A1*, *DBP/GC*, *VDR, CASR* [120], while the genome-wide association (GWAS) approach confirmed the association between the SNPs in the genes such as *DHCR7*, *CYP2R1*, *DBP/GC*, and *CYP24A1* and

25(OH)D status [123]. Genetic association studies examine genetic markers and disease status to identify genes that contribute to a certain disease [124]. If an allele or a genotype is found to be at a higher frequency in a group of people with a certain disease, then this could be interpreted as a direct association, meaning that the examined variant increases the risk of that disease. However, other interpretations such as indirect association, which can occur when the examined SNP and the true causal SNP are in linkage disequilibrium (LD) is also possible. LD measures the correlation of the non-random association of alleles at two or more nearby loci on the same chromosome [125]. When two SNPs are in complete LD, it is not possible to distinguish the causative SNP [126]. Another interpretation of the association of a certain disease with a high allele frequency is a false-positive finding, which could be caused by a chance finding or by systematic confounding such as population stratification [124]. Population stratification can arise when cases and controls in a study are from genetically different populations that have different SNP allele frequencies, causing associations due to sampling differences rather than the disease of interest [127]. Furthermore, genetic heterogeneity, which is the presence of different genetic variants or genetic mechanisms that produce similar phenotypic characteristics, is another issue that could affect the interpretations of findings in genetic association studies and could lead to inaccurate conclusions about the role of a SNP in disease risk [128].

It is important to identify potential gene-environment interactions involving vitamin D genetic variants and disease outcomes in order to understand the genetic architecture underlying vitamin D status. Analysing the effect of lifestyle factors with multiple vitamin D associated markers across the genome is important to identify interactions between the environmental factors, such as diet, physical activity etc with genetic factors on disease risk outcomes [129]. Given that vitamin D status is influenced by several genetic variants, with each having a small effect, combining the effect of several variants as a polygenic score can

provide a better understanding of disease risk than the single SNP approach [121]. The use of a cumulative genetic risk score (GRS) from individual SNPs capturing the variance in a trait has been used in metabolic disease risk prediction [130, 131]. A GRS is constructed by summing up the number of risk alleles (0, 1, or 2) of multiple genetic variants across all genotype data [132]. The use of the GRS approach for predicting disease risk is favourable over analysing the effect of individuals SNPs as it maximises statistical power, reduces the drawback of multiple testing, and improves disease risk prediction [131, 133].

1.11 Heritable Disorders of Vitamin D Deficiency

Genetic disorders in vitamin D metabolic pathway are responsible for causing vitamin D-dependant rickets in children. There are five known genetic mutations in the genes involved in vitamin D synthesis and metabolism affecting proper bone mineralization in children (**Table 1.7**) [134]. The first category of heritable disorders include vitamin D-dependent rickets type 1A (VDDR1A) and vitamin D dependent rickets type 1B (VDDR1B), both of which are due to failure in activation of vitamin D. VDDR1A, also known as pseudovitamin D deficiency rickets, is caused by a mutation of the renal 1α-hydroxylase gene (*CYP27B1*), that encodes the $1,25(OH)_2D$ enzyme. This mutation causes reduction or lack of activation and renal synthesis of 25(OH)D into $1,25(OH)_2D$ leading to persistent rickets in infants. VDDR1A presents similar phenotype as nutritional vitamin D dependent rickets and it can be treated with a high dose of vitamin D or its analogues, however, the active $1,25(OH)_2D$ gives faster results [135]. VDDR1B, is caused by an extremely rare mutation in the 25-hydroxylase gene (*CYP2R1*) that encodes the 25(OH)D enzyme and causes reduced synthesis of serum 25(OH)D in the liver. In VDDR1B the only treatment option is administering $1,25(OH)_2D$ [136].

The second category of heritable vitamin D disorders include vitamin D-dependent rickets type 2A (VDDR2A) and vitamin D-dependent rickets type 2B (VDDR2B), which are

characterised by partial or complete resistance to $1,25(OH)_2D$ activity. VDDR2A, also known as, vitamin D resistant rickets, is caused by a loss-of-function mutation of the gene encoding vitamin D receptor gene (*VDR)* which results in partial or complete resistance to $1,25(OH)_2D$ action, leading to elevated levels of $1,25(OH)_2D$. Phenotypic features include classical presentation of rickets and about half of the patients have alopecia. Treatment requires administration of high doses of calcium [137]. VDDR2B, is not caused by a mutation in the *VDR* gene, however, there is an unknown molecular defect causing an unusual expression and overproduction of hormone responsive-element binding proteins that interferes with *VDR* function, and prevents the action of $1,25(OH)_2D$ in transcription, causing target-cell resistance and elevated levels of $1,25(OH)_2D$. Clinical presentations and treatments of VDDR2B are similar to VDDR2A [134].

The third category is vitamin D dependent rickets type 3 (VDDR3), which is caused by increased inactivation of vitamin D and its metabolites. VDDR3 is caused by gain-of-function missense mutation in the Cytochrome P450 Family 3 Subfamily A Member 4 gene (*CYP3A4*), which encodes CYP3A4 enzyme. This is an extremely rare form of rickets and to date only 2 reported cases were diagnosed with this genetic mutation. Both children exhibited classical features of rickets and had low serum 25(OH)D and $1,25(OH)_2D$. Due to the rapid and extensive inactivation of vitamin D metabolites, patients require daily high doses of vitamin D or $1,25(OH)_2D$ to maintain normal levels of serum 25(OH)D. Other types of hereditary rickets exist which are related to genes associated with phosphorus levels known as hypophosphate rickets [138].

Table 1. 7: Inborn error of Vitamin D Metabolism: Vitamin D dependent Rickets.

Disorder	Inheritance	Gene	Location	25(OH)D	$1,25(OH)_2D$

Vitamin D dependent rickets Type 1A (VDDR1A)	Autosomal Recessive	*CYP27B1*	12q14.1	Normal or Increased	Decreased
Vitamin D dependent rickets Type 1B (VDDR1B)	Autosomal Recessive	*CYP2R1*	12p15.2	Decreased	Decreased
Vitamin D dependent rickets Type 2A (VDDR2A)	Autosomal Recessive	*VDR*	12q13.11	Normal or Increased	Normal or Increased
Vitamin D dependent rickets Type 2B (VDDR2B)	Autosomal Recessive	Unknown	---	Normal or Increased	Normal or Increased
Vitamin D dependent rickets Type 3 (VDDR3)	Autosomal Dominant	*CYP3A4*	7q22.1	Decreased	Decreased

1.12 Nutrigenetics Approach

Nutrigenetics is a field of science that studies the link between genes and nutrition. While it is closely related to the field of nutrigenomics, which focusses on examining the effect of nutrient intake on gene expression; nutrigenetics examines the effect of genetic variation in human DNA in response to dietary nutrient intake in relation to health and disease risk [139]. The field of nutrigenetics is progressing rapidly and providing evidence of gene-diet interactions that have great potential in changing the field of nutrition to provide tailored nutritional advice for genetic subgroups and individuals [140].

1.12.1 Genetic Variations and Ethnicity

The complex metabolic pathway and biological actions of vitamin D involve multiple genes [1]. Genetic variants can alter vitamin D status by affecting the synthesis of enzymes involved in vitamin D synthesis, metabolism, transport, and nuclear receptor response [6]. Genetic heritability studies showed that genetic factors play a role in vitamin D deficiency [121]. With advances in the field of genetic sequencing, candidate gene studies and genome wide association studies (GWAS), several single nucleotide polymorphisms (SNPs) have been shown to be associated with vitamin D levels in the metabolic pathway genes such as, 7-Dehydrocholesterol reductase gene (*DHCR7*), *CYP2R1*, vitamin D binding protein gene/group-specific component gene (*DBP/GC*), *CYP27B1*, 24-hydroxylase gene (*CYP24A1*), and *VDR* [120, 121].

Multiple studies have examined the association of vitamin D genetic variants with metabolic diseases. The studies examining vitamin D SNPs and the metabolic diseases such as obesity and T2D in various ethnicities were reviewed and discussed in detail in a published review article [1] (detailed in **chapter 2)**. The frequencies of genetic variants vary from one population to another, leading to differences in the prevalence of diseases across multiple ethnic groups [141, 142]. Therefore, it is important to study the influence of genetic factors on metabolic risk across various ethnicities.

1.12.2 Rationale for Studying Gene-Diet Interactions

Genetic contributions have been shown to influence vitamin D status and many SNPs in vitamin D-related genes have been identified to be associated with low serum 25(OH)D concentration [120, 121]. However, evidence on the relative role of genetics, lifestyle, and phenotypic factors on vitamin D status is scarce. Vitamin D deficiency is widespread in many populations and is often implicated in increasing the risk of several diseases [12]. Association

studies have examined the link between common genetic variants of vitamin D and metabolic diseases to shed some light on the suggested relationship between vitamin D concentration and risk of metabolic disease [1]. However, disease risk prediction is complicated by the environmental and lifestyle factors [143]. Therefore, it is important to examine gene-diet interactions that contribute to vitamin D deficiency as well as identifying the interactions of vitamin D-related genes with diet on the risk of metabolic diseases. This knowledge is essential for developing effective individualized dietary strategies for the prevention of vitamin D deficiency and its related metabolic outcomes based on the genetic makeup.

1.12.3 Importance of Examining Gene-Diet Interactions in Diverse Ethnic Groups

Large scale genetic studies have investigated the associations of vitamin D genetic variants with metabolic diseases primarily in Caucasian populations [144-147]. Multiple diverse ethnic groups from around the world are underrepresented in vitamin D genetic studies which hinders the understanding of the influence of vitamin D genes on disease risk [148]. Given the wide prevalence of vitamin D deficiency around the world and its implication in several metabolic diseases, it is imperative to investigate the influence of vitamin D genetic variants across different populations to understand the full scope of the influence of vitamin D genetics on vitamin D status and its links with metabolic diseases [149]. Furthermore, translating genetic research for use in clinical settings would be hindered by the underrepresentation of diverse ethnic groups and impede the potential of providing personalised intervention aimed at reducing disease risk [148]. As vitamin D status can also be influenced by lifestyle factors like dietary intake, and that ethnic groups respond differently to dietary interventions, it is necessary to examine gene-diet interactions in different ethnic populations to be able to provide personalised nutritional advice based on ethnicity [141].

Therefore, it is necessary that genetic risk of vitamin D deficiency is investigated in different ethnic groups.

1.12.4 Study Designs and their Role in Identifying Gene-Diet Interactions

Evidence indicate that genetic variants can influence gene expression and influence metabolic traits. Moreover, genetic variants can interact with lifestyle factors and modulate this influence on metabolic disease outcomes [17]. Multiple genes are involved in the metabolism and biological actions of vitamin D and several SNPs in these genes may be involved in modifying metabolic disease outcomes [120, 121]. The aim of vitamin D gene-diet interaction studies is to provide data for dietary modification and disease prevention based on the genetic susceptibilitly for vitamin D deficiency in an individual or a subgroup in the population. For such studies, observational and experimental study designs may be used.

The cross-sectional observational design is the most used study design, and it involves studying a group of people, chosen based on a set of inclusion and exclusion criteria, at one point in time when the exposure and outcome variables are collected, examined, and interpreted [150]. The case-control observational design involves studying two groups of participants, cases and controls, based on an outcome of choice, such as a specific disease, to determine if an exposure is associated with the outcome [151]. Observational studies can be used to collect large sample sizes as they can be conducted relatively faster and are inexpensive. They can also be used to generate a hypothesis for future studies [152]. Unfortunately, observational design cannot be used to explain causal relationships between risk factors and diseases as they are one-time measurements, and they can be limited by confounding effects, which have to be adjusted for in statistical analyses. In addition, lack of replication in many observational studies make it difficult to draw definitive conclusions on whether the findings are true or due to chance [152]. However, the cross-sectional design is successfully used in GWAS studies,

which analyse numerous genetic variants in the human genome, and have identified several genetic variants associated with disease risk, which are most likely unaffected by confounding variables [129]. Nutrigenetics research using observational design can have several limitations such as the inherent recall bias in dietary intake assessment methods. Dietary collection methods used in observational studies include single or multiple 24 hour-recall and food frequency questionnaires (FFQ). These methods are prone to recall errors, omissions, and errors in estimating portion sizes [153]. Another limitation of observational design is that phenotypes can vary over different periods of time, for example fasting serum glucose levels can vary over time, thus relying on one measurement at one point in time could be a constraint [154].

Randomised control trials (RCTs) are considered the gold standard for evaluating an intervention in experimental design. In a RCT, two groups are created by random selection process, one group receives the desired intervention or treatment, and the other group receives a placebo (the control group), with the aim of assessing intervention-related changes in biomarker measurements [152]. The main reasons that RCTs are considered superior is that the intervention is clearly defined and that both researcher and participant can be double blinded to minimise the bias for treatment group. Another factor is the randomisation in assigning participant to study groups which can reduce confounding in gene-diet interactions [152]. However, RCTs have challenges with high cost, adherence to treatment, participant dropouts, and often have small sample size [153]. Another type of experimental study is “cross-over” design, in which half of the participants are assigned to a treatment and the other half to a placebo for a period of time, then the groups undergo a washout period, followed by a swich of the participants to the other group (treatment or placebo) for the same period of time [152]. The main advantage of cross-over study design is the verification of findings of the first phase by reproducing it in the second phase within the same group of participants, thus minimising

confounding, and reinforcing the outcomes of the study. However, concerns of dietary cross-over studies are similar to RCTs with the addition of the requirement of a washout period which may decrease the power of observing effect sizes for gene-diet interactions and can decrease the rate of participant adherence to the assigned diet of the study [129].

1.12.5 GeNuIne Collaboration

Over the past few years, multiple studies have investigated gene-lifestyle interactions in the European populations. Although some studies have reported that dietary intake and physical activity could modify the association of genetic variants with metabolic disease, other studies reported contradictory results [155-160]. This could be due to differences in dietary and lifestyle pattens within different populations, and/or could be due to genetic heterogeneity, which refers to the differences in the allele frequencies in different ethnic groups [18]. Nevertheless, most available gene-diet interaction studies are focused on metabolic disease-related traits with only few studies focusing on vitamin D, which has been shown to have significant associations with metabolic diseases [161, 162]. Several genetic variants have been identified to be associated with vitamin D deficiency [121] and several genetic studies have investigated vitamin D-related variants with metabolic diseases reporting conflicting results [163, 164]. Thus, there is a need for assessing the interactions between vitamin D-related genes and dietary intake and examining their effect on metabolic disease outcomes. Investigating gene-diet interactions in various ethnic groups would generate information that could elucidate the relationship between vitamin D and metabolic diseases and would ultimately enable the personalization of dietary recommendations based on ethnicity. To facilitate these objectives, a large-scale collaborative project has been initiated, the **Ge**ne–**Nu**trient **Inte**ractions (**GeNuIne**) Collaboration [17] with the aim of examining the interplay of gene-diet interactions

in lower middle-income countries (LMIC), such as Indonesia, India, Sri Lanka,Brazil, Turkey, Ghana, Peru, and Malaysia.

1.12.6 From Nutrigenetics to Personalised Nutrition

It is a well-known fact that dietary factors can contribute to the development of chronic diseases such as obesity, T2D, and cardiovascular disease. The development of these chronic diseases involves an interplay between genetic, environmental, and lifestyle factors [165]. Although, genetic factors in different populations are important in determining susceptibility to metabolic diseases, lifestyle habits such as physical activity and dietary intake, can also influence the risk of developing these diseases [166]. Given that the risk of most chronic diseases can be significantly reduced by environmental factors, modification of lifestyle is the main approach for preventing metabolic diseases in public health practice. Nevertheless, the current approach is that of "one-size-fits-all" which is outdated and relatively ineffective in disease management as evident by the continued rise in the prevalence of chronic metabolic diseases [167, 168]. It is therefore important to revisit the dietary prevention strategies and include personalised dietary recommendations according to phenotypic and genotypic data [169].

Two decades ago, the completion of the Human Genome Project (HGP), provided a wealth of new genetic data to be explored. Subsequently, advances in DNA sequencing, genotyping, and the "omics" technologies provided knowledge that nutrients have the ability to interact at a molecular level to modulate genomic actions [170]. The emerging "omics" technologies investigations include, the entire genome analysis (genomics), the entire mRNA transcripts in a cell (transcriptomics), the entire proteins in a cell or tissue (proteomics), and the entire metabolites in a cell or organism (metabolomics) [171]. Capitalising on the multi-omics fields drove the creation of two new distinct areas in nutritional research, nutrigenetics

and nutrigenomics with the goal of personalised / precision nutrition. Nutrigenomics aims to study the effect of dietary nutrients on gene expression, consequently on the proteins (enzymes) and metabolites produced, relating the results to how nutrients affect the metabolic pathways and result in different phenotypes. On the other hand, nutrigenetics aims to study the influence of genetic makeup, using genetic polymorphisms, in response to dietary nutrients intake on the risk of developing chronic diseases [172]. Future prospects for using the data generated form these fields will change the face of nutritional intervention to prevent diseases.

At present, tailoring dietary recommendations to an individual's exact biological requirement is the outlook with the approach of personalised or precision nutrition. Precision nutrition is a more recent term used in the literature describing similar prospect as personalised nutrition with a further degree of scientific certainty [167, 173]. To effectively implement this prospect, more gene-diet interaction data is required highlighting the urgent need for the nutrigenetics research to explore the effects of nutrient interaction with genetic variants and their influence on disease risk [174]. Furthermore, evidence suggests that gut microbiota profile should also be considered in precision nutrition, as it has been shown to play a role in nutrient absorption, storage, and regulation, and also, it could affect gene-diet interactions to modify the risk of developing metabolic diseases [175, 176]. In the following years, measurement of nutritional biomarkers could be used for early detection of susceptibility to disease risk and in identifying individuals who could benefit from personalised dietary interventions [177]. However, the journey is at the beginning with required validation for the gene-diet interaction findings in functional and prospective studies to elucidate biological mechanism and to confirm results before they can be applied in clinical health practice [17].

1.13 Hypothesis, Aims and Outline of the Thesis

I hypothesised that there could be an interplay between metabolic disease-related genetic variants and vitamin D genetic variants on metabolic trait outcomes and vitamin D concentration and that these associations could be modulated by dietary factors in various ethnic groups. Based on this hypothesis, this thesis aimed to:

1. Examine the association between GRSs devised from selected SNPs for metabolic diseases and selected SNPs for vitamin D deficiency with metabolic traits and vitamin D concentration in different ethnic groups.

2. Examine the interaction between these GRSs and lifestyle factors (dietary fat, protein, carbohydrate, and fibre intakes; and physical activity levels) on vitamin D concentrations and metabolic outcomes in multiple ethnic groups (**Figure 1.3**).

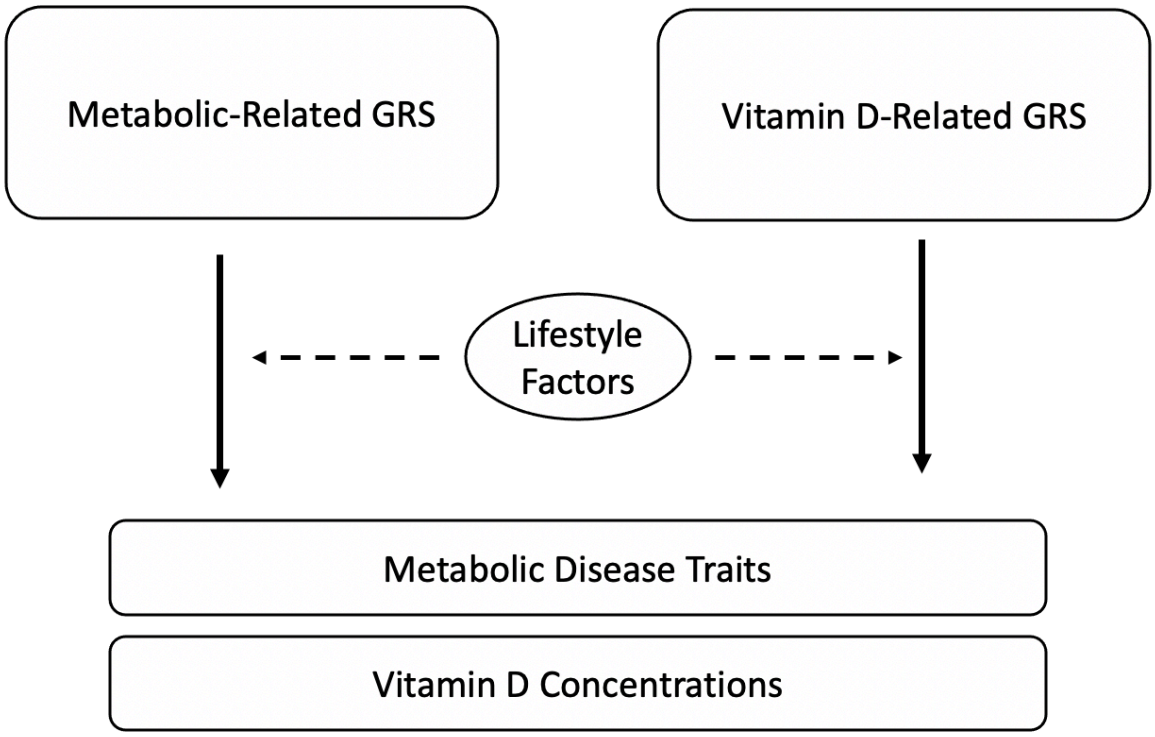


Figure 1. 3: **Diagram representing the main aims of this thesis.**

Genetic associations are represented by solid arrows and interactions between GRSs and lifestyle factors on clinical and biochemical measurements are indicated by broken arrows. The association of the metabolic-related GRS with metabolic traits and 25(OH)D concentrations,

and the association of vitamin D-related GRS with metabolic traits and 25(OH)D levels were tested. In addition, the effect of lifestyle factors on these genetic associations was investigated.

Background information and the aims of the thesis chapters are listed below:

Chapter 2: This chapter is a literature review study focusing on the relationship between vitamin D genetic variants and obesity and T2D. Given that several studies have reported a link between low vitamin D status and various metabolic diseases, this review aimed to evaluate all available studies that examined the relationship between vitamin D genetic variants and the metabolic diseases such as obesity and T2D. The review identified the gene variants that have been shown to be associated with serum 25(OH)D concentration and described the role of genes involved in vitamin D synthesis and metabolism. Additionally, the review reported the associations between the metabolic diseases and vitamin D-related SNPs in specific ethnic groups.

Chapter 3: This chapter is a nutrigenetic study in South Asian Indian population. Studies in Asian Indians have examined the association of metabolic traits with vitamin D status. However, findings have been quite inconsistent. The aim of this study was to explore the relationship between metabolic traits and 25(OH)D concentrations and to investigate whether this relationship can be modified by lifestyle factors in South Asian Indian population using a nutrigenetic approach.

Chapter 4: Vitamin D deficiency is widespread in Indonesian women with an estimated prevalence of 60% to 95%. Metabolic diseases have been shown to be associated with low vitamin D status. The objective of this study was to use a genetic approach to investigate the

relationship between vitamin D status and metabolic disease-related traits in healthy Southeast Asian Minangkabau women and to examine whether this relationship was modified by lifestyle factors.

Chapter 5: The metabolic diseases such as obesity and T2D are on the rise in Brazil. Moreover, recent studies reported vitamin D deficiency and insufficiency to be at 28.2% and 45.3%, respectively in the Brazilian population. Given the relationship between vitamin D deficiency (VDD) and adverse outcomes of metabolic diseases, we investigated the interplay of lifestyle and genetic components on vitamin D levels and metabolic traits in healthy young adults from Brazil.

Chapter 6: The Ghanaian population is experiencing an upsurge in obesity and T2D due to rapid urbanization. Besides lifestyle, vitamin D-related genetic factors have also been shown to contribute to the development of these metabolic diseases. Hence, this study aimed to examine the interaction between vitamin D genetic risk and lifestyle factors on obesity and T2D related outcomes in a Ghanaian population.

Chapter 7: In Turkey, the prevalence of vitamin D deficiency is reported to be between 58.9-66.6% in adults. Previous studies have pointed out a link between vitamin D status and metabolic traits, however, consistent evidence has not been provided yet. This study investigated the interaction between metabolic genetic risk and lifestyle factors on serum 25(OH)D concentrations in unrelated healthy Turkish adults.

Chapter 8: This chapter presents the discussion of all the findings from the six chapters of this thesis. In addition, it lists the limitations, and strengths, and suggests possible future investigations related to this PhD project.

Chapter 2: A Review of Vitamin D Pathway-Related Gene Polymorphisms and their Association with Metabolic Diseases

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

Alathari BE, Sabta AA, Kalpana CA, Vimaleswaran KS. Vitamin D pathway-related gene polymorphisms and their association with metabolic diseases: A literature review. J Diabetes Metab Disord. 2020;19(2):1701-1729. doi: 10.1007/s40200-020-00561-w. PMID: 33553043. *https://doi.org/10.1007/s40200-020-00561-w*

Buthaina AlAthari's contribution: For this literature review, I extracted and assessed articles that had examined the association of vitamin D-related genetic variants with obesity and/or T2D. I conducted a search on PubMed database (up to December 2019) and I identified 1,455 articles. After excluding irrelevant and duplicate titles and applying the exclusion criteria, 112 articles were chosen. Following this, I carefully evaluated the chosen papers to determine relevance to the topic and further exclusions were applied and as a result, only 73 articles were included in this review paper. I contacted corresponding authors to provide additional information when needed. I wrote the manuscript and revised the manuscript based on comments from the co-authors and I drafted responses to the comments from the reviewers.

2.1 Abstract

Purpose: Given that the relationship between vitamin D status and metabolic diseases such as obesity and type 2 diabetes (T2D) remains unclear, this review will focus on the associations, which are less prone to confounding, between vitamin D-related single nucleotide polymorphisms (SNPs) and metabolic diseases.

Methods: A literature search of relevant articles was performed on PubMed up to December 2019. Those articles that had examined the association of vitamin D-related SNPs with obesity or T2D were included. Two reviewers independently evaluated the eligibility for the inclusion criteria and extracted the data. In total, 73 articles were included in this review.

Results: There is a lack of research focussing on the association of vitamin D synthesis-related genes with obesity and T2D; however, the limited available research, although inconsistent, is suggestive of a protective effect on T2D risk. While there are several studies that investigated the vitamin D metabolism-related SNPs, the research focussing on vitamin D activation, catabolism and transport genes is limited. Studies on *CYP27B1*, *CYP24A1* and *GC* genes demonstrated a lack of association with obesity and T2D in Europeans; however, significant associations with T2D were found in South Asians. *VDR* gene SNPs have been extensively researched; in particular, the focus has been mainly on BsmI (rs1544410), TaqI (rs731236), ApaI (rs7975232) and FokI (rs2228570) SNPs. Even though the association between *VDR* SNPs and metabolic diseases remain inconsistent, some positive associations showing potential effects on obesity and T2D in specific ethnic groups were identified.

Conclusion: Overall, this literature review suggests that ethnic-specific genetic associations are involved. Further research utilizing large studies is necessary to better understand these ethnic-specific genetic associations between vitamin D deficiency and metabolic diseases.

2.2 Introduction

Vitamin D is a fat-soluble vitamin and a secosteroid prohormone that plays a crucial role in bone mineralization through the absorption and regulation of calcium and phosphate levels [178]. The vitamin D endocrine system regulates calcium homoeostasis and a range of physiological functions such as cell growth, proliferation, differentiation, immune function, inflammation, and apoptosis [5]. A broad spectrum of diseases has been related to vitamin D deficiency and research to date suggests that vitamin D deficiency is a marker of ill health with effective connection to all-cause mortality, obesity, diabetes, cardiovascular risk, hypertension, dyslipidaemia, multiple sclerosis, Alzheimer, and some types of cancer [20, 24, 26, 179, 180]. However, causality is yet to be proven for any disease that is associated with vitamin D deficiency.

Vitamin D_3 (cholecalciferol) is the natural form of vitamin D and the body can synthesise it in the skin in response to the sunlight exposure. Ultraviolet-B (UVB) (290-315 nm wavelength) skin irradiation initiates the photochemical conversion of 7DHC (provitamin D_3) to previtamin D_3 by breaking the 9,10 carbon-carbon bond which is then quickly thermally isomerized to vitamin D_3. Diet is another source of vitamin D_3, which can be obtained from animal foods such as oily fish, egg yolk, liver, butter, and fortified milk and cheese. Vitamin D_2 (ergocalciferol) originates from conversion of a plant sterol ergosterol and is solely obtained from the diet which includes plant-sourced foods such as yeast and mushrooms [4, 25]. Vitamin D_3 has a superior bioavailability than vitamin D_2; nonetheless they both go through the same metabolic pathway to produce the active hormonal forms [19, 24]. Vitamin D is biologically inert and has to undergo hydroxylation twice before it can perform its physiological functions. Vitamin D binding protein (DBP/GC) is the key transport protein which binds over 85% of the circulating 25(OH)D, and most vitamin D metabolites, and it transports theses metabolites to

target cells. In the liver, vitamin D (cholecalciferol and ergocalciferol) is converted by the enzyme 25-hydroxylase (*CYP2R1*) into 25-hydroxyvitamin D [25(OH)D], also known as calcidiol, which is the primary circulating form of vitamin D. Subsequently, the kidney, acting as an endocrine gland, converts 25(OH)D by the action of the enzyme 1α-hydroxylase (*CYB27B1*) to the active hormonal form 1α, 25-dihydroxyvitamin D [1,25$(OH)_2$D], also known as calcitriol, which then binds to *VDR* and regulates calcium homeostasis and bone metabolism (**Figure 2.1**). The *VDR*, a member of the nuclear receptor family, is a receptor specific to vitamin D through which vitamin D exerts its function, and it has been discovered in a multitude of cell membranes of tissues that have no musculoskeletal function, this implies the involvement of vitamin D in various extra skeletal biological functions [24, 26, 180].

Heritability of vitamin D deficiency has been reported by twin and family studies to range between 20% to 85% [121]. Although there is a great variation in the estimation of the heritability results, they do show that genetic factors play a role in circulating serum 25(OH)D levels. Candidate gene studies have reported several single nucleotide polymorphisms (SNPs) related to serum 25(OH)D levels mainly with genes that are involved in synthesis and metabolism of vitamin D such as *DHCR7*, *CYP2R1*, *CYP27B1*, *CYP24A1, DBP/GC*, *VDR* [120]. Genome wide association studies (GWAS) have confirmed the association between genetic polymorphisms in the genes such as *GC*, *DHCR7*, *CYP2R1* and *CYP24A1* and 25(OH)D concentrations [121].

There exists a plethora of studies that have reported association of genetic variants with low vitamin D levels and a wide spectrum of associated diseases [181-184]. This article aims to evaluate the results of the associations between vitamin D-related genetic variants and metabolic diseases such as obesity and type 2 diabetes (T2D). Understanding the possible underlying genetic factors of vitamin D metabolism will lead to an increased understanding of

the biological mechanisms underlying vitamin D deficiency and its effects on metabolic diseases.

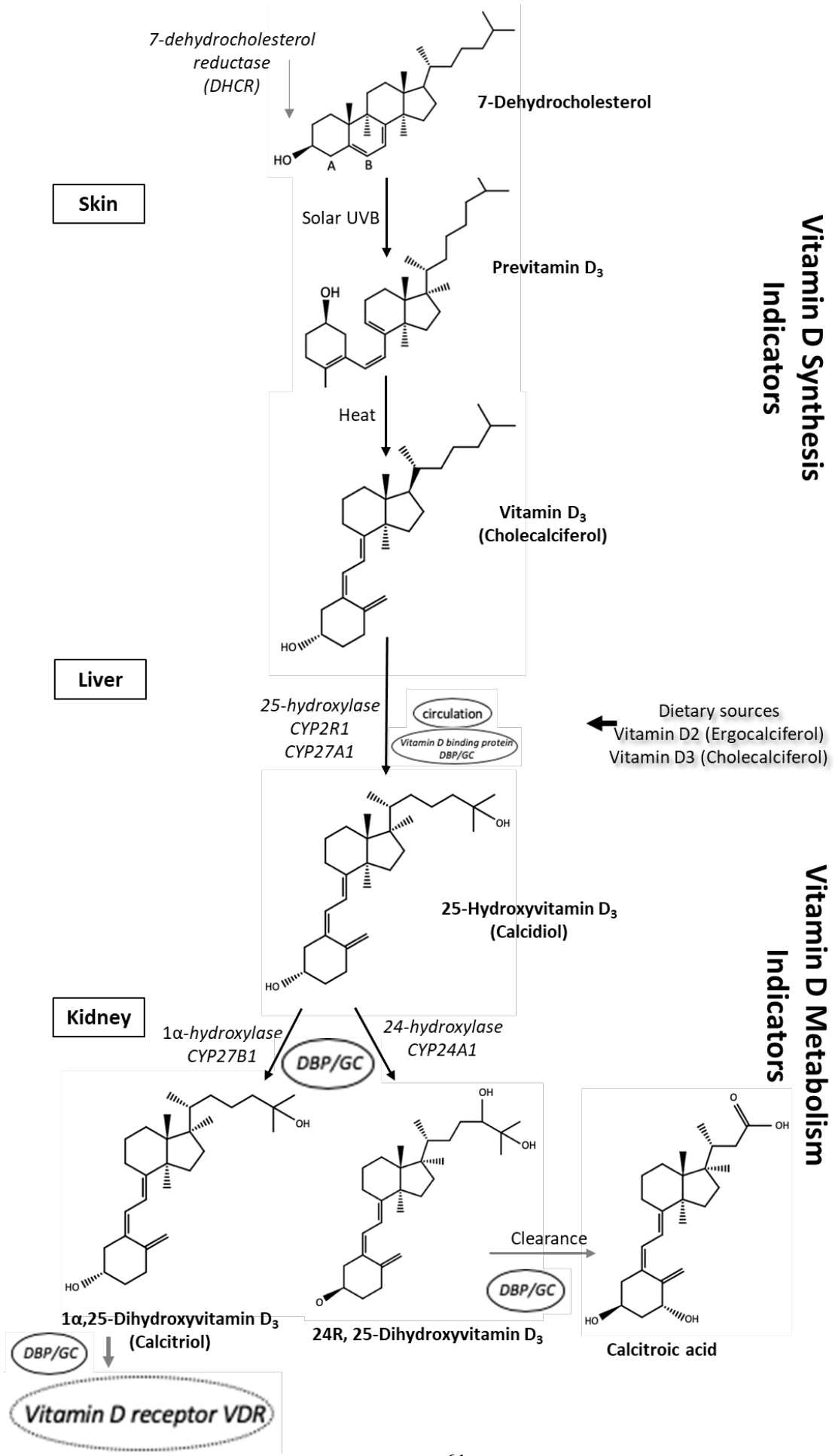

7-dehydrocholesterol reductase (DHCR)
7-Dehydrocholesterol
Skin
Solar UVB
Previtamin D3
Heat
Vitamin D3 (Cholecalciferol)
Liver
25-hydroxylase CYP2R1 CYP27A1
circulation
Vitamin D binding protein DBP/GC
Dietary sources
Vitamin D2 (Ergocalciferol)
Vitamin D3 (Cholecalciferol)
25-Hydroxyvitamin D3 (Calcidiol)
Kidney
1α-hydroxylase CYP27B1
24-hydroxylase CYP24A1
DBP/GC
1α,25-Dihydroxyvitamin D3 (Calcitriol)
24R, 25-Dihydroxyvitamin D3
Clearance
DBP/GC
Calcitroic acid
DBP/GC
Vitamin D receptor VDR
Vitamin D Synthesis Indicators
Vitamin D Metabolism Indicators

Figure 2. 1: Vitamin D synthesis and metabolism.

Upon exposure to Ultraviolet-B (290-315 nm wavelength) skin irradiation, 7-dehydrocholesterol produces previtamin D3, by breaking the C (9–10) bond at the B ring, which then undergoes a thermally induced rearrangement to form vitamin D_3. Vitamin D can also enter the body from dietary sources in two forms: vitamin D_3 (cholecalciferol) from fish, eggs, fortified milk and supplements and vitamin D_2 (ergocalciferol) from mushrooms and yeast. Once transported to the liver, vitamin D is hydroxylated to 25(OH)D (calcidiol) by 25-hydroxylase enzymes (*CYP2R1* & *CYP27A1*). In the kidneys, 25(OH)D is further hydroxylated by two enzymes to activate or inactivate vitamin D. For activation, the 1α-hydroxylase enzyme (*CYP27B1*) converts 25(OH)D to 1α,25-dihydroxyvitamin D (calcitriol), which is transported by vitamin D binding protein (*DBP*/GC). Finally, calcitriol binds to vitamin D receptor (*VDR*) to perform its biological function. For inactivation, the 24-hydroxylase enzyme (*CYP24A1*) catabolizes 25(OH)D to 24,25-dihydroxy vitamin D. Control of metabolism of vitamin D is exerted primarily by biliary excretion.

2.3 Methods and Materials

2.3.1 Study Identification

To review published research articles relevant to the topic, a literature search of PubMed (National Library of Medicine) https://www.ncbi.nlm.nih.gov/pubmed/ was performed up to December 2019. The following key terms were used to search for research articles: “vitamin D genetics and diabetes” (n = 543), “vitamin D genetics and obesity” (n = 202), “vitamin D gene polymorphisms and diabetes” (n = 308), “vitamin D gene polymorphisms and obesity” (n = 85), “Genetic variants of vitamin D and diabetes” (n = 79), “Genetic variants of vitamin D and obesity” (n = 31), “vitamin D SNPs and diabetes” (n =

150), “vitamin D SNPs and obesity” (n = 57). As a result of all the search combinations, a total of 1,455 articles were obtained. Citations from relevant papers and review papers were examined to identify additional relevant articles for inclusion.

2.3.2 Study Selection

Any study that was published in PubMed and written in English was included. Only genetic association studies examining the association of vitamin D-related SNPs with diabetes and/or obesity were included. Studies were excluded if they were (1) animal studies; (2) studies in pregnant women; (3) studies on humans identified with disease other than metabolic diseases or traits; (4) randomized controlled trials; (5) gene-vitamin D interaction studies, (6) haplotype studies, (7) studies with outcome as serum 25(OH)D, bone disease, metabolic syndrome, type 1 diabetes, diabetic complications or any other disease except for obesity and T2D.

The article titles were reviewed to eliminate duplication and relevant papers were chosen (n = 112). Abstracts of the chosen articles were read to further determine their relevance to our topic. After reading the full text of these papers, 73 articles were considered relevant and were included to extract the data for this review (**Figure 2.2**).

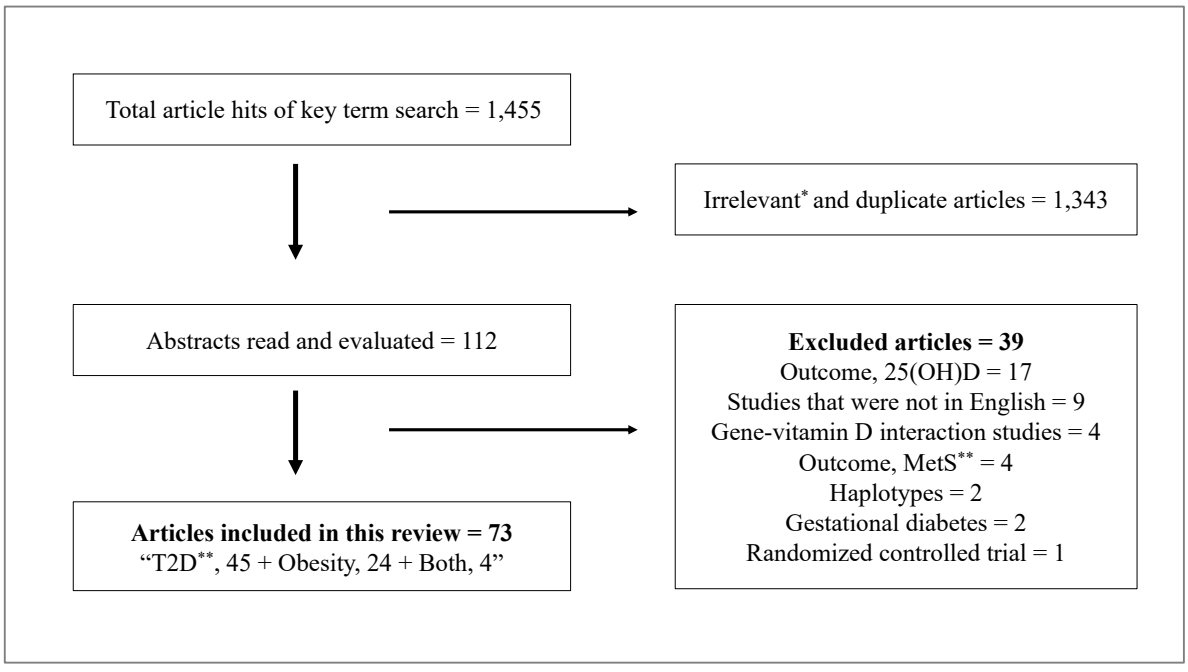


Figure 2. 2: Flow chart describing the literature search and the steps involved in selecting the articles.

* Irrelevant articles are those that did not meet the inclusion criteria. ** MetS: Metabolic Syndrome; T2D: Type 2 diabetes.

2.3.3 Data Extraction

The studies were identified by a single investigator (BA), and the following data were double-extracted independently by two reviewers (VK and AS): first author, publication year, location or ethnicity of participants, sample size, mean age, study design, SNP position, name and reference SNP (rs) ID, genotype and allele distribution for vitamin D. Corresponding authors were contacted to provide any additional information where needed.

This review will look at the genes that function upstream and influence 25(OH)D synthesis (*DHCR7, CYP2R1)* and genes that function downstream and play a role in 25(OH)D metabolism (*CYP24A1, CYP27B1, GC/DBP, VDR)* (**Figure 2.1**). The following sections will focus on the SNPs in the vitamin D pathway-related genes and their associations with obesity traits and T2D.

2.4 Genes Responsible for Vitamin D Synthesis

Despite several studies that have examined the association between vitamin D deficiency and metabolic diseases [181-184], the literature is remarkably scarce in studies investigating the association of genes involved in the synthesis of 25(OH)D with metabolic diseases such as obesity and T2D.

2.4.1 7-Dehydrocholesterol Reductase (*DHCR7*)

7-Dehydrocholesterol reductase enzyme is encoded by the *DHCR7* gene and is located on chromosome 11q13.4. The main function of *DHCR7* is to convert 7DHC to cholesterol [120]. 7DHC is a substrate of the enzyme *DHCR7* and is the precursor of vitamin D (specifically vitamin D_3); the conversion of 7DHC to vitamin D_3 in the skin is facilitated by exposure to UVB light from the sun which causes the cleavage of the C (9–10) bond in 7DHC to form vitamin D_3 [185]. The production of cholesterol from 7DHC in the skin reduces the availability of 7DHC for vitamin D synthesis which limits vitamin D production [186].

2.4.2 Cytochrome P450 Family 2 Subfamily R Member 1 / 25-Hydroxylase (*CYP2R1*)

The *CYP2R1* gene is located on chromosome 11q15.2. *CYP2R1* encodes 25-hydroxylase enzyme in the liver, which is the main enzyme responsible for the conversion of vitamin D_3 and vitamin D_2 into the main circulating form of vitamin D [25(OH)D] [187].

2.4.3 Obesity

Two cross-sectional studies investigated SNPs in the vitamin D synthesis-related genes; *DHCR7* SNP was examined in a small study (n = 323) of African ethnicity and *CYP2R1* SNP was studied in nearly 7,000 Chinese women [188, 189]. Nominal significant associations were reported with obesity traits in the Chinese study for *CYP2R1* rs10832313 polymorphism; however this did not remain significant after correction for multiple testing [188] and no significant associations were reported for *DHCR7* SNP rs12785878 in the African population [189]. There are two notable large studies by Vimaleswaran et. al. that examined SNPs from the *DHCR7* and *CYP2R1* vitamin D synthesis-related genes in relation to obesity using data from multiple Caucasian cohorts [145, 147]. One study was a bi-directional Mendelian Randomization study (n = 42,024) which showed that 10% genetically higher BMI was associated with 4.2% lower concentrations of 25(OH)D but no significant effect of vitamin D

allelic scores on obesity was reported [145]. The other study was a genetic association analysis that analyzed 5,224 participants from the 1958 British birth cohort (1958BC) and 123,865 individuals from the GIANT (Genetic Investigation of Anthropometric Traits) consortium. None of the vitamin D synthesis SNPs was significantly associated with obesity traits [147]. Although the number of studies that investigated the association between vitamin D synthesis genes and obesity were small; the lack of association found in the two very large meta-analysis studies [145, 147] does suggest that the vitamin D synthesis-related gene polymorphisms may not be a contributing factor to the development of obesity. However, further studies with large number of samples are required to confirm the role of these SNPs in obesity and its related traits.

2.4.4 Type 2 Diabetes

Studies using vitamin D synthesis-related gene polymorphisms suggest an association with low serum 25(OH)D concentrations and increased diabetes risk [190]. A recent prospective observational study in Italians (n = 2,163) demonstrated an association between *DHCR7* (rs12785878) and 25(OH)D concentrations (p = 1 x 10^{-4}) in T2D patients [191]. Furthermore, in a recent large Mendelian Randomization meta-analysis of 10 studies from European and Chinese populations (n = 58,312 cases and 370,000 controls), the allelic score of two SNPs from the vitamin D synthesis-related genes, *DHCR7* (rs12785878) and *CYP2R1* (rs10741657), were shown to be significantly associated with lower risk of T2D (p = 0.01) where a 25-nmol/l higher 25(OH)D concentration was associated with a 14% lower risk of diabetes [192]. A Mendelian Randomization analysis in 96,423 Danish individuals examined four genetic polymorphisms in the *DHCR7* and *CYP2R1* genes in relation to T2D [190], where the *DHCR7* allele score (rs11234027 + rs7944926) showed significant association with increased risk of T2D (p for trend = 0.04); but there were no significant associations between

CYP2R1 SNPs or allele scores and risk of diabetes [190]. In the Chinese Han population (n = 794), the 'G' allele carriers of the *CYP2R1* SNPs rs10766197 and rs1993116 had 1.64 and 1.76 times increased risk of developing T2D compared with 'AA' homozygotes, respectively (p = 0.024 and p = 0.048, respectively) [193]. However, the studies in 53,088 Germans and 4,877 Norwegians failed to show an association of SNPs in the *DHCR7* (rs12785878, rs3829251, rs3794060) and *CYP2R1* (rs10741657) genes with T2D [194, 195]. Although there is inconsistency in the results across various studies, a recent large Mendelian Randomization meta-analysis (n = 428,312) [192] has provided evidence using synthesis SNPs that genetically instrumented higher 25(OH)D has a protective effect against diabetes risk. However, more studies are needed to confirm this finding and understand the functional significance of vitamin D synthesis-related genes in T2D.

2.5 Genes Responsible for Vitamin D Metabolism

Several studies have examined the association of vitamin D metabolism-related SNPs with metabolic diseases; however, majority of the studies have been restricted to the *VDR* SNPs and only a few studies have investigated the association of genes involved in activation, catabolism and transport of 25(OH)D with metabolic diseases. Hence, *VDR*–related SNPs are discussed in a separate section.

2.5.1 I. Activation, Catabolism, and Transport Genes:

2.5.1.1 Cytochrome P450 Family 27 Subfamily B Member 1 / 1α-Hydroxylase (*CYP27B1*)

The *CYP27B1* gene is located on chromosome 12q14.1. The activating enzyme 1α-hydroxylase is encoded by the *CYP27B1* gene in the kidney where 25(OH)D is converted to the active 1α,25$(OH)_2$D which binds to the *VDR* to perform its biological functions [196, 197].

2.5.1.2 Cytochrome P450 Family 24 Subfamily A Member 1 / 24-Hydroxylase (*CYP24A1*)

The *CYP24A1* gene, which is located on chromosome 20q13.2, codes for the vitamin D inactivating enzyme 24-hydroxlase. This enzyme controls the levels of vitamin D in blood serum by breaking down the active form to biliary excretory products and by reducing intestinal absorption of calcium and phosphate. Mutations in the gene have been shown to result in hypercalcemia and nephrolithiasis [198, 199].

2.5.1.3 Vitamin D Binding Protein (*DBP*) / Group-Specific Component (*GC*)

The *DBP*/*GC* gene is located on chromosome 4q13.3. The *GC* gene encodes for vitamin D binding protein (DBP), which is a glycoprotein secreted by the liver that binds to vitamin D and its metabolites from the gut and skin and transports them to target tissues and organs and, hence, factors affecting DBP levels can also affect vitamin D concentrations. Nearly 85% of serum 25(OH)D is bound to DBP and the remainder 15% binds to albumin. Approximately 0.4% of total $1\alpha,25(OH)_2D_3$ and 0.03% of total $25(OH)D_3$ exist in the unbound, free form in the serum of healthy individuals (excluding pregnant women) [200, 201].

2.5.1.4 Obesity

To date, there has been only one study in Europeans (n = 5,224) [147] that has investigated the association of *CYP27B1* SNPs, rs1048691 and rs10877012, with obesity and this study did not find any significant association of these SNPs with obesity-related outcomes. The same study in = 5,224 Europeans also failed to show an association of 22 *CYP24A1* SNPs with obesity [147]. This is in line with another study in up to 700 Chinese women [188] which also did not show a significant association of the *CYP24A1* SNP rs2248359 with obesity traits after correction for multiple testing. For the *GC* gene, while a study in 5,224 participants from the 1958 British Birth Cohort failed to show an association of the 13 SNPs in the *GC* gene with

obesity outcomes [147], studies in Caucasian nuclear families [202] (n = 1,837), Bahraini population [203] (n = 406) and African population [189] (n = 323) showed significant associations of SNPs in the *GC* gene with obesity-related outcomes. Based on the Quantitative Transmission Disequilibrium Test in the Caucasian nuclear family study, it was shown that the *GC* SNP rs17467825 increased percent fat mass (PFM) by 1.42 times and the haplotype 'GAA' in the *GC gene* increased PFM by 1.19 times [202]. In the African population, the 'TT' genotype of the *GC* SNP rs2298849 was associated with 1.76 times increased risk of overweight [189]. Furthermore, the vitamin D metabolism-related genes were also analysed as a risk score in a Mendelian Randomization study in 42,024 Caucasians and there was no significant causal effect of the vitamin D metabolism-risk score on obesity [145].

Only a few studies have focused on the genes involved in activation, catabolism and transport of 25OHD of which there has been only one study on activation gene *CYP27B1* in relation to obesity [147]. Three studies have examined the catabolism gene *CYP24A1* SNPs; however, none of them showed a significant association with obesity traits [145, 147, 188]. Five studies included SNPs from the transport gene *GC* [145, 147, 189, 202, 203] where only three studies showed significant associations with obesity [189, 202, 203]. Given that majority of the studies failed to find an association of these SNPs with obesity outcomes, it is quite unlikely for the SNPs in the *CYP27B1*, *CYP24A1* and *GC* to have a significant functional role in obesity-related metabolic pathways.

2.5.1.5 Type 2 Diabetes

There have been only two studies [194, 204] that examined SNPs from vitamin D activating *CYP27B1* gene in relation to T2D and both the studies, the prospective case-cohort study in 53,088 Germans [194] and a cross-sectional study in 522 individuals from a Polish population [204], failed to show an association of *CYP27B1* SNP rs10877012 and SNP rs184712 with T2D, respectively. Five studies have explored the association of SNPs in

vitamin D catabolism *CYP24A1* gene with T2D [192, 194, 195, 205, 206]. Two Chinese case-control family-based studies (n = 1,560 & n = 1,556) examined *CYP24A1* SNPs rs2248359 and rs4809957 [205, 206]; while the study in 1,556 individuals showed no association of the SNP rs4809957 with T2D [206], the study in 1,560 individuals demonstrated an association of the SNP rs2248359 with T2D in women ($p = 0.036$) but not in men ($p = 0.816$) [205]. It was shown that the 'T' allele of the SNP rs2248359 was transmitted 1.39 times more in offspring of T2D participant compared to non-T2D participant ($p = 0.035$) suggesting that 'T' allele might be a risk factor for T2D.

Despite large sample size, three studies, one in Germans (n = 53,088) [194], the other in Norwegians (n = 4,877) [195], and a study in Chinese (n = 5,566) [192] failed to find an association between *CYP24A1* SNP rs6013897 and T2D. In the vitamin D transport gene, *GC*, significant associations were reported in Asians [207-209] but not in European populations such as Germans, Polish and Norwegians [192, 194, 195, 210]. However, a recent prospective observational study in Europeans recruited from Italian outpatient clinics did provide an evidence of association between *GC* (rs4588) and 25(OH)D concentrations ($p = 1 \times 10^{-6}$) in T2D patients [191]. The *GC* SNPs, rs7041 (codon 416) and rs4588 (codon 420), showed a significant association with T2D in Bangladeshi population (n = 211) [208]. The participants with Glu/Glu at codon 416 had 2.87 times increased risk of T2D and the participants with Lys/Lys genotype at codon 420 had 8.9 times increased risk of T2D. Furthermore, the combined allele score of these two SNPs, rs7041 and rs4588, was significantly associated with T2D in a case-control study in a Pakistani population (n = 330) [207]. In a meta-analysis of studies of Caucasians and Asians, there was no association in the overall analysis of SNPs at codon 416 and codon 420 with the risk of T2D, however, after stratification based on ethnicity, a significant association was found in Asians at codon 420, where the allele 'Lys' had a 1.49 times increased risk of T2D. In addition, a 1.36 times increased T2D risk was observed for

those with ‘Asp/Asp’ genotype at codon 416 compared to those with ‘Glu/Asp’ and ‘Glu/Glu’ genotype [209].

To date, most of the large studies failed to demonstrate a significant association of the SNPs in the *CYP27B1* and *CYP24A1* gene with T2D suggesting that these genes are unlikely to play a potential role in the pathogenesis of T2D. However, based on the published studies, genetic variants in *GC* gene may have an impact on T2D among Asians but not in Europeans, which could be due to the existence of genetic heterogeneity across the two ethnicities.

2.5.2 II. The Vitamin D Receptor (*VDR*):

The *VDR* gene is located on chromosome 12q13.11. Vitamin D receptor, encoded by *VDR* gene, is a member of the nuclear receptor of transcription factors. The secosteroid $1\alpha,25(OH)_2D_3$, a natural ligand to *VDR*, enters the target cell and binds to its *VDR* receptor. The $1\alpha,25(OH)_2D_3$-*VDR* complex heterodimerizes with the retinoid X receptor (*RXR*) and binds to the vitamin D response element (VDRE), a sequence of DNA nucleotides in the promoter region of the vitamin D regulated genes. The VDR/RXR-VDRE complex attracts coactivators and gene transcription is initiated to produce mRNA, which is then translated to the corresponding protein [211-213]. The *VDR* gene is predominantly expressed in kidneys, bones and the intestinal tract for bone homeostasis but further expression has been discovered in almost all human tissues and organs including adipose tissue and cells involved in the regulation of glucose metabolism, such as muscle and pancreatic cells [214]. Several functional *VDR* SNPs are known: BsmI, ApaI and Tru9I in intron 8, TaqI in exon 9 and FokI in exon 2. These genetic variants are named after their restriction enzyme sites [214]. Another *VDR* SNP is Cdx2, which is found in the promoter region [26]. Some SNPs in the *VDR* are restriction fragment length polymorphisms such as BsmI, ApaI and TaqI. They are closely linked to a microsatellite poly A repeat of variable length in the 3′UTR region which is thought to affect

VDR translation and may affect mRNA stability [26, 215]. *VDR* SNPs are the most commonly studied genetic variants with non-skeletal outcomes mainly in BsmI, ApaI and TaqI and FokI SNPs [164]. Genetic variants in the *VDR* gene have also shown to contribute to the genetic susceptibility of T2D by modulating insulin secretion and affecting cellular insulin sensitivity [162]. Allelic differences in the *VDR* gene are also possible contributor to obesity through modulating adipocyte function and affecting adipocyte inflammation [162, 164, 216].

2.5.2.1 Obesity

BsmI SNP rs1544410

Thirteen studies have examined the association between the *VDR* BsmI SNP and obesity-related traits (**Table 2.1**), of which nine of them have reported a significant association in the Arab, Brazilian, Polish, French, Swedish, and Vietnamese populations (n = 140-891) [217-225]. In the Arab population, associations have been consistent in four studies (n = 198 – n = 891) [217-219, 224] suggesting that the presence of the BsmI risk allele could be a risk factor for obesity in this ethnic group. Nevertheless, the sample sizes were relatively small; hence, larger studies are required in this population to confirm the risk of BsmI polymorphism on obesity.

ApaI SNP rs7975232

Twelve Studies have investigated the association between the *VDR* ApaI polymorphism and obesity traits (**Table 2.1**) and four of them have reported significant associations [220, 226-228] in the Chinese, Vietnamese and Czech populations (n = 140-882). Two studies in Asian postmenopausal women (n = 140; n = 260) reported significant associations with the ApaI variant (p = 0.036; p = 0.049, respectively) [220, 228], where the study in the postmenopausal Vietnamese women found that the ApaI risk allele ‘a’ had a 3 times increased risk of overweight and obesity [220]. Hence, ApaI risk allele may be an important factor predisposing individuals to adult onset obesity among Asian postmenopausal women;

however, further large studies are warranted in men and women to validate the role of the ApaI variant in this group.

TaqI SNP rs731236

Fourteen studies have examined the association between the *VDR* TaqI polymorphism and obesity traits (**Table 2.1**), of which six have shown significant association in several populations including Saudi, Czech, Greek, French, and Chinese (n = 184 - 891) [218, 219, 225, 226, 229, 230]. In a case-control study in the Chinese Han population (n = 529), the TaqI polymorphism showed a strong association with obesity ($p < 0.001$) [229] where the 't' allele was 2.67 times more prevalent in the obese group compared to the control group and the 'tt' genotype showed a 3.79 times increased risk of obesity. For the European and Arab populations, results have been largely inconsistent. Of the five studies in European population (n = 184 – 123,865), three reported an association between obesity traits and the TaqI polymorphism [225, 226, 230], while other studies failed to report a significant association (n = 701–123,865) [147, 231]. Similarly, in the Arab population, two studies reported an association of the TaqI polymorphism with obesity in Saudi individuals (n = 891; n = 300) [218, 219], where the minor 't' allele was significantly more frequent in the obese group compared to the control group ($p = 0.009$; $p = 0.041$, respectively). While three other studies in Arabs from Saudi, Bahrain, and UAE (n = 198 – 570) reported no significant association [203, 217, 224]. Although the results are inconsistent and conflicting, given that the majority of large studies failed to find an association, it is unlikely for the TaqI polymorphism to have a significant impact on obesity in Europeans or Arabs. However, due to existence of genetic heterogeneity, the polymorphism may have an effect on obesity in other ethnic groups such as Chinese population.

FokI SNP rs2228570

Twelve Studies have investigated the association between the *VDR* FokI polymorphism and obesity traits (**Table 2.1**) and only three of these have been consistent in reporting a significant association in Caucasian men (n = 302) and Czech (n = 517) and Chinese (n = 882) populations [226, 227, 232]. Studies in other ethnic groups such as Europeans, Asians, and Arabs (n = 140–1,773) failed to find an association of the FokI variant with obesity traits [217, 220, 222, 224, 225, 229, 231, 233, 234]. The overall evidence from these genetic epidemiological studies failed to support a consistent association of this polymorphism with obesity traits.

Cdx2 SNP rs11568820

Three Studies have investigated the association between the *VDR* Cdx2 SNP and obesity traits (**Table 2.1**). Two of these studies reported significant associations between Cdx2 SNP and obesity and its traits [233, 234]. A significant association of Cdx2 SNP with waist circumference (WC) and abdominal height (AH) ($p = 0.03$; $p = 0.05$, respectively) was shown in a cross-sectional study in American Caucasian women (n = 1,773) [234]; however, the association did not remain significant after Bonferroni correction. On the other hand, a family-based study of 400 nuclear Chinese families (n = 1,215) [233] reported significant associations with body mass index (BMI) ($p = 0.046$), fat mass (FM) ($p = 0.004$) and PFM ($p = 0.02$). Furthermore, the analysis in 415 sons showed that those with 'AA' genotype had 5.4% higher BMI, 18.8% higher FM and 14.8% higher PFM compared to those with 'AG' genotype. Nonetheless, data from two large cohorts, the 1958 British Birth Cohort (n = 5,224) and the GIANT Consortium (n = 123,865), failed to find an association between the Cdx2 polymorphism and obesity-related traits [147]. Even though the results are inconsistent regarding the effect of the *VDR* Cdx2 polymorphism on obesity traits, majority of the large studies in Caucasians have failed to find significant associations and hence it could be concluded that this polymorphism is unlikely to have an impact among the Caucasian

population. However, such large studies in other ethnic groups are required to confirm the role of this polymorphism in obesity.

Other *VDR* SNPs

Four studies have investigated other *VDR* SNPs; of which, two have shown significant association [226, 234], while the other two failed to report a significant association with obesity traits [147, 227]. A study in American Caucasian women (n = 1,773) showed a significant association of five *VDR* SNPs (rs739837, rs2239179, rs3819545, rs3782905, rs4760648) with obesity outcomes [234]. Another *VDR* SNP, EcoRV rs4516035 showed a significant association with sum of skin fold thickness (SSFT) (p = 0.02), where there was a 7.7 times decrease in SSFT among those with the 'GG' genotype compared to those with 'AA' genotype in 882 Czech individuals [226]. Given that the studies have been conducted in small number of samples, large studies are required to further elucidate the role of these SNPs in obesity.

2.5.2.2 Type 2 Diabetes

BsmI SNP rs1544410

Twenty-six studies have examined the association between the *VDR* BsmI polymorphism and T2D (**Table 2.2**), of which only seven have demonstrated a significant association in Arab, Indian, Chinese and German populations (n = 80–627) [97, 235-240]. The remaining fifteen studies failed to show a significant association in populations of similar ethnicities (n = 57–4,563) [217, 225, 241-253]. The meta-analysis studies have also shown inconsistent findings, where of the four meta-analyses in Asian and Caucasian populations (n = 2,608–6,274) [163, 254-256], two of the studies (n = 4,578; n = 6,274) showed a marginal association between BsmI SNP and risk of T2D (p = 0.033; p = 0.038, respectively) [254, 255]. Despite several studies have been carried out in multiple ethnic groups, the association between the *VDR* BsmI variant and T2D is still questionable. It is possible that the effect of gene-lifestyle interactions might mask the genetic effect in some of the populations and hence studies

focusing on gene-diet and gene-physical activity interactions are required to confirm this association.

ApaI SNP rs7975232

None of the fifteen studies have demonstrated a significant association between ApaI polymorphism and risk of T2D (n = 171–4,563) (**Table 2.2**) including the two meta-analyses that investigated the association in up to 3,871 individuals [163, 254]. However, there was one study that reported a borderline association (p = 0.058) in a Caucasian US population (n = 1,545) [247]. Furthermore, a study in 171 Bangladeshi participants had shown a significant association (p = 0.006) between ApaI SNP and insulin secretion index (ISI) [244]. Based on these studies, it can be concluded that it is unlikely that the *VDR* ApaI SNP has a significant role in the development of T2D.

TaqI SNP rs731236

Despite twenty-three studies have been carried out to explore the association between *VDR* TaqI polymorphism and T2D (**Table 2.2**), only one small study has reported a significant association in the Indian population (n = 80) where 't' allele had a 1.5 times increased risk of T2D [237]. Even the two large meta-analyses in Caucasians and Asians (n up to 3,826) have failed to show an association [163, 254]. Given that majority of the large studies failed to demonstrate a significant association, the *VDR* TaqI SNP might not be a strong candidate for T2D.

FokI SNP rs2228570

To date, eighteen studies have examined the association between the *VDR* FokI SNP and T2D (**Table 2.2**); five studies in the Saudi, Emirati, Egyptian and the Chilean populations (n = 100–4,077) [97, 217, 241, 243, 245] and three meta-analyses (n = 2,070 – 4,077) in Asians and Caucasians [163, 254, 255] have reported significant associations. Given that large meta-analysis studies have confirmed the association of the SNP with T2D, the *VDR* FokI SNP might

have an important role to play in T2D among Asians and Caucasians. Future meta-analyses should focus on other ethnic groups to identify the existence of genetic heterogeneity in the association between *VDR* FokI SNP and T2D.

Cdx2 SNP rs11568820

Only two studies have examined the association between the *VDR* Cdx2 SNP and T2D (**Table 2.2**); one in the Italian population (n = 1,788) where individuals with 'AA' genotype of the SNP had a 1.43 times increased risk of T2D compared to those with 'GG + GA' genotypes ($p = 0.002$) [257] and the other one in the Norwegian populations (n = 4,563), which failed to show an association with T2D and its related traits [253]. More studies in different ethnic groups are required to understand the role of the *VDR* Cdx2 SNP in T2D.

BglI SNP rs739837

Of the four studies that examined the association between the *VDR* BglI polymorphism and T2D (**Table 2.2**), two large case-control studies in the Chinese Han population (n = 1,191 and n = 3,714) reported a significant association of the BglI polymorphism with increased risk of T2D [252, 258]. However, there was no association between the BglI variant and risk of T2D in a smaller study in the Chinese Han (n = 420) and Chinese Hui (n = 269) populations [239] as well as no association was observed in the Caucasian population (1958 British Birth Cohort, n = 5,160) [160]. This inconsistency can be attributed to the sample size and the existence of genetic heterogeneity between the Caucasian and Chinese ethnic groups; but additional large studies are warranted to confirm or refute these findings.

Other *VDR* SNPs

A few studies have investigated other SNPs in the *VDR* gene; three studies reported a significant association of the *VDR* SNPs, rs2239179, rs7968585, rs2189480 and rs3847987, with T2D risk [252, 253, 259]. The two *VDR* SNPs, rs2189480 and rs3847987, were reported to have significant association ($p < 0.003$ and $p = 0.032$, respectively) with T2D in a Chinese

Henan population (n = 574) [259]. The *VDR* SNP rs2239179 was found to be significantly associated with increased risk of T2D (p = 0.049) in Chinese Han men who were above 55 years (n = 1,191) [252] and the *VDR* SNP rs7968585 showed a significant association with T2D (p = 0.044) in the Norwegian population (n = 4,563) [253]. These SNPs might have a functional importance in the pathogenesis of T2D which needs to be further investigated and evaluated by large scale studies in different populations.

2.6 Conclusion

In summary, our review has pooled all available data related to the association of vitamin D pathway genes with metabolic diseases such as obesity and T2D and has identified 57 significant associations of vitamin D pathway genes with obesity and its related traits such as BMI, WC, FM, SSFT, and AH in SNPs from 5 genes in the vitamin D pathway. Of the 57 associations, only one was from the vitamin D synthesis-related genes (*DHCR7*, *CYP2R1)*. The vast majority of the associations (56 associations) were identified in the vitamin D metabolism-related genes (*CYP24A1, GC* and *VDR*) and in particular the *VDR* gene SNPs showed 48 significant associations with obesity outcomes. In addition, our review has identified 35 significant associations in relation to T2D in SNPs from 7 genes in the vitamin D pathway. A similar pattern of association was seen where only five significant associations were reported for vitamin D synthesis-related genes (*DHCR7* and *CYP2R1)* as compared to the vitamin D metabolism-related genes (*CYP24A1, GC* and *VDR*), where 30 significant associations were observed, and in particular, 26 significant associations with T2D was seen for the *VDR* SNPs. The vitamin D synthesis-related genes *DHCR7* and *CYP2R1* have not been adequately investigated in relation to obesity and T2D. There is a gap in the research pertaining to the effect of vitamin D synthesis-related gene polymorphisms on obesity and T2D. For vitamin D metabolism-related genes, the literature is still lacking in several ethnic groups, and available

results are inconsistent. Understanding how genetics influence serum 25(OH)D levels is important for identifying persons at risk of vitamin D deficiency and improving the understanding of the observed association between vitamin D deficiency and several diseases. Large well-designed genetic association studies considering gene-environment interactions in multiple ethnic populations are necessary to improve the understanding of the role of vitamin D-related polymorphisms in metabolic diseases. Furthermore, functional characterization of the vitamin D-related SNPs is highly warranted to facilitate the understanding of the pathogenetic mechanisms of obesity and diabetes, which will provide the platform for developing strategies to prevent and treat metabolic diseases.

Table 2. 1: Studies that have investigated the association between Vitamin D-related gene polymorphisms and obesity-related traits.

Gene Symbol and Name/Locus	Chromosomal Location	SNP and nucleotide change (as per dbSNP)	Minor Allele Frequency	Study Design	Ethnicity and Sample Size	Age	Outcome Measure	Association *p*-Value	References
DHCR7 / NADSYN1 7-Dehydrocholesterol reductase / NAD Synthetase 1	11q13.4	rs12785878 G/A,T	T = 0.21	Cross-sectional	African n = 323	46 ± 12	Obesity	0.93 *	Foucan et al. [189]
			G = 0.22 – 0.40[ψ]	Meta-analysis	Caucasians n = 42,024	31.16 ± 0.4 – 74.86 ± 2.9	BMI	0.78 *	Vimaleswaran et al.[145]
CYP2R1 Cytochrome P450 Family 2 Subfamily R Member 1 (25-Hydroxylase)	11p15.2	rs10832313 A/G	G = 0.05	Cross-sectional	Chinese Women n = 6922	25 – 70	BMI	0.02 [α] (not significant after multiple testing)	Dorjgochoo et al. [188]
		rs10741657 A/G	G = 0.31 – 0.43[ψ]	Meta-analysis	Caucasians n = 42,024	31.16 ± 0.4 – 74.86 ± 2.9	BMI	0.30 *	Vimaleswaran et al.[145]
Synthesis Score *DHCR7 + CYP2R1*		rs12785878 + rs10741657		Meta-analysis	Caucasians n = 42,024	31.16 ± 0.4 – 74.86 ± 2.9	BMI	0.57 *	Vimaleswaran et al.[145]
CYP24A1 Cytochrome P450 Family 24 Subfamily A Member 1 (24-Hydroxylase)	20q13.2	rs2248359 C/T	T = 0.34	Cross-sectional	Chinese Women n = 6922	25 – 70	BMI	0.02 [α] (not significant after multiple testing)	Dorjgochoo et al. [188]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.26 [α]/0.16 [α]/ 0.48 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.29 [α]/ 0.86 [α]	Vimaleswaran et al. [147]
		rs6013897 T/A	A = 0.19 – 0.26[ψ]	Meta-analysis	Caucasian n = 42,024	31.16 ± 0.4 – 74.86 ± 2.9	BMI	0.61 *	Vimaleswaran et al.[145]
GC / VDBP	4q13.3	rs2282679 T/G	T = 0.30	Cross-sectional	Bahraini n = 406	34.07 ± 10.86	BMI	0.36 [α]	Almesri et al. [203]

Group-Specific Component / Vitamin D Binding Protein			G = 0.19 – 0.38[ψ]	Meta-analysis	Caucasian n = 42,024	31.16 ± 0.4 – 74.86 ± 2.9	BMI	0.91 *	Vimaleswaran et al.[145]
			G = 0.07	Cross-sectional	African n = 323	46 ± 12	Obesity	0.20 *	Foucan et al. [189]
		rs4588 (codon 420) G /A,T	G = 0.22	Cross-sectional	Bahraini n = 406	34.07 ± 10.86	BMI	0.43 α	Almesri et al. [203]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.32 α/0.72 α/ 0.72 α	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	--/ --	Vimaleswaran et al. [147]
		rs7041 (codon 416) A/C,T	C = 0.46	Cross-sectional	Bahraini n = 406	34.07 ± 10.86	BMI	0.007 α	Almesri et al. [203]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.89 α/0.24 α/ 1.00 α	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.66 α/ 0.43 α	Vimaleswaran et al. [147]
		rs2298849 A/G	A = 0.38	Cross-sectional	Bahraini n = 406	34.07 ± 10.86	BMI	0.993 α	Almesri et al. [203]
			G = 0.41	Cross-sectional	African n = 323	46 ± 12	Obesity	0.04 *	Foucan et al. [189]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	1.00 α/1.00 α/ 0.16 α	Vimaleswaran et al. [147]

			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.51 [α]/ 0.97 [α]	Vimaleswaran et al. [147]
		rs1491711 C/G	C = 0.33	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/ PFM	0.23 [α]/0.30 [α]/ 0.047 [α]	Jiang et al. [202]
		rs17467825 A/G	G = 0.27	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/ PFM	0.048 [α]/0.006 [α]/ 0.001 [α]	Jiang et al. [202]
		rs705117 C/T	C = 0.16	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/ PFM	0.32 [α]/0.037 [α]/ 0.11 [α]	Jiang et al. [202]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.53 [α]/0.78 [α]/ 0.72 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.51 [α]/ 0.65 [α]	Vimaleswaran et al. [147]
		rs222042 G/A	A = 0.07	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/ PFM	>0.05 [α]/>0.05 [α]/ >0.05 [α]	Jiang et al. [202]
		rs222040 G/A	G = 0.42	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/ PFM	>0.05 [α]/>0.05 [α]/ >0.05 [α]	Jiang et al. [202]
		rs222035 T/G	T = 0.43	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/ PFM	>0.05 [α]/>0.05 [α]/ >0.05 [α]	Jiang et al. [202]
		rs222003 C/A,G,T	C = 0.08	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/ PFM	>0.05 [α]/>0.05 [α]/ >0.05 [α]	Jiang et al. [202]

		rs16846971 T/A	T = 0.002	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/ PFM	>0.05 [α]/>0.05 [α]/ >0.05 [α]	Jiang et al. [202]
		rs222020 C/T	C = 0.16	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/PFM	>0.05 [α]/>0.05 [α]/ >0.05 [α]	Jiang et al. [202]
			——	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	1.00 [α]/1.00 [α]/ 0.16 [α]	Vimaleswaran et al. [147]
			——	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.15 [α]/ 0.44 [α]	Vimaleswaran et al. [147]
		rs16847015 C/A,T	A = 0.04	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/PFM	>0.05 [α] />0.05 [α]/ >0.05 [α]	Jiang et al. [202]
		rs1352843 T/C	C = 0.12	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/PFM	>0.05 [α]/>0.05 [α]/ >0.05 [α]	Jiang et al. [202]
		rs222029 G/A	G = 0.17	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/PFM	>0.05 [α] />0.05 [α]/ >0.05 [α]	Jiang et al. [202]
		rs3733359 G/A	A = 0.05	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/PFM	>0.05 [α]/>0.05 [α] / >0.05 [α]	Jiang et al. [202]
		rs16847036 A/G	G = 0.05	Cross-sectional (family-based)	US Caucasian n = 1,873	47.49 ± 16.41	BMI/FM/PFM	>0.05 [α]/>0.05 [α]/ >0.05 [α]	Jiang et al. [202]
Synthesis Score *GC + CYP24A1*		rs2282679 + rs6013897		Meta-analysis	Caucasian n = 42,024	31.16 ± 0.4 – 74.86 ± 2.9	BMI	0.67 [*]	Vimaleswaran et al.[145]
VDR	12q13.11	BsmI [φ]	b = 0.42	Case-control	Saudi	45.9 ± 14.5	Obesity/BMI/	0.04 [*]/0.08 [γ]/	Al-Daghri et al. [217]

Vitamin D Receptor		rs1544410 C/A,G,T (B/b)			n = 570		WC	0.57 $^{\gamma}$	
			b = 0.40	Case-control	Saudi n = 891	39.6 ± 12.8	Obesity	0.028 $^{\beta}$	Al-Daghri et al. [218]
			b = 0.28	Case-control	Saudi Men n = 300	27.25 ± 4.22	Obesity/BMI	0.04 $^{\beta}$/0.02 $^{\alpha}$	Al-Hazmi et al. [219]
			b = 0.37	Case-control	Czechs n = 882	48.3 ± 14.2	Obesity/WC/ SSFT/TBF	0.65 */0.055 $^{\alpha}$/ 0.71 $^{\alpha}$ /0.20 $^{\alpha}$	Bienertova-Vasku et al. [226]
			b = 0.37	Cross-sectional	Spanish n = 701	20.41 ± 2.48	BMI/FM/ PFM/VFL	0.87 $^{\alpha}$/0.86 $^{\alpha}$ / 0.90 $^{\alpha}$/0.93 $^{\alpha}$	Correa-Rodriguez et al. [231]
			b = 0.39	Cross-sectional	Brazilian n = 319	10.6 ± 1.4	BMI/BFM	0.03 $^{\alpha}$/0.24 $^{\alpha}$	Ferrarezi et al. [221]
			B = 0.41	Cross-sectional	Polish Men n = 176	51.99 ± 10.73	BM/BMI/ WHR/ WC	0.23 $^{\alpha}$/0.048 $^{\alpha}$/ 0.75 $^{\alpha}$/0.03 $^{\alpha}$	Filus et al. [222]
			B = 0.44	Cross-sectional	Arabs n = 198	21 ± 9	WC/ BMI/ WHR/PBF	0.08 $^{\delta}$/ 0.04 $^{\delta}$/ 0.1 $^{\delta}$/0.1 $^{\delta}$	Hasan et al. [224]
			b = 0.19	Cross-sectional	Malaysian n = 941	13	Obesity/Wt./ BMI/WC/ WHR/PBF	0.40 */0.18 $^{\alpha}$/ 0.26 $^{\alpha}$/0.16 $^{\alpha}$/ 0.69 $^{\alpha}$ /0.31 $^{\alpha}$	Rahmadhani et al. [215]
			B = 0.37	Cross-sectional	Polish Postmenopausal Women n = 351	55.43 ± 2.75	BMI/WC/ PTF/PVD	0.90 $^{\alpha}$/ 0.86 $^{\alpha}$/ 0.76 $^{\alpha}$/ 0.92 $^{\alpha}$	Tworowska-Bardzinska et al. [260]
			B = 0.39	Case-control	French n = 452	61.5 ± 14	BMI/PO	0.01 $^{\gamma}$/ 0.02 $^{\gamma}$	Ye et al. [225]
			B = 0.09	Cross-sectional	Vietnamese Postmenopausal Women	55.6 ± 3.8	Obesity	0.039 *	Binh et al. [220]

					n =140					
			B = 0.38	Cross-sectional	Swedish n = 153	29.6 ± 5.9	BMI/FM	0.09 [γ]/0.049 [γ]	Grundberg et al. [223]
		ApaI [φ] rs7975232 C/A (A/a)	a = 0.37	Case-control	Saudi n = 570	45.9 ± 14.5	Obesity/BMI/ WC	0.27[*]/0.18 [γ]/ 0.93 [γ]	Al-Daghri et al. [217]
			a = 0.38	Case-control	Saudi n = 891	39.6 ± 12.8	Obesity	0.10 [α]	Al-Daghri et al. [218]
			a = 0.41	Case-control	Saudi Men n = 300	27.25 ± 4.22	Obesity/BMI	0.32 [α]/0.42 [α]	Al-Hazmi et al. [219]
			a = 0.49	Case-control	Czechs n = 882	48.3 ± 14.2	Obesity/WC/ SSFT/TBF	0.2 [*]/0.007 [α]/ 0.31 [α]/0.56 [α]	Bienertova-Vasku et al. [226]
			A = 0.36	Case-control	Chinese Han n = 529	54.38 ± 11.08	Obesity	0.21 [β]	Fan et al. [229]
			a = 0.41	Cross-sectional	Brazilian n = 319	10.6 ± 1.4	BMI/BFM	>0.05 [α]/>0.05 [α]	Ferrarezi et al. [221]
			A = 0.28	Cross-sectional (family-based)	Chinese 415 sons	30.4 ± 6.1	BMI/ FM/PFM	0.99 [α]/0.83 [α]/ 0.57 [α]/0.38 [α]	Gu et al. [233]
			a = 0.40	Case-control	Mexican n = 250	47.3 ± 7.8	BMI	0.36 [β]	Rivera-Leon et al. [261]
			a = 0.46	Case-control	French n = 452	61.5 ± 14	BMI/PO	0.09 [γ]/0.09 [γ]	Ye et al. [225]
			A = 0.33	Cross-sectional	Vietnamese Postmenopausal Women n =140	55.6 ± 3.8	Obesity	0.036 [*]	Binh et al. [220]

			A =0.28	Cross-sectional	Chinese Postmenopausal Women n = 260	57.9	BMI	0.049 [ε]	Xu et al. [228]
			a = 0.25	Cross-sectional	Chinese Han n = 517	18 – 90	BMI/WC/ PBF/TSFT	>0.05 [*]/>0.05 [*]/ 0.02 [α]/<0.001 [α]	Shen et al. [227]
		TaqI [φ] rs731236 A/G (T/t)	t = 0.44	Case-control	Saudi n = 570	45.9 ± 14.5	Obesity/BMI/ WC	0.32 [*]/0.26 [γ]/ 0.94 [γ]	Al-Daghri et al. [217]
			t = 0.41	Case-control	Saudi n = 891	39.6 ± 12.8	Obesity	0.009 [α]	Al-Daghri et al. [218]
			t = 0.42	Case-control	Saudi Men n = 300	27.25 ± 4.22	Obesity/BMI	0.04 [α] /0.048 [α]	Al-Hazmi et al. [219]
			t = 0.35	Cross-sectional	Bahraini n = 406	34.07 ± 10.86	BMI	0.98 [α]	Almesri et al. [203]
			t = 0.37	Case-control	Czechs n = 882	48.3 ± 14.2	Obesity/WC/ SSFT/TBF	0.034 [*]/0.035 [α]/ 0.35 [α]/0.88 [α]	Bienertova-Vasku et al. [226]
			t = 0.36	Cross-sectional	Spanish n = 701	20.41 ± 2.48	BMI/FM/ PFM/VFL	0.90 [α]/0.83 [α]/ 0.88 [α] /0.93 [α]	Correa-Rodriguez et al. [231]
			t = 0.10	Case-control	Chinese Han n = 529	54.38 ± 11.08	Obesity	<0.001 [β]	Fan et al. [229]
			t = 0.40	Cross-sectional	Brazilian n = 319	10.6 ± 1.4	BMI/BFM	>0.05 [α]/>0.05 [α]	Ferrarezi et al. [221]
			t = 0.39	Cross-sectional	Arabs n = 198	21 ± 9	WC/ BMI/ WHR/PBF	0.55 [δ]/ 0.58 [δ]/ 0.9 [δ]/0.55 [δ]	Hasan et al. [224]
			t = 0.44	Case-control	Mexican n = 250	47.3 ± 7.8	BMI	0.80 [β]	Rivera-Leon et al. [261]

			t = 0.39	Case-control	Greek n = 184	68.23 ± 8.99	Obesity/Waist	0.019 β/0.87 α	Vasilopoulos et al. [230]
			t = 0.39	Case-control	French n = 452	61.5 ± 14	BMI/PO	0.017 γ/0.015 γ	Ye et al. [225]
			t = 0.06	Cross-sectional	Vietnamese Postmenopausal Women n =140	55.6 ± 3.8	Obesity	0.12 *	Binh et al. [220]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.39 α/0.70 α/ 0.72 α	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.10 α/ --	Vimaleswaran et al. [147]
		FokI φ rs2228570 A/C,G,T (F/f)	F = 0.27	Case-control	Saudi n = 570	45.9 ± 14.5	Obesity/BMI/ WC	0.23 */0.42 γ/ 0.08 γ	Al-Daghri et al. [217]
			F = 0.43	Case-control	Czechs n = 882	48.3 ± 14.2	Obesity/WC/ SSFT/TBF	0.055 */0.06 α/ 0.046 α/0.003 α	Bienertova-Vasku et al. [226]
			f = 0.37	Cross-sectional	Spanish n = 701	20.41 ± 2.48	BMI/FM/PFM/ VFL	0.07 α/0.34 α/ 0.02 α/0.43 α	Correa-Rodriguez et al. [231]
			f = 0.35	Case-control	Chinese Han n = 529	54.38 ± 11.08	Obesity	0.36 β	Fan et al. [229]
			f = 0.39	Cross-sectional	Polish men n = 176	51.99 ± 10.73	BM/BMI/ WHR/ WC	0.37 α/0.87 α/ 0.52 α/0.47 α	Filus et al. [222]
			f = 0.48	Cross-sectional (family-based)	Chinese n = 415 sons	30.4 ± 6.1	BMI/FM/ PFM	0.42 α/0.24 α/ 0.05 α	Gu et al. [233]
			f = 0.23	Cross-sectional	Arabs n = 198	21 ± 9	WC/ BMI/ WHR/PBF	0.35 δ/ 0.68 δ/ 0.86 δ/0.66 δ	Hasan et al. [224]

			f = 0.46	Cross-sectional	Vietnamese Postmenopausal Women n =140	55.6 ± 3.8	Obesity	0.15 *	Binh et al. [220]
			f = 0.38	Cross-sectional	Caucasians Men n = 302	72.8 ± 0.8	BMI/FM/ PBF	0.01 ε /0.07 ε / 0.27 ε	Roth et al. [232]
			F = 0.38	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.43 α /0.67 α / 0.27 α	Ochs-Balcom et al. [234]
			f = 0.47	Cross-sectional	Chinese Han n = 517	18 – 90	BMI/WC/ PBF/TSFT	>0.05 */>0.05 */ 0.007 α /0.05 α	Shen et al. [227]
		Cdx2 rs11568820 C/T	T = 0.48	Cross-sectional (family-based)	Chinese 415 sons	30.4 ± 6.1	BMI/FM/ PFM	0.006 α/0.004 α/ 0.002 α	Gu et al. [233]
			T = 0.20	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.09 α/0.03 α/ 0.05 α (WC and AH did not remain significant after Bonferroni correction)	Ochs-Balcom et al. [234]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.16 α/0.31 α/ 0.08 α	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.57 α/ 0.72 α	Vimaleswaran et al. [147]
		EcoRV rs4516035 T/C	C = 0.46	Case-control	Czechs n = 882	48.3 ± 14.2	Obesity/WC/ SSFT/TBF	0.67 */0.49 α/ 0.02 α /0.39 α	Bienertova-Vasku et al. [226]

			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.39 [α]/0.49 [α]/ 0.48 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.19 [α]/ 0.11 [α]	Vimaleswaran et al. [147]
		BglI rs739837 G/C,T	G = 0.47	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.20 [α]/0.16 [α]/ 0.03 [α]	Ochs-Balcom et al. [234]
			G = 0.46	Cross-sectional	British n = 5,160	45	Obesity/BMI/ WC/WHR	0.83 [*]/0.43 [α]/ 0.84 [α]/0.30 [α]	Vimaleswaran et al. [160]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.26 [α]/0.29 [α]/ 0.72 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.94 [α]/ 0.28 [α]	Vimaleswaran et al. [147]
		Tru9I rs757343 C/T	T = 0.13	Case-control	French n = 452	61.5 ± 14	BMI/PO	0.15 [γ]/0.49 [γ]	Ye et al. [225]
		rs1540339 C/T	T = 0.37	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.56 [α]/0.23 [α]/ 0.24 [α]	Ochs-Balcom et al. [234]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.48 [α]/0.48 [α]/ 0.16 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.25 [α]/ 0.54 [α]	Vimaleswaran et al. [147]
		rs2239179 T/C	C = 0.43	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.10 [α]/0.04 [α]/ 0.02 [α] (WC and AH did not remain	Ochs-Balcom et al. [234]

								significant after Bonferroni correction)	
			C = 0.44	Cross-sectional	British n = 5,160	45	Obesity/BMI/ WC/WHR	0.83 [*]/0.23 [α]/ 0.42 [α]/0.63 [α]	Vimaleswaran et al. [160]
			G = 0.22	Cross-sectional	Chinese Han n = 517	18 – 90	BMI/WC/ PBF/TSFT	>0.05 [*]/>0.05 [*]/ 0.56 [α]/0.001 [α]	Shen et al. [227]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.48 [α]/0.70 [α]/ 0.29 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.47 [α]/ 0.64 [α]	Vimaleswaran et al. [147]
		rs12721377 T/C	T = 0.08	Cross-sectional	Bahraini n = 406	34.07 ± 10.86	BMI	0.32 [α]	Almesri et al. [203]
		rs2189480 G/T	G = 0.34	Cross-sectional	Chinese Han n = 517	18 – 90	BMI/WC/ PBF/TSFT	>0.05 [*]/>0.05 [*]/ 0.15 [α]/0.07 [α]	Shen et al. [227]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	1.00 [α]/0.11 [α]/ 0.08 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.61[α]/ 0.70 [α]	Vimaleswaran et al. [147]
		rs3819545 A/G	G = 0.39	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.04 [α]/0.02 [α]/ 0.05 [α]	Ochs-Balcom et al. [234]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.67 [α]/0.59 [α]/ 0.48 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.65 [α]/ 0.55 [α]	Vimaleswaran et al. [147]

		rs3782905 G/C	G = 0.33	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.001 [α]/0.001 [α]/ 0.001 [α]	Ochs-Balcom et al. [234]
		rs2239186 A/C,G	G = 0.21	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.23 [α]/0.07 [α]/ 0.58 [α]	Ochs-Balcom et al. [234]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.64 [α]/0.37 [α]/ 0.29 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.73 [α]/ 0.85 [α]	Vimaleswaran et al. [147]
		rs2853564 G/A	G = 0.39	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.84 [α]/0.70 [α]/ 0.19 [α]	Ochs-Balcom et al. [234]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.12 [α]/0.22 [α]/ 0.48 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.09 [α]/ 0.70 [α]	Vimaleswaran et al. [147]
		rs4760648 C/A,G,T	T = 0.42	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.30 [α]/0.16 [α]/ 0.04 [α]	Ochs-Balcom et al. [234]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	1.00 [α]/0.98 [α]/ 0.16 [α]	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.09 [α]/ 0.58 [α]	Vimaleswaran et al. [147]

		rs3890734 G/A	A = 0.33	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.53 $^{\alpha}$/0.36 $^{\alpha}$/ 0.08 $^{\alpha}$	Ochs-Balcom et al. [234]
		rs7136534 C/T	T = 0.24	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.07 $^{\alpha}$/0.07 $^{\alpha}$/ 0.11 $^{\alpha}$	Ochs-Balcom et al. [234]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	0.21 $^{\alpha}$/0.29 $^{\alpha}$/ 0.08 $^{\alpha}$	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.44 $^{\alpha}$/ 0.77 $^{\alpha}$	Vimaleswaran et al. [147]
		rs10783210 T/A,G	T = 0.36	Cross-sectional	American Women n = 1,773	57.2	BMI/WC/AH	0.19 $^{\alpha}$ /0.26 $^{\alpha}$/ 0.66 $^{\alpha}$	Ochs-Balcom et al. [234]
		rs7299460 C/T	T = 0.29	Cross-sectional	American Caucasian Women n = 1,773	57.2	BMI/WC/AH	0.10 $^{\alpha}$/0.12 $^{\alpha}$/ 0.09 $^{\alpha}$	Ochs-Balcom et al. [234]
			____	Cross-sectional	British n = 5,224	45	BMI/WC/ WHR	1.00 $^{\alpha}$ /0.08 $^{\alpha}$ / 1.00 $^{\alpha}$	Vimaleswaran et al. [147]
			____	Cross-sectional	Europeans n = 123,865	30 -79	BMI/WHR	0.59 $^{\alpha}$/ 0.91$^{\alpha}$	Vimaleswaran et al. [147]

Abbreviations: BMI: Body mass index, WC: Waist circumference, AH: Abdominal Height, SSFT: Sum of skin fold thickness, TBF: Total body fat, FM: Fat mass, PFM: Percentage fat mass, VF: Visceral fat, BM: Body mass, WHR: Waist hip ratio, PBF: Percent body fat, PTF: Percent total fat, PVD: Percent visceral deposit, TSFT: Triceps skinfold thickness. 81 Tag SNPs that were investigated in the study by Vimaleswaran et al.

[147] were not listed in this table because they showed no significant association with obesity traits and were not examined in other studies. $^{\varphi}$ Through literature reviewing we found that describing the genotype of *VDR* was very confusing. We decided on using the initial letter of the restriction enzyme to name the different alleles instead of using the nucleotide base letter for BsmI, ApaI, TaqI and FokI. *Logistic regression; $^{\alpha}$ Linear regression; $^{\beta}$ Chi square test; $^{\gamma}$ ANOVA; $^{\delta}$ Kruskal-Wallis test; $^{\varepsilon}$ ANCOVA; $^{\Psi}$ Indicates range value.

Table 2. 2: Studies that have investigated the association between Vitamin D-related gene polymorphisms and diabetes-related traits.

Gene Symbol and Name/Locus	Chromosomal Location	SNP and nucleotide change	Minor Allele Frequency	Study Design	Ethnicity and Sample Size	Age	Outcome Measure	Association *p*-Value	References
DHCR7 / NADSYN1 7-Dehydrocholesterol reductase / NAD Synthetase 1	11q13.4	rs11234027 G/A	A = 0.20	Cross-sectional	Danish n = 96,423	20-100	T2D	0.11 *	Afzal et al. [190]
		rs7944926 A/G	A = 0.32	Cross-sectional	Danish n = 96,423	20-100	T2D	0.03 *	Afzal et al. [190]
		rs12785878 G/A,T	G = 0.27	Case-cohort	German n = 53,088	35-65	T2D	0.28 *	Buijsse et al. [194]
			T = 0.46	Cross-sectional	Chinese n = 82,464 (sub-group)	51 ±10.6	T2D	>0.05 *	Lu et al. [192]
		rs3829251 G/A	A = 0.17	Case-cohort	German n = 53,088	35-65	T2D	0.22 *	Buijsse et al. [194]
		rs3794060 C/T	C = 0.39	Case-cohort	Norwegians n = 4,877 (sub-group)	62.9 ± 12.45	T2D	> 0.05 *	Jorde et al. [195]
***DHCR7* allele score**		rs11234027 + rs7944926		Cross-sectional	Danish n = 96,423	20-100	T2D	0.04 *	Afzal et al. [190]
CYP2R1 Cytochrome P450 Family 2 Subfamily R Member 1 (25-Hydroxylase)	11p15.2	rs10741657 A/G	A = 0.42	Cross-sectional	Danish n = 96,423	20-100	T2D	0.78 *	Afzal et al. [190]

			A = 0.38	Case-control	Chinese Han n = 794	59.53 ± 11.95	T2D	0.60 $^{\beta}$	Wang et al. [193]
			A = 0.39	Case-cohort	German n = 53,088	35-65	T2D	0.72 *	Buijsse et al. [194]
			A= 0.42	Case-cohort	Norwegians n = 4,877 (sub-group)	62.9 ± 12.45	T2D	> 0.05 *	Jorde et al. [195]
			A = 0.36	Cross-sectional	Chinese n = 82,464 (sub-group)	51 ± 10.6	T2D	>0.05 *	Lu et al. [192]
		rs12794714 G/A	A = 0.41	Cross-sectional	Danish n = 96,423	20-100	T2D	0.93 *	Afzal et al. [190]
			A = 0.41	Case-control	Chinese Han n = 794	59.53 ± 11.95	T2D	0.09 $^{\beta}$	Wang et al. [193]
		rs10766197 G/A,C	A = 0.39	Case-control	Chinese Han n = 794	59.53 ± 11.95	T2D	0.024 $^{\beta}$	Wang et al. [193]
		rs1993116 A/G	A = 0.38	Case-control	Chinese Han n = 794	59.53 ± 11.95	T2D	0.048 $^{\beta}$	Wang et al. [193]
***CYP2R1* allele score**		rs10741657 + rs12794714		Cross-sectional	Danish n = 96,423	20-100	T2D	0.84 *	Afzal et al. [190]
Synthesis Score *DHCR7 + CYP2R1*		rs12785878 + rs10741657		Cross-sectional	Chinese n = 82,464 (sub-group)	51 ±10.6	T2D	>0.05 *	Lu et al. [192]
Synthesis Score *DHCR7 + CYP2R1*		rs12785878 + rs10741657		Meta-analysis	Chinese & European n = 428,904	____	T2D	0.01*	Lu et al. [192]
CYP27B1	12q14.1	rs10877012 G>C / G>T	T = 0.33	Case-cohort	German n = 53,088	35-65	T2D	0.77 *	Buijsse et al. [194]

Cytochrome P450 Family 27 Subfamily B Member 1 (1α-Hydroxylase)									
		rs184712 C/T	C = 0.18	Case-control	Polish n = 522	56.9 ± 11.8	T2D	0.65 [β]	Malecki et al. [204]
		Intron 6	C = 0.34	Case-control	Polish n = 522	56.9 ± 11.8	T2D	0.67 [β]	Malecki et al. [204]
CYP24A1 Cytochrome P450 Family 24 Subfamily A Member 1 (24-Hydroxylase)	20q13.2	rs6013897 T/A	A = 0.20	Case-cohort	German n = 53,088	35-65	T2D	0.56 [*]	Buijsse et al. [194]
			A = 0.23	Case-cohort	Norwegians n = 4,877 (sub-group)	62.9 ± 12.45	T2D	>0.05 [*]	Jorde et al. [195]
			T = 0.84	Cross-sectional	Chinese n = 82,464 (sub-group)	51 ±10.6	T2D	>0.05 [*]	Lu et al. [192]
		rs4809957 A/G	A = 0.34	Case-control (family-based)	Chinese n = 1,556	59.4	T2D	0.65 [β]	Yu et al. [206]
		rs2248359 C/T	T = 0.38	Case-control (family-based)	Chinese n = 1,560	50.77 ± 17.07	T2D	0.036 [η] (women)	Yu et al. [205]
GC / VDBP Group-Specific Component / Vitamin D Binding Protein	4q13.3	rs2282679 T/G	G = 0.28	Case-cohort	German n = 53,088	35-65	T2D	0.99 [*]	Buijsse et al. [194]

			G = 0.30	Cross-sectional	Chinese n = 82,464 (sub-group)	51 ±10.6	T2D	>0.05 *	Lu et al. [192]
		rs1155563 T/A,C	C = 0.28	Case-cohort	German n = 53,088	35-65	T2D	0.61 *	Buijsse et al. [194]
		rs7041 (codon 416) A/C,T	C = 0.43	Case-control	Bangladeshi n = 211	39.7 ± 1.5	T2D	< 0.05 β	Rahman et al. [208]
			G = 0.43	Case-control	Polish n = 393	56.4 ± 13.4	T2D	0.28 β	Malecki et al. [210]
			____	Meta-analysis	Asian & Caucasian n = 2,073	____	T2D	>0.05 *	Wang et al. [209]
		rs4588 (codon 420) G/A,T	A = 0.27	Case-control	Bangladeshi n = 211	39.7 ± 1.5	T2D	< 0.01 β	Rahman et al. [208]
			A = 0.30	Case-control	Polish n = 393	56.4 ± 13.4	T2D	0.52 β	Malecki et al. [210]
			____	Meta-analysis	Asian & Caucasian n = 2,073	____	T2D	>0.05 *	Wang et al. [209]
			____	Meta-analysis	Asian n = 922 (sub-group)	____	T2D	<0.05 *	Wang et al. [209]
		rs2298850 G/C	C = 0.24	Case-cohort	Norwegians n = 4,877 (sub-group)	62.9 ± 12.45	T2D	> 0.05 *	Jorde et al. [195]

***GC* allele score**		rs7041 (codon 416) + rs4588 (codon 420)		Case-control	Pakistani n = 330	47.6 ± 9	T2D	< 0.05 *	Iqbal et al. [207]
Synthesis & metabolism Score *DHCR7 + CYP2R1 + CYP24A1 + GC*		rs12785878 + rs10741657 + rs6013897 + rs2282679		Cross-sectional	Chinese n = 82,464 (sub-group)	51 ±10.6	T2D	>0.05 *	Lu et al. [192]
Synthesis & metabolism Score *DHCR7 + CYP2R1 + CYP24A1 + GC*		rs12785878 + rs10741657 + rs6013897 + rs2282679		Meta-analysis	Chinese & European n = 428,904	____	T2D	0.07 *	Lu et al. [192]
VDR Vitamin D Receptor	12q13.11	BsmI φ rs1544410 C/A,G,T (B/b)	b = 0.41	Case-control	Saudi n = 627	47.8 ± 9.3	T2D	<0.001 *	Al-Daghri et al. [235]
			b = 0.42	Case-control	Saudi n = 570	45.9 ± 14.5	T2D/BG	0.11*/0.15 γ	Al-Daghri et al. [217]
			b = 0.30	Case-control	Chilean n = 310	60-79	T2D	0.92 *	Angel et al. [241]
			B = 0.41	Case-control	North Indian n = 260	49.32 ± 10.97	T2D	0.21 *	Bid et al. [242]
			B = 0.45	Case-control	Bangladeshi n = 171	45.9± 10.3	ISI	0.23 *	Hitman et al. [244]
			b = 0.24	Case-control	Egyptian n = 190	47.84 ± 6.75	T2D	0.95 β	Mackawy et al. [245]
			b = 0.31	Case-control	Kashmiri n = 200	48.1 ± 9.9	T2D	0.0001 *	Malik et al. [236]

			B = 0.48	Case-control	German n = 293	61.5 ± 9.9	T2D	0.002 β	Ortlepp et al. [238]
			B = 0.36	Case-control	Polish n = 548	56.9 ± 12.2	T2D	0.29 β	Malecki et al. [246]
			b = 0.47	Case-control	Emirati n = 355	54.1 ± 11.9	T2D	0.031 *	Safar et al. [97]
			B = 0.42	Case-control	Caucasian n = 1,545	70.3 ± 8.9	T2D	> 0.05 β	Oh et al. [247]
			b = 0.39	Case-control	Indian n = 60	49.05 ± 9.26	T2D	0.44 β	Sarma et al. [248]
			____	Case-control	Czechs n = 234 (sub-group)	56 ± 9.88	T2D	> 0.05 *	Vedralova et al. [251]
			Hui b = 0.12 Han b = 0.09	Case-control	Chinese Hui n = 269 Chinese Han n = 420	____	T2D	0.68 β 0.028 β	Xu et al. [239]
			B = 0.39	Case-control	French n = 452	61.5 ± 14	T2D	0.96 β	Ye et al. [225]
			b = 0.05	Case-control	Chinese Han n = 1,191	59.55 ± 11.96	T2D	0.84 β	Yu et al. [252]
			b = 0.13	Case-control	Chinese Han n = 404	56.5 ± 10.7	T2D	0.015 β	Zhang et al [240]
			____	Case-control	Norwegians n = 4,563 (sub-group)	62.5 ± 12.5	T2D	> 0.05 ζ	Zostautiene et al. [253]
			b = 0.34	Case-control	Indian n = 80	45.5 ± 11.5	T2D	< 0.05 *	Mukhopadhyaya et al. [237]

			B = 0.46	Case-control	Indian Gujarati n = 57	> 45	T2D	> 0.05 $^{\beta}$	Shah et al. [249]
			B = 0.40	Case-control	Caucasians n = 187	23 - 83	T2D	> 0.05 $^{\beta}$	Speer et al [250]
			b = 0.40	Case-control	Egyptian n = 100	51.74 ± 7.38	T2D	0.11 *	Gendy et al. [243]
			B = 0.06 – b = 0.46 $^{\Psi}$	Meta-analysis	Asian & Caucasian n = 2,608	____	T2D	0.23 *	Zhu et al. [256]
			b = 0.05 – B = 0.42 $^{\Psi}$	Meta-analysis	Caucasian & East Asian n = 4,578 (sub-group)	36.0 ± 4.9 – 71.7 ± 8.6	T2D	0.033 * (reported marginally significant)	Wang et al. [254]
			B = 0.10 – b = 0.46 $^{\Psi}$	Meta-analysis	Asian & Caucasian n = 3,314 (sub-group)	40 - 62	T2D	0.70 *	Li et al. [163]
			b = 0.05 – 0.42 $^{\Psi}$	Meta-analysis	Chinese & European n = 6,274 (sub-group)	36.0 ± 4.9 – 71.7 ± 8.6	T2D	0.038 * (reported marginally significant)	Yu et al. [255]
		ApaI $^{\varphi}$ rs7975232 C/A (A/a)	a = 0.38	Case-control	Saudi n = 627	47.8 ± 9.3	T2D	0.05 * (not reported significant)	Al-Daghri et al. [235]
			a = 0.37	Case-control	Saudi n = 570	45.9 ± 14.5	T2D/BG	0.58 */0.42 $^{\gamma}$	Al-Daghri et al. [217]

			a = 0.39	Case-control	Turkish n = 241	56.6 ± 8.8	T2D/FPG /HbA1c	0.48 [*]/0.11 [α]/ 0.43 [α]	Dilmec et al. [262]
			a = 0.42	Case-control	Bangladeshi n = 171	45.9± 10.3	ISI	0.006 [*]	Hitman et al. [244]
			A = 0.49	Case-control	Polish n = 548	56.9 ± 12.2	T2D	0.33 [β]	Malecki et al. [246]
			a = 0.40	Case-control	Mexican n = 250	47.3 ± 7.8	T2D/BG	0.98 [β]/0.12 [β]	Rivera-Leon et al. [261]
			a = 0.42	Case-control	Caucasian n = 1,545	70.3 ± 8.9	T2D	0.058 [β] (reported marginally significant)	Oh et al. [247]
			____	Case-control	Czechs n = 234 (sub-group)	56 ± 9.88	T2D	> 0.05 [*]	Vedralova et al. [251]
			a = 0.46	Case-control	French n = 452	61.5 ± 14	T2D	0.20 [β]	Ye et al. [225]
			a = 0.36	Case-control	Chinese Han n = 404	56.5 ± 10.7	T2D	0.39 [β]	Zhang et al [240]
			____	Case-control	Norwegians n = 4,563 (sub-group)	62.5 ± 12.5	T2D	> 0.05 [ζ]	Zostautiene et al. [253]
			A = 0.47	Case-control	Indian Guadeloupe n = 189	51 ± 9.7	T2D	> 0.05 [*]	Boullu-Sanchis et al. [263]
			A = 0.43	Case-control	Iranian n = 200	40 ± 8	T2D	0.54 [β]	Nosratabadi et al. [264]

			A = 0.27 – 0.49[ψ]	Meta-analysis	Caucasian & East Asian n = 3,871 (sub-group)	36.0 ± 4.9 – 71.7 ± 8.6	T2D	0.98 [*]	Wang et al. [254]
			A = 0.28 – 0.49[ψ]	Meta-analysis	Asian & Caucasian n = 3,381 (sub-group)	40 - 62	T2D	0.80 [*]	Li et al. [163]
		TaqI [φ] rs731236 A/G (T/t)	t = 0.43	Case-control	Saudi n = 627	47.8 ± 9.3	T2D	0.07 [*]	Al-Daghri et al. [235]
			t = 0.44	Case-control	Saudi n = 570	45.9 ± 14.5	T2D/BG	0.40 [*]/0.70 [γ]	Al-Daghri et al. [217]
			T = 0.38	Case-control	North Indian n = 260	49.32 ± 10.97	T2D	0.70 [*]	Bid et al. [242]
			t = 0.36	Case-control	Turkish n = 241	56.6 ± 8.8	T2D/FPG /HbA1c	0.76 [*]/0.11 [α]/ 0.40 [α]	Dilmec et al. [262]
			t = 0.32	Case-control	Bangladeshi n = 171	45.9± 10.3	ISI	0.06 [*]	Hitman et al. [244]
			t = 0.32	Case-control	Brazilian n = 200	65.4 ± 8.18	T2D	1.00 [*]	Maia et al. [265]
			t = 0.38	Case-control	Kashmiri n = 200	48.1 ± 9.9	T2D	0.67 [*]	Malik et al. [236]
			t = 0.35	Case-control	Polish n = 548	56.9 ± 12.2	T2D	0.09 [β]	Malecki et al. [246]
			t = 0.38	Case-control	Emirati n = 355	54.1 ± 11.9	T2D	0.84 [*]	Safar et al. [97]

			t = 0.44	Case-control	Mexican n = 250	47.3 ± 7.8	T2D/Glucose	0.06 $^{\beta}$/0.74 $^{\beta}$	Rivera-Leon et al. [261]
			t = 0.39	Case-control	Caucasian n = 1,545	70.3 ± 8.9	T2D	> 0.05 $^{\beta}$	Oh et al. [247]
			t = 0.28	Case-control	Indian n = 60	49.05 ± 9.26	T2D	0.15 $^{\beta}$	Sarma et al. [248]
			____	Case-control	Czechs n = 234 (sub-group)	56 ± 9.88	T2D	> 0.05 *	Vedralova et al. [251]
			Hui t = 0.07 Han t = 0.06	Case-control	Chinese Hui n = 269 Chinese Han n = 420	____	T2D	0.82 $^{\beta}$ 0.32 $^{\beta}$	Xu et al. [239]
			t = 0.39	Case-control	French n = 452	61.5 ± 14	T2D	0.94 $^{\beta}$	Ye et al. [225]
			____	Case-control	Norwegians n = 4,563 (sub-group)	62.5 ± 12.5	T2D	> 0.05 $^{\zeta}$	Zostautiene et al. [253]
			T = 0.32	Case-control	Indian Guadeloupe n = 189	51 ± 9.7	T2D	> 0.05 *	Boullu-Sanchis et al. [263]
			t = 0.39	Case-control	Indian n = 80	45 ± 11.5	T2D	< 0.05 *	Mukhopadhyaya et al. [237]
			T = 0.36	Case-control	Iranian n = 200	40 ± 8	T2D	1.00 $^{\beta}$	Nosratabadi et al. [264]
			t = 0.33	Case-control	Turkish n = 200	____	T2D	> 0.05 $^{\beta}$	Vural et al. [266]
			t = 0.32	Case-control	Egyptian	51.74 ± 7.38	T2D	0.56 *	Gendy et al. [243]

					n = 100				
			T = 0.04 – t = 0.39[ψ]	Meta-analysis	Caucasian & East Asian n = 3,826 (sub-group)	36.0 ± 4.9 – 71.7 ± 8.6	T2D	0.53 [*]	Wang et al. [254]
			T = 0.32 – t = 0.39[ψ]	Meta-analysis	Asian & Caucasian n = 3,435 (sub-group)	40 - 62	T2D	>0.05 [*]	Li et al. [163]
		FokI [φ] rs2228570 A/C,G,T (F/f)	f = 0.26	Case-control	Saudi n = 627	47.8 ± 9.3	T2D	0.14 [*]	Al-Daghri et al. [235]
			F= 0.27	Case-control	Saudi n = 570	45.9 ± 14.5	T2D/BG	0.02 [*]/0.30 [γ]	Al-Daghri et al. [217]
			f = 0.47	Case-control	Chilean n = 310	60-79	T2D	0.04 [*]	Angel et al. [241]
			f = 0.28	Case-control	North Indian n = 260	49.32 ± 10.97	T2D	0.10 [*]	Bid et al. [242]
			f = 0.33	Case-control	Italians N = 1,713	49.5 ± 15.8	T2D	> 0.05 [β]	Bertoccini et al. [267]
			f = 0.45	Case-control	Chinese Han n = 3,714	59.99 ± 9.90	T2D	0.24 [*]	Jia et al. [258]
			f = 0.31	Case-control	Egyptian n = 190	47.84 ± 6.75	T2D	0.001 [β]	Mackawy et al. [245]
			f = 0.36	Case-control	Brazilian n = 200	65.4 ± 8.18	T2D	0.13 [*]	Maia et al. [265]
			f = 0.46	Case-control	Polish n = 548	56.9 ± 12.2	T2D	0.54 [β]	Malecki et al. [246]

			f = 0.19	Case-control	Indian n = 60	49.05 ± 9.26	T2D	0.74 $^{\beta}$	Sarma et al. [248]
			f = 0.43	Case-control	Czechs n = 234 (sub-group)	56 ± 9.88	T2D	0.57 *	Vedralova et al. [251]
			f = 0.29	Case-control	Emirati n = 355	54.1 ± 11.9	T2D	0.0007 *	Safar et al. [97]
			F = 0.45	Case-control	Chinese Han n = 1,191	59.55 ± 11.96	T2D	0.69 $^{\beta}$	Yu et al. [252]
			____	Case-control	Norwegians n = 4,563 (sub-group)	62.5 ± 12.5	T2D	> 0.05 $^{\zeta}$	Zostautiene et al. [253]
			f = 0.40	Case-control	Egyptian n = 100	51.74 ± 7.38	T2D	<0.001 *	Gendy et al. [243]
			f = 0.27 – 0.46$^{\psi}$	Meta-analysis	Caucasian & East Asian n = 3,023 (sub-group)	36.0 ± 4.9 – 71.7 ± 8.6	T2D	<0.001 *	Wang et al. [254]
			f = 0.28 – 0.46$^{\psi}$	Meta-analysis	Asian & Caucasian n = 2,070 (sub-group)	40 - 62	T2D	0.001 *	Li et al. [163]
			f = 0.26 – 0.48$^{\psi}$	Meta-analysis	Chinese & European n = 4,077 (sub-group)	36.0 ± 4.9 – 71.7 ± 8.6	T2D	<0.001 *	Yu et al. [255]
		Cdx2 rs11568820 C/T	T = 0.25	Case-control	Italian n = 1,788	47.85 ± 14.9	T2D	0.002 *	Sentinelli et al. [257]

			____	Case-control	Norwegians n = 4,563 (sub-group)	62.5 ± 12.5	T2D	> 0.05 ζ	Zostautiene et al. [253]
		BglI rs739837 G/C,T	C = 0.28	Case-control	Chinese Han n = 3,714	59.99 ± 9.9	T2D	0.002 *	Jia et al. [258]
			Hui T = 0.31 Han T = 0.35	Case-control	Chinese Hui n = 269 Chinese Han n = 420	____	T2D	0.65 β 0.69 β	Xu et al. [239]
			T = 0.26	Case-control	Chinese Han n = 1,191	59.55 ± 11.96	T2D	0.02 β	Yu et al. [252]
			G = 0.46	Cross-sectional	European n = 5,160	45	T2D/HbA1c	0.18 */0.76 α/	Vimaleswaran et al. [160]
		Tru9I rs757343 C/T	Hui T = 0.21 Han T = 0.20	Case-control	Chinese Hui n = 269 Chinese Han n = 420	____	T2D	0.19 β 0.91 β	Xu et al. [239]
			T = 0.13	Case-control	French n = 452	61.5 ± 14	T2D	0.21 β	Ye et al. [225]
		rs2239179 T/C	C = 0.23	Case-control	Chinese Han n = 1,191	59.5 ± 11.9	T2D	0.049 β	Yu et al. [252]
			C = 0.44	Cross-sectional	European n = 5,160	45	T2D/HbA1c	0.15 */0.57 α/	Vimaleswaran et al. [160]
		rs7968585 C/G,T	C = 0.45	Case-control	Norwegians n = 4,563 (sub-group)	62.5 ± 12.5	T2D	0.044 ζ	Zostautiene et al. [253]
		rs11574129	G = 0.18	Case-control	Chinese Han	59.99 ± 9.9	T2D	0.15 *	Jia et al. [258]

		A/G			n = 3,714				
		rs2189480 G/T	G = 0.36	Case-control	Chinese Henan n = 574	59.23 ± 12.31	T2D	< 0.003 *	Han et al. [259]
		rs3847987 C/A	A = 0.20	Case-control	Chinese Henan n = 574	59.23 ± 12.31	T2D	0.03 *	Han et al. [259]

Abbreviations: BG: Blood glucose, ISI: Insulin secretion index, FPG: Fasting plasma glucose, HbA1c: Haemoglobin A1c, FSG: Fasting serum glucose; φ Through literature reviewing we found that describing the genotype of *VDR* was very confusing. We decided on using the initial letter of the restriction enzyme to name the different alleles instead of using the nucleotide base letter for BsmI, ApaI, TaqI and FokI. *Logistic regression; α Linear regression; β Chi square test; γ ANOVA; ζ Cox regression; η Family-based association testing (FBAT); Ψ Indicates range value.

Chapter 3: A Nutrigenetic Approach to Investigate the Relationship between Metabolic Traits and Vitamin D Status in an Asian Indian Population

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

Alathari BE, Bodhini D, Jayashri R, Lakshmipriya N, Shanthi Rani CS, Sudha V, Lovegrove JA, Anjana RM, Mohan V, Radha V, Pradeepa R, Vimaleswaran KS. A Nutrigenetic Approach to Investigate the Relationship between Metabolic Traits and Vitamin D Status in an Asian Indian Population. Nutrients. 2020;12(5):1357. doi: 10.3390/nu12051357. PMID: 32397403. *https://doi.org/10.3390/nu12051357*

Buthaina AlAthari's contribution: For this study, I assessed, organized, and evaluated the variables in the dataset. I developed an analysis plan compatible with my objectives which was approved by the primary supervisor. I performed the statistical analysis using the Statistical Package for the Social Sciences (SPSS) software (v24; SPSS Inc., Chicago, IL, USA) and interpreted the results. Then, I carried out a literature search and reviewed all relevant papers and wrote the first draft of the manuscript. I revised the manuscript based on co-authors' comments. The final draft was formatted based on the journal's guidelines. Comments from the reviewers were addressed and responded.

3.1 Abstract

Purpose: Studies in Asian Indians have examined the association of metabolic traits with vitamin D status. However, the findings have been quite inconsistent. Hence, we aimed to explore the relationship between metabolic traits and 25-hydroxyvitamin D (25(OH)D) concentrations.

Methods: We investigate whether this relationship was modified by lifestyle factors using a nutrigenetic approach in 545 Asian Indians randomly selected from the Chennai Urban Rural Epidemiology Study (219 normal glucose tolerant individuals, 151 with pre-diabetes and 175 individuals with type 2 diabetes). A metabolic genetic risk score (GRS) was developed using five common metabolic disease-related genetic variants.

Results: There was a significant interaction between metabolic-GRS and carbohydrate intake (energy%) on 25(OH)D ($p_{interaction} = 0.047$). Individuals consuming a low carbohydrate diet (≤ 62%) and those having lesser number of metabolic risk alleles (GRS ≤ 1) had significantly higher levels of 25(OH)D ($p = 0.033$). Conversely, individuals consuming a high carbohydrate diet despite having lesser number of risk alleles did not show a significant increase in 25(OH)D ($p = 0.662$).

Conclusion: In summary, our findings show that individuals carrying a smaller number of metabolic risk alleles are likely to have higher 25(OH)D levels if they consume a low carbohydrate diet. These data support the current dietary carbohydrate recommendations of 50-60% energy suggesting that reduced metabolic genetic risk increases 25(OH)D.

3.2 Introduction

Interaction between genetic and lifestyle factors have been shown to contribute to the development of metabolic disorders such as obesity and type 2 diabetes (T2D) [16, 268]. The prevalence of metabolic diseases is increasing worldwide, and Asian Indians have a greater predisposition [269, 270]. The Asian Indian population have a unique clinical phenotype characterized by increased visceral fat and waist circumference (WC), increased susceptibility to type 2 diabetes at a younger age, hyperinsulinemia, insulin resistance and dyslipidemia with raised triglycerides and low high density lipoprotein-cholesterol cholesterol (HDL-c) levels at normal ranges of body mass index (BMI) collectively known as "Asian Indian Phenotype" [271, 272]. Furthermore, several studies have demonstrated that metabolic diseases are associated with micronutrient deficiencies, such as vitamin D deficiency [273-276].

Vitamin D is a fat-soluble vitamin, known for its impact on skeletal and extra-skeletal physiological processes. Vitamin D deficiency exists in endemic proportions all over India, with a prevalence ranging from 80%-90% [277]. Adequate levels of vitamin D are important for calcium absorption, bone mineralization and skeletal growth as well as a multitude of biologic functions at the cellular level such as cell growth, proliferation, differentiation, inflammation, and apoptosis. Additionally, vitamin D has been linked to cancer, cardiovascular diseases, inflammation and autoimmune diseases [20, 187, 278]. Several observational studies have associated vitamin D deficiency with increased obesity and reported inverse relationship between 25(OH)D concentration and BMI, WC, and total body fat; however, the causal effect was not established [182, 183]. Nevertheless, a Mendelian Randomization analysis in 42,024 participants of European ancestry concluded that increased BMI leads to reduced 25(OH)D concentrations while there was no causal association between lower 25(OH)D concentrations and higher BMI [145]. Given that observational studies are often prone to bias and confounding, a genetic approach to explain the relationship between metabolic diseases and vitamin D

deficiency may be a better option to reduce any influence from unmeasured confounding factors.

Association of several genetic variants with metabolic diseases has been identified by candidate gene and genome-wide association studies (GWAS) studies [16, 279-281]. Currently, the fat mass and obesity-associated (*FTO*) gene is the strongest risk loci for obesity [268, 282]. The *FTO* gene is the first obesity susceptibility gene to be identified by two GWAS in European populations [283, 284]. A study in an Asian Indian population has shown that lifestyle factors can influence the association of *FTO* gene with obesity traits [282]. Besides the *FTO* gene, Melanocortin 4 Receptor (*MC4R*) and Transcription Factor 7-Like 2 (*TCF7L2*) genes are the two commonly studied candidate genes for obesity and T2D [285-299]. In the present study, we examined the association of a metabolic-genetic risk score (GRS) developed from five single-nucleotide polymorphisms (SNPs) [*FTO* (rs8050136 and rs2388405), *MC4R* (rs17782313), and *TCF7L2* (rs12255372 and rs7903146)] with metabolic traits and vitamin D concentrations. In addition, we investigated the link between metabolic traits and vitamin D status by exploring the interactions between the metabolic-GRS and lifestyle factors such as diet and physical activity on metabolic traits and vitamin D concentrations in an Asian Indian population.

3.3 Methods

3.3.1 Study Population

Five hundred forty-five study participants were recruited randomly from the Chennai Urban Rural Epidemiology Study (CURES) follow-up study **Figure 3.1**, aged 29-85 years old [269]. CURES is an epidemiological cross-sectional study conducted on participants from Chennai city population in southern India, which is the fourth largest city in India. Details of the methodology have been previously published [300]. In brief, the CURES study was

conducted in three phases. Phase 1: 26,001 adult participants (> 20 years of age) were recruited using a systematic random sampling method covering the whole Chennai city and all participants were screened for diabetes. Phase 2: all 1,382 diabetic participants were invited for further investigation (90.4% compliance). Phase 3: 2207 adult participants designated by way of every tenth participant from Phase 1, excluding diabetics, underwent further detailed investigations. Phases 2 & 3 constitutes the CURES follow-up cohort (n = 3589) [301]. For present study, 545 individuals were randomly selected from the follow-up cohort, which included: 219 normal glucose tolerant (NGT), 151 prediabetic, and 175 T2D individuals. Three exclusion criteria were applied in this study: known cases of type 1 diabetes, diabetes secondary to other causes, and intake vitamin D supplements. The Madras Diabetes Research Foundation Institutional Ethics Committee granted ethical approval, and informed consent was obtained from the study participants. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki (ICH GCP).

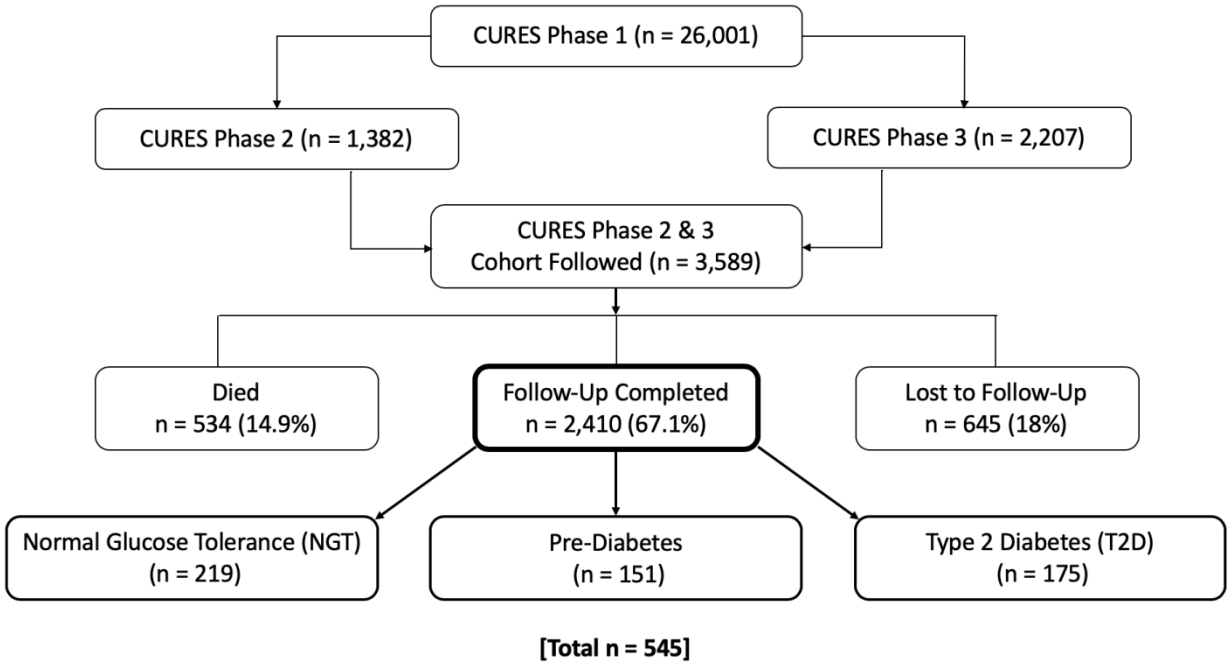


Figure 3. 1: Selection of study participants from the Chennai Urban Rural Epidemiological Study (CURES follow-up study).

3.3.2 Anthropometric and Biochemical Measurements

Standardized methods were used to measure weight, height, and WC. BMI was calculated based on the body weight in kilograms divided by the square of body height in meters. Generalized obesity was defined according to the World Health Organization Asia Pacific Guidelines for Asians (The Asia Pacific perspective 2000) as non-obese (BMI < 25 kg/m2) and obese (BMI ≥ 25 kg/m2) [302]. The following biochemical measurements were performed using kits supplied by Roche Diagnostics (Mannheim) on a Hitachi-912 Auto Analyzer (Hitachi, Mannheim, Germany) to measure fasting plasma glucose (glucose oxidase–peroxidase), serum total cholesterol (cholesterol oxidase-phenol-4-amino-antipyrene peroxidase), serum triglycerides (glycerol phosphatase oxidase-phenol-4-amino-antipyrene peroxidase), and HDL-c (direct method; polyethylene glycol-pretreated enzymes) [303]. The Friedewald formula was used to calculate low-density lipoprotein cholesterol (LDL-c). Glycated hemoglobin (HbA1c) was determined by high-performance liquid chromatography using a Variant™ machine (Bio-Rad, Hercules, CA, USA). Serum insulin and serum 25(OH)D vitamin D concentrations were estimated using the electrochemiluminescence (ECLIA) using a Roche e601Cobas immunoassay analyzer (Roche Diagnostics, Indianapolis, IN, USA) [282]. The intra- and inter-assay coefficients of variation for vitamin D assay was 3.62% and 6.38% respectively.

3.3.3 Assessment of Dietary Intake and Physical Activity

A validated, interviewer administered semi-quantitative food frequency questionnaire (FFQ) consisting of a list of 222 different foods was used in order to assess dietary intake for the previous year [304]. In brief, participant had an interview ranging from 20 and 30 min where they had to estimate their usual portion size and usual frequencies (per day, week, month, year, never) with the help of visual aids of measurement equipment and food sizes. Description of

the development of FFQ and the data on reproducibility and validity was previously published [304]. Daily average food and nutrient intake including macronutrient and total energy intake were analyzed and estimated by the EpiNu database system. A validated self-report questionnaire was used to assess physical activity levels of the participants [305]. Individuals were classified into three groups: 1. Vigorously active: where the participants both exercised and engaged in demanding work activities; 2. Moderately active: where the participants either exercised or performed heavy physical work; 3. Sedentary: those participants who did not exercise or have physically demanding work.

3.3.4 SNP Selection and Genotyping

For the present study, five SNPs were chosen from three different genes based on their previous associations with obesity and T2D in several populations: *FTO* (rs8050136 & rs2388405) [282, 286, 287, 299, 306-311], *TCF7L2* (rs12255372 & rs7903146) [285, 289, 312-316], and *MC4R* (rs17782313) [286, 288, 295, 298, 299]. *FTO* gene variants are known to be the strongest genetic predictors of obesity to date [317, 318]. The *FTO* SNP rs8050136 has shown a strong association with obesity and T2D [288, 310, 311, 318, 319]. Furthermore, the *FTO* SNPs, rs8050136 and rs2388405, have also been reported as intronic enhancers, as they may have an influence on the gene expression [306, 320, 321]. *MC4R* SNP rs17782313 was shown to be associated with obesity in European populations [295, 299] and this finding then replicated in other populations including South Asians [288, 298, 322]. *TCF7L2* SNPs, rs12255372 and rs7903146, were shown to be associated with increased susceptibility to T2D in two large multiethnic meta-analyses [313, 316]. Some studies have reported that the *TCF7L2* SNPs are involved in modulating and reducing adiposity through changes in the lifestyle [323-325]. Based on the previous studies, the above mentioned five SNPs were chosen for the present study.

Phenol-chloroform method of DNA extraction from whole blood was performed. The genotyping methodology for the five SNPs have previously published [270, 285, 289]. Direct sequencing by an ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA) was performed to confirm the efficiency of the genotyping; there was 99% concordance based on random duplicates of 20% of the samples.

3.3.5 Statistical Analysis

Statistical analyses were performed out by using SPSS statistical software (version 24; SPSS Inc., Chicago, IL, USA). Allele frequencies were calculated by gene counting and chi-square test was carried out to compare the proportions of genotypes or alleles. The genotypic frequencies of the five SNPs were in the Hardy Weinberg Equilibrium (HWE) ($p > 0.05$) (**Table S3.1**). To obtain normal distribution, all metabolic outcomes and vitamin D values were log transformed. The difference in the means of continuous variables between the participants with NGT vs. pre-diabetes and NGT vs. T2D was analyzed by independent sample t test. Descriptive statistics for continuous variables are presented as means and standard deviation (SD). The chi-squared test was used to analyze and compare physical activity levels (vigorously active, moderately active, and sedentary) between individuals with NGT vs. those with pre-diabetes and individuals with NGT vs. those with T2D. Unweighted metabolic-GRS was calculated for each participant by adding the number of risk alleles for metabolic diseases. The SNPs, rs8050136, rs2388405, rs12255372, rs7903146, and rs17782313, were used to generate the GRS. A value of zero, one and two was assigned to each SNP, which indicates the number of metabolic disease-related risk alleles. These values were then calculated by adding the number of metabolic disease-related risk alleles across each SNP. The risk allele score was then divided by the median into those carrying ≤ 1 risk allele (lower risk) vs. those with > 1 risk allele (higher risk).

A schematic representation of the study objectives is presented in **Figure 3.2**. Association analysis between the GRS and continuous and categorical variables were carried out using general linear and binary logistic regression models, respectively, adjusting for age, BMI, T2D, and month of sample collection, wherever appropriate. The variable 'month of sample collection' was created based on the three seasons in India: summer (March to June), autumn/monsoon (July to October), and winter (November to February) [326]. Linear and logistic regression analyses were also used for investigating the interaction between SNPs and lifestyle factors (dietary intake and physical activity), where the interaction terms were incorporated into the models and adjusted for age, gender, BMI, T2D, total energy intake and month of sample collection wherever appropriate. Further tertile stratification of the lifestyle factor (diet/physical activity) was performed when there was a significant interaction between metabolic-GRS and lifestyle factors on 25(OH)D concentrations and metabolic traits. Study participants were split into three equal groups based on nutrient intake and designated as 'low intake', 'medium intake' or 'low intake' and further analysed. Power calculation was not performed, given that there are no studies on metabolic-GRS and no previously reported effect sizes for South Asians.

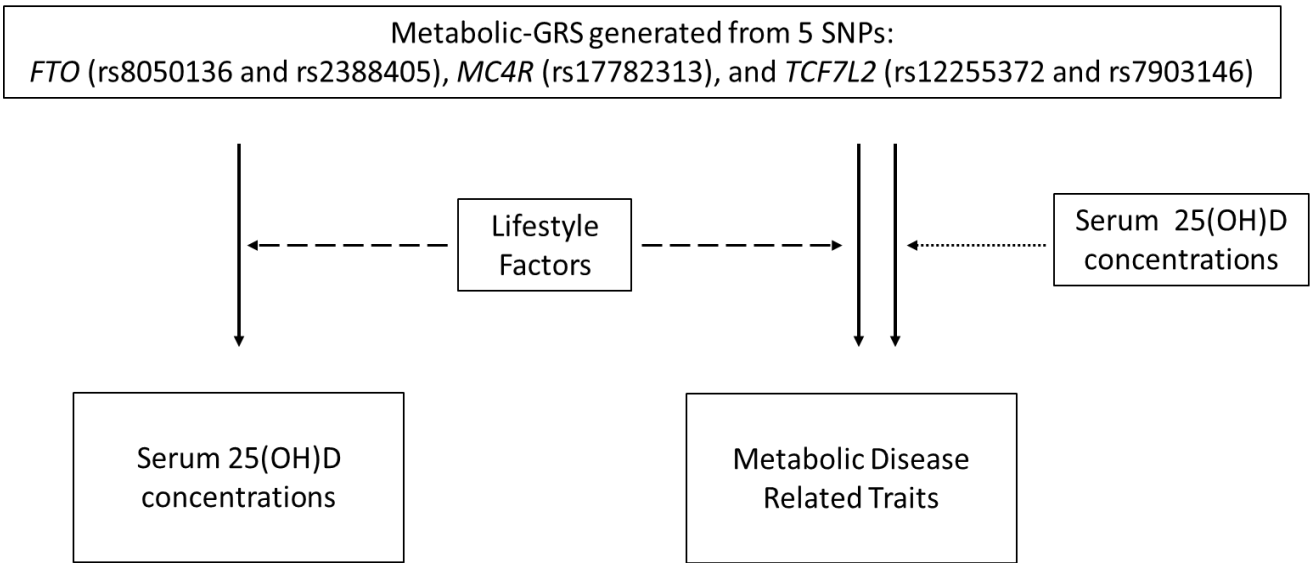


Figure 3. 2: Study objectives.

The unbroken one-sided arrows indicate the associations that were tested between the metabolic-GRS and vitamin D concentrations and metabolic disease related traits. The broken one-sided arrows represent the interactions that were investigated between the GRS and lifestyle factors (diet and physical activity levels) on serum vitamin D and metabolic disease related traits. The one-sided dotted arrow indicates the interaction that was examined between metabolic-GRS and 25(OH)D concentrations on metabolic disease -related traits.

3.4 Results

3.4.1 Characteristics of Study Participants

The anthropometric, biochemical and lifestyle characteristics of the CURES participants are presented in **Table 3.1**. Significant differences were found between individuals with NGT, pre-diabetes and T2D, where individuals with T2D were older ($p < 0.001$), had higher WC ($p < 0.001$), fasting plasma insulin ($p < 0.001$), systolic and diastolic blood pressure ($p < 0.001$) and serum triglycerides ($p < 0.001$). However, individuals with pre-diabetes had higher BMI ($p = 0.001$) and LDL-c ($p = 0.004$) than individuals with NGT and T2D. No significant differences were observed in the levels of vitamin D, diastolic blood pressure, total cholesterol, HDL-c and dietary intakes across the three groups ($p > 0.05$).

Table 3. 1: Baseline characteristics of the study participants.

Characteristics of study participants	n	Normal Glucose Tolerance	n	Pre-Diabetes	N	Type 2 Diabetes	*p* Value
Age (years)	219	46.82±10.54	151	47.79±11.5	175	54.19±11.04	< 0.001 $^{\alpha\,\gamma}$
BMI (kg/m^2)	219	26.10±5.15	151	27.95±5.22	174	26.56±4.58	0.001 $^{\beta}$
WC (cm)	219	86.04±11.73	151	89.54±11.2	173	90.11±10.27	< 0.001 $^{\alpha\,\beta}$
Vitamin D (ng/mL)	219	19.55±13.5	151	19.14±10.47	175	17.8±10.03	0.381
Fasting Plasma Glucose (mg/dL)	201	89.74±6.54	144	103.43±11.59	172	156.28±64.43	< 0.001 $^{\alpha\,\beta\,\gamma}$
HbA1c (%)	219	5.61±0.47	151	5.91±0.59	175	8.19±2.07	< 0.001 $^{\alpha\,\beta\,\gamma}$
Fasting Plasma Insulin (µIU/mL)	216	7.76±5.13	139	8.13±4.73	132	11.48±7.69	< 0.001 $^{\alpha\,\gamma}$
Systolic BP (mmHg)	219	125.77±20.97	151	126.54±17.77	175	134.53±19.6	< 0.001 $^{\alpha\,\gamma}$
Diastolic BP (mmHg)	219	79.17±12.84	151	79.79±10.78	175	80.67±10.95	0.320
Total Cholesterol (mg/dL)	219	181.07±35.81	151	187.72±35.28	175	181.2±38.77	0.126
LDL Cholesterol (mg/dL)	219	114.58±31.58	151	119.17±31.43	175	107.74±34.63	0.004 $^{\alpha\,\gamma}$
HDL Cholesterol (mg/dL)	219	42.06±9.57	151	40.10±7.82	175	40.32±8.58	0.093
Serum Triglycerides (mg/dL)	219	122.15±63.7	151	142.25±83.08	175	165.71±95.93	< 0.001 $^{\alpha\,\beta\,\gamma}$
Total Energy Intake (kcal)	185	2620.04±752.02	83	2535.29±803.78	93	2585.85±787.79	0.609
Protein Energy %	185	11.28±1.19	83	11.31±0.89	93	11.38±1.2	0.758
Fat Energy %	185	23.91±4.76	83	23.33±4.51	93	24±4.72	0.582
Carbohydrate Energy %	185	64.09±6.69	83	64.89±5.51	93	64.36±5.97	0.556
Protein (g)	185	73.47±21.39	83	71.59±23.74	93	72.78±21.2	0.704
Fat (g)	185	69.62±25.15	83	65.92±26.97	93	67.94±22.15	0.407
Carbohydrate (g)	185	417.24±115.73	83	409.91±125.98	93	418.82±142.37	0.847
Dietary Fibre (g)	185	32.18±10.91	83	30.77±11.4	93	33.01±11.85	0.235
Physical Activity Level	171	Sedentary (80.1%) Moderate (18.7%)	73	Sedentary (83.6%) Moderate (13.7%)	81	Sedentary (84.0%) Moderate (13.6%)	0.676$^{\delta}$

	Vigorous (1.2%)	Vigorous (2.7%)	Vigorous (2.5%)

Data shown are represented as means ± SD, *p* values were calculated using one-way ANOVA, $^{\delta}$ *p* values were calculated using the chi-square test. $^{\alpha}$ indicates significance between non-diabetics and T2D individuals, $^{\beta}$ indicates significance between normal glucose tolerance and pre-diabetics, $^{\gamma}$ indicates significance between pre-diabetes and Type 2 diabetes. Abbreviations: CURES: Chennai Urban Rural Epidemiological Study, BMI: body mass index, WC: waist circumference, BP: blood pressure, LDL: low density lipoprotein, HDL: high density lipoprotein.

3.4.2 Association of 25(OH)D Concentrations with Obesity and Type 2 Diabetes

There was a significant association between 25(OH)D concentrations with BMI (p = 0.017), and WC (p = 0.047) after adjusting for age, gender, month of sample collection and T2D. However, there was no association of 25(OH)D concentrations with fasting plasma glucose (p = 0.739), HbA1c (p = 0.823) and fasting plasma insulin (p = 0.387) after adjusting for age, gender, month of sample collection and BMI.

3.4.3 Association of the Metabolic-GRS with 25(OH)D Level and Metabolic-Related Traits

No significant associations were observed between metabolic-GRS and 25(OH)D concentrations (p = 0.34). None of the clinical and biochemical parameters such as BMI, WC, fasting plasma glucose, insulin, HbA1c, systolic and diastolic blood pressure, total cholesterol, HDL-c, LDL-c and triglycerides, showed a significant association with metabolic-GRS (p > 0.19 for all comparisons). (**Table S3.2**).

3.4.4 Interaction between Metabolic-GRS and 25(OH)D Concentrations on Metabolic Traits

There was a borderline interaction between the metabolic-GRS and 25(OH)D concentrations on HbA1c level (p = 0.048) after adjusting for age, gender, BMI, T2D and month of sample collection. However, no association was detected between the metabolic-GRS and HbA1c when participants were grouped in tertiles of 25(OH)D concentrations (tertile 1, p = 0.471; tertile 2, p = 0.870; tertile 3, p = 0.486).

3.4.5 Interaction between Metabolic-GRS and Lifestyle Factors on 25(OH)D Concentrations

After adjusting for age, gender, BMI, T2D and month of sample collection, there was a significant interaction between the GRS and dietary carbohydrate intake on 25(OH)D

concentrations ($p_{interaction}$ = 0.047). Tertile analysis was performed where individuals were grouped based on the tertiles of carbohydrate intake (energy %) [low ≤ 62%, medium = 62% - 67%, and high > 67%]. There were significant differences between the two GRS groups only among those who were in the first tertile of carbohydrate intake (p = 0.003), where individuals with lesser number of risk alleles (GRS ≤ 1) had greater 25(OH)D concentrations compared to those with higher number of risk alleles (GRS > 1) (**Figure 3.3**). Among individuals who had a higher carbohydrate intake (> 67.28%), despite having lesser number of metabolic risk alleles, did not show a significant higher 25(OH)D concentrations (p = 0.66) compared to those with higher number of risk alleles (GRS > 1) (**Figure 3.3**).

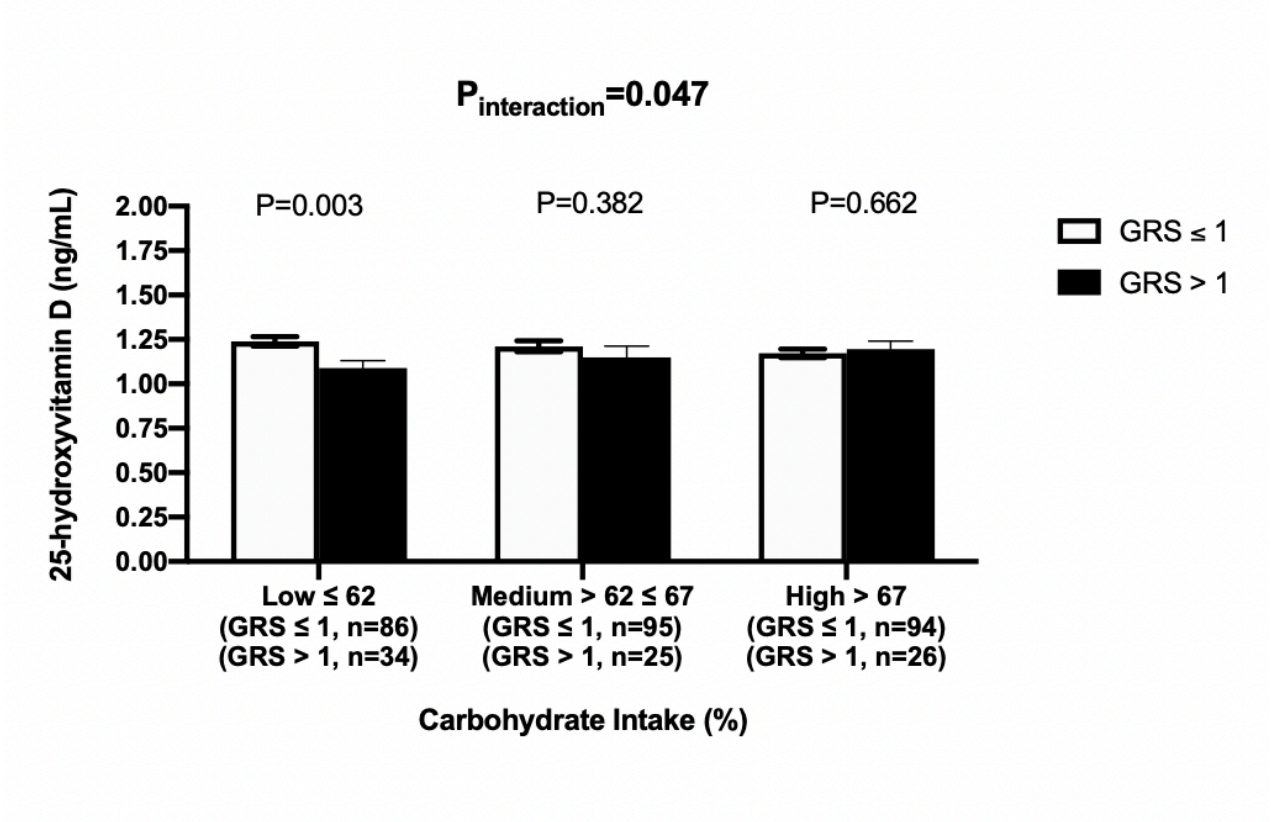


Figure 3. 3: Interaction between metabolic-GRS and log carbohydrate intake (%) on log 25 hydroxyvitamin D.

White bars indicate individuals with GRS ≤ 1 risk allele; Black bars indicate individuals with GRS > 1 risk allele. Among individuals with low carbohydrates intake, those with ≤ 1 risk

allele had significantly higher 25 hydroxyvitamin D concentrations compared to those with > 1 risk allele ($p = 0.003$).

3.4.6 Interaction between the GRS and Lifestyle Factors on Clinical and Biochemical Parameters

There were significant interactions of metabolic-GRS with dietary total fat intake and carbohydrates intake on LDL-c concentrations ($p_{interaction} = 0.032$ and $p_{interaction} = 0.028$; respectively) after adjusting for age, gender, BMI, and T2D. However, no significant interaction was found between GRS and saturated, polyunsaturated, and monounsaturated fat intake on LDL-c concentrations ($p > 0.05$, for all comparisons). After individuals were split into tertiles based on their fat intake (energy %) [low ≤ 22%, medium = 22% - 25%, and high > 25%], there was a significant association of metabolic-GRS with LDL-c in the low-fat intake group ($p = 0.033$), where individuals despite having a higher genetic risk (> 1 risk allele) had significantly lower LDL-c concentrations (**Figure 3.4**).

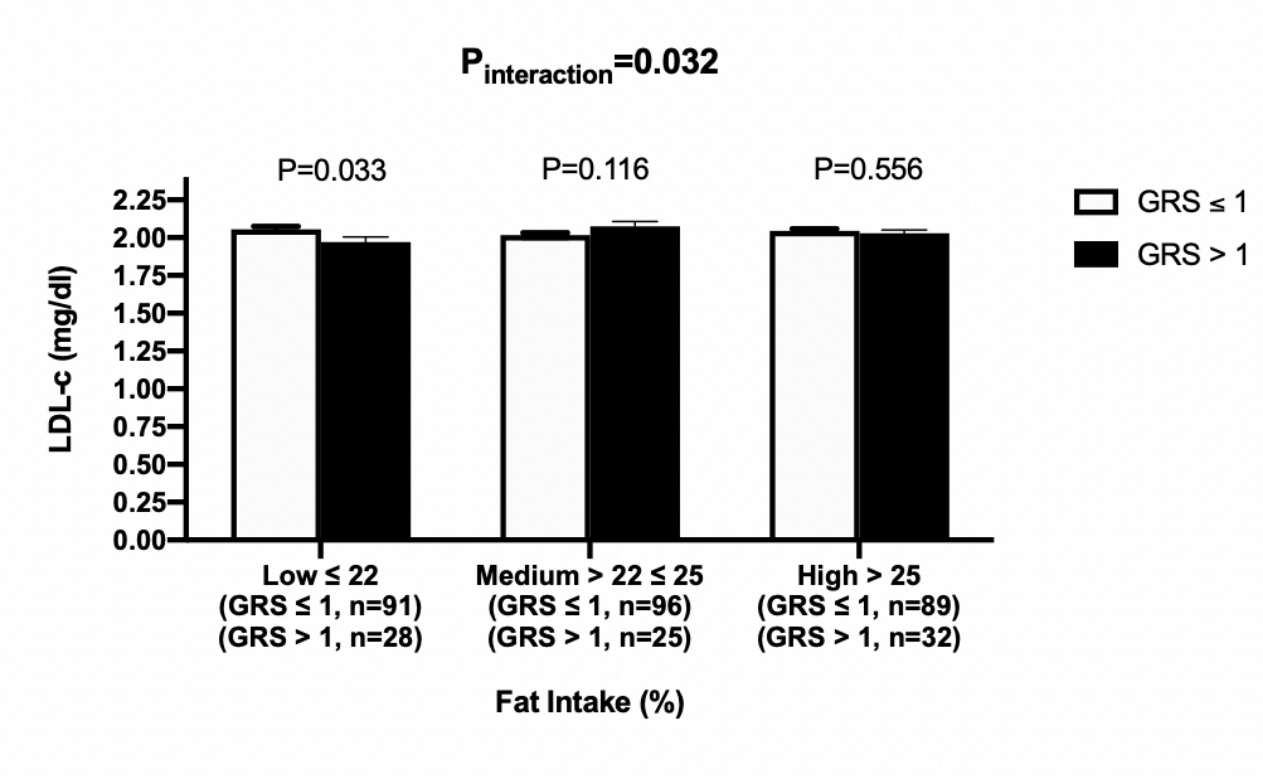


Figure 3. 4: Interaction between metabolic-GRS and fat intake (%) on log LDL-c.

White bars indicate individuals with GRS ≤ 1 risk allele; black bars indicate individuals with GRS > 1 risk allele. Among individuals consuming a low-fat diet, despite having a higher genetic risk (> 1 risk allele), they had significantly lower LDL-c concentrations ($p = 0.033$).

On the other hand, the tertile analysis of carbohydrate intake did not show any significant association between GRS and LDL-c concentrations (tertile 1, $p = 0.453$; tertile 2, $p = 0.146$; tertile 3, $p = 0.460$). None of the other lifestyle factors including physical activity showed a significant interaction with metabolic-GRS on metabolic traits ($p > 0.11$ for all comparisons) **(Table 3.2)**.

Table 3. 2: Interaction between Genetic Risk score and Lifestyle Factors on Clinical and Biochemical Parameters.

Outcome Measures	Physical Activity Levels	Protein %	Fat %	Carbohydrates %	Saturated Fatty Acids g/d*	Polyunsaturated Fatty Acids g/d*	Monounsaturated Fatty Acids g/d*
Body Mass Index	0.89	0.94	0.16	0.20	-	-	-
Waist Circumference	0.45	0.70	0.54	0.47	-	-	-
25(OH)D **	0.90	0.69	0.32	0.047	-	-	-
Fasting Plasma Glucose	0.12	0.90	0.16	0.09	-	-	-
Glycated Haemoglobin	0.13	0.52	0.44	0.32	-	-	-
Fasting Plasma Insulin	0.84	0.41	0.14	0.76			
Systolic Blood Pressure	0.72	0.19	0.96	0.62	-	-	-
Diastolic Blood Pressure	0.93	0.93	0.22	0.54	-	-	-
Total Cholesterol	0.80	0.40	0.47	0.55	-	-	-
Low density lipoprotein Cholesterol	0.90	0.12	0.032	0.028	0.21	0.28	0.27
High density lipoprotein cholesterol	0.68	0.55	0.72	0.80	-	-	-
Fasting serum triglycerides	0.87	0.11	0.26	0.11	-	-	-

p value for interactions obtained by general linear univariate analysis. All interactions were adjusted for age, gender, type 2 diabetes, and BMI (except BMI); *Adjusted for log-total energy intake; **Adjusted for month of sample collection

3.5 Discussion

Our study is the first to investigate gene-lifestyle interactions on 25(OH)D concentrations in Asian Indians. The main finding of our study is the interaction between carbohydrate intake and metabolic-GRS, generated from five common metabolic-disease-related genetic variants, on 25(OH)D concentrations, where individuals who had less number of risk alleles (GRS $\leq$ 1) and consumed lower amounts of carbohydrates ($\leq$ 62%) had significantly higher levels of 25(OH)D concentrations. Achieving and maintaining adequate levels of vitamin D is a desirable outcome as vitamin D deficiency is linked to several chronic diseases [5]. Given that previous studies have reported that Asian Indians have lower 25(OH)D concentrations [277, 327], our findings suggest that, even if the genetic risk is lower, following the dietary carbohydrate recommendations (50 - 60%) is required to improve the vitamin D status in this Asian Indian population.

Epidemiological studies have demonstrated a link between metabolic diseases such as obesity and T2D and vitamin D deficiency; however, it remains uncertain whether improving the metabolic status would reduce the risk of vitamin D deficiency [328-331]. The association between obesity and vitamin D status was consistent across different populations in several meta-analyses [330, 331]. A large meta-analysis of 23 studies (n = 65,445) of mixed races reported that 35% of obese individuals suffer from vitamin D deficiency [330]. Several longitudinal studies have shown an inverse association between 25(OH)D status and T2D [5]. A meta-analysis in 2,320 Caucasians showed that participants with adequate 25(OH)D concentrations had a 43% reduced risk of T2D [328]. However, unlike observational studies, vitamin D supplementation (4000 IU/day intake of vitamin D_3) did not show beneficial effects on glycaemic measures in two randomized controlled trials (RCTs). In the first trial, well-controlled patients with T2D (n = 127) did not show any improvement in β-cell function,

insulin secretion rate, nor in HbA1c levels after 48 weeks of supplementation [332]. In the second trial, 2,423 prediabetic adults were evaluated for the development of diabetes for an average of 2.5 years; at the end of the study, 293 out of 1,211 participants (24.2%) in the vitamin D supplementation group developed diabetes compared to 323 out of 1,212 (26.7%) in the placebo group [72]. Furthermore, majority of RCTs also did not show an effect of vitamin D supplementation on weight loss [333].

Genetic studies are considered to be an effective approach in investigating the relationship between vitamin D and metabolic outcomes [144], as they are free from confounding and bias, which have been shown to affect the association between vitamin D levels and metabolic diseases. To our knowledge, there are no previous studies that have investigated the effect of metabolic disease-related genetic variants on vitamin D status. However, there are genetic association studies that have investigated vitamin D-related genetic variants on metabolic disease outcomes; but, the findings have been quite inconsistent [219, 231, 236, 261]. A large bidirectional meta-analysis 42,024 Europeans reported that there was no association between vitamin D-related genetic score and higher BMI; however, there was a significant association between genetically instrumented BMI and low vitamin D status [145]. In our study, the phenotypic associations of 25(OH)D concentrations with BMI and WC were statistically significant; but the metabolic-GRS did not show any association with 25(OH)D concentrations suggesting that the phenotypic associations are highly confounded.

To understand whether the genetic risk of metabolic diseases was influenced by vitamin D status, we tested for the interaction between the metabolic-GRS and 25(OH)D concentrations on metabolic-disease related traits. None of the interactions were significant, except for a borderline interaction between the metabolic-GRS and 25(OH)D concentrations on HbA1c level ($p = 0.048$); however, there was no association between metabolic-GRS and HbA1c levels when participants were grouped into tertiles of 25(OH)D concentrations suggesting that there

was no evidence for metabolic genetic risk acting as effect modifiers of the association between vitamin D status and metabolic traits. A study in 5,160 participants of European ancestry also provided no evidence of vitamin D-related genetic variants acting as major modifiers of the association between 25(OH)D levels and cardio-metabolic risk [160]. Hence, these findings including the results from the present study indicate that vitamin D status is unlikely to have a significant impact on metabolic disease risk.

In the present study we found an interaction between the metabolic-GRS and carbohydrate intake on vitamin D levels where lower consumption of carbohydrates was shown to be associated with higher 25(OH)D concentrations in the presence of reduced genetic risk. In the CURES, the carbohydrate intake included cereal grains, pulses, legumes, tubers, fruits, sweets, sweet beverages, carbonated beverages, junk food and added sugar, where consumption of refined cereals (i.e., mainly white rice) accounted for 78.1% of total calories [334]. This is a high intake compared to the recommended carbohydrate intake 50-60% of total calories for Asian Indians [335], and the WHO (2002) recommendations of total carbohydrate intake at 55-75% of total dietary energy [336]. The lowest tertile of the carbohydrate intake (≤ 62%), where we observed the positive association with vitamin D status, was close to the recommended dietary intake for Asian Indians (50-60%), which supports the benefits of the current carbohydrate recommendations for Asian Indians. Our findings are also in line with a five-week intervention study [337] which used a reduced carbohydrate diet (43% carbohydrate; 27% fat) in 28 obese African American girls (9–14 years). The study showed that 25(OH)D concentrations were inversely associated with fasting glucose levels providing evidence that vitamin D may exert on alterations in the biologic response to macronutrients such as dietary carbohydrates.

A further interesting finding in our study is the significant interaction between metabolic-GRS and fat intake (%) on LDL-c concentrations, where despite having higher

metabolic risk alleles, individuals who consumed a low-fat diet (≤ 21.89%) had significantly lower LDL-c levels. This suggests that lower dietary fat intake may influence the genetic risk of higher serum LDC-c concentrations, although mechanisms of action are unclear. This finding is in accordance with a GWAS on lipids in 541 individuals from the Quebec Family Study which reported an interaction between GRS (29 SNPs) and total fat intake on LDL-c concentrations ($p << 10^{-5}$) [338]. The recommended dietary fat intake for Asian Indians is < 30% [335]; however, in our study, only those individuals consuming total fat < 21.8% demonstrated a significantly lower serum LDL-c concentrations, despite higher genetic susceptibility. Hence, our findings, if replicated using larger cohorts and dietary intervention studies, may have significant implications in providing dietary recommendations for those with higher metabolic-risk alleles.

The present study has several strengths, which include the use of a representative sample of Chennai [300] and an extensive and a validated semi-quantitative FFQ for dietary assessment [304] In addition, the semi-quantitative FFQ has demonstrated high reproducibility and validity for macronutrient intakes such as dietary carbohydrate and fibre intake. Furthermore, the use of a metabolic GRS, which combines the effect of multiple SNPs, has been shown to increase the statistical power and an effective approach to study metabolic diseases [131, 339]. However, there are some underlying limitations that need to be acknowledged. The measurement bias that is associated with self-reported FFQ and physical activity questionnaire cannot be ruled out. The study used a cross-sectional design and hence, no cause and effect conclusions can be established. Even though potential confounders were adjusted in all our statistical analyses, confounding factors such as sun exposure cannot be ruled out; however, we have adjusted for month of sample collection to overcome this limitation [326]. Finally, small sample size could be considered as another limitation in our

study; nevertheless, we have identified significant findings which suggest that the study is statistically powered to identify gene-diet interactions.

3.6 Conclusion

The present study has identified a novel interaction between the metabolic-GRS and carbohydrate intake on 25(OH)D levels in an Asian Indian population where individuals carrying a lesser number of metabolic risk alleles are likely to have higher 25(OH)D concentrations, only if they have a carbohydrate intake < 62% energy. This is broadly in line with current dietary recommendations in India (50-60% energy). This finding needs to be replicated in a larger cohort before these data can be confirmed. Mechanistic links also need to be identified.

Chapter 4: Interaction between Vitamin D-Related Genetic Risk Score and Carbohydrate Intake on Body Fat Composition: A Study in Southeast Asian Minangkabau Women

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

Alathari BE, Aji AS, Ariyasra U, Sari SR, Tasrif N, Yani FF, Sudji IR, Lovegrove JA, Lipoeto NI, Vimaleswaran KS. Interaction between Vitamin D-Related Genetic Risk Score and Carbohydrate Intake on Body Fat Composition: A Study in Southeast Asian Minangkabau Women. Nutrients. 2021;13(2):326. doi: 10.3390/nu13020326. PMID: 334986189. *https://doi.org/10.3390/nu13020326*

Buthaina AlAthari's contribution: Prior to running the statistical analysis, I cleaned the dataset where outliers were removed. Then I developed the statistical analysis plan for the study. Statistical Package for the Social Sciences (SPSS) software (v24; SPSS Inc., Chicago, IL, USA) was used to conduct the statistical analysis. I interpreted the results of the study and wrote the first draft of the paper. The manuscript was revised based on co-authors' comments and suggestions and prepared according to the journal's guidelines and a graphical abstract was created for this study.

4.1 Abstract

Purpose: Metabolic diseases have been shown to be associated with low vitamin D status; however, the findings have been inconsistent. Hence, the objective of our study was to investigate the relationship between vitamin D status and metabolic disease-related traits in healthy Southeast Asian women and examine whether this relationship was modified by dietary factors using a nutrigenetic study.

Methods: The study included 110 Minangkabau women (age: 25–60 years) from Padang, Indonesia. Genetic risk scores (GRS) were constructed based on five vitamin D-related single nucleotide polymorphisms (SNPs) (vitamin D-GRS) and ten metabolic disease-associated SNPs (metabolic-GRS).

Results: The metabolic-GRS was significantly associated with lower 25-hydroxyvitamin D (25(OH)D) concentrations ($p = 0.009$) and higher body mass index (BMI) ($p = 0.016$). Even though the vitamin D-GRS had no effect on metabolic traits ($p > 0.12$), an interaction was observed between the vitamin D-GRS and carbohydrate intake (g) on body fat percentage (BFP) ($p_{interaction} = 0.049$), where those individuals who consumed a high carbohydrate diet (mean ± SD: 319 g/d ± 46) and carried > 2 vitamin D-lowering risk alleles had significantly higher BFP ($p = 0.016$).

Conclusion: In summary, we have replicated the association of metabolic-GRS with higher BMI and lower 25(OH)D concentrations and identified a novel interaction between vitamin D-GRS and carbohydrate intake on body fat composition.

4.2 Introduction

Over a billion people in the world have vitamin D deficiency (VDD) and race and ethnicity have been shown to be strong predictors of vitamin D status, as measured by 25-hydroxyvitamin D (25(OH)D) concentrations. Low 25(OH)D concentrations have been shown to be associated with metabolic diseases, such as obesity and type 2 diabetes (T2D) [340]. Hereditary factors play a large role in VDD affecting up to 85% of serum concentrations of 25(OH)D [121, 341] and the development of metabolic disorders [16, 177, 342-348]. However, we cannot overlook the effect of environment, lifestyle, nutrition and dietary factors as major contributors to metabolic diseases [349, 350]. Hence, it is of great importance to examine the lifestyle factors in distinct regions and varied environments in relation to their genetic susceptibility.

Indonesia is a country in Southeast Asia consisting of more than seventeen thousand islands that are split by the equator resulting in a tropical climate rich in sunlight with typically even temperatures year-round. Nevertheless, VDD rates are high in Indonesian women ranging between 60 to 95% [351]. Furthermore, incidence of obesity and T2D are high in Indonesia [352], with estimates of obesity and central obesity prevalence of 23.1% and 28%, respectively based on data from Indonesia's national health survey [353] and 10.3 million people are living with T2D which constitutes as a major public health concern [354]. The prevalence of T2D has also been shown to be higher among Indonesian women compared to men (7.7 vs. 5.6%) [355, 356].

Since only a few studies have examined the influence of single nucleotide polymorphisms (SNPs) on 25(OH)D levels in populations within Southeast Asia, our study focused on Minangkabau women from Padang, the capital of West Sumatra. The Minangkabau ethnic group is of particular interest given that the Minangkabau people have the largest matrilineal family structure in the world where the family line is inherited from the mother's

side and where women have higher status than men in the family and society [357, 358]. We used a genetic study to investigate the association between vitamin D status and metabolic traits in a cohort of Minangkabau women from both urban and rural areas of Padang and examined whether associations were modified by environmental and dietary factors using a nutrigenetic approach. The application of a genetic approach to establish the link between vitamin D status and metabolic diseases is favored over observational studies since genetic associations are less affected by confounding. Furthermore, we analyzed the combined effects of multiple genetic variants using two genetic risk scores (GRS) instead of the common single gene variant method in order to increase the statistical power to detect gene-diet interactions [339, 359].

4.3 Methodology

4.3.1 Study Population

This study included 110 Indonesian women from the Minangkabau Indonesia Study on Nutrition and Genetics (MINANG), a cross-sectional study that was conducted in the city of Padang, Indonesia. The MINANG study is a part of the ongoing GeNuIne (Gene-Nutrient Interactions) Collaboration, the main aim of which is to investigate the impact of gene-nutrient interactions on cardiometabolic traits using population-based data from diverse ethnic groups [17, 360]. The methodology has been published previously [357]. In brief, one hundred and thirty-three women were recruited from community health centers in two sub districts in Padang City: Padang Timur (urban area) and Kuranji (rural area) of Padang population. The inclusion criteria included: (i) healthy women, (ii) age between 25-60 years old, and (iii) Minangkabau ethnicity. After applying exclusion criteria (pregnancy or lactating, history of metabolic diseases or communicable disease, taking supplements, or being a relative to another

participant), 117 women completed the MINANG study. For the present study, we excluded an additional seven participants, six had incomplete genetic data and one had incomplete dietary information.

The MINANG study was conducted in line with the principles of the Declaration of Helsinki and the study was approved by the Ethical Review Committee of the Medical Faculty, Andalas University (No.311/KEP/FK/2017). The study participants provided written informed consent prior to the start of the study.

4.3.2 Anthropometric Measures

Anthropometric measurements including height, weight, and waist circumference were obtained using standardized techniques. Body mass index (BMI) was calculated as weight (in kg) over height (in m^2) and we used Asia-Pacific classification for defining obesity [361]. Body fat percentage (BFP) was assessed using Tanita MC780 (TANITA, Tokyo, Japan) multi-frequency segmental body composition analyzer.

4.3.3 Biochemical Measures

Fasting blood samples (5 mL) were taken, and serum was separated and stored at -20 °C until the assays were performed. Samples were analyzed for 25(OH)D, glucose, insulin, glycated hemoglobin A1c (HbA1c), total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c). All biochemical assays were conducted using the Mark Microplate Spectrophotometer (Bio-Rad Laboratories Inc., Hercules, CA, USA). Serum 25(OH)D concentrations, and fasting glucose, insulin and HbA1c were analyzed using enzyme-linked immunosorbent assay (ELISA) kits from Bioassay Technology Laboratory (Shanghai, China). The intra and inter-assay coefficients of variation for 25(OH)D were 5% and 8.1%, respectively. Fasting lipid levels were analyzed using

enzymatic colorimetric procedures, namely glycerine phosphate oxidase peroxidase (GPO-PAP) for triglycerides, and cholesterol oxidase phenol 4-aminoantipyrine peroxidase (CHOD-PAP) for total cholesterol, and HDL-c. LDL-c was calculated using Friedewald's formula.

4.3.4 Assessment of Dietary Intake

A proficient nutritionist collected dietary intake data from participants either at home or at an integrated health service post. For assessing dietary intake, a validated interviewer administered semi-quantitative food frequency questionnaire (SQ-FFQ) was used [362]. All data provided by participants were analyzed with Indonesian Food Database and Nutrisurvey (EBISpro, Willstätt, Germany) to estimate the total energy intake and macronutrient intakes.

4.3.5 SNP Selection and Genotyping

Five vitamin D-related SNPs were selected for investigation based on previously published studies that have shown association with 25(OH)D concentrations [363-371]: 7-dehydrocholesterol reductase (*DHCR7*) rs12785878 [364, 369-371], 25-hydroxylase (*CYP2R1*) rs12794714 [363, 371], 24-hydroxylase (*CYP24A1*) rs6013897 [367, 369], vitamin D binding protein (*DBP*)/group-specific component (*GC*) rs2282679 [363, 364], and calcium sensing receptor (*CASR*) rs1801725 [365, 366, 368]. Ten metabolic disease-related SNPs were selected based on previously published candidate gene studies and genome-wide association studies (GWAS) [342-344, 348, 372-376]: Fat mass and obesity-associated (*FTO*) SNPs rs8050136, rs9939609 and rs10163409 [343, 374, 375], transcription factor 7-like 2 (*TCF7L2*) SNPs rs12255372 and rs7903146 [372], melanocortin 4 receptor (*MC4R*) SNPs rs17782313 and rs2229616 [372, 373], potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) SNPs rs2237895 and rs2237892 [376], cyclin dependent kinase inhibitor 2A/B (*CDKN2A*/*B*)

SNP rs10811661 [348]. In this study, the minor alleles of the SNPs were in line with the minor alleles reported in the dbSNP for all the ethnic groups except for *DHCR7* SNP rs12785878, where the minor allele is the "T" allele which is in line with the South Asian and the East Asian populations as per the dbSNP; but, in the European population, the "G" allele is the minor allele (**Table S4.1**).

DNA was extracted from whole blood leukocytes using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) using spin column methods. A NanoDrop spectrophotometer was used to determine DNA concentration. Genotyping was conducted using the competitive allele specific PCR-KASP® assay at LGC Genomics, London, UK.

4.3.6 Statistical Analysis

SPSS statistical software (v24; SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses. All 15 gene variants were in the Hardy-Weinberg equilibrium (HWE) ($p > 0.05$), which was tested using a simple goodness-of-fit chi square test (**Table S4.1**). Baseline characteristics (continuous variables) are presented as means and standard deviations (SD) and comparisons between groups were tested using one-way analysis of variance (ANOVA). Test of normality was performed on all continuous variables and verified by the Shapiro-Wilk test. Log transformation was created for variables that were not normally distributed, which included age (years), waist circumference (WC) (cm), BFP (%), 25(OH)D level (ng/mL), glucose (mg/dL), HbA1c (ng/mL), insulin (nmIU/L), total cholesterol (mg/dL), HDL-c (mg/dL), LDL-c (mg/dL), triglycerides (mg/dL), total energy intake (Kcal), carbohydrate (g), protein (g), fat (g), fibre (g), saturated fatty acids (SFA) (g), monounsaturated fatty acids (MUFA) (g), and polyunsaturated fatty acids (PUFA) (g). Two separate Genetic risk scores (GRSs) were developed from the summation of the counts of the risk allele counts across each SNP. The vitamin D-related GRS was calculated from 5 SNPs and the metabolic disease-

related GRS was calculated from 10 SNPs. A value of 0, 1, or 2 was assigned to each SNP, which denotes the number of risk alleles for each GRS. Then, these values were then calculated by adding the number of risk alleles across each SNP. For each GRS, risk allele scores were then divided by the median and classified into a "low genetic risk group" and a "high genetic risk group". Using the median of vitamin D- related GRS, low risk and high risk were classified as individuals carrying ≤ 2 (n = 67) and those carrying > 2 (n = 43) risk alleles, respectively. For the metabolic disease-related GRS using the median, low risk corresponds to individuals carrying < 4 (n = 54) and high risk corresponds to those carrying ≥ 4 (n = 56). **Figure 4.1** represents the study design of the analyses performed.

The effect of both the vitamin D-related GRS and the metabolic disease-related GRS on anthropometric and biochemical outcomes (BMI, WC, BFP, 25(OH)D, glucose, HbA1c, fasting insulin, total cholesterol, HDL-c, LDL-c and triglycerides) were analyzed using linear regression models. Furthermore, the interaction between the two GRSs and dietary factors on clinical and biochemical variables were tested using linear regression models by including the interaction term (GRS*dietary factor) in these models. Models were adjusted for age, BMI, location (rural or urban), and total energy intake, wherever appropriate. Dietary factors included carbohydrate, protein, fat and fibre intake in grams. Statistically significant interactions were examined further where study participants were stratified by the tertiles of dietary consumption. Power calculations were not performed as there are no studies in relation to GRS and vitamin D status and no previously reported effect sizes are available for the Southeast Asian populations.

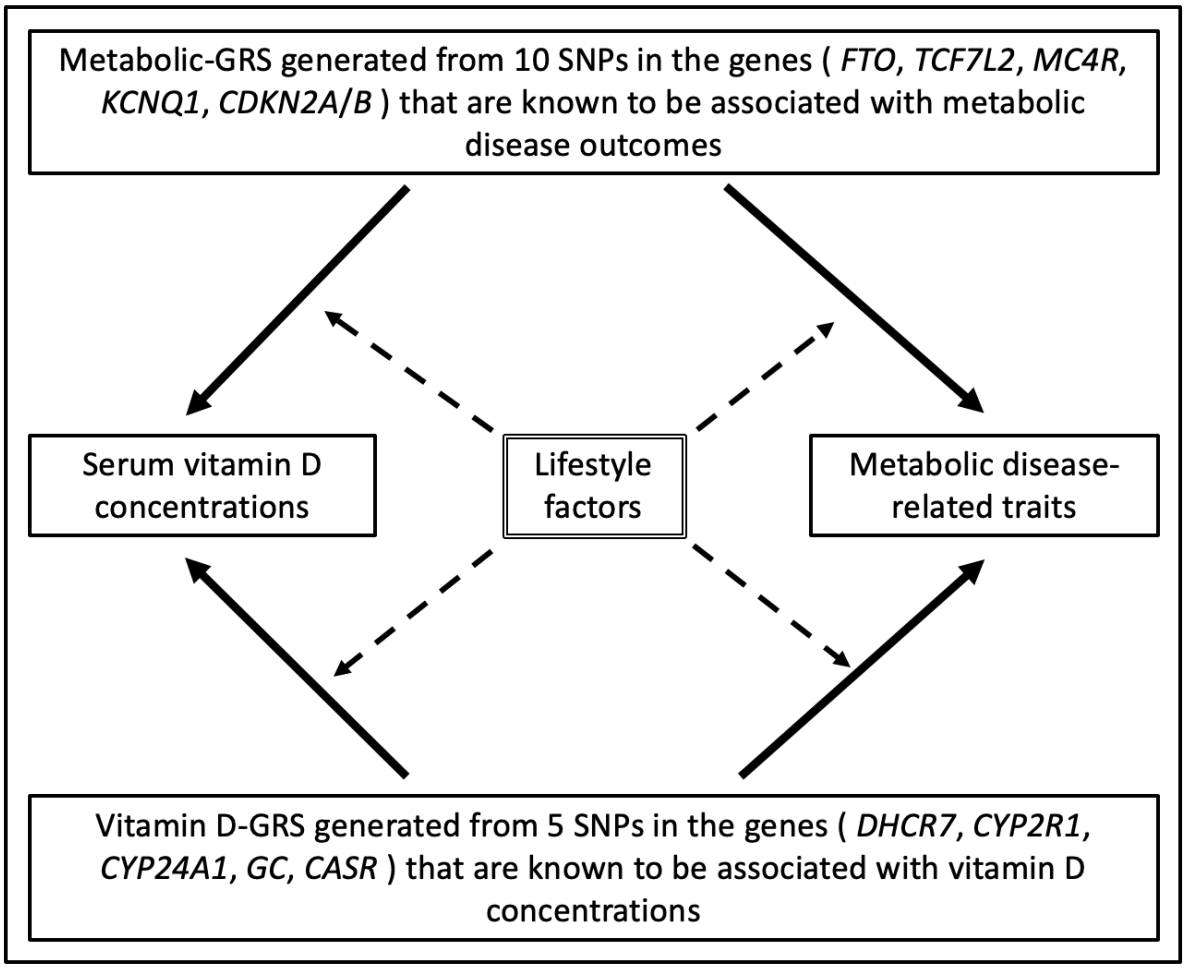


Figure 4. 1: Study design.

Genetic associations are represented by one-sided arrows with unbroken lines and interactions between GRS and diet on clinical and biochemical measurements are shown as one-sided arrows with broken lines. The association of the metabolic-GRS with 25(OH)D levels and metabolic traits and the association of vitamin D-GRS with 25(OH)D levels and metabolic traits were tested. In addition, the effect of dietary factors on these genetic associations was examined. Abbreviations: GRS: genetic risk score, SNP: single nucleotide polymorphism, FTO: fat mass and obesity-associated gene, TCF7L2: transcription factor 7-like 2 gene, MC4R: melanocortin 4 receptor gene, KCNQ1: potassium voltage-gated channel subfamily Q member 1, CDKN2A/B: cyclin dependent kinase inhibitor 2A/B, DHCR7: 7-dehydrocholesterol

reductase, CYP2R1: 25-hydroxylase, CYP24A1: 24-hydroxylase, GC: group-specific component, CASR: calcium sensing receptor.

4.4 Results

4.4.1 Characteristics of Participants

A total of 110 women (mean age 40.46 ± 9.38 years) were included, where 51% were from an urban area (Padang Timur) and 49% were from a rural area (Kuranji) in the city of Padang, West Sumatra, Indonesia. In this study, 20% of the women were vitamin D deficient and 40% were vitamin D insufficient (**Table 4.1**). There was a significant difference in the mean age across the three vitamin D groups ($p = 0.001$) where women with mean age 34.3±10.3 years were vitamin D deficient and women with mean age 43.8±7.8 years were vitamin D sufficient. There were no significant differences in the clinical and biochemical parameters across the three groups classified based on vitamin D status (**Table 4.1**).

Table 4. 1: Anthropometric and biochemical characteristics of the study participants stratified based on Vitamin D status.

Characteristics of study participants	Vitamin D sufficiency (≥ 20 ng/mL) (n=44)	Vitamin D insufficiency (≥ 12 ≤19 ng/mL) (n=44)	Vitamin D deficiency < 12 ng/mL (n=22)	*p* Value
Age (years)	43.8±7.8	40.0±10.9	34.3±10.3	0.001
BMI (kg/m^2)	25.7±4.4	25.3±4.0	24.1±4.3	0.358
WC (cm)	86.5±9.9	82.6±11.3	81.0±8.6	0.070
BFP (%)	36.2±7.8	36.4±6.5	34.0±6.3	0.392
Glucose (mg/dL)	96.1±30.4	92.1±9.0	86.4±10.6	0.198
HbA1c (ng/mL)	710±703	670±573	440±209	0.178
Fasting Insulin (nmIU/L)	36020±29916	32915±24107	22261±8092	0.101
Total Cholesterol (mg/dL)	209±46	216±44	194±37	0.142
HDL Cholesterol (mg/dL)	57.4±8.4	59.9±11.4	61.2±11.4	0.295
LDL Cholesterol (mg/dL)	127.3±40.3	135.4±41.9	113.8±31.8	0.115
Serum Triglycerides (mg/dL)	103±44.9	93.8±41.7	96.7±44.0	0.601

Total Energy Intake (kcal)	1797.0±645.0	1695.0±545.0	1894.3±675.4	0.442
Carbohydrate (g)	233.7±75.1	225.9±58.9	246.0±86.5	0.563
Protein (g)	77.2±41.7	74.2±30.4	82.2±39.2	0.707
Fat (g)	61.2±36.1	54.7±31.6	64.1±32.1	0.498
Dietary Fibre (g)	8.6±4.3	8.3±3.5	10.2±5.5	0.218

Data are presented as means ± SD. *p* values were calculated by using one-way analysis of variance (ANOVA). Vitamin D cut-off points were based on the suggestions of the Institute of Medicine for vitamin D levels [377]. Abbreviations: BMI body mass index; WC waist circumference; BFP body fat percentage; 25(OH)D 25-hydroxyvitamin D; HbA1C glycated haemoglobin; HDL-c high-density lipoprotein cholesterol; LDL-c low-density lipoprotein cholesterol.

4.4.2 Association between Metabolic-GRS and Anthropometric and Biochemical Measurements

A significant association was found between the metabolic-GRS and BMI ($p = 0.016$), where participants who carried ≥ 4 risk alleles had higher BMI levels (mean ± SD: 26.10 ± 3.87) compared to those with < 4 risk alleles (mean ± SD: 24.25 ± 4.43), **Figure 4.2a**. Moreover, a significant association between the metabolic-GRS and serum 25(OH)D concentrations ($p = 0.009$) was observed where individuals who carried ≥ 4 risk alleles had lower 25(OH)D levels (mean ± SD: 1.20 ± 0.19) compared to those with < 4 risk alleles (mean ± SD: 1.28 ± 0.21), **Figure 4.2b.**

4.4.3 Association between Vitamin D-GRS and Anthropometric and Biochemical Measurements

There was no statistically significant association between the vitamin D-GRS and 25(OH)D levels ($p = 0.93$). Furthermore, there was no significant association of the clinical and biochemical parameters such as BMI, WC, BFP, glucose, HbA1c, insulin, total cholesterol,

HDL-c, LDL-c, and triglycerides with vitamin D-GRS ($p > 0.12$ for all comparisons) (**Table S4.2**).

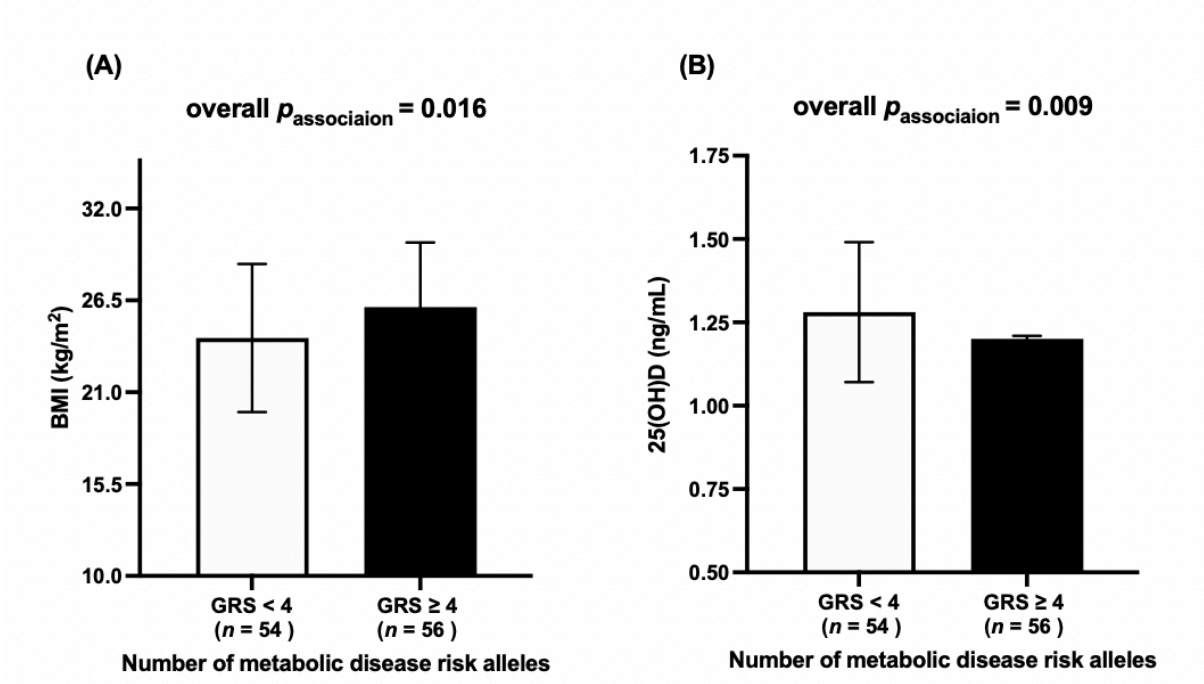


Figure 4. 2: Associations between metabolic-GRS with log BMI and log 25(OH)D.

(A) Association between metabolic-GRS and BMI in Indonesian women. Those who carried ≥ 4 metabolic risk alleles had higher BMI compared to individuals with < 4 risk alleles (p = 0.016). (B) Association between metabolic-GRS and 25(OH)D concentrations in Indonesian women. Those who carried ≥ 4 metabolic risk alleles had lower 25(OH)D concentrations compared to individuals with < 4 risk alleles (p = 0.009). Abbreviations: GRS: genetic risk score, BMI: body mass index, 25(OH)D: 25-hydroxyvitamin D.

4.4.4 Interaction between the Vitamin D-GRS and Dietary Factors on Biochemical and Anthropometric Parameters

There was a statistically significant interaction between the vitamin D-GRS and carbohydrate intake on log BFP ($p_{interaction}$ = 0.049), where participants who consumed high

amounts of carbohydrates (mean ± SD: 319g/d ± 46) and carried > 2 risk alleles (mean ± SD: 1.60 ± 0.04, $p = 0.016$) had significantly higher log BFP than those with ≤ 2 risk alleles (mean ± SD: 1.53 ± 0.11, $p = 0.016$) **Figure 4.3**.

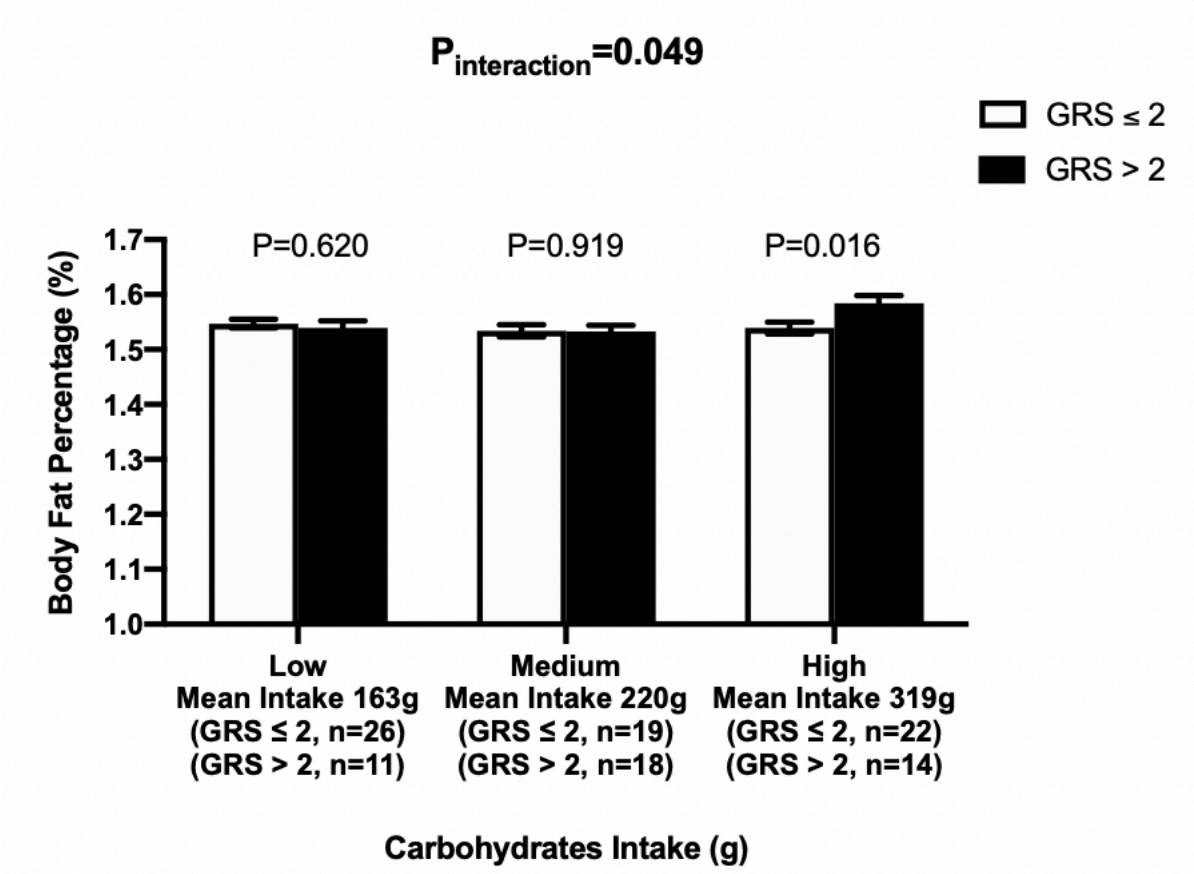


Figure 4. 3: Interaction between the Vitamin D-GRS and dietary carbohydrate intake (g) on log BFP.

Those who were on the highest tertile of carbohydrate intake and carried > 2 risk alleles had significantly higher BFP compared to individuals carrying ≤ 2 risk alleles ($p = 0.016$). Abbreviations: GRS: genetic risk score, BFP: body fat percentage.

4.4.5 Interaction between the Metabolic-GRS and Dietary Factors on Clinical and Biochemical Characteristics

No statistically significant interactions were found between metabolic-GRS and dietary intake on serum 25(OH)D concentrations and clinical and biochemical parameters ($p > 0.997$ for all comparisons), (**Table S4.3**).

4.5 Discussion

To date, this is the first study to utilize a nutrigenetic approach to examine the relationship between vitamin D status and metabolic traits in a Southeast Asians. Our study has confirmed the association of metabolic disease-related GRS with higher BMI and lower serum 25(OH)D concentrations. Additionally, the study has shown an impact of genetically instrumented vitamin D status on BFP, a marker of body fat composition, through the influence of dietary carbohydrate intake, where women consuming high amounts of carbohydrates (mean ± SD: 319 g/d ± 46) and those with higher genetic risk of VDD had higher BFP. Given that percent body fat is a better predictor of cardiovascular risk factors than BMI, if the results are replicated in future studies, our findings may have a significant public health implication in preventing cardiovascular diseases in Minangkabau women by developing dietary intervention strategies to reduce the intake of carbohydrates.

In the present study, we created a metabolic-GRS consisting of 10 SNPs known to be associated with obesity and type 2 diabetes [342-344, 348, 372-376] was created; this GRS was used as a genetic instrument to explore the link between metabolic disease-related traits and vitamin D status. The study showed that participants carrying ≥ 4 metabolic risk alleles had higher BMI and lower 25(OH)D concentrations compared to those carrying < 4 risk alleles. There are several mechanisms that have been proposed for the effect of obesity on vitamin D status, such as, vitamin D dilution due to increased fat stores, having more vitamin D within adipose tissue, and lifestyle variations between obese and lean individuals as well as differences in the activity of the vitamin D activating enzymes between obese and lean

individuals [187]. Furthermore, a Mendelian Randomization (MR) study in up to 42,024 Caucasians had demonstrated a causal relationship between obesity and vitamin D status, where a 10% higher genetically instrumented BMI was associated with 4.2% lower 25(OH)D concentrations [145]. These findings suggest that increased obesity can lead to lower vitamin D status, which was also further confirmed in the present study where a significant association between metabolic-GRS and lower 25(OH)D concentrations was observed.

Vitamin D-GRS was constructed as a genetic instrument for vitamin D status using five common vitamin D pathway-related SNPs that have been identified by the GWA scans [123, 369, 378-380]. However, our study did not find any significant association of vitamin D-GRS with 25OHD concentrations and other clinical and biochemical parameters. The lack of association implies that linear decreases in vitamin D may not have an impact on the metabolic disease-related outcomes which is in line with the findings from the MR study [145]. Furthermore, a genetic association study in two large European cohorts (n = 5,224, 123, 865; respectively) failed to demonstrate a significant impact of vitamin D-related gene variants on obesity traits, suggesting that vitamin D pathway genes are unlikely to have an important impact on the obesity-related outcomes.[147]. Animal studies have convincingly illustrated the role of the vitamin D genes in contributing to adiposity phenotypes [381, 382]; however, it is possible that there is no direct effect of 25(OH)D concentrations on adiposity in humans.

Even though there was no association of vitamin D-GRS with metabolic disease risk, there was an interaction of vitamin D-GRS with dietary carbohydrate intake on BFP, where those who consumed a high carbohydrate diet and had high genetic risk of VDD had significantly higher BFP than those with low genetic risk. The total carbohydrate intake of our study participants ranged from 121–436 g/day (mean ± SD: 233.08 ± 71.34) and there was no significant difference in carbohydrate intake between those living in urban (mean ± SD: 238.74 ± 77.35) and rural (mean ± SD: 227.21 ± 64.73) areas (p = 0.40). The intake of carbohydrates in the

highest tertile (mean ± SD: 319 ± 46 g/d) is above the Indonesian dietary guidelines [383, 384], which recommends consuming 50% of total energy from carbohydrates. In line with these findings, a recent study [372] from our team in 545 Asian Indians had also demonstrated an interaction of metabolic-GRS with dietary carbohydrate intake on 25(OH)D concentrations, where individuals consuming a low carbohydrate diet (≤ 62%, equivalent to ~346 g/d) and having lower metabolic genetic risk had significantly higher concentrations of 25(OH)D. Increased carbohydrate intake, in particular refined carbohydrates and sugars, have been linked to obesity and T2D [385], and the use of restricted carbohydrate diets have shown to be effective in reducing body weight and WC, as well as, fasting glucose, HbA1c, and plasma insulin levels [386, 387]. The interaction of vitamin D-GRS with dietary carbohydrate intake on BFP that was observed in our study is biologically possible, given that vitamin D has been shown to mediate the impact of reduced consumption of carbohydrate through its direct action on pancreatic beta-cell function. Vitamin D has a protective effect on beta cells rendering them more resistant to cellular stress [337, 388]. While studies have shown that there might be a failure to expand beta-cell mass in response to obesity [389], it is possible that lower levels of vitamin D due to genetic susceptibility can lead to obesity in the presence of high carbohydrate diet. However, the mechanism by which the carbohydrates interact with vitamin D-related genetic variants and affect body composition is unclear and requires further exploration. Given that the main source of energy for the Minangkabau is carbohydrates, where rice, banana, cassava, corn, sweet potato, sago, noodles, glutinous rice, and mung bean are part of their daily meals [390], our findings will have public health relevance if replicated in larger cohorts.

BMI is the most common indicator of obesity; however, BMI does not distinguish between lean mass and adiposity. Given that BFP can distinguish between fat and lean body mass [391] and increased BFP has been shown to be associated with cardiometabolic diseases and mortality [392], BFP is considered as a better indicator of obesity. Thus, detecting excess

adiposity and body fat composition has become particularly important to make accurate conclusions with regards to establishing causal relationships and identifying risk to prevent the onset of metabolic and cardiometabolic diseases [391, 392]. There are ethnic differences in body fat composition as we explore the complex interaction between the genes, lifestyle and culture [357]. Understanding of ethnic differences may lead to the implementation of effective approaches to recognize and prevent metabolic diseases across different ethnic groups [393]. It is important that the findings from this study are replicated before consideration is given to personalized dietary advice for Indonesian women carrying a higher genetic risk of VDD.

The current study has strengths including being the first nutrigenetic study to evaluate the relationship of vitamin D status with metabolic disease risk in Southeast Asians. The construction of the GRSs instead of a single SNP approach increases the statistical power to identify gene-diet interactions [339, 359]. Additionally, the use of a comprehensive, validated SQ-FFQ [362] collected by a well-trained nutritionist increases the accuracy of dietary data collection. The study does have several limitations that should be acknowledged. The sample size of the study is small; however, the study was sufficiently powered to identify significant associations and gene-diet interactions. Even though we used a validated SQ-FFQ, bias due to self-reported dietary intake information cannot be excluded. The study did not include data on specific categories of foods that were included in the total carbohydrate count and it did not quantify different classes of carbohydrates, such as complex carbohydrates and simple disaccharides and monosaccharides. Finally, the study was limited to Minangkabau women, and hence, our findings cannot be generalized to the Indonesian population.

4.6 Conclusion

In summary, our study has replicated the association of metabolic-GRS with higher BMI and lower 25(OH)D concentrations and identified a novel interaction of vitamin D-GRS with carbohydrate intake on BFP, where women with increased genetic risk of vitamin D deficiency and who consumed higher amounts of carbohydrates have increased body fat composition. This finding is potentially valuable in introducing nutritional recommendations to reduce carbohydrate intake for Indonesian women belonging to the Minangkabau community, as a third of our study participants had a mean intake of 319 g/d. This is equivalent to 71.8% dietary intake of carbohydrates, based on the average intake of our participants of 1,776 kcal/day, which is very high compared to the Indonesian dietary guidelines that recommends obtaining 50% of total energy from carbohydrates [383, 384]. However, before dietary interventions can be developed and recommended to improve vitamin D status and reduce carbohydrate intake in Indonesian women, replication of our findings in a larger cohort is required.

Chapter 5: Impact of Genetic Risk Score and Dietary Protein Intake on Vitamin D Status in Young Adults from Brazil

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

Alathari BE, Cruvinel NT, da Silva NR, Chandrabose M, Lovegrove JA, Horst MA, Vimaleswaran KS. Impact of Genetic Risk Score and Dietary Protein Intake on Vitamin D Status in Young Adults from Brazil. Nutrients. 2022;14(5):1015. PMID: 35267990. *https://doi.org/10.3390/nu14051015*

Buthaina AlAthari's contribution: I started this study with data cleaning which included the removal of duplicates, outliers, and missing variables prior to running the statistical analysis. Then, I generated the analysis plan, and performed the statistical analysis using the Statistical Package for the Social Sciences (SPSS) software (v27; SPSS Inc., Chicago, IL, USA), and interpreted the results of the study. 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 addressed.

5.1 Abstract

Purpose: Given the relationship between vitamin D deficiency (VDD) and adverse outcomes of metabolic diseases, we investigated the interplay of dietary and genetic components on vitamin D levels and metabolic traits in young adults from Brazil.

Methods: Genetic analysis, dietary intake, and anthropometric and biochemical measurements were performed in 187 healthy young adults (19–24 years). Genetic risk scores (GRS) from six genetic variants associated with vitamin D (vitamin D-GRS) and 10 genetic variants associated with metabolic disease (metabolic-GRS) were constructed.

Results: High vitamin D-GRS showed a significant association with low 25(OH)D concentrations ($p = 0.001$) and high metabolic-GRS showed a significant association with high fasting insulin concentrations ($p = 0.045$). A significant interaction was found between vitamin D-GRS and total protein intake (g/day) (adjusted for non-animal protein) on 25(OH)D ($p_{interaction} = 0.006$), where individuals consuming a high protein diet (≥ 73 g/d) and carrying > 4 risk alleles for VDD had significantly lower 25(OH)D ($p = 0.002$) compared to individuals carrying ≤ 4 risk alleles.

Conclusion: Even though our study did not support a link between metabolic-GRS and vitamin D status, our study has demonstrated a novel interaction, where participants with high vitamin D-GRS and consuming ≥ 73 g of protein/day had significantly lower 25(OH)D levels. Further research is necessary to evaluate the role of animal protein consumption on VDD in Brazilians.

5.2 Introduction

An increased prevalence of vitamin D deficiency (VDD) has been reported worldwide [8, 394, 395]; however, in South America, studies reporting the prevalence of vitamin D deficiency are scarce [396-398]. Brazil, the largest country in South America, has a low-latitude and elevated ultraviolet rays (UVB) [399] and mostly tropical weather conditions with high temperatures throughout the year [400]. Despite having abundant year-round sunshine, vitamin D deficiency and insufficiency have been reported to be at 28.2% and 45.3%, respectively, regardless of age or gender [397]. Dietary consumption of vitamin D is generally low in the Brazilian populace and the amount of vitamin D in foods in Brazil is not accurately known. Moreover, many food products do not list information about vitamin D quantity in the food composition table and this hinders any studies trying to assess the influence of dietary intake of vitamin D on serum 25(OH)D levels. In general, dietary sources of vitamin D are limited and the small bioavailable amounts found in foods are inadequate for the phycological requirements of the human body [397, 401].

In Brazil, overweight and obesity are important health concerns. Overweight was reported at 43% in 2006, 53.8% in 2016 [402] and has increased in prevalence to 57.5% in 2020 [190]. Obesity was reported at 11.8% in 2006, increased to 18.9% in 2016 [402] and increased in prevalence to 21.5% in 2020 in the Brazilian population [190]. The concern over the increased frequency of overweight and obesity is due to their poor health consequence and being a key risk factor to several chronic diseases such as diabetes, cardiovascular disorders and some types of cancers [403]. Type 2 diabetes (T2D) is another health issue of concern in the Brazilian population and the country is ranked 5th in the world for highest number of people with T2D [404]. Furthermore, a little over 75% of T2D patients are either overweight or obese [405].

Several epidemiological studies have demonstrated a relationship between low vitamin D status and metabolic traits [333, 406-408]. Using genetic polymorphisms to explore the relationship between VDD and metabolic diseases can help minimize the inherent limitations of nutritional epidemiological studies [372, 409]. The use of genotypic information limits the effect of residual confounding from unconsidered factors or uncollected data [410]. Given that there are limited nutrigenetic studies in the Brazilian young adult population, this study will examine the association of the vitamin D-related genetic risk score (GRS) and metabolic disease-related GRS with clinical, biochemical and anthropometric parameters [130, 339], and examine the interactions between the GRSs and lifestyle factors on metabolic disease outcomes in young Brazilian adults using a nutrigenetic approach.

5.3 Methodology

5.3.1 Study Population

Obesity, Lifestyle and Diabetes in Brazil (BOLD) is a cross-sectional study in young healthy Brazilian adults aged 19–24 years enrolled at the Federal University of Goiás (UFG) between the months of March and June 2019. The study took place as part of Gene-Nutrient Interactions ("GeNuIne" Collaboration) which is an ongoing collaboration aiming to examine the effect of genes and lifestyle on chronic diseases in ethnically diverse populations [360, 411-413]. A baseline questionnaire was completed by all participants completed baseline questionnaires regarding health status, socioeconomic, and demographics status. Exclusion criteria included: use of hypoglycemic or lipid-lowering drugs; use of vitamin or mineral supplements; undergoing any dietary intervention in the past 6 months; engaging in vigorous physical activity; having acute medical conditions such as fever, inflammation, infection, diarrhea, or being diagnosed with chronic diseases such as hypertension, diabetes mellitus, rheumatoid

arthritis, cancer, or cardiovascular disease. In total, 200 individuals completed the BOLD study; however, for the present analysis after excluding 13 participants with incomplete genetic data the total number of participants included was 187. Ethical approval was granted by the Ethics Committee of the Federal University of Goiás (protocol number 3.007.456, 8 November 2018), and performed according to the ethical principles in the Declaration of Helsinki. All study participants signed an informed consent form.

5.3.2 Anthropometric Measures

Anthropometric measurements were taken by trained investigators using validated methods. Measurements of body weight, height and waist circumference (WC) were taken using identical techniques [414, 415]. A Tanita® (Tanita Corporation, Itabashi, Tokyo, Japan) portable electronic scale was used to weigh participants, the maximum capacity was 150 kg. Height measurements were taken using a stadiometer with a mobile rod. Measurements of WC were obtained by using a non-stretchable measuring tape. Calculation of body mass index (BMI) was performed using the following formula: weight (kg)/height (m2). Dual Energy Radiological Absorptiometry scan (DXA) was used to measure body composition, using the Lunar DPX NT model (General Electric Medical Systems Lunar®; Madison, WI USA). Body fat percentage (BFP) was considered elevated when it was 30.0% for women and 20.0% for men [416-418].

5.3.3 Biochemical Measures

Blood samples were collected by a qualified professional, through a peripheral venous puncture in the morning after a 12-h fast (not eating or drinking anything but water), with additional advice not to consume alcohol 72 h before the blood collection. Ethylene-diaminetetra acetic acid (EDTA) tubes using a BD Vacutainer® were used to collect blood

samples and the samples were immediately processed after pooling at Romulo Rocha Laboratory (Goiânia, Brazil) to obtain plasma and serum, respectively. Serum vitamin D concentration was measured by chemiluminescence, using the model Architect i1000, Abbott Diagnostics[419]. The fasting blood glucose and insulin were analyzed by the enzymatic colorimetric technique, with an automatic System Vitros Chemistry 950 XRL (Johnson & Johnson, New Brunswick, NJ, USA). Glycated hemoglobin (HbA1c) measurements were undertaken using high-pressure liquid chromatography (HPLC-Bio-Rad Laboratories, Hercules, CA, USA).

5.3.4 Assessment of Sun Exposure and Dietary Intake

Our study was conducted in the state of Goiás, which is in the central-west region of Brazil, during the fall season between the months of March and June 2019 where the average annual temperature is 24.6 °C [420] and the sunlight hours range between 11 and 12 h [421]. Sun exposure was assessed using a specific validated questionnaire adapted from the MIT-UV study protocol, which includes the usual time of daily exposure, commonly exposed body parts, use of sunscreen and sun protection factor (SPF) and skin type [422]. Daily sun exposure was recorded for 7 days and determined in 3 categories and assigned a numerical value: 1 = under 5 min of sun exposure, 2 = between 5 and 30 min of sun exposure, 3 = over 30 min of sun exposure. Total sun exposure for the week was determined by summing up the numerical values for each day which resulted in a range of 7-21 representing sun exposure for each participant. A median value of 16 was used to define participants, where individuals with value < 16 and ≥ 16 were categorized as those with low and high sun exposures, respectively [422]. Intake of food were assessed by experienced nutritionists using a three-day food diary (two weekdays and one weekend day) [423]. Food measuring equipment such as measuring spoons and measuring cups were provided to participants to aid them in approximating portion sizes

of foods. To determine exact amounts of consumed foods the software Avanutri Online® diet calculation (Avanutri Informática Ltd., Rio de Janeiro, Brazil) was used to convert food intakes into grams. The protein consumption was categorized as animal (including all kind of meat, fish, eggs, milk, and dairy products), and non-animal protein source in grams.

5.3.5 SNP Selection and Genotyping

We selected a total of 16 SNPs, of which 6 SNPs previously showed associations with vitamin D levels and 10 SNPs previously showed associations with metabolic traits in several ethnic groups. Six vitamin D-related SNPs were included in the analysis: vitamin D receptor (*VDR*) SNPs rs2228570 and rs7975232 [424, 425]; 7-dehydrocholesterol reductase (*DHCR7*) SNP rs12785878 [189, 369-371]; 25-hydroxylase (*CYP2R1*) SNP rs12794714 [363, 371]; 24-hydroxylase (*CYP24A1*) SNP rs6013897 [367, 369]; and vitamin D binding protein (*DBP*)/group-specific component (*GC*) SNP rs2282679 [189, 363]. Ten metabolic disease-related SNPs were included in the analysis: Fat mass and obesity-associated (*FTO*) SNPs rs8050136 and rs9939609 [282, 318, 343, 345, 372, 375, 426]; transcription factor 7-like 2 (*TCF7L2*) SNPs rs12255372 and rs7903146 [285, 344, 346, 348, 372, 427-429]; melanocortin 4 receptor (*MC4R*) SNP rs17782313 [177, 288, 372, 373, 430]; potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) SNPs rs2237895 and rs2237892 [342, 431]; cyclin dependent kinase inhibitor 2A/B (*CDKN2A*/B) SNPs rs10811661 [348, 376, 432, 433]; Peroxisome Proliferator Activated Receptor Gamma (*PPARG*) SNP rs1801282 [347, 348, 434, 435]; calpain 10 (*CAPN10*) SNP rs5030952 [436, 437].

DNA analysis was performed by collecting blood samples (3 mL each) in an EDTA tubes BD Vacutainer® tubes which were transferred in a (- 80ºC) temperature-controlled environment by the World Courier Company to perform genotyping at the LGC Genomics, London, UK (http://www.lgcgroup.com/services/genotyping, accessed on 26 February 2022).

5.3.6 Statistical Analysis

Statistical software SPSS (v27; SPSS Inc., Chicago, IL, USA) was used to conduct statistical analyses. The selected 16 SNPs were in Hardy–Weinberg equilibrium (HWE) ($p > 0.05$), which was tested using a goodness-of-fit chi square test (**Table S5.1**). Descriptive features of the study population were given as means and standard deviations (SD) for continuous variables and comparisons between groups were tested using independent samples t-test. Shapiro–Wilk test of normality was conducted on all continuous variables to verify the normality of the data in the variables. Log-transformation was performed on all non-normally distributed variables, and these variables included age (years), BMI (kg/m^2), waist circumference (WC) (cm), glucose (mg/dL), insulin (uIU/mL), HbA1c (ng/mL), total energy intake (Kcal), total carbohydrate (g), total protein, animal protein, non-animal protein (g), total fat (g), fibre (g) and 25(OH)D level (ng/mL).

Vitamin D cut-offs were decided based on the 2020 revised reference values of 'The Brazilian Society of Endocrinology and Metabolism and The Brazilian Society of Clinical Pathology/Laboratory Medicine'. The vitamin D values for the general Brazilian population were defined as normal when 25(OH)D levels were between 20 and 60 ng/mL, and deficient when 25(OH)D levels were below 20 ng/mL [438].

Two independent genetic risk scores (GRSs) were created by the addition of the sums of the risk allele across each SNP. The vitamin D-GRS was computed from six SNPs and the metabolic disease- GRS was computed from 10 SNPs. Each SNP had a value of 0, 1, or 2 and this value indicates the number of risk alleles for each GRS. Subsequently, these values were calculated by adding the number of risk alleles across each SNP. For each individual GRS, risk allele scores were then divided by the median and categorized into a "low genetic risk group" and a "high genetic risk group." Using the median of vitamin D-related GRS, low risk and high risk were categorized as individuals carrying ≤ 4 ($n = 112$) and those carrying > 4 ($n = 71$) risk

alleles, respectively. For the metabolic disease-GRS, low risk relates to individuals carrying ≤ 5 (n = 123) and high risk relates to those carrying > 5 (n = 60) risk alleles. **Figure 5.1** denotes the study design of the performed analyses. Statistically significant interactions were examined further with binary analysis based on nutrient dietary consumption. Study participants were split at midpoint of intake into two groups and designated as 'low intake' or 'high intake'. Given that there were no previously reported effect sizes for Brazilians, we were unable to perform a power calculation.

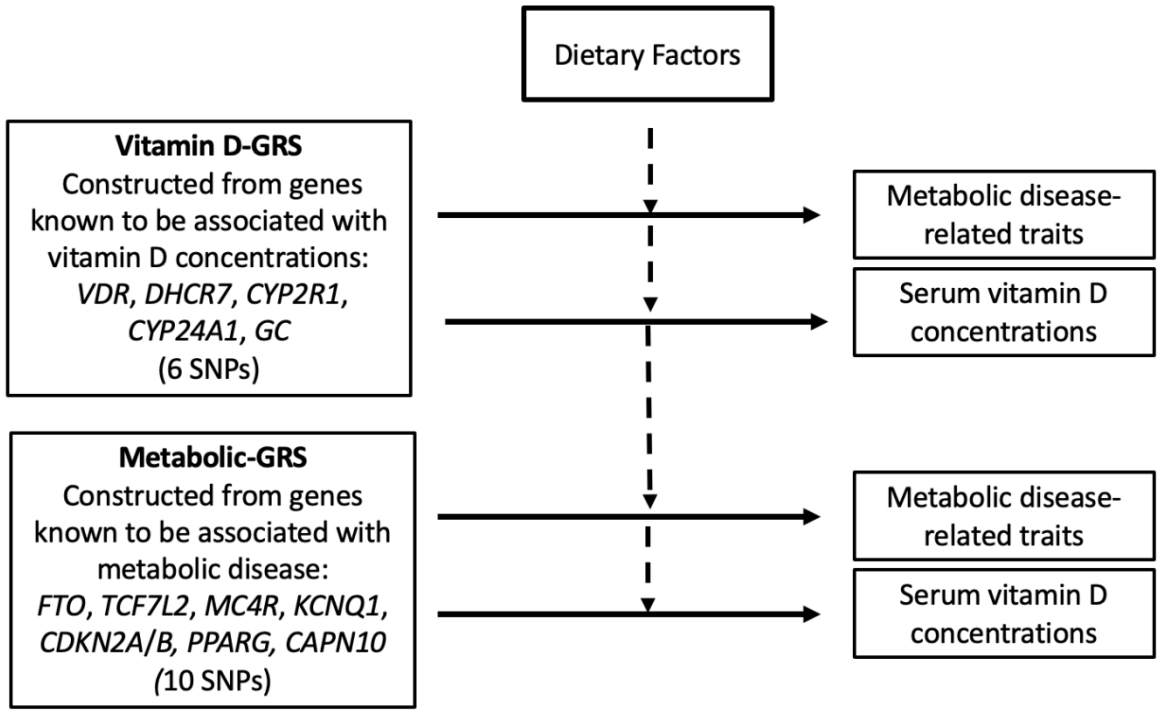


Figure 5. 1: Study design.

The one-sided horizontal arrows with solid lines represent the genetic associations and the one-sided vertical arrows with dotted lines represent the interactions between GRS and diet on clinical and biochemical measurements. The association of vitamin D-GRS with 25(OH)D levels and metabolic traits and the association of the metabolic-GRS with 25(OH)D levels and metabolic traits and were tested. Furthermore, analyses of the effect of dietary factors on these genetic associations were performed. Abbreviations: GRS: genetic risk score, SNP: single nucleotide polymorphism, *VDR*: Vitamin D Receptor, *DHCR7*: 7-dehydrocholesterol reductase,

CYP2R1: 25-hydroxylase, *CYP24A1*: 24-hydroxylase, *GC*: group-specific component, *FTO*: fat mass and obesity-associated gene, *TCF7L2*: transcription factor 7-like 2 gene, *MC4R*: melanocortin 4 receptor gene, *KCNQ1*: potassium voltage-gated channel subfamily Q member 1, *CDKN2A*/B: cyclin dependent kinase inhibitor 2A/B, *PPARG*: Peroxisome Proliferator Activated Receptor Gamma, *CAPN1*: calpain 10.

Linear regression models were used to analyze the association of vitamin D-related GRS and the metabolic disease-related GRS on anthropometric and biochemical outcomes (BMI, WC, 25(OH)D, glucose, HbA1c, fasting insulin), respectively. Additionally, the interaction between GRSs and dietary factors on clinical and biochemical variables were tested using linear regression models by including the interaction term (GRS x dietary factor). Regression models were adjusted for age, BMI, sun exposure and total energy intake, wherever appropriate. Dietary factors included total carbohydrate, total protein (animal protein, non-animal protein), total fat, and fibre intake in grams. Furthermore, in cases where interactions were statistically significant, study participants were split by the tertiles of dietary consumption and further analyzed.

5.4 Results

5.4.1 Characteristics of Participants

Anthropometric, biochemical, and dietary parameters of the BOLD study participants were compared based on 25(OH)D status and are summarized in **Table 5.1**. No significant differences were found between participants with normal vitamin D levels and participants with 25(OH)D deficiency except for total protein intake (g) ($p = 0.008$).

Table 5. 1: Baseline characteristics of study participants stratified by Vitamin D status.

Characteristics of study participants	*n*	Normal vitamin D status 25(OH)D ≥ 20 ng/mL	*n*	Deficient vitamin D status 25(OH)D < 20 ng/mL	*p* Value
Age (years)	154	21.32±1.71	31	21.35±1.56	0.928
BMI (kg/m^2)	154	23.01±3.87	31	23.76±5.66	0.370
WC (cm)	154	74.05±11.89	31	76.60±14.04	0.291
BFP (%)	154	33.76±10.65	31	34.57±11.05	0.702
Glucose (mg/dL)	156	86.74±6.79	31	88.35±7.29	0.235
HbA1c (%)	156	4.73±0.26	31	4.72±0.22	0.911
Fasting Insulin (uIU/mL)	156	8.72±3.69	31	8.80±4.09	0.911
Total Energy Intake (kcal)	156	1793±591	31	2024.12±676.96	0.054
Total Protein (g)	156	75.20±28.17	31	90.43±33.48	0.008
Total Carbohydrate (g)	156	230.67±84.32	31	258.08±99.59	0.111
Total Fat (g)	156	63.34±23.43	31	70.017±24.88	0.153
Dietary Fibre (g)	156	14.45±8.48	31	16.39±9.68	0.258

Data is presented as means ± SD, *p* values were calculated by using the independent t test. Vitamin D cut-off points were created on the recommendations of the Brazilian Society of Endocrinology and Metabolism and the Brazilian Society of Clinical Pathology/Laboratory Medicine vitamin D levels [438]. Abbreviations: BMI: body mass index, WC: waist circumference, BFP: body fat percentage, HbA1c: glycated hemoglobin.

5.4.2 Association between Vitamin D-GRS and Anthropometric and Biochemical Measurements

There was a statistically significant association between vitamin D-GRS and serum 25(OH)D concentrations ($p = 0.001$). Individuals who carried > 4 vitamin D risk alleles (mean ± SE: 1.38 ± 0.02) had significantly lower 25(OH)D levels compared to participants with ≤ 4 risk alleles (mean ± SE: 1.45 ± 0.01) (**Figure 5.2A)**.

5.4.3 Association between Metabolic-GRS and Anthropometric and Biochemical Measurements

A statistically significant association was observed between metabolic-GRS and fasting insulin ($p = 0.045$), where individuals who carried > 5 metabolic risk alleles (mean ± SE: 0.94

± 0.02) had significantly higher fasting insulin levels compared to individuals with ≤ 5 risk alleles (mean ± SE: 0.89± 0.02) (**Figure 5.2B**).

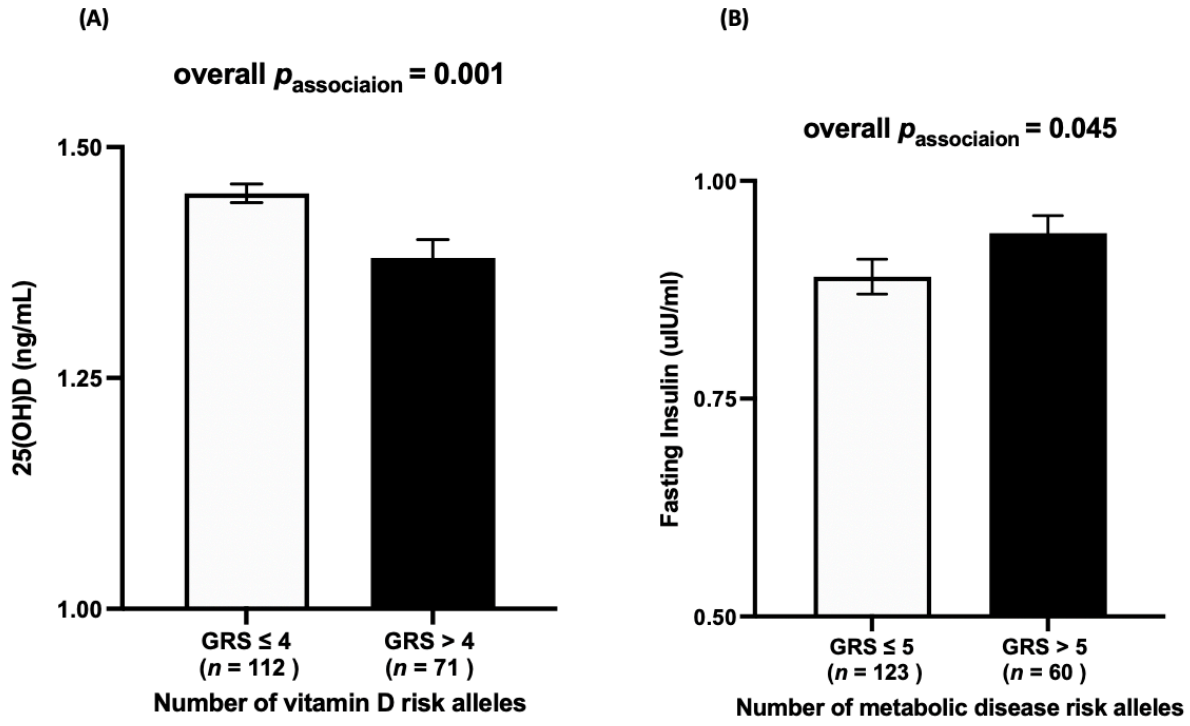


Figure 5. 2: Associations between Vitamin D-GRS with log 25(OH)D and between metabolic-GRS with log fasting insulin.

(A) The association between vitamin D-GRS and log 25(OH)D. Participants carrying > 4 vitamin D risk alleles (mean ± SE: 1.38 ± 0.02) had lower 25(OH)D levels compared to participants with ≤ 4 risk alleles (mean ± SE: 1.45 ± 0.01). (B) The association between metabolic-GRS and log fasting insulin. Participants carrying > 5 metabolic risk alleles (mean ± SE: 0.94 ± 0.02) had higher fasting insulin levels compared to participants with ≤ 5 risk alleles (mean ± SE: 0.89± 0.02).

5.4.4 Interaction between the Vitamin D-GRS and Dietary Factors on Biochemical and Anthropometric Measurements

A statistically significant interaction was found between the vitamin D-GRS and total protein intake (g) on log 25(OH)D concentrations ($p_{interaction}$ = 0.006), where participants who had high protein intake (≥ 73 g/d) and > 4 risk alleles, had significantly lower log 25(OH)D concentrations (mean ± SE 1.36 ± 0.021, p = 0.001) than participants with ≤ 4 risk alleles (mean ± SE: 1.46 ± 0.019, p = 0.001). Even after adjusting for non-animal protein sources, the interaction of vitamin D-GRS with protein intake (g) was statistically significant, where a participants with > 4 risk alleles had significantly lower log 25(OH)D concentrations (mean ± SE 1.36 ± 0.021, p = 0.002) than participants with ≤ 4 risk alleles (mean ± SE: 1.46 ± 0.019, p = 0.002), (**Figure 5.3)**. In addition, there was also a significant interaction of vitamin D-GRS with total protein intake (g) on BFP ($p_{interaction}$ = 0.049), and with fat intake (g) on fasting glucose ($p_{interaction}$ = 0.019), and with MUFA intake (g) on fasting glucose concentrations ($p_{interaction}$ = 0.027).

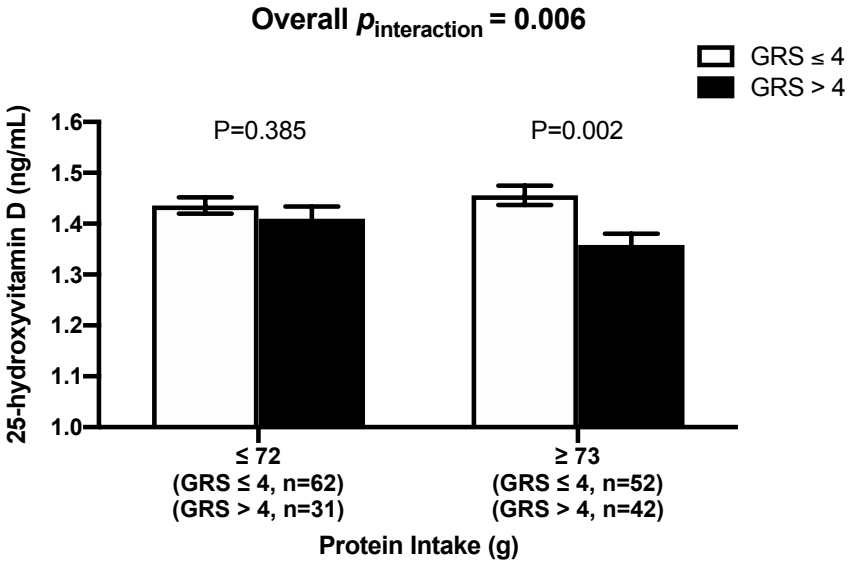


Figure 5. 3: Interaction between Vitamin D-GRS and total dietary protein intake (g) on log 25(OH)D adjusted for non-animal protein intake.

Participants who had high protein intake (≥ 73 g/d) and GRS > 4 (mean ± SE 1.36 ± 0.021) had significantly lower 25(OH)D ($p = 0.002$) than participants with GRS ≤ 4 (mean ± SE: 1.46 ± 0.019).

5.4.5 Interaction between the Metabolic-GRS and Dietary Factors on Clinical and Biochemical Measurements

There was no significant interaction between metabolic-GRS and dietary intake on serum 25(OH)D concentrations and clinical and biochemical factors ($p > 0.079$ for all comparisons), respectively (**Table S5.2**).

5.5 Discussion

As fa as we know, this study is the first to examine the link between vitamin D status and metabolic disease related traits using a nutrigenetic approach in young healthy Brazilian adults. Our study has demonstrated the association of the high vitamin D-GRS with lower serum vitamin D concentrations and the association of metabolic-GRS with fasting insulin concentration. The main finding of this study is a significant interaction between the vitamin D-GRS and total protein intake on serum vitamin D levels after adjusting for non-animal protein intake. The association of total protein intake in participants with higher genetic risk of VDD on 25(OH)D levels is an interesting finding; further investigation of the impact of higher protein intakes (from animal and non-animal sources) on vitamin D status using a randomized controlled trial would be helpful to illustrate whether this is a true finding.

In our study, we generated a GRS based on six vitamin D-related SNPs in genes involved in vitamin D metabolism. The vitamin D-GRS was associated with low vitamin D levels indicating that it is an ideal instrument for vitamin D deficiency. Even though we were unable to provide statistical evidence for the association between genetically instrumented

vitamin D-GRS and metabolic disease traits in our study, we identified that individuals who had higher genetic risk alleles (GRS > 4) and consumed higher amounts of protein (≥ 73 g/d) had significantly lower 25(OH)D than participants with lesser risk alleles (GRS ≤ 4). Studies looking into the effect of dietary intake in people with high genetic risk for vitamin D deficiency are uncommon and studies reporting on the effect of dietary protein intake on vitamin D status for individuals with high genetic risk of vitamin D deficiency are non-existent. We previously investigated the effect of genetic factors and dietary intake in the Indonesian Minangkabau women and discovered a significant interaction between dietary carbohydrate intake and high vitamin D genetic risk on body fat composition ($p_{interaction}$ = 0.049) [409]. This implicates the significance of genetic and dietary heterogeneity that exists across multiple ethnic groups. A randomized weight-loss intervention trial [439] in 118 overweight and obese participants in the United States, where the participates were randomly assigned to a weight-loss diet for two years with different percentages of caloric energy from macronutrients, showed a significant interaction between vitamin D-GRS and dietary fat intake on two-year changes in whole-body bone mineral density ($p_{interaction}$ = 0.02). Nevertheless, this study did not examine vitamin D status [440]. In comparison to the previous studies, our findings are novel and hence further studies relating to vitamin D GRS – diet interactions on vitamin D status are necessary to corroborate our findings.

The mechanism of the possible effect of high protein intake on vitamin D concentrations particularly in genetically VDD susceptible individuals is not clear, however, it could be specifically driven by animal protein sources as the findings in our study remained significant after adjustments for non-animal protein sources. Our finding is contrary to the results from a couple of studies on bone and skeletal health where it was reported that high protein intake positively interacts with vitamin D metabolism through the production of insulin-like growth factor-I (IGF-I) and enhances renal production of 1,25 dihydroxyvitamin

D [441, 442]. Nevertheless, these studies did not specify the sources of the dietary protein that enhanced vitamin D metabolism. In a cross-sectional study investigating vitamin D concentrations in 176 healthy vegetarian vs non-vegetarian Pakistani individuals, a significantly lower serum 25(OH)D (p = 0.001) was found in individuals who were non-vegetarians (n= 9; mean ± SD: 9.39 ± 2.45) compared to vegetarians (n= 83; mean ± SD: 13.78 ± 3.48) [443], but whether the reduced 25(OH)D levels were influenced by increased animal protein intake was not investigated. The effect of dietary animal protein intake on vitamin D concentrations requires future investigations to confirm or refute our findings and to determine the molecular mechanisms of action.

According to the 2008 - 2009 data from the Brazilian Household Expenditure Surveys (HES), dietary protein and fat intakes have increased in Brazil while carbohydrates content has decreased [444]. Increase in protein intake was due to increased consumption of animal flesh and animal products. The mean daily caloric intake from a nationwide cross-sectional survey using the HES data of 2008-2009 was estimated to be 1,902 kcal. The total carbohydrate intake contributed to 56% of the total energy, total fat intake contributed to 27% of total energy and total protein intake contributed to 17% of total energy with animal protein providing 10% of the total energy intake [444]. According to internationally accepted dietary guidelines [445-447] the total daily recommended protein intake is between 10-15% of total daily energy intake; this translates into 48-71 g of daily protein intake from the estimated caloric intake of the Brazilian population (1,902 kcal). In our study, the protein intake of our participants ranged between 73 and 217 g/day which is higher than the daily protein intake recommendations. Hence, adherence to the dietary protein intake recommendations may be an effective strategy to overcome the genetic risk of vitamin D deficiency in Brazilians.

The main strengths of the study are that it is the first nutrigenetics analysis to investigate interactions between vitamin D-GRS and metabolic related traits in a healthy young Brazilian

population. A genetic approach is favorable to observational studies as the genotype is not modified by the disease and is free from confounding [144, 410]. Additionally, the use of a GRS analysis instead of a single SNP analysis is a favorable approach as GRS method has a greater statistical power in predicting genetic predisposition over the single-locus approach [339, 359]. Furthermore, biochemical, and anthropometric measures were determined using validated techniques by skilled staff which improved the precision of these estimates. However, some limitations need to be acknowledged. One of the main limitations of the study is the small sample size, nevertheless, significant gene-diet interactions were identified suggesting that the study is well powered. Another limitation is that dietary intake was assessed using a three-day self-reported food record; despite being a validated and widely used method, we cannot discount the effect of reporting and recall bias. Furthermore, the Brazilian population is an admixture of many genetic ancestries from all over the world which makes it genetically heterogenous and could cause biased estimates of disease risk because of population stratification [127, 448-450]. Finally, the study population included healthy young adults which may not be demonstrative of the total Brazilian population.

5.6 Conclusion

In conclusion, our study has discovered a novel interaction between vitamin D-GRS and total protein intake on serum 25(OH)D levels after adjusting for non-animal protein intake in a young Brazilian adult population, where individuals with high GRS consuming more than 73 g of protein/day had significantly lower 25(OH)D levels. The finding is of particular interest for setting public health recommendations for preventing 25(OH)D deficiency in genetically susceptible young healthy Brazilian individuals given the increase in the dietary intake of animal protein in recent years [444]. Further investigations and randomized controlled trials are required to shed more light on the effect of increased animal protein intake on vitamin D

levels especially in individuals genetically susceptible to VDD to enable effective public health interventions to prevent VDD.

Chapter 6: Interactions between Vitamin D Genetic Risk and Dietary Factors on Metabolic Disease-Related Outcomes in Ghanaian Adults

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

Alathari BE, Nyakotey DA, Bawah AM, Lovegrove JA, Annan RA, Ellahi B, Vimaleswaran KS. Interactions between Vitamin D Genetic Risk and Dietary Factors on Metabolic Disease-Related Outcomes in Ghanaian Adults. Nutrients. 2022;14(13):2763. PMID: 35807945. *https://doi.org/10.3390/nu14132763*

Buthaina AlAthari's contribution: I developed the statistical analysis plan based on the dataset. Once after it was approved by the supervisors, cleaning of the dataset was performed before undertaking the statistical analysis using Statistical Package for the Social Sciences (SPSS) software (v27; SPSS Inc., Chicago, IL, USA). Based on the study findings, 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.

6.1 Abstract

Purpose: The Ghanaian population is experiencing an upsurge in obesity and type 2 diabetes (T2D) due to rapid urbanization. Besides dietary factors, vitamin D-related genetic determinants have also been shown to contribute to the development of obesity and T2D. Hence, we aimed to examine the interactions between dietary factors and vitamin D-related genetic variants on obesity and T2D related outcomes in a Ghanaian population.

Methods: Three hundred and two healthy Ghanaian adults (25–60 years old) from Oforikrom, Municipality in Kumasi, Ghana were randomly recruited and had genetic tests, dietary consumption analysis, and anthropometrical and biochemical measurements of glucose, HbA1c, insulin, cholesterol, and triglycerides taken.

Results: A significant interaction was discovered between vitamin D-GRS and fibre intake (g/day) on BMI ($p_{interaction} = 0.020$) where those consuming low fibre (≤ 16.19 g/d) and carrying more than two risk alleles for vitamin D deficiency ($p = 0.01$) had a significantly higher BMI. In addition, an interaction between vitamin D-GRS and fat intake (g/day) on HbA1c (total fat, $p_{interaction} = 0.029$) was found, where participants who had a lower total fat intake (≤ 36.5 g/d), despite carrying more than two risk alleles, had significantly lower HbA1c ($p = 0.049$).

Conclusion: In summary, our study has identified novel gene–diet interactions of vitamin D-GRS with dietary fibre and fat intakes on metabolic traits in Ghanaian adults.

6.2 Introduction

There is an observed increase in the prevalence of obesity and type 2 diabetes (T2D) in Sub-Saharan African (SSA) countries, and their related chronic diseases are becoming a rising cause of morbidity and mortality [451-453]. Ghana is a West Africa country with a public health concern of an increase in obesity and overweight which is mainly attributed to rapid urbanization along with increased industrialization, use of motorised transport, increased income, westernised diet and reduced physical activity [454-456]. The prevalence of the combined percentage of overweight and obesity in Ghana was reported to be around 43% in a systemic review meta-analysis in 48,966 adults (mean age range: 23 – 56.2 years old) from ten regions in Ghana [457]. Likewise, the prevalence of T2D in Ghanaian adults has been estimated in the year 2018 to be in the range of 6.2% - 13.9% [458] with a substantial proportion of undiagnosed cases that typically get identified with the onset of diabetic complications [459, 460]. Various factors predispose individuals to develop obesity and T2D including older age, diet, and inactivity. Additionally, genome-wide association studies (GWAS) in diverse populations have discovered more than 1,100 loci to be associated with obesity traits and nearly 600 loci to be associated with T2D risk, suggesting the role of genetic factors in metabolic diseases [461, 462].

Vitamin D deficiency has been demonstrated to be influenced by several genetic factors, including genes involved with its synthesis and metabolism [120, 145]. The 7-dehydrocholesterol reductase gene (*DHCR7*), encodes the enzyme that converts 7-dehydrocholesterol (7DHC) to cholesterol, [144, 147, 185], and 25-hydroxylase gene (*CYP2R1*), encodes the enzyme that converts vitamin D to the circulating form 25(OH)D [144, 147, 187]. Vitamin D metabolism genes include the 24 hydroxylase gene (*CYP24A1*), which encodes the enzyme that catabolises the biologically active form of vitamin D (1,25-dihydroxyvitamin D) to the inactive and water-soluble calcitroic acid, the excretory product of

vitamin D metabolism, thus, controlling the amount of active vitamin D in the blood [144, 147, 198], the vitamin D binding protein (*DBP*)/group-specific component (*GC*) which binds to 25-hydroxyvitamin D and its plasma metabolites to transports them to target tissues [144, 147, 200], the vitamin D receptor gene (*VDR*), which encodes the nuclear vitamin D receptor and heterodimerize with the retinoid X receptor (*RXR*) to enables the biological activities of 1,25-dihydroxyvitamin D [463, 464]. Finally, the calcium sensing receptor gene (*CASR*), which is important in regulating calcium homeostasis and synthesis of the parathyroid hormone (PTH), has been shown to stimulate the synthesis of 1,25-dihydroxyvitamin D [465, 466]. Numerous studies have identified genetic variants associated with vitamin D status and those genetic markers have been validated as genetic instruments for low vitamin D concentrations [120, 144, 463].

Several epidemiological studies investigating the link between vitamin D status and metabolic traits have reported inconsistent findings where some studies found an association between vitamin D deficiency and metabolic outcomes [328-330] while other studies failed to find any connection [328-331]. Using genetic variants to examine the relationship between vitamin D status and metabolic disease outcomes has been shown to be an effective method to overcome the effects of confounding [467]. Therefore, the aims of this study were to use a nutrigenetic approach to determine whether a genetic risk score (GRS) of eight single nucleotide polymorphisms (SNPs) from six selected candidate genes related to vitamin D deficiency was associated with metabolic disease-related traits and whether these associations were modified by dietary intake in 302 randomly chosen healthy adults from Oforikrom, Ghana.

6.3 Methodology

6.3.1 Study Population

Study population was taken from the Genetics of Obesity and Nutrition in Ghana (GONG) study, which is a cross-sectional study in healthy Ghanaian adults aged 25–60 years. The study which took place in Oforikrom Municipality in Kumasi, Ashanti region, Ghana. The GONG study is a part of the Gene–Nutrient Interactions (GeNuIne). Collaboration, the main objective of which is to explore gene–nutrient interactions on metabolic disease outcomes in different ethnicities using population-based studies from various countries [17, 18, 412, 468]. The Oforikrom Municipal Assembly is one of the 43 districts in the Ashanti region in Ghana. Oforikrom was a part of the KumasiMetropolitan Assembly until 2018 when it was elevated to a municipal assembly district. In the OforikromMunicipal Assembly there are seventeen recognized communities with an estimated total population of 360,254. Five communities (Oforikrom, Ayeduase, Ayigya, Kotei, and Bomso) were randomly selected from the list of communities in the Oforikrom Municipal Assembly. In each community, a central point was located (automobile station, marketplace, or a landmark). An assigned field investigator chose to enter the first house that is facing either East,West, North or South of that central point. After selecting the house, the investigator requested to randomly recruit one person from the household. If no one agreed to participate in the household, the investigator moved on to the next household. Subsequently, the fieldworker entered the next house, and the selection process was repeated. All participants freely agreed to participate using a written informed consent.

A total of three hundred and two healthy free-living and adult volunteers were included in this study. Participants had no prior diagnosis of disease or physical complaints; all participants were screened and recruited for the study by trained investigators (**Figure 6.1**). A pre-questionnaire was developed and used where participants were asked if they had been diagnosed with cancer, diabetes, high cholesterol, high blood pressure, inflammatory, respiratory, gastrointestinal, thyroid, renal, liver or heart diseases, and about medication use and recent surgeries, to eliminate any unhealthy volunteers. The inclusion criteria were: (a) age

from 25 to 60 years old; (b) healthy adults; and (c) both parents to be Asante ethnicity. The exclusion criteria were: (a) age less than 25 years or above 60 years; (b) pregnant women; (c) current diagnosis or having a history of communicable disease or any non-communicable diseases such as, cardiovascular diseases, T2D, and hypertension; and (d) use of medication for controlling diabetes, and hypertension, or lipid lowering drugs.

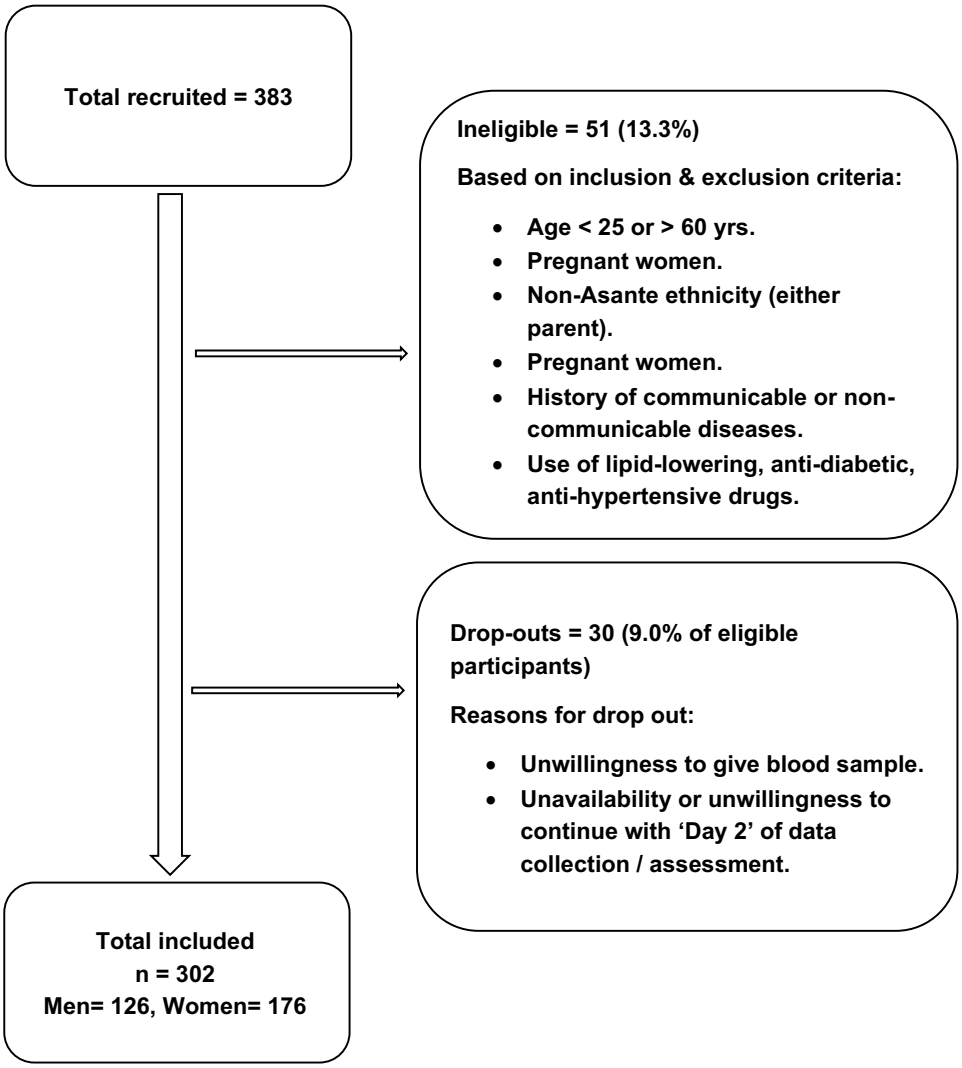


Figure 6. 1: Flow chart showing the recruitment of the study participants.

Ethical approval was obtained from the Council for Scientific and Industrial Research (CSIR) Institutional Review Board (IRB) (Ref: RPN 003/CSIR-IRB/2018). Furthermore, this study was approved by the Metro Director of Health Services, Kumasi (KMHD/MPHs/13). An informed consent form was signed by each participant prior to their participation in the study.

6.3.2 Data Collection

Questionnaires were used to gather information about the participants' demographic characteristics, dietary intakes, sleep, sunshine exposure patterns, and medical history, and demographic characteristics. Proper training was provided to the field investigators prior to the start of data collection. The instruments used for the survey were pre-tested to make sure that investigators had complete understanding of the questionnaires. The study was conducted from July to September 2018.

Anthropometric data [weight, height, hip circumference (HC), waist circumference (WC), visceral fat, and body fat percentage (BFP)] were measured with participants wearing light clothing. Height was measured to the nearest 0.1 cm, with a stadiometer (Seca 213 mobile stadiometer, Hamburg, Germany) with participants standing upright without shoes. Weight was measured to the nearest 0.1 kg, using an OMRON Body Composition Analyzer which also provided the values for the visceral fat, BFP, and body mass index (BMI). Nonextensible measuring tape was used to measure HC and WC. The HC was measured at the level of the greater trochanter to the nearest 0.1 cm whereas the WC was measured just above the naval to the nearest 0.1 cm. Waist-to-hip ratio (WHR) was calculated by dividing WC by HC.

6.3.3 Biochemical Measurements

Blood samples were collected via venipuncture after an 8–12 h fast from each participant by a trained phlebotomist. Fasting plasma glucose, glycated haemoglobin (HbA1c), fasting insulin, and lipid profile were analyzed. Fluoride tubes and ethylene–diaminetetra acetic acid (EDTA) tubes were used to collect blood samples which were stored in ice packs for temporary storage and transported to the Clinical Analyses Laboratory, KNUST for analysis. Blood glucose was analyzed using a semi-automated spectrophotometer (Biolabo

Diagnostic Kenza Biochemistry Try, France). Fasting serum insulin was analyzed using an enzyme-linked immunosorbent assay (ELISA) kit. Ion-exchange chromatography method was used to determine total glycated hemoglobin. Total cholesterol, HDL-c, and serum triglycerides concentrations were determined by using a semi-automated spectrophotometer (Humalyzer Junior, Human GmBH Germany). The Friedewald formula was used to compute concentration of LDL-c in all the samples. All test kits were from Medsource Ozone Biomedicals Pvt. Ltd. Haryana, India

6.3.4 Assessment of Dietary Intake

A repeated three-day 24-h dietary recall (one weekend day and two weekdays) was used to extract the participants' dietary intake data. The participants were asked to recall time of all meals taken and all the content of the consumed meals. Common household measures were used to help participants accurately estimate the actual amounts of foods and drinks consumed. An African specific dietary software (Nutrient Analysis Template; Food Science and Nutrition Department, University of Ghana, Accra, Ghana, 2010) was then used to analyse the nutritional composition of the participants' diet.

6.3.5 SNP Selection, GRS Construction and Genotyping

The following 8 SNPs were selected based on their known association with vitamin D concentrations: *VDR* SNPs rs2228570 and rs7975232 [122, 469]; *DHCR7* SNP rs12785878 [122, 364, 369-371]; *CYP2R1* SNPs rs12794714 and rs10741657 [122, 363, 369, 371, 463]; *CYP24A1* SNP rs6013897 [122, 367, 369]; *DBP*/*GC* SNP rs2282679 [363, 364]; and *CASR* SNP rs1801725 [122, 470]. Selected gene variants were tested using goodness-of-fit chi square test and all were in Hardy–Weinberg equilibrium (HWE) ($p > 0.05$) (**Supplementary Table S6.1).**

The collected blood samples for the DNA analyses were transported in dry ice to the United Kingdom (UK). Subsequently, genomic DNA extraction took place from a 5 mL whole blood sample from each participant, and genotyping was performed using the competitive allele-specific PCR-KASP assay at the LGC Genomics (http://www.lgcgroup.com/services/genotyping, accessed on 26 June 2022).

Vitamin D-related GRS was constructed by the addition of the sums of the risk allele across each of the eight SNPs. Given that there are no studies pertaining to vitamin D risk allele data in the West African population, the risk alleles for the present study were determined based on studies that were carried out in Caucasian populations [469, 471-481]. Each single SNP was assigned a value of 0, 1, or 2 and this value designates the number of risk alleles. Subsequently, these values were calculated by the addition of the total risk alleles across each SNP and the score ranged from 0 to 6 risk alleles. Risk allele scores were then divided by the median, which was 2 risk alleles, and categorized into a "low genetic risk group" and a "high genetic risk group." By use of the median of vitamin D-related GRS, low risk and high risk were categorized as individuals carrying less than 2 risk alleles (n = 68) and those carrying 2 or more risk alleles (n = 211), respectively. The risk alleles were not weighted, since no previously reported effect sizes were available for these SNPs for Ghanaians, and weighting of risk alleles has shown to have only limited effects [482]. Sample size calculations could not be performed because there were no previously reported effect sizes available for these SNPs for Ghanaians.

6.3.6 Statistical Analysis

Analyses of data were performed using Statistical Package for the Social Sciences (SPSS) software (version 27; SPSS Inc., Chicago, IL, USA). Descriptive characteristics of study population were given as means and standard deviations (SD) for continuous variables,

and between group comparisons were tested using an independent samples t-test. The Shapiro–Wilk test of normality was performed on continuous variables to test if the variables were in normal distribution. All variables were non-normally distributed; therefore, natural log transformation values were used in association and interaction analyses for all variables. General linear models (GLM) were used to analyze the association between vitamin D related GRS and biochemical and clinical metabolic outcomes (BMI, WC, WHR, BFP, glucose, HbA1c, fasting insulin). The vitamin D-GRS and dietary intake interactions were also analyzed using GLM by incorporating the interaction terms in these models. Models were adjusted for age, gender, and BMI (when BMI was not an outcome), and total energy intake (only in the nutrient–GRS interaction analysis). The dietary factors investigated in our study included total dietary intake of carbohydrate, protein, fat, and fibre. If the interaction of the GRS with total fat intake was significant, further analyses were performed to assess the influence of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). All vitamin D-GRS and dietary interactions reaching a nominal level of significance were further investigated using a binary and tertile stratification of dietary factors. The two-tailed value of $p < 0.05$ was considered statistically significant. Given that there are no studies on GRS and no previously reported effect sizes for the West Africans, we were unable to perform a power calculation.

6.4 Results

6.4.1 Characteristics of Study Participants

GONG study participants were stratified by gender, and the baseline characteristics of clinical, anthropometric, biochemical, and dietary measurements were compared and summarized in **Table 6.1**. The mean BMI and total energy intake of all participants were 26.6

± 5.0 kg/m^2 and 1645 ± 688 kcal, respectively. Several significant differences in clinical, anthropometric, and dietary measurements were found between men and women. Women had significantly higher age, BMI, WC and BFP than men ($p < 0.003$). Conversely, men had significantly higher total energy intake and higher dietary intake of all measured nutrients; protein, carbohydrates, fat, SFA, MUFA, PUFA and fibre ($p < 0.006$). However, there were no significant differences in biochemical measurements (glucose, HbA1c and fasting insulin) between men and women.

Table 6. 1: Baseline characteristics of study participants.

	n	**Total**	***n***	**Men**	***n***	**Women**	*p* Value
Age (years)	279	38 ± 10	115	36 ± 9	164	40 ± 10	0.003
BMI (kg/m^2)	279	26.6 ± 4.91	115	23.6 ± 3.02	164	28.7 ± 4.92	< 0.001
WC (cm)	279	88.4 ± 12.22	115	81.8 ± 9.92	164	93 ± 11.59	< 0.001
WHR	279	1.5 ± 7.24	115	0.9 ± 0.1	164	1.9 ± 9.43	0.15
BFP (%)	279	32.9 ± 13.55	115	20.5 ± 10.01	164	41.6 ± 7.58	< 0.001
Glucose (mg/dl)	278	4.4 ± 0.91	115	4.3 ± 0.59	163	4.4 ± 1.09	0.33
HbA1c (%)	275	5.3 ± 0.58	111	5.3 ± 0.5	164	5.3 ± 0.62	0.94
Fasting Insulin (µIU/mL)	270	12.6 ± 14.38	109	13.1 ± 16.08	161	12.3 ± 13.15	0.62
Total Cholesterol (mg/dL)	276	212.7 ± 58	113	208.8 ± 41.76	163	216.6 ± 39.06	0.07
HDL-c (mg/dL)	276	69.6 ± 7.70	113	69.6 ± 7.35	163	65.7 ± 0.7.73	0.12
LDL-c (mg/dL)	276	127.6 ± 41.76	113	123.7 ± 42.54	163	131.5 ± 40.99	0.06
Serum Triglycerides (mg/dL)	276	87.3 ± 32.78	113	86.8 ± 29.23	163	87.7 ± 36.32	0.98
Total Energy Intake (kcal)	279	1645 ± 688	115	1901 ± 714	164	1465 ± 610	< 0.001
Protein (g)	279	53 ± 23	115	63 ± 24	164	46 ± 19	< 0.001
Carbohydrate (g)	279	240 ± 98	115	281 ± 104	164	211 ± 81	< 0.001
Fat (g)	279	51 ± 27	115	57 ± 29	164	47 ± 24	0.001
Saturated fat (g)	279	16 ± 10	115	18 ± 11	164	15 ± 9	0.006
Monounsaturated fat (g)	279	18 ± 10	115	20 ± 11	164	16 ± 9	0.002
Polyunsaturated fat (g)	279	9 ± 5	115	10 ± 6	164	8 ± 5	0.002
Dietary Fibre (g)	279	22 ± 11	115	25 ± 12	164	19 ± 10	< 0.001

Data is presented as means ± SD, *p* values for the differences in the means between the two groups were calculated by using the independent samples t-test, Abbreviations: BMI: body mass index, WC: waist circumference, WHR: wait-hip ratio; BFP: body fat percentage; HbA1c: glycated haemoglobin; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol.

6.4.2 Genetic Associations between Vitamin D-GRS and Metabolic Traits

No statistically significant associations were found between vitamin D-GRS and anthropometric and biochemical measurements (p > 0.12 for all comparisons) (**Supplementary Table S6.2**).

6.4.3 Interactions between Dietary Factors and Vitamin D-GRS on Metabolic Traits

There was a statistically significant interaction between vitamin D-GRS and fibre intake (g/day) on BMI ($p_{interaction}$ = 0.020). Participant who consumed low fibre (≤ 16.2 g/d) and carried more than two risk alleles (Mean ± SE: 1.45 ± 0.009, p = 0.010) had significantly higher BMI than participants with less than two vitamin D risk alleles (Mean ± SE: 1.40 ± 0.015, p = 0.010) (**Figure 6.2A**).

There was also a significant interaction between vitamin D-GRS and fat intake (g/day) on HbA1c ($p_{interaction}$ = 0.029), where participants who had lower consumption of dietary fat (≤ 36.5 g/d) and carried more than two risk alleles (Mean ± SE: 0.72 ± 0.005, p = 0.049) had significantly lower HbA1c than participants with less than risk alleles (Mean ± SE: 0.74 ± 0.010, p = 0.049) (**Figure 6.2B**). Total fat was stratified into SFA, MUFA, and PUFA and we found that SFA showed a significant interaction ($p_{interaction}$ = 0.044) with vitamin D-GRS on HbA1c (**Table 6.2**). However, after dividing participants based on SFA intake (low SFA, medium SFA, and high SFA), no significant differences were detected between participants with high or low vitamin D deficiency genetic risk in any of the fat intake groups.

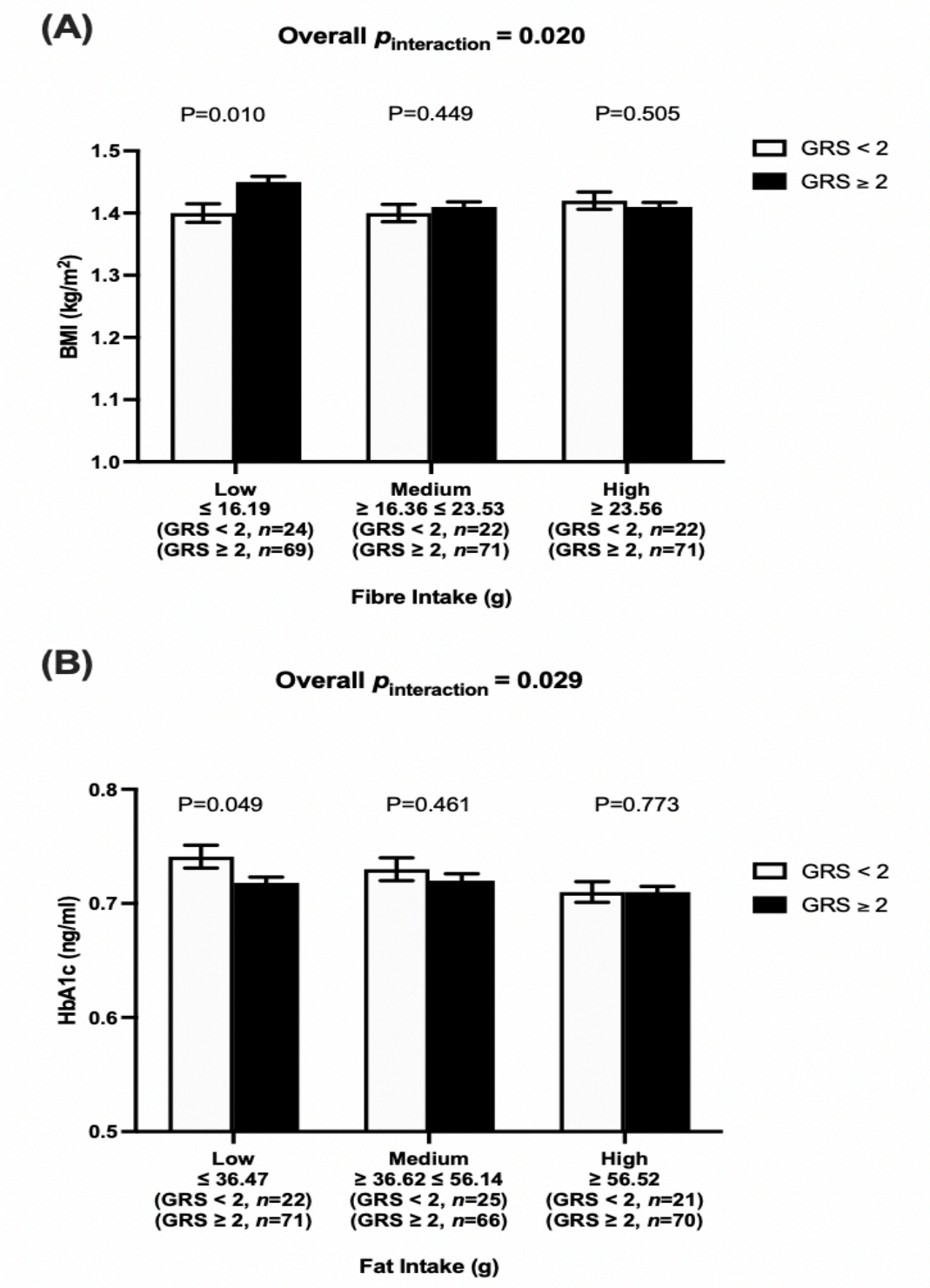


Figure 6. 2: Interactions of Vitamin D-GRS with fibre intake (g) on log BMI and with fat intake (g) on log HbA1c.

(A) Interaction between vitamin D-GRS and total dietary fibre intake (g) on log BMI ($p_{interaction}$ = 0.020); Participant who consumed lower fibre (≤ 16.19 g/d) and carried ≥ 2 risk alleles (Mean

± SE: 1.45 ± 0.009) had significantly higher BMI than participants with < 2 vitamin D risk alleles (Mean ± SE: 1.40 ± 0.015). (B) Interaction between vitamin D-GRS and total fat intake (g) on log HbA1c ($p_{interaction}$ = 0.020); Participant who consumed lower fat intake (≤ 36.47 g/d) and carried ≥ 2 risk alleles (Mean ± SE: 0.72 ± 0.005) had significantly lower HbA1c than participants with < 2 risk alleles (Mean ± SE: 0.74 ± 0.010).

Table 6. 2: Interaction between dietary factors and Vitamin D-GRS on clinical and metabolic traits.

	Carbohydrates (g)	Protein (g)	Fat (g)	Fibre (g)	SFA (g)	PUFA (g)	MUFA (g)
BMI (kg/m2)	0.05	0.16	0.99	0.02			
WC (cm)	0.16	0.07	0.22	0.13			
WHR	0.72	0.76	0.85	0.87			
BFP (%)	1.00	0.27	0.22	0.12			
Glucose (mg/dL)	0.98	0.83	0.88	0.52			
HbA1c (ng/mL)	0.06	0.12	0.03	0.10	0.04	0.13	0.84
Fasting Insulin (µIU/mL)	0.35	0.68	0.43	0.13			

GLM was used to perform interaction analysis. All variables were log transformed. All associations were adjusted for age, gender, BMI (except BMI which was not adjusted for when the outcome was BMI) and total energy. The analysis was performed on log-transformed variables. Abbreviations: GRS: genetic risk score, BMI: body mass index, WC: waist circumference, WHR: waist-hip ratio, BFP: body fat percentage, HbA1c: glycated haemoglobin.

6.5 Discussion

To the best of our knowledge, our study is the first to use a nutrigenetic approach to examine the interaction between vitamin D genetic variants and dietary factors on metabolic outcomes in healthy West African adults from Ghana. This study demonstrated the association of vitamin D-GRS on BMI, a diagnostic marker of obesity [483, 484], through the interaction

with dietary fibre intake. Additionally, this study provided evidence for an interaction between vitamin D-GRS and fat intake on HbA1c levels, an indicator of glycaemic control [485, 486]. Both these findings are in agreement with general dietary recommendations addressing dietary fibre and total dietary fat consumption [487] to decrease the risk of obesity and T2D [488-492]. Our results carry public health implications where individuals with genetic risk of vitamin D deficiency might benefit from adhering to general dietary recommendations to prevent obesity and T2D.

According to the Institute of Medicine (IOM), the dietary guidelines for total daily fibre intake is 14 g/1,000 kcal/day and for total daily fat intake, the recommendation is between 20-35% of total energy intake [487]. In our cohort, the average total energy intake was 1,645 kcal/d; hence the recommended average fibre intake should be 23 g/day, and the total recommended fat intake should be between 36.5-64.0 g/day. The findings from our study confirms the importance of adhering to the dietary fibre and fat intake guidelines and emphasises their importance to individuals with higher genetic predisposition to vitamin D deficiency to prevent obesity. Our study has demonstrated a significant impact of vitamin D-GRS on BMI which was detected under the influence of a low fibre diet (≤ 16.2 g/day); hence increasing the dietary fibre consumption will benefit those individuals who are genetically susceptible. Likewise, reducing the consumption of dietary fat intake to 20% of total calories might be beneficial in improving the glycaemic control in genetically susceptible individuals.

The results of our study emphasizes the importance of a high fibre diet for individuals with genetic risk of vitamin D deficiency. The role of dietary fibre in relation to the vitamin D-GRS and BMI is not clear; however, both fibre and vitamin D are involved in the health and regulation of gut microbiome [493]. Dietary fibres are known to have beneficial effects on body weight management due to their bulking effect and fermentation by the gut microbiota [494, 495]. Prebiotics specifically, when consumed induce changes in intestinal microbiota

diversity and increase the release of short-chain fatty acids (SCFAs), such as butyrate, which has the capacity to regulate anti-inflammation processes [496, 497]. Obesity is known to be associated with chronic Inflammatory markers, most notably the overexpression of pro-inflammatory cytokines produced in adipose tissues [498]. Furthermore, obesity is associated with altered gut microbiota composition and/or activity in humans [499]. As for vitamin D, studies have demonstrated that vitamin D and its receptor, VDR, can regulate and influence gut microbiota [500, 501]. Vitamin D has immune-modulatory properties such as inhibiting inflammation and infections and hence might be important in modifying gut microbiota [493]. As both fibre and vitamin D have anti-inflammatory properties, and both are potential modifiers of gut microbiome, therefore, our gene-fibre intake interaction on BMI could be possibly a result of the influence of microbiome modulation brought by reduced dietary fibre intake in participants with increased genetic susceptibility to vitamin D deficiency. Furthermore, some studies have examined the impact of dietary fibre and genetic markers on obesity outcomes. One study in Asian Indian adults (n = 1,618) reported that fibre intake and fat mass obesity gene (*FTO*) SNPs had a significant influence on BMI and WC ($p_{\text{interaction}}$ = 0.0008) where participants with high fibre intake (44 g/day) had lower BMI (p = 0.07) and lower WC (p = 0.02) [282]. Another study reported a significant interaction between metabolic-related GRS and fibre intake on BFP where participants with higher fibre intake (> 19 g/day) had lower BFP [502].

A recent longitudinal observational study (n = 2500) reported that dietary fibre consumption in Ghana (24.9 ± 9.7 g/day) was higher than Jamaica, Seychelles, South Africa and Africans from the United States, which could be attributed to the high consumption of cassava in Ghana [503]. Nevertheless, only 43% of the study participants [503] met the recommended dietary guidelines for fibre intake (14 g/1,000 kcal/day) established by the Institute of medicine (IOM) [487], and fibre intake continue to decline with rapid urbanization

in Ghana [504]. It is established that higher total fibre intake is associated with lower inflammation, obesity and BMI [487, 505]. However, studies focusing on the relationship between fibre intake and obesity in the African population are limited. A recent cross-sectional study in 406 Ghanaian men (20-29 years old) demonstrated a significant inverse relationship between fibre intake and abdominal obesity [506]. Furthermore, a randomised trial in 107 African American women investigating fibre intake after 6- and 18-month dietary weight loss intervention found that the consumption of fibre at the 6-month follow-up was significantly and negatively associated with BMI and stronger negative association was found at the 18-month follow-up BMI [507]. It is important to emphasise the importance of increasing fibre intake for individuals at risk of vitamin D deficiency to meet the recommended dietary guidelines to help tackle the current rise in obesity in Ghana.

Nutritional transition from traditional diet to westernized diet plays a role in increasing dietary fat consumption in SSA countries and Ghana is no exception [504, 508]. The effects of the changing diet from a low fat rural diet to a high fat urban diet have already translated in to increased obesity and T2D in the Ghanaian population [453, 509]. A recent nutrigenetics study in healthy Ghanaian adults (n = 302) [502] found a significant interaction between metabolic-related GRS and high total fat intake (> 47 g/day) and SFA (> 14 g/day) on increased WC suggesting the existence of an interplay between fat intake and genetic susceptibility on obesity outcomes and supporting the general dietary recommendations to reduce total fat intake and SFA to prevent obesity. Our study showed that lower fat intake was associated with a better glycaemic control for people with increased genetic risk of vitamin D deficiency. Links between total fat intake and glycaemic control have been reported in previous studies on diabetic participants where one crossover clinical trial in diabetic adults (n = 7) from Israel reported that higher dietary fat intake increased glucose concentrations [510]. Additionally, a cross-sectional study in 150 adult participants from New-Zealand found that increased SFA

was associated with 6% (95% CI 2–10%; $p = 0.004$) increase in HbA1c and concluded that reducing SFA maybe helpful in improving glycaemic control [511]. Total fat intake seems to have an effect on HbA1c levels this could be caused by reduced insulin sensitivity, or it could be because higher fat intake is causing an increased hepatic glucose production which can cause an increase in the peak time and amount of the glucose response [512, 513]. Evidence that dietary fat intake can affect glycaemic control in vitamin D susceptible individuals has important implications for developing strategies to prevent T2D in this subgroup.

Our study has several strengths of which being the first nutrigenetic study to examine the interaction between genetic variants of vitamin D and metabolic traits in healthy West Africans from Ghana is the main strength. In addition, we used the GRS approach to increased the statistical power of our analysis, and reduce the negative impact of multiple testing [121, 133]. Furthermore, anthropometric, and biochemical measurements were determined using validated techniques by skilled staff. Population stratification was also reduced as the participants were strictly Asante Ghanaians (both parents). Limitations include small sample size; however, we were still able to identify significant gene-diet interactions indicating that the study is sufficiently powered to detect significant interactions. We used a repeated 24-hour dietary recall method to assess nutrient intake which could be biased or have some recall errors. The study design is cross-sectional which is inherently prone to residual confounding [514, 515]. Finally, the study population were of a specific ethnic group in Ghana (Asante) which may not represent other ethnic groups in Ghana. Hence, more studies are required to confirm our findings to establish the clinical significance and possible applications as part of metabolic disease prevention.

6.6 Conclusion

In conclusion, the current study has identified novel gene-diet interactions in the West African Ghanaian population. Our study has shown that low fibre intake is associated with higher obesity and low-fat intake is associated with greater glycaemic control in vitamin D genetically susceptible individuals. Given that both obesity and T2D are on the rise in Ghana [457, 459], our study highlights the importance of implementing strategies to follow IOM dietary guidelines to increase dietary fibre intake to 14 g/1,000 kcal/day and to decrease total fat intake to 20% of total energy for genetically susceptible individuals. These gene-diet interaction findings need to be replicated in a larger cohort before any dietary recommendations can be implemented for genetically susceptible individuals.

Chapter 7: Interaction between Dietary Fat Intake and Metabolic Genetic Risk Score on 25-Hydroxyvitamin D Concentrations in a Turkish Adult Population

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

Isgin-Atici K*, **Alathari BE***, Turan-Demirci B, Sendur SN, Lay I, Ellahi B, Alikasifoglu M, Erbas T, Buyuktuncer Z, Vimaleswaran KS. Interaction between Dietary Fat Intake and Metabolic Genetic Risk Score on 25-Hydroxyvitamin D Concentrations in a Turkish Adult Population. Nutrients. 2022;14(2):382. PMID: 35057563. *https://doi.org/10.3390/nu14020382*. *** Equal contribution.**

Buthaina AlAthari's contribution: For this study, I developed the analysis plan according to our objectives and the available data. I performed the statistical analysis using the Statistical Package for the Social Sciences (SPSS) software (v27; SPSS Inc., Chicago, IL, USA) and interpreted the results. Then, I carried out a literature search and reviewed all relevant papers and contributed to writing the first draft of the manuscript. I contributed to updating the manuscript several times based on the comments of co-authors. The final draft was formatted based on the journal's guidelines. Comments from the reviewers were addressed and responded.

7.1 Abstract

Purpose: Previous studies have pointed out a link between vitamin D status and metabolic traits, however, consistent evidence has not been provided yet.

Methods: This cross-sectional study has used a nutrigenetic approach to investigate the interaction between metabolic-genetic risk score (GRS) and dietary intake on serum 25-hydroxyvitamin D (25(OH)D) concentrations in 396 unrelated Turkish adults, aged 24–50 years.

Results: Serum 25(OH)D concentration was significantly lower in those with a metabolic-GRS ≥ 1 risk allele than those with a metabolic-GRS < 1 risk allele (p = 0.020). A significant interaction between metabolic-GRS and dietary fat intake (energy %) on serum 25(OH)D levels was identified ($p_{interaction}$ = 0.040). Participants carrying a metabolic-GRS ≥ 1 risk allele and consuming a high fat diet (≥ 38% of energy = 122.3 ± 52.51 g/day) had significantly lower serum 25(OH)D concentration (p = 0.006) in comparison to those consuming a low-fat diet (< 38% of energy = 82.5 ± 37.36 g/d).

Conclusion: In conclusion, our study suggests a novel interaction between metabolic-GRS and dietary fat intake on serum 25(OH)D level, which emphasises that following the current dietary fat intake recommendation (< 35% total fat) could be important in reducing the prevalence of vitamin D deficiency in this Turkish population. Nevertheless, further larger studies are needed to verify this interaction, before implementing personalized dietary recommendations for the maintenance of optimal vitamin D status.

7.2 Introduction

Nearly one billion people suffer from vitamin D deficiency (VDD) globally [400]. The prevalence of VDD among adults has been reported as ~40% in Europe [516] and 44-96% in the Asia, the Middle East, North Africa, and 30-90% in West Asia [517-521]. Despite having high levels of sun exposure, VDD remains a significant problem in Turkey [522, 523]. A meta-analysis of data from 111,582 Turkish participants reported that the prevalence of VDD was 63.5% (58.9-66.6%) in adults, 76% in pregnant women, 39.8% in children and 86.6% in infants [522]. In addition to the genetic determinants of vitamin D status, personal characteristics such as age, gender, skin colour, race, religious beliefs and clothing style, and lifestyle factors including physical activity level have been suggested as potential factors that can affect the levels of vitamin D in the Turkish population [522, 524, 525].

As a member of secosteroid hormones, vitamin D plays essential roles in both calcium and phosphorus metabolism, cell proliferation and differentiation, muscle contraction, nerve transmission, and function of the immune system [526]. Due to the immunomodulatory, anti-inflammatory, antifibrotic, and antioxidant roles of vitamin D, its deficiency has associations with several diseases including obesity, diabetes, cardiovascular diseases, bone metabolic disorders, cancers, neuropsychiatric disorders and autoimmune diseases, and more recently with increased risk of SARS-CoV-2 infection [526-528]. The link between VDD and the risk of cardiometabolic diseases has been extensively studied [529-531], and it has been shown that vitamin D exhibits anti-adipogenic activity in 3T3-L1 preadipocytes [532, 533] and has potential roles in inducing the expression of insulin receptor, regulation of insulin secretion, and glucose homoeostasis, and inflammation [534, 535].

Despite the current evidence for the link between VDD and cardiometabolic diseases, a causal effect has not been established [536]. Furthermore, previous studies investigating this link are inconsistent due to the unmeasured confounding factors [537, 538]. A genetic approach may

provide a better understanding to the potential association between VDD and metabolic diseases by eliminating any unclear confounding factors [539]. The heritability of circulating vitamin D levels has been reported between 20-85%, and a number of genetic variants in genes for vitamin D pathway have been associated with metabolic diseases [121, 526]. Furthermore, several genetic variants associated with cardiometabolic health have also been linked to one's vitamin D level status. Melanocortin 4 Receptor (*MC4R*) and Transcription Factor 7-Like 2 (*TCF7L2*) genes are commonly studied candidate genes for obesity and diabetes [294, 295, 309, 409, 539-548], and the interactions of *MC4R* and *TCF7L2* genotypes with dietary intakes on obesity [409, 546] and diabetes related traits [539, 543, 547] have been investigated in multiple ethnic groups. However, to our knowledge, the potential effects of the interaction between metabolic-genetic risk score (GRS) and dietary intake on vitamin D status have not been investigated in a Turkish population. Hence, in the present study, we have explored the association of the metabolic-GRS with metabolic traits and vitamin D status and explored the interaction between metabolic-GRS and dietary intake on the vitamin D status of a Turkish population.

7.3 Materials and Methods

7.3.1 Study Population

This cross-sectional study was performed with 396 Turkish adults, aged 24-50 years. The study participants were enrolled following a physical examination by the research endocrinologists at the outpatient clinic of the Department of Endocrinology and Metabolism at the Hacettepe University Hospitals, in Ankara, Turkey between June and November 2017. Criteria for inclusion required a routine visit to the outpatient clinic, being 24-50 years old with a Body Mass Index (BMI) of $\geq$ 18.50 kg/m^2. Those who had diagnosed liver and kidney diseases, mental and psychological disorders, cancers and severe endocrine abnormalities

(hypothyroidism, hyperthyroidism, hypopituitarism, etc.), as well as those who were pregnant or breastfeeding, using drugs or dietary supplements that affect the body weight, or have a history of bariatric surgery were excluded from the study. Following physical examination, all participants underwent a nutritional assessment and biochemical and genetic analysis. The study was approved by the Non-interventional Clinical Research Ethics Board of Hacettepe University (GO 15/612-11) in compliance with the Declaration of Helsinki, and written informed confirmation was obtained from all the participants. The details of the study, including the procedure for taking blood samples and transport to the laboratory have been previously published [549]. The study was performed as a part of the GeNuIne (Gene-Nutrient Interactions) Collaboration [17, 360].

7.3.2 Anthropometrical Measurements

Height and body weight were measured using standardised methods with a digital scale (Seca 220 Scale, Germany). BMI was calculated with the formula: "Body weight (in kilograms) divided by the square of height (in meters)" [400]. Waist circumference (WC) and hip circumference (HC) were measured by standard methods, and the waist-to-hip ratio was calculated by dividing WC (cm) to HC (cm) [550]. Body composition was determined by bioelectrical impedance (Tanita MC- 980 MA). Fat mass index (FMI) was estimated as fat mass (kg)/height squared of (m^2) [551].

7.3.3 Biochemical and Clinical Measures

Fasting lipid profile including triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol), and both fasting and postprandial plasma glucose and insulin concentrations were analysed by the routine methods at Hacettepe University Biochemistry Laboratory. Plasma adiponectin and

serum 25(OH)D concentrations were analysed in Hacettepe University Clinical Pathology Laboratory using ELISA kits (Ebioscience, Austria; and Dia Source, Belgium, respectively). According to the Institute of Medicine recommendation (IOM) [552], ≥ 20 ng/mL was considered as an optimal concentration for serum 25(OH)D concentration. Insulin resistance (HOMA-IR) was calculated using the formula: 'Fasting insulin level (μIU/mL) x fasting glucose level (nmol/L) / 22.5 [553]. The systolic (SBP) and diastolic (DBP) blood pressure was measured as a part of the physical examination [554].

7.3.4 Dietary Assessment

Two trained research dietitians assessed the dietary intake using the 24-h-dietary recall method. The amount of food items consumed by the participants were confirmed using the food portion size photographic atlas [555], replicas of food items, and household measurement tools. Dietary energy and nutrient intakes were estimated using a dietary analysis computer program (BeBIS, Nutrition Information System, Version 8).

7.3.5 Assessment of Physical Activity Level

A Turkish version of the International Physical Activity Questionnaire (IPAQ) was used to determine the physical activity level of the participants [556]. The physical activity level was categorized into 3 groups based on metabolic equivalent of task (MET) values suggested by IPAQ protocol: sedentary (< 600 MET/min/w), moderate (600-3000 MET/min/w), and vigorous (> 3000 MET/min/w) [377].

7.3.6 Single Nucleotide Polymorphism (SNP) Selection and Genotyping

SNPs, *TCF7L2* rs7903146, and MC4R rs571312, were selected because of their associations with metabolic diseases that have been suggested previously in different

populations [294, 295, 309, 409, 539-548]. The genomic DNA was isolated from the whole blood in K2EDTA containing tubes by the salting-out method. The details of this method have been described previously [549]. The genotypes of the *TCF7L2* rs7903146 and *MC4R* rs571312 SNPs were in the Hardy–Weinberg equilibrium (p = 0.101 and p = 0.176, respectively). Genotype distributions and MAFs for the SNPs of *TCF7L2* and *MC4R* are given in **Table S7.1**.

7.3.7 Statistical Analysis

The statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) software (version 24) was used for the statistical analysis. Descriptive data for continuous variables were given as the mean and standard deviation, and groups were compared using the independent sample t test. Allele and genotype frequencies of two SNPs were computed by gene counting, and the chi-squared test was used to calculate the percentages of alleles/genotypes. The SNPs of *TCF7L2* rs7903146 and *MC4R* rs571312 were used to create the GRS. A value varying from zero to two was given to each SNP, indicating the number of metabolic disease-associated risk alleles. The GRS was determined via the addition of the number of risk alleles through each SNP. The median value (1 risk allele) was used to classify the participants into two groups: Those with < 1 risk allele (lower risk) and ≥ 1 risk allele (higher risk). The association analysis between the GRS and categorical and continuous variables was performed using logistic regression and general linear models, respectively. Logistic and linear regression analyses were performed to examine the interaction between lifestyle factors and SNPs. The models were adjusted for age, gender, obesity status, and energy intake, and months of measurements, wherever appropriate. The variable 'month of measurement' was created based on the months (June–November) in which the participants were enrolled in the study. The participants included in June, July, and August were coded as

'Summer' (n = 192 for this group) while the participants included in September, October, and November were coded as 'Autumn' (n = 204 for this group). p value < 0.05 was considered to be statistically significant. Moreover, the dietary factors and metabolic traits were assessed according to the vitamin D status classified by the IOM recommendation [552]. Statistically significant interactions were examined further with binary analysis based on nutrient dietary consumption. A power calculation was not conducted, given that there are no available effect sizes from studies focusing on metabolic GRS and vitamin D levels in the Turkish population.

7.4 Results

7.4.1 Characteristics of the Study Participants

The mean of serum 25(OH)D concentration was 24.6 ± 1.66 ng/mL in the study population, and the prevalence of VDD was 25% (**Table 7.1**). The general characteristics of the study participants including anthropometric measurements, biochemical parameters, dietary intake, and physical activity level are given in **Table 7.1** stratified based on serum vitamin D levels (deficient/insufficient < 20 ng/mL and optimal ≥ 20 ng/mL). No significant difference in clinical, anthropometric, and biochemical parameters was obtained between the groups (p > 0.05, for each).

Table 7. 1: Basic characteristics of the study participants according to serum Vitamin D levels.

	Serum 25(OH)D Concentration*		
	Deficient/Insufficient (n=182)	**Optimal (n=214)**	***p* Value**
Anthropometric measurements			
Body mass index (kg/m^2)	25.7±4.21	25.8±4.11	0.271[a]
Waist circumference (cm)	87.0±10.79	88.8±12.04	0.938[a]
Hip circumference (cm)	101.7±8.27	101.8±7.41	0.127[a]
Waist-to-hip ratio	0.86±0.09	0.87±0.08	0.404[a]
Fat mass index	6.84±2.96	6.94±2.85	0.559[a]

Body fat mass (%)	25.7±7.90	26.0±7.29	0.890[a]
Body fat mass (kg)	19.1±7.55	19.6±7.48	0.556[a]
Visceral fat percentage (%)	5.59±3.15	5.89±3.25	0.628[a]
Biochemical parameters			
Glucose (mg/dL)	88.1±8.21	87.5±8.48	0.305[a]
Insulin (μIU/mL)	8.1±0.39	7.3±0.29	0.055[a]
Postprandial glucose (mg/dL)	84.9±17.21	84.7±15.72	0.408[a]
Postprandial insulin (μIU/mL)	29.3±2.69	24.9±1.95	0.091[a]
VLDL cholesterol (mg/dL)	24.1±15.25	23.1±13.76	0.453[a]
Total cholesterol (mg/dL)	190.2±40.12	188.0±37.12	0.977[a]
HDL cholesterol (mg/dL)	48.6±11.55	48.8±11.57	0.440[a]
LDL cholesterol (mg/dL)	123.9±31.20	122.2±28.72	0.913[a]
Triglyceride (mg/dL)	120.7±76.35	115.7±68.74	0.440[a]
Adiponectin (ng/mL)	10480.1±6217.49	10626±6692.54	0.556[a]
Insulin resistance (HOMA-IR)	1.8±0.09	1.6±0.07	0.058[a]
Dietary intake			
Total energy (kcal)	2429.3±1093.98	2368.0±992.98	0.675[a]
Carbohydrate (%)	46.7±8.90	45.3±9.73	0.073[a]
Protein (%)	15.5±3.68	15.7±4.83	0.207[a]
Fat (%)	37.5±7.66	38.9±8.41	0.098[a]
Total fibre (g)	23.9±10.95	23.7±11.31	0.382[a]
Physical activity level, n (%)			
Sedentary	68 (37.4)	84 (39.3)	0.306[b]
Moderate	90 (49.5)	112 (52.3)	
Vigorous	24 (13.1)	18 (8.4)	

Data are represented as means ± SD for anthropometric measurements, biochemical parameters, and dietary intake; and as number (percentage) for physical activity level. [a] Independent Sample t test, [b] Pearson chi-square test. * Cut-off point for serum vitamin D level was based on the recommendation of Institute of Medicine

7.4.2 Association of Vitamin D Status with Metabolic Traits

After adjusting for potential confounders, the serum 25(OH)D concentration was significantly associated with the fasting insulin ($p = 0.011$) and HOMA-IR ($p = 0.010$) (**Figure S7.1**) and none of the other phenotypic associations were statistically significant (**Table S7.2**).

7.4.3 Genetic Association of Metabolic-GRS with Metabolic Traits and Serum 25(OH)D Concentrations

Metabolic-GRS was significantly associated with the serum 25(OH)D concentration ($p = 0.020$), where participants carrying ≥ 1 risk allele had lower serum 25(OH)D levels (23.5 ±

0.89 ng/mL) compared to those carrying < 1 risk allele (27.9 ± 1.96 ng/mL) (**Figure 7.1**). None of the other characteristics differed significantly between the two GRS groups (< 1 risk allele vs. ≥ 1 risk allele) ($p > 0.05$, for all associations) (**Table S7.3**).

7.4.4 Interaction between Metabolic-GRS and Serum 25(OH)D Concentration on Clinical and Biochemical Outcomes

There was no significant interaction between metabolic-GRS and vitamin D concentrations on metabolic traits ($p > 0.05$) (**Table S7.4**).

7.4.5 Interaction between Metabolic-GRS and Dietary Intake on Serum Vitamin D Concentration

There was a significant interaction between metabolic-GRS and dietary energy from fat intake on serum 25(OH)D concentrations after adjusting for age, gender, and obesity status and months of measurement ($p = 0.040$, **Figure 7.2**). Participants in the highest tertile of fat intake (122.3 ± 52.51 g/d) and carrying ≥ 1 risk allele had significantly lower serum 25(OH)D concentrations compared to the participants having the highest tertile of fat intake and carrying < 1 risk allele ($p = 0.006$) (**Figure 7.2**). No significant interactions between metabolic-GRS and dietary intakes of other macronutrients on serum 25(OH)D were obtained ($p > 0.05$, for each|) (**Table S7.5**).

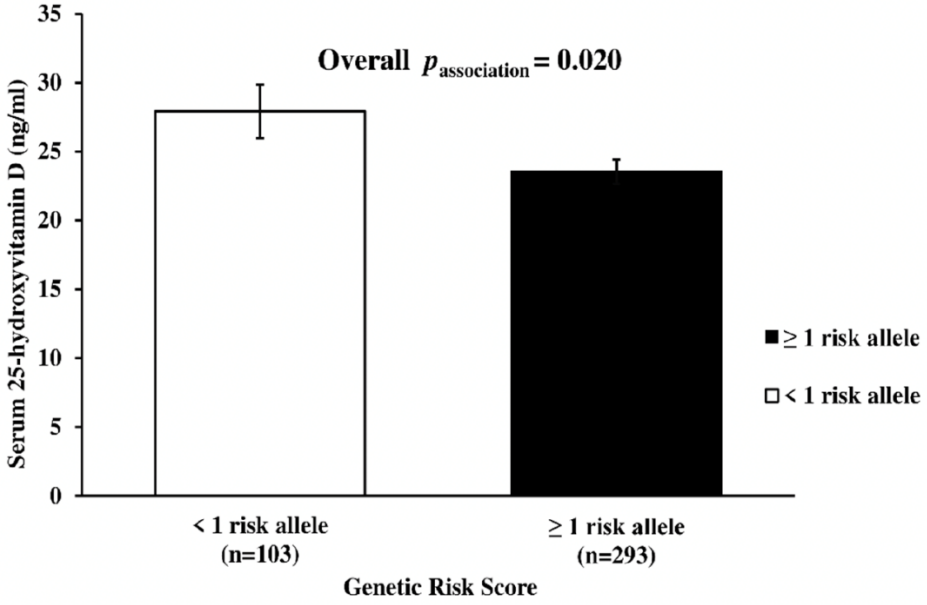


Figure 7. 1: Association between serum 25-hydroxyvitamin D level and metabolic-GRS.

Individuals having 1 or more risk allele had lower serum 25(OH)D concentrations compared to participants with < 1 risk allele. The mean and standard deviation for serum 25(OH)D level was 27.9 ± 1.96 ng/mL in participants with < 1 risk allele, while it was 23.5 ± 0.89 ng/mL in participants with ≥ 1 risk allele. *p* value was calculated using linear regression analysis after adjusting for age, gender, obesity status and months of measurement.

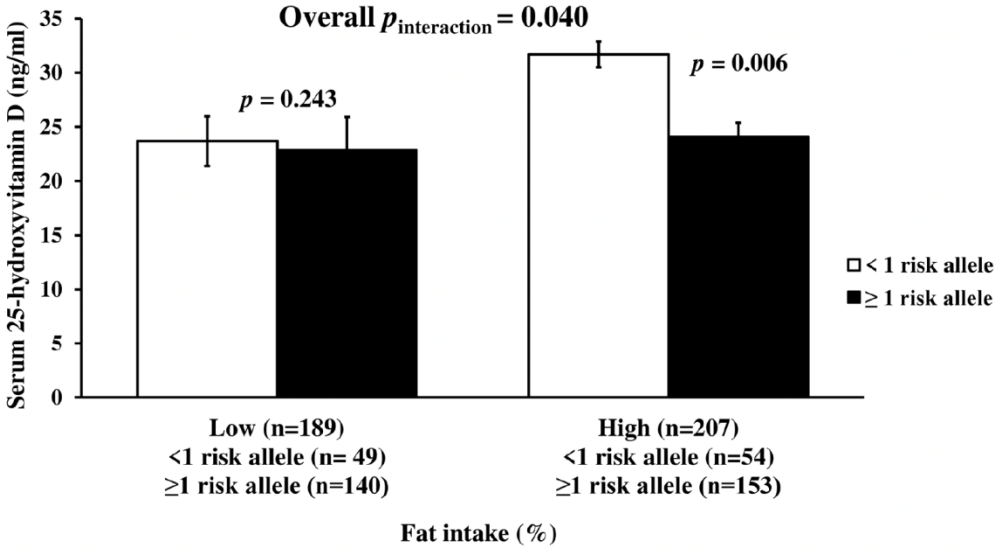


Figure 7. 2: Interaction between metabolic-GRS and fat intake (%) on serum 25(OH)D concentration.

There was a significant interaction of the GRS with dietary fat intake on serum 25-hydroxyvitamin D level. Among those with ≥ 1 risk alleles, individuals with high of fat intake had lower serum 25-hydroxyvitamin D level (p = 0.006). Vitamin D level was 23.1 ± 1.06 ng/mL among those with low fat intake: For individuals without risk allele: 23.7 ± 2.29; for individuals with risk allele: 22.9 ± 1.19 ng/mL. It was 26.1 ± 1.26 ng/mL among those with high fat intake (for < 1 risk allele: 31.7 ± 3.03; for ≥ 1 risk alleles: 24.1 ± 1.30 ng/mL). The median value of dietary fat intake was 38%. The mean intake of low-fat intake was 31.6 ± 4.61% (for individuals without risk allele: 31.7 ± 4.89%; for individuals having ≥ 1 risk alleles: 31.6 ± 4.52%). The mean intake of high fat intake was 44.4 ± 5.32% (for < 1 risk allele: 45.1 ± 5.46%; for ≥ 1 risk alleles: 44.1 ± 5.26%). p values were derived from linear regression analysis and adjusted for age, gender, obesity status and months of measurement.

7.5 Discussion

To date, our study is the first to use a nutrigenetic approach to investigate the interaction between metabolic-GRS and dietary intakes on serum 25(OH)D levels in a Turkish population. This study has proposed a novel interaction between metabolic-GRS and dietary fat intake on serum 25(OH)D concentrations by demonstrating that participants with high metabolic-GRS and higher dietary fat intake had significantly lower serum 25(OH)D levels compared to the participants with high metabolic-GRS but lower dietary fat intake. Given the high prevalence of VDD in Turkey [522, 523], these results might have public health significance in preventing VDD in those with high metabolic genetic risk. Therefore, following the current dietary fat intake recommendations (< 35%) [115, 557] might be important to maintain the optimal vitamin D status, especially in individuals who have a genetic risk of VDD.

Studies that examined the link between metabolic disease associated gene variants and vitamin D status are limited and the findings have been conflicting [409, 539]. A recent study conducted in 545 Asian Indians showed no significant association between metabolic-GRS and serum 25(OH)D concentration [539]. On the other hand, Alathari et al. [409] found that Southeast Asian women carrying < 4 metabolic risk alleles had higher serum 25(OH)D concentration compared to the individuals carrying four or more risk alleles. Similarly, our study has shown that individuals having ≥ 1 metabolic risk allele had lower serum 25(OH)D concentrations than the individuals not having any risk allele. Despite the limited evidence on the link between metabolic disease-associated gene variants and vitamin D levels, many genetic association studies investigated the associations of vitamin D-related SNPs that can modify the activation, catabolism and transport of vitamin D, with metabolic traits. However, the findings of these studies were also inconsistent [192, 209, 536, 558]. For instance, a couple of studies conducted in European populations showed no association between the gene variants of the vitamin D binding protein/group-specific component (DBP/GC) and the risk of diabetes [192, 558], while significant associations have been demonstrated in Asian populations [209]. The discrepancies in the findings of different studies could be explained by the diversity in the number of SNPs, ethnicity, culture, and socioeconomic status.

The present study examined whether the genetic risk of metabolic diseases has been affected by VDD and found no significant interaction between metabolic-GRS and the serum 25(OH)D level on metabolic traits. Similarly, a study that examined the interactions between the vitamin D receptor SNPs and serum vitamin D level on metabolic disease related traits in 5,160 Europeans failed to show any evidence of vitamin D-related gene variations modifying the interaction between 25(OH)D concentrations and metabolic traits [160]. Other studies also confirmed the lack of associations between genetically instrumented serum 25(OH)D concentrations and metabolic traits, such as BMI [227, 409, 559], waist circumference [227,

409, 559, 560], glycated hemoglobin [245, 409], fasting insulin [245, 409], and glucose levels [245, 409, 561].

The World Health Organization Noncommunicable Diseases Progress Monitor (2017) declared that Non-Communicable Diseases (NCDs) have been responsible for 88% of deaths in the Turkish population [562]. Targeting modifiable risk factors for NCDs including the dietary modifications for obesity could prevent mortality [546, 563, 564]. The present study found that dietary fat intake and metabolic-GRS had an interaction on vitamin D concentrations, and the level of serum 25(OH)D was lower in those carrying risk allele and consuming a high amount of dietary fat. The high amount (≥ 38% = 122.3 ± 52.51 g/d) was defined according to the median of total dietary fat intake in the study population. This cut off value also meets the high dietary fat intake as defined by the recommendations of WHO (15 - 30%), IOM (20 - 35%) and Turkish Dietary Guidelines (20 - 35%) [115, 557, 565]. Vitamin D is a fat-soluble vitamin and absorbed with dietary fat by passive diffusion therefore, dietary fat can have a potential to modify the interaction between the genetic risk of metabolic disease and vitamin D status [377]. Similar to current findings, it was shown that high fat diet-induced obesity resulted in lower serum 25(OH)D levels in an animal study [566]. To date, there have been only two studies that have examined the metabolic-GRS-diet interactions on serum 25(OH)D concentrations [409, 539]. The first study examined whether any dietary factor could modify the relationship between the serum 25(OH)D concentration and metabolic traits in 545 Asian Indians. In discordance with the findings of our study, they showed that individuals with low GRS (GRS ≤ 1) and lower dietary carbohydrate intake (≤ 62%) had higher serum 25(OH)D concentrations [539]. Furthermore, the study generated the GRS using five SNPs from three genes (*FTO*, *TCF7L2* and *MC4R*), and the energy from carbohydrate, protein and fat was 64%, 11% and 23%, respectively. The second study tested a similar hypothesis in Southeast Asian Minangkabau women using two GRSs constructed based on 15 SNPs from vitamin D and

metabolic disease-associated genes, respectively, and showed no significant interaction between metabolic-GRS and dietary intake on one's vitamin D status [409]. Some of the reasons for the discrepancy in the findings across the studies might be the number of SNPs that were used in the GRS, ethnicity, and the diversity in the dietary macronutrient intake patterns. Given these ethnic-specific findings, meeting the current dietary recommendations for macronutrient intake might be more essential in individuals with a known genetic risk to help maintain a healthy vitamin D status [115, 557, 565].

Several hypotheses have been proposed to define the potential mechanisms of the associations between metabolic diseases including obesity and one's vitamin D status [12, 328, 567-574]. These include the volumetric dilution of serum vitamin D levels, as a significant amount of vitamin D is stored in adipose tissue; thus excess body adiposity could contribute to relatively low serum 25(OH)D level [566, 570, 571], the adipocyte hypertrophy contributing to overexpression of proinflammatory cytokines [574, 575], modifications of vitamin D-related enzymes [572, 573] affected by high fat diet-induced obesity, and lower endogenous vitamin D synthesis in the skin as a consequence of the less outdoor activity [572, 576], less physical activity [328], and less exposure to sunlight in obese individuals [12, 570-576]. In addition, the bi-directional Mendelian randomisation analysis conducted in 42,024 Europeans showed a relationship between vitamin D status and obesity, suggesting that higher BMI leads to lower vitamin D levels where a 4.2% decrease in serum 25(OH)D concentrations was observed for every 10% increase in BMI [536]. The Framingham Study also showed that the prevalence of VDD was higher among individuals with a higher BMI [577]. Furthermore, a lifestyle intervention study conducted in obese individuals demonstrated that serum 25(OH)D concentrations were significantly increased as a consequence of weight loss [578]. Despite these findings, some studies failed to show any association between vitamin D status and metabolic disease related traits [579-581]. For instance, Larsen et al. [579] showed no or

marginal associations between the serum 25(OH)D level and biomarkers of adiposity in 10,898 individuals comprising Danish, British, and Finnish participants. Similarly, independent of the genetic associations, our study also has not shown either any difference in metabolic traits by vitamin D status, or any association between obesity-related traits and the serum 25(OH)D level. The inconsistencies among the studies might depend on the potential predisposition to bias and confounding factors (e.g. the time and amount of sunlight exposure, physical activity level, more clothing, skin colour and ethnicity) in observational study designs conducted in different populations. Furthermore, the differences in the categorisation of vitamin D status and the measures of obesity including BMI, body weight, and waist circumference might be the other reason for the inconsistency [582]. Genetic studies can provide more consistent findings in the exploration of the association between vitamin D status and metabolic traits, because the bias and confounding factors can be partly eliminated with this approach [121, 144].

The main strengths of this study were the use of several biochemical markers related to metabolic traits and a well-characterised study cohort. In addition, the construction and use of a GRS method rather than a single SNP approach enhances the statistical power, and presents an efficient perspective for metabolic outcomes [339, 515, 583]. However, there are some limitations that need to be acknowledged. Firstly, the study did not measure exposure to sunlight, and the data collection period only covered summer and autumn seasons. For overcoming this limitation, the months of measurement was adjusted as a confounding factor in all the analyses. Secondly, the small sample size might be considered as a further limitation of the study however, our study has been able to confirm previously reported associations and identify gene-diet interactions. Thirdly, dietary intake was assessed using a 24-h dietary recall method, which is prone to self-reporting bias however, this method is used commonly in nutrigenetic studies, and the method could be applied to diverse groups with a wide range of

eating habits. Fourthly, we could not examine the causative effects due to limitations of the cross-sectional study design. Lastly, although analysis undertaken was adjusted for potential confounders, we cannot rule out the impact of residual confounders caused by unknown variables.

7.6 Conclusion

In summary, our study has provided evidence for a novel interaction between metabolic-GRS and dietary fat intake on serum vitamin D concentrations, suggesting that following the current dietary fat intake recommendations (< 35%) might be effective to prevent any consequences of the genetic risk of VDD. However, further larger studies are needed to endorse this interaction before generalising the findings to the Turkish population and implementing any personalized dietary recommendations for the maintenance of one's optimal vitamin D status.

Chapter 8: Discussion and Conclusion

8.1 Discussion

The study of nutrigenetics has generated novel gene-diet interaction knowledge which will provide scientific rational for tailoring dietary intake to reduce risk of chronic diseases, such as obesity, type 2 diabetes (T2D) and cardiovascular diseases. Personalising nutrition advice according to the individual's genetic susceptibility is a promising prospect with the objective of delaying or preventing the development of diseases related to vitamin D deficiency. Findings from this thesis have contributed to the field of nutrigenetics with data demonstrating the existence of genetic heterogeneity in gene-diet interactions related to vitamin D and metabolic traits across different ethnic groups. The results of this thesis will help our understanding of how genetic variants related to vitamin D concentrations interact with lifestyle factors to affect the development of metabolic disorders.

Several gene-diet interaction studies have investigated the relationship between genetic variants and dietary intake on chronic metabolic disease outcomes, however, these studies reported inconsistent findings. This could be due to two factors, firstly genetic heterogeneity and secondly small sample size of the studies with limited replication. Thus, these findings could not be used to develop a personalised dietary recommendation for each ethnic group [17, 18, 412]. Until recently, gene-diet interaction studies were only investigated in developed countries. In the low and middle income countries (LMICs), nutrigenetics studies were limited by poor funding, lack of expertise and inadequate infrastructure [17, 18, 412]. In this thesis, a genetic approach was used to examine the association of vitamin D-related single nucleotide polymorphisms (SNPs) and metabolic disease-related SNPs with vitamin D concentrations and metabolic outcomes in different ethnic groups. Additionally, a nutrigenetic approach was used to investigate the interaction between these SNPs and lifestyle factors including physical

activity and dietary factors (protein, fat, carbohydrate, and fibre) on vitamin D concentrations and metabolic traits.

In this project, I explored gene-lifestyle interactions in diverse populations with varied genetic make-up. Five studies were undertaken in five distinctive populations [South Asian Indian adults, Indonesian women, Brazilian young adults, Ghanaian adults, and Turkish adults] to examine my objectives and to generate data for personalised dietary recommendations based on ethnicity. These studies included: two case-control studies [the Chennai Urban Rural Epidemiology Study (CURES; Asian Indians, n = 545) and a study in Turkish adults (n = 396)] and three cross-sectional cohort studies [the Minangkabau Indonesia Study on Nutrition and Genetics (MINANG study; Indonesian women; n = 110), the Obesity, Lifestyle and Diabetes in Brazil (BOLD study; Brazilian young adults; n = 187), and the Genetics of Obesity and Nutrition in Ghana (GONG study; Ghanaian adults; n = 302)]. Association and interaction analyses were performed using binary logistic regression and general linear models using the Statistical Package for the Social Sciences (SPSS) software (version 24 and 27; SPSS Inc., Chicago, IL, USA). Regression models were adjusted for age, sex, body mass index (BMI), T2D, sun exposure and total energy intake whenever appropriate. Thesis findings are summarised below.

8.2 A Review of Vitamin D Pathway-Related Gene Polymorphisms and their Association with Metabolic Diseases

Vitamin D is a fat-soluble prohormone that plays a crucial role in bone mineralization and is involved in a diverse range of biological actions [26]. A broad spectrum of diseases has been related to vitamin D deficiency including metabolic diseases [5]. Though observational studies have reported an association of the 25-hydroxyvitamin D (25(OH)D) concentrations with metabolic diseases such as obesity and T2D [183, 276, 584, 585], this relationship remains

unclear. Given that genetic associations are less prone to confounding, the understanding of genetic associations of 25(OH)D status with obesity and T2D will provide a better clarification of the relationship between vitamin D and metabolic diseases.

The objective of this review was to identify and discuss reports of associations between vitamin D-related SNPs and their associations with obesity and T2D. A literature search of PubMed was performed up to September 2019. Total articles extracted from initial search was 1,455 and after eliminating irrelevant observations and duplicates and applying the exclusion criteria, the final number of articles included in this review was seventy-three. All articles that examined associations of vitamin D-related SNPs with obesity or T2D were included.

There is a lack of research focussing on the association of vitamin D synthesis-related genes with obesity and T2D; however, the available research, although inconsistent, is suggestive of a protective effect on T2D risk [190, 192, 193]. While there are several studies that investigated the vitamin D metabolism-related SNPs, the research focussing on the vitamin D activation, catabolism and transport genes is limited. Available research on 1α-hydroxylase (*CYP27B1*), 24-hydroxylase (*CYP24A1*) and vitamin D binding protein (*DBP*)/group-specific component (*GC*) genes suggests that there was no effect on obesity and T2D in Europeans [194, 195, 210] but there was an effect on T2D in South Asians [207-209]. SNPs from the vitamin D receptor (*VDR*) gene have been extensively researched and the focus has been mainly on the BsmI (rs1544410), TaqI (rs731236), ApaI (rs7975232) and FokI (rs2228570) SNPs. Even though the association between *VDR* SNPs and metabolic diseases remain inconsistent, some positive associations with obesity and T2D in specific ethnic groups have been reported [97, 163, 220, 225, 226, 229, 237, 240].

8.3 Interaction between Metabolic Genetic Risk Score and Carbohydrate Intake on Serum 25(OH)D Levels in Asian Indians

Asian Indians have a high prevalence of T2D with some unique clinical and biochemical characteristics referred to as the 'Asian Indian Phenotype' represented by lower BMI, higher central obesity with high waist circumference (WC) and waist hip ratio (WHR), higher insulin resistance, and increased plasma insulin levels [272, 586]. In the past year, the International Diabetes Federation reported that Indians account for 1 in 7 of all adults living with diabetes around the world, with a total of 74.2 million adults diagnosed with diabetes [587]. Although the Asian Indian ethnicity appears to be genetically predisposed to T2D, unhealthy lifestyle choices can further contribute to this disease [588-590]. Chronic metabolic diseases are linked to increased risk of vitamin D deficiency as reported by numerous studies [5, 14, 179, 591, 592], however, findings have been inconsistent [59, 60, 182, 183]. In Asian Indians, vitamin D deficiency is a condition that is highly prevalent in all age groups [277, 593]. Thus, in this study I used a nutrigenetic approach to investigate the link between metabolic-related genetic variants and vitamin D concentrations and to explore the effect of lifestyle factors on this relationship.

In the current study, participants were randomly recruited from the CURES study, a cross-sectional case-control epidemiological study conducted on a representative population of Chennai in Southern India [300]. I investigated five SNPs from Fat mass and obesity-associated (*FTO*) gene (rs8050136 and rs2388405), Melanocortin 4 receptor (*MC4R*) gene (rs17782313) and Transcription factor 7-like 2 (*TCF7L2*) gene (rs12255372 and rs7903146) which are known to be associated with obesity and T2D in several populations and created a metabolic-related genetic risk score (GRS). I examined the associations between the metabolic-GRS with metabolic trait outcomes and vitamin D concentrations and explored whether dietary intake modified these associations. After adjusting for age, gender, BMI, T2D and month of sample collection, there was no significant association between the metabolic-GRS and vitamin D concentrations, however, there were two statistically significant interactions between the

metabolic-GRS and dietary carbohydrate intake on serum 25(OH)D levels and dietary fat intake on low density lipoprotein cholesterol (LDL-c).

I observed that within the participants with lower carbohydrate intake (≤ 62%) those with less risk alleles (GRS ≤ 1) had greater serum 25(OH)D concentrations compared to those with higher number of risk alleles (GRS > 1). This finding is supported by a recent study in a Polish population evaluating 25(OH)D concentration in participants consuming low-carbohydrate-high-fat (LCHF) (n = 41) and Eastern European (EE) diet (n = 67). It was reported that 25(OH)D concentration was significantly higher in the group on the LCHF diet (34.9±15.9 ng/mL) than in the group on the EE diet (22.6±12.1 ng/mL), providing evidence that low carbohydrate diet had a positive influence on plasma vitamin D concentration [594]. In my study, the average carbohydrate intake for the study participants was 78.1% of total energy, which is higher than the recommended guidelines (50%-60%) for Asian Indians [335].This represents the carbohydrate consumption in the Indian population as indicated by the Indian national household consumption data which reported that Indians consume an excess amount of grains and less proteins than recommended [595]. My study findings showed that individuals carrying a lesser number of metabolic risk alleles are likely to have higher 25(OH)D concentrations, only if they have a carbohydrate intake < 62% of total energy intake. Given that dietary carbohydrates are the major source of energy in the Asian Indian diet, my study highlights the importance of following the Indian dietary guidelines of consuming 50%-60% of energy from carbohydrates. Following such advice could substantially reduce vitamin D deficiency among Asian Indians. Furthermore, the study showed that participants consuming a low-fat diet (≤ 22%), despite having a higher genetic risk (> 1 risk allele) had significantly lower LDL-c concentrations. This result is in line with a GWAS that was performed in Canada (n = 541) which identified interactions between GRS (from 29 SNPs) and total fat intake on LDL-c concentrations ($p < 10^{-5}$) [338]. My study findings if confirmed in larger cohorts and

intervention studies may have significant implications in providing dietary recommendations for those with higher metabolic-risk alleles.

8.4 Interaction between Vitamin D Genetic Risk Score and Carbohydrate Intake on Body Fat Percentage in Southeast Asian Women

Although Indonesia is a tropical country, located across the equator, with plenty of sunlight exposure all year round, vitamin D deficiency is widespread in Indonesian women with an estimated prevalence of 60% to 95% [596]. Sufficient vitamin D level is essential for women during pregnancy and lactation to support the growth and development of the fetus and for milk production [41]. Suboptimal levels of vitamin D during pregnancy have been linked to adverse pregnancy outcomes such as, preeclampsia, gestational diabetes, preterm birth, cognitive impairments, respiratory infections, and asthma [38, 351]. Indonesia is home to the largest matrilineal society in the world, the Minangkabau ethnic group, mostly living in West Sumatra. This ethnic group is reported to have high rates of generalised and central obesity and diabetes [393, 597-599]. Thus, the current study was conducted in a sample from the Minangkabau women using a genetic approach to investigate the relationship between metabolic outcomes and vitamin D status and to examine whether these relationships were modified by dietary factors.

Fifteen SNPs were selected and two GRSs were constructed for analysis in this study. A vitamin D-related GRS from five SNPs in genes known to be associated with vitamin D synthesis and metabolism: 7-dehydrocholesterol reductase (*DHCR7*) rs12785878, 25-hydroxylase (*CYP2R1*) rs12794714, *CYP24A1* rs6013897, *DBP*/*GC* rs2282679, and calcium sensing receptor (*CASR*) rs1801725; and a metabolic-related GRS from ten SNPs from genes associated with obesity and T2D: *FTO* (rs8050136, rs9939609, and rs10163409), *TCF7L2* (rs12255372 and rs7903146), *MC4R* (rs17782313 and rs2229616), potassium voltage-gated

channel subfamily Q member 1 (*KCNQ1*) gene (rs2237895 and rs2237892) and cyclin dependent kinase inhibitor 2A/B (*CDKN2A*/*B*) gene (rs10811661).

This study revealed two statistically significant associations of the metabolic-GRS with lower 25(OH)D levels and with higher BMI. I found that individuals carrying greater than 4 risk alleles had lower 25(OH)D levels than those with less than 4 risk alleles, suggesting that having a higher metabolic-GRS is a strong risk factor for lower 25(OH)D levels. Additionally, I found that individuals with greater than 4 risk alleles had higher BMI than those with less than 4 risk alleles, implying that a higher metabolic-GRS increases the risk of generalised obesity. Also, a statistically significant interaction was detected between vitamin D-GRS and carbohydrate intake on body fat percentage (BFP), where women with more than 2 risk alleles had significantly higher BFP than those with 2 or less risk alleles, when consuming high amounts of dietary carbohydrates (mean ± SD: 319 g/d ± 46). The average energy intake of the participants in this study was 1776 kcal/day, therefore, the mean carbohydrate intake would be equivalent to 71.8% of total energy intake. This percentage carbohydrate intake is very high compared to the Indonesian dietary guidelines that recommends obtaining 50% of total energy from carbohydrates [383, 384]. My results showed modulating effect of high carbohydrate consumption on the obesity indicator, BFP, which is a better marker than BMI because it can distinguish between lean body mass and adiposity and can better predict the risk of cardiometabolic disease [391, 392]. While some studies link increased obesity and T2D with increased consumption of refined carbohydrates and sugars due to their effects on insulin elevation [385, 600]. Some studies have demonstrated that restricting carbohydrate intake results in positive outcomes such as weight reduction, normalizing insulin levels, reducing fasting glucose and glycated haemoglobin (HbA1c) levels [386, 387]. Vitamin D has been reported to improve insulin sensitivity and enhance beta cell function [388, 601]. Since lower levels of vitamin D has been associated with obesity, the finding that high carbohydrate intake

can lead to increased BFP in the vitamin D susceptible individuals is possible, nevertheless, the mechanism is unclear and requires further investigation. The Indonesian dietary guidelines recommend consuming 50% of total energy from carbohydrates [384], however, the Minangkabau diet is still typically high in carbohydrates [390]; the findings if replicated in larger cohorts and interventions studies could have public health relevance in encouraging the reduction of total carbohydrate intake in Indonesians.

8.5 Interaction between Vitamin D Genetic Risk Score and Protein Intake on Serum 25(OH)D Levels in Young Adults from Brazil

The metabolic diseases, obesity and T2D are public health problems, with health consequences and economic burden that have raised concern all over the world and in particular in LMICs [602]. Over the past decade, obesity and T2D have been on the rise in Brazil [404, 603]. Additionally, recent studies reported that vitamin D deficiency and insufficiency to be at 28.2% and 45.3%, respectively in the Brazilian population despite having abundant sunshine throughout the year [397]. Low vitamin D status is often linked with metabolic diseases; however, findings are inconsistent. Therefore, the study used a nutrigenetic approach to investigate the association between vitamin D status and metabolic outcomes and whether this relationship can be modified in response to dietary intake.

Two GRSs were created from genetic variants associated with vitamin D (vitamin D-GRS) and genetic variants associated with metabolic diseases (metabolic-GRS). Six SNPs were used to create the vitamin D-GRS: SNPs rs2228570 and rs7975232 from *VDR*, SNP rs12785878 from *DHCR7*, SNP rs12794714 from *CYP2R1*, SNP rs6013897 from *CYP24A1* and SNP rs2282679 from *DBP*/*GC* gene. Ten SNPs were used to create the metabolic-GRS: SNPs rs8050136 and rs9939609 from *FTO*, SNPs rs12255372 and rs7903146 from *TCF7L2*, SNP rs17782313 from *MC4R*, SNPs rs2237895 and rs2237892 from *KCNQ1* SNP rs10811661

from *CDKN2A*/B, SNP rs1801282 from Peroxisome Proliferator Activated Receptor Gamma (*PPARG*), and SNP rs5030952 from calpain 10 (*CAPN10*) gene.

Results from this study showed that vitamin D-GRS was significantly associated with low 25(OH)D concentrations indicating that the GRS is an ideal instrument for vitamin D deficiency. The study also showed that the metabolic-GRS was significantly associated with high fasting insulin concentrations. The novel finding of this study is a significant interaction between vitamin D-GRS and total protein intake (g/day) (adjusted for non-animal protein) on 25(OH)D, where individuals consuming a high protein diet (≥ 73 g/d) and carrying > 4 risk alleles for vitamin D deficiency had significantly lower 25(OH)D compared to individuals carrying ≤ 4 risk alleles. The estimated mean daily caloric intake for the Brazilian population is 1,902 kcal [444]. The dietary guidelines for protein intake are between 10-15% of total daily energy intake [604]; this would translate to a range between 48–71 g of daily protein intake. In this study, the protein intake of participants with the high protein intake ranged from 73 to 217 g/day which is considerably higher than the daily protein intake recommendations illustrating that following the dietary guidelines for protein intake will be an effective strategy to overcome the genetic risk of vitamin D deficiency in Brazilians.

There were no previous studies that reported an effect of dietary protein intake on vitamin D levels in the literature and the mechanism of the possible effect of high protein intake on vitamin D concentrations particularly in the genetically susceptible individuals for vitamin D deficiency is not clear. However, it could be specifically driven by animal protein sources as the findings in the study remained significant after adjustments for non-animal protein sources. This gene-diet interaction finding is interesting and requires further investigation of the impact of higher protein intakes (from animal and non-animal sources) on vitamin D status using a randomized controlled trial to elucidate the modulating effect exerted on vitamin D levels.

8.6 Interaction between Vitamin D Genetic Risk Score and Fibre intake on BMI in Ghanaian adults

Ghana is a rapidly urbanizing country with increasing economic development and changes in lifestyle involving a shift from the consumption of traditional staple foods low in fat and rich in fibre, to energy-dense processed and refined foods which are high in fats and sugar. Urbanisation is also associated with increased industrialization, sedentary lifestyle, and use of motorised transport. All of these factors may have contributed to the increased prevalence of obesity and T2D noticed in Ghana in recent years [451, 453, 457]. Vitamin D-related genetic determinants have also been shown to contribute to the development of these metabolic diseases [162, 164, 605]. Using genetic variants to investigate the relationship between vitamin D status and metabolic disease outcomes has been shown to be an effective approach to overcome the effects of confounding [467]. Thus, in this study, I aimed to use a nutrigenetic approach to determine whether selected candidate genes involved in vitamin D deficiency were associated with metabolic disease-related traits and whether these associations were modified by dietary intake.

A vitamin D-related GRS was constructed from 8 SNPs that were selected based on their known association with vitamin D concentrations: *VDR* SNPs rs2228570 and rs7975232, *DHCR7* SNP rs12785878 [364, 369-371]; *CYP2R1* SNPs rs12794714 and rs10741657, *CYP24A1* SNP rs6013897, *DBP*/*GC* SNP rs2282679, and *CASR* SNP rs1801725.

A statistically significant interaction was found between vitamin D-GRS and fibre intake (g/day) on BMI where participants who consumed low fibre (≤ 16.19 g/d) and carried ≥ 2 risk alleles for vitamin D deficiency had a significantly higher BMI. This finding suggests that lower fibre intake is associated with higher obesity in vitamin D genetically susceptible individuals. It is recognised that higher total fibre intake is associated with lower inflammation, obesity and BMI [487, 505] but previous studies have demonstrated an inverse relationship

between fibre intake and obesity outcomes [506, 507]. The effect of high dietary fibre on BMI in vitamin D susceptibility is not clear. Vitamin D was shown to have anti-inflammatory effects and gut microbiota modifying properties [493, 500, 501], thus, the gene-fibre interaction on increased BMI could be due to the compounded effect of low fibre intake and genetic susceptibility to vitamin D deficiency through reduced anti-inflammatory action and the influence on microbiome modulation. According to the Institute of Medicine (IOM), the dietary guidelines for total daily fibre intake is 14 g/1000 kcal/day [487]. In our cohort, the average total energy intake was 1,645 kcal/d, hence the recommended average fibre intake should be 23 g/day. This confirms the importance of adhering to the guidelines for dietary fibre and emphasizes their importance to individuals with a higher genetic predisposition to vitamin D deficiency to prevent obesity.

In addition, an interaction between vitamin D-GRS and fat intake (g/day) on HbA1c was found, where participants who had lower total fat intake ($\leq$ 36.5 g/d), despite carrying $\geq$ 2 risk alleles had significantly lower HbA1c. This finding suggests that low-fat intake is associated with greater glycaemic control in vitamin D genetically susceptible individuals. Increased total fat intake has been shown to increase insulin requirements for diabetic patients [510], also, higher total fat intake as well as SFA and MUFA intake has been shown to be associated with increased HbA1c levels [606]. Fat intake seems to have an effect on HbA1c levels and this could be caused by impairment of insulin sensitivity, or it could be because higher fat intake causes an increase in the peak time and amount of the glucose response [512, 607]. Further studies are needed to clarify this effect. The IOM recommendations for total daily fat intake are between 20–35% of total energy intake [487], which is 36.5–64.0 g/day for this study cohort. This finding suggests that individuals with genetic risk for vitamin D deficiency should adhere to 20% fat intake, the lower figure of the total fat recommendation, to improve glycaemic control. Given that both obesity and T2D are on the rise in Ghana [457, 459], the

study highlights the importance of implementing strategies to follow IOM dietary guidelines [487] to increase dietary fibre intake to 14 g/1,000 kcal/day and to decrease total fat intake to 20% of total energy for genetically susceptible individuals.

8.7 Interaction between Metabolic Genetic Risk Score and Fat intake on Serum 25(OH)D Levels in a Turkish Adult Population

Vitamin D deficiency is a public health problem with significant health consequences all over the world. In Turkey, the prevalence of vitamin D deficiency is reported to be between 58.9 and 66.6% in adults [522]. Vitamin D has versatile functions in the body and due to the immunomodulatory, anti-inflammatory, and antifibrotic roles of vitamin D, its deficiency has associations with several metabolic diseases including obesity and T2D [528, 608]. Vitamin D has been shown to exhibit anti-adipogenic activity in preadipocytes, and to have an effect in the regulation of insulin secretion and glucose homoeostasis [532-534]. Although the link between vitamin D deficiency and the risk of metabolic diseases has been extensively studied, causal connections have not been established [145]. Thus, in this study, I aimed to use a genetic approach that eliminates confounding factors to investigate the potential association between vitamin D deficiency and metabolic traits.

A metabolic-related GRS was created from the SNPs, rs7903146 of *TCF7L2*, and rs571312 of *MC4R*, which have shown association with metabolic diseases in different populations. In agreement with the Indonesian study, I found a statistically significant association between the metabolic-GRS and serum 25(OH)D concentration. Participants who had 1 or more risk alleles had significantly lower 25(OH)D concentration than those with less than 1 risk allele. Nonetheless, in the Indian and Brazilian studies I was unable to find such association. The inconsistencies in the findings could be explained by difference in effect allele

frequencies, genetic heterogeneity across different ethnic groups, variations in sample sizes of the study, and the diversity in the number of SNPs assessed.

The main finding of this study was the novel statistically significant interaction between the metabolic-GRS and dietary fat intake (%) on serum 25(OH)D levels. I identified that participants carrying 1 or more risk alleles and consuming a high fat diet (≥ 38% of energy = 122.3 ± 52.51 g/day) had significantly lower serum 25(OH)D concentration in comparison to those consuming a lower fat diet. In my previous studies that examined the interactions of the metabolic-GRS and vitamin D concentrations, only the study in the Asian Indians detected a significant interaction which showed that individuals with lower metabolic risk and lower dietary carbohydrate intake had higher levels of 25(OH)D. The result in my Turkish study suggested that a higher intake of dietary fat might have the potential to lower serum 25(OH)D concentration in the people with metabolic risk susceptibility. Several mechanisms could be responsible for the effect of high fat intake and metabolic diseases including obesity and one's vitamin D status including the volumetric dilution of serum vitamin D levels, overexpression of proinflammatory cytokines, modification of vitamin D-related enzymes affected by high fat diet-induced obesity, and lower endogenous vitamin D synthesis in the skin as a consequence of less outdoor activity [566, 570, 571, 573, 574]. The finding suggests that following current Turkish Dietary fat intake recommendations (< 35%) [565] might be effective to prevent any consequences of the genetic risk of vitamin D deficiency. However, further larger studies are needed before implementing personalized dietary recommendations for the maintenance of optimal vitamin D status.

8.8 General Trends Observed Across Different Ethnicities

The five ethnic groups examined in this thesis had differences in macronutrient intakes as listed in **Table 8.1**. This emphasises the need for investigating the effects of dietary factors

and gene-diet interactions on metabolic diseases in diverse populations. The percent of energy from carbohydrate was higher in the Indian (64 ± 6%) and Ghanaian populations (63 ± 10%) and lowest in the Turkish population (46 ± 9%) than other ethnic groups (Brazilian young adults: 51 ± 7% and Indonesian adults: 54 ± 9%). The acceptable macronutrient distribution range (AMDR) for carbohydrate is 45-65% of total energy intake [609].The carbohydrate intake for Turkish and Indian populations was very close to the lower and upper limits of the AMDR, respectively. The percent of energy from protein intake was higher in Brazilian young adults (17 ± 4%) and Indonesian women (17 ± 3%) and lowest in Indian adults (11 ± 1%) than other populations (Turkish: 16 ± 4 % and Ghanaian: 14 ± 4%). Protein intake was within the AMDR of 10-35% in all the populations [609]. The percent of energy from fat was highest in the Turkish population (38 ± 8%) and lowest in Indian adults (23 ± 5%) and Ghanaian adults (23 ± 9%) than other populations (Indonesian women: 29 ± 8% and Brazilian young adults: 32 ± 6%). The Fat AMDR is 20-35% of total energy intake [609]. The Turkish population consumed fat above the AMDR, whereas the fat intake of all other groups was within the AMDR.

Sampling strategy across these five studies could have affected the comparisons between the groups. The Brazilian population included young adults aged 19 to 24 years, whereas the Indian population included adults and elderly participants from age 29 to 85 years. Indonesian, Turkish and Ghanaian populations included participants with age ranging between 24-60 years. It is important to note that younger populations are more likely to adopt new dietary patterns than older populations, thus, targeting younger population might be an effective strategy to prevent chronic metabolic diseases. Additionally, Indians and Indonesian women were sampled in both urban and rural areas, whereas the Brazilian, Ghanaian, and Turkish population-based studies were conducted in urban areas. Further research looking at both urban and rural populations and controlling for confounding factors such as

socioeconomic status is needed [610]. Moreover, some of the studies did not specify what days of the week sampling took place; this is important to know because the amount of dietary intake may vary in the weekend compared to weekdays [611]. Another point that could have affected the assessment between groups is the methodological differences in dietary collection methods used. The FFQ was used to estimate dietary intake in the CURES and MINANG studies, whereas a repeated three day 24-hour dietary recall was used in the BOLD and GONG studies, while a single day 24-hour recall was used in the Turkish study. Thus, dietary differences across populations could have been due to the different dietary assessment tools used in studies along with cultural reasons.

The anthropometric and biochemical parameters of the study participants are listed in **Table 8.1**. The highest mean BMI was observed in the Indian and Ghanaian populations (26.8 ± 5 and 26.6 ± 4.9 kg/m^2), whereas Brazilians reported the lowest mean (23.1 ± 4.2 kg/m^2) and Turkish and Indonesians reported similar means of BMI (25.8 ± 4.2 and 25.2 ± 4.2 kg/m^2, respectively). Indonesian women reported the highest BFP (35.8 ± 7%), whereas the Turkish population reported the lowest mean (25.9 ± 7.6%) and similar means of BFP were observed in Ghanaian and Brazilian populations (32.9 ± 13.6 and 33.9 ± 10.7%, respectively). Indians reported the highest means of fasting glucose (115.7 ± 47.8 mg/dL) compared to the other four populations. Vitamin D levels also showed population-based differences where the Brazilian young adults and the Turkish adults had the highest vitamin D concentrations (27.6 ± 8.8 and 24.6 ± 16.6 ng/mL) whereas the Indonesian women and the Indian adults had lower vitamin D status (19.4 ± 8.8 and 18.9 ± 11.7 ng/mL, respectively). It is difficult to generalise these findings given the heterogeneity that exists across these ethnic groups and the limited sample size of the populations.

Table 8. 1: Macronutrient Intakes, and Anthropometric and Biochemical Parameters: A Comparison of the CURES, MINANG, BOLD, GONG and Turkish studies.

Parameters	Indian CURES study	Indonesian MINANG study	Brazilian BOLD study	Ghanaian GONG study	Turkish study
	(n = 545)	(n = 110)	(n = 187)	(n = 302)	(n = 396)
Total energy (Kcal/day)	2592 ± 772	1775 ± 612	1832 ± 611	1645 ± 688	2416 ± 1064
Carbohydrate (%)	64 ± 6	54 ± 9	51 ± 7	63 ± 10	46 ± 9
Protein (%)	11 ± 1	17 ± 3	17 ± 4	14 ± 4	16 ± 4
Fat (%)	23 ± 5	29 ± 8	32 ± 6	23 ± 9	38 ± 8
Fibre (g)	32 ± 11	8.8 ± 4.3	15.4 ± 9.1	22 ± 11	23.8 ± 12.1
Age (yrs)	49.5 ±11.4	40.4 ± 10.2	21.3 ± 1.7	38.3 ± 9.8	34.8 ± 7.1
BMI (kg/m^2)	26.8 ± 5.0	25.2 ± 4.2	23.1 ± 4.2	26.6 ± 4.9	25.8 ± 4.2
WC (cm)	88.3 ± 11.3	83.8 ±10.4	74.5 ± 12.3	88.4 ± 12.2	88.1 ± 11.6
WHR	0.91 ± 0.08	N/A	N/A	0.90 ± 0.11	0.87 ± 0.09
BFP (%)	N/A	35.8 ± 7	33.9 ± 10.7	32.9 ± 13.6	25.9 ± 7.6
Vitamin D (ng/mL)	18.9 ± 11.7	19.4 ± 8.8	27.6 ± 8.8	N/A	24.6 ± 16.6
FPG (mg/dL)	115.7 ± 47.8	92.5±20.7	87.0 ± 6.9	4.4 ± 0.9	87.8 ± 8.3
HbA1c (%)	6.5 ± 1.7	6.6 ± 6.0	4.7 ± 0.26	5.3 ± 0.6	N/A
Fasting insulin (µIU/mL)	8.9 ± 6.0	32026.1± 24918.8	8.7 ± 3.8	12.6 ± 14.4	7.7 ± 0.3
Total Cholesterol (mg/dL)	183 ± 36.7	208.9 ± 44	178 ± 36.8	142.1 ± 107.9	189 ± 38.6
LDL-C (mg/dL)	113.7 ± 32.8	127.8 ± 39.9	103.6 ± 29.3	N/A	123.1 ± 30
HDL-C (mg/dL)	41 ± 8.8	59.1 ± 10.3	56.5 ± 14.3	N/A	48.7 ± 11.6
Triglycerides (mg/dL)	141.7 ± 82.5	98 ± 43.3	85.3 ± 45.4	N/A	118.2 ± 72.5

Abbreviations: CURES Chennai Urban Rural Epidemiology Study; MINANG, Minangkabau Indonesia Study on Nutrition and Genetics; BOLD, Obesity, Lifestyle and Diabetes in Brazil; SNP single nucleotide polymorphisms; GONG, Genetics of obesity and nutrition in Ghana; BMI, body mass index; WC, waist circumference; WHR, waist hip ratio; BFP, Body Fat Percentage; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

Several ethnic differences exist for the metabolic traits and vitamin D status within the five populations studied in this thesis, which could be driven by variations at several genetic loci. An example of genetic heterogeneity is the *FTO* SNP rs8050136 which is one of the strongest BMI associated variants in several populations. The frequency of minor allele 'A' was 13% in the Indian population, 23% in the Indonesian population and in the Brazilian

population it was as high as 40% which was in agreement with previously reported values for the Latin American population (*https://www.ncbi.nlm.nih.gov/snp/rs8050136*). Another example is the *MC4R* SNP rs17782313, where the minor allele 'C' was found at a frequency of 13%, 17%, and 24%, in the Indonesian, Brazilian, and Indian populations, respectively. *CYP2R1* rs12794714 and *DHCR7* rs12785878 are variants in genetic loci associated with vitamin D synthesis, which also demonstrated variation in the frequencies in different ethnicities, as their prevalence was lowest in the Ghanaian population (9% and 11%, respectively) and highest in the Brazilian population (40% and 50%, respectively). In the Indonesian population, the prevalence was 26% and 22% respectively (**Table 8.2**).

Table 8. 2: Frequencies of the SNPs: A Comparison of the CURES, MINANG, BOLD, GONG and Turkish studies.

Gene	rs number	Major allele	Minor allele	Common Homozygotes n (%)	Heterozygotes n (%)	Rare Homozygotes n (%)	Minor allele frequency	HWE	Ethnicity
FTO	rs8050136	C	A	290 (75.1)	90 (23.3)	6 (1.6)	0.13	0.74	Indian
	rs8050136	C	A	69 (60)	39 (34)	7 (6.0)	0.23	0.64	Indonesian
	rs8050136	C	A	71 (35.5)	96 (48)	30 (15)	0.40	0.79	Brazilian
	rs10163409	A	T	106 (90.6)	11 (9.4)	-	0.05	0.59	Indonesian
	rs10163409	A	T	117 (58.5)	64 (32)	17 (8.5)	0.25	0.06	Brazilian
	rs2388405	T	C	340 (83.5)	62 (15.2)	5 (1.2)	0.09	0.26	Indian
	rs9939609	T	A	70 (60.4)	39 (33.6)	7 (6.0)	0.23	062	Indonesian
TCF7L2	rs12255372	G	T	163 (64.4)	75 (29.6)	15 (5.9)	0.21	0.12	Indian
	rs12255372	G	T	97 (83)	20 (17)	-	0.09	0.31	Indonesian
	rs12255372	G	T	85 (42.5)	92 (46)	23 (11.5)	0.35	0.80	Brazilian
	rs7903146	C	T	131 (51.8)	98 (38.7)	24 (9.5)	0.29	0.37	Indian
	rs7903146	C	T	95 (81.9)	21 (18.1)	-	0.09	0.28	Indonesian
	rs7903146	C	T	90 (45)	87 (43.5)	23 (11.5)	0.33	0.78	Brazilian
	rs7903146	C	T	180 (45.5)	163 (41.2)	53 (13.3)	0.34	0.10	Turkish
MC4R	rs17782313	T	C	144 (57.1)	93 (36.9)	15 (6.0)	0.24	1.00	Indian
	rs17782313	T	C	89 (76.1)	26 (22.2)	2 (1.7)	0.13	0.95	Indonesian
	rs17782313	T	C	139 (69.5)	55 (27.5)	6 (3)	0.17	0.84	Brazilian
	rs2229616	G	A	116 (99.1)	1 (0.9)	-	0	0.96	Indonesian
	rs571312	C	A	196 (49.5)	157 (39.6)	43 (10.9)	0.31	0.18	Turkish
KCNQ1	rs2237895	A	C	58 (50.4)	46 (40)	11 (9.6)	0.30	0.67	Indonesian
	rs2237895	A	C	85 (42.5)	91 (45.5)	23 (11.5)	0.34	0.86	Brazilian
	rs2237892	C	T	44 (37.6)	53 (45.3)	20 (17.1)	0.40	0.56	Indonesian
	rs2237892	C	T	160 (80)	35 (17.5)	5 (2.5)	0.11	0.08	Brazilian
CDKN2A/B	rs10811661	T	C	50 (43.1)	57 (49.1)	9 (7.8)	0.32	0.19	Indonesian
	rs10811661	T	C	151 (75.5)	45 (22.5)	4 (2)	0.13	0.76	Brazilian
PPARG	rs1801282	C	G	170 (85)	30 (15)	-	0.08	0.25	Brazilian

CAPN10	rs5030952	C	T	128 (64)	66 (33)	6 (3)	0.20	0.47	Brazilian
VDR	rs2228570	C	T	188 (67.4)	87 (31.2)	4 (1.4)	0.17	0.08	Ghanaian
	rs2228570	C	T	83 (42.6)	89 (45.6)	23 (11.8)	0.35	0.91	Brazilian
	rs7975232	A	C	127 (45.5)	123 (44.1)	29 (10.4)	0.32	0.92	Ghanaian
	rs7975232	A	C	72 (36)	92 (46)	35 (18)	0.41	0.49	Brazilian
DHCR7	rs12785878	G	T	221 (79.2)	55 (19.7)	3 (1.1)	0.11	0.84	Ghanaian
	rs12785878	G	T	48 (24)	106 (53)	46 (23)	0.50	0.40	Brazilian
	rs12785878	G	T	73 (62.4)	36 (30.8)	8 (6.8)	0.22	0.23	Indonesian
CYP2R1	rs12794714	G	A	230 (82.4)	46 (16.5)	3 (1.1)	0.09	0.68	Ghanaian
	rs12794714	G	A	72 (36.2)	95 (47.7)	32 (16.1)	0.40	0.94	Brazilian
	rs12794714	G	A	62 (53)	50 (42.7)	5 (4.3)	0.26	0.19	Indonesian
	rs10741657	G	A	158 (56.6)	100 (35.8)	21 (7.6)	0.25	0.35	Ghanaian
CYP24A1	rs6013897	T	A	157 (56.3)	100 (35.8)	22 (7.9)	0.26	0.28	Ghanaian
	rs6013897	T	A	115 (57.5)	73 (36.5)	12 (6)	0.24	0.93	Brazilian
	rs6013897	T	A	54 (46.5)	48 (41.4)	14 (12.1)	0.33	0.51	Indonesian
DBP/GC	rs2282679	A	C	263 (94.3)	16 (5.7)	-	0.03	0.62	Ghanaian
	rs2282679	A	C	134 (67.3)	59 (29.6)	6 (3.1)	0.18	0.87	Brazilian
	rs2282679	A	C	77 (67)	33 (28.7)	5 (4.3)	0.19	0.55	Indonesian
CASR	rs1801725	G	T	274 (98.2)	5 (1.8)	-	0.01	0.88	Ghanaian
	rs1801725	G	T	96 (82.1)	19 (16.2)	2 (1.7)	0.10	0.36	Indonesian

8.9 Limitations and Strengths

The studies conducted for this thesis project had some limitations that need to be considered. First, all five studies had relatively small sample sizes; which could have resulted in insufficiently powered analyses, however, I used the GRS approach in my investigations to maximise power and I was able to detect significant associations and interactions. Second, all the studies used a cross-sectional design in which all measurements were taken at a single point in time, which hinders the ability to examine the causal relationship between the GRSs and lifestyle interactions on vitamin D concentrations and on metabolic disease outcomes. Third, dietary intake and physical activity were assessed using self-reported measures which are prone to recall bias which could have affected the results, nevertheless, validated questionnaires were used to minimize potential errors in some studies, furthermore, food models and common household measures were utilised to help participants accurately estimate the actual amounts of foods and drinks consumed. Finally, dietary intakes were analysed based on macronutrient component without the effect of food sources except in the CURES and BOLD studies where the effect of plant vs animal protein source was available to examine GRS-diet interactions, which led to a better understanding of the observed interaction in the BOLD study. Nevertheless, the impact of different carbohydrate sources (sugars and starch) and different fibre sources (soluble and insoluble) which could have added valuable input to my findings were not available in any of the studies.

The main strengths of this thesis include the use of five diverse and distinct ethnic populations and the creation and use of different GRSs using multiple genetic variants. The use of a GRS approach is favorable to using a single genetic variant analysis because the GRS can increase the statistical power of the study, reduce the negative impact of multiple testing, and can provide better identification for disease risk [130, 133]. Additionally, two studies, CURES

and MINANG, used validated food frequency questionnaires (FFQs) [304, 362] to measure the long-term macronutrient consumption of the population and all studies used well trained staff to measure outcomes and to ensure accuracy of data. Furthermore, the studies of this thesis are the first to report gene-diet interactions (metabolic-related and/or vitamin D-related genes) on vitamin D concentrations and metabolic traits in Asian Indian adults, Indonesian women, Brazilian young adults, Ghanaian adults, and Turkish adults.

8.10 Future Prospects

In this thesis I found that genetic risk of low vitamin D status may be associated with metabolic outcomes through the dietary influence of carbohydrates, fibre, and fat intake; and with 25(OH)D levels in response to dietary protein intake. Also, metabolic genetic risk may be associated with low vitamin D concentrations through the dietary influence of carbohydrates and fat intake, and with LDL cholesterol levels in response to dietary fat intake. These gene-diet interaction findings need to be replicated in larger cohorts and intervention studies before they can be implemented for population-based health recommendations. If the genetic risk of low vitamin D concentrations has an effect on metabolic disease traits in response to diet, and if low vitamin D status is affected by genetic risk of metabolic diseases through a dietary influence, then it is important that mechanistic studies are conducted to determine how these interactions affect metabolism or how epigenetic mechanisms contribute to the risk of metabolic diseases.

Validation of my findings in future studies is essential, however, isolating the macronutrient accountable for any nutrigenetic effects is difficult, particularly fat and carbohydrates, as they tend to compensate for each other [612]. Thus, for validation, conducting dietary intervention trials would be important as they clearly define the experimental dietary component and would eliminate any recall bias that could occur with

common dietary data collection methods. Additionally, longitudinal studies would be of great value to examine the causal relationship between the gene-diet interactions on vitamin D concentrations and metabolic traits. Future studies should also consider assessing body composition using advanced measures such as dual-energy X-ray absorptiometry, magnetic resonance imaging, and/or computed tomography scans [613]. Measuring and analysing the various vitamin D metabolites in human serum, examining the regulatory effects of vitamin D on gut microbiota, scrutinising the effect of different doses of vitamin D supplementation on different clinical outcomes such as, BMI, WC, WHR, BFP, glucose, insulin, and HbA1c, are other avenues that could be of interest for future investigations. Furthermore, examining gene-lifestyle interactions in the groups that are at-risk of vitamin D deficiency, such as the elderly, people with dark skin, pregnant and lactating women, vegetarians, and obese individuals as well as ethnic minorities should be considered.

Insights from this PhD thesis show promise for the use of personalised nutrition in the area of vitamin D and metabolic disease, whereby certain vitamin D-related genetic variants may be used to predict an individual's risk of developing metabolic traits and may be modified according to an individual's dietary pattern. Gene-diet interactions have been investigated extensively in the European population, however, very few research studies have been performed in the LMICs, therefore, the Gene–Nutrient Interactions (GeNuIne) Collaboration has been established to focus on this missing gap of research in these countries [412]. Although the field of nutrigenetics has made great progress in identifying several gene-diet interactions, little is known about the underlying metabolic pathways of these interactions which need to be explored further [614, 615]. The fields of nutrigenetics and nutrigenomics will ultimately assist in implementing personalised nutrition and changing the face of nutritional practice by tailoring dietary recommendations based on an individual's genetic makeup for supporting health and preventing metabolic diseases [170]. Nevertheless, a large gap does exists between nutrition

recommendations and a person's eating behaviour, but evidence suggests that using genetic information for personalising dietary advice does promote positive behavioural changes in dietary intakes [168, 614].

At present, there is a need to study nutrigenetics in diverse ethnic groups, in order to translate our understanding of genetic disease architecture into clinical practice. Obtaining reliable phenotype information is important for generating quality genetic associations. Studying ethnically diverse populations is challenging in many settings, and this might be due to mistrust in the biomedical studies based previous exploitative experiences. Also, in many low middle-income countries that are characterised by great diversity, investment in professional training and adequate facilities is required [148]. Nutrigenetic research is urgently required, given the importance of developing public health strategies to decrease the prevalence and impact of metabolic diseases.

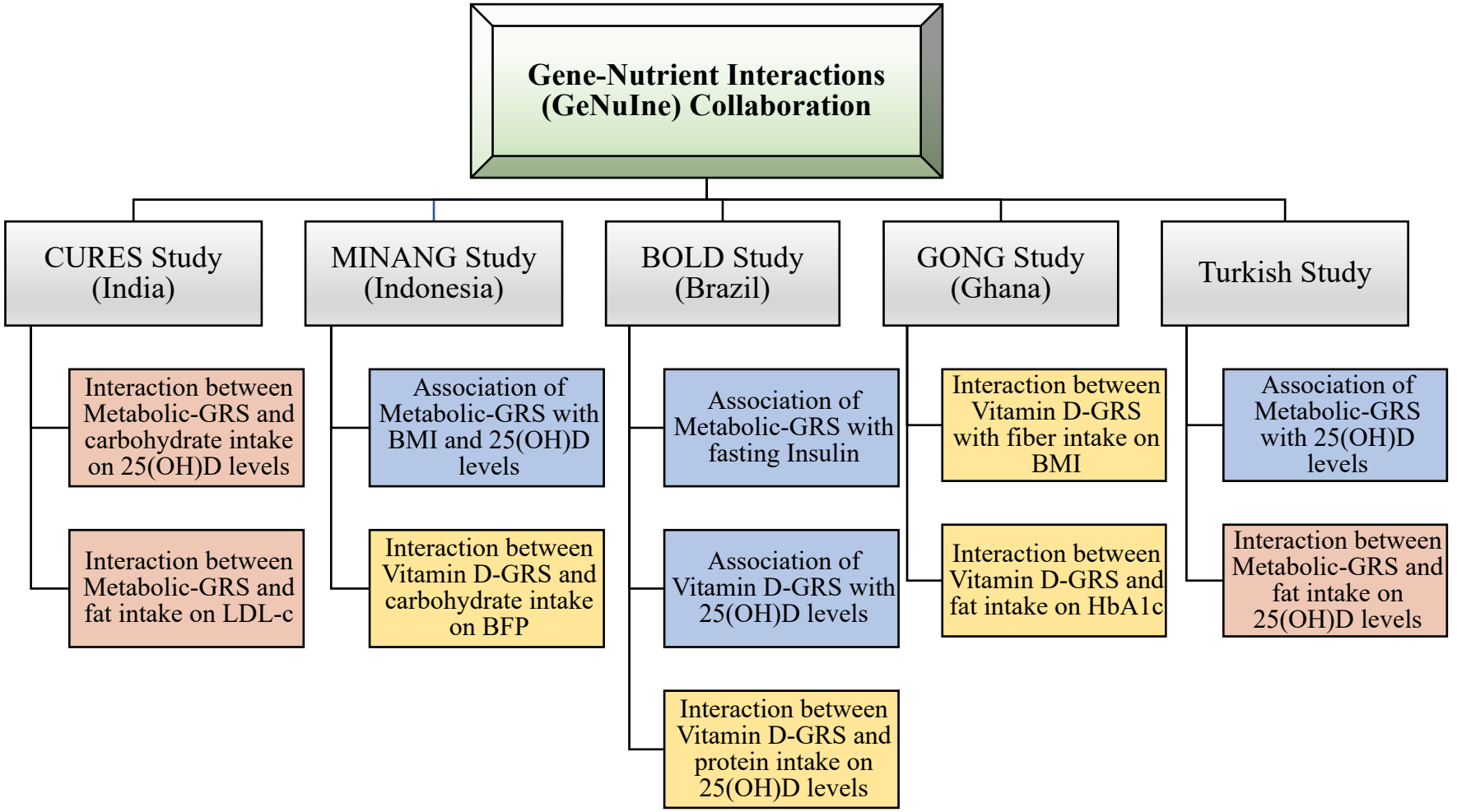
In summary, advancements in the field of nutrigenetics holds a promising future in preventing the development of diet-related chronic metabolic diseases. Though, there are many studies which show interactions between genes and lifestyle factors, there is still a need for larger, well-powered, and intervention studies in diverse ethnic groups in order to implement personalised nutrition.

8.11 Conclusion

In conclusion, my research has identified novel interactions between the vitamin D-GRS and dietary nutrient intake on obesity indicators, glycaemic control indicator HbA1c, and on serum 25(OH)D concentrations in specific ethnic populations. Additionally, this thesis identified novel interactions between the metabolic-GRS and nutrient intakes on 25(OH)D levels and LDL cholesterol. The findings of this thesis provides a better understanding of how

genetic variants related to vitamin D synthesis and metabolism interacts with dietary factors and contribute to metabolic outcomes (**Figure 8.1**).

The gene-nutrient interactions identified in this thesis may have significant public health implications. Nevertheless, it is essential to replicate the findings from this thesis in randomised control trials with larger sample sizes of different ethnicities and more precise and objective measures of lifestyle factors. 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-related and vitamin D-related SNPs, thus there is a need to utilise a comprehensive panel of genetic variants with vitamin D or metabolic disease related traits to create a polygenic risk score. In summary, this thesis contributes to a better understanding of the complex interplay between genetic and dietary factors in the variation of metabolic traits across multiple ethnic groups, which is essential for implementing precision nutrition in the future. Findings of this thesis have demonstrated significant GRS-nutrient interactions on metabolic traits and vitamin D status. However, these interactions need to be replicated in larger cohorts and functional studies are also required to understand the molecular aspects of these interactions before applying personalised dietary strategies to prevent or treat metabolic diseases.



⇒ **Genetic risk of low vitamin D status may be associated with metabolic outcomes through the dietary influence of carbohydrates, fibre, and fat intake; and with 25(OH)D levels in response to dietary protein intake.**

⇒ **Metabolic genetic risk may be associated with low vitamin D concentrations through the dietary influence of carbohydrates and fat intake.**

	Genetic associations
	Interactions with Metabolic-GRS
	Interactions with Vitamin D-GRS

Figure 8. 1: Main findings of the studies included in the thesis.

Abbreviations**:** GeNuIne, Gene–Nutrient Interactions; CURES, Chennai Urban Rural Epidemiology Study; MINANG, Minangkabau Indonesia Study on Nutrition and Genetics; BOLD, Obesity, Lifestyle and Diabetes in Brazil; GONG, Genetics of Obesity and Nutrition in Ghana; GRS, Genetic Risk Score; 25(OH)D, 25 hydroxyvitamin D; BMI, Body Mass Index; WC, Waist Circumference; LDL-c, Low Density Lipoprotein cholesterol; BFP, Body Fat Percentage; HbA1c, glycated Haemoglobin and HOMA-IR, Homeostasis Model Assessment of insulin resistance.

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Appendix

10.1 Research Analysis Plan: A Nutrigenetic Approach to Investigate the Relationship Between Metabolic Traits and Vitamin D Status in an Asian Indian Population

Hypothesis:

We hypothesise that metabolic disease-related gene variants are associated with vitamin D concentrations and that this association could be modulated by dietary factors in South Asian Indians.

Main aim:

To identify whether metabolic traits are associated with vitamin D levels using a genetic approach in South Asian Indian population (n = 545) [269, 300, 301], and to examine whether these associations are modified by lifestyle factors.

Objectives:

1. Create a metabolic-GRS from 5 SNPs from three genes.
2. Examine the association between vitamin D levels with metabolic traits.
3. Examine the association between the metabolic-GRS with vitamin D levels and metabolic traits.
4. Examine the interaction between the metabolic-GRS and vitamin D levels on metabolic traits.
5. Examine the interaction between the metabolic-GRS and lifestyle factors on vitamin D levels and on metabolic traits.

Metabolic disease-related outcomes:

- Body Mass Index (BMI) [kg/m^2]
- Waist Circumference (WC) [cm]
- Fasting Blood Glucose (FBG) [mg/dL]
- Glycated Haemoglobin (HbA1c) [%]
- Fasting serum Insulin [µIU/mL]
- Total Cholesterol [mg/dL]
- High Density Lipoprotein (HDL) [mg/dL]
- Low Density Lipoprotein (LDL) [mg/dL]
- Triglyceride (TG) [mg/dL]

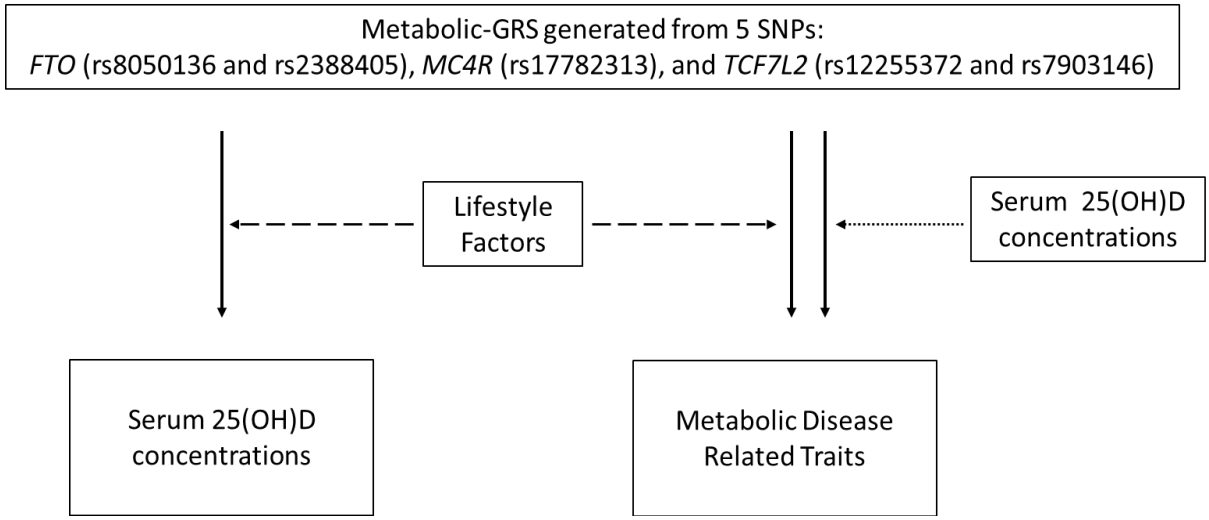

Figure 1: Diagram describing study design. The unbroken one-sided arrows indicate the associations that were tested between the metabolic-GRS and vitamin D concentrations and metabolic disease related traits. The broken one-sided arrows represent the interactions that were investigated between the GRS and lifestyle factors (diet and physical activity levels) on serum vitamin D and metabolic disease related traits. The one-sided dotted arrow indicates the interaction that was examined between metabolic-GRS and 25(OH)D concentrations on metabolic disease-related traits.

Disease cut off-values:

1. In CURES 2003 study, diabetes was diagnosed 'based on the past medical history, drug treatment for diabetes, and/or using the ADA fasting criteria' [300].
2. Generalized obesity was defined according to the World Health Organization Asia Pacific Guidelines for Asians as non-obese (BMI < 25 kg/m^2) and obese (BMI ≥ 25 kg/m^2) [302].
3. Vitamin D status is defined according to the Institute of Medicine Recommendations [616] and the Indian Academy of Paediatrics (IAP) [100]:

	Nanograms/mL	Status	Nanomoles/L
25(OH)D	< 12 ng/mL	Deficient	< 30 nmol/L
	12-20 ng/mL	Insufficient	30-50 nmol/L
	> 20 ng/mL	Sufficient	> 50 nmol/L

Previous Studies that have investigated the association of the genetic variants with metabolic traits:

Gene	SNP rs number	studies
Fat mass and obesity-associated (*FTO*)	rs8050136	[282, 288, 318, 319]
	rs2388405	[306, 307]
Transcription factor 7-like 2 (*TCF7L2*)	rs12255372	[285, 289, 294, 314]
	rs7903146	[285, 287, 289, 294, 312-314]
Melanocortin 4 receptor (*MC4R*)	rs17782313	[215, 286, 288, 298, 299]

Objectives	Statistical test used	Reason for statistical test used	Outcome of statistical test used	Covariates (When appropriate)
1: To check whether the selected SNPs are in Hardy-Weinberg equilibrium				
Determine whether the observed genotype counts are in Hardy-Weinberg equilibrium (HWE).	Chi-Squared test	To compare observed genotype counts with the values expected under Hardy-Weinberg	To test whether a population is in HWE at a locus [215, 282]	
2: To produce descriptive statistics for the study participants who had completed an assessment on demographics and biochemical and anthropometric measurements				
a. Generate the descriptive statistics of the participants who completed the study assessment on demographics, anthropometric and biochemical outcomes of interest.	Descriptive statistics descriptive Or Descriptive statistics frequencies	To determine baseline measures for continuous variables. To determine baseline frequencies for categorical variables.	To determine the mean and standard deviation of the anthropometric and biochemical variables: Age (year), BMI (kg/m^2), waist circumference (cm), Vitamin D (nmol/L), Fasting plasma glucose (mg/dL), Fasting serum insulin (µIU/mL), Glycated Haemoglobin (%), Systolic BP (mmHg), Diastolic BP (mmHg), Total Cholesterol (mg/dL), LDL Cholesterol (mg/dL), HDL Cholesterol (mg/dL), Serum Triglycerides (mg/dL), Total Energy (Kcal), Protein energy (%), Fat energy (%), Carbohydrate energy (%), Protein (g), Fat (g), Carbohydrate (g), Dietary fibre	

			(g), PAL [Sedentary (%); Moderate (%); Vigorous (%)].	
b. To stratify the descriptive statistics table into individuals with type 2 diabetes (T2D), pre-diabetes and normal glucose tolerance (NGT).	One way analysis of variances (ANOVA)	To compare the mean and standard deviations of participants, anthropometric and biochemical variables between each group.	To identify if there are any statistically significant differences in the participants, anthropometric and biochemical variables between each group.	
3: To test the association between the metabolic-GRS thought to have a role in metabolic disease with serum vitamin D levels and metabolic traits (BMI, WC, fasting plasma glucose, HbA1c, fasting plasma insulin, total cholesterol, HDL-c, LDL-c, triglycerides).				
To test for the association between the metabolic-GRS and vitamin D levels and metabolic traits.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variable (vitamin D levels and metabolic traits) each is a continuous variable.	To identify the impact of the variants on vitamin D levels and metabolic traits.	-Age, gender, BMI, and T2D (BMI will not be adjusted for when BMI/obesity is the outcome) [617].
4: To test the interaction between the metabolic-GRS and lifestyle factors on vitamin D levels and metabolic traits (BMI, WC, fasting plasma glucose, HbA1c, fasting plasma insulin, total cholesterol, HDL-c, LDL-c, triglycerides).				
a. Testing the interaction between the metabolic-GRS and lifestyle factors on vitamin D levels and metabolic traits.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variables (vitamin D levels and metabolic traits) are continuous variables.	To identify the impact of the gene variants and the macronutrients: carbohydrate, protein, fat on vitamin D levels and metabolic traits.	-Age, gender, BMI, and T2D (BMI will not be adjusted for when BMI/obesity is the outcome) [617].

b. If there are significant interactions then test to find if high, medium or low consumption of macronutrients are causing the interaction.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variables (vitamin D levels and metabolic traits) are continuous variables.	To identify the impact of gene variants and the consumption of different quantities of the macronutrient on vitamin D levels and metabolic traits. (Data split into binary and tertile groups of carbohydrate, protein and fat intake)	-Age, gender, BMI, and T2D (BMI will not be adjusted for when BMI/obesity is the outcome) [617] - Total energy (when dietary variable is measured in grams).
5: To test the interaction between the metabolic-GRS and vitamin D levels on metabolic traits (BMI, WC, fasting plasma glucose, HbA1c, fasting plasma insulin, total cholesterol, HDL-c, LDL-c, triglycerides).				
a. Testing the interaction between the metabolic-GRS and vitamin D levels on metabolic disease-related traits.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variables (metabolic traits) are continuous variables.	To identify the impact of the gene variants and serum vitamin D levels on metabolic traits.	-Age, gender, BMI, and T2D (BMI will not be adjusted for when BMI/obesity is the outcome) [617]. - Total energy (when dietary variable is measured in grams).
b. If there are significant interactions then test to find if high, medium or low consumption of macronutrients are causing the interaction.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variables (vitamin D levels and metabolic traits) are continuous variables.	To identify the impact of gene variants and the consumption of different quantities of the macronutrient on vitamin D levels and metabolic traits. (Data split into binary and tertile groups of carbohydrate, protein and fat intake)	-Age, gender, BMI, and T2D (BMI will not be adjusted for when BMI/obesity is the outcome) [617] - Total energy (when dietary variable is measured in grams).

NOTES				
When looking at carbohydrates, proteins and fat in grams, I will adjust for total energy intake. When using the percentage energy intake of the macronutrients, there is no need to adjust for Kcal, as it has already been adjusted for.	Compute variables	For carbohydrate interactions: 1g of carb = 4 kcal For fat interactions: 1g of fat =9 kcal For protein interactions: 1g protein = 4 kcal		
When significant interactions with macronutrients are detected, further investigation will take place to underline the specific type of macronutrient responsible for the interaction.	Univariate linear regression	If interaction with fat intake is significant: Test for the interaction between the GRS with saturated fatty acid intake, monounsaturated fatty acid intake and polyunsaturated fatty acid intake. Tertiles will be made for each of these sub-groups.		

10.2 Research Analysis Plan: Interaction between Vitamin D-Related Genetic Risk Score and Carbohydrate Intake on Body Fat Composition: A Study in Southeast Asian Minangkabau Women

Hypothesis:

We hypothesise that low vitamin D concentrations caused by genetic variants are associated with an increased risk of obesity and type 2 diabetes (T2D) and that metabolic disease-related gene variants effects can be modified by controlling serum vitamin D concentrations in Southeast Asian Minangkabau Women.

Main objectives:

The aim of the present study was to explore the relationships between metabolic traits and vitamin D status in a cohort of healthy Indonesian women (n = 110) and to investigate whether these relationships were modified by dietary intake using a genetic approach [357].

Objectives:

1. Create a vitamin D-related GRS from 5 SNPs from five genes.
2. Create a metabolic-GRS from 10 SNPs from five genes.
3. We will test for the association between metabolic-GRS and vitamin D concentrations and metabolic disease-related traits

4. We will then test for the associations between vitamin D–GRS and vitamin D status and metabolic disease-related traits.
5. Lastly, we will test whether these genetic associations are modified by dietary factors.

Metabolic disease-related outcomes:

- Body Mass Index (BMI) [kg/m^2]
- Waist Circumference (WC) [cm]
- Body Fat Percentage (BFP) [%]
- Fasting Blood Glucose (FBG) [mg/dL]
- Glycated Haemoglobin (HbA1c) [%]
- Fasting serum Insulin [μIU/mL]
- Total Cholesterol [mg/dL]
- High Density Lipoprotein (HDL) [mg/dL]
- Low Density Lipoprotein (LDL) [mg/dL]
- Triglyceride (TG) [mg/dL]

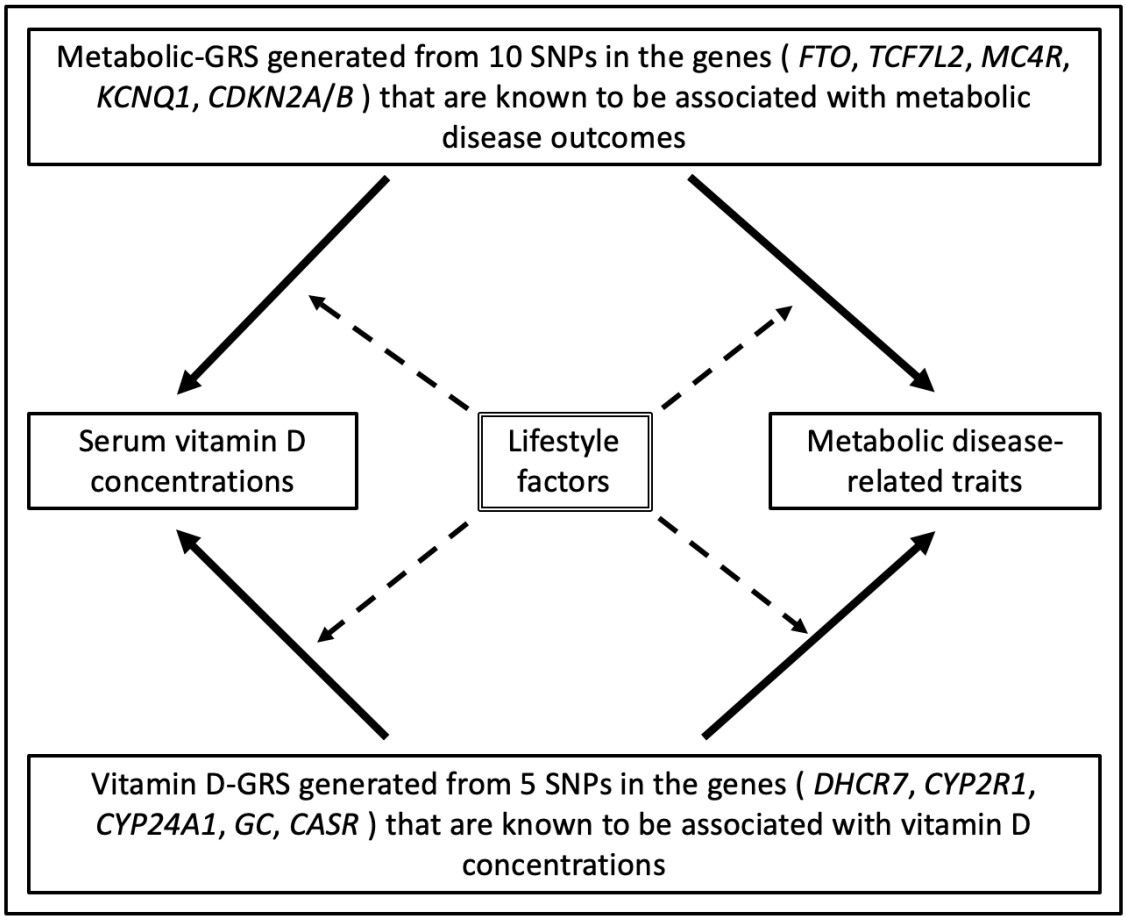

Figure 1. Diagram representing the study design. Genetic associations are represented by one-sided arrows with unbroken lines and interactions between GRS and diet on clinical and biochemical measurements are shown as one-sided arrows with broken lines. The association of the metabolic-GRS with 25(OH)D levels and metabolic traits and the association of vitamin D-GRS with 25(OH)D levels and metabolic traits were tested. In addition, the effect of dietary factors on these genetic associations was examined. Abbreviations: GRS: genetic risk score, SNP: single nucleotide polymorphism, FTO: fat mass and obesity-associated gene, TCF7L2: transcription factor 7-like 2 gene, MC4R: melanocortin 4 receptor gene, KCNQ1: potassium voltage-gated channel subfamily Q member 1, CDKN2A/B: cyclin dependent kinase inhibitor 2A/B, DHCR7: 7-dehydrocholesterol reductase, CYP2R1: 25-hydroxylase, CYP24A1: 24-hydroxylase, GC: group-specific component, CASR: calcium sensing receptor.

Disease cut off-values:

1. Generalized obesity was defined according to the World Health Organization Asia Pacific Guidelines for Asians as non-obese (BMI < 25 kg/m^2) and obese (BMI ≥ 25 kg/m^2) [302].
2. Vitamin D status is defined according to the Institute of Medicine Recommendations [616]:

25(OH)D	Nanograms/mL	Status	Nanomoles/L
	< 12 ng/mL	Deficient	< 30 nmol/L
	12-20 ng/mL	Insufficient	30-50 nmol/L
	> 20 ng/mL	Sufficient	> 50 nmol/L

Genes and SNPs under investigation:

1. *FTO*: Fat mass and obesity-associated
    - rs8050136 (associated with obesity & type 2 diabetes)
    - rs9939609 (associated with obesity & type 2 diabetes)
    - rs10163409 (associated with obesity)
2. *TCF7L2*: Transcription factor 7-like 2
    - rs12255372 (associated with type 2 diabetes)
    - rs7903146 (associated with obesity & type 2 diabetes)
3. *MC4R*: Melanocortin 4 receptor
    - rs17782313 (associated with obesity)
    - rs2229616 (associated with obesity)
4. *KCNQ1*: Potassium Voltage-Gated Channel Subfamily Q Member 1
    - rs2237895 (associated with type 2 diabetes)
    - rs2237892 (associated with type 2 diabetes)
5. *CDKN2A/B*: Cyclin Dependent Kinase Inhibitor 2A/B
    - rs10811661(associated with type 2 diabetes)

6. *DHCR7*: 7-Dehydrocholesterol Reductase
    - rs12785878 (associated with serum vitamin D levels)
7. *CYP2R1*: Cytochrome P450 Family 2 Subfamily R Member 1 / 25-Hydroxylase
    - rs12794714 (associated with serum vitamin D levels)
8. *CYP24A1*: Cytochrome P450 Family 24 Subfamily A Member 1 / 24-Hydroxylase
    - rs6013897 (associated with serum vitamin D levels)
9. *GC*: Vitamin D Binding Protein
    - rs2282679 (associated with serum vitamin D levels)
10. *CASR*: Calcium Sensing Receptor
    - rs1801725 (associated with serum vitamin D levels)

Previous Studies looking at the association of the 11 metabolic disease-related SNPs with obesity and T2D traits:

Gene	SNP	Studies which show its association with obesity	Studies which show its association with type 2 diabetes
FTO	rs8050136	[282, 318, 343, 372]	[282, 343, 345]
	rs9939609	[375, 426]	[375, 426]
	rs10163409	[374, 618]	
TCF7L2	rs12255372		[285, 344, 372]
	rs7903146	[346, 372, 429]	[285, 344, 348, 372, 427, 428]
MC4R	rs17782313	[177, 288, 372, 619]	
	rs2229616	[373]	
KCNQ1	rs2237895		[342, 431]
	rs2237892		[342, 431]
CDKN2A/B	rs10811661		[348, 376, 432, 433]

Previous Studies looking at the association of the 5 vitamin D-related SNPs with vitamin D concentrations:

Gene	SNP	Studies which show an association with vitamin D levels
DHCR7	rs12785878	[364, 369-371]
CYP2R1	rs12794714	[363, 371]
CYP24A1	rs6013897	[369, 620]
GC	rs2282679	[363, 364]
CASR	rs1801725	[365, 366, 368]

Objectives	Statistical test used	Reason for statistical test used	Outcome of statistical test used	Covariates (when appropriate)
1: To check whether the selected SNPs are in Hardy-Weinberg equilibrium				
Determine whether the observed genotype counts are in Hardy-Weinberg equilibrium (HWE).	Chi-Squared test	To compare observed genotype counts with the values expected under Hardy-Weinberg	To test whether the study population is in HWE for the loci under study [215, 282]	
2: To produce descriptive statistics for the study participants who had completed an assessment on demographics and biochemical and anthropometric measurements				
a. Generate the descriptive statistics of the participants who completed the study assessment on demographics, anthropometric and biochemical outcomes of interest.	Descriptive statistics Or Descriptive statistics frequencies	Descriptive for continuous variables. Frequencies for categorical variables.	To determine the mean and standard deviation of the anthropometric and biochemical variables: Age (year), BMI (kg/m^2), waist circumference (cm), body fat percentage (5), Fasting plasma glucose (mg/dL), Fasting serum insulin (μIU/mL), Glycated Haemoglobin (%), Total Cholesterol (mg/dL), LDL Cholesterol (mg/dL), HDL Cholesterol (mg/dL), Serum Triglycerides (mg/dL), Total Energy (Kcal), Protein energy (%), Fat energy (%), Carbohydrate energy (%), Protein (g), Fat (g), Carbohydrate (g), Dietary fibre (g).	

b. To stratify the descriptive statistics table into three groups based on participants vitamin D concentrations into sufficient, insufficient, and deficient vitamin D status.	One way analysis of variances (ANOVA)	To compare the mean and standard deviations of participants, anthropometric and biochemical variables between groups.	To identify if there are any statistically significant differences in the participants, anthropometric and biochemical variables between groups [282].	
3: To test the association between the metabolic-GRS thought to have a role in metabolic disease with vitamin D concentrations and anthropometric and biochemical measurements (BMI, WC, body fat percentage, fasting plasma glucose, HbA1c, fasting plasma insulin, total cholesterol, HDL-c, LDL-c, triglycerides).				
To test for the association between the metabolic-GRS and vitamin D levels and anthropometric and biochemical measurements while adjusting for covariates.	Univariate linear regression	The exposure (independent) variable (GRS) is a categorical variable and the outcome (dependant) variable (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the genetic variants on anthropometric and biochemical measurements.	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617]. - Location.
4: To test the association between the vitamin D-GRS thought to have a role in vitamin D level with vitamin D concentrations and anthropometric and biochemical measurements (BMI, WC, body fat percentage, fasting plasma glucose, HbA1c, fasting plasma insulin, total cholesterol, HDL-c, LDL-c, triglycerides).				
To test for the association between the vitamin D-GRS and vitamin D levels and anthropometric and biochemical measurements while adjusting for covariates.	Univariate linear regression	The exposure (independent) variable (GRS) is a categorical variable and the outcome (dependant) variable (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the genetic variants on anthropometric and biochemical measurements.	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617]. - Location.

5: To test the interaction between the two GRSs (Vitamin D-GRS and metabolic-GRS) and dietary factors on vitamin D concentrations and anthropometric and biochemical measurements (BMI, WC, body fat percentage, fasting plasma glucose, HbA1c, fasting plasma insulin, total cholesterol, HDL-c, LDL-c, triglycerides).				
a. To test the interaction between macronutrients and the GRSs on vitamin D levels and anthropometric and biochemical measurements.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variable (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the gene variants and the macronutrients: carbohydrate, protein, fat on anthropometric and biochemical measurements.	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617]. - Total energy (when dietary variable is measured in grams). - Location.
b. To test to find out if high, low or medium consumption of these macronutrients are causing the interaction.	Univariate linear regression	exposure variable (GRS) is a categorical variable and outcome variable (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the gene variants and the macronutrients: carbohydrate, protein, fat on anthropometric and biochemical measurements. (Data split into binary and tertile groups of carbohydrate, protein, and fat intake)	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617]. - Total energy (when dietary variable is measured in grams). - Location.
NOTES				
When looking at carbohydrates, proteins and fat in grams, I will adjust for total energy intake. When using the percentage energy intake of the macronutrients,	Compute variables	For carbohydrate interactions: 1g of carb = 4 kcal For fat interactions: 1g of fat =9 kcal For protein interactions: 1g protein = 4 kcal		

there is no need to adjust for Kcal, as it has already been adjusted for.		
When significant interactions with macronutrients are detected, further investigation will take place to underline the specific type of macronutrient responsible for the interaction.	Univariate linear regression	If interaction with fat intake is significant: Test for the interaction between SNPs with saturated fatty acid intake, monounsaturated fatty acid intake and polyunsaturated fatty acid intake. Tertiles/ binary will be made for each of these sub-groups.

10.3 Research Analysis Plan: Impact of Genetic Risk Score and Dietary Protein Intake on Vitamin D Status in Young Adults from Brazil

Hypothesis:

We hypothesise that low vitamin D concentrations caused by genetic variants are associated with an increased metabolic disease risk and that metabolic disease-related gene variants effects can be modified by controlling serum vitamin D concentrations in South American Brazilians.

Main aim:

We aim to investigate the relationships between metabolic traits and vitamin D status in a cohort of young healthy individuals from Brazil (n = 187) and to investigate whether these relationships are modified by lifestyle factors using a nutrigenetic approach.

Objectives:

1. Create a vitamin D-related GRS from 6 SNPs from five genes.
2. Create a metabolic-GRS from 10 SNPs from seven genes.
3. Examine the association between metabolic-related GRS and vitamin D concentrations and metabolic disease-related traits.
4. Examine the associations between vitamin D–GRS and vitamin D status and metabolic disease related traits.

5. Examine the interaction between the GRSs and lifestyle factors (diet and physical activity) on the metabolic-GRS and the vitamin D-GRS

Metabolic disease-related outcomes:

- Body Mass Index (BMI) [kg/m^2]
- Waist Circumference (WC) [cm]
- Body Fat Percentage (BFP) [%]
- Fasting Blood Glucose (FBG) [mg/dL]
- Glycated Haemoglobin (HbA1c) [%]
- Fasting serum Insulin [μIU/mL]
- Total Cholesterol [mg/dL]
- High Density Lipoprotein (HDL) [mg/dL]
- Low Density Lipoprotein (LDL) [mg/dL]
- Triglyceride (TG) [mg/dL]

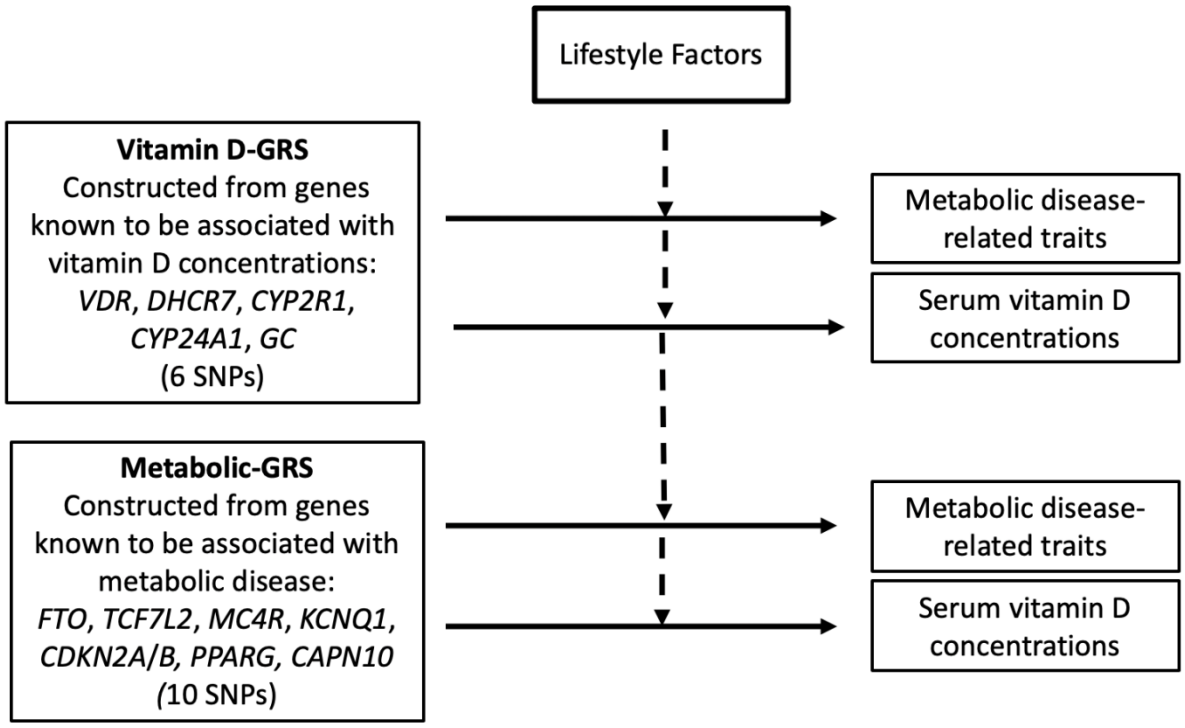

Figure 1. Diagram representing the study design. The one-sided horizontal arrows with solid lines represent the genetic associations and the one-sided vertical arrows with dotted lines represent the interactions between GRS and diet on clinical and biochemical measurements. The association of vitamin D-GRS with 25(OH)D levels and metabolic traits and the association of the metabolic-GRS with 25(OH)D levels and metabolic traits and were tested. Furthermore, analyses of the effect of dietary factors on these genetic associations were performed.

Disease cut off-values:

1. Generalized obesity was defined according to the World Health Organization Asia Pacific Guidelines for Asians as non-obese (BMI < 25 kg/m^2) and obese (BMI ≥ 25 kg/m^2) [302].
2. Vitamin D cut-off points were based on the recommendations of the Brazilian Society of Endocrinology and Metabolism and the Brazilian Society of Clinical Pathology/Laboratory Medicine vitamin D levels (normal = 20-60 ng/mL) and (deficient < 20 ng/mL) [438].

Genes and SNPs under investigation:

1. *FTO*: Fat mass and obesity-associated
    - rs8050136 (associated with obesity & type 2 diabetes)
    - rs9939609 (associated with obesity & type 2 diabetes)
2. *TCF7L2*: Transcription factor 7-like 2
    - rs12255372 (associated with type 2 diabetes)
    - rs7903146 (associated with obesity & type 2 diabetes)
3. *MC4R*: Melanocortin 4 receptor
    - rs17782313 (associated with obesity)
    - rs2229616 (associated with obesity)
4. *KCNQ1*: Potassium Voltage-Gated Channel Subfamily Q Member 1
    - rs2237895 (associated with type 2 diabetes)
    - rs2237892 (associated with type 2 diabetes)
5. *CDKN2A*/*B*: Cyclin Dependent Kinase Inhibitor 2A/B
    - rs10811661(associated with type 2 diabetes)
6. *PPARG*: Peroxisome Proliferator Activated Receptor Gamma
    - rs1801282 (rs1801282 also known as Pro12Ala, has been reported to be associated with metabolic syndrome)
7. *CAPN10*: Calpains 10
    - rs5030952 (associated with type 2 diabetes)
8. *VDR*: Vitamin D Receptor
    - rs2228570 (associated with serum vitamin D levels)
    - rs7975232 (associated with serum vitamin D levels)
9. *DHCR7*: 7-Dehydrocholesterol Reductase

- rs12785878 (associated with serum vitamin D levels)

10. *CYP2R1*: Cytochrome P450 Family 2 Subfamily R Member 1 / 25-Hydroxylase
    - rs12794714 (associated with serum vitamin D levels)
11. *CYP24A1*: Cytochrome P450 Family 24 Subfamily A Member 1 / 24-Hydroxylase
    - rs6013897 (associated with serum vitamin D levels)
12. *GC*: Vitamin D Binding Protein
    - rs2282679 (associated with serum vitamin D levels)

Previous Studies looking at the association of the 11 metabolic disease-related SNPs with obesity and type 2 diabetes traits:

Gene	SNP	Studies which show its association with obesity	Studies which show its association with type 2 diabetes
FTO	rs8050136	[282, 318, 343, 372]	[282, 343, 345]
	rs9939609	[375, 426]	[375, 426]
TCF7L2	rs12255372		[285, 344, 372]
	rs7903146	[346, 372, 429]	[285, 344, 348, 372, 427, 428]
MC4R	rs17782313	[177, 288, 372, 619]	
	rs2229616	[373]	
KCNQ1	rs2237895		[342, 431]
	rs2237892		[342, 431]
CDKN2A/B	rs10811661		[348, 376, 432, 433]
PPARG	rs1801282	[347]	[348, 434, 435]
CAPN10	rs5030952		[436, 437]

Previous Studies looking at the association of the 6 vitamin D SNPs with vitamin D concentrations:

Gene	SNP	Studies which show an association with vitamin D levels
VDR	rs2228570 (FokI)	[425, 621]
	rs7975232 (ApaI)	[622, 623]
DHCR7	rs12785878	[364, 369-371]

CYP2R1	rs12794714	[363, 371]
CYP24A1	rs6013897	[367, 369]
GC	rs2282679	[363, 364]

Objectives	Statistical test used	Reason for statistical test used	Outcome of statistical test used	Covariates (when appropriate)
1: To check whether the selected SNPs are in Hardy-Weinberg equilibrium				
Determine whether the observed genotype counts are in Hardy-Weinberg equilibrium (HWE).	Chi-Squared test	To compare observed genotype counts with the values expected under Hardy-Weinberg	To test whether the study population is in HWE for the loci under study [215, 282]	
2: To produce descriptive statistics for the study participants who had completed an assessment on demographics and biochemical and anthropometric measurements				
a. Generate the descriptive statistics of the participants who completed the study assessment on demographics, anthropometric and biochemical outcomes of interest.	Descriptive statistics	Descriptive for continuous variables. Frequencies for categorical variables.	To determine the mean and standard deviation of the anthropometric and biochemical variables: Age (year), BMI (kg/m^2), waist circumference (cm), body fat percentage (5), Fasting plasma glucose (mg/dL), Fasting serum insulin (μIU/mL), Glycated Haemoglobin (%), Total Energy (Kcal), Protein energy (%), Fat energy (%), Carbohydrate energy (%), Protein (g), Fat (g), Carbohydrate (g), Dietary fibre (g).	
b. To stratify the descriptive statistics table into two groups based on participants vitamin	Independent samples t-test	To compare the mean and standard deviations of participants, anthropometric	To identify if there are any statistically significant differences in the participants, anthropometric	

D levels into normal status and deficient status.		and biochemical variables between the two groups.	and biochemical variables between the two groups [282].	
3: To test the association between the metabolic-GRS with metabolic traits (BMI, WC, body fat percentage, glucose, insulin, HbA1c) and serum vitamin D concentrations				
To test for the association between the metabolic-GRS and vitamin D levels and anthropometric and biochemical measurements while adjusting for covariates	Univariate linear regression	The exposure (independent) variable (GRS) is a categorical variable and the outcome (dependant) variable (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the genetic variants on anthropometric and biochemical measurements	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617].
4: To test the association between vitamin D-GRS with metabolic traits (BMI, WC, body fat percentage, glucose, insulin, HbA1c) and serum vitamin D concentrations				
To test for the association between the vitamin D-GRS and vitamin D levels and anthropometric and biochemical measurements while adjusting for covariates.	Univariate linear regression	The exposure (independent) variable (GRS) is a categorical variable and the outcome (dependant) variable (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the genetic variants on anthropometric and biochemical measurements.	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617].
5: To test the interaction between the two GRSs (vitamin D-GRS and metabolic-GRS) and dietary factors on metabolic traits (BMI, WC, body fat percentage, glucose, insulin, HbA1c) and serum vitamin D concentrations				
a. To test the interaction between macronutrients and GRSs on anthropometric and biochemical measurements	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variable (anthropometric and	To identify the impact of the gene variants and the macronutrients: carbohydrate, protein, fat on	- Age, gender, and BMI. (BMI will not be adjusted for when

		biochemical measurements) is a continuous variable.	anthropometric and biochemical measurements	BMI/obesity is the outcome) [617]. - Total energy (when dietary variable is measured in grams).
b. To test to find out if high, low or medium consumption of these macronutrients are causing the interaction.	Univariate linear regression	exposure variable (GRS) is a categorical variable and outcome variable (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the gene variants and the macronutrients: carbohydrate, protein, fat on anthropometric and biochemical measurements (Data split into binary and tertile groups of carbohydrate, protein, and fat intake)	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617]. - Total energy (when dietary variable is measured in grams).
NOTES				
When looking at carbohydrates, proteins and fat in grams, I will adjust for total energy intake. When using the percentage energy intake of the macronutrients, there is no need to adjust for Kcal, as it has already been adjusted for.	Compute variables	For carbohydrate interactions: 1g of carb = 4 kcal For fat interactions: 1g of fat =9 kcal For protein interactions: 1g protein = 4 kcal		
When significant interactions with macronutrients are detected, further investigation will take place to underline the specific type of	Univariate linear regression	If interaction with fat intake is significant: Test for the interaction between SNPs with saturated fatty acid intake, monounsaturated fatty acid intake and polyunsaturated fatty acid intake. Tertiles/ binary will be made for each of these sub-groups.		

macronutrient responsible for the interaction.		

10.4 Research Analysis Plan: Interactions between Vitamin D Genetic Risk and Dietary Factors on Metabolic Disease-Related Outcomes in Ghanaian Adults.

Hypothesis:

We hypothesise that vitamin D genetic variants are associated with increased metabolic disease risk and that this association could be modulated by dietary factors in West African Ghanaians.

Main aim:

We aim to investigate the relationships between vitamin D genetic variants and metabolic traits in a cohort of healthy individuals from Ghana (n = 302) and to investigate whether these relationships are modified by lifestyle factors using a nutrigenetic approach.

Objectives:

1. Create a vitamin D-related GRS from 8 SNPs from six genes.
2. Examine the associations between vitamin D–GRS and metabolic disease related traits.
3. Examine the interaction between the vitamin D-GRSs and dietary factors on metabolic disease-related traits.

Metabolic disease-related outcomes:

- Body Mass Index (BMI) [kg/m^2]

- Waist Circumference (WC) [cm]
- Body Fat Percentage (BFP) [%]
- Fasting Blood Glucose (FBG) [mg/dL]
- Glycated Haemoglobin (HbA1c) [%]
- Fasting serum Insulin [µIU/mL]
- Total Cholesterol [mg/dL]
- High Density Lipoprotein (HDL) [mg/dL]
- Low Density Lipoprotein (LDL) [mg/dL]
- Triglyceride (TG) [mg/dL]

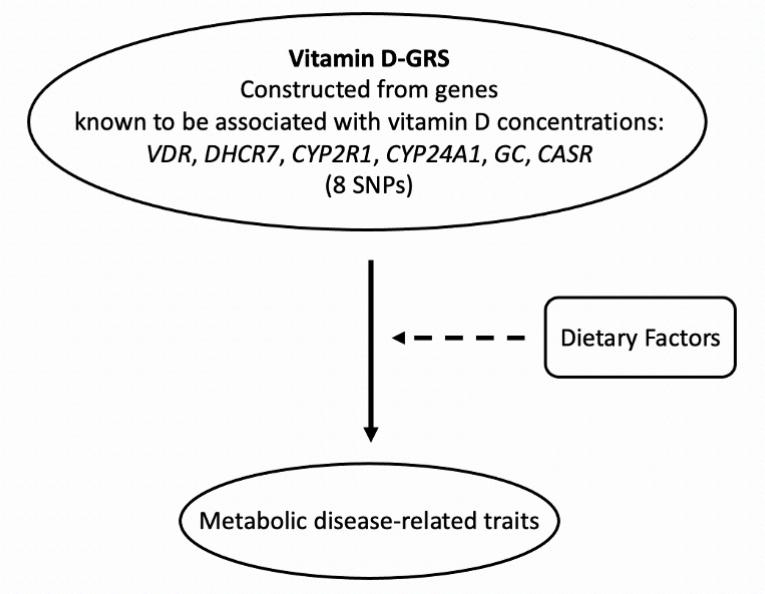

Figure 1: Diagram describing study design Study design. The solid arrow denotes genetic association analysis, and the dashed arrow represents genetic interaction analysis between vitamin D-GRS and dietary factors on biochemical and clinical measurements outcomes. Abbreviations: GRS: genetic risk score; SNP: single nucleotide polymorphism; *VDR*: Vitamin D Receptor; *DHCR7*: 7-

dehydrocholesterol reductase; *CYP2R1*: 25-hydroxylase; *CYP24A1*: 24-hydroxylase; *GC*: group-specific component; *CASR*: Calcium Sensing Receptor.

1. *VDR*: Vitamin D Receptor
    - rs2228570 (associated with serum vitamin D levels)
    - rs7975232 (associated with serum vitamin D levels)
2. *DHCR7*: 7-Dehydrocholesterol Reductase
    - rs12785878 (associated with serum vitamin D levels)
3. *CYP2R1*: Cytochrome P450 Family 2 Subfamily R Member 1 / 25-Hydroxylase
    - rs12794714 (associated with serum vitamin D levels)
    - rs10741657 (associated with serum vitamin D levels)
4. *CYP24A1*: Cytochrome P450 Family 24 Subfamily A Member 1 / 24-Hydroxylase
    - rs6013897 (associated with serum vitamin D levels)
5. *GC*: Vitamin D Binding Protein
    - rs2282679 (associated with serum vitamin D levels)
6. *CASR*: Calcium Sensing Receptor
    - rs1801725 (associated with serum vitamin D levels)

<u>Previous Studies looking at the association of the 8 vitamin D SNPs with vitamin D concentrations:</u>

Gene	SNP	Studies which show an association with vitamin D levels
VDR	rs2228570 (FokI)	[425, 621]
	rs7975232 (ApaI)	[622, 623]
DHCR7	rs12785878	[364, 369-371]
CYP2R1	rs12794714	[363, 371]
	rs10741657	[121, 369]
CYP24A1	rs6013897	[369, 620]

GC	rs2282679	[363, 364]
CASR	rs1801725	[365, 366, 368]

Objectives	**Statistical test used**	**Reason for statistical test used**	**Outcome of statistical test used**	**Covariates** (when appropriate)
1: To check whether the selected SNPs are in Hardy-Weinberg equilibrium				
Determine whether the observed genotype counts are in Hardy-Weinberg equilibrium (HWE).	Chi-Squared test	To compare observed genotype counts with the values expected under Hardy-Weinberg	To test whether the study population is in HWE for the loci under study [215, 282]	
2: To produce descriptive statistics for the study participants who had completed an assessment on demographics and biochemical and anthropometric measurements				
a. Generate the descriptive statistics of the participants who completed the study assessment on demographics, anthropometric and biochemical outcomes of interest.	Descriptive statistics	Descriptive for continuous variables. Frequencies for categorical variables.	To determine the mean and standard deviation of the anthropometric and biochemical variables: Age (year), BMI (kg/m^2), waist circumference (cm), waist-hip ratio (WHR), body fat percentage (5), Fasting plasma glucose (mg/dL), Fasting serum insulin (μIU/mL), Glycated Haemoglobin (%), Total Cholesterol (mg/dL), LDL Cholesterol (mg/dL), HDL Cholesterol (mg/dL), Serum Triglycerides (mg/dL),Total Energy (Kcal), Protein energy (%), Fat energy (%), Carbohydrate energy (%), Protein (g), Fat (g), Carbohydrate (g), Dietary fibre (g).	

b. To stratify the descriptive statistics table into men and women.	Independent samples t-test	To compare the mean and standard deviations of participants, anthropometric and biochemical variables between the men and women.	To identify if there are any statistically significant differences in the participants, anthropometric and biochemical variables between the men and women [282].	
3: To test the association between the vitamin D-GRS thought to have a role in vitamin D level with anthropometric and biochemical measurements (BMI, WC, waist-hip ratio, body fat percentage, fasting plasma glucose, HbA1c, fasting plasma insulin).				
To test for the association between the vitamin D-GRS and anthropometric and biochemical measurements while adjusting for covariates.	Univariate linear regression	The exposure (independent) variable (GRS) is a categorical variable and the outcome (dependant) variable (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the genetic variants on anthropometric and biochemical measurements.	- Age & BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617].
5: To test the interaction between the GRSs (Vitamin D-GRS and metabolic-GRS) and dietary factors on metabolic traits (BMI, WC, Body Fat Percentage, glucose, insulin, HbA1c, Chol, HDL, LDL, Trig) and serum vitamin D concentrations				
To test the interaction between macronutrients and the vitamin D-GRS on anthropometric and biochemical measurements.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variable (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the gene variants and the macronutrients: carbohydrate, protein, fat on anthropometric and biochemical measurements.	- Age & BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617]. - Total energy (when dietary variable is measured in grams).
To test to find out if high, low or medium consumption of these macronutrients are causing the interaction.	Univariate linear regression	exposure variable (GRS) is a categorical variable and outcome variable (anthropometric and	To identify the impact of the gene variants and the macronutrients: carbohydrate, protein, fat on on	- Age & BMI. (BMI will not be adjusted for when

		biochemical measurements) is a continuous variable.	anthropometric and biochemical measurements. (Data split into binary and tertile groups of carbohydrate, protein, and fat intake)	BMI/obesity is the outcome) [617]. - Total energy (when dietary variable is measured in grams).
NOTES				
When looking at carbohydrates, proteins and fat in grams, I will adjust for total energy intake. When using the percentage energy intake of the macronutrients, there is no need to adjust for Kcal, as it has already been adjusted for.	Compute variables	For carbohydrate interactions: 1g of carb = 4 kcal For fat interactions: 1g of fat =9 kcal For protein interactions: 1g protein = 4 kcal		
When significant interactions with macronutrients are detected, further investigation will take place to underline the specific type of macronutrient responsible for the interaction.	Univariate linear regression	If interaction with fat intake is significant: Test for the interaction between SNPs with saturated fatty acid intake, monounsaturated fatty acid intake and polyunsaturated fatty acid intake. Tertiles/ binary will be made for each of these sub-groups.		

10.5 Research Analysis Plan: Interaction between Dietary Fat Intake and Metabolic Genetic Risk Score on 25-Hydroxyvitamin D Concentrations in a Turkish Adult Population

Hypothesis:

1. We hypothesise that metabolic disease-related gene variants are associated with vitamin D concentrations and that this association could be modulated by dietary factors in the Turkish populations.

Main aim:

To identify whether metabolic traits are associated with vitamin D levels using a genetic approach in adult Turkish population (n = 396), and to examine whether these associations are modified by lifestyle factors.

Objectives:

6. Create a metabolic-GRS from 2 SNPs from two genes.
7. Examine the association between the metabolic-GRS with vitamin D levels and metabolic traits.
8. Examine the interaction between the metabolic-GRS and lifestyle factors on vitamin D levels and on metabolic traits.

Metabolic disease-related outcomes:

- Body Mass Index (BMI) [kg/m^2]
- Waist Circumference (WC) [cm]
- Hip circumference (HC) (cm)
- Waist-to-hip ratio (WHR)
- Fasting Blood Glucose (FBG) [mg/dL]
- Glycated Haemoglobin (HbA1c) [%]
- Fasting serum Insulin [μIU/mL]
- Total Cholesterol [mg/dL]
- High Density Lipoprotein (HDL) [mg/dL]
- Low Density Lipoprotein (LDL) [mg/dL]
- Triglyceride (TG) [mg/dL]

Disease cut off-values:

4. Generalized obesity was defined according to the World Health Organization Asia Pacific Guidelines for Asians as non-obese (BMI < 25 kg/m^2) and obese (BMI ≥ 25 kg/m^2) [302].
5. Vitamin D status is defined according to the Institute of Medicine Recommendations [616]:

	Nanograms/mL	Status	Nanomoles/L
25(OH)D	< 12 ng/mL	Deficient	< 30 nmol/L
	12-20 ng/mL	Insufficient	30-50 nmol/L
	> 20 ng/mL	Sufficient	> 50 nmol/L

Studies that have investigated the association of the genetic variants with metabolic traits:

Gene	SNP rs number	studies
Transcription factor 7-like 2 (*TCF7L2*)	rs7903146	[285, 287, 289, 294, 312-314]
Melanocortin 4 receptor (*MC4R*)	rs571312	[295, 309, 624]

Objectives	Statistical test used	Reason for statistical test used	Outcome of statistical test used	Covariates (When appropriate)
1: To check whether the selected SNPs are in Hardy-Weinberg equilibrium				
Determine whether the observed genotype counts are in Hardy-Weinberg equilibrium (HWE).	Chi-Squared test	To compare observed genotype counts with the values expected under Hardy-Weinberg	To test whether a population is in HWE at a locus [215, 282]	
2: To produce descriptive statistics for the study participants who had completed an assessment on demographics and biochemical and anthropometric measurements				
a. Generate the descriptive statistics of the participants who completed the study assessment on demographics, anthropometric and biochemical outcomes of interest.	Descriptive statistics descriptive Or Descriptive statistics frequencies	To determine baseline measures for continuous variables. To determine baseline frequencies for categorical variables.	To determine the mean and standard deviation of the anthropometric and biochemical variables: BMI (kg/m^2), waist circumference (cm), hip circumference (cm), waist-to-hip ratio, fat mass index, body fat mass (%), body fat mass (kg), visceral fat percentage (%), glucose (mg/L), insulin (µIU/mL), Postprandial glucose (mg/dL), Postprandial insulin (µIU/mL), VLDL cholesterol (mg/dL), Total cholesterol (mg/dL), HDL cholesterol (mg/dL), LDL cholesterol (mg/dL), Triglyceride (mg/dL), Insulin resistance (HOMA-IR), Total Energy (kcal), Protein energy (%), Fat energy (%), Carbohydrate energy (%),	

			Dietary fibre (g), PAL [Sedentary (%); Moderate (%); Vigorous (%)].	
b. To stratify the descriptive statistics table into two groups based on participants vitamin D levels into optimal status and deficient/insufficient status.	Independent samples t-test	To compare the mean and standard deviations of participants, anthropometric and biochemical variables between the two groups.	To identify if there are any statistically significant differences in the participants, anthropometric and biochemical variables between the two groups.	
3: To test the association between the metabolic-GRS thought to have a role in metabolic disease with serum vitamin D and metabolic traits.				
To test for the association between the metabolic-GRS and vitamin D levels and metabolic traits.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variable (anthropometric and biochemical measurements) each is a continuous variable.	To identify the impact of the variants on anthropometric and biochemical measurements	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617].
4: To test the interaction between the metabolic-GRS of and lifestyle factors on vitamin D levels and metabolic traits.				
a. Testing the interaction between the metabolic-GRS and lifestyle factors on vitamin D levels and metabolic traits.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variables (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of the gene variants and the macronutrients: carbohydrate, protein, fat on anthropometric and biochemical measurements	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617]. - Total energy (when dietary variable is measured in grams).

b. If there are significant interactions then test to find if high, medium or low consumption of macronutrients are causing the interaction.	Univariate linear regression	The exposure variable (GRS) is a categorical variable and the outcome variables (anthropometric and biochemical measurements) is a continuous variable.	To identify the impact of gene variants and the consumption of different quantities of the macronutrient on vitamin D levels and metabolic traits. (Data split into binary and tertile groups of carbohydrate, protein and fat intake)	- Age, gender, and BMI. (BMI will not be adjusted for when BMI/obesity is the outcome) [617]. - Total energy (when dietary variable is measured in grams).
NOTES				
When looking at carbohydrates, proteins and fat in grams, I will adjust for total energy intake. When using the percentage energy intake of the macronutrients, there is no need to adjust for Kcal, as it has already been adjusted for.	Compute variables	For carbohydrate interactions: 1g of carb = 4 kcal For fat interactions: 1g of fat =9 kcal For protein interactions: 1g protein = 4 kcal		
When significant interactions with macronutrients are detected, further investigation will take place to underline the specific type of macronutrient responsible for the interaction.	Univariate linear regression	If interaction with fat intake is significant: Test for the interaction between the GRS with saturated fatty acid intake, monounsaturated fatty acid intake and polyunsaturated fatty acid intake. Tertiles will be made for each of these sub-groups.		