Exercise, appetite and cardiometabolic risk markers in South Asian and European Men

by

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GENERAL ABSTRACT

South Asians have a higher risk of cardiovascular disease (CVD) and type 2 diabetes (T2D) than white Europeans. The mechanisms responsible remain partially understood with physical activity and cardiorespiratory fitness possibly playing a role. Thus, this thesis examined ethnic differences in traditional and unconventional CVD and T2D risk markers including appetite-related hormones and inflammatory markers as well as free fatty acids based on metabolomics methods, between South Asian and white European men. Furthermore, this thesis explored associations with objectively-measured physical activity/cardiorespiratory fitness and examined effects of acute exercise on subjective appetite ratings, appetite-related hormones and *ad libitum* energy intake.

In study 1 (Chapter 3), South Asians exhibited lower fasting acylated ghrelin, high-density lipoprotein and $\dot{V}O_2$ max than white Europeans but higher body fat percentage, C-reactive protein, leptin, triacylglycerol, glucose and insulin concentrations. Study 2 (Chapter 4), revealed higher laurate, myristate, palmitate, γ-linolenate and linoleate concentrations in South Asians than white Europeans. Free fatty acids were strongly correlated with body fat percentage and total area under the curve for glucose in South Asians and with total step counts in white Europeans. Study 3 (Chapter 5) revealed similar appetite perceptions, energy intake and appetite-related hormones between ethnic groups in response to exercise although subtle differences were identified with South Asians exhibiting lower appetite perceptions 2 h after the buffet meal, delta acylated ghrelin concentrations at 4 h and lower carbohydrate intake after the exercise. Acute exercise increased concentrations of delta acylated ghrelin and total peptide YY and induced a transient suppression in appetite perceptions at 4.5 h and stimulation at 6.5 h, without provoking energy compensation in both groups.

These data demonstrate that South Asian have an adverse cardiometabolic risk profile than white European men which may be linked to their lower physical activity and cardiorespiratory fitness. Furthermore, these show that South Asians exhibit different levels of acylated ghrelin and FFAs metabolic profile than white Europeans and provide evidence that acute exercise induces a short-term energy deficit irrespective of ethnicity.

Key words: cardiovascular disease, type 2 diabetes, South Asians, exercise, cardiorespiratory fitness, appetite, energy balance, appetite hormones, free fatty acids

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LMM (linear mixed model)

AUC (area under the curve)

BMI (body mass index)

ES (effect size)

SD (standard deviation)

SEM (standard error of the mean)

PCA (principle components analysis)

OPLS-DA (orthogonal partial least squares discriminant analysis)

OPLSA (orthogonal partial least square)

CV-ANOVA (cross validated analysis of variance)

LMM (linear mixed model)

CVD (cardiovascular disease)

T2D (type 2 diabetes)

OGTT (oral glucose tolerance test)

TAG (triacylglycerol)

HDL-C (high density lipoprotein cholesterol)

LDL-C (low density lipoprotein cholesterol)

CRP (C-reactive protein)

IL-6 (interleukin-6)

FFAs (free fatty acids)

PARQ (Physical activity readiness questionnaire)

VAS (visual analogue scale)

$\dot{V}O_2$ max (maximum oxygen uptake)

SST (serum separator tube)

POMC (proopiomelanocortin)

AgRP (agouti related peptide)

ARC (arcuate nucleus)

PVN (paraventricular nucleus)

LHA (lateral hypothalamic area)

CRF (corticotrophin releasing factor)

TRH (thyrotropin releasing factor)

NPY (neuropeptide Y)

CART (cocaine and amphetamine regulated transcript)

MCH (melanin concentrating hormone)

CCK (cholecystokinin)

GLP-1 (glucagon-like peptide 1)

PYY (peptide YY)

Oxm (oxyntomodulin)

PP (pancreatic polypeptide)

Chapter 1 – Literature review

1.1 Cardiovascular disease and Type 2 diabetes: definition and aetiology

1.1.1 Cardiovascular disease

Cardiovascular disease (CVD) or heart disease is a general term which relates to those conditions that affects the heart (cardio) and blood vessels (vascular) and comprises several types of disorders including coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease and deep vein thrombosis and pulmonary (WHO, 2017). Among all the CVD categories, CHD and cerebrovascular diseases are the primary causes of cardiovascular death in both developed and developing countries in men and women (Figure 1.1) (Wong, 2014).

Coronary heart disease refers to a reduced blood flow to the heart mostly due to a progressive shrinkage and obstruction of the coronary arteries, condition known as cardiac ischemia. If ischemia is severe or lasts too long, it can cause chest pain (angina pectoris) or heart attack (myocardial infarction) which eventually leads to heart tissue death. Symptoms of a heart attack are various and may include pain or discomfort in the centre of the chest or in other areas including arms, left shoulder, elbows, jaw or back (WHO, 2017).

Cerebrovascular disease includes a group of disorders causing limited or no blood supply to different areas of the brain. Vessel narrowing (e.g., carotid stenosis) refers to a decreased blood flow to the brain causing cerebral hypoxia, condition known as cerebral ischemia, which may lead to serious impairment of brain function if the lack of oxygen and nutrition is not restored promptly.

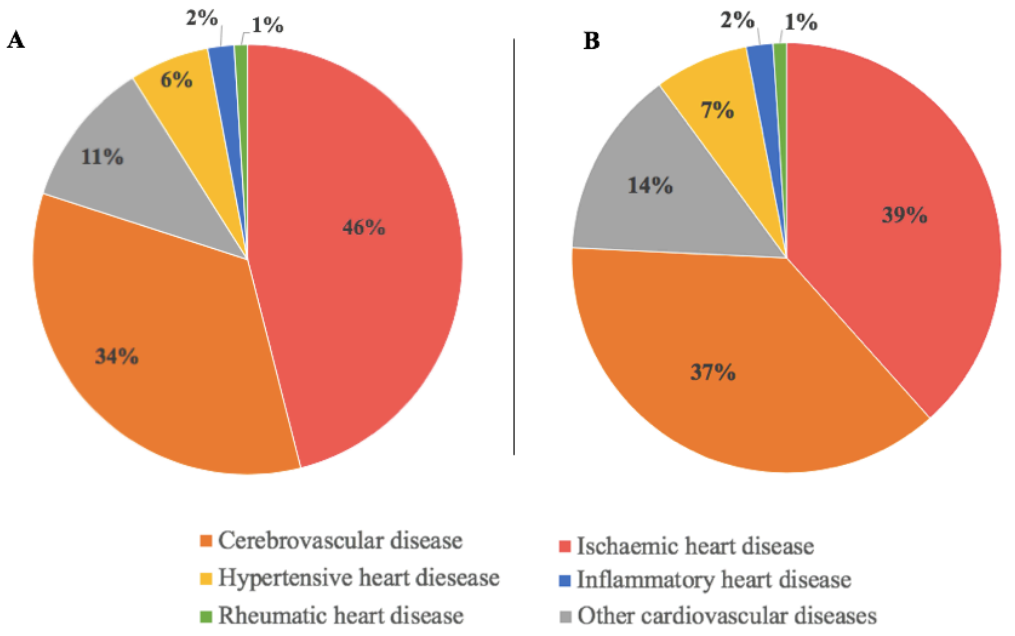

Figure 1.1. The proportions of cardiovascular deaths caused by ischaemic heart disease, cerebrovascular disease, inflammatory heart disease, rheumatic heart disease, hypertensive heart disease, and other cardiovascular diseases in 2011 in men (A) and in women (B) worldwide. Adapted from Wong (2014).

Additionally, clot formation (cerebral thrombosis), blockage (cerebral embolism), and blood vessel rupture (cerebral haemorrhage) are other types of cerebrovascular disorders which may provoke a prolonged ischemia and death of brain tissue known as stroke or brain attack (WHO, 2017). The most common symptom of a stroke is sudden weakness or numbness of the face, arm or leg, most often on one side of the body. Nevertheless, other symptoms may include confusion, difficulty speaking or understanding speech, dizziness and loss of balance or coordination, severe headache with no known cause and fainting or unconsciousness. The effects of cerebrovascular disease depend on which part of the brain is injured and how severely it is affected. A very severe stroke, for instance, can cause sudden death (WHO, 2017).

Coronary heart disease and cerebrovascular disease are predominantly associated to a chronic low-grade inflammatory condition of the blood vessels known as "atherosclerosis" (Mallika et al. 2007). Atherosclerosis comes from the Greek words "athero" (meaning gruel or paste) and "sclerosis" (meaning hardness) and refers to the thickened and hardened lesions known as atherosclerotic plaques characterised by the progressive accumulation of lipids and fibrous elements in the intimae and media of elastic and muscular arteries (Mallika et al. 2007). As a consequence, arteries can be narrowed due to the build-up of fatty plaque, which can result in insufficient blood flow to organs such as heart and brain causing myocardial infarction or stroke (Lusis, 2000). Although a number of hypothesis have been made to explain the genesis of atherosclerosis, this condition may be initiated by damage to the endothelium known as "injury hypothesis" (Ross, 1993). According to this hypothesis, an intact endothelium releases antithrombotic and fibrinolytic factors as well as the vasodilator nitric oxide (NO). However, CVD risk factors such as hyperlipidaemia, hyperglycaemia, hypertension and smoking may contribute to induce an injury or damage to the endothelium cells of the arteries leading to endothelium disfunction and plaque formation. Particularly, the damaged endothelium causes abnormal responses such as increasing the production of vasoconstricting agents including thromboxane A2 and prostaglandins (Ross, 1993). Furthermore, endothelial damage triggers platelets to adhere and aggregate at the site of the damage that causes monocytes to enter the tunica intima and proliferate within the tunica-media junction of the artery, which contributes to the atherogenic changes within the arteries (Ross, 1993). Atherosclerosis is a slow and progressive disease that begins early in life with early lesions (fatty streaks) visible in childhood (Lusis, 2000). Age progression and prolonged exposure to atherosclerotic risk

factors result in the development of fatty streaks into more advanced lesions responsible for the artery stenosis and reduced blood flow to organs (Lusis, 2000).

1.1.2 Type 2 diabetes

Diabetes refers to a group of metabolic disorders characterised by higher levels of plasma glucose resulting from defects in insulin secretion, insulin action, or both (ADA, 2013). Diabetes is categorised into type 1 diabetes (T1D) and type 2 diabetes (T2D). Type 1 diabetes, also known as insulin-dependent diabetes or juvenile-onset diabetes, accounts for only 10% of those with diabetes and results from a cellular-mediated autoimmune destruction of the pancreatic ß-cells (ADA, 2013). Type 2 diabetes (T2D), known as non–insulin-dependent diabetes or adult-onset diabetes, accounts for 90% of those with diabetes and includes people with insulin resistance (e.g. the pancreas produces insulin, but the tissues are unresponsive to it) (WHO, 2016). Apart from these, there is gestational diabetes which relates to elevated blood glucose concentrations induced during pregnancy. The majority of patients with T2D are obese, and obesity itself is responsible for some degree of insulin resistance (ADA, 2013). However, individuals who are not obese by conventional weight criteria may have greater percentage of body fat distributed predominantly in the abdominal area, which is associated with greater insulin resistance (WHO, 2016). Type 2 diabetes leads to complications including blindness, amputation and kidney failure and represents a well-established risk factor for CHD and cerebrovascular disease with endothelial dysfunction reported to be greater in individuals with insulin resistance and associated hyperglycaemia compared with normoglycemic individuals (Huang et al. 2016). The mechanism responsible for the increased risk for endothelial dysfunction and atherosclerosis in diabetes remain controversial (Kuusisto et al. 1994). However, T2D and impaired glucose tolerance (IGT) are often associated with

other risk factors such as hypertension, dyslipidaemia and obesity, each of which may cause endothelial dysfunction (Meigs et al. 1997).

1.2 Cardiovascular disease and Type 2 diabetes: statistics

1.2.1 Global burden

Cardiovascular disease accounts for more than 17.9 million deaths worldwide each year, (~ 31% of all deaths) with the majority of which occurring in low and middle-income countries and this figure is estimated to increase to 23.6 million by 2030 (WHO, 2017). In the United States, CVD is the leading cause of death killing more than 2000 people per day, an average of one person every 40 seconds (AHA, 2016). In addition, one or more types of CVD affect an estimated 82.6 million American adults and out of these, an estimated 40 400 000 are ≥ 60 years of age. CVD is responsible for half of all deaths in the United States with cancer (second largest killer) accounting for only half as many deaths. The American Heart Association also estimated that 68% of patients with diabetes die of some form of CVD (AHA, 2016). In Europe, CVD represents also the main cause of death causing each year 3.9 million deaths and over 1.8 million deaths in the European Union (EU) (EHN, 2017). Particularly, death rates from both CHD and stroke are generally greater in central and eastern Europe such as Germany, Hungary, Poland and Slovakia compared with northern countries such as Denmark, Finland, and Sweden and southern Europe including, Italy, Greece and Spain (EHN, 2017). However, CVD risk factors such as age-standardised rates of diabetes prevalence, as well as obesity and physical inactivity have gone up in Nordic and Southern Europe countries (EHN, 2017).

The prevalence of CHD and stroke, once thought to be a disease of the 'rich' is growing in low - and middle - income countries which are experiencing a rapid increase of mortality and morbidity (Gijsberts et al. 2015). In this regard, CVD events have been reported to be more common in South Asia than in high income countries with acute myocardial infarction occurring six years earlier in South Asians than in Europe individuals, probably due to an earlier onset of risk factors including hyperinsulinemia and dyslipidaemia (Misra et al. 2017). Furthermore, the proportion of death, particularly in younger adults, is greater in South Asia which increases the years of life lost as a result of CVD (Yusuf et al. 2004). According to the Global Burden of disease study in 2015, CVD and diabetes in South Asia account for 27% and 4.0% of deaths, respectively, with CHD representing the leading cause of death in India, Pakistan, Nepal, and Sri Lanka, whereas stroke is the leading cause in Bangladesh (Misra et al. 2017). In India, the largest country of South Asia, CHD in the urban population was estimated to grow from 7% to 13% and in the rural population from 2% to 7% (Prabhakaran and Yusuf, 2011). Increasing rates of urbanisation and major economic changes (such as improved transportation) have led to changes in lifestyle patterns for a large proportion of people in India resulting in lower physical activity and greater body fat and weight leading to diabetes, hypertension and dyslipidaemia (Reddy, 1999).

Type 2 diabetes represents the main CVD risk factors and the proportion of people with T2D is alarmingly growing in most countries (Figure 2.2). According to the International Diabetes Federation, approximately 463 million adults (20-79 years) worldwide are living with diabetes, predominantly in low and middle-income countries and it is expected to grow to 700 million by 2045 (Figure 1.2) (IDF, 2019).

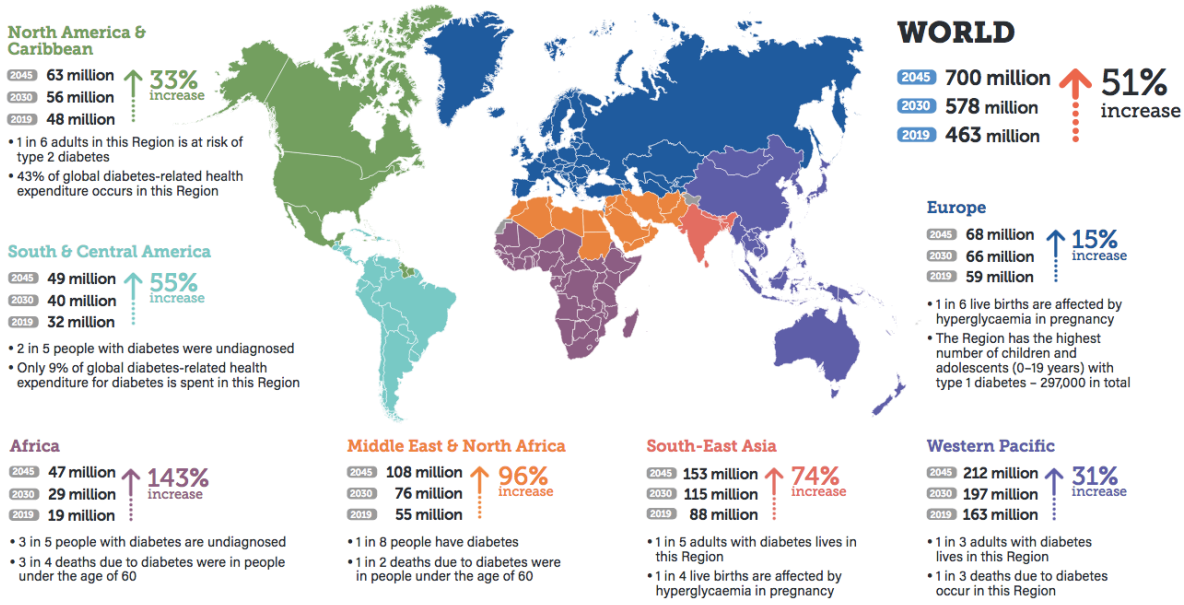

Figure 1.2. Number of adults (20-79 years) with diabetes worldwide. International Diabetes Federation, Diabetes Atlas, 9th edition. Copyright permission authorised by IDF (2019).

In South Asia, 88 million of people (1 in 11 adults) have diabetes and this figure is estimated to increase to 153 million by 2045 (IDF, 2019). Furthermore, the actual number of deaths due to diabetes in South Asia has been estimated to be 1 150 3000 people, which is significantly greater compared to other western countries including North America (301 700 people) and Europe (465 900 people) (IDF, 2019).

Prevalence data in South Asia, however, change according to the country or area of residence (urban vs. rural). The prevalence of T2D in urban areas in India is nearly five times higher than in rural areas, and this has been observed also in Pakistan, Bangladesh and Nepal (Hall et al. 2008). However, the increase rate of diabetes over the years was greater in the rural compared to the urban areas. For instance, between 1989 and 2005 prevalence of diabetes in the urban areas of South India increased from 8.3% to 18.6%,

whereas in the rural populations the increase of diabetes was more than 3 times (from 2.2% to 9.2%) (Misra et al. 2014). Furthermore, amongst all the Indian subcontinent countries, India exhibits 77 million cases of diabetes and occupies the first position for number of people with diabetes (20-79 years) followed by Bangladesh (8.4 million), Sri Lanka (1.2 million) and Nepal (0.7 million) (IDF, 2019).

1.2.2 United Kingdom

In the United Kingdom (UK), CVD is responsible for more deaths than any other single cause according to the British Heart Foundation (BHF, 2019). Particularly, 7.4 million people in the UK are living with CVD which cause 170,000 deaths every year, an average one death every three minutes (BHF, 2019). Half of all CVD cases in the UK are due to CHD and one-third are due to stroke. Coronary heart disease was responsible for 64,000 deaths in 2016, one in seven in men and one in twelve in women (BHF, 2019). Additionally, CHD is the most common cause of premature death (under the age of 75) in the UK (18% in men and 10% in women) with nearly all deaths from CHD caused from a heart attack. The mortality rate from CHD, however, has been decreasing since the 1970s, but CHD incidence has been on the rise from the 1980s, particularly in men aged 75 and older.

The number of people experiencing complications or dying because of their diabetes in the UK is also growing. Type 2 diabetes accounts for around 90% of all diabetes cases whereas T1D accounts for only 10% (Diabetes UK, 2019). Approximately 4.7 million people in the UK have diabetes, and this include around one million of people who have diabetes but have not been diagnosed. The number of people diagnosed with diabetes in the UK has more than doubled in 20 years. In 1996 there were 1.4 million people

diagnosed while in 2019 there were 3.8 million, and this figure is estimated to increase to 5.5 million by 2030 (Diabetes UK, 2019). Compared to individuals without diabetes, people with diabetes are two times more likely to have a heart attack, heart failure or a stroke. Particularly, diabetes causes 27 000 heart attacks and almost 100 000 cases of heart failure (Diabetes UK, 2019). In the UK, diabetes is more common in men than women (9.6% compared with 7.6% women) and individuals with a South Asian background are two to four times more likely to develop T2D compared with white people (Diabetes UK, 2019).

1.2.3 South Asian population in the UK

The term South Asian refers to a heterogeneous group who have ancestral origin from the Indian subcontinent which includes India, Pakistan, Bangladesh, Nepal, Sri Lanka and Bhutan, representing almost a quarter of the world's population (Sattar and Gill, 2015). Many South Asians live outside the Indian subcontinent with a large representation living in western countries including the United Kingdom (UK), United States (USA), Canada and Europe (Sattar and Gill, 2015). According to the 2011 UK census, individuals classifying themselves as South Asians born in the UK or in one of the South Asian countries represented the largest ethnic minority group (Office for National Statistics 2013). Particularly, 4.9% of the entire population in the UK (~ 3 million people) originates from one of the Indian subcontinent countries.

Migrant South Asians as well as those living in the Indian subcontinent, exhibit greater CVD and T2D susceptibility than their western counterparts. Although there is not explicit data in the literature reporting comparison of CVD or T2D risk in South Asian native versus south Asian migrants, previous data report an increased cardiometabolic

risk in the Indian Subcontinent compared with western countries (Misra 2017, IDF, 2019) or a greater prevalence of CVD and T2D in South Asian migrants compared with the white populations in the countries where they move (Johns and Sattar, 2017). Indeed, in the UK, people from south Asian backgrounds are more likely to suffer from CVD and have an elevated risk of T2D compared with white European individuals or other ethnic groups (BHF, 2019; Johns and Sattar, 2017; SAHF, 2014). According to the South Asian Health Foundation (SAHF), 11.2% of the total South Asian population in the UK (~ 431, 000 people) have diabetes, with T2D including 90% of all diabetes cases (~ 388, 000 people) (SAHF, 2014). The higher prevalence of T2D amongst the South Asian population living in the UK was formally described for the first time in 1985 by Mather and Keen in the Southall Diabetes Survey showing a 5-fold higher prevalence of diabetes in South Asian than white European individuals living in London (Mather and Keen, 1985). Nonetheless, more recent estimates revealed a decrease in the prevalence of diabetes in South Asians although the risk remains approximately 2 to 4-fold greater compared with the white population (Ntuk et al. 2014). Previous studies provide evidence of a trend towards greater insulin resistance in South Asians at a younger age than white Europeans (Sattar and Gill, 2015). A report from a prospective pregnancy cohort, showed 10% greater umbilical cord insulin levels on South Asian neonates born in the UK, despite showing a lower birthweight, indicating elevated insulin resistance at birth (Lawlor et al. 2014). Similar results were also shown in an earlier report comparing South Asian babies born in India with white European babies born in the UK (Yajnik et al. 2002). The greater risk of insulin resistance in South Asians compared to white European individuals continues throughout childhood and adulthood with glucose intolerance , T2D and CVD typically diagnosed 5 to 10 years earlier and at a lower BMI in South Asian than white European individuals (Sattar and Gill, 2015; Khunti et al. 2013; Gholap

et al. 2011). South Asians exhibit higher incidence of T2D also into later life than white Europeans with 30-40% of UK South Asians diagnosed with T2D by the age of 70 years old, at least twice the prevalence of their white European counterpart (Sattar and Gill, 2015). South Asians have also shown higher prevalence of impaired glucose tolerance (IGT) and elevated fasting glucose concentrations compared with whites (Tziomalos et al. 2008). Furthermore, it has been reported that the transition from impaired glucose tolerance (IGF) to overt T2D is typically quicker in South Asian individuals which may explain, at least partially, the earlier onset on T2D in this population (SAHF, 2014).

The higher prevalence of insulin resistance and T2D in the South Asian population appears to be the most relevant contributor for their elevated CVD risk (Tziomalos et al. 2008). Cardiovascular complications such as myocardial infarction or stroke remain appreciably higher in the UK South Asian men and women than the white population, although the risk differences in diabetes-related cardiovascular complications between South Asians and white Europeans have decreased compared to a few decades ago (Johns and Sattar, 2017). The SABRE cohort study included 20-year follow-up data from patients recruited from the Southall study between 1988 and 1991 and reported greater risk of myocardial infarction and stroke in South Asian compared to white European participants (Tillin et al. 2013a). Likewise, over 2 million patients with diabetes in England and Wales were included in the UK National Diabetes Audit 2010-2011 that reported elevated myocardial infarction, angina, heart failure and stroke in South Asian than white European individuals (Health and Social care Information Centre, 2012). Furthermore, previous studies have reported a narrower gender difference in CHD risk between South Asian women and men living in the UK compared to the overall UK population (Hall et al. 2008). Specifically, UK South Asian women experience 46%

higher CHD mortality than women in the general UK population whereas the CHD mortality in South Asian men is 36% greater men compared with men of other ethnic groups (Hall et al. 2008). As with cardiovascular complications, previous population-based studies investigated ethnic differences in cardiovascular mortality, which consistently reported an elevated risk of mortality in South Asian than white European individuals (Mather et al. 1998). However, more recent studies have indicated a lower mortality risk in the South Asian diabetic population than their European counterparts probably due to an earlier detection and treatment of diabetes and CVD risk factors such as obesity, hypertension and dyslipidaemia (Johns and Sattar, 2017). Therefore, these data suggest that cardiovascular morbidity continues to be elevated compared to the white European counterpart whereas there has been a shift in the cardiovascular mortality pattern, which has been reversed positively in South Asians.

In the context of describing the greater risk of CVD and T2D amongst South Asian individuals, there is not clear evidence suggesting genetic differences between south Asians and white Europeans (Bakker 2013; Sattar and Gill, 2015). Please refer to subsection 1.3.1.3.1, where an overview of the actual data has been reported. However, it is relevant to mention that migration to urban areas from rural areas in the Indian subcontinent or from south Asian to western countries, has been reported to induce changes in dietary intake and lifestyle which have been associated with greater CVD and T2D risk. For instance, assessment of dietary intake in South Asian migrated to western countries has shown an elevated consumption of products rich in energy, fat and refined carbohydrate in the host than in the country of origin (Shah and Kanaya, 2014). Culture also seems to have an impact on the low levels of physical activity amongst South Asian people. In this regard, Caperchione et al. (2009) reported that exercise is a foreign

concept to the cultural identity of South Asians, and it is seen as marginal in regard to the disease process. Additionally, cultural restrictions against South Asian women, language barriers, fear of racism or the view of exercise beyond daily work as selfish have all been cited as barriers to physical activity among South Asians (Caperchione et al. 2009).

Finally, an additional consideration in the context of comparing the risk of CVD and T2D in UK South Asians versus white Europeans, is that the term "South Asian" refers to a heterogeneous group with significant differences in diet, culture, religion and lifestyle within the South Asian population. Thus, although South Asians generally have a greater risk of CHD and T2D than other groups in the UK, there is also a variation in risk for these conditions within the South Asian populations. Specifically, the prevalence of CVD and T2D risk has been reported to be higher in Bangladeshi men, followed by Pakistani and Indian men living in the UK compared with the other UK South Asian populations (BHF, 2019; Diabetes UK, 2012). However, for blood pressure, Indians have grater prevalence of hypertension followed by Pakistanis and Bangladeshis (Agyemang et al. 2002). Furthermore, Pakistani and Bangladeshi men born in the Indian subcontinent but living in the UK now are more than two times more likely to die from CHD than the national average whereas Pakistani women are two and half times more susceptible to CHD than the national average (BHF 2019).

1.3 Risk factors for cardiovascular disease and Type 2 diabetes in South Asians

The term risk factors refer to those conditions that increase the chances of developing a specific disease and are typically classified into modifiable and non-modifiable risk factors. Concerning CVD and T2D, examples of conventional modifiable risk factors are smoking, physical inactivity, dyslipidaemia, obesity or hypertension whereas age, sex

and ethnicity are examples of non-modifiable risk factors. Conventional risk factors for CVD and T2D have been reported to not fully explain the heightened cardiometabolic risk in South Asian compared with white European individuals (Forouhi et al. 2006; Enas et al. 2007). For instance, data from South Asian and white European patients recruited from the Southall and Brent population-based study, both conducted between 1988 and 1991, reported higher CHD mortality in South Asian than white European men after adjusting for conventional risk factors including smoking, age, total cholesterol, blood pressure, hyperglycaemia or insulin resistance, measured by homeostatic model assessment (HOMA) (Forouhi et al. 2006). Additionally, it is well-established that South Asians have greater levels of total and abdominal adiposity, for a given BMI, than white Europeans and this has been linked to their excess insulin resistance and T2D (Sattar and Gill, 2015; Gholap et al. 2011). Nonetheless, South Asian individuals have shown to be more insulin resistant and greatly exposed to T2D even after adjustment for total and visceral fat (Chandalia et al. 1999; Sattar and Gill, 2015; Hall et al. 2010) suggesting that additional factors may contribute the elevated cardiometabolic risk in this population. Thus, continuous effort amongst the scientific community has been observed to examine unconventional markers, which may add further information of the elevated CVD and T2D susceptibility amongst the South Asian community. In this regard, given chronic surplus of energy intake as a possible contributor for body fat accumulation, it is plausible that ethnic differences in the short-term regulation of appetite and energy intake may underlie the elevated adiposity and associated cardiometabolic risk in the South Asian compared with the white population. Particularly, it is possible that the innate phenotype of increased adiposity in South Asians is linked with ethnic differences in appetite-related hormones such as acylated ghrelin and peptide YY (PYY) concentrations which play a key role in the acute regulation of appetite perceptions and food intake (Hussain and

Bloom, 2013). Furthermore, circulating concentrations of fasting ghrelin have been observed to be lower in individuals with elevated adiposity and exhibiting insulin resistance (Le Roux et al. 2005; McLaughlin et al. 2004). However, it is not known whether circulating acylated ghrelin and PYY concentrations are different between South Asian and white European individuals. Additional parameters including plasma leptin, C-reactive protein (CRP), interleukin-6 (IL-6) and free fatty acids (FFAs) have also been implicated as mechanisms, which may contribute to increase and accelerate the risk of T2D and CVD in South Asians compared with white Europeans (Bakker et al. 2013). It is well established that exercise can improve insulin sensitivity and cardiometabolic risk profile (ADA, 2013) and the vast majority of published literature advocates moderate-to-vigorous exercise in promoting short-term energy deficit without inducing compensatory effects on appetite feelings, which may relate to the modulation of appetite gut-hormones including acylated ghrelin and PYY (Deighton and Stensel, 2014). Thus, physical activity may represent an effective strategy to enhance fat loss in South Asians and ameliorate their health outcomes if exercise is performed frequently. However, it remains unknown how differences in individual ethnicity background modulate appetite perceptions, energy intake and appetite-related hormones in response to exercise. Thus, the following sections will begin by defining the most relevant conventional risk factors related to the greater risk for CVD and T2D in South Asian than white European individuals, with a particular focus on those factors likely responsible for the greater insulin resistance and T2D risk in South Asians. After this, unconventional risk factors will be explored with a particular focus on studies that have been performed in South Asian individuals. Finally, the last section will examine appetite-related hormones involved in the regulation of appetite and energy intake such as leptin, acylated ghrelin and total PYY and review the evidence relating to the modulation of appetite perceptions,

energy intake and appetite-related hormones in response to exercise in the general population.

1.3.1 Conventional risk factors

According to the INTERHEART report, a large epidemiological case-control study of risk factors for CVD conducted in 52 Countries (Asia, Europe, the Middle East, Africa, Australia, North America and South America), more than 80% of the person's attributable risk for CHD can be explained by 9 modifiable risk factors, irrespective of ethnic background (Yusuf et al. 2004). They comprise low consumption of fruit and vegetables, smoking, alcohol consumption, psychological factors, sedentary lifestyle, hypertension, dyslipidaemia, abdominal adiposity and diabetes. Age, sex, hereditary and ethnicity are the non-modifiable CHD risk factors. Compared with white Europeans, South Asians have a higher prevalence of conventional risk factors for CHD, which largely explained their earlier onset of CHD (Tziomalos et al. 2008). Thus, we will examine some of the most relevant ones which have been examined in this thesis.

1.3.1.1 Dyslipidaemia

Dyslipidaemia is a well-established CVD risk factor and refers to abnormal levels of blood lipids including high concentrations of triacylglycerol (TAG), low-density lipoprotein cholesterol (LDL-C) and low levels of high-density lipoprotein cholesterol (HDL-C) (AHA, 2016). South Asians appear to have an elevated atherogenic lipid profile compared with white European individuals. Particularly, they exhibit greater circulating TAG and lower HDL-C predisposing them to atherogenesis which may also reflect underlying insulin resistance (Chowdhury and Lasker, 2002; Anand et al, 2000; McKeigue et al, 1991; Chambers et al, 2001). Additionally, according to the Framingham

Offspring Study, concentrations of large HDL particles (more cardio protective) were lower in South Asians than white Europeans (Bhalodkar et al. 2004). In contrast, concentrations of LDL-C in South Asians are comparable with other populations, although LDL particles size has been reported to be smaller in South Asians (Bhalodkar et al. 2004). This is critical, as small LDL particles are more susceptible to oxidation, hence more atherogenic (Kulkarni et al, 1999).

1.3.1.2 Hypertension

Hypertension or elevated blood pressure may be related to different factors such as age, family history, obesity, physical inactivity, high sodium intake or ethnicity (AHA, 2016) and substantially contributes to high death rates from CHD, cerebrovascular disease and renal failure (Joshi et al, 2006). Hypertension is highly prevalent amongst the South Asian population (McKeigue et al, 1991; Agyemang and Bhopal, 2002). Specifically, the prevalence of hypertension in individuals aged between 18-75, is higher in South Asian than in European men whereas inconclusive results were found in relation to the prevalence rates of hypertension between South Asian and European women (Agyemang and Bhopal, 2002). Furthermore, mean blood pressure and the prevalence of hypertension also differed within the South Asian population (Bhopal et al, 1999). According to the Newcastle Heart Project study and the 1999 Health Survey of England, Bangladeshi males and females aged 25-74, exhibit lower mean systolic and diastolic blood pressure than their Pakistani and Indian counterparts (Bhopal et al, 1999). The same study also reported lower prevalence of hypertension in Bangladeshi men and women compared with Pakistani and Indian males and females.

1.3.1.3 Insulin resistance and Type 2 diabetes: current hypothesis for the elevated risk in South Asians

Insulin is an anabolic peptide hormone produced by the β-cells of the pancreatic islets, which promotes glucose uptake and utilisation by muscle cells and other tissues. Insulin action is the consequence of the binding to its plasma membrane receptor (tyrosine kinase), which in turn activates a complex intracellular signalling network (e.g. insulin receptor substrate 1 or IRS-1) generating its biological response (Taylor et al. 2012). The inability of the target tissues to respond to a known quantity of exogenous or endogenous insulin, results in a condition known as insulin resistance which inhibits glucose transportation from the bloodstream into most tissues leading to hyperglycaemia and, ultimately, to T2D (Taylor et al. 2012). Insulin resistance is another well-established risk factor for CHD and ischaemic stroke with evidence showing greater risk of CVD in patients with insulin resistance and T2D compared with normoglycemic individuals (WHO, 2016).

The elevated prevalence of insulin resistance and T2D amongst the South Asian population seems to be a key contributor to the observed elevated CVD risk in this population (Tziomalos et al. 2008). The reason explaining the increased prevalence of insulin resistance and T2D in individuals with South Asian background remain unclear, although it probably results from the combination of innate and environmental factors. Current hypothesis for the mechanisms responsible for increased insulin resistance and diabetes in South Asian individuals are summarised in the following sections.

1.3.1.3.1 Genetic predisposition to diabetes

Type 2 diabetes is considered as a polygenic condition that involves polymorphisms of different genes with a high gene–environment interaction (Radha and Mohan, 2007).

Many loci associated with T2D in white Europeans have been verified in studies with South Asian individuals but only few differences between the ethnic groups have been identified and the differences were not all consistently reported (Bakker et al. 2013). For example, an attractive difference may lie in the fat-mass and obesity-associated (FTO) gene, which continues to be the strongest known obesity-susceptibility locus in white Europeans. Relationships between FTO and T2D have also been identified, although this appears to be secondary to obesity (Bakker et al. 2013). In South Asians, however, the FTO polymorphism was previously reported to be associated with T2D, independently of BMI (Sanghera et al. 2008; Li et al. 2012), suggesting that in South Asians there may be a distinctive association between BMI and T2D. Nonetheless, associations between FTO and T2D mediated by obesity have been also shown in South Asian individuals (Ramya et al. 2011), whereas data from another study conducted in India showed no associations between the FTO variants tested and T2D (Chauhan et al. 2011). Therefore, to date no clear genetic differences between south Asian and white European individuals have been found.

1.3.1.3.2 Fetal programming

The fetal programming theory is based on previous studies reporting strong associations between low birth size and risk to develop impaired glucose tolerance, T2D and CVD in adult life, known as thrifty phenotype or Barker's hypothesis (Hales and Barker, 1992). According to the thrifty phenotype hypothesis, there is a mismatch between intrauterine and adult life environments. Particularly, an intrauterine disadvantageous environment such as maternal malnutrition, induces thrifty mechanisms that sets the metabolism to cope with potential future food deficiencies such as reduced capacity for inulin synthesis and secretion (Hales and Barker, 1992). While this may be beneficial to cope with

potential future food shortages, it increases the risk of T2D later in life in a nutrient rich environment typical of the modern society (Hales and Barker, 1992). This theory is based on the association between low birth weight and increased risk of T2D later in life observed in different ethnic populations (Bakker et al. 2013). In this regard, a meta-analysis has shown low birthweight (a marker of fetal undernutrition) to be associated with greater risk of T2D with each kg increase associated with roughly a 25% decrease in diabetes risk (Sattar andGill, 2015). Although South Asians have lower birthweights than European populations, an analysis from the Child Heart and Health Study (CHASE) conducted in the United Kingdom in people with different ethnicities did not support low birthweight per se as an explanation for the emerging ethnic difference in risk markers for diabetes (Nightingale et al. 2015). Specifically, in the CHASE study, which examined associations between birthweight and risk markers for T2D and CVD in UK-resident white European, South Asian and black African-Caribbean children, adjustment for birthweight had no effect on ethnic differences in diabetes and CVD markers (Nightingale et al. 2015). However, South Asian children demonstrate a greater body fat percent at birth compared with white European children (based on skin-folds or cord leptin levels, or both) often accompanied by elevated cord insulin levels commensurate with greater insulin resistance (Lawlor et al. 2014). In this study, when adjustment was made for maternal fasting glucose concentrations, which were greater in the South Asian than white European women, the difference between ethnicities in cord leptin halved and became non-significant (Lawlor et al. 2014). Therefore, because evidence supporting the fetal programming role on diabetes in South Asians remain inconclusive, future studies investigating the effects of lifestyle intervention in South Asian compared with white European pregnant women would seem worthwhile, with key endpoints including

incidence rate of gestational diabetes, birthweight, and importantly, neonatal body composition.

1.3.1.3.3 Pancreatic ß-cell capacity

Previous evidence has identified ethnic differences in pancreatic ß-cell capacity with South Asians experiencing earlier declines in ß-cell function compared with other ethnic groups, which may contribute to the greater T2D susceptibility in South Asians (Bakker et al. 2013). Petersen and colleagues (2006) investigated both insulin resistance and β-cell function in young adults East Asian, South Asian, Black, and white Caucasian individuals and reported a threefold to fourfold greater prevalence of insulin resistance in South Asian men than men of other ethnic groups, despite being matched on lifestyle factors and BMI (Petersen et al. 2006). In the same study, the assessment of β-cell function in a subgroup of South Asian and white Caucasian men revealed a 30% increase in basal β-cell responsiveness in the South Asians group. Nonetheless, this increase in β-cell function was not enough to compensate for the degree of insulin resistance, as shown by a 60% decrease in the insulin sensitivity index (ISI) (a measure of β-cell response to insulin resistance), in South Asian men (Petersen et al. 2006). Data from the Whitehall study in the UK, examining 230 South Asian and 5749 white European individuals (39-79 years old), also suggested greater β-cell function in South Asians at age of 50 years than white Europeans (Ikehara et al. 2015). Similar findings were also observed in the UK Southall and Brent revisited (SABRE) study (Tillin et al. 2013b). However, although in the Whitehall study β-cell function increased with age in white Europeans to compensate for increasing insulin resistance, this pattern was not observed in South Asians who exhibited a decline in β-cell capacity by the age of 60 years onwards (Ikehara et al. 2015). These findings were corroborated by a cross-sectional study conducted in

the USA showing lower β-cell function in South Asians (mean age 57 years old) than white Europeans (mean age 63 years old) (Kanaya et al. 2014). Thus, taken together these data suggest greater β-cell insulin production in South Asians at a younger age to compensate their peripheral insulin resistance. This may lead with age to a subsequent early β-cell exhaustion, which seems to accompany the transition to dysglycaemia and eventually to diabetes (Sattar and Gill, 2015). However, it remains unknown whether the decline in β-cell function in South Asians results primarily from the adiposity-induced insulin resistance or reflects lower inherent β-cell capacity (Sattar and Gill, 2015).

1.3.1.3.4 Dietary changes

Urbanization across Asia and migration of South Asians to western countries resulted in changes in dietary intake and physical activity levels, which have been associated with the rising rates of CVD and T2D diabetes in South Asian individuals (Shah and Kanaya, 2014). A previous study conducted in southern India reported lower prevalence of diabetes in villages (9.2%) compared with 18.6% in the main cities, although villages are experiencing an increase in diabetes rates due to the rapid urbanization (Ramachandran et al. 2008). Furthermore, previous evidence showed that people living in urban areas in India have greater prevalence of diabetes compared to people living in rural areas (7.9% vs 2.5%), which is comparable to those South Asians who have migrated to the UK (Cheema et al. 2014).

A nutrition transition, which is mainly characterised by increased intakes of energy dense and ultra-processed foods rich in fats and refined carbohydrates, has initially started in high-income countries to then spread to low-income countries in the last decades, including the Indian subcontinent, as a result of the rapid urbanization in both urban and rural areas (Popkin, 2009). Assessment of dietary intake in South Asian migrants to

western countries has also shown elevated consumption of products rich in energy, fat and refined carbohydrate in the host than in the country of origin (Shah and Kanaya, 2014). Particularly, the most remarkable changes after migration appear to be a substantial increase in energy and fat intake and a switch from whole grains to more refined sources of carbohydrates, resulting in a low intake of fibre, with these data suggesting that these dietary changes may contribute to their greater cardiometabolic risk (Holmboe-Ottesen and Wandel, 2012). A previous study investigating the impact of migration on CHD risk factors in British Gujaratis versus their counterparts living in Gujarat in India, also reported greater energy intake in the UK group, both in men and women (Patel et al. 2006). Furthermore, the high fat intake in South Asian migrants in the UK has also been reported in other studies with fat intakes providing 35 to 40% of the total energy intake (Landman and Cruickshank, 2001). Additionally, Bakker and colleagues (2014) demonstrated greater perturbations in insulin resistance in response to short-term high fat overfeeding than European men, which may contribute to exacerbating the excess insulin resistance and T2D risk in South Asians (Bakker et al. 2014). Nonetheless, a few older investigations have shown reduced energy intake in UK South Asians than their European counterparts (Vyas et al. 2003; Smith et al. 1993) which confirms that the dietary intake patterns of South Asians living in high-income countries remain sparse (Holmboe-Ottesen and Wandel, 2012). A further consideration in the context of dietary intake assessment in South Asians, is that the aforementioned studies based their energy intake investigations on self-reported questionnaires, which represents a limitation due to issues of participant recall bias which makes it difficult to accurately correspond self-reported intake with actual intake (Dhurandhar et al. 2015).

1.3.1.3.5 Physical activity and fitness

Physical activity refers to any bodily movement produced by skeletal muscles that results in energy expenditure. Numerous studies have examined the associations between levels of physical activity and risk of T2D, with all of them reporting consistently that regular physical activity markedly reduces the risk of this condition (Gill and Cooper, 2008), independent of BMI. In this regard, controlling for differences in BMI between active and inactive groups has shown to attenuate the magnitude of risk reduction, although greater levels of physical activity were still associated with a reduction in T2D risk of 20–30%, even after adjustment for BMI (Jeon et al. 2007). Furthermore, data from interventional studies reported the potential for increasing physical activity to reduce the risk of diabetes even in individuals exhibiting no significant weight loss (Pan et al. 1997).

Several studies conducted in UK South Asians reveal lower levels of physical activity than their white European counterparts, suggesting that South Asian individuals living in high-income countries engage in low habitual physical activity, which is likely to contribute to the excess T2D and CHD risk in this population (Fischbacher et al. 2004; Williams et al. 2011a; Williams et al. 2011b; Yates et al. 2010; Ghouri et al. 2013; Afaq et al. 2019). Lower levels of physical activity were also observed in UK South Asian children compared to their white European counterparts (Duncan et al. 2012). The reasons explaining the lower physical activity level among South Asian communities remain unclear, however it can be postulated that there may be barriers to engage in physical activities amongst South Asians. Particularly, physical activity is a foreign concept to the cultural identity of South Asians, and it is seen as marginal in regard to the disease process (Caperchione et al. 2009). Furthermore, cultural restrictions against South Asian women, language barriers, fear of racism or the view of exercise beyond

daily work as selfish have all been cited as barriers to physical activity among South Asians (Caperchione et al. 2009).

Similarly to dietary intake, it is important to consider that the existing evidence on habitual physical activity levels in South Asians has largely been gleaned from self-report questionnaires, although data using objective measures is emerging (Afaq et al. 2019; Ghouri et al. 2013; Iliodromiti et al. 2016; Duncan et al. 2012). Yates and colleagues (2015) in their cross-sectional study investigated differences in levels of physical activity between South Asians and white Europeans in the UK, using both objective accelerometer and self-report questionnaires, and reported similar levels of physical activity between groups when measured objectively although self-reported estimates were 40% lower in the South Asian population (Yates et al. 2015). This highlights the limitations of using self-reported lifestyle measures and indicates the need of future studies using objectively-measured physical activity in South Asians.

Although recent evidence reported a variation in physical activity levels in UK South Asians with second-generation South Asians engaging in greater physical activity than the first-generations, South Asians remain still less active than their white counterparts (Bhatnagar et al. 2015). According to the physical activity guidelines from the WHO, 30 min of moderate physical activity per day, for a total of cumulative 150 min per week, shows a protective effect on cardiometabolic health in adults (WHO, 2016). However, recent findings suggest that South Asians may need to undertake approximately 230 minutes of moderate intensity physical activity per week, an addition of 10 to15 minutes per day, to achieve a comparable CHD risk factor profile of white Europeans who are meeting the current WHO physical activity recommendations (Sattar and Gill, 2015). Therefore, given the heightened cardiometabolic risk and lower levels of physical activity

in South Asian individuals, educational programmes should aim to develop culturally suitable strategies to enhance physical activity levels in this ethnic group to optimise health outcomes.

Although lower levels of physical activity are likely to contribute to the elevated insulin resistance and T2D risk, South Asians have been reported to be more insulin resistant than white Europeans even after adjustment for differences in physical activity level (Ghouri et al. 2013). A contributing factor that may relate to ethnic differences in cardiometabolic risk is the association between physical activity and cardiorespiratory fitness. Cardiorespiratory or cardiovascular fitness, assessed by $\dot{V}O_2$ max, refers to the capability of the cardiovascular and respiratory systems to supply oxygen to working muscles during sustained physical activity. Increasing evidence suggests that South Asian have lower cardiovascular fitness levels compared white European people (Hall et al. 2010; Ghouri et al. 2010; Arjunan et al. 2013; Arjunan et al. 2015). Although levels of physical activity have shown to be strongly associated with cardiorespiratory fitness, there is further evidence that the lower $\dot{V}O_2$ max in South Asians is independent of physical activity levels (Hall et al. 2010; Ghouri et al. 2013). Additionally, previous studies suggest that cardiorespiratory fitness is an important T2D risk factor (Ghouri et al. 2013; Hall et al. 2010). In this regard, Ghouri and colleagues (2013) demonstrated that low cardiorespiratory fitness was the single strongest predictor of the excess insulin resistance and fasting glycaemia in middle-aged South Asian compared with white European men living in the UK (Ghouri et al. 2013). Particularly, this study revealed that the lower $\dot{V}O_2$ max explained 68% of the ethnic difference in HOMA-IR. However, similar studies are warranted in women. There is also evidence indicating that fat oxidation during exercise may be a key feature of the insulin resistance phenotype in

South Asians (Hall et al. 2010). Indeed, Hall and colleagues (2010) reported lower fat oxidation during submaximal exercise, but not at rest, in South Asian than white European men and demonstrated positive associations between lower fat oxidation and insulin sensitivity index (Hall et al. 2010). The same study also reported lower insulin sensitivity index in South Asians than white European participants, which was abolished after adjusting for fat oxidation during exercise. This difference in fat oxidation during submaximal exercise observed by Hall and colleagues, however, was not related to the reduced skeletal muscle expression of oxidative and lipid metabolism genes, which is in agreement with Nair and colleagues (Nair et al. 2008), demonstrating that mitochondrial dysfunction cannot account for the observed insulin resistance in South Asians. Although the lower fat oxidation seems promising and may contribute to the increased insulin resistance in South Asians, so far only two small studies (Hall et al. 2010; Nair et al. 2008) have examined skeletal muscle oxidation in South Asians. Furthermore, the mechanisms underlying the lower fat oxidative capacity in South Asians and how this relates to their lower insulin sensitivity remain unknown. Thus, future studies are needed before any conclusion can be drawn.

1.3.1.3.6 Lean and adipose tissue mass

Skeletal muscle is quantitatively the most relevant site of insulin-mediated glucose uptake, most of which is directed towards glycogen synthesis when is not oxidised. Previous studies have shown have lower lean tissue in South Asians compared with white Europeans, and this may represent an important factor contributing to the elevated insulin resistance in this population (Sattar and Gill, 2015). Lear and co-workers (2009), in their study demonstrated lower lean mass, greater fat mass and fat-to-lean mass ratio in both South Asian male and female, compared with other ethnic groups, including white Europeans (Lear et al. 2009). In the same study, higher insulin concentrations and

HOMA-IR were observed in South Asians compared with other ethnic groups, even after adjusting for body fat, whereas the same ethnic difference disappeared after controlling for fat-to-lean mass ratio, suggesting a contribution of lower lean mass to the excess insulin resistance in this population (Lear et al. 2009). Similar findings were observed by previous studies suggesting that South Asians typically have higher body fat and lower skeletal muscle mass at the same or lower BMI in comparison with white Europeans, which is known as 'high body fat-normal BMI-low muscle mass phenotype' (Misra et al. 2019).

It is well-established that greater levels of total and abdominal adiposity contribute to the pathogenesis of insulin resistance increasing the risk to develop T2D and CHD (Diabetes UK, 2019; AHA, 2019). Many studies reported greater percent body fat and accumulation of visceral adipose tissue, for a given BMI, in South Asian than white European individuals, which contributes to their elevated cardiometabolic risk (Sattar and Gill, 2015; Bakker et al. 2013; Misra et al. 2014). Such differences in adiposity have been also observed in childhood and adolescence (Gujral et al. 2013). Raj and colleagues (2001) reported greater amounts of total adiposity, visceral fat (measured by abdominal computed tomography scan), fasting insulin and lower insulin sensitivity (using the insulin clamp technique) in healthy South Asian than white Europeans, matched for age and BMI (Raj et al. 2001). In the same study, insulin-mediated glucose disposal was negatively associated with both total and visceral fat (VAT) suggesting that total and regional adiposity may account for the greater insulin resistance and hyperinsulinemia in this population (Raj et al. 2001). These findings corroborated previous data from Banerji and colleagues (1999), showing negative correlations between insulin-mediated glucose disposal and visceral adipose tissue in a cohort of healthy Asian Indians (Banerji et al.

1999). Therefore, considering the greater adiposity in South Asians than BMI-matched white Europeans and their greater risk of developing obesity related comorbidities (e.g. glucose intolerance and T2D) at lower levels of BMI and waist circumference (Tziomalos et al. 2008), the proposed cut-offs values for defining overweight and obesity appear to be unsuitable in South Asians. In this regard, the WHO proposed BMI cut off points of 23 to 24.9 $kg \cdot m^2$ for overweight and ≥25 $kg \cdot m^2$ for obesity, endorsed as 'public health action point' for South Asian individuals (Tziomalos et al. 2008). These are lower than the general guidelines for overweight, (25 to 29.9$\cdot kg\ m^2$) and obesity (≥30 $kg\ m^2$) (Misra et al. 2014; Tziomalos et al. 2008). Likewise, cut off points of ≥ 90 cm and ≥80 cm for waist circumference in South Asian men and women, respectively, have been proposed instead of the actual cut-off points of ≥102 cm in men and and ≥88 cm in women (Misra et al. 2014; Tziomalos et al. 2008).

However, although South Asians have greater adiposity and central fat distribution, additional studies shown that South Asians remain more insulin resistant and have higher insulin concentrations (both fasting and after glucose load) than white Europeans after controlling for BMI, total and abdominal adiposity (Sattar and Gill, 2015). For example, the cross-sectional study from Chandalia and colleagues (1999), reported lower glucose disposal rate in South Asian than white European men, even after adjusting for total body fat and truncal skinfold thickness (Chandalia et al. 1999). These findings were also in agreement with other studies, suggesting that South Asians are more insulin resistant independently of generalised or truncal adiposity (Forouhi et al. 1999, Davey et al. 2000). However, according to previous studies, these crude adjustments without discriminating between superficial subcutaneous adipose tissue (SSAT) and deep subcutaneous adipose tissue (DSAT), may not explain the whole story (Bakker et al. 2013). Sniderman and colleagues (2007) suggest that the adipose tissue is divided into primary and secondary

compartments, with SSAT being the primary adipose tissue compartment and DSAT and VAT representing the secondary compartment, which has been linked with insulin resistance and associated adverse metabolic effects (Sniderman et al. 2007). According to the overflow hypothesis, South Asians have smaller SSAT compartment than white Europeans, resulting to the overflow of fat in the secondary compartments, which leads to an earlier and greater accumulation of fat in the DSAT and VAT, elevating the risk of T2D and CVD in South Asians (Sniderman et al. 2007). In support of this hypothesis, previous cross-sectional studies reported lower or similar SSAT and higher DSAT and VAT in South Asian than white European individuals (Anand et al. 2011; Kohli et al. 2010; Chandalia et al. 2007; Lear et al. 2012). Besides the different adipose tissue compartments theorized by Sniderman et al. (2007), it is possible that ethnic differences in brown adipose tissue (BAT) volume or activity may also underlie the disadvantageous metabolic phenotype and susceptibility to T2D in south Asian individuals. Brown adipose tissue has been shown to have a role in energy homoeostasis in humans (Bakker et al. 2014). Particularly, in contrast to white adipose tissue, BAT burns triglycerides and glucose to generate heat through a process called mitochondrial uncoupling (Bakker et al. 2014). However, a limited number of studies have investigated potential differences in brown adipose tissue between south Asians and white Europeans with findings remaining sparse (Admiral et al. 2013; Bakker et al. 2014). Not only the total and distribution of body fat varies between South Asian and white Europeans, previous studies report ethnic differences in adipocyte size and functions (Bakker et al. 2013) with large adipocytes being shown to predict insulin resistance and type 2 diabetes independent of obesity (Weyer et al. 2000). Chandalia and co-workers (2007) reported greater abdominal subcutaneous adipocyte size in South Asian compared with white European men, with this difference remaining significant even after adjusting for total

body fat (Chandalia et al. 2007). In the same study, adipocyte size was also negatively correlated with glucose disposal rate (Chandalia et al. 2007). Likewise, Anand and co-workers (2011) revealed increased adipocyte diameter in healthy South Asian than white Caucasian men, and adjustment for adipocyte area reduced the ethnic differences in insulin levels (Anand et al. 2011). Furthermore, previous evidence shown that South Asians exhibit a higher ratio of small-to-larger adipocytes than individuals of European descent (Balakrishnan et al. 2012). Taken together, these data have been interpreted as an incapability for small adipocytes to differentiate into mature cells, which leads to an increased size of the small pool of large adipose cells, reduced storage capacity of TAG in the adipose tissue and early storage in ectopic depots such as skeletal muscle and liver (Sattar and Gill, 2015; Bakker et al, 2013). Defect in adipose tissue cells maturation and hypertrophic adipocytes are considered dysfunctional which has been associated with insulin resistance and appears to be an independent predictor of T2D (Bakker at al. 2013). In this regard, ectopic fat can accumulate in the liver as a result of excess circulating free fatty acids via the portal vein and systemic circulation, which can lead to non-alcoholic fatty liver disease (NAFLD) and hepatic insulin resistance (Hall et al. 2008). Although the mechanisms underlying this are not fully clarified, it has been hypothesised that the elevated fatty acid delivery to hepatocytes results in hepatic accumulation of fatty acid intermediates including ceramides and diacylglycerol. This interferes with insulin signalling pathway and inhibits insulin action leading to a reduction of the hepatic glycogen synthase and stimulation of gluconeogenesis (Samuel et al. 2004). Limited evidence suggest that hepatic lipid accumulation can contribute to insulin resistance in South Asians. Particularly, a previous study shown that South Asian men display a three- to four-fold increase in the prevalence of insulin resistance than White, East Asian, Black and Hispanic groups and this was associated with a two-fold increase in hepatic

triglyceride content in South Asians than the other ethnic groups (Petersen et al. 2006). Therefore, given the possible causal relationship between hepatic lipid accumulation and hepatic insulin resistance in South Asians, this represents an area where further research is needed.

Dysfunctional adipocytes have been also related to greater levels of leptin and FFAs in South Asians (Bakker et al. 2013). In support of this, previous studies observed greater concentrations of plasma leptin and free fatty acids in healthy nondiabetic South Asian men compared with their white European counterparts of similar age and total and abdominal adiposity, suggesting abnormalities of the adipose tissue metabolism (Abate et al. 2004; Chandalia et al. 2007). Furthermore, elevated concentrations of the proinflammatory cytokines IL-6 and CRP, associated with greater CHD risk, have been observed to be higher in South Asians than Caucasians with a similar degree of adiposity, which may suggest associations with abnormalities in the adipose tissue metabolism (Bakker et al. 2013; Tziomalos et al. 2008).

1.3.2 Unconventional risk markers

Along with conventional risk markers, emerging or unconventional risk markers may represent significant predictors for CHD and T2D risk in South Asians. These are numerous, but we will describe CRP, IL-6, and FFAs which were examined in the present thesis.

1.3.2.1 Inflammatory cytokines

Inflammation seems to play a key role in all stages of the atherosclerosis process, from emergence of the lesion to occurrence of a coronary event (Verma et al. 2006). Particularly, elevated levels of inflammatory cytokines including CRP, a circulating acute phase protein of hepatic origin and IL-6, a pro-inflammatory cytokine produced in

different tissues, such as adipocytes and endothelial cells, can cause down regulation of NO production, by inhibiting endothelial nitric oxide synthase (eNOS). This causes a reduction in vasodilation increasing the risk of endothelial dysfunction (Hein et al. 2009). Furthermore, elevated plasma CRP concentrations, which levels increase in response to primary stimulation from IL-6, reduces the concentration of tissue plasminogen activator (tPA), responsible for lysing clots at the vessel wall, and increases plasminogen activator inhibitor-1 (PAI-1) levels, which inhibits the fibrinolysis process. This facilitates synthesis of thrombi on the endothelial wall, which also increases the risk of cardiovascular events (Devaraj et al. 2003).

The majority of previous studies report elevated plasma CRP concentrations in South Asian men and women compared with their white European counterparts (Gujral et al. 2013; Tziomolas et al. 2008; Anand et al. 2004; Arjunan et al. 2015; Bastard et al. 1999), although this finding is not universal (Chatha et al. 2002). Furthermore, previous studies have reported elevated CHD risk in South Asian individuals associated with greater concentrations of CRP compared with white Europeans (Gujral et al. 2013; Tziomolas et al. 2008). In this regard, Chambers and colleagues estimated that higher CRP concentrations and related inflammation are associated with a 14% increase in CHD risk in Indian Asians compared with white Europeans (Chambers et al. 2001). Previous evidence also suggests that low-grade chronic inflammation in South Asians likely reflect their greater adiposity and visceral adipose tissue (Chambers et al. 2001; Forouhi et al. 2006). However, it has also been demonstrated previously that South Asian individuals exhibit greater CRP concentrations than Caucasians despite similar levels of body fat (Chandalia et al. 2003). Therefore, it is possible that ethnic differences in CRP concentrations may reflects abnormalities in the adipose tissue functions described in Chapter 1.3.1.3.6. However, additional work is required to determine independent

contribution of adiposity on CRP and associations with adipose cells dysfunction in South Asians before any conclusion can be drawn.

Concentrations of IL-6 have been shown to be higher in individuals with IGT or type 2 diabetes (Pradhan et al. 2001) and have been shown to modulate insulin resistance either by affecting directly the insulin signalling pathway or indirectly via stimulation of inflammatory pathways (Tilg et al. 2008). Furthermore, studies in rodents have shown that acute infusions of IL-6 induced insulin resistance in both liver and skeletal muscle (Kim et al. 2004). Previous studies have reported higher concentrations of IL-6 in South Asians than white Europeans (Arjunan et al. 2015) suggesting a possible implication of the increased prevalence of insulin resistance in South Asians, although this is not a universal finding (Peters et al. 2013). Considering IL-6 released from adipose tissue has been identified as a precursor for hepatic CRP secretion and that the greater IL-6 production from the adipose tissue seems to be more associated with the increase of total adiposity (Bastard et al. 1999), it is possible that the ethnic differences in inflammation may be mediated by the higher body fat levels in South Asian individuals. In this regard, Arjunan and co-workers demonstrated greater plasma IL-6 concentrations in South Asian compared with white European, with the ethnic difference disappearing after adjusting for percent of body fat (Arjunan et al. 2015). Peters and colleagues (2013) did not observe ethnic differences in IL-6 between South Asian and white European men, although the same study revealed greater IL-6 in South Asian than white European women (Peters et al. 2013). In the same study, additionally, adjustment for visceral fat area or percentage body fat eliminated the differences in IL-6 between South Asian and white European women, with visceral fat area and body fat percentage explaining up to 30% of the difference in IL-6 (Peters et al. 2013). However, data on IL-6 in South Asian compared

with white European individuals remain limited and future work is required to ascertain the independent contribution of ethnicity and adiposity on IL-6 in South Asians.

1.3.2.2 Free fatty acids

Elevated concentrations of FFAs or non-esterified fatty acids (NEFA) has been proposed to be an important link between adipose tissue and skeletal muscle/liver insulin resistance and may contribute to β-cell dysfunction (Boden, 2002; Boden and Shulman, 2002; Abate et al. 2004; Karpe et al. 2011). Despite the mechanisms explaining the link between FFAs and insulin remain partly understood, FFAs have been reported to inhibit insulin action, particularly by inhibiting insulin-induced glucose transport and/or by inhibiting insulin-signalling pathways (Boden et al. 2011). Previous evidence also reported elevated circulating FFAs in individuals with T2D and obesity compared with individuals who are healthy and lean (Boden, 2011; Capurso and Capurso, 2012). In this regard, the contribution of chronically elevated FFA concentration on insulin resistance has been demonstrated by acutely normalising the elevated levels of FFA in obese patients with T2D, which ameliorated insulin sensitivity from approximately 25% to 50% (Boden et al. 2011). Furthermore, a considerable part of the insulin resistance lowering effect of thiazolidinediones (TZDs), typically prescribed for reducing insulin resistance and improving insulin sensitivity, can be attributed to their lowering of plasma FFA levels (Boden et al. 2006), which they do by increasing FFA oxidation (Boden et al. 2005).

Previous evidence reported higher total FFAs concentration in South Asian than white European individuals, which has been linked with the greater insulin resistance and T2D risk amongst the South Asian population (Chandalia et al. 2007; Abate et al. 2004). In respect of this, the study conducted by Chandalia and co-workers revealed higher total

non-esterified fatty acids (NEFA) in non-diabetic South Asian men compared with Caucasian men, with the South Asian group exhibiting also larger subcutaneous adipocytes size, independent of total or subcutaneous abdominal fat (Chandalia et al. 2007). Furthermore, the same study exhibited a negative correlation between adipocyte size and glucose disposal rate, used as a measure of insulin sensitivity (Chandalia et al 2007). In a similarly designed cross-sectional study, non-diabetic insulin resistant Asian Indian exhibited elevated total FFA concentrations compared with Caucasian men, with the ethnic difference in FFA remaining significant after adjusting for total adiposity, waist and truncal skinfold thickness (Abate et al. 2004). Thus, the elevated concentrations of FFAs and larger adipocyte size in South Asians seems to be irrespective of total body fat and adipose tissue distribution, suggesting possible abnormalities in the adipose tissue metabolism which may contribute to the elevated insulin resistance and T2D in South Asians (Bakker et al. 2013; Sattar and Gill, 2015) as described in Chapter 1.3.1.3.6. Previous evidence also suggests that adiposity dysfunction and elevated circulating FFAs may induce intracellular lipid deposition in ectopic depots (e.g. skeletal muscle and liver), which seems to play a causal role in insulin resistance and T2D in South Asians. As reported in Chapter 1.3.1.3.6, ectopic fat can accumulate in the liver as a result of excess circulating FFAs via the portal vein and systemic circulation, which can lead to non-alcoholic fatty liver disease (NAFLD) and hepatic insulin resistance (Hall et al. 2008). This interferes with insulin signalling pathway and inhibits insulin action leading to a reduction of the hepatic glycogen synthase and stimulation of gluconeogenesis (Samuel et al. 2004). Likewise, high levels of circulating FFAs have been reported to cause an oversupply of lipid to skeletal muscle leading to an accumulation of intramuscular triglyceride and fatty acids metabolites such as long-chain acyl-CoA, diacylglyceride and ceramide, which inhibit insulin action (Hall et al. 2008). Evidence

from Forouhi and co-workers (1999) shown 30% greater intramuscular triglyceride concentrations in South Asian than BMI-matched white European men (Forouhi et al, 1999). Although it is now accepted that intramuscular triglyceride does not induce directly insulin resistance (as opposed to lipid intermediates), it represents a marker of cytosolic lipid accumulation (Hall et al. 2010). Therefore, the fact that South Asians have exhibited elevated intramuscular triglyceride suggests an impaired skeletal muscle lipid metabolism. Furthermore, it has been suggested that the accumulation of lipid metabolites in insulin-resistant skeletal muscle is not only induced by elevated FFAs concentration and subsequent lipid oversupply, but also by a reduced lipid oxidation in skeletal muscles (Hall et al. 2008). The findings by Hall and colleagues (2010) (described in Chapter 1.3.1.3.5) reporting lower fat oxidation during submaximal exercise in South Asians and positive associations between lower fat oxidation and insulin sensitivity index, appear to corroborate the causal role of lipid accumulation in skeletal muscle and insulin resistance in South Asians (Hall et al. 2010).

Although elevated concentrations of total FFAs may mediate the insulin resistance in South Asian individuals (Abate et al. 2004; Chandalia et al. 2007), not all FFAs contribute equally to the insulin resistance process. Indeed, high concentrations of saturated fatty acids (SFAs) and omega-6 polyunsaturated fatty acids (PUFAs) have been associated with increased levels of glucose and insulin whereas specific PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to improve insulin sensitivity (Rasic-Milutinovic et al. 2012). While previous cross-sectional investigations have explored the association between individual fatty acids and markers of cardiometabolic risk (Imamura et al. 2012; Ferrucci et al. 2006), these studies have not investigated ethnic-specific between individual free fatty acids and markers of insulin resistance. To the author's knowledge, only one study investigated ethnic-specific

associations between plasma fatty acids and fasting glucose and insulin resistance, identifying significant associations in Caucasian, but not in South Asian individuals (Ralston et al. 2013). However, this previous study looked at esterified fatty acids (EFS), instead of free fatty acids, by carrying out hydrolysis of plasma TAG post-extraction and this ignores the fact the rate of lipolysis of triglycerides, which is controlled by glucocorticoids and catecholamines, might be important (Xu et al. 2009). Furthermore, while this study reported baseline levels of FFAs in South Asian and Caucasians, ethnic-differences in FFAs were not explicitly examined while associations between fatty acids and physical activity/fitness level were not explored. Thus, the examination of individual FFAs, instead of total levels of FFAs, in relation to insulin resistance markers in South Asians needs further investigation.

A further important consideration in the context of examination of individual plasma FFAs concerns the methods used for the quantification and identification of these metabolites. In this regard, the techniques employed to measure concentrations of FFAs has progressively grown over the years ranging from microfluorometric and colorimetric enzymatic methods, titrimetric determination, thin-layer chromatography (TLC) to metabolomics advanced technologies such as gas chromatography (GC) and liquid chromatography (LC) based approaches (Song et al. 2019). However, although the majority of previous studies examined total NEFA in South Asian compared with Caucasian individuals using colorimetric enzymatic methods (Abate et al. 2004; Chandalia et a. 2007), actual data suggest that metabolomics-based approaches are more appropriate when an accurate measurement of individual FFA concentrations is required (Song et al. 2019).

1.3.2.2.1 Application of metabolomics for the quantification of free fatty acids

Metabolomics represents an evolving technology that enables the identification and quantification of low-molecular weight metabolites in an organism that are intermediates and products in metabolic pathways including lactate, fatty acids and branched-chain- and aromatic amino acids (Gonzales-Franquesa et al. 2016). The use of this approach is growing in popularity and has been successfully applied to different fields such as nutrition research, biomarker screening and disease aetiology (Liu et al. 2010). In this regard, the applications of metabolomics appear to be particularly relevant for the quantification of known metabolites and for the identification of new metabolites which may contribute to diagnose earlier specific diseases and understand the underlying mechanisms in addition to standard clinical biomarkers (Lu et al. 2016).

The metabolic changes linked with a specific condition or disease can be examined by using mainly two different metabolomic approaches: targeted and untargeted approach (Dunn et al. 2011). Targeted metabolomic is a quantitative approach typically employed as a result of a specific question or hypothesis which aims to quantify known metabolites related to one or more pathways (Patti et al. 2012). Conversely, untargeted approaches are less quantitative as their main purpose is to detect as many metabolites/compounds as possible in a given biological sample having little or no knowledge of the expected metabolic profiles in order to generate hypothesis (Patti et al. 2012).

Metabolites include a variety of chemical compounds of different molecular weights and functional groups. Although their examination may be achieved using similar techniques used in routine chemical analyses, the identification and quantification of individual metabolites in complex mixtures requires higher sensitivity and selectivity techniques (Dunn and Ellis, 2005). In this regard, LC and GC, coupled with mass spectrometers

(MS) represent the most advanced and frequently used techniques (Patti et al. 2012). Particularly, previous research examining FFAs in human plasma and associations with metabolic disorders such as insulin resistance and T2D, has made considerable use of GC and LC coupled with MS (Binbin et al. 2010; Yi et al. 2007; Dai et al. 2015; Liu et al. 2010; Ma et al. 2018; Lu et al. 2016; Serafim et al. 2019; Park et al. 2015; Gonzales-Franquesa et al. 2016; Feng et al. 2017). For example, in the study from Lu and co-workers (2016), baseline serum FFAs profile using LC-MS approach was compared between patients with T2D and healthy control with diabetic patients exhibiting elevated myristic, palmitic and stearic acid (Lu et al. 2016). The same study also demonstrated positive associations between diabetes risk with different FFAs including palmitic, stearic, oleic and linoleic acid (Lu et al. 2016). These studies, however, have not investigated ethnic-specific differences in plasma FFAs metabolic profile using between South Asian and white European individuals, which was a purpose of the present thesis.

1.4 Regulation of appetite and energy intake

This section will initially describe the physiological regulation of appetite, illustrating the role of peripheral signals and their integration with central mechanisms to regulate appetite and food intake. Furthermore, this section will describe leptin and its role in regulating long-term changes in energy homeostasis and body fat, but also its associations with insulin resistance in South Asian individuals. The roles of acylated ghrelin and total PYY in the short-term appetite and energy intake regulation will be subsequently described with the last section examining the acute effects of exercise on appetite feelings, energy intake and concentrations of acylated ghrelin and total PYY.

1.4.1 Physiological regulation of appetite and energy intake

The regulation of appetite and energy intake results from the integration of central and peripheral signals in the hypothalamus and brainstem, which are the main regions of the brain involved in the regulation of energy homeostasis (Druce and Bloom, 2006). Particularly, neuro-hormonal signals from the gut and adipose tissue converge on the hypothalamus providing information about adiposity and acute nutritional state of the body (Wren and Bloom, 2007). A vital region of the hypothalamic regulatory system is the arcuate nucleus (ARC), which receives different inputs from other hypothalamic regions as well as from peripheral hormones which cross the blood-brain barrier at the median eminence (Figure 1.3). The neurons in the ARC of the hypothalamus, can be grouped into two main populations. They include orexigenic neurons neuropeptide Y (NPY) and agouti-related peptide (AgRP), which stimulate food intake and promote weight gain, and anorexigenic neurons pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), that inhibit feeding and promote weight loss (Wren and Bloom, 2007). Stimulation of POMC/CART neurons induces the expression of the neuropeptide α-melanocyte-stimulating hormone (α-MSH), which acts on melanocortin-3 receptor (MC3) and melanocortin-4 receptor (MC4) receptors in the paraventricular nucleus (PVN) (another region of the hypothalamic area), to reduce appetite. Conversely, AgRP blocks the actions of α-MSH by inducing antagonistic effects on MC3 and MC4 receptors while NPY stimulates feeding mostly through the activation of Y1 and Y5 receptors in the PVN (Neary et al. 2004). The nucleus of the solitary tract (NTS) is an additional key area in the control of appetite located within the brainstem receiving hormonal and neural inputs from the circulation and from vagal afferent nerves located in the gastrointestinal tract (Druce and Bloom, 2006) (Figure 1.3). In addition to the ARC, NTS and PVN, which have prominent roles in the central

regulation of appetite, other integrated pathways also exist (Schwartz et al. 2000). Nonetheless, a discussion of these additional pathways is beyond the aim of this thesis, but the reader is referred to Schwartz et al. (2000) for an expanded review of the hypothalamic neurocircuits regulating energy balance. Different hormones within the peripheral circulation provide information about the nutritional and adiposity state of the body and can be divided into two main groups: episodic and tonic hormones. Tonic hormones (leptin and insulin) produce chronic signals proportionally to adiposity, while episodic hormones, such as ghrelin or PYY, change acutely in response to food intake.

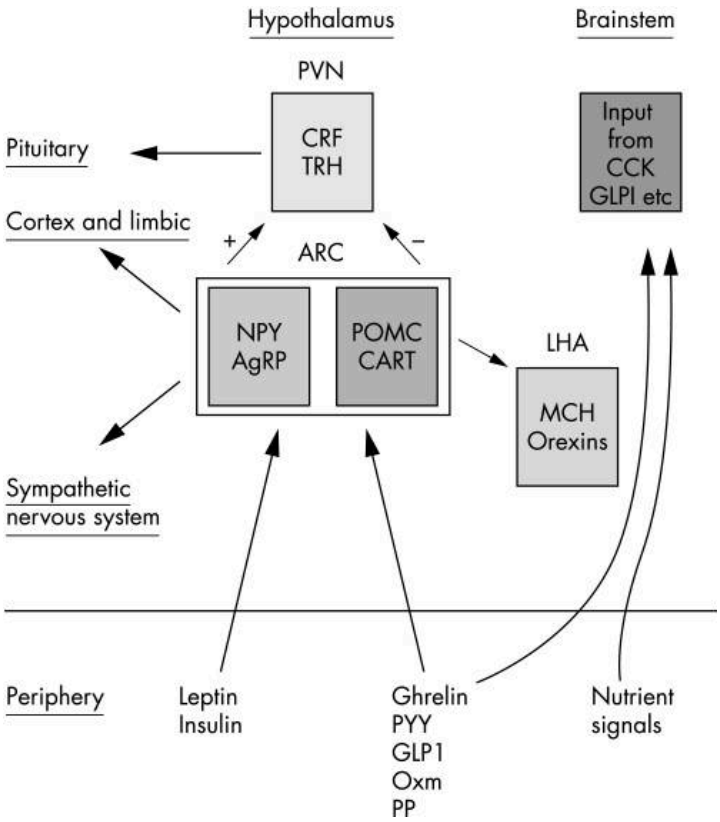

Figure 1.3. Schematic representation of appetite and energy intake control resulting from the integration of central and peripheral signals in the hypothalamus and brainstem. ARC, arcuate nucleus; *PVN*, paraventricular nucleus; *LHA*, lateral hypothalamic area; *CRF*, corticotroph releasing factor; *TRH*, thyrotropin releasing factor (form part of integration with energy expenditure); *NPY*, neuropeptide Y; *AgRP*, agouti related peptide; *POMC*, proopiomelanocortin; *CART*, cocaine and amphetamine regulated transcript; *MCH*, melanin concentrating hormone; *CCK*, cholecystokinin; *GLP-1*, glucagon-like peptide 1; *PYY*, peptide YY; *Oxm*, oxyntomodulin; *PP*, pancreatic polypeptide. Druce and Bloom (2006). Copyright permission authorised by Professor S. R Bloom.

1.4.2 Tonic signals

Tonic signals are long-acting signals which reflect the levels of energy stores and control body weight as well as the amount of energy stored as fat over the long term.

Together with leptin, insulin is the only other hormone regarded as a tonic appetite regulator and although insulin is not secreted directly from the adipose tissue, its concentrations circulate in proportion with body fat mass, with phasic increments occurring during meals (Woods and Seeley, 1998). Insulin has shown to exert anorexigenic effects via the stimulation of POMC and inhibition of NPY and AgRP neurones in the ARC of the hypothalamus (Schwartz et al. 2000). Structure and function of this hormone as well as differences in circulating insulin concentrations and associations with cardiometabolic risk in South Asian and white European men have been described in detail in Chapters 1.2.3 and 1.3.1.3.

1.4.2.1 Leptin

Leptin is a peptide hormone principally secreted by adipocytes which circulates at concentrations proportional to body fat mass. In this regard, obese subjects typically exhibit elevated leptin concentrations than normal weight individuals with most of the previous evidence reporting positive associations of circulating leptin levels with BMI and adiposity (Considine et al. 1996). Leptin plays a key role in providing information about nutritional status and subcutaneous fat mass to neural centres located in the hypothalamus that control feeding behaviour and energy expenditure (Considine et al. 1996). Particularly, leptin exerts anorexigenic effects in the ARC of the hypothalamus via stimulating POMC neurones and inhibiting NPY and AgRP neurones (Sahu 2003). Furthermore, leptin has shown to play a key role in glucose homeostasis, independent of body weight, food intake and energy expenditure, promoting insulin-sensitising effect on

the whole-body level and glucose uptake (Denroche et al. 2012). It is therefore plausible that differences in leptin levels explain, at least partly, the ethnic variations in insulin resistance and T2D in South Asian compared with white European individuals.

Previous studies reported higher leptin levels in South Asian men, women and neonates compared with their white European counterparts (Kalhan et al. 2001; Abate et al. 2004; Lilja et al. 2010; Mente et al. 2010). The SHARE study, a cross-sectional study of CVD risk factors conducted between 1996 and 1998 in 1176 Canadians of South Asian, Chinese, Aboriginal and European origin, reported elevated plasma leptin in South Asian, men and women, compared with individuals of European descent and revealed positive associations of plasma leptin with insulin resistance (Mente et al. 2010). These findings were similar with smaller studies conducted previously (Abate et al. 2004; Chandalia et al. 2007; Liew et al. 2003). For example, in the report from Liew and colleagues (2003) plasma leptin concentrations were elevated in Asian Indian than white European men and significantly associated with insulin clearance and fasting insulin (Liew et al. 2003).

Considering circulating leptin levels are directly proportional to body fat mas (Considine et al. 1996), it has been suggested that the greater leptin concentrations in South Asian than white Europeans is mediated, at least partly, by differences in adiposity (Bakker et al. 2013). In support of this, Banerji and colleagues (1999) revealed positive associations of plasma leptin with BMI and subcutaneous adipose tissue in healthy Asian Indian male (Banerji et al. 1999). Likewise, Liew and colleagues (2003) reported that fasting leptin reflected the higher body fat percentage as evidenced by the positive associations between leptin and body fat (Liew et al. 2003). However, BMI, waist circumference as well as total adiposity and fat distribution appear to not exhaustively explain the higher leptin levels amongst South Asians (Gujral et al. 2013). In the cross-sectional study

conducted by Abate and colleagues (2004), South Asian revealed elevated fasting plasma leptin concentrations compared with white European men exhibiting similar total and abdominal fat (truncal skinfold thickness) than white European men suggesting elevated leptin production from adipose tissue irrespective of total/abdominal adiposity (Abate et al. 2004). Furthermore, in the SHARE study the waist-to-hip ratio (WHR) and leptin showed similar correlation values in both South Asian and European groups (Mente et al. 2010), suggesting the possibility of abnormalities in the adipose tissue metabolism in South Asians that extend beyond the greater total and abdominal fat (Gujral et al. 2013; Mente et al. 2010; Abate et al. 2004). Elevated plasma leptin levels in obesity appear to be linked with leptin resistance and may be involved in the pathogenesis of obesity-related insulin resistance (Abate et al. 2004). Leptin induces lipid oxidation in cells, therefore decreased leptin action may predispose to lipids accumulation in skeletal muscle, which has been associated with impaired skeletal muscle insulin resistance (Hall et al. 2008). While the mechanisms explaining the link between hyperleptinemia and insulin resistance is not completely understood, prolonged hyperinsulinemia may induce an increase in leptin levels suggesting that insulin resistance and higher concentrations of insulin may have a role in promoting hyperleptinemia (Wang et al. 1999). In support of this, Abate et al. (2004) demonstrated positive correlation between leptin levels and AUC insulin during OGTT in South Asians and white European men, suggesting that the elevated insulin concentrations in the South Asian group may have contributed to the greater leptin concentrations in South Asian than white European men.

1.4.3 Episodic signals

Despite the fact that leptin and insulin play an important role in the tonic regulation of appetite, changes in the concentrations of these hormones cannot explain the increase or

decrease in appetite perceptions around the meal, which control initiation and termination of the meal. In this regard, acute appetite-regulation seems to be mostly mediated by neuroendocrine signalling from the gastrointestinal (GI) tract (Druce and Bloom, 2006). Short-acting GI signals are characterised by gut hormones such as cholecystokinin (CCK) and mechanical factors, such as gastric distension, which typically convey a sense of "fullness" leading to postprandial satiation and meal cessation. Additional appetite regulating hormones from the gut have been identified, including the appetite-stimulating hormone ghrelin and a variety of appetite inhibiting hormones such as PYY, pancreatic polypeptide (PP), glucagon-like peptide 1 (GLP-1) or oxyntomodulin (OXM) (Wren and Bloom, 2007). The studies in this thesis focused on the measurement of ghrelin and PYY and, thus the following sections will describe the structure and function of these two hormones, particularly in relation to appetite and energy intake control.

1.4.3.1 Ghrelin

Ghrelin is a 28-amino-acid hormone which is mainly synthesised by the endocrine X/A cells of the oxyntic glands of the gastric mucosa, responsible for approximately 50 to 70% of the systemic ghrelin production (Kojima and Kangawa, 2005). To exert its biological function and to cross the blood-brain barrier, this peptide requires post-translational acylation with a medium chain fatty acid, typically octanoic acid, which reaction is catalysed by the ghrelin-O-acyltransferase (GOAT) (Ghigo et al. 2005). Ghrelin circulates in the bloodstream predominantly in a non-acylated form (80-90% of total circulating ghrelin), which is likely to be explained by the abundance of ghrelin than GOAT within the ghrelin-producing cells (Ghigo et al. 2005). However, the orexigenic actions of ghrelin are exclusively mediated by the acylated form of this gut peptide (Neary et al. 2004) whereas the measurement of total ghrelin has been reported to mask changes in acylated ghrelin (Hosoda et al. 2004). Therefore, acylated ghrelin is typically

targeted in studies examining the effect of exercise on circulating plasma ghrelin concentrations in humans (Hosoda et al. 2004).

Ghrelin is the only known orexigenic gut hormone identified so far and it is well-established that feeding represents the main factor regulating ghrelin secretion considering that its circulating levels decline with food intake and rise in response to short-term fasting (Cummings et al. 2001). Ghrelin is the natural ligand for the growth hormone secretagogue type 1a receptor (GHS-R1a) and appears to be a potent stimulus for the growth hormone release (Ukkola et al. 2005). However, ghrelin has received specific attention as the only peripheral hormone to stimulate appetite and food intake. Particularly, when administered into the central nervous system (CNS), ghrelin stimulates food intake more powerfully compared with any other substance investigated including NPY, considered previously the most powerful orexigenic factor (Wren and Bloom, 2007). Although its signalling mechanisms remain partly unknown, previous evidence suggest that ghrelin acts predominantly via arcuate NPY/AgRP neurons, which express the GHS-R1a (Wren and Bloom, 2007). Additionally, ghrelin has shown to increase appetite and food intake when administered also systemically in both rodents and humans (Wren and Bloom, 2007).

Ghrelin has been shown to affect chronic energy homeostasis as frequent central and peripheral injections of ghrelin significantly induced body weight and fat mass gain in rodents as a result of an increase in food intake and decrease in fat oxidation (Tschöp et al. 2000). Furthermore, circulating ghrelin concentrations also appear to reflect body weight changes over the longer term, which may suggest a possible role of ghrelin in body weight regulation as an adiposity signal in humans. In this regard, ghrelin has been

shown to respond to chronic changes in energy balance as circulating ghrelin levels are inversely associated with adiposity (William and Cummings, 2005) with previous studies reporting low levels in obese populations and elevated in individuals with anorexia nervosa (Tschöp et al. 2001). Furthermore, Ravussin and co-workers (2001) reported a down-regulation of ghrelin concentrations in response to approximately three months of overfeeding and an up-regulation in response to an energy deficit induced via exercise for a similar duration (Ravussin et al. 2001). In a different study conducted in female mice, Moesgaard and co-workers (2004) reported that obesity induced by 10 weeks of high-fat diet markedly suppressed fasting plasma ghrelin concentrations and ghrelin mRNA expression in the GI tract, suggesting that feeding and/or weight gain reduced ghrelin release (Moesgaard et al. 2004). However, it remains difficult to establish whether the changes in plasma ghrelin in these studies are related to weight gain or to increased fat in the diet. Nonetheless, although the mechanisms explaining the fluctuation of ghrelin concentrations related to changes in body weight are not completely understood, there is evidence that the low concentrations of ghrelin observed in obese individuals may be related more to insulin resistance than to higher BMI or fat mass (Flanagan et al. 2003; McLaughlin et al. 2004). In this regard, McLaughlin and colleagues (2004) compared ghrelin concentrations in obese individuals classified as either insulin-resistant or insulin-sensitive, based on steady-state plasma glucose concentrations (McLaughlin et al. 2004). The results of this study revealed significantly lower ghrelin concentrations in the insulin-resistant group and exhibited negative correlations of ghrelin with insulin resistance and insulin concentrations. Additionally, in the same study multivariate analysis confirmed that both insulin resistance and hyperinsulinemia, independently, predicted low ghrelin levels (McLaughlin et al. 2004),

with these data suggesting that insulin resistance and hyperinsulinemia may be associated with ghrelin suppression independently of adiposity.

1.4.3.2 Peptide YY

Peptide YY is a 36-amino-acid peptide which is primarily synthesised and secreted from entero-endocrine L cells of the distal GI tract with concentrations increasing from the pylorus to the rectum (Adrian et al. 1985). Peptide YY exists in two different forms: the integral 36 amino acid peptide PYY_{1-36}, which is cleaved after secretion to give the truncated and active 34 amino acid form PYY_{3-36}. In the circulation, PYY_{3-36} is the abundant form of circulating hormone (~65%) whereas PYY_{1-36} represents ~35% of the total circulating PYY (Batterham et al. 2006). In terms of biological effect, PYY_{1-36} binds with similar affinity to all of the functional Y receptor subtypes in humans (Y1-5) while PYY_{3-36} favourably binds to the Y2 receptor (Cabrele and Beck-Sickinger, 2000). In this regard, although the appetite-suppressing effects of PYY are thought to be mediated particularly by PYY_{3-36} (Sloth et al. 2007), previous studies have reported strong positive correlations between changes in total PYY and PYY_{3-36} in response to food intake and exercise (Tsilchorozidou et al. 2008; Broom et al. 2009). Thus, consistently with the available evidence suggesting that total PYY measurements reflect changes in PYY_{3-36} (Broom et al. 2009), total PYY was examined in the studies presented in this thesis.

PYY secretion is mainly stimulated by food ingestion, with concentrations starting to increase within 15 min of food ingestion, reaching a peak at ~90 min and remaining high for approximately 6 hours (Adrian et al. 1985). However, PYY exerts a variety of effects on the GI tract. Administration of PYY, for instance, has been shown to increase the absorption of water and electrolytes from the ileum after food ingestion and inhibits

secretions from the pancreas, stomach and gallbladder as well as delaying gastric emptying (Wren and Bloom, 2007). Additionally, peripheral administration of PYY also exerts effects on other body systems including reduction of cardiac output, glomerular filtration rate, plasma renin, and aldosterone activity (Adrian et al. 1985). However, the significance of these numerous physiological actions has not been elucidated. The pattern of PYY secretion after a meal suggests that it may be a satiety signal, inducing termination of the meal and promoting coordinated GI responses to assist digestion and absorption. In humans, intravenous administration of $PYY_{3–36}$ has demonstrated to inhibit food intake in both lean and obese individuals (Batterham et al. 2002). For instance, in normal weight men and women, intravenous injections of 0.8 $pmol.kg^{-1}.min^{-1}$ PYY_{3-36} for 90 min exhibited a significant decrease in hunger feelings and an approximate 36 % decline in energy intake at an *ad libitum* buffet meal given two hours after the infusion (Batterham et al. 2006). In the same study, examination of food diaries revealed inhibition of food intake throughout the 12 h after infusion without any compensatory increases thereafter, which resulted in a substantial lower 24 h energy intake in comparison with a saline infusion control trial (Batterham et al. 2002). These findings have been confirmed to obese participants (Batterham et al. 2003). However, the same physiological effects do not occur at similar doses of PYY_{1-36}, which suggests that the appetite suppressing effects are mainly mediated by the active form PYY_{3-36} (Sloth et al. 2007). The mechanisms whereby $PYY_{3–36}$ inhibits appetite and food intake is thought to be mediated predominantly by acting directly on Y2 receptors (Y2R) in the ARC of the hypothalamus (Karra & Batterham 2010). The binding of PYY to the Y2R seems to reduce appetite and food intake by inhibiting NPY neurones, which reduces orexigenic signalling and also disinhibits POMC neurones to increase anorexigenic outputs (Batterham et al. 2002).

Peptide YY may also influence chronic energy homeostasis as repeated peripheral infusions of PYY_{3-36} has revealed to significantly decrease adiposity and body weight rodents as a result of a reduction in food intake as well as an increase in fat oxidation at whole body (Adams et al. 2006). In humans, PYY also responds to chronic changes in energy balance as obese individuals have been shown to exhibit reduced fasting and postprandial circulating PYY than lean individuals (Le Roux et al. 2006; Batterham et al. 2003), although this is not a universal finding (Cahill et al. 2011; Stock et al. 2005). The mechanisms underlying a blunted PYY response in obesity, however, are unclear but studies conducted in obese mice suggests that circulating levels are decreased as a result of impaired postprandial secretion, rather than synthesis, of PYY (Chandarana et al. 2011). Regardless of the mechanisms, the blunted PYY and satiety response to food consumption may contribute to the exacerbation of obesity.

Given the implication of PYY and ghrelin in the energy homeostasis and body weight/fat mass control, it is possible that ethnic differences in these hormones as well as in the regulation of appetite and energy intake may underlie the well-established elevated adiposity and associated cardiometabolic risk in South Asian compared with white European individuals. However, no studies to the author's knowledge have been conducted to compare the levels of these hormones between South Asian and white European individuals.

1.5 Effect of exercise on appetite, food intake and appetite related hormones

1.5.1 Appetite and energy intake assessment

Appetite represents the qualitative aspect of feeding behaviour and can be described as the momentary disposition of an individual to ingest food and is experienced as perceived hunger and desire to eat. Alternatively, although food intake responds to the same stimuli,

it refers to the quantitative aspect of the feeding behaviour. The relationship between appetite and food intake, however, is not always perfect and actual recommendations suggest that both parameters are examined within the same experiment (Gregersen et al. 2008; Stubbs et al. 2000).

Appetite feelings are normally assessed using visual analogue scales (VAS) measuring four different aspects of appetite: hunger, satisfaction, fullness and prospective food consumption (Flint et al. 2000). These scales require participants to mark with a horizontal line, that is typically 100 mm or 150 mm in length with specific statements at each end (e.g. 'not at all full'/ 'totally full'). On the other hand, acute appetite studies typically measure *ad libitum* food intake using either a free choice buffet meal or a single meal with a predetermined macronutrient composition. Both options can be used within the laboratory setting, which allows precise quantifications of food intake by weighing food items before and after ingestion. However, some intervention studies may monitor food intake using food diaries although self-report measures are particularly prone to individual bias, which limits the validity of this method (Livingstone & Black 2003).

Interest in the effects of exercise on appetite and energy intake arises from the acknowledgement that physical activity may enhance weight and fat loss (Donnelly et al. 2009), representing a good strategy for the management of obesity and associated complications. Nonetheless, the weight/fat loss response to physical activity is determined by the subsequent energy intake, as an increase in food ingestion may negate the energy deficit of exercise. Likewise, any compensatory increases in appetite perceptions after exercise may contribute to the difficult of maintaining a negative energy balance and weight loss. In this regard, although previous evidence suggest that South

Asian individuals engage in less habitual physical activity than white Europeans (Sattar and Gill, 2015), which is likely to contribute to the excess of adiposity and associated cardiometabolic risk in this population (Fischbacher et al. 2004; Williams et al. 2011a; Williams et al. 2011b; Sattar and Gill, 2015), no studies have investigated ethnic differences in appetite perceptions and food intake responses to acute exercise in South Asian and white European individuals.

The following sections will discuss the most relevant studies that investigated the acute and chronic effects of exercise on appetite perceptions, food intake and appetite-related hormones in the general population

1.5.1.1 Appetite perceptions in response to exercise

A plethora of studies have examined the effects of single bouts of continuous aerobic exercise (≥ 60 % $\dot{V}O_2$ max) on appetite perceptions, with the majority of these studies performed in lean and physically active males. The majority of these study have shown a transient suppression of appetite perceptions during and shortly after exercise, which typically returns to resting control values within 30 to 60 min of exercise cessation (Broom et al. 2007; Deighton et al. 2013a; King et al. 2010a); a phenomenon known as 'exercise-induced anorexia' (King et al. 1994). Appetite suppression has been also observed during a variety of exercise modes including cycling (Deighton et al. 2013a; Douglas et al. 2017), running (Broom et al. 2017; King et al. 2010a) and resistance exercise (Broom et al. 2009).

Most of the studies that have examined appetite feelings in response to exercise have adopted an observation period of 2 h post exercise. The majority of these studies have shown no differences in appetite during the post-exercise period, after the recovery from

exercise-induced anorexia, compared with the resting control trial in both males and females (Martins et al. 2007; Hagobian et al. 2013; Larson-Meyer et al. 2012). Additional studies have employed longer observation period post exercise (2 – 9 h), and the consensus amongst these studies is that appetite does not increase above control values (Wasse et al. 2012; Wasse et al. 2013; Broom et al. 2009), although this is not universal (King et al. 2011a). Particularly, King and colleagues (2011a) observed higher appetite perceptions after 60 min of intermittent swimming compared with the resting control trial from 1.5 to 6 h post exercise (King et al. 2011a). These data, however, differed from previous studies from the same author using a similar study protocol and participant population, but employing running or brisk walking as exercise type (King et al. 2010a; King et al. 2010b), suggesting an influence of the exercise mode on the subsequent appetite response.

Excess in adipose tissue is typically characterised by a chronic excess of energy intake over energy expenditure, thus it is plausible that appetite regulation in response to exercise may differ obese compared with lean individuals. Nonetheless, current evidence suggests that aerobic exercise fails to induce acute compensatory changes in appetite perceptions in overweight and obese men and women (Martins et al. 2015; Douglas et al 2017).

The majority of the aforementioned studies investigated the effects of exercise on subsequent appetite and food intake following an overnight fast. This probably stems from the fact that performing aerobic exercise following an overnight fast has been shown to create a favourable lipolytic hormonal environment, such as reduced plasma insulin levels and elevated cortisol and epinephrine concentrations, which enhance weight and fat loss (Maughan et al. 2010). However, postprandial exercise has been

suggested as more beneficial for weight control, which seems to exhibit more favourable effects on appetite regulation than fasted exercise. In this regard, Deighton and colleagues demonstrated that 60 min of running at ~70% of $\dot{V}O_2$ max resulted in more prolonged hunger suppression when performed ~2 h after a high carbohydrate breakfast (72.9% carbohydrate, 9.5% protein, 17.6% protein) rather than after a 10 h overnight fast (Deighton et al. 2012). Similar findings were observed by Cheng and colleagues (2009) who demonstrated that 50 min of cycling at ~60% of $\dot{V}O2$ max resulted in a longer appetite suppression when performed after breakfast than an overnight fast (Cheng et al. 2009). Therefore, although more studies are needed before definitive conclusions can be drawn, exercising after a meal may represent a viable strategy, in alternative to exercise in fasted conditions, for the treatment and management of weight loss.

1.5.1.2 Energy intake in response to exercise

Most of the studies mentioned in the previous section also examined the food intake response to exercise in a laboratory setting by providing participants with an *ad libitum* meal within 2 h after the exercise test. In agreement with the appetite feeling responses reported in the previous section, most of these studies reported no significant changes in absolute energy intake after performing aerobic exercise (Douglas et al. 2017; Alajmi et al. 2015; King et al. 2010a) or resistance exercise (Broom et al. 2009; Jokisch et al. 2012; Balaguera-Cortes et al. 2011) compared with the control trial, in males and females. Furthermore, additional studies have also shown no changes in energy intake even when the *ad libitum* meal was provided in a laboratory setting closer (≤ 1 h) (Balaguera-Cortes et al. 2011; Gonzalez et al. 2013; Kelly et al. 2012) or more than 2 h after the exercise bout (King et al. 2010a; King et al. 2010b; Wasse et al. 2012) in males and females. With respect to the latter, previous studies suggest that exercise does not induce energy intake

compensation up to 22.5 h after exercise (King et al. 2010a) and this finding is also in agreement with those studies that used self-reported measures of food intake which have failed to identify any changes in energy intake up to 72 h after exercise (Pomerleau et al. 2004). The examination of food intake in response to exercise in a laboratory setting also allows the quantification of macronutrient intakes which represents an important factor considering that an over consumption of a high calories fat may overturn the energy deficit of exercise (Lluch et al. 1998). However, a plethora of studies in this regard did not observe changes in macronutrients in response to exercise (Shorten et al. 2009; Douglas et al. 2017; Kelly et al. 2012; Deighton et al. 2014; King et al. 2010a; King et al. 2010b).

Considering the weight loss response to physical activity depends on subsequent energy intake, as elevated food intake may negate the energy deficit induced by exercise, there has been a growing interest in examining food intake responses to exercise in overweight and obese individuals. In this regard, Douglas and co-workers (2017) demonstrated no change in *ad libitum* absolute energy and macronutrient intake in lean and overweight/obese, men and women, aged on average between 37.5 and 45 years old, 6 h after performing 60 min running at 60% of peak oxygen uptake (Douglas et al. 2017), with this finding corroborating previous evidence (Martins et al. 2015; Hopkins et al. 2014). Additionally, previous evidence comparing lean and overweight/obese individuals have demonstrated that energy intake remains unchanged even when food intake is examined closely after exercise (≤ 1 h). In this regard, a previous study reported no energy compensations in overweight obese and normal weight females aged on average 35 years old, when an *ad libitum* meal was provided within 1 h after walking on a treadmill at moderate intensity (60% maximum heart rate) (George and Morganstein,

2003), suggesting that acute exercise induces a short-term energy deficit regardless of weight and adiposity status (Dorling et al. 2018).

Evidence from cross-sectional studies have suggested that energy and macronutrient intake may vary after acute exercise according to individual physical activity level (Dorling et al. 2018). Although studies comparing energy intake between inactive and active individuals after exercise bouts are sparse, low active individuals may increase their energy and fat intakes after acute exercise (Dorling et al. 2018). In this regard, Larson-Meyer and colleagues (2012) observed that ad libitum energy as well as fat intakes after 60 min moderate-to-vigorous running were higher in the low active than the high active group (Larson-Mayer et al. 2012). These findings were also in agreement with Finlayson and co-workers (2009) who demonstrated higher energy intake and greater preference for energy-dense foods in low self-reported physically active individuals after performing 60 min moderate-to-vigorous cycling (Finlayson et al. 2009). Although the reasons explaining the greater energy compensation in low active people in response to acute exercise is not completely understood, it may be possible that low active individuals perceive exercise as a less enjoyable activity, thus the acutely increased food intake post exercise can be experienced as a reward (Dorling et al 2018). Alternatively, it is possible that active individuals are better informed on nutrition guidelines and opt for more sensible and healthier food choices (Dorling et al. 2018). Additional studies have also examined energy intake responses between individuals with different habitual physical activity levels, with previous evidence suggesting better energy intake adjustment in response to energy balance perturbations in habitual active individuals (Dorling et al. 2018). In this regard, different authors reported that individuals who habitually exercise exhibit reduced satiety and increased hunger in response to a standardised meal than low active individuals (Gregersen et al. 2011; Van Walleghen et

al. 2007). This seems to suggest that physically active individuals may ingest greater amount of food to compensate for the energy expenditure induced by the habitual exercise (Dorling et al. 2018).

1.5.2 Effects of exercise on ghrelin and PYY

Observations of the exercise-induced anorexia phenomenon and the short-term energy deficit in response to exercise has stimulated considerable interest in in the underlying mechanisms explaining these effects. In this regard, an increasing number of studies have examined a variety of episodic appetite-regulated gut hormones, which appear to regulate feelings of hunger and satiety including ghrelin, known as the only orexigenic gut peptide and PYY due to its effects on appetite suppression. However, there are no studies to the author's knowledge that investigated whether differences in individual ethnicity background may modulate differently appetite-related hormones in response to exercise. In the next section, we will examine the studies that investigated the effects of acute and chronic exercise on these hormones in the general population.

1.5.2.1 Ghrelin

Earlier research investigating the underlying mechanisms linking physical activity, appetite and food intake examined the effects of exercise on total ghrelin concentrations which has produced inconsistent findings with studies reporting elevation (Borer et al. 2009; Christ et al. 2006; Cheng et al. 2009), decrease (Vestergaard et al. 2007; Toshinai et al. 2007) and no change (Dall et al. 2002; Martins et al. 2007) of total ghrelin concentrations in response to a single bout of exercise. However, total ghrelin has been shown to not reflect changes in the active acylated form which is more susceptible to acute energy deficit induced by exercise or reduction in food intake (Mackelvie et al. 2007). In this regard, Marzullo and co-workers (2008) examined differences in acylated

ghrelin and total ghrelin concentrations in response to an incremental cycle test to exhaustion with acylated ghrelin being suppressed immediately after the cycle test whereas total ghrelin remained unchanged (Marzullo et al. 2008). Many studies that examined the effect of acute moderate-to-vigorous exercise (≥ 60 % $\dot{V}O_2$ max) on acylated ghrelin demonstrated acylated ghrelin suppression during exercise with perturbations typically returning to control values within 30 min (Deighton et al. 2013; Broom et al. 2009; Kawano et al. 2013; Wasse et al. 2013). However, this is not a universal finding with other studies observing no changes in acylated ghrelin in response to acute moderate-to-vigorous exercise (Douglas et al. 2017; Douglas et al. 2015; Hagobian et al. 2009) with only one study, to our knowledge, exhibiting an elevation in acylated ghrelin during the two hours post exercise (Larson-Meyer et al. 2012). On the other hand, the acylated ghrelin responses to acute resistance training seem less definitive, with limited studies showing either suppression or no change in circulating acylated ghrelin concentrations (Dorling et al. 2018).

Furthermore, many studies demonstrated a simultaneous suppression of acylated ghrelin and appetite which may suggest a mediating influence of this episodic gut hormone on appetite feelings (King et al. 2010b; Kawano et al. 2013; Unick et al. 2010; Ueda et al. 2009; Wasse et al. 2012). However, although these studies observed a concordance between appetite perceptions and acylated ghrelin in response to exercise, additional studies observed different patterns (Wasse et al. 2013; Douglas et al. 2017; Broom et al. 2017). For example, in their investigation Douglas and colleagues (2017) did not observe acylated ghrelin suppression in response to 60 min of moderate intensity running (59% peak $\dot{V}O_2$), whereas there was a marked suppression of appetite perceptions during and immediately after exercise (Douglas et al. 2017). The lack of acylated ghrelin suppression in this study may be linked to the insufficient intensity of the exercise with previous

research identifying exercise intensity as an important determinant of the acylated ghrelin response to exercise (Broom et al. 2009; King et al. 2010a). In this regard, acylated ghrelin suppression is typically reported in response to exercise intensity above 60% of $\dot{V}O_2$ max (Shubert et al. 2014). Conversely, Broom and colleagues (2017) observed a reduction of acylated ghrelin during a single bout of moderate-to-vigorous running (75% $\dot{V}O_2$ max) but hunger did not differ between the exercise and control trial (Broom et al. 2017), which contrasts with previous studies reporting a simultaneous reductions in hunger and acylated ghrelin previously described. These dissociations between acylated ghrelin and hunger perceptions, however, highlights the complexity of the appetite regulation in which different appetite-related hormones and physiological factors are involved (Broom et al. 2017).

The majority of the aforementioned studies investigated the effects of exercise on acylated ghrelin following an overnight fast. However, considering the longer hunger suppression induced by exercise performed after a meal compared with exercising after an overnight fast previously reported (Cheng et al. 2009; Deighton et al. 2012), it is possible that this effect may be mediated, at least partly, by the different concentrations of acylated ghrelin. In this regard, however, Cheng and colleagues (2009) did not observe any difference in acylated ghrelin between fast and fed exercise whereas Deighton and colleagues (2012) did not examine differences in this gut peptide in their study. Although the evidence from Cheng and colleagues (2009) appears to suggest no differences in acylated ghrelin responses after manipulation of the study protocol, additional investigations comparing the appetite responses to fed versus fast exercise may be useful for a greater understanding of acylated ghrelin in response to exercise.

Very few investigations have explored the effects of chronic exercise on ghrelin concentrations by examining differences in acylated ghrelin levels between habitual physical active and inactive individuals. For example, Lund et colleague (2013) examined appetite-related hormone changes after the ingestion of a standardised liquid meal between young inactive men, who had not performed exercise in the last six months, and endurance-trained athletes (Lund et al. 2013). In this report, concentrations of fasting acylated ghrelin were greater in the active group than the inactive group, whereas concentrations of postprandial acylated ghrelin did not differ between groups (Lund et al. 2013). In a previous study, middle aged sedentary postmenopausal women, who typically exhibit similar fasting ghrelin concentrations than premenopausal women (Stojiljkovic-Drobnjak et al. 2018; Iwamoto et al. 2005), exhibited 18% increase in fasting total ghrelin concentrations after one year of a regular exercise programme (45 min of moderate intensity aerobic exercise, for 5 days per week) compared with a group of women who only performed 45 min of stretching sessions once per week for one year (Foster-Schubert et al. 2004). However, in the same study the authors did not observe a significant association between increased physical activity with plasma ghrelin, and the elevation of ghrelin was concomitant with the reduction of body weight which may have confounded the results. Furthermore, in both studies levels of habitual physical activity were measured using self-reported physical activity, which represents a limitation due to issues of participant recall bias which makes it difficult to accurately correspond self-reported with actual physical activity (Prince et al. 2008). Thus, because only a few studies have examined acylated ghrelin concentrations in relation to chronic exercise and because the actual evidence are based on self-reported physical activity, future studies investigating relations between acylated ghrelin concentrations and physical activity objectively-measured are needed.

1.5.2.2 Peptide YY

As stated previously, peptide YY is an anorexigenic gastrointestinal hormone acting in opposition to acylated ghrelin. The first study to investigate circulating concentrations of PYY in response to exercise was performed by Martins and colleagues (2007). In this study, 60 min of continuous cycling at 65 % of maximum heart rate induced an elevation in plasma total PYY concentrations during and upon completion of cycling in healthy males and females. In the same study, a reduction in hunger occurred in parallel with the increase of PYY with both parameters returning to control values within 30 min after exercise, which suggested a causal role of this satiety hormone in exercise-induced anorexia. Similar transient increases in total PYY have been then replicated by other studies which have examined the effect of moderate-to-vigorous aerobic exercise (≥ 60% $\dot{V}O_2$ max) on total PYY (Broom et al. 2009; Deighton et al. 2013a; Douglas 2015; Wasse et al. 2012; Kawano et al. 2013; Douglas et al. 2017). Such transient increases in circulating total PYY levels have been also shown in other studies which employed a lower exercise intensity (≤ 60% $\dot{V}O_2$ max) (Cooper et al. 2011; Ueda et al. 2009).

In contrast to the aforementioned studies, other studies have reported no changes in total PYY levels after 40 - 45 min of exercise at 70 % of VO_2 max (Balaguera-Cortes et al. 2011; Kelly et al. 2012; Shorten et al. 2009). Although the reasons for such disparity are unclear, exercise duration may cause this as these studies used shorter exercise protocols than those that have shown an increase in PYY after exercise. Additionally, Balaguera-Cortes and colleagues (2011) confirmed previous studies in which concentrations of total PYY do not change in response to intermittent resistance exercise (Broom et al. 2009). Although these findings appear to suggest that a prolonged exercise protocol is needed to elevate circulating PYY concentrations, Kawano et al. (2013) demonstrated that 30

min of skipping and cycling exercise at approximately 65% of VO_2 max induced a transient elevation in total PYY upon completion of exercise. Additionally, according with the anorexigenic effect of PYY, appetite perceptions appear to decrease during exercise when PYY levels are increased (Kawano et al. 2013) but remain unchanged when circulating concentrations of PYY are unaffected (Kelly et al. 2012).

Increase in total PYY in response to exercise have been also observed in overweight/obese individuals. In this regard, Ueda and co-workers (2009) investigated whether changes in total PYY levels in response to acute aerobic exercise differ between obese and normal weight males. Levels of total PYY were reported higher in response to exercise in both groups, suggesting an effect of exercise on PYY independent of bodyweight. However, this contrasts with a more recent study which reported greater total PYY elevation in lean compared to overweight/obese, males and females, in response to 60 min running at 59 % peak oxygen uptake (Douglas et al. 2017). The implications of this disparity, however, remain unclear and additional work is definitely required to ascertain whether total PYY in response to exercise may be mediated by differences in adiposity (Dorling et al. 2018).

A further important consideration is that the aforementioned investigations measured total PYY which includes concentrations of both forms: PYY_{1-36} and PYY_{3-36}. Nonetheless, the appetite-suppressing effects of PYY are thought to be mediated particularly by PYY_{3-36} (Sloth et al. 2007). Thus, although strong correlations between changes in total PYY and PYY_{3-36} have been reported (Tsilchorozidou et al. 2008), some studies have specifically examined the effect of exercise on PYY_{3-36}. In this regard, the effect of exercise on PYY_{3-36} seems to be similar to total PYY as transient elevations have been shown upon completion of prolonged exhaustive running (Russel et al. 2009)

but also during and immediately after 60 min of walking and running exercise at 70 % of VO_2 max (Larson-Meyer et al. 2012). Furthermore, the association between PYY_{3-36} and appetite feelings during exercise also needs additional investigation as an inverse temporal pattern has been shown in some (King et al. 2011b; Ueda et al. 2009a) but not all studies (Hagobian et al. 2013; Larson-Meyer et al. 2012).

A limitation concerning the examination of the effect of exercise on circulating PYY concentrations, is that the majority of previous work has focused merely on the effect of acute exercise with only one study to our knowledge examining the impact of chronic exercise on this gut peptide (Lund et al. 2013). In this report, concentrations of fasting and post-prandial total PYY were similar between active and inactive individuals (Lund et al. 2013). Therefore, future studies investigating the influence of chronic exercise on plasma PYY levels may be relevant in determining how different levels of physical activity modulate this gut hormone and appetite and energy intake responses.

1.6 Summary

The elevated risk of CVD and T2D in South Asians has been linked to the higher prevalence of different traditional risk factors including greater adiposity, insulin resistance or dyslipidaemia. However, these risk factors do not exhaustively explain the excess cardiometabolic risk in South Asians compared with white Europeans. Considering chronic surplus of energy intake as a possible contributor leading to body fat accumulation, we theorized that the elevated adiposity and associated cardiometabolic risk in South Asians than white Europeans may be linked with ethnic differences in eating behaviour and appetite-regulating hormones such as acylated ghrelin and peptide YY (PYY) concentrations. Furthermore, fasting ghrelin concentrations have been shown to be lower in individuals with elevated adiposity and exhibiting insulin resistance (Le Roux

et al. 2005; McLaughlin et al. 2004). Thus, we hypothesised ethnic differences in circulating appetite-regulating hormones concentrations, particularly lower levels of acylated ghrelin in South Asian than white European men. Furthermore, the examination of additional parameters including appetite-related hormones, CRP, IL-6, leptin as well as FFAs, based on advanced metabolomics analytical methods, may add further information of the elevated CVD and T2D susceptibility amongst the South Asian community. Low levels of physical activity and fitness levels in South Asians may contribute to exacerbate the elevated adiposity and associated cardiometabolic risk, which may be also linked to ethnic differences in appetite regulation and food intake. Conversely, acute moderate-to-vigorous exercise has shown to promote short-term energy deficit suggesting an important role of physical activity to enhance weight and fat loss if exercise is performed frequently; however, the effects of exercise on short-term energy balance in South Asians remain unknown. Thus, the aims of the present thesis were threefold: *(1)* investigate ethnic differences in traditional risk markers and unconventional parameters for CVD and T2D including acylated ghrelin, PYY, leptin, glucose tolerance, insulin, TC, TAG, HDL-C, LDL-C, CRP and IL-6 and explore relationships of these parameters with objectively-measured physical activity and cardiorespiratory fitness; *(2)* examine ethnic differences in the FFA metabolic profile based on advanced metabolomics analytical methods such as GC-MS and LC-MS and explore relationships with objectively-measured physical activity and cardiorespiratory fitness and risk markers for CVD and T2D; and *(3)* investigate the effects of acute exercise on subjective appetite ratings, appetite-related hormones and ad libitum energy intake in healthy South Asian and white European men.

Chapter 2 – General Methods

This chapter describes the experimental methods employed in the three studies presented within this thesis. Specifically, the aims of the present thesis were to:

- investigate ethnic differences in unconventional parameters for CVD and T2D including fasting concentrations of plasma acylated ghrelin and total PYY (primary aim). Study 1 (Chapter 3);
- investigate ethnic differences in traditional risk markers for CVD and T2D such as metabolic markers (i.e. plasma concentrations of glucose and insulin) and inflammatory markers (i.e. plasma concentrations of CRP and IL-6) (secondary aim). Study 1 (Chapter 3);
- explore associations of unconventional and traditional risk markers for CVD and T2D with adiposity and physical activity/cardiorespiratory fitness in South Asian and white European men (secondary aim). Study 1 (Chapter 3);
- examine ethnic differences in the FFA metabolic profile based on metabolomics methods (primary aim). Study 2 (Chapter 4);
- explore relationships of FFA concentrations with adiposity and physical activity/cardiorespiratory fitness in South Asian and white European men (secondary aims). Study 2 (Chapter 4);
- investigate the effects of acute exercise on subjective appetite-related hormones, appetite feelings and *ad libitum* energy intake in healthy South Asian and white European men (primary aims). Study 3 (Chapter 5);
- explore associations of appetite measures with adiposity and physical activity/ cardiorespiratory fitness in South Asian and white European men (secondary aims). Study 3 (Chapter 5).

All studies were conducted following the approval of Kingston University's Ethics Advisory Committee, and all volunteers were fully informed about the aims, procedures and potential risks before participating in these experiments

2.1 Participants

Participants were voluntarily recruited from Kingston University and the general public via general notices such as posters, university notice board, social media and by word of mouth. Study 1 (Chapter 3) and study 2 (Chapter 4) included the same participant cohort whereas in study 3 (Chapter 5) different participants were recruited. Before attending the laboratory, participants received by email or by post the following documents:

- participation informed consent form (Appendix A)
- health screening questionnaire (Appendix B)
- pre-screening form for blood sampling (Appendix C)
- physical activity readiness questionnaire (PARQ) for exercise screening (Appendix D) which is routinely used in exercise studies prior undertaking an exercise testing (Thomas et al. 1992)
- Standardised breakfast and buffet meal food list (Appendix E)

Participants were asked to sign and fill the above documents prior to participating in the experimental studies to ensure they were suitable to take part in these experiments. Furthermore, participants were free to contact the investigators for any clarification related to the study before attending the laboratory and it was also reiterated to them that they were free to withdraw from the study at any point during the study without giving reason.

As described in subsection 1.2.2, males are reported to experience greater cardiometabolic risk than female individuals in the United Kingdom thus, in all studies we targeted male volunteers and aged 19 – 50 years to ensure health suitability for undertaking the incremental exercise test to volitional exhaustion for the determination of cardiorespiratory fitness ($\dot{V}O_2$ max). Additionally, the inclusion criteria for participation were as follows:

- non-smoker
- no personal history of cardiovascular disease or metabolic disorders
- not taking anticoagulant or anti-inflammatory medication
- fit and well to participate in the maximal testing following Physical Activity Readiness Questionnaire clearance
- not dieting or undertaking any extreme dietary habits
- weight stable for the last three months, i.e. < 2.3 kg change in body weight (St Jeor et al. 1997).

2.2 Anthropometry

Body mass was measured to the nearest 0.1 kg using a digital scale (Seca Ltd, Hamburg, Germany), and stature was measured to the nearest 0.1 cm using a portable stadiometer (Seca Ltd, Birmingham, UK). Participants wore light clothing and removed shoes, jewellery and all items from pockets for body mass and height measurements. Body mass index (BMI) was subsequently calculated as mass (kg) divided by stature squared (m^2). Waist circumference was measured in duplicate to the nearest 0.1 cm at the midpoint between the xiphoid process and the iliac crest using a standard anthropometric measuring tape (HaB International Ltd., Southam, UK), and the mean of the two

measurements was recorded. Body composition was assessed using air displacement plethysmography (BodPod; software version 5.2.0, COSMED, Rome, Italy).

2.3 Heart rate measurement

During preliminary tests and main trials, heart rate was monitored using a short-range radio telemetry system (Polar FT1, Polar Electro, Kempele, Finland).

2.4 Rating of perceived exertion

Rating of perceived exertion was assessed periodically during preliminary exercise tests and main trials using the Borg scale (Borg 1973) to determine participant's level of exertion. This scale ranges from six (no exertion) to 20 (maximal exertion).

2.5 Blood pressure measurement

Arterial blood pressure was measured using a digital monitor (Omron M10-IT, Omron Healthcare Co. Ltd., Japan) during preliminary screening and measurements were taken after a 10 min of seated rest in a semi-supine position in duplicate from the left arm. The mean of these measurements was used as the final value.

2.6 Cardiorespiratory tests

In all studies, after familiarisation with the electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands), participants performed an incremental exercise test to volitional exhaustion for the determination of maximum oxygen consumption ($\dot{V}O_2$ max). In Study 1 (Chapter 3), participants cycled at a self-selected pedal rate between 70 to 90 revolutions per minute for 3 min at 50 watts (warm up), followed by increments of 6 watts every 15 s until volitional fatigue. However, using this protocol most of participants reached their $\dot{V}O_2$ max beyond twelve minutes whereas

existing research in the literature suggests that measurement of maximal responses should last between 8 and 12 minutes (Pierce et al. 1999). Therefore, the $\dot{V}O_2$ max protocol in Study 3 (Chapter 5) was optimised by increasing the initial work rate to 80 watts per 3 min (warm up), instead of 50 watts, followed by increments of 30 watts every 3 min until volitional fatigue, which provided $\dot{V}O_2$ max readings within recommended protocols time.

Expired air samples were monitored continuously using an online breath-by-breath gas analysis system (Oxycon Pro, Viasys Healthcare Gmbh, Höchberg, Germany). An average of the breath-by-breath $\dot{V}O_2$ data was calculated every 15 s, and $\dot{V}O_2$ max was recorded as the highest 15 s average. Prior to testing, the gas analysis system was calibrated using a 3 L syringe (series 5530, Hans Rudolph Inc, Shawnee, KS, USA) with certified reference gases according to the manufacturer's instruction. Throughout the $\dot{V}O_2$ max test, heart rate and rating of perceived exertion were monitored. Oxygen consumption, heart rate and peak watts were used to determine the appropriate intensity of work during the exercise trial in Study 3.

2.7 Environmental temperature and humidity

Prior commencement and throughout each trial, environmental temperature and humidity were assessed using a hand-held sensor (THGR810, Oregon Scientific UK Ltd., Berkshire, UK).

2.8 Calculation of energy expenditure

Oxygen consumption and carbon dioxide production values during exercise trials in Study 3 were used to determine energy expenditure and substrate oxidation using the equations described by Weir (1990).

2.9 Physical activity and dietary control

Participants were asked to refrain from consuming alcohol, caffeinated drinks and from participating in strenuous exercise during the 24 h prior to each visit. Participants were also asked to exert themselves minimally when travelling to the laboratory, using motorised transport where possible. Prior to visit 1 in Study 1 (Chapter 3), and prior to the main trials in Study 3 (Chapter 5), volunteers fasted overnight (no food or drink except water) for 9 hours whereas they were required to fast for 3 hours before performing the incremental exercise test on the second visit in Study 1. Participants were encouraged to consume at least 500 mL of plain water the night before the experimental trials to ensure euhydration.

In Study 3 (Chapter 5), a food diary (Appendix F) was completed in the 24 h prior to the first trial, with participants required to replicate food and drink intake as closely as possible for the 24 h prior to the subsequent trial. Participants were instructed to consume identical amounts of food and drink items at identical times during this period to ensure dietary standardisation before each trial.

2.10 Blood sample collection

On the morning of visit 1 in Study 1 (Chapter 3 and 4) and main trials in Study 3 (Chapter 5), a fasting venous blood sample was collected from volunteers by a trained phlebotomist after resting in a semi-supine position for 15 min.

In Study 1, blood samples were collected from the antecubital vein using a 25 g butterfly needle (BD Vacutainer®, Plymouth, UK) whilst participants were in a semi-supine position for the measurement of total cholesterol, high density lipoprotein cholesterol,

low density lipoprotein cholesterol, triacylglycerol, C-Reactive protein, interleukin 6, leptin, acylated ghrelin and total PYY concentrations. Samples were collected into four pre-cooled vacutainers: 10.0 mL EDTA, 4.0 mL EDTA, 5.0 mL SST and 6.0 mL heparin (BD Vacutainer®, Plymouth, UK). To prevent the degradation of acylated ghrelin, a 40 µL solution containing potassium phosphate buffer (PBS), p-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH) was added immediately to the 4.0 mL EDTA vacutainer which was then centrifuged at 1500 × *g* for 10 min at 4°C (Rotina 420R, Andreas Hettich GmbH & Co., Berlin, Germany). The plasma supernatant was dispensed into a storage tube and 100 µL of 1 M hydrochloric acid was added per millilitre of plasma to preserve acylated ghrelin (Hosoda et al. 2004). Thereafter, samples were spun at 1500 × *g* for 5 min at 4°C prior to storage at -80ºC. The 6.0 mL heparin and 10 mL EDTA vacutainers were centrifuged immediately, while the 5.0 mL SST vacutainer was left at room temperature for 30 min before centrifugation using the same conditions. During the OGTT, whole blood was collected using the finger-prick technique into a 20 µL heparin capillary tube (Sanguis Counting, Nümbrecht, Germany) for glucose analysis and into a 300 µL EDTA Microvette tube (Microvette® CB 300 K2E, Starstedt, Leicester, UK) for insulin analysis. The heparin tube was immediately mixed into a separate 1 mL haemolysing solution and then analysed. The EDTA tube was immediately centrifuged at 1500 × *g* for 10 min at 4°C (Eppendorf® Microcentrifuge 5415R, Eppendorf AG, Hamburg, Germany) and the plasma supernatant was then dispensed into aliquots and stored at -80 °C for later analysis.

In Study 3, venous blood samples were collected via a cannula (Vasofix® Safety, B. Braun, Melsungen, Germany) inserted into an antecubital vein for the determination of acylated ghrelin, PYY, insulin and glucose. During each trial, all blood samples were

collected with the participants rested in a semi supine position with the exception of the sample at 3 h time point in the exercise trial, which was taken with the participant seated, but not pedalling, on the cycle ergometer at the end of the exercise. Samples were collected into four pre-cooled vacutainers: 6.0 mL heparin, 10.0 mL EDTA and two, 4.0 mL EDTA (BD Vacutainer®, Plymouth, UK). To prevent the degradation of acylated ghrelin, same procedure as above was followed for one 4.0 mL EDTA vacutainer. The 6.0 mL heparin and 10 mL EDTA vacutainers were centrifuged immediately at 1500 × *g* for 10 min at 4°C prior to storage at -80ºC. The other 4.0 mL EDTA vacutainer was immediately analysed for determination of haemoglobin and haematocrit.

To avoid blood clot, the cannula was kept cleaned by flushing it with 10 mL 0.9% Sodium Chloride syringe (Becton Dickinson UK Ltd., Berkshire, UK) after each blood sample. To avoid dilution of subsequent samples, residual saline was drawn off immediately prior to blood collection.

2.11 Blood analysis

2.11.1 Haemoglobin and haematocrit

Blood haemoglobin and haematocrit concentrations were analysed in duplicate using a haematology blood counter (Yuminez H500-CT, HORIBA ABX Diagnostic, Northampton, UK) to determine plasma volume changes over time (Dill and Costill, 1974).

2.11.2 Free fatty acids

The examination of the free fatty acid metabolic profile was initially conducted at Kingston University based on gas chromatography–mass spectrometry (GC-MS). The blood samples for the FFA analysis were obtained from the same set of participants recruited in Study 1 (Chapter 3). Full details of the FFA analysis based on GC-MS is

described in Chapter 4.2.7.1. However, due to inadequate results, liquid chromatography–mass spectrometry (LC-MS) was subsequently employed for the identification and quantification of FFAs in South Asian and white European participants and the analysis was conducted at the University of Strathclyde. Details of the FFA analysis based on LC-MS are fully described in Chapter 4.2.7.2.

2.11.2.1 Chemicals and Solvents

High-performance liquid chromatography (HPLC) grade acetonitrile (ACN), water, acetic acid and hexane were obtained from Fisher Scientific (Leicestershire, UK). A mixture of fatty acid methyl ester standards (Supelco 37-component fatty acid methyl ester mix) was obtained from Sigma Aldrich (Dorset, UK). The methyl esters were hydrolysed with 1 M KOH by heating at 60ºC for 15 minutes, the mixture was acidified and extracted into hexane. The hexane stock solution was diluted to the levels required for the calibration curves with ethanol. 31H2-palmitic acid which was used as an internal standard was obtained from Sigma Aldrich (Dorset, UK). Plasma samples were prepared by mixing aliquots of plasma (0.3 ml) with 0.2 ml of acetonitrile containing 8 $\mu g \cdot mL^{-1}$ of internal standard. A calibration series was prepared by mixing the diluted fatty acid stock solution with the internal standard to give an internal standard concentration of 4.8 $\mu g \cdot mL^{-1}$ mixed with fatty acid standards in the range 0.8-38. $\mu g \cdot mL^{-1}$ (the original standard mixture contained fatty acids at different concentrations: 0.2, 0.4 or 0.6 $mg \cdot mL^{-1}$).

2.11.2.2 Instrumental techniques and column

Free fatty acids were profiled by using a Dionex 3000 HPLC system interfaced to an Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) with the aid of a reversed phase column (ACE C4, 150 × 3.0 mm, 3 µm, HiChrom, Reading

UK). The mobile phase for the elution of the ACE C4 column consisted of 1 mM acetic acid in water (A) and 1 mM acetic acid in acetonitrile (B) at a flow rate of 0.4 mL · min^{1}. The elution gradient was as follows: A:B ratio 40:60 at 0 min, 0:100 at 30 min, 0:100 at 36 min, 40:60 at 37 min and 40:60 at 41 min.

2.11.2.3 Mass spectrometry run conditions

The nitrogen sheath and auxiliary gas flow rates were maintained at 50 and 17 arbitrary units. The electrospray ionisation (ESI) interface was operated in both positive and negative modes. The spray voltage was 4.5 kV for the positive mode and 4.0 kV for negative mode, while the ion transfer capillary temperature was 275°C. Full scan data was obtained in the mass-to-charge range of m/z 75 to m/z 1200 for both ionisation modes. The MS system was fully calibrated prior to running the samples according to the manufacturer's guidelines. The resulting data was acquired using the XCalibur 2.1.0 software package (Thermo Fisher Scientific, Bremen, Germany).

2.11.3 Appetite-related hormones

Commercially available enzyme-linked immunosorbent assay kits were used to determine concentrations of key appetite regulating hormones such as appetite-stimulating plasma acylated ghrelin (Bertin Bioreagent, Montigny le Bretonneux, France for Study 1; Sceti K.K., Tokyo, Japan for Study 3) and appetite inhibiting hormones including total PYY and leptin (Millipore, Billerica, USA). Absorbance was measured using a plate reader (Infinite M200 PRO, Tecan Group Ltd., Männedorf, Switzerland) at specific wavelengths as specified by the manufacturer. Precision of analysis for acylated ghrelin was ensured by the quantification of an internal quality control and for total PYY and leptin by the quantification of internal quality controls exhibiting low and high

values. The within-batch coefficients of variation for the assays are reported in detail in Chapter 3 and 5.

2.11.4 Metabolic parameters

Plasma glucose concentrations were analysed in singular using a glucose analyser (Biosen C-Line Clinic, EKF Diagnostic, Germany) whereas plasma concentrations of insulin was measured using a commercially available enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden) with the aid of a plate reader (Infinite M200 PRO, Tecan Group Ltd., Männedorf, Switzerland) to measure absorbance at specific wavelengths as specified by the manufacturer. Precision of analysis for insulin was ensured by the quantification of internal quality controls (Mercodia diabetic antigen control) exhibiting low and high values. The within-batch coefficients of variation relating to the assays are reported in Chapters 3 and 5.

2.11.5 Lipid parameters

Plasma total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triacylglycerol concentrations were analysed in duplicate by enzymatic, colorimetric methods using an automated bench top analyser (Pentra 400; HORIBA ABX Diagnostics, Montpellier, France). To ensure precision of analysis, internal quality controls exhibiting low and high values were run prior to sample analysis. The within-batch coefficients of variation for the assays are reported in detail in Chapter 3.

2.11.6 Inflammatory markers

Commercially available enzyme-linked immunosorbent assay kit was used to determine concentrations of plasma C-Reactive protein and Interleukin 6 (high sensitivity kit, IBL International, Hamburg, Germany) with the aid of a plate reader (Infinite M200 PRO,

Tecan Group Ltd., Männedorf, Switzerland) to measure absorbance at specific wavelengths as specified by the manufacturer. Precision of analysis was ensured by the quantification of internal quality controls exhibiting low and high values. The within-batch coefficients of variation relating to the assays are reported in Chapter 3.

2.12 Data processing and statistical analysis

The Quan Browser in Xcalibur was used to plot calibration curves (weighted with 1/x) and quantify the responses for the samples against the calibration curves in Study 2 (Chapter 4). Then the levels of FFAs in the samples were calculated from the calibration curves by Quan Browser. *P* values and ratios of the mean values for the fatty acids were determined by using Microsoft Excel (Microsoft Office 2013). SIMCA-P version 14.1 (Umetrics, Umeå, Sweden) was used for multivariate analysis (Trivedi et al. 2012) which included Principle Components Analysis (PCA), Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) and Orthogonal Partial Least Square (OPLSA). PCA and OPLS-DA models are powerful statistical modelling tools that provide insights into separations between experimental groups and are routinely used in metabolomics studies to better support the visualization and interpretation of the data (Yamamoto et al. 2009). Particularly, PCA is an unsupervised model employed to explore how variables cluster and is considered the main tool used for data reduction to extract meaningful information which is achieved by combining variables that correlate with each other into few latent variables (components) (Kirwan et al. 2012). PCA is normally employed as the first step in the analysis of metabolomics data in order to visualise data and detect outliers (Kirwan et al. 2012). OPLS-DA is an unsupervised model used as an alternative method when PCA fails to expose group separation and is employed to identify reliable biomarkers that have a strong association with separation between groups (Kirwan et al. 2012). The

quality of the supervised model is typically assessed via cross-validation procedures to determine the degree of significance of the model using internal and external diagnosis tools (Triba et al. 2015). In internal validation, R^2 represents the percentage of variation explained by the model whereas Q^2 indicates the percentage of variation in response to cross validation. In addition to internal validation tools, the permutation test and cross validated analysis of variance (CV-ANOVA) as external diagnosis tools are performed to validate supervision model (Worley and Powers, 2013).

Statistical analyses for all the other data in were conducted using the analytical software SPSS version 23.0 for Windows (SPSS 23.0, IBM Corp, Armonk, NY, USA). Normality of these data were checked using Shapiro-Wilk tests before statistical analysis. Normally distributed data are presented as mean (SD) in text and tables whereas data were natural log transformed before analysis if they were not normally distributed. These data are presented as geometric mean (95% confidence interval) in text and tables and analysis is based on ratios of geometric means and 95% confidence intervals (CI) for ratios. Graphical representation of results is presented as mean (SEM) for clarity. Linear mixed model (LMM) was used as a statistical model instead of two-way analysis of variance (ANOVA). An important assumption of ANOVA is that the covariate is independent from the treatment effect (Field, 2017). In Study 1 (Chapter 3) this assumption was violated because body fat percentage, used as a covariate in the analysis of the fasting plasma concentrations, was significantly different between South Asian and white European men. Thus, because the use of this model may not be robust enough when assumptions are violated, any effects from the ANOVA such as ethnic differences between groups are more likely to disappear (Field, 2017). Conversely, LMM are less reliant on assumptions and more robust, thus in study 1 (Chapter 3) it was decided to

present both the unadjusted and adjusted models for transparency. The other data were also analysed based on the LMM modelling for consistency. Particularly, LMM including fixed factors such as ethnicity in Study 1 (Chapter 3) and ethnicity, time and trial in Study 3 (Chapter 5) were used to examine differences between ethnic groups. Detailed description of the statistical tests used for data analysis are presented in Chapter 3, 4 and 5, respectively. In study 3 (Chapter 5), appetite perceptions and appetite-related hormones concentrations were reported as delta changes instead of absolute values. Given the great day to day variability in appetite-related hormones, the use of delta changes appears to be more meaningful and indicative of appetite changes throughout the day than the raw absolute values themselves. In study 3 (Chapter 5), glucose concentrations were also reported as delta changes for consistency. Pearson's correlation (Study 1, Chapter 3) and Spearman's correlation (Study 3, Chapter 5) coefficients were used to examine relationships between variables and statistical significance was accepted as $P < 0.05$.

Chapter 3 – Study 1: Ethnic differences in appetite-related hormones and risk markers for cardiovascular disease and type 2 diabetes in healthy South Asian and white European men

3.1 Introduction

South Asians represent the largest ethnic minority group in the United Kingdom (UK) (~3 million, 4.9% of the population) (Office for National Statistics 2013), and comprise individuals originating from the Indian subcontinent. It is well established that South Asians have an elevated risk of CVD and T2D both on the Indian subcontinent and after migration to Western nations (Sattar and Gill, 2015; Gholap et al. 2011; Khunti et al. 2013). Furthermore, CVD and T2D manifest 5 to 10 years earlier, at a lower body mass index, and are associated with premature complications and mortality in South Asian compared with white European individuals (Sattar and Gill, 2015; Gholap et al. 2011).

The elevated risk of CVD and T2D in South Asians has been linked to the higher prevalence of insulin resistance and associated CVD risk factors including differences in adiposity as well as markers of inflammation and metabolic health (Gholap et al. 2011; Joshi et al. 2007). Specifically, South Asians have a greater percent body fat and accumulation of visceral adipose tissue for a given BMI compared with white Europeans (Lear et al. 2007). Furthermore, compared with other ethnic groups, South Asians are more insulin resistant, glucose intolerant, dyslipidaemic and exhibit a less favourable inflammatory profile CRP and IL-6 (Gholap et al. 2011; Tziomalos et al. 2008). However, these risk markers seem to explain only partially the greater cardiometabolic risk in the South Asian population and the mechanisms underlying increased

susceptibility of CVD and T2D remain poorly understood (Forouhi et al. 2006). Therefore, profiling a greater array of parameters may provide a more holistic insight into cardiometabolic health outcomes in South Asian and white European individuals.

A well-established postulation for the increased CVD and T2D risk in South Asians is their higher levels of body fatness which may be linked with differences in appetite between South Asian and other ethnicities. Several appetite-related hormones have been implicated in the short-term regulation of food intake, including acylated ghrelin and PYY which exert orexigenic and anorexigenic effects, respectively (Hussain and Bloom, 2013). However, it is not known whether circulating acylated ghrelin and PYY concentrations are different between South Asian and white European individuals. Previous evidence has identified ethnic differences in circulating adipokines with individuals of South Asian descent exhibiting elevated leptin concentrations compared with white European individuals (Mente et al. 2010). Leptin circulates at concentrations proportional to body fatness (Considine et al. 1996) and plays a central role in regulating long-term changes in energy homeostasis and body fat.

Physical inactivity is estimated to explain >20% of the excess coronary heart disease (CHD) mortality in UK South Asians after adjustment for potential confounding factors such as socioeconomic status, smoking, diabetes and existing CVD (Williams et al. 2011a). Low levels of physical activity and cardiorespiratory fitness amongst South Asians are likely to contribute to exacerbating the excess insulin resistance and CVD risk in this population (Yates et al. 2015; Williams et al. 2011b). Previous observational evidence suggests that a higher level of self-reported physical activity is associated with a lower risk of CVD and T2D in South Asian individuals (Rastogi et al. 2004; Mohan et

al. 2005). However, there is limited evidence examining cardiorespiratory fitness and objectively-measured physical activity in South Asians whereas associations between these factors and appetite-related hormones remain unknown.

Therefore, the aim of this study was to investigate ethnic differences in appetite-related hormones and a variety of traditional risk markers for CVD and T2D such as glucose tolerance, insulin, TAG, HDL-C, CRP, IL-6 and leptin in South Asian compared with white European men. In addition, this study aimed to quantify objectively the levels of physical activity and cardiorespiratory fitness in South Asian and white European men and to examine relationships with appetite-related hormones and risk markers for CVD and T2D.

3.2 Methods

3.2.1 Participants

A total of 16 South Asian and 16 white European men matching the inclusion criteria listed in Chapter 2.1, volunteered to participate in this study. The study was approved by Kingston University's Faculty Ethics Committee (Ethic approval code: 1516/017) and written informed consent was provided by participants before the study commenced. The sample size was calculated using G*Power (Faul et al. 2007). Based on previous data (Chandalia et al. 2007), it was estimated that a sample size of 16 participants per group would have 89% power at the 0.05 level to detect a between-group difference in fasting leptin of 1.05 between-subject SDs. The South Asian group comprised eight British Asians born in the UK (UK Indian n=5; UK Pakistani n=1; UK Sri Lankan n=2) and eight individuals born in South Asia (India n=5; Pakistan n=1; Sri Lanka n=1; Nepal n=1). Conversely, the white European group comprised nine British born participants and seven individuals originating from European countries (Spain n=1; Italy n=2; France n=1; Germany n=1; Poland n=1; Check Republic n=1). Groups were matched for BMI.

3.2.2 Study design

Using a cross-sectional observational design, participants attended the laboratory on two occasions separated by an interval of 7 to 14 days (Figure 3.1). As described in Chapter 2.9, participants were asked to avoid strenuous exercise and not to consume caffeine or alcohol in the 24 h period prior to visits 1 and 2. Participants were also asked to exert themselves minimally when travelling to the laboratory, using motorised transport when possible. Participants also consumed 500 mL of plain water the night before visit 2 to ensure euhydration before the exercise test.

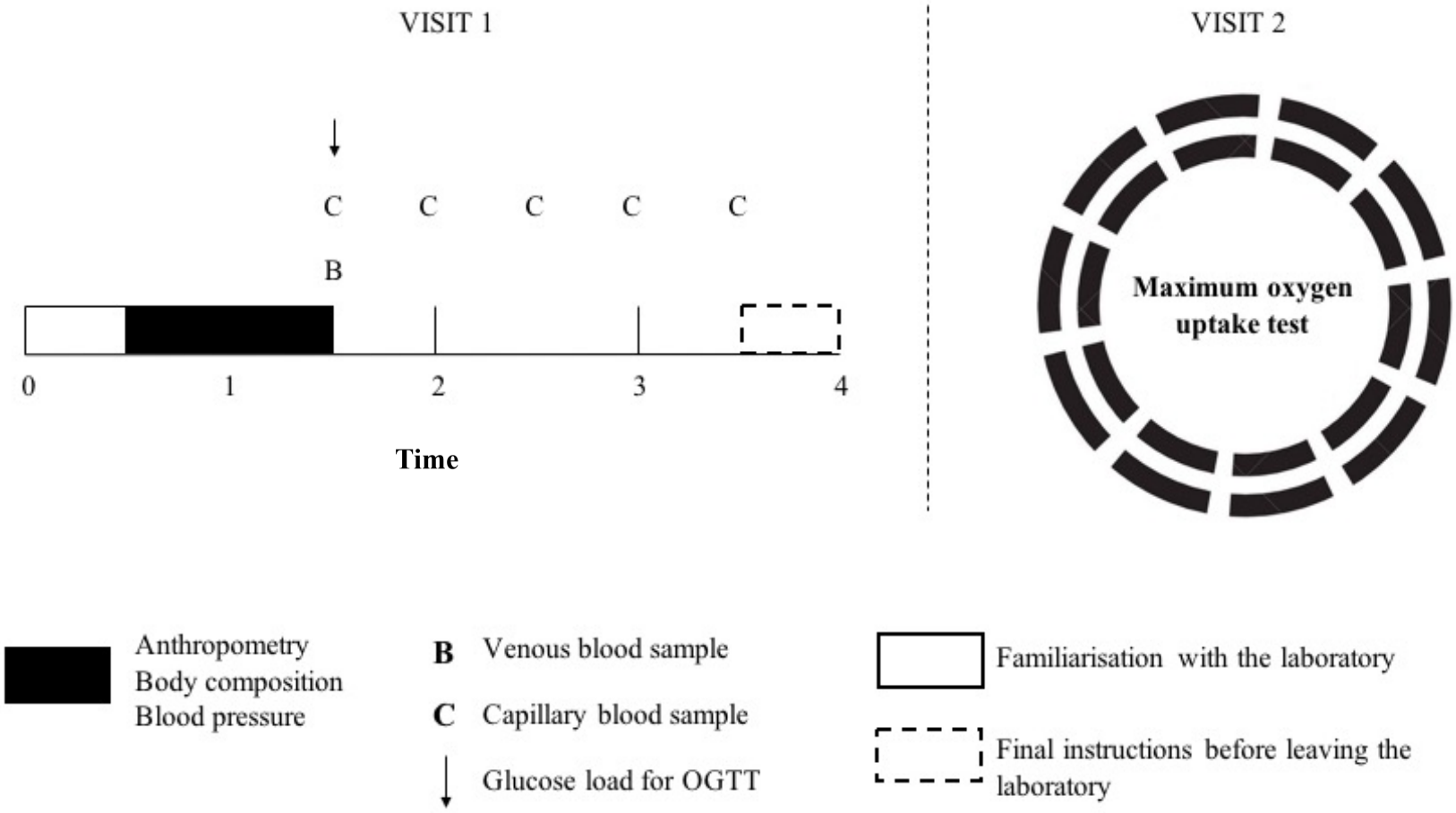

Figure 3.1. Schematic representation of the study design.

3.2.3 Visit 1

3.2.3.1 Anthropometry and blood pressure

Participants arrived at the laboratory between 08:00 and 09:00 after a 9 h overnight fast and completed a 4 h trial. After familiarisation with the laboratory equipment and completing the written informed consent form (Appendix A) and screening questionnaires (Appendix B, C and D) as detailed in Chapter 2.1, volunteers were asked to undergo anthropometric measurement and blood pressure measurements. Body mass, stature, waist circumference, BMI, body composition and blood pressure were then measured as described in Chapter 2.2 and 2.5.

3.2.3.2 Fasting metabolic assessment and oral glucose tolerance test

After completion of the anthropometric and blood pressure measurements, a fasting venous blood sample was obtained from the antecubital vein by a trained phlebotomist for the measurement of appetite-related hormones, inflammatory markers and lipid profiling. A fasting fingertip capillary blood sample was taken to determine insulin and glucose concentrations. Participants then consumed a 75 g glucose load (100% dextrose, BulkPowderTM, Colchester, UK) dissolved in 300 mL of water, marking the start of the oral glucose tolerance test (OGTT). Subsequent fingertip capillary blood samples were collected every 30 minutes for two hours (0.5, 1, 1.5 and 2 h) to quantify glucose and insulin concentrations whilst participants rested in the semi-supine position. Before leaving the laboratory, participants were given an accelerometer, food diary and food scale and instructed how to use them to record their physical activity and food intake before returning for the second visit.

3.2.4 Visit 2

3.2.4.1 Maximum oxygen uptake test

After a 3 h fast, participants performed an incremental exercise test to volitional exhaustion on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) for the determination of maximum oxygen consumption ($\dot{V}O_2$ max) as described in Chapter 2.6.

3.2.5 Habitual physical activity and sedentary time

Between visits 1 and 2, participants wore an ActiGraph GT3X+ accelerometer (ActiGraph, Pensacola, USA) on the right hip (posterior to the anterior superior iliac spine), attached to an elastic belt supplied by the manufacturer for seven consecutive days during waking hours (except water-based activities). All devices were initialised to record counts and steps and raw data files were analysed using the manufacturer's software (ActiLife v6.2; ActiGraph, Pensacola, USA). Data from participants with at least 10 h of daily wear time for at least four days were included in the analysis. A 60 s sampling epoch was used throughout and non-wear time, defined as ≥ 60 min of consecutive zero counts, was removed from the analysis (Troiano et al. 2008). Physical activity was expressed as average counts per minute (CPM) and standard cut-points for adults were applied to quantify sedentary time (<100 CPM), light activity (100–1951 CPM) and moderate-to-vigorous activity (> 1951 CPM) (Freedson et al. 1998).

3.2.6 Dietary intake

Participants weighed and recorded their dietary intake on three consecutive days including two weekdays and one weekend day. Participants were also asked to take a digital photograph of all food and drink items consumed during the three-day assessment

period which were matched to the diet record. Three-day diet records were analysed using Dietplan 6 software (Forestfield Software Ltd, Horsham, UK).

3.2.7 Blood sample collection

Fasting venous blood samples were collected for the measurement of total cholesterol (TC), HDL-C, LDL-C, TAG, CRP, IL-6, leptin, acylated ghrelin and total PYY concentrations. Full details of blood sample collection are reported in Chapter 2.10.

A fasting fingertip capillary blood sample was collected to determine baseline insulin and glucose concentrations before participants consumed a 75 g glucose load dissolved in 300 mL of water, marking the start of the OGTT. Full details of blood sample collection for the OGTT are reported in Chapter 2.10.

3.2.8 Blood analysis

Plasma concentrations of insulin, acylated ghrelin, total PYY and leptin were measured using commercially available enzyme-linked immunosorbent assays. Specifications of the analysis and details of the commercially available enzyme-linked immunosorbent assay kits are reported in Chapter 2.11.3 and 2.11.4. Data for plasma insulin concentrations were analysed in a sub-sample of 8 South Asian and 8 white European participants matched for BMI. Plasma TC, HDL-C, LDL-C and TAG concentrations were determined by enzymatic, colorimetric methods using a bench top analyser (specifications of the analysis and details of the automated bench top analyser reported in Chapter 2.11.5). Plasma glucose concentrations were analysed immediately in singular using a glucose analyser (specifications of the analysis and details of the glucose analyser are reported in Chapter 2.11.4). Samples from each participant were analysed in the same run to avoid inter-assay variation. Coefficients of variation for the assay duplicates were

as follows: 3.8% acylated ghrelin, 5.1% total PYY, 4.0% leptin, 4.6% CRP, 8.8% IL-6, 0.4% total cholesterol, 0.9% HDL-C, 0.8% LDL-C, 2.0% TAG and 6.8% insulin.

3.2.9 Statistical analysis

Data analyses were conducted using the analytical software SPSS version 23.0 for Windows (SPSS 23.0, IBM Corp, Armonk, NY, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) (Matthews et al. 1985) and insulin sensitivity index (Matsuda et al. 1999) were calculated. Normality of the data was checked using Shapiro-Wilk tests. Normally distributed data are presented as mean (SD). Data for appetite-related hormones, inflammatory markers and metabolic markers were not normally distributed and were natural log transformed before analysis. These data are presented as geometric mean (95% confidence interval) and analysis is based on ratios of geometric means and 95% confidence intervals (CI) for ratios.

Physical and physiological characteristics and dietary intake were compared between the South Asian and white European men using linear mixed models with ethnic group included as a fixed factor. Habitual physical activity levels and sedentary time were compared between ethnic groups using linear mixed models with wear time included as a covariate. The trapezium rule was used to calculate the total area under the curve (AUC) for glucose and insulin during the OGTT. Linear mixed models, both unadjusted and adjusted for percentage body fat, were employed to examine between-group differences in fasting plasma constituents, 2 h glucose and insulin concentrations and AUC values. Differences in glucose and insulin concentrations over the 2 h OGTT were examined using 2 x 5 (group x time) linear mixed models (unadjusted and adjusted for percentage body fat). Absolute standardised effect sizes (ES) (Cohen's d) were calculated for each

variable by dividing the difference between the mean values (South Asian versus white European) with the pooled SD. An ES of 0.2 was considered the minimum important difference, 0.5 moderate and 0.8 large (Cohen et al. 1988). Ethnicity-specific Pearson's correlation coefficients were used to examine the magnitude of linear association between the various predictors (age, body fat percentage, $\dot{V}O_2$ max, sedentary time, MVPA) and outcome measures (acylated ghrelin, leptin, insulin sensitivity index, CRP, HDL-C). Statistical significance was accepted as $P < 0.05$.

3.3 Results

3.3.1 Participants characteristics

The physical and physiological characteristics of the South Asian and white European participants are shown in Table 3.1. There were no significant differences between groups in stature, body mass, BMI, waist circumference, resting systolic blood pressure and resting diastolic blood pressure (all $P \geq 0.172$). Compared with white European participants, South Asian participants exhibited higher fat mass (ES = 0.66, $P = 0.071$) and body fat percentage (ES = 0.86, $P = 0.021$). Fat free mass (ES= 1.29, $P = 0.001$), age (ES = 0.72, P = 0.052) and $\dot{V}O_2$ max expressed in absolute (ES = 1.79, $P < 0.001$) and relative (ES = 1.36, $P = 0.001$) terms were lower in South Asians compared with white European participants.

3.3.2 Habitual physical activity and sedentary time

Habitual physical activity levels and sedentary time in the South Asian and white European participants are displayed in Table 3.2. No significant differences were seen between the groups for wear time adjusted sedentary time, light activity or MVPA (all $P \geq 0.169$). Wear time adjusted average CPM (ES = 0.65, $P = 0.109$) and total step counts (ES = 0.71, $P = 0.142$) were meaningfully, albeit not significantly, lower in the South Asian compared with white European participants.

3.3.3 Dietary intake

Average protein intake tended to be lower in South Asian compared with white European participants (ES = 0.64, $P = 0.079$), resulting in a lower contribution of protein (ES = 0.70, $P = 0.064$) and a higher contribution of carbohydrate (ES = 0.65, $P = 0.069$) to total energy intake in the South Asian men. No other significant differences in energy,

macronutrient or micronutrient intakes were observed between the South Asian and white European participants (all $P \geq 0.083$) (Table 3.3).

3.3.4 Fasting plasma concentrations of appetite-related hormones, inflammatory markers and lipid parameters

Fasting plasma concentrations of appetite-related hormones, inflammatory markers and lipid parameters are shown in Table 3.4. Linear mixed models revealed higher fasting plasma concentrations in the South Asian compared with white European participants for CRP (113%, ES = 0.87, $P = 0.019$), leptin (187%, ES = 1.11, $P = 0.004$), TC/HDL-C ratio (22%, ES = 0.80, $P = 0.030$) and TAG (43%, ES = 0.74, $P = 0.044$). Compared with white European participants, South Asian participants exhibited lower concentrations of fasting acylated ghrelin (-47%, ES = 1.00, $P = 0.008$) and HDL-C (-17%, ES = 0.78, $P = 0.035$). Fasting plasma concentrations of IL-6 were meaningfully, albeit not significantly, higher in South Asian compared with white European participants (57%, ES = 0.86, $P = 0.074$). No between-group differences were seen in fasting plasma total PYY, TC or LDL-C concentrations (all $P \geq 0.215$). Between-group differences in fasting plasma constituents were attenuated after adjustment for body fat percentage ($P \geq 0.080$), although a tendency for a higher leptin concentration in South Asians remained (37%; ES = 0.33, $P = 0.061$).

Table 3.1. Participant characteristics.

	South Asians (n=16)	White Europeans (n=16)	White Europeans vs. South Asians 95% CI[a]	Effect size
Age (years)	30 (8)	36 (8)	-11 to 0.04	0.72
Stature (cm)	176.3 (6.9)	179.3 (4.7)	-7.2 to 1.3	0.49
Body mass (kg)	79.4 (14.6)	80.5 (8.4)	-9.7 to 7.5	0.09
Body mass index ($kg \cdot m^{-2}$)	25.7 (5.2)	25.2 (3.3)	-2.6 to 3.7	0.12
Fat free mass (kg)	57.4 (5.3)	64.5 (5.7)	-11.2 to -3.2[b]	1.29
Fat mass (kg)	22.1 (11.0)	16.0 (6.9)	-0.5 to 12.7	0.66
Body fat (%)	26.4 (9.0)	19.5 (7.0)	1.1 to 12.8[b]	0.86
Waist Circumference (cm)	87.8 (13.4)	85.5 (6.6)	-5.4 to 9.9	0.21
Resting sBP (mmHg)	120 (11)	122 (10)	-10 to 5	0.23
Resting dBP (mmHg)	78 (8)	77 (11)	-6 to 7	0.05
$\dot{V}O_2$ max ($L \cdot min^{-1}$)	2.97 (0.55)	4.00 (0.60)	-1.44 to -0.61[b]	1.79
$\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	38 (9)	50 (8)	-18 to -5[b]	1.36

All values are mean (SD). Data were analysed using linear mixed models.

sBP, systolic blood pressure; *dBP,* diastolic blood pressure; $\dot{V}O_2$ *max,* maximum oxygen uptake.

[a] 95% confidence interval of the mean absolute difference between groups.

[b] Significant difference between South Asians and white Europeans ($P < 0.05$).

Table 3.2. Habitual physical activity levels and sedentary time in South Asian and white European men.

	South Asians (n=13)	White Europeans (n=13)	White Europeans vs. South Asians 95% CI[a]	Effect size
Wear time (min·day^{-1})	828 (87)	858 (64)	-92 to 32	0.39
Total activity (counts·min^{-1}·day^{-1})	396 (115)	474 (124)	-182 to 19	0.65
Sedentary time (min·day^{-1})	546 (82)	528 (66)	-16 to 87	0.25
Light physical activity (min·day^{-1})	172 (73)	197 (47)	-62 to 32	0.41
MVPA (min·day^{-1})	53 (15)	57 (24)	-21 to 14	0.18
Total steps (per day)	8759 (1935)	10157 (1981)	-2725 to 417	0.71

All values are mean (SD). Data were analysed using linear mixed models. Models for total activity, sedentary time, light physical activity, MVPA and total steps included wear time as a covariate.

MVPA moderate-to-vigorous physical activity.

[a] 95% confidence interval of the mean absolute difference between groups.

Table 3.3. Average daily energy, macronutrient and micronutrient intakes in South Asian and white European men.

	South Asians (n=16)	White Europeans (n=16)	White Europeans vs. South Asians 95% CI[a]	Effect size
Energy (kcal·day^{-1})	2136 (527)	2060 (603)	-333 to 486	0.12
Carbohydrate (g·day^{-1})	252 (75)	217 (105)	-31 to 100	0.38
Fat (g·day^{-1})	81.5 (30.9)	77.4 (37.5)	-20.6 to 29.0	0.12
Protein (g·day^{-1})	98.7 (28.5)	123.7 (46.8)	-53.0 to 3.1	0.64
% energy from carbohydrate	48 (19)	41 (10)	-0.5 to 13	0.65
% energy from fat	34 (8)	33 (12)	-7 to 8	0.02
% energy from protein	19 (6)	26 (13)	-14 to 0.4	0.70
Dietary fibre (g·day^{-1})	15.9 (7.6)	18.8 (13.1)	-10.6 to 4.9	0.21
Calcium (mg·day^{-1})	723 (470)	691 (311)	-256 to 319	0.08
Magnesium (mg·day^{-1})	262 (113)	311 (81)	-120 to 22	0.50
Sodium (mg·day^{-1})	1976 (1066)	2154 (884)	-885 to 529	0.18
Folate (µg·day^{-1})	256 (142)	229 (66)	-53 to 107	0.24
Vitamin D (µg·day^{-1})	2.2 (1.9)	5.9 (8.1)	-8.0 to 0.5	0.63
Vitamin C (mg·day^{-1})	116.3 (83.0)	132.4 (169.9)	-112.7 to 80.4	0.12

All values are mean (SD). Data were analysed using linear mixed models. Energy, macronutrient and micronutrient values were recorded for 3 days (2 weekdays and 1 weekend).

[a] 95% confidence interval of the mean absolute difference between group.

Table 3.4. Fasting plasma concentrations in South Asian and white European men.

	South Asians (n=16)	White Europeans (n=16)	Model 1[a]		Model 2[b]	
			White Europeans vs. South Asians 95% CI[c]	Effect size	White Europeans vs. South Asians 95% CI[c]	Effect size
Acylated ghrelin ($pg \cdot mL^{-1}$)	35.7 (25.8 to 49.4)	67.7 (48.8 to 93.7)	-67 to -16%[e]	1.00	-59 to 5%	0.65
Total peptide YY ($pg \cdot mL^{-1}$)	90.3 (80.0 to 101.8)	83.8 (74.3 to 94.5)	-9 to 28%	0.31	-9 to 33%	0.39
Leptin ($ng \cdot mL^{-1}$)	6.11 (3.76 to 9.92)	2.13 (1.31 to 3.46)	44 to 470%[e]	1.11	-1 to 91%	0.33
C-reactive protein ($\mu g \cdot mL^{-1}$)	0.89 (0.57 to 1.38)	0.42 (0.27 to 0.65)	14 to 298%[e]	0.87	-17 to 197%	0.52
Interleukin-6 ($pg \cdot mL^{-1}$)[d]	0.71 (0.49 to 1.03)	0.45 (0.32 to 0.64)	-5 to 161%	0.86	-19 to 119%	0.54
TC ($mmol \cdot L^{-1}$)	4.37 (4.04 to 4.73)	4.28 (3.96 to 4.63)	-9 to 14%	0.13	-11 to 14%	0.03
HDL-C ($mmol \cdot L^{-1}$)	1.10 (0.98 to 1.24)	1.32 (1.17 to 1.48)	-29 to -1%[e]	0.78	-22 to 8%	0.36
TC/HDL-C ratio	3.97 (3.50 to 4.51)	3.25 (2.86 to 3.69)	2 to 47%[e]	0.80	-8 to 30%	0.35
LDL-C ($mmol \cdot L^{-1}$)	2.72 (2.42 to 3.06)	2.45 (2.18 to 2.76)	-6 to 31%	0.45	-12 to 25%	0.20
Triacylglycerol ($mmol \cdot L^{-1}$)	1.16 (0.91 to 1.48)	0.81 (0.63 to 1.03)	1 to 102%[e]	0.74	-18 to 48%	0.20

All values are geometric mean (95% confidence interval). Statistical analyses are based on log-transformed data. Data were analysed using linear mixed models.

TC, total cholesterol; *HDL-C,* high-density lipoprotein cholesterol; *LDL-C,* low-density lipoprotein cholesterol.

[a] Model 1: unadjusted, [b] Model 2: adjusted for body fat percentage; [c] 95% confidence interval for the ratio of geometric means

[d] Data for interleukin-6 available for n = 9 South Asian and n = 10 white European; [e] Significant difference between South Asian and white European ($P < 0.$

3.3.5 Plasma glucose and insulin concentrations during the OGTT

Plasma glucose concentrations in the fasted state and 2 h post-challenge were not significantly different between South Asian and white European participants in the unadjusted or body fat adjusted models (all $P \geq 0.190$) (Table 3.5). South Asian participants exhibited higher insulin concentrations in the fasted state (71%; ES = 1.06, $P = 0.053$) and at 2 h post-challenge (303%; ES = 1.28, $P = 0.022$) (Table 5). Between-group differences in fasting (34%; ES = 0.58, $P = 0.218$) and 2 h post-challenge (110%; ES = 0.68, $P = 0.082$) insulin were diminished after controlling for body fat percentage (Table 5). The HOMA-IR was meaningfully, albeit not significantly, higher in the South Asian compared with white European participants (66%; ES = 0.94, $P = 0.081$) (Table 3.5). The insulin sensitivity index was lower in the South Asian compared with white European participants in the unadjusted (61%; ES = 1.22, $P = 0.016$) and body fat adjusted (41%; ES = 0.68, $P = 0.055$) models (Table 3.5).

Linear mixed model for glucose OGTT identified a main effect of time ($P < 0.001$) and group-by-time interaction ($P = 0.047$) but not a main effect of group (95% CI -2 to 26%, $P = 0.086$) (Figure 3.2). Post-hoc analysis of the group-by-time interaction revealed higher glucose concentrations in the South Asian compared with white European participants at 1.5 h (22%; 95% CI 4 to 44%, ES = 0.77, $P = 0.014$). The total area under the curve for glucose was higher in the South Asian compared with white European participants (14%; 95% CI -1 to 30%, ES = 0.69, $P = 0.060$), but this difference was attenuated after adjusting for body fat percentage (5%; 95% CI -8 to 20%, ES = 0.27, $P = 0.434$) (Figure 3.2).

Linear mixed models for insulin OGTT identified a main effect of group ($P = 0.010$), time ($P < 0.001$) and a group-by-time interaction ($P = 0.025$) (Figure 3.2). The main

effect of group revealed higher insulin concentrations in the South Asian compared with white European participants (211%; 95% CI 38 to 602%, ES = 1.11). The ethnic group difference was diminished but remained significant after adjustment for body fat percentage (100%; 95% CI 8 to 269%, ES = 0.68, $P = 0.030$). Post hoc analysis of the group-by-time interaction revealed higher insulin concentrations in the South Asian compared with white European participants at 0.5, 1, 1.5 and 2 h (all ES $\geq$ 1.28, $P \leq$ 0.013). The total area under the curve for insulin was higher in the South Asian compared with white European participants in unadjusted (245%; 95% CI 51 to 690%, ES = 1.61, $P = 0.006$) and body fat adjusted (123%; 95% CI 17 to 326%, ES = 1.04, $P = 0.019$) models (Figure 3.2).

Table 3.5. Plasma glucose and insulin concentrations during the OGTT in South Asian and white European men.

	South Asians (n=16)	White Europeans (n=16)	Model 1[a]		Model 2[b]	
			White Europeans vs. South Asians 95% CI[c]	Effect size	White Europeans vs. South Asians 95% CI[c]	Effect size
Glucose						
Fasted ($mmol \cdot L^{-1}$)	4.50 (4.28 to 4.74)	4.71 (4.47 to 4.96)	-11 to 3%	0.45	-12 to 3%	0.51
2 h ($mmol \cdot L^{-1}$)	4.78 (4.11 to 5.56)	4.16 (3.58 to 4.83)	-7 to 42%	0.47	-14 to 35%	0.25
Insulin						
Fasted ($\mu U \cdot mL^{-1}$)	7.21 (4.90 to 10.60)	4.22 (2.87 to 6.20)	-1 to 195%	1.06	-18 to 118%	0.58
2 h ($\mu U \cdot mL^{-1}$)	23.49 (10.32 to 53.49)	5.84 (2.56 to 13.29)	26 to 1188%[d]	1.28	-10 to 391%	0.68
HOMA-IR	1.50 (1.00 to 2.26)	0.91 (0.60 to 1.36)	-7 to 195%	0.94	-23 to 109%	0.44
Insulin sensitivity index	5.62 (3.32 to 9.49)	14.41 (8.53 to 24.36)	-81 to -18%[d]	1.22	-65 to 1%	0.68

All values are geometric mean (95% confidence interval) for n=32 (glucose) and n=16 (insulin, HOMA-IR, insulin sensitivity index). Statistical analyses are based on log-transformed data. Data were analysed using linear mixed models.

OGTT, oral glucose tolerance test; *HOMA-IR*, homeostasis model assessment of insulin resistance.

[a] Model 1: unadjusted, [b] Model 2: adjusted for body fat percentage.

[c] 95% confidence interval for the ratio of geometric means.

[d] Significant difference between South Asians and white Europeans ($P < 0.05$).

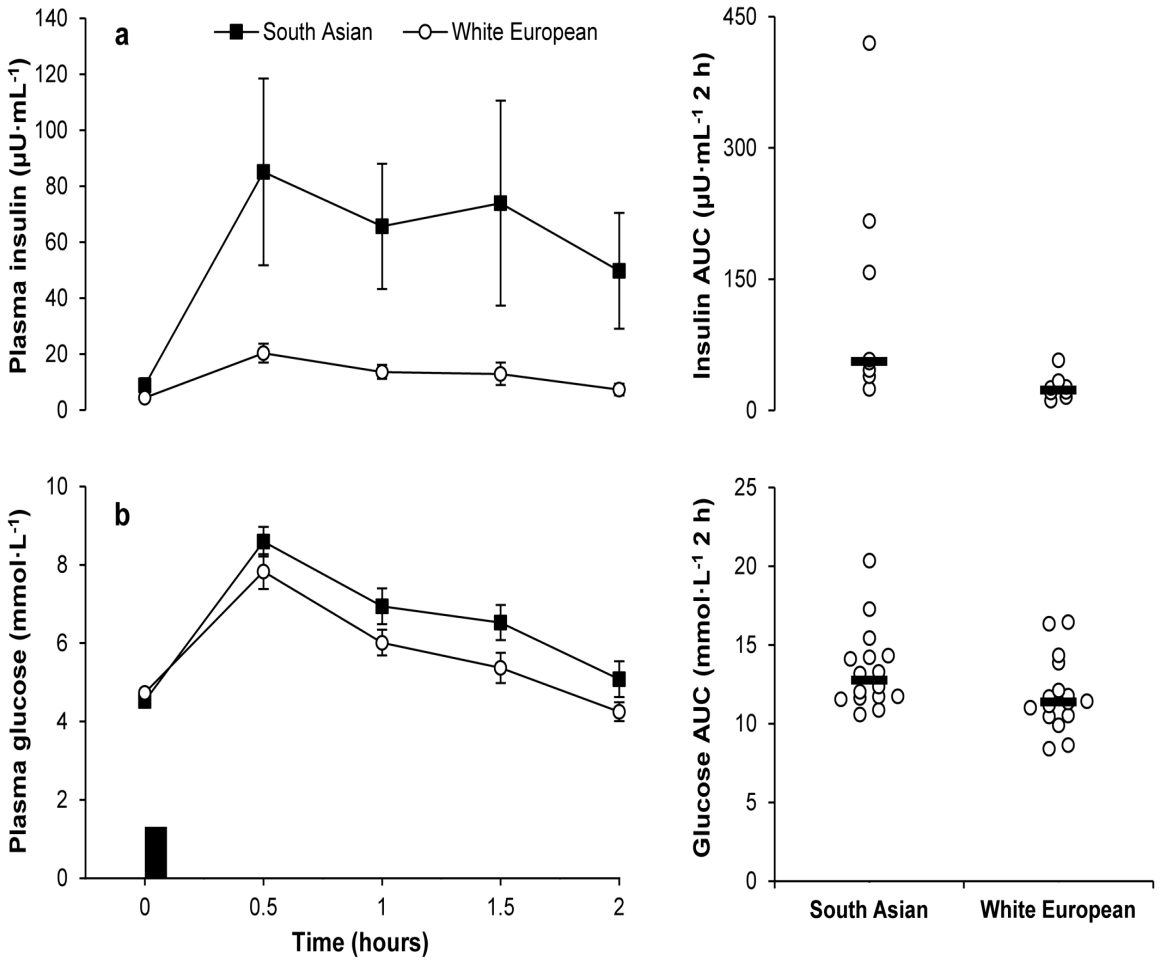

Figure 3.2. Plasma concentrations of (a) insulin (top panels; South Asian n=8, white European n=8) and (b) glucose (bottom panels; South Asian n=16; white European n=16) during the oral glucose tolerance test. Data points on left panels represent mean (SEM) for the South Asian (■) and white European (○) men. Black rectangle indicates consumption of glucose load. Data points on right panels represent individual data values (○) and the solid line indicates the median (—).

3.3.6 Correlations

Body fat percentage was positively associated with leptin (r = 0.84 in white Europeans and r = 0.88 in South Asians, $P \leq 0.001$), and negatively associated with insulin sensitivity index in South Asian and white European men (r = -0.76 in white Europeans and r = -0.83 in South Asians, $P \leq 0.024$) (Table 3.6). Body fat percentage was negatively associated with acylated ghrelin (r = -0.73, $P = 0.002$) and HDL-C (r = -0.81, $P = 0.001$) in white European men, and positively associated with CRP in South Asian men (r = 0.52, $P = 0.035$) (Table 3.6). A positive association was also identified between $\dot{V}O2$ max and insulin sensitivity index in white European men (r = 0.80, $P = 0.016$), whereas MVPA was negatively associated with leptin in white European men (r = -0.50, $P = 0.044$) (Table 6). Sedentary time was positively associated with CRP in South Asian men only (r = 0.50, $P = 0.041$).

Table 3.6. Ethnicity-specific Pearson's correlation coefficients between the various predictors and metabolic risk markers.

	Acylated ghrelin[a]		**Leptin**		**Insulin sensitivity index**		**C-reactive protein[a]**		**HDL-C[a]**	
	South Asian	**White European**	**South Asian**	**White European**	**South Asian**	**White European**	**South Asian**	**White European**	**South Asian**	**White European**
Age	0.05	0.40	0.07	-0.03	0.11	-0.14	0.45	-0.01	-0.09	0.38
Body fat	-0.25	**-0.73**[b]	**0.88**[c]	**0.84**[c]	**-0.83**[b]	**-0.76**[b]	**0.52**[b]	0.16	-0.28	**-0.81**[b]
$\dot{V}O_2$ max	0.37	0.27	-0.01	-0.12	0.28	**0.80**[b]	0.29	0.38	-0.06	0.24
Sedentary time	0.20	-0.13	0.47	0.05	0.04	-0.27	**0.50**[b]	0.11	-0.06	-0.19
MVPA	0.05	0.04	-0.18	**-0.50**[b]	0.14	0.42	-0.43	-0.02	0.39	0.18

[a] Statistical analysis are based on log-transformed data.

HDL-C, high-density lipoprotein cholesterol; $\dot{V}O_2$ *max,* maximum oxygen uptake; *MVPA* moderate-to-vigorous physical activity.

[b] $P < 0.05$; [c] $P < 0.001$.

3.4 Discussion

The novel finding of this study is that healthy South Asian men exhibited lower fasting acylated ghrelin concentrations compared with BMI-matched white European men. Furthermore, South Asian men exhibited impaired CVD and T2D risk markers compared with white European men comprising: *(1)* elevated fasting concentrations of insulin, leptin, CRP, IL-6 and TAG and a higher TC/HDL-C ratio; *(2)* lower fasting concentrations of HDL-C; and *(3)* higher glucose and insulin concentrations during the OGTT. A further key finding is that cardiorespiratory fitness was substantially lower in the South Asian men than in the white European men, but no differences between the ethnicities was observed in objective levels of physical activity or sedentary behaviour.

The lower fasting acylated ghrelin concentration in the South Asian compared with white European men represents a novel finding of this study. Although the reason for this finding is unclear, it may be linked to the ethnic group differences in adiposity. Previous research has demonstrated that individuals with obesity exhibit lower circulating concentrations of fasting ghrelin than lean individuals (Le Roux et al. 2005). Although our findings only revealed a large inverse correlation between body fat percentage and acylated ghrelin in the white European men, the ethnic group difference in fasting acylated ghrelin concentrations was mitigated after controlling for body fat percentage. Therefore, it seems plausible that the lower acylated ghrelin concentration in the South Asian men may be linked to the higher body fat levels. These findings appears to support the data in Study 3 (Chapter 5), where concentrations of fasting acylated ghrelin were not statistically different between South Asian and white European men, with both groups exhibiting similar percentage of body fat.

Similar to previous findings, circulating concentrations of plasma leptin were substantially elevated in the South Asian compared with white European men (Mente et al. 2010; Abate et al. 2004). Considering circulating leptin concentrations are directly proportional to body fat mass (Considine et al. 1996), it is likely that the elevated leptin concentrations in South Asian individuals is, at least partly, mediated by differences in body fat. In support of this, plasma leptin concentrations were positively associated with body fat percentage in the present study, replicating previous findings in South Asian individuals (Banerji et al. 1999; Abate et al. 2004). However, South Asian individuals have also been shown to exhibit higher concentrations of leptin than Caucasian individuals despite a similar body fat percentage (Abate et al. 2004), and the between-group difference in leptin concentrations in this study was diminished, but not eliminated completely, after controlling for body fat percentage. Consequently, it is possible that irregularities in adipose tissue metabolism concomitant with insulin resistance may contribute to the elevated CVD and T2D risk in South Asians. Despite the between-group differences in acylated ghrelin and leptin, the current study did not find any significant differences in plasma total PYY between South Asian and white European participants.

In support of previous findings, higher fasting insulin and elevated glucose and insulin OGTT concentrations were also observed in South Asian participants compared with white European men (Chandalia et al. 1999; Raji et al 2001; Peters et al 2013). These differences are indicative of a greater degree of insulin resistance and are further supported by the higher HOMA-IR and lower insulin sensitivity index observed in the South Asian participants. It is well established that insulin resistance is a primary determinant of the elevated propensity for CVD and T2D in individuals of South Asian descent (Sattar and Gill, 2015; Gholap et al. 2011; Tziomalos et al. 2008). One factor suggested to contribute to the excess insulin resistance in South Asian individuals

represents differences in adiposity and body fat distribution (Sattar and Gill, 2015). In accord with our findings, South Asian individuals exhibit a higher body fat percentage and lower lean body mass for a given BMI compared with white European individuals (Lear et al. 2007). Furthermore, it has been suggested previously that insulin-mediated glucose disposal is inversely correlated with total and regional body fat in South Asian individuals (Raji et al. 2001; Banerji et al. 1999). However, the greater insulin resistance in South Asian individuals has also been shown to persist after adjustment for adiposity (Chandalia et al. 1999), and the lower insulin sensitivity index and insulin OGTT in the South Asian participants in this study remained after controlling for body fat percentage, albeit the magnitude of difference was diminished. This is also in agreement with our findings reported in Study 3 (Chapter 5) where South Asians exhibited markedly higher fasting insulin and elevated glucose and insulin post-prandial despite having similar levels of body fat than white European men.

The present study also measured circulating concentrations of fasting IL-6 and CRP which represent key indicators of chronic low-grade inflammation and have been implicated in explaining the excess CHD risk in South Asian individuals (Tziomalos et al. 2008). The elevated fasting IL-6 and CRP concentration in South Asian compared with white European men supports several previous studies (Anand et al. 2004; Arjunan et al. 2015; Bastard et al. 1999), although this finding is not universal (Peters et al. 2013). Considering pro-inflammatory IL-6 released from adipose tissue has been identified as a precursor for hepatic CRP secretion (Bastard et al. 1999), it is possible that the ethnic differences in inflammation may be mediated by the higher body fat levels in South Asian individuals. In this regard, the divergent inflammatory profiles between ethnicities in the present study were diminished after controlling for body fat percentage, supporting previous findings in South Asian and white European individuals (Chambers et al. 2001;

Arjunan et al. 2015). However, it has also been demonstrated previously that South Asian individuals exhibit higher CRP concentrations than Caucasians despite similar levels of body fat (Chandalia et al. 2003). Future work is required to determine the independent contribution of ethnicity and adiposity on inflammatory markers in South Asians.

Consistent with previous studies (Arjunan et al. 2013; Arjunan et al. 2015; Anand et al. 2000), the South Asian men exhibited a more unfavourable fasted lipid profile compared with the white European men encompassing lower concentrations of HDL-C coupled with an elevated TC/HDL-C ratio and higher TAG concentration. The reasons explaining the adverse lipid profile in South Asian participants have not been fully elucidated, but it is proposed that the greater insulin resistance experienced by South Asians may be implicated (Gholap et al. 2011; Tziomalos et al. 2008). Specifically, hyperinsulinaemia associated with insulin resistance appears to downregulate skeletal muscle lipoprotein lipase activity (Pollare et al. 1991), thereby diminishing the clearance of TAG from the circulation. Furthermore, insulin resistance is suggested to impair the ability of insulin to suppress hepatic release of very low-density lipoprotein (Malmström et al. 1997). Regardless of the mechanism, our findings contribute to existing knowledge regarding the differential lipid profiles between individuals of South Asian and white European descent.

Previous research suggests that South Asian individuals engage in less habitual physical activity than white European individuals, which is likely to contribute to the excess CHD and T2D risk in this population (Williams et al. 2011a; Yates et al. 2015; Ghouri et al. 2013). The existing evidence on habitual physical activity levels in South Asians has largely been gleaned from self-report questionnaires (Yates et al. 2015; Williams et al. 2011b), but data using accelerometry is emerging (Ghouri et al. 2013; Iliodromiti et al.

2016) and the objective accelerometer measurement represents a strength of this study. Although the similar levels of MVPA and sedentary time between the ethnicities in this study appears to contradict the aforementioned studies, the South Asian participants accumulated less total activity (CPM) and fewer steps, and stark differences in CVD and T2D risk markers were still apparent between the ethnic groups. This is in line with previous evidence suggesting that South Asian individuals are more insulin resistant than white European individuals even after adjustment for habitual physical activity levels (Ghouri et al. 2013). Furthermore, it has been suggested recently that South Asians may need to accumulate higher levels of moderate physical activity equating to an additional 10 to 15 minutes per day to achieve a comparable CHD risk factor profile of white Europeans who are meeting the current physical activity recommendations (Iliodromiti et al. 2016).

Despite the similar levels of habitual physical activity between the ethnicities, cardiorespiratory fitness assessed by $\dot{V}O_2$ max was markedly lower in the South Asian compared with white European participants. This corroborates previous findings (Arjunan et al. 2013; Bastard et al. 1999; Hall et al. 2010), and there is further evidence that the lower $\dot{V}O_2$ max in South Asian individuals is independent of physical activity levels (Ghouri et al. 2013). Given the importance of physical activity as a method of enhancing $\dot{V}O_2$ max, these findings add further weight to the proposition that South Asians may need to engage in greater physical activity levels than white Europeans to optimise health outcomes (Iliodromiti et al. 2016). In addition, it has been demonstrated that low $\dot{V}O_2$ max was the strongest predictor of the excess insulin resistance seen in UK South Asian compared with white European men (Ghouri et al. 2013), although our findings only revealed a positive association between $\dot{V}O_2$ max and insulin sensitivity index in the white European men. Nevertheless, it is likely that the lower $\dot{V}O_2$ max in the

South Asian individuals may contribute to the heighted cardio-metabolic health risk in this population considering that low cardiorespiratory fitness is a well-established and strong predictor of all-cause mortality and CVD events (Kodama et al. 2009).

A further important consideration in the context of chronic disease risk concerns dietary intake. Although studies comparing dietary intake patterns between South Asian and white European individuals are sparse, South Asians typically increase energy and fat intake but also exhibit a switch from whole grains and pulses to more refined sources of carbohydrates, which results in a low intake of fibre after migration to European countries (Holmboe-Ottesen and Wandel, 2012). In addition, South Asian men have been shown to respond to short-term high fat overfeeding with greater perturbations in insulin resistance than European men (Bakker et al. 2014). Whilst the current study identified lower protein intake in the South Asian men, the assessment of dietary intake using self-report represents a limitation due to issues of participant recall bias which makes it difficult to accurately correspond self-reported intake with actual intake (Dhurandhar et al. 2015).

A limitation of this study concerns the potentially confounding effects of body fat percentage which may have accentuated the differences in CVD and T2D risk markers between the ethnicities. Although South Asians are known to exhibit a higher body fat percentage for a given BMI (Lear et al. 2007), further research is needed to clarify the role of adiposity and ethnicity in modulating CVD and T2D risk in South Asians. Furthermore, the number of participants in the study was small and the South Asian and white European men were not matched for age. However, our findings revealed marked differences in CVD and T2D risk markers between the ethnicities despite the South Asian men being, on average, six years younger than the white European men, and age was not

significantly associated with any of the outcome variables in either ethnic group. Finally, the population sample was mostly limited to South Asian men originating from India and, therefore, further investigations are required in other South Asian groups (e.g., Bangladeshis, Sri Lankan and Bhutanese) and in South Asian women.

In conclusion, the present study provides evidence that healthy South Asian men exhibit lower concentrations of acylated ghrelin and an adverse CVD and T2D risk marker profile compared with BMI-matched white European men including higher concentrations of insulin, TAG, leptin and CRP, and lower concentrations of HDL-C. Although objectively assessed physical activity levels and sedentary time were similar between the ethnic groups, the lower cardiorespiratory fitness in the South Asian men may contribute to the heightened cardiometabolic heath risk in this population. Future research that targets the identification of additional parameters of CVD and T2D risk in South Asians should be prioritised.

Chapter 4 – Study 2: Investigation of Plasma Free Fatty Acids metabolic profile based on GC-MS and LC-MS in healthy South Asian compared with white European men and association with adiposity, physical activity and cardiorespiratory fitness

4.1 Introduction

Cardiovascular disease (CVD) and type 2 diabetes (T2D) have emerged as major causes of morbidity and mortality worldwide (WHO, 2017; IDF, 2019) particularly among individuals with South Asian background (Johns and Sattar, 2015). In this regard, South Asians who have migrated to western countries, including European or North American countries, as well as those living in the Indian subcontinent, exhibit a greater cardiometabolic risk compared to their western counterparts (Sattar and Gill, 2015; Khunti et al. 2013).

The elevated adiposity, higher prevalence of insulin resistance and associated CVD risk factors including differences in markers of inflammation and metabolic health may act as catalysts inducing the elevated risk of CVD and T2D in South Asians (Benedetti et al. 2019; Gholap et al. 2011; Joshi et al. 2007). However, these risk markers do not exhaustively explain the greater CVD and T2D susceptibility in South Asian than white European individuals and the mechanisms underlying progression to T2D remain unclear (Chandalia et al. 2003; Hall et al. 2010).

In this regard, there is evidence that exercise related factors such as low levels of physical activity and reduced cardiorespiratory fitness play an important role in the insulin resistance phenotype in South Asians (Biddle et al. 2019; Williams et al. 2011b; Ghouri

et al. 2013; Yates et al. 2015; Hall et al. 2010). Additionally, physical activity and cardiorespiratory fitness are key factors associated with CVD and T2D with previous studies reporting lower physical activity engagement and fitness level amongst UK South Asians compared with white Europeans (Ghouri et al. 2013; Williams et al. 2011a; Benedetti et al. 2019; Yates et al. 2015). Nonetheless, a great deal of the data available on physical activity in South Asians have been acquired from self-reported questionnaires which have limited validity, while evidence examining the association of objectively-measured physical activity and cardiorespiratory fitness with fatty free acids in South Asian individuals remains limited.

Elevated circulating free fatty acids (FFAs) play a central role in liver and skeletal muscle insulin resistance and may contribute to β-cell dysfunction (Boden, 2002; Boden and Shulman, 2002; Karpe et al. 2011). Previous evidence reported higher total FFAs concentration in South Asian than white European individuals, irrespective of total or abdominal adiposity (Chandalia et al. 2007; Abate et al. 2004) which suggests possible abnormalities in the adipose tissue in South Asians (Bakker et al. 2013). However, not all FFAs contribute equally to the insulin resistance process. Indeed, high concentrations of saturated fatty acids (SFA) and omega-6 polyunsaturated fatty acids (PUFA) have been associated with increased levels of glucose and insulin whereas specific PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to improve insulin sensitivity (Rasic-Milutinovic et al. 2012). However, while previous cross-sectional investigations have explored the association between individual fatty acids and markers of cardiometabolic risk (Imamura et al. 2012; Ferrucci et al. 2006), these studies have not investigated ethnic-specific between individual free fatty acids and markers of insulin resistance. To the author's knowledge, only one study examined ethnic-specific associations between plasma fatty acids and fasting glucose and insulin

resistance, identifying significant associations in Caucasian, but not in South Asian individuals (Ralston et al. 2013). This study, however, looked at esterified fatty acids (EFS), instead of free fatty acids, by carrying out hydrolysis of plasma TAG post-extraction and this ignores the fact the rate of lipolysis of triglycerides, which is controlled by glucocorticoids and catecholamines, might be important (Xu et al. 2009). Furthermore, while this study reported baseline levels of FFAs in South Asians and Caucasians, ethnic-differences in FFAs were not explicitly examined while associations between fatty acids and body composition and physical activity/fitness levels were not explored.

Metabolomics method represents a promising approach to identify and quantify a large variety of metabolites within biological systems that are substrates and products in different metabolic or pathological pathways, including fatty acids (Gonzales-Franquesa et al. 2016). Particularly, previous research examining concentrations of individual free fatty acids in human plasma and associations with insulin resistance and T2D, has made considerable use of different analytical techniques for metabolomics analysis including gas chromatography (GC) and liquid chromatography (LC) coupled with mass-spectrometry (MS) (Binbin et al. 2010; Yi et al. 2006; Dai et al. 2015; Liu et al. 2010; Ma et al. 2018; Lu et al. 2016; Feng et al. 2017). However, the examination of baseline individual concentrations of FFAs based on metabolomics methods between South Asian and white European remain unknown.

Therefore, the aim of this study was to investigate ethnic differences in the free fatty acid metabolic profile based on GC-MS and LC-MS in South Asian compared with white European men. Furthermore, this study aims to explore associations of FFAs with adiposity, glycaemia, levels of physical activity and cardiorespiratory fitness.

4.2 Methods

4.2.1 Participants and Study Design

The present study was conducted in collaboration with the University of Strathclyde where the free fatty acids analysis based on LC-MS was performed. The plasma samples used for this analysis were collected at Kingston University from the same set of participants recruited in Study 1 (Chapter 3). Therefore, ethic code, full detail of participants including body composition, $\dot{V}O_2$ max and physical activity data and study design are reported in Chapters 3.2.1 and 3.2.2. Approval of the study was obtained prior commencement by the Kingston University's and University of Strathclyde's Ethics Advisory Committee (Ethic approval code: 1516/017).

4.2.2 Body composition

Details of the body composition assessment is reported in Chapter 3.2.3.1.

4.2.3 Maximum oxygen uptake test

Details of the protocol, cycle ergometer and gas analysis system used for the determination of the maximum oxygen uptake in South Asian and white European volunteers are reported in Chapter 3.2.4.1.

4.2.4 Habitual physical activity and sedentary time

An Actigraph GT3X+ accelerometer (ActiGraph, Pensacola, USA) was wore in the right hip for one week to examine habitual physical activity and sedentary time in South Asian and white European participants. Full detail of the device wearing condition, initialisation and data analysis are described in Chapter 3.2.5.

4.2.5 Plasma samples

The details of blood sampling and collection have been reported in Chapters 3.2.3.2 and 3.2.7. Briefly, after completion of the anthropometric and blood pressure measurement in Visit 1, a fasting venous blood sample was obtained for the measurement of FFAs. The blood sample was collected from the antecubital vein via venepuncture using a 25 g butterfly needle (BD Vacutainer, Plymouth, UK) into a precooled 6.0 mL heparin vacutainers (BD Vacutainer®, Plymouth, UK). The blood sample was immediately centrifuged at 1500 × *g* for 10 min at 4°C and the plasma supernatant was then dispensed into aliquots and stored at -80°C for later analysis. Using the finger-prick technique, a whole blood was collected during the OGTT every 30 min for two hours into a 20 µL heparin capillary tube (Sanguis Counting, Nümbrecht, Germany) and immediately mixed into a separate 1 mL haemolysing solution for glucose analysis.

4.2.6 Glucose analysis

Analysis of plasma glucose is described in Chapter 3.2.8.

4.2.7 Free fatty acids analysis

The examination of the free fatty acid metabolic profile was based initially on gas chromatography–mass spectrometry (GC-MS). However, due to inadequate results, liquid chromatography–mass spectrometry (LC-MS) was subsequently employed for the identification and quantification of FFAs in South Asian and white European participants. Therefore, this subsection describes *(1)* full details of the methods used for FFAs analysis in South Asian and white European men, based on GC-MS and LC-MS; and *(2)* explanation for employing LC-MS, instead of GC-MS for the metabolomics analysis.

4.2.7.1 Free fatty acids analysis based on GC-MS - Developmental work

Heparin samples selected randomly from three participants were used for evaluating the validity of the sample preparation method and GC-MS conditions for the analysis of FFAs.

The sample preparation included 300 µL aliquot of plasma which was added to 900 µL of chloroform/methanol solution (3/1, v/v). The mixture was then vortexed for 60 s and centrifuged for 10 min at 4000 *g* at 4°C to separate the upper organic phase and the lower chloroform layer containing the FFAs. The upper phase was transferred to a different tube whereas the phase containing the FFAs was left in the same tube and dried under N_2 gas flow before the addition of the derivate reagent (0.5 mL of 5% H_2SO_4/HCl/CH_3OH). The dried tubes were then sealed and placed in a water bath at 70°C for 30 min with the tubes mixed every 5 min to optimise the reaction. Then, 500 µL of hexane was added to obtain the fatty acid methyl esters (FAMEs), and the tubes were centrifuged for 3 min at 4000 *g* at 4°C. The hexane phase containing the FAMEs was transferred to a clean glass tube for GC-MS analysis.

GC-MS analysis was performed using an Agilent 6890N gas chromatograph coupled to an Agilent 5793 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). In the gas chromatography system, a BPX5 capillary column (30 m x 0.255 mm I.D. film thickness 0.25 µm; Trajan Scientific and Medical, Melbourne, Australia) was used and the GC-MS conditions were as follows: *(1)* initial temperature 50 °C for 1 min; *(2)* temperature was increased to 180°C at the rate of 25°C/min; *(3)* temperature was increased at a rate of 4°C/min to 230°C. The total GC run time was 28 min. Helium carrier gas was used at a constant flow rate of 1.0 ml · min^{-1} and temperature of the

injector was 250°C. A sample of 1 μL was injected at a split ratio of 1:5 and the mass conditions were as follows: electron energy, 70 eV; ion source temperature, 230°C; full scan mode (m/z 45-600) with a scan time of 0.6 s.

The results following the experiments based on the aforementioned methods were unsatisfactory as the chromatogram did not reveal any compounds of interest. Although the reasons for this were not clear, the poor results were ascribed to either a lack of concentration of fatty acids in the sample injected into the GC or a low sensitivity of the MS detection. Therefore, the GC-MS method was optimised by changing the injection mode from split to splitless, and subsequently by increasing the sensitivity of the MS detection by using SIM (m/z 55, 67, 74 and 79) instead of full SCAN mode (m/z 45-600), in splitless mode (Vasconcellos et al. 2015; Dai et al. 2015). However, although the attempts in changing the GC-MS methods, fatty acids were still not detected with the scarcity of the results linked possibly to the poor extraction yield. Therefore, to increase free fatty acid extraction, the sample preparation method was optimised by: *(1)* increasing the aliquot of plasma to 500 μL, instead of 300 μL; *(2)* mixing aliquots of 500 μL with 0.5 mL of 0.4 mol/L KOH/CH_3OH, in addition to 1500 μL of chloroform/methanol solution; and *(3)* extracting the methyl esters products of FFAs by adding 2 mL of hexane, instead of 500 μL. The remaining part of the sample preparation method was the same to the initial method described above. However, even after changing some aspects of the sample preparation method, results did not exhibit significant improvement with the FFAs eluding detection.

Thus, efficacy of the sample preparation and GC-MS conditions were excluded as possible reasons causing unsatisfactory results which, instead, were most likely explained by the inadequacy of the BPX5 capillary column used in our experiments.

Particularly, previous studies examining fatty acids profile reported the use of different capillary columns including DB-23, DB-WAX or Agilent select FAMEs (Yi et al. 2006; Ma et al. 2018). However, given the paucity of the outcomes by the GC-MS and the uncertainty to achieve better results even changing the capillary column, LC-MS was then employed for the quantitation of FFAs in South Asians and white European participants. The free fatty acid analysis performed using LC-MS produced excellent results, reported in 4.3.4, which were subsequently published (Benedetti et al. 2019).

4.2.7.2 Free fatty acids analysis based on LC-MS

4.2.7.2.1 Chemicals and Solvents

Full description of the chemical and solvents used for the free fatty acids analysis based on LC-MS is reported in Chapter 2.11.2.1. Briefly, HPLC grade acetonitrile (ACN), water, acetic acid and hexane were obtained from Fisher Scientific (Leicestershire, UK) whereas a mixture of fatty acid methyl ester standards (Supelco 37-component fatty acid methyl ester mix) was obtained from Sigma Aldrich (Dorset, UK). The methyl esters were hydrolysed with 1 M KOH by heating at 60ºC for 15 min, the mixture was acidified and extracted into hexane.

4.2.7.2.2 Sample preparation and calibration series

Full description of the LC-MS sample preparation and calibration series used for profiling the FFAs is reported in Chapter 2.11.2.1.

4.2.7.2.3 Instrumental technique and LC-MS conditions

LC-MS analysis was carried out using a Dionex 3000 HPLC interfaced with and an Orbitrap Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The column characteristics and details for the mobile phase have been reported in Chapter 2.11.2.2. The nitrogen sheath and auxiliary gas flow rates were maintained at 50 and 17 arbitrary units. The electrospray ionisation (ESI) interface was operated in both positive and negative modes. The spray voltage was 4.5 kV for the positive mode and 4.0 kV for negative mode, while the ion transfer capillary temperature was 275°C. Full description of the LC-MS condition are displayed in Chapter 2.11.2.3.

4.2.8 Data processing and statistical analysis

Statistical analysis for participants characteristics, physical activity and plasma glucose concentrations is described in Chapter 3.2.9.

With regard to the free fatty acid analysis, the Quan Browser in Xcalibur was used to plot calibration curves (weighted with 1/x) and quantify the responses for the samples against the calibration curves. Then the levels of FFAs in the samples were calculated from the calibration curves by Quan Browser. P values and ratios of the mean values for the fatty acids were determined by using Microsoft Excel (Microsoft Office 2013). SIMCA-P version 14.1 (Umetrics, Umeå, Sweden) was used for multivariate analysis (Trivedi et al. 2012) which included Principle Components Analysis (PCA), Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) and Orthogonal Partial Least Square (OPLS). Specifically, the quantitative values for the samples were then mean centred, and Pareto scaled for PCA, OPLS-DA and OPLS to generate S-plots for visualisation of the components.

4.3 Results

4.3.1 Participants Characteristics

The physical and physiological characteristics of the South Asian and white European participants are reported in Table 3.1, Chapter 3.3.1.

4.3.2 Habitual physical activity and sedentary time

The habitual physical activity and sedentary time in South Asian and white European participants are reported in Table 3.2, Chapter 3.3.2.

4.3.3 Plasma glucose concentrations during the OGTT

Plasma glucose concentrations during the OGTT are displayed Table 3.5 and Figure 3.2, Chapter 3.3.5.

4.3.4 Free Fatty Acids metabolic profile based on LC-MS

Figure 4.4 shows a comparison between the fatty acid standard mixture and the fatty acids present in a plasma sample. Out of the 37 fatty acids present in the standard, 19 of these could be detected in plasma and quantitative values are given in Table 4.1.

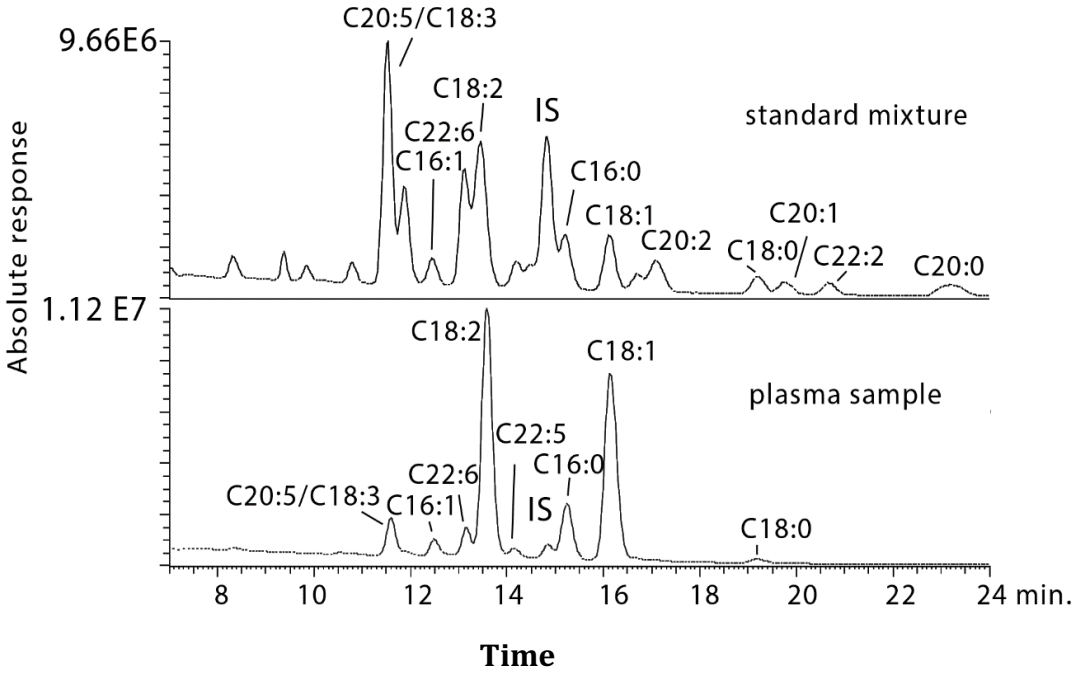

Figure 4.4. Chromatogram for the fatty acids detected in plasma compared with a chromatogram for the calibration standards (concentration range 3.2 - 9.6 $\mu g \cdot ml^{-1}$).

Table 4.1. Concentration of 19 FFAs in plasma from South Asian and white European men.

Free fatty acid	South Asians (n=16)	White Europeans (n=16)	RT (min)	m/z	P value SA/WE	Ratio of Mean conc. SA/WE
Laurate (12:0)	29.8 (12.2)	28.3 (14.5)	8.3	199.169	0.040[a]	1.092
Myristate (14:0)	48.0 (44.8)	33.5 (34.3)	11.5	227.200	0.011[a]	1.478
Pentadecenoate (16:0)	12.5 (7.9)	12.3 (4.5)	10.9	239.200	0.224	1.032
Pentadecanoic (15:0)	13.5 (21.5)	12.5 (26.7)	13.3	241.216	0.214	1.111
Palmitoleic (16:1*n*-7)	51.3 (66.8)	36.3 (60.5)	12.4	253.216	0.137	1.371
Palmitate (16:0)	81.0 (33.3)	54.8 (30.6)	15.2	255.232	0.004[a]	1.487
γ-Linolenic (18:3*n*-6)	17.8 (30.1)	13.8 (35.0)	11.6	277.216	0.017[a]	1.328
Linoleate (18:2*n*-6)	322.3 (31.2)	206.0 (44.7)	13.6	279.232	0.005[a]	1.621
Oleate (18:1*n*-9)	633.8 (43.4)	473.5 (42.5)	16.1	281.247	0.081	1.317
Stearic (18:0)	193.5 (29.8)	155.3 (28.6)	19.2	283.263	0.095	1.227
Eicosapentaenoic (20:5*n*-3)	9.3 (19.7)	10.3 (37.9)	11.5	301.216	0.449	0.899
Eicosatetraenoic (20:4*n*-3)	25 (30.9)	25.3 (35.9)	13.4	303.232	0.958	0.972
Eicosatrienoic (20:3*n*-3)	7.0 (27.2)	6.5 (27.0)	14.8	305.247	0.554	1.059
Eicosadienoic (20:2*n*-6)	12.2 (12.3)	11.5 (14.7)	17.1	307.263	0.215	1.068
Eicosenoic (20:1*n*-9)	14.0 (24.9)	13.3 (37.6)	19.8	309.279	0.666	1.022
Arachidate (20:4*n*-6)	35.3 (59.2)	49.0 (53.4)	23.1	311.294	0.074	0.681
Docosahexaenoic (22:6*n*-3)	18.3 (43.0)	25.3 (69.9)	13.1	327.232	0.100	0.683
Tricosanoate (23:0)	20.0 (53.3)	16.5 (47.2)	28.1	353.341	0.456	1.179
Lignocerate (24:0)	13.4 (49.5)	10.8 (29.1)	29.6	367.357	0.250	1.225

All values are mean (RSD %).

RT, retention time; *m/z*, mass-to-charge ratio.

[a] Significant difference between South Asians and white Europeans ($P < 0.05$).

The *P* values for the FFAs were validated using correction for multiple comparisons (Benjamini et al. 1995), which indicated for the number of variables used that all *P* values <0.05 could be regarded as significant. Linoleic and oleic acid were by some way the most abundant FFAs in plasma. Figure 4.5 shows the principal components analysis (PCA) plot for 16 South Asian and 16 white European samples based on peak quantities of 19 free fatty acids in plasma. PCA was employed as the first step in the analysis to explore how the free fatty acids clusters provide insights into separations between South Asian and white European men for a better interpretation of the data. However, as shown in figure 4.5, many samples were scattered but could not be clearly separated between the two ethnic groups. This is because PCA finds a lower dimensional space capturing the maximum amount of variance in an input data matrix without losing any useful data. Nonetheless, it is also possible the elevated number of samples could have resulted in confusion during the samples discrimination between the two groups (Liu et al. 2010). After the first screening by PCA, orthogonal partial least squares discriminant analysis (OPLS-DA) was used as an alternative method to identify reliable free fatty acids that have a strong association with separation between groups (Figure 4.6). Specifically, a strong OPLS-DA model (CVANOVA 5.9 x 10-7) could be built which differentiated clearly 13 of the South Asian samples from 13 of the white European samples based on four of the FFAs (Figure 4.6). The OPLS-DA model explained 54.5% of the variance which was greater than that explained by the PCA model (18.5%). As shown in figure 4.6, the two groups were clearly separated in the OPLS-DA plot, with South Asian samples being clustered on the right side whereas the white European samples grouped on the left side of the plot. Furthermore, the distance between each participant within the same group along the Y axis represents the concentrations for the four fatty acids used in this model; participants with similar free fatty acid concentrations are displayed close to

each other in the OPLS-DA plot. The cross-validation plot for this model can be seen in Figure 4.7 and the loadings plot in Figure 4.8. The loadings are mainly towards the South Asian group and the majority of the FFAs in the model showed a higher trend or were significantly higher (laurate, myristate, palmitate, γ-linolenic and linoleate) in the South Asian group (Table 4.1).

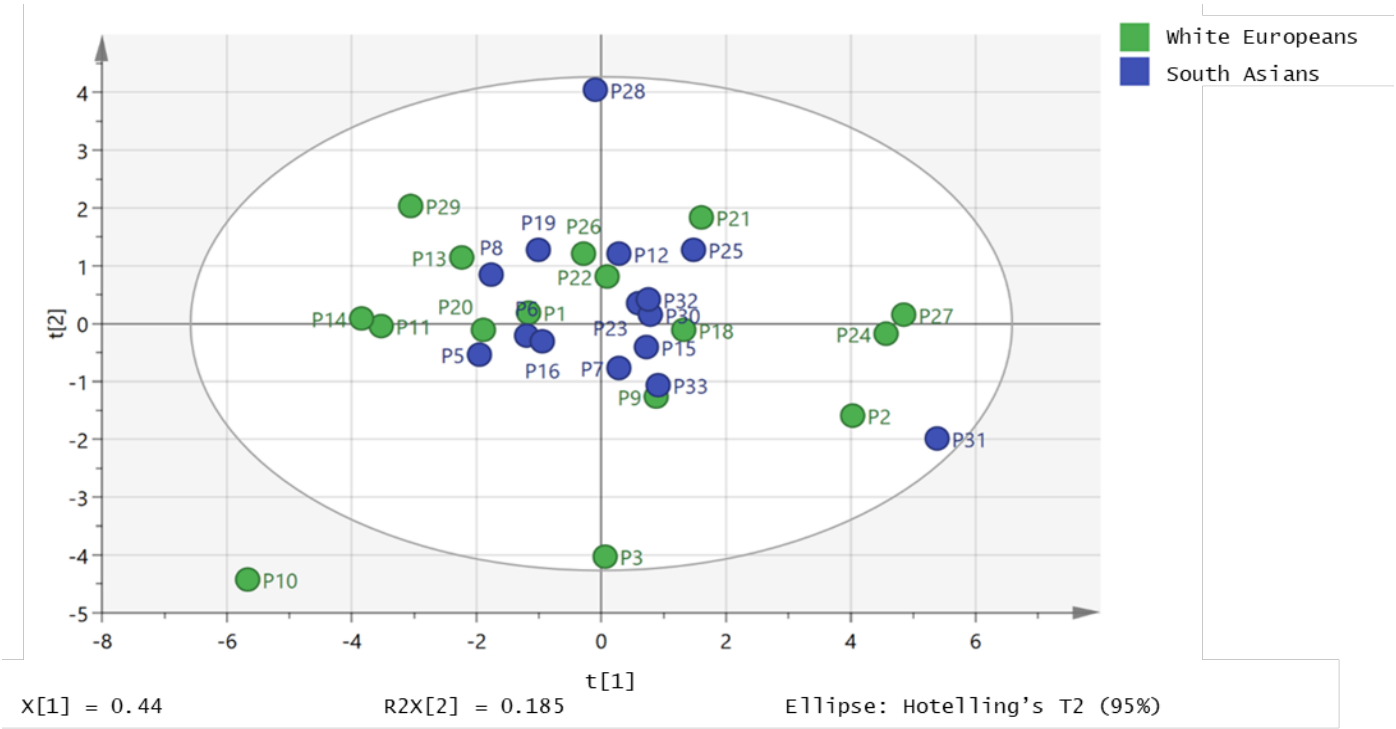

Figure 4.5. Principle components analysis (PCA) plot for South Asian (n = 16) and white European (n = 16) samples based on peak quantities of 19 fatty acids in plasma. The Y axis reflects the variation within the groups while the X axis reflects the variation between the groups.

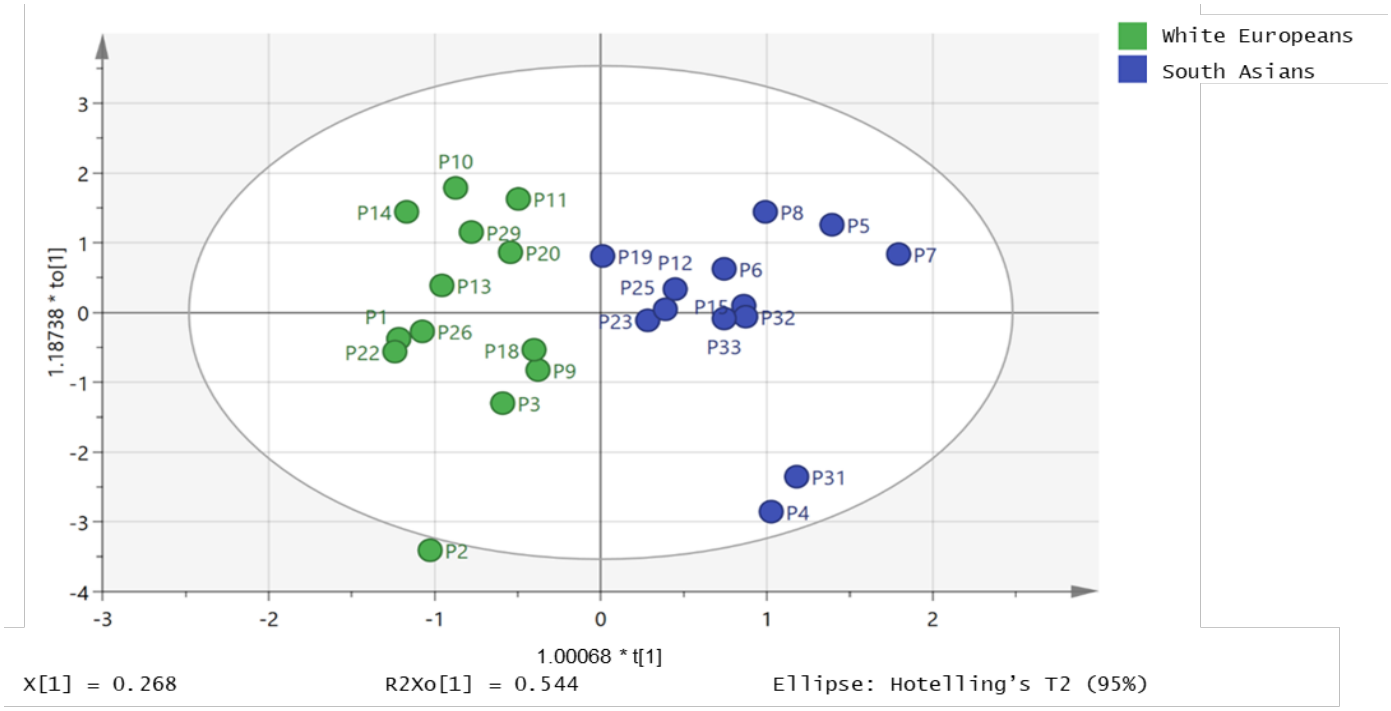

Figure 4.6. Orthogonal partial least squares (OPLS-DA) plot showing separation of 13 South Asian samples from 13 white European samples based on the concentrations for four fatty acids (myristate, linoleate, linolenate and docosapentenoate) in plasma. The Y axis reflects the variation within the groups while the X axis reflects the variation between the groups.

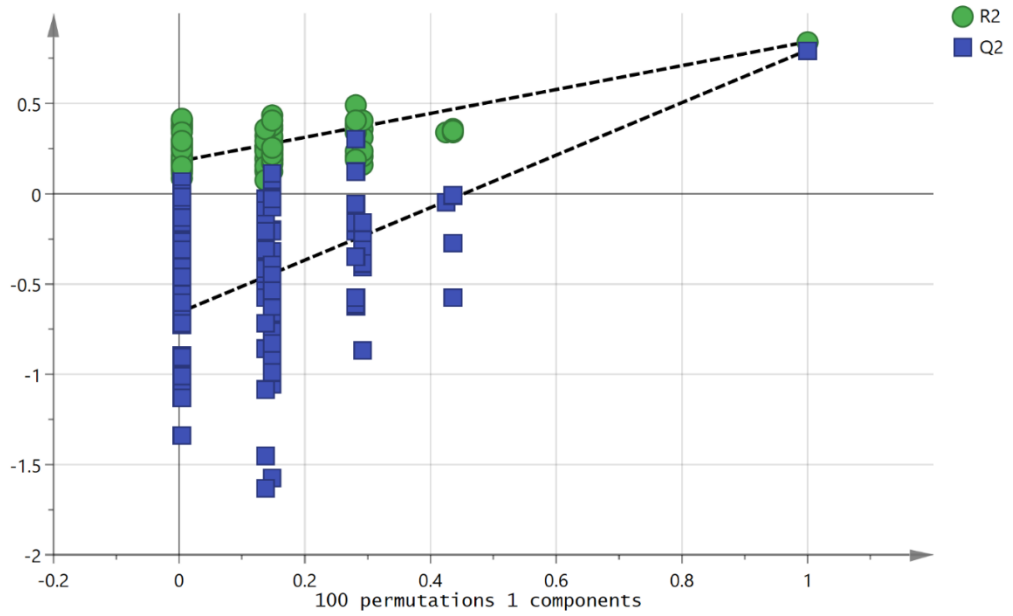

Figure 4.7. Cross-validation plot corresponding to Figure 4.6. The Y axis represents R^2 (circles) and Q^2 (square) for the model, and the X axis designates the correlation between original and permuted responses data.

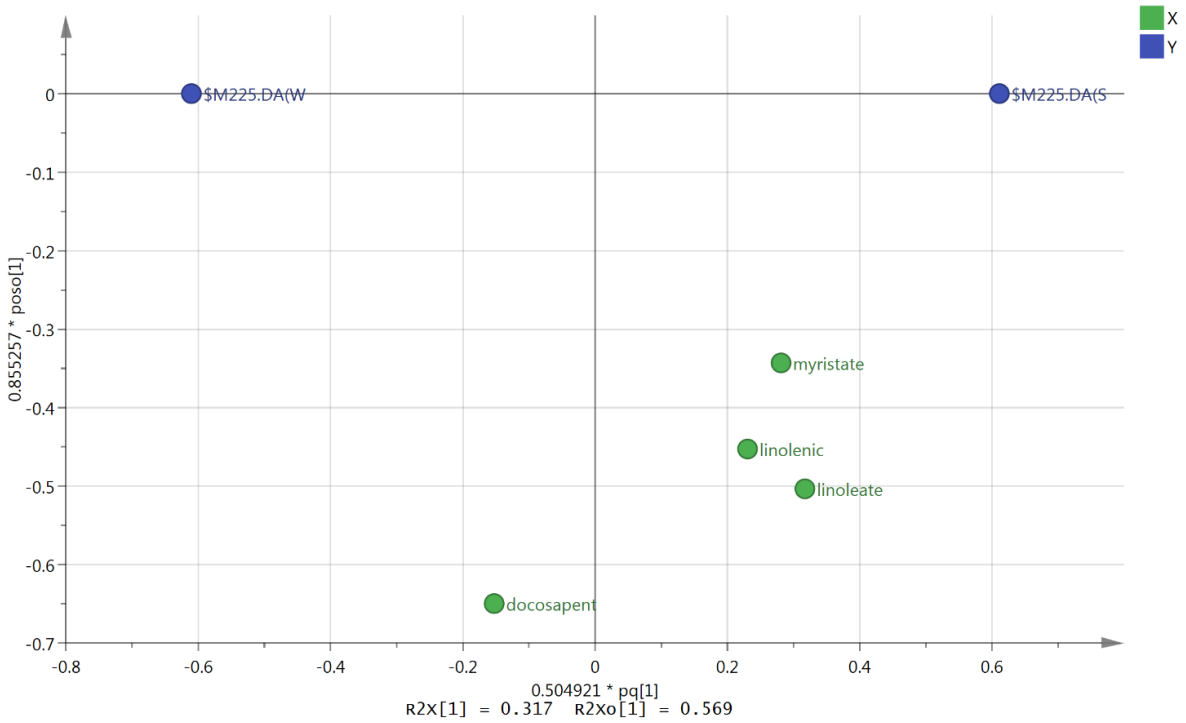

Figure 4.8. Loadings plot corresponding to the OPLS-DA plot shown Figure 4.6. The Y axis displays the loading of the predictive component while the X axis displays the loading of the orthogonal component.

4.3.5 Correlations

It was not possible to produce strong OPLS models when trying to fit the participant characteristics such as body mass, BMI or body fat percentage (full details of participant characteristics are reported in Table 3.1, Chapter 3.3.1) to the combined white European and South Asian groups. In addition, when the white European group was modelled in isolation, it was not possible to produce a strong model for any of the parameters in Table 3.1 presented in Chapter 3.3.1. However, when the South Asian group was modelled on its own, valid models could be produced for body mass, BMI, body fat percentage and AUC glucose (Figures 4.9, 4.10, 4.11 and 4.14). Conversely, in white European but not in South Asian men there was a strong correlation between total step counts and fatty acids profile (Figure 4.15).

4.3.5.1 Correlations between fatty acids and body mass, BMI, body fat percentage and AUC glucose

The strongest correlation was produced for body fat percentage ($r^2 = 0.92$; Figure 4.11) where the loadings indicated that that the highest body fat percentage correlated with the highest levels of palmitic, linoleic, docosahexenoic and eicosatetraenoic acids (Figure 4.12). Figure 4.13 shows extracted ion traces for palmitic and eicosatetraenoic acids for participants P31 and P8 (both South Asians) who had the highest and lowest body fat percentage, respectively. Body mass could also be modelled using an OPLS model and just three FFAs to produce a strong model ($r^2 = 0.86$; Figure 4.9). Two of the FFAs, docosahexenoic acid and stearic acid, which correlated with body fat percentage were also used in the OPLS-DA model (Figure 4.6) for actual against predicted body mass and in addition the long chain fatty acid lignoceric acid was included. It was also, perhaps not surprisingly, possible to fit an OPLS model for predicted BMI against actual BMI using four FFAs; stearate, palmitate, myristate and arachidate (Figure 4.10) although the correlation ($r^2 = 0.79$) between predicted and actual BMI was weaker than for OPLS plots for body fat percentage and body mass. In addition, for the South Asian group it was possible to correlate AUC glucose to four FFAs (palmitate, oleate, eicosatrienoic and docosahexenoic acid) ($r^2 = 0.89$; Figure 4.14).

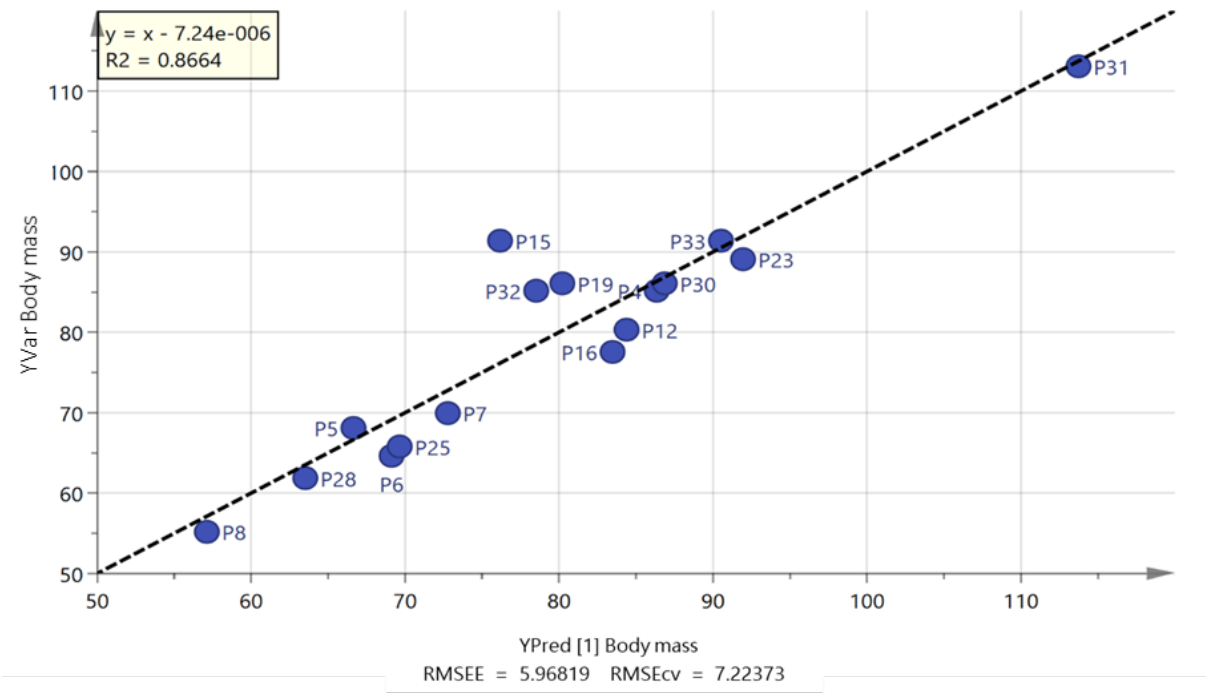

Figure 4.9. OPLS of predicted (X axis) vs. actual body mass (Y axis) based on three free fatty acids (lignoceric, docosahexenoic and stearic acid) for South Asian men.

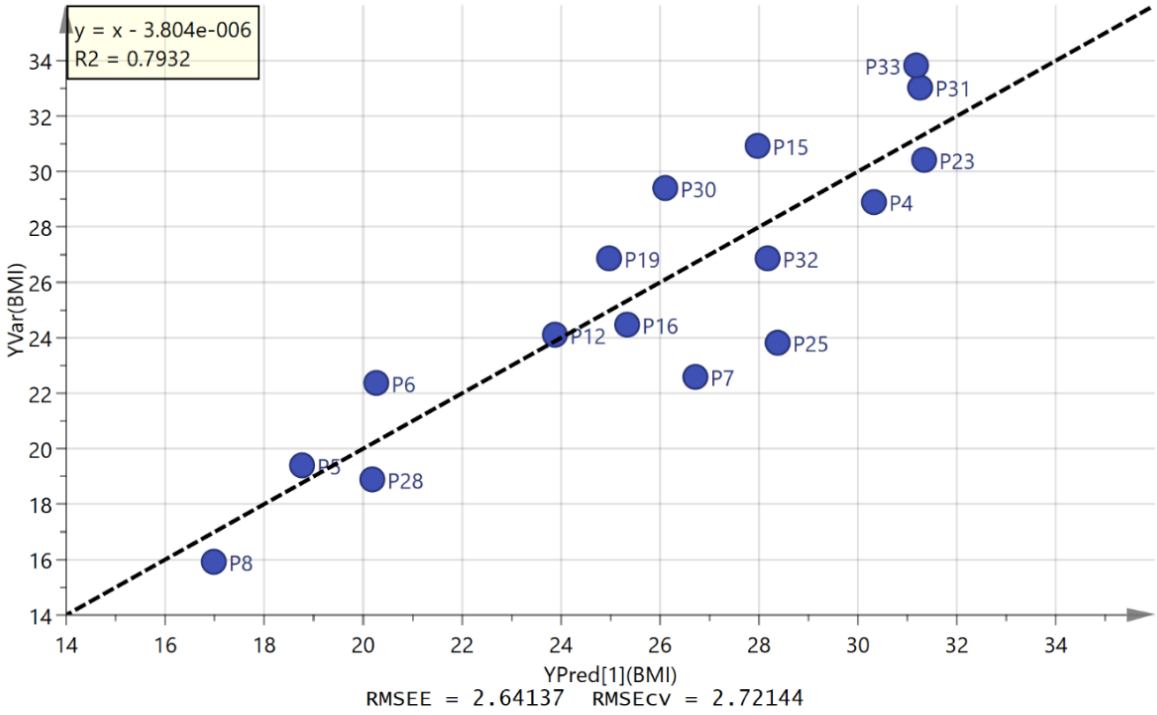

Figure 4.10. OPLS plot of predicted (X axis) vs. actual BMI (Y axis) based on four free fatty acids (stearate, palmitate, myristate and arachidate) for South Asian men.

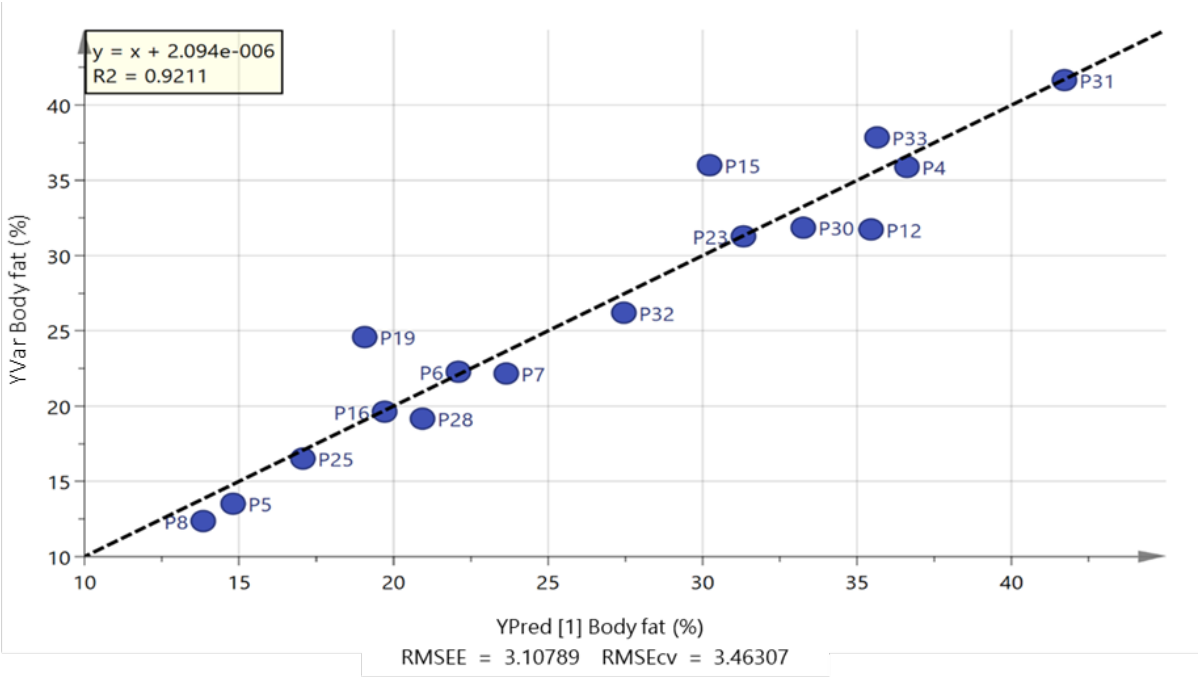

Figure 4.11. OPLS plot of predicted (X axis) vs. actual body fat percentage (Y axis) based on five free fatty acids (palmitate, stearate, linoleate, eicosatetraenoic acid and docosahexaenoic acid) for South Asian men.

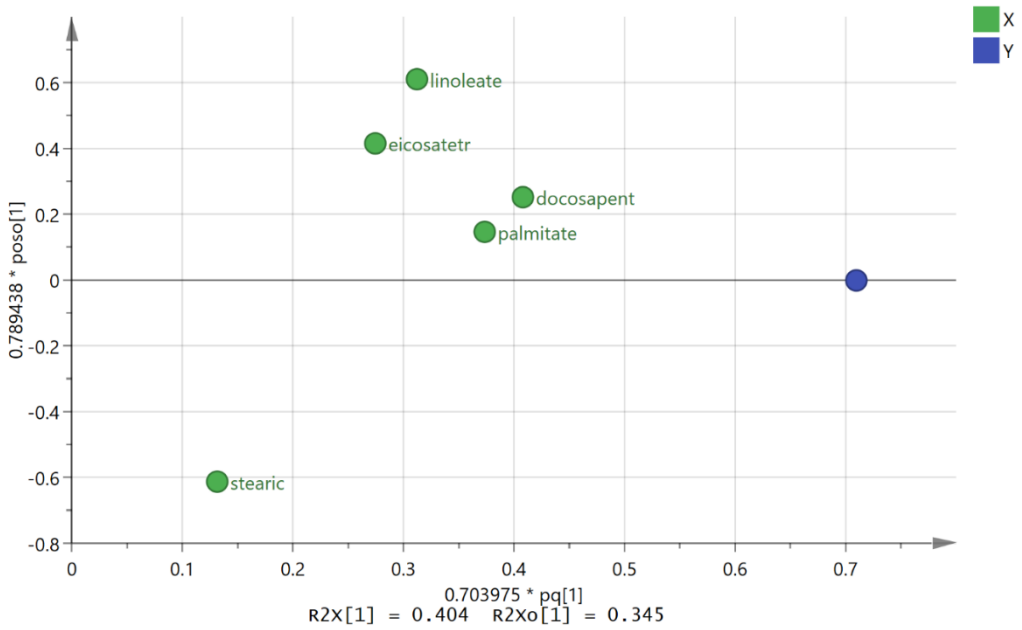

Figure 4.12. Loadings plot for Figure 4.11. The Y axis displays the loading of the predictive component while the X axis displays the loading of the orthogonal component.

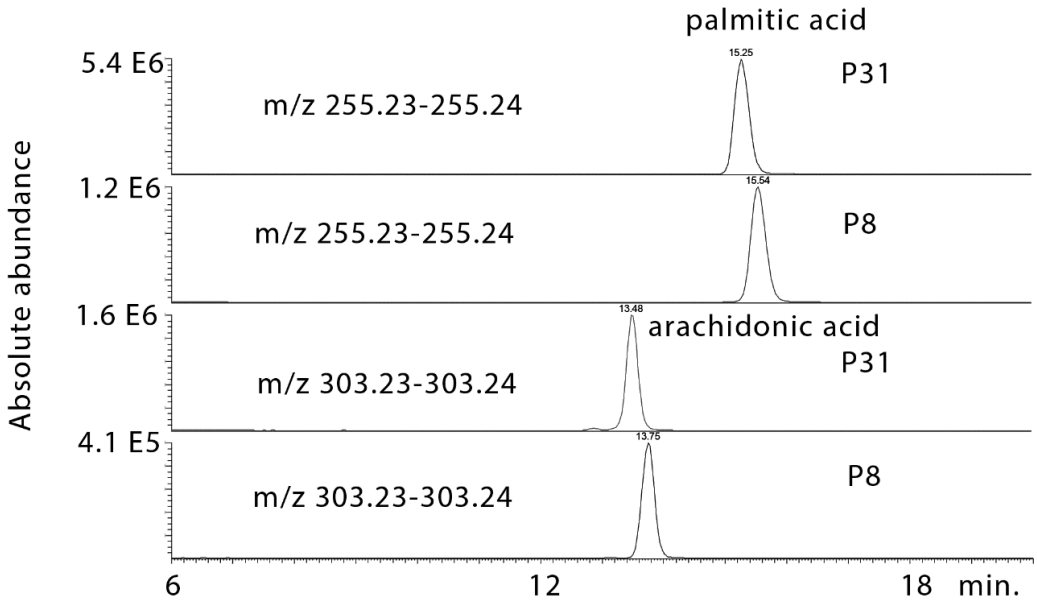

Figure 4.13. Extracted ion traces for two markers of higher body fat percentage, palmitic acid and eicosatetraenoic acid. The levels of the acids are highest in participant P31 (South Asian) who had the highest body fat percentage and lowest in participant P8 (South Asian) who had the lowest body fat percentage.

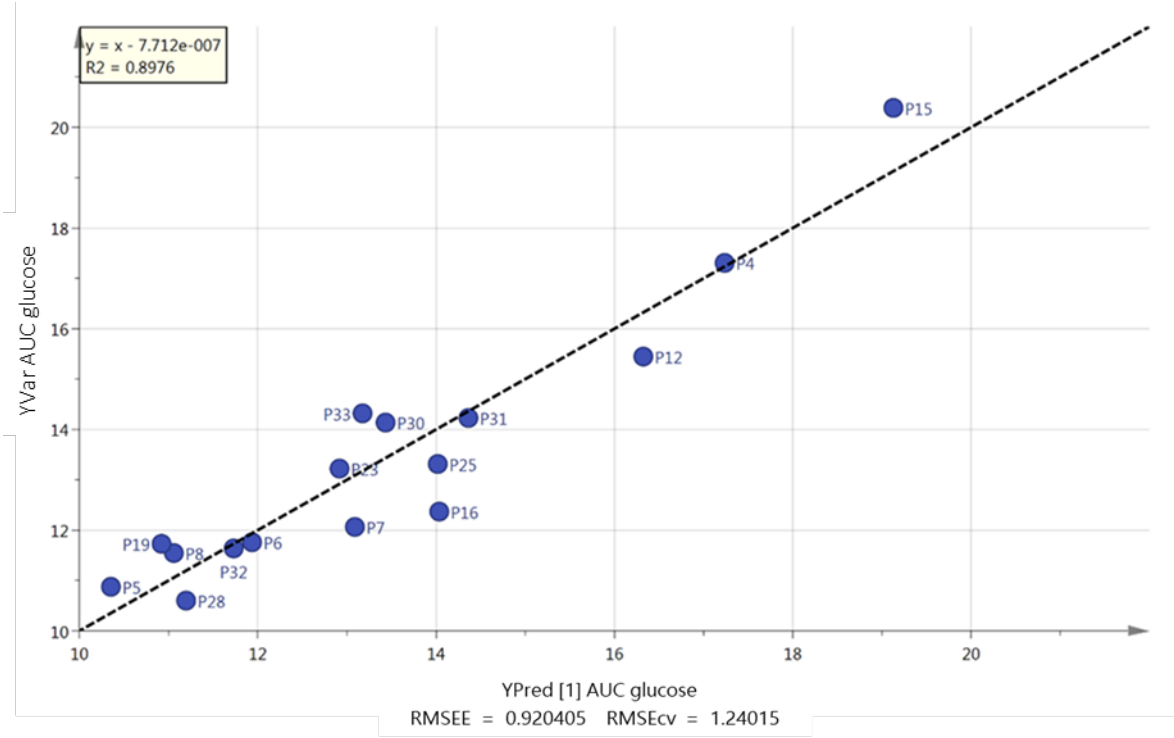

Figure 4.14. OPLS of predicted (X axis) vs. actual area under the curve (Y axis) (AUC) for glucose based on four free fatty acids (palmitate, oleate, eicosatrienoic acid and docosahexenoic acid) for South Asian men.

It was not possible to produce a strong OPLS model predicting $\dot{V}O_2$ max, waist circumference or systolic and diastolic blood pressure for the South Asian or white European group. Partial Least Square (PLS) models could also be fitted to the variables used for modelling body fat percentage, body mass and AUC glucose, but the fit was not quite as strong as for the OPLS models.

4.3.5.2 Correlations between free fatty acids and total step counts

In white European but not in South Asian men there was a strong correlation between total step counts and fatty acids profile (r^2 = 0.96; Figure 4.15) based on six FFAs: palmitoleic, linoleic, arachidate, laurate, erucate and nervonate (Figure 4.16). Lower step counts were also associated with elevated levels of FFAs, particularly palmitoleic and linoleic acids (Figure 4.17).

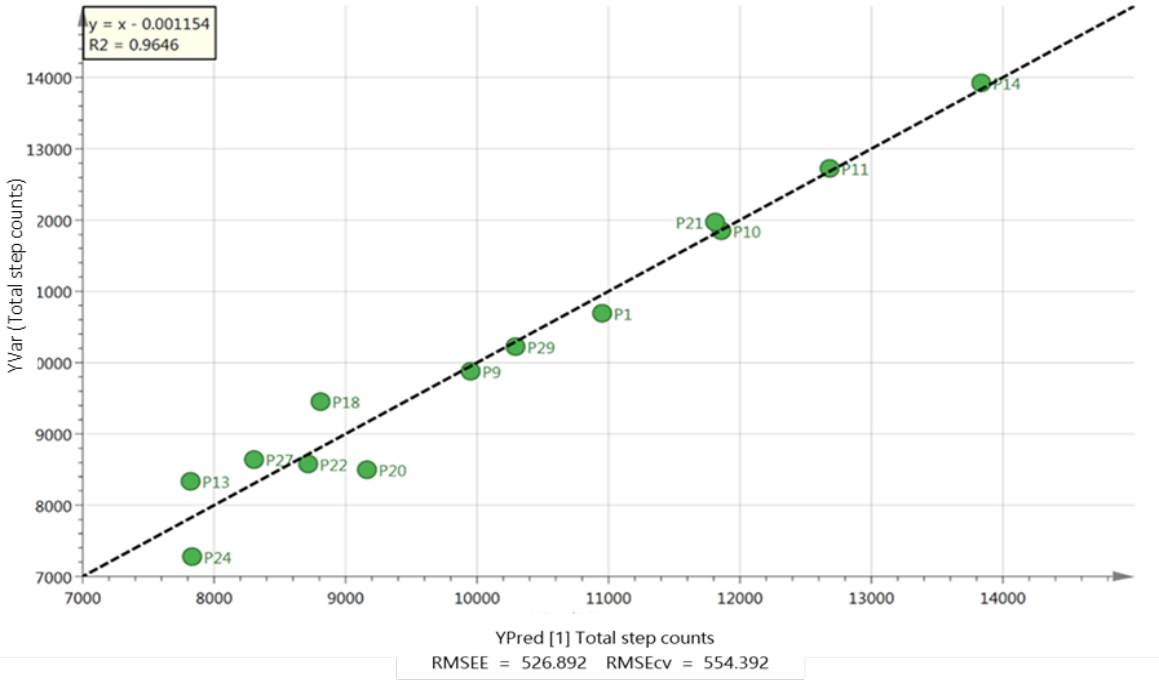

Figure 4.15. OPLS of predicted (X axis) vs. actual total step counts (Y axis) based on six fatty acids (palmitoleic, linoleic, arachidate, laurate, erucate and nervonate) for white European men.

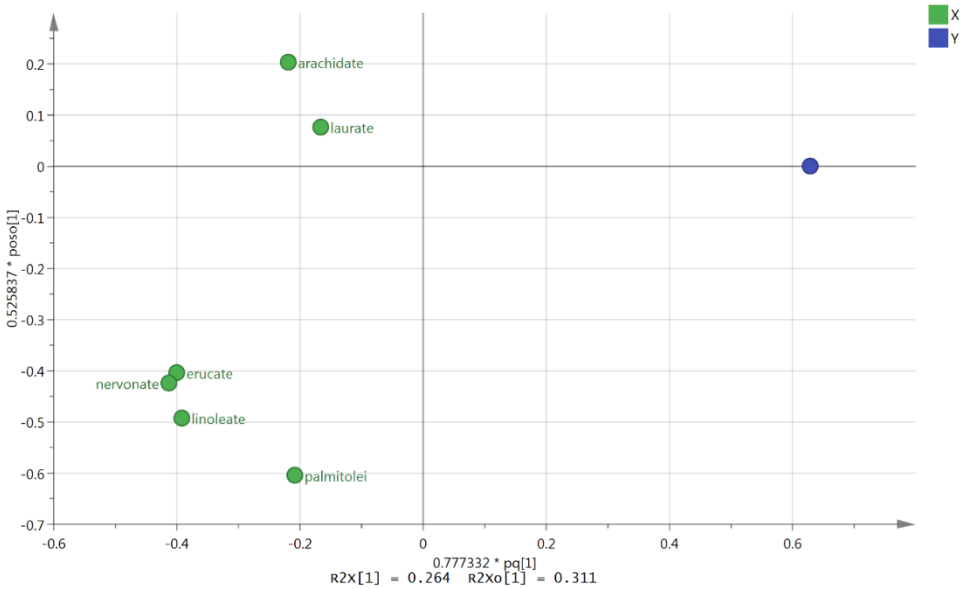

Figure 4.16. Loadings plot for Figure 4.15. The Y axis displays the loading of the predictive component while the X axis displays the loading of the orthogonal component.

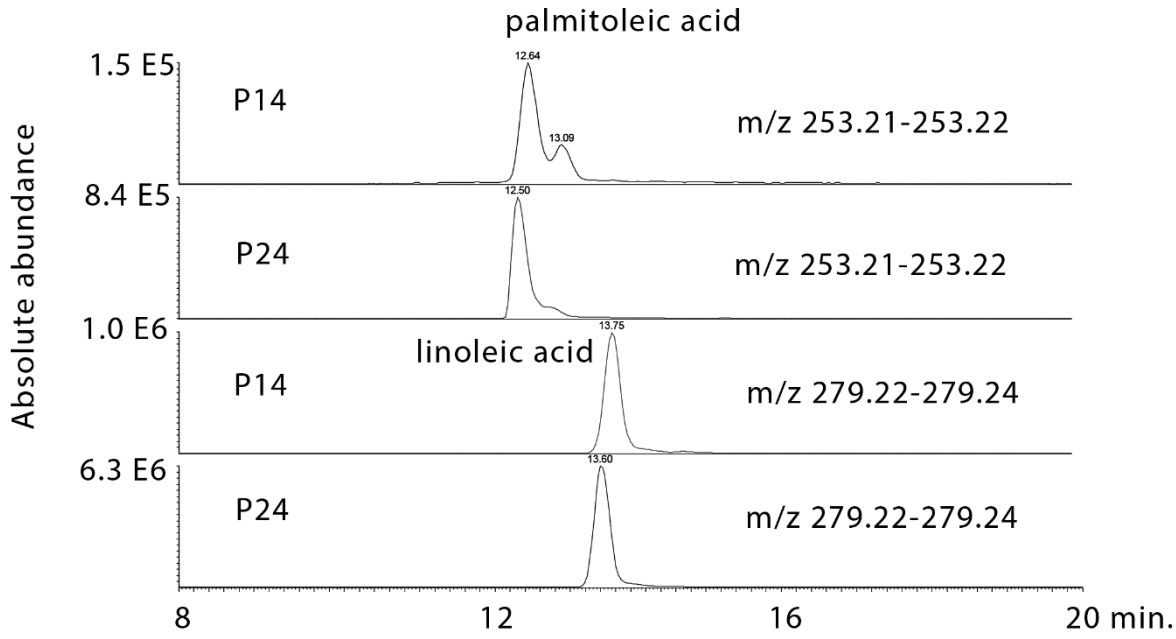

Figure 4.17. Extracted ion traces for palmitoleic acid and linoleic acid for participant P24 (white European) who had the lowest step count and participant P14 (white European) who had the highest total step counts.

4.4 Discussion

In this study, we conducted a cross-sectional analysis to determine whether the free fatty acid metabolic profile differed between healthy South Asian men and with white European men. This study also examined associations with physical activity and cardiorespiratory fitness levels, in addition to other T2D and CVD risk factors.

The initial results based on the GC-MS method were unsatisfactory as we could not identify any compounds of interest. The sample preparation protocol initially employed for the lipid extraction as well as the GC-MS conditions were subsequently optimised to exclude that the unsatisfactory results were ascribed to either a lack of concentration of fatty acids in the sample injected into the GC or a low sensitivity of the MS detection. However, after these adjustments results did not exhibit significant improvement with the FFAs eluding detection, which most likely reflect the inadequacy of the capillary column used in our experiments.

Conversely, we could separate majority of the South Asian from most of the white European men based on LC-MS method using a strong OPLS-DA model, which provided some confidence that these participants had markedly different fatty acid profiles. Furthermore, it was possible to fit OPLS models for the South Asian men to predict body mass, BMI, body fat percentage and AUC glucose whereas the same models were not a good fit for the white European men. Release of fatty acids from adipocytes is under the control of glucocorticoids and it might be that there are differences in either glucocorticoid concentration or glucocorticoid sensitivity within the two ethnic groups, although it has been found that cortisol levels are actually lower in South Asians in comparison with white Europeans (Reynolds et al. 2006). However, the lack of fit with

the data for the white European group lends some additional confidence that the data was not over-fitted which is possible when the sample size is small.

The plasma free fatty acid composition has been reported to be linked to the risk of T2D and CVD (Lu et al. 2016; Warensjö et al. 2005). In the present study, lauric, myristic, palmitic, γ-linolenic and linoleic acid were significantly higher in South Asian compared with white European participants, which represents a novel finding of this study. Previous studies reported that individuals with insulin resistance typically exhibit higher levels of SFAs (i.e. palmitate, laurate or stearate), omega-6 PUFAs (i.e. linoleic acid) and low concentrations of omega-3 PUFAs such as eicosapentaenoic and docosahexaenoic acid (Lu et al. 2016; Warensjö et al. 2005). Given the greater risk of CVD and T2D in South Asian than white Europeans (Sattar and Gill, 2015), it was surprising that baseline concentrations of γ-linolenic and linoleic acid were higher in the South Asian group. To the author's knowledge, only one previous study explored differences in baseline levels of FFAs between South Asians and other ethnic groups, in which γ-linolenic and linoleic acid were displayed to be also higher in the South Asian compared with the Caucasian group (Ralston et al. 2013). However, the study from Ralston and co-workers focused mainly on ethnic-specific associations between individual plasma FFAs and markers of insulin resistance, without explicitly examining ethnic differences in baseline concentrations of FFAs (Ralston et al. 2013). Although the reasons for this finding remain unclear it is possible that differences in habitual food intake between ethnic groups in this study may have influenced the results. South Asians typically increase consumption of fat intake when moving to western countries, particularly saturated fat-rich food such as coconut oil, palm oil or semifluid clarified butter (ghee), which is reflected in the concentrations in plasma and cell membranes (Holmboe-Ottesen and Wandel, 2012).

The present study also identified positive associations between AUC glucose and four FFAs such as palmitate, oleate, eicosatrienoic and docosahexenoic acid in South Asian participants (Figure 4.14). The positive association between palmitate and AUC glucose in our study is in agreement with previous research, despite these studies being conducted in populations with different ethnic background (Lu et al. 2016; Ebbeson et al. 2010; Kusunoki et al. 2007). Although the mechanisms of saturated fatty acids-induced insulin resistance remain elusive, altered membrane phospholipid fatty acid composition and membrane fluidity and stability may play a role (Ebbeson et al. 2010). We also reported positive associations between AUC glucose and oleic acid, which seems to confirm the data from Ralston et al. (2013) who identified significant correlations between oleate and insulin markers, although in Caucasians but not in South Asian men (Ralston et al. 2013).

The present study also revealed a strong correlation of FFAs with body fat percentage in the South Asian group and between FFAs and physical activity in the white European group. Particularly, in the South Asian group greater levels of palmitate, stearate, linoleate, eicosatetraenoic and docosahexaenoic acid were positively associated with body fat percentage (Figure 4.11). Higher levels of FFAs have been associated with both obesity and insulin resistance (Boden, 2011; Langin et al. 2005) thus, it may be possible that in the South Asian group a greater body fat percentage resulted in higher levels of FFAs, but not in the white European group. However, ethnic differences in factors involved in myocellular lipid mobilisation may underlie this response. Two key lipolytic enzymes involved in the intracellular triglyceride mobilisation in the body include the hormone sensitive lipase (HSL), which is regulated by catecholamine release, and the adipose triacylglycerol lipase (ATGL) which is not under the same hormonal control (Schreiber et al. 2018). In muscle cells, for example, lipolytic activity of ATGL is stimulated by perilipin 5 (PLIN-5), a lipid droplet-associated protein which regulates

basal lipolysis (Schreiber et al. 2018). According to previous research, South Asians exhibit higher PLIN-5 protein content in skeletal muscle in response to a 5-day high fat diet, compared with Caucasian males (Gemmink et al. 2017), which may reflect higher lipolysis rates in South Asian individuals. Thus, it is plausible that in our study ethnic differences in ATGL/PLIN-5-induced lipolysis may have contributed to the higher levels of FFAs linked to body fat in the South Asian group, although further research is required before any conclusion can be drawn. Conversely, six FFAs (palmitoleic, linoleic, arachidate, laurate, erucate and nervonate) were strongly associated with total step counts in the white European group only, and palmitoleic and linoleic acid were also associated with lower step counts suggesting that fatty acid metabolism is less responsive to physical activity in South Asian than white European men. This finding might reflect the fact that the FFAs release is promoted more by HSL, which responds to corticosteroids and catecholamine release (Schreiber et al. 2018). Catecholamines also promote peroxisome proliferation (Russel et al. 2013) which could result in lower levels of FFAs correlating with higher total step counts.

The present study was limited by the small number of participants and the South Asian population was mostly limited to men originating from India. Additionally, the potential confounding effects of body fat percentage may have accentuated the differences in FFAs between ethnic groups. Therefore, further work should be performed to investigate differences in FFAs metabolic profile in other South Asian groups (e.g., Bangladeshis, Sri Lankan and Bhutanese) and in South Asian women compared with body fat-matched white European individuals. Furthermore, investigating FFAs in response to exercise and/or food intake and how and explore how these responses correlate with cardiometabolic risk markers may elucidate the role of exercise and energy intake in

modulating individual plasma FFAs concentrations and in optimising health outcomes in South Asians.

In conclusion, the current study provides evidence that levels of circulating FFAs are different between South Asians and white European men. Using an OPLS models, FFAs were strongly correlated with body fat percentage and AUC for glucose in South Asian, whereas total step counts were strongly correlated with lower levels of FFAs in white European men. This may suggest that the different FFAs metabolic profile may be linked with the elevated T2D risk in South Asians than white Europeans and that fatty acid metabolism is less responsive to physical activity in South Asian men in comparison to white European men.

Chapter 5 – Study 3: Effects of moderate-vigorous cycling on appetite, ad libitum energy intake and appetite-related hormones in healthy South Asian and white European men

5.1 Introduction

Cardiovascular disease (CVD) represents the main cause of death globally (WHO, 2017) as well as in the United Kingdom (BHF, 2019) and type 2 diabetes (T2D) represents the main CVD risk factors (IDF, 2019). South Asians have a heightened risk of CVD and T2D compared to white Europeans, irrespective of their place of living, with both conditions manifesting at younger age in the South Asian population (Sattar and Gill, 2015; Gholap et al. 2011; Tziomalos et al. 2008). Although there seems to be a shift in the pattern of mortality risk between ethnicities with diabetic UK South Asians experiencing lower cardiovascular and all-cause mortality rate than their white European counterparts, the rate of cardiovascular complications such as myocardial infarction or stoke in British South Asians with diabetes continue to be higher than white Europeans (Johns and Sattar, 2017).

There is clear evidence that South Asians have a greater percentage of body fat and intra-abdominal adipose tissue for a given body mass index (BMI) compared with white European individuals which contributes to the elevated CVD and T2D risk in this population (Lear et al. 2012; Hall et al. 2010). The reasons for these are uncertain. It is possible that this innate phenotype of increased adiposity may be linked with differences in food intake and appetite regulation between South Asian and white European individuals. Several appetite-related hormones play a key role in energy homeostasis and weight control, including acylated ghrelin and peptide YY (PYY) which are mediators

of hunger and satiety, respectively (Hussain et al. 2013). Recent work from our laboratory reported lower concentrations of fasting acylated ghrelin, but not differences in fasting total PYY levels, in South Asian than white European men, which may be linked to the greater adiposity in South Asians (Study 1, Chapter 3), but further work is required to confirm this.

Given the important role of physical activity in the management of obesity and weight control, there has been considerable interest in the effects of exercise on appetite perception and energy intake in response to exercise and in the underlying mechanisms linking physical activity, appetite and weight management. In this regard, a plethora of studies have investigated the appetite and energy intake responses during and after acute bouts of continuous aerobic exercise (50-70% $\dot{V}O_2$ max, 30-90 min), with the majority of these studies showing a transient suppression of appetite perceptions during and shortly after exercise, known as 'exercise-induced anorexia' (Deighton and Stensel, 2014). Furthermore, single sessions of exercise have consistently been shown to suppress acylated ghrelin concentrations and increasing levels of anorexigenic appetite-related hormones including total PYY, without stimulating subsequent changes in absolute energy intakes (Shubert et al. 2014).

However, it remains unknown how differences in individual ethnicity background modulate appetite perceptions, energy intake and appetite-related hormones in response to exercise. Furthermore, since observational evidence suggests that South Asian individuals engage in less habitual physical activity than white European individuals, which is likely to contribute to their elevated CVD and T2D risk (Rastogi et al. 2004; Williams et al. 2011b; Yates et al. 2015), research to examine the impact of exercise on appetite regulation, energy intake in this population is warranted.

Therefore, the aim of this study was to investigate the effects of acute exercise on subjective appetite ratings, appetite-related hormones and ad libitum energy intake in healthy South Asian and white European men.

5.2 Methods

5.2.1 Participants

Following approval from Kingston University's Ethics Advisory Committee (approval ethic code: 1617/034), 15 South Asian and 15 white European men aged 19–50 years provided written informed consent to participate in this study. The present study has been also registered as a clinical trial according to the protocol registration and results system (PRS) (registration number: NCT03698786). Based on previous preliminary data reported in Study 1 (Chapter 3), it was estimated that a sample size of 15 participants per group would have 83% power at the 0.05 level to detect a between-group difference in fasting acylated ghrelin of 1.11 between-subject standard deviations (SDs). The sample size was calculated using G*Power (Faul et al. 2007). Groups were matched for age and BMI. The South Asian group comprised seven British Asians born in the UK (UK Indian n=4; UK Pakistani n=2; UK Bangladeshi n=1) and eight individuals born in South Asia (India n=4; Pakistan n=2; Bangladesh n=1; Nepal n=1). Conversely, the white European group comprised nine British born participants and six individuals originating from European countries (Germany n=3; Spain n=1; Italy n=1; France n=1). One South Asian participant dropped out without giving any specific reason, thus it was excluded from analysis. This participant was therefore replaced with another South Asian man in order to have 15 South Asians and 15 white Europeans. All participants were non-smokers, had no personal history of cardiovascular/metabolic disease, were not taking any anticoagulant or anti-inflammatory medication and were not dieting. Before physical testing, the Physical Activity Readiness Questionnaire (PAR-Q) (Thomas et al. 1992) was completed by all participants to screen for possible contraindications to exercise. Table 1 shows the key participant characteristics.

5.2.2 Preliminary testing

Before the main trials, participants visited the laboratory to undergo preliminary assessments and to be familiarised with the laboratory environment and study procedures. Specifically, participants completed questionnaires assessing general health status, contraindications for blood sampling and habitual physical activity levels (International Physical Activity Questionnaire (IPAQ)) (Craig et al. 2003). At this visit, the participants verbally confirmed acceptability of the standardised breakfast and ad libitum buffet meal subsequently provided during the main experimental trials.

Body mass, stature, waist circumference, BMI, body composition and blood pressure were then measured as described in Chapter 2.2 and 2.5. Participants then completed an incremental exercise test to volitional exhaustion on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) for the determination of peak oxygen uptake ($\dot{V}O_2$ peak) as described in Chapter 2.6. Throughout the cardiorespiratory test, heart rate was monitored every 3 min using a HR monitor (Polar FT1, Polar Electro, Kempele, Finland). Oxygen consumption, HR and peak watts were used to determine the exercise intensity of the main trial.

5.2.3 Experimental procedure

Participants completed two, 7 h trials (control and exercise) in a randomised crossover design with at least 7 days between each trial. Figure 5.1 shows the trial protocol. Participants were asked to refrain from consuming alcohol, caffeinated drinks and from participating in strenuous exercise during the 24 h prior to each trial. Participants were also asked to consume 500 mL of plain water the night before the exercise trial to ensure euhydration. A food diary was completed in the 24 h prior to the first trial, with

participants required to replicate food and drink intake as closely as possible for the 24 h prior to the subsequent trial.

On the morning of each trial, volunteers arrived at the laboratory at approximately 08:30 after a 9 h overnight fast. Particularly, all participants were informed to not ingest any food or drink, apart from water, after 11 pm of the night before attending the laboratory. Furthermore, participants were informed to exert themselves minimally when travelling to the laboratory, using motorised transport where possible. Upon arrival, participants rested in a semi-supine position whilst a cannula was inserted into the antecubital vein by a trained phlebotomist, and a standardised breakfast meal was then consumed within 15 min. The 7 h trial commenced at the start of the breakfast (0 h). In the exercise trial, participants rested throughout apart from completing 60 min of continuous cycling at 70% of $\dot{V}O_2$ peak between 2 and 3 h. Samples of expired air were collected at 15, 30, 45 and 60 min during exercise using an online breath-by-breath gas analysis system (as described above) to monitor the exercise work-load. Heart rate and ratings of perceived exertion (RPE) (Borg, 1973) were also recorded at this time. Energy expenditure and non-protein respiratory exchange ratio (RER) for the estimation of substrate utilisation were calculated from oxygen uptake and carbon dioxide production (Weir, 1990) during the exercise bout. A buffet meal was provided at 4 h and participants were free to consume ad libitum for 30 min. Identical procedures were followed in the control trial except that no exercise was performed.

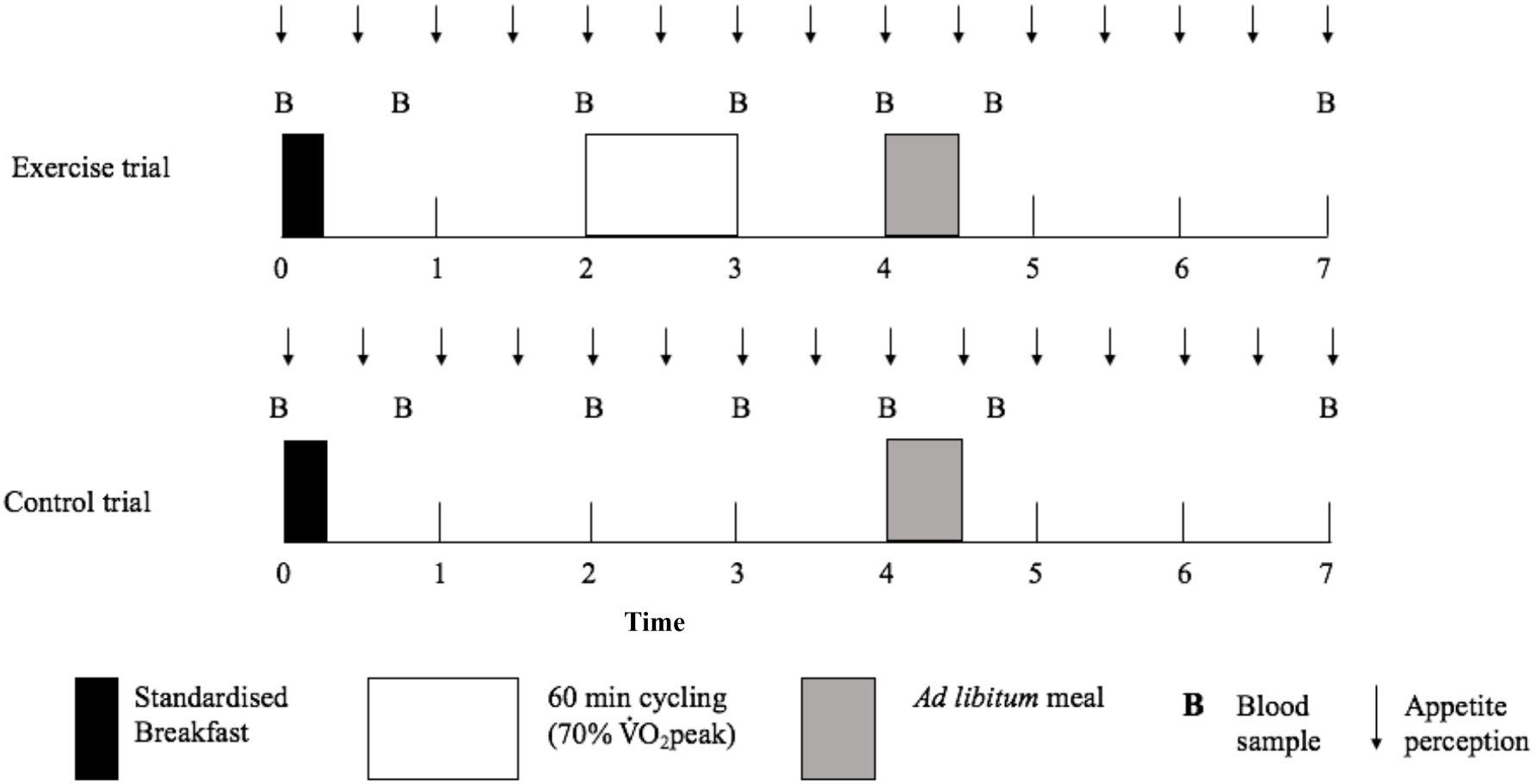

Figure 5.1. Schematic illustration of the study protocol

5.2.4 Self-reported physical activity

Raw data from the long form IPAQ were assessed following the questionnaire guidelines (Craig et al. 2003) and expressed as metabolic equivalent (MET) minutes per day spent in walking, moderate-to-vigorous physical activity (MVPA) and total physical activity. Total time spent sitting expressed as minutes per day was also reported. Data from participants with the sum total of all walking, moderate and vigorous time greater than 960 minutes (16 hours) were excluded from the analysis (Craig et al. 2003).

5.2.5 Appetite perceptions

During each trial (exercise and control) perceptions of appetite (hunger, satisfaction, fullness and prospective food consumption) were assessed before starting the standardised breakfast meal and at 30 min intervals using 100 mm visual analogue scales (VAS) (Flint et al. 2000). An overall appetite rating was calculated as the mean value of the four appetite perceptions after reversing the values for satisfaction and fullness (Stubbs et al. 2000).

5.2.6 Standardised breakfast and *ad libitum* meal

The standardised breakfast consisted of a sandwich (55 g white bread, 23 g cheese, 10g mayonnaise and 40 g ham), 60 g chocolate muffin and 250 ml orange juice. For five South Asian participants ham was replaced with isocaloric tuna due to religious beliefs. The energy and macronutrient content of this meal was 2853 kJ, 46% carbohydrate, 14% protein and 40% fat.

The *ad libitum* buffet meal was set up identically for each trial and consisted of granola, oats, corn flakes, white bread, semi skimmed milk, orange juice, cheese, ham/tuna, butter, margarine, mayonnaise, salted crisps, chocolate bars, cereal bars, cookies,

muffins, apples, oranges and bananas. All food pre-weighted and being presented in excess of expected consumption. Participants were told to eat until satisfied and that additional food was available if required. The buffet meal was consumed in isolation with no distraction and the use of computers or mobile phones was prohibited to minimise any influence on food consumption. At the end of the buffet meal, leftover food was weighed, and absolute energy intake and macronutrient composition of the food consumed was determined by calculating the weighted difference of each food item before and after each meal. In the exercise trial, relative energy intake was calculated as follows: absolute energy intake minus the net energy expenditure of exercise. Water was available *ad libitum* throughout the trials.

5.2.7 Blood sampling

Venous blood samples were collected via a cannula (Vasofix® Safety, B. Braun, Melsungen, Germany) inserted into an antecubital vein. Blood samples were collected at 0, 0.75, 2, 3, 4, 4.75 and 7 h for the determination of acylated ghrelin, PYY, insulin and glucose concentrations. Full details of blood sample collection are reported in Chapter 2.10.

5.2.8 Analytical methods

Plasma concentrations of acylated ghrelin, total PYY, and insulin were measured using commercially available enzyme-linked immunosorbent assays. Specifications of the analysis and details of the commercially available enzyme-linked immunosorbent assay kits are reported in Chapter 2.11.3 and 2.11.4. Plasma glucose concentrations were analysed immediately in singular using a glucose analyser (specifications of the analysis and details of the glucose analyser are reported in Chapter 2.11.4). Haemoglobin and haematocrit were analysed immediately in duplicate using a haematology blood counter

(Yuminez H500-CT, HORIBA ABX Diagnostic, Northampton, UK). The within batch coefficients of variation for the assays were as follows: 7.3 % acylated ghrelin, 3.2 % total PYY, 2.4 % insulin and 3.3 % glucose.

5.2.9 Statistical analysis

All statistical analyses were conducted using the analytical software SPSS version 23.0 for Windows (SPSS 23.0, IBM Corp, Armonk, NY, USA). Normality of the data was checked using Shapiro-Wilk tests. Normally distributed data are presented as mean (SD). Data for self-reported physical activity and fasting plasma concentrations were not normally distributed and were natural log-transformed before analysis. These data are presented as geometric mean (95% confidence interval) and analysis is based on ratios of the geometric mean and 95% confidence intervals (CI) for ratios. The trapezium rule was used to calculate the time averaged total under the curve (tAUC). Concentrations of plasma appetite-related hormones and metabolic markers are presented relative to baseline concentrations (delta) and time-averaged total area under the curve (tAUC) for these markers are also presented as delta values for consistency. Correction of plasma values for plasma volume changes did not alter the interpretation of the results and the unadjusted data are reported for simplicity. Habitual physical activity expressed as walking, MVPA, total physical activity and total sitting were compared between ethnic groups using linear mixed models including awake time as a covariate.

Participants characteristics, exercise responses and self-reported physical activity levels were compared between the South Asian and white European men using linear mixed models with ethnic group included as a fixed factor. Differences in fasting plasma concentrations, baseline appetite perceptions, time-averaged tAUC and energy/macronutrient intakes were examined using linear mixed models with ethnic

group (South Asian, white European) and trial (exercise, control) modelled as fixed factors. Differences in appetite perceptions, appetite-related hormones, glucose and insulin concentrations over time were examined using linear mixed models with ethnic group, trial and time as fixed factors. Ethnicity-specific Spearman's correlation coefficients using the mean difference between trials were used to examine the magnitude of linear association between the various predictors (age, body fat percentage, $\dot{V}O_2$ peak, appetite ratings) and outcome measures (acylated ghrelin, total PYY and insulin). Bivariate Spearman's correlations were also used to determine associations between total carbohydrate oxidation during exercise and absolute energy intake in South Asian and white European men. Absolute standardised effect sizes (ES) (Cohen's d) were calculated for each variable by dividing the difference between the mean values (South Asian versus white European) with the pooled SD. An ES of 0.2 was considered the minimum important difference, 0.5 moderate and 0.8 large (Cohen, 1988). Statistical significance was accepted as $P < 0.05$.

5.3 Results

5.3.1 Participant characteristics

The physical and physiological characteristics of the South Asian and white European men are shown in Table 5.1. The 95% CI for age, body mass, BMI, fat mass, body fat percentage, waist circumference and resting diastolic blood pressure overlapped zero (all $P \geq 0.064$). However, standardised ESs were small-to-moderate for age, body fat percentage, waist circumference and resting diastolic blood pressure (ES = 0.26–0.44) and a moderate-to-large ES was observed for body mass (ES = 0.73). Compared with white European men, South Asian men had lower stature (ES = 1.16, $P = 0.003$), fat free mass (ES = 1.09, $P = 0.006$), resting systolic blood pressure (ES = 0.90, P = 0.020) and $\dot{V}O_2$ peak expressed in absolute (ES = 1.70, $P < 0.001$) and relative (ES = 0.99, $P = 0.007$) terms.

5.3.2 Self-reported physical activity

Awake time adjusted total physical activity was lower in South Asian than white European men (ES = 0.78, $P = 0.030$) whereas total MVPA was meaningfully, albeit not significantly, lower in South Asians than white Europeans (ES = 0.78, $P = 0.051$) (Table 5.2). South Asian men exhibited, on average, greater sitting time than white Europeans (ES = 0.72, $P = 0.064$), although the difference did not reach significance (Table 5.2). Walking (ES = 0.54, $P = 0.116$) and awake time (ES = 0.52, $P = 0.165$) were similar between groups (Table 5.2).

Table 5.1. Participant characteristics.

	South Asians (n=15)	White Europeans (n=15)	White Europeans vs. South Asians Mean difference (95% CI[a])	Effect size
Age (years)	29 (8)	33 (10)	-4 (-11, 3)	0.44
Stature (cm)	173.7 (6.8)	181.5 (6.6)	-7.8 (-12.9, -2.8) [b]	1.16
Body mass (kg)	76.0 (12.5)	85.5 (14.4)	-9.5 (-19.5, 0.6)	0.73
Body mass index ($kg \cdot m^{-2}$)	25.4 (4.5)	26.1 (3.8)	-0.6 (-3.7, 2.4)	0.17
Fat free mass (kg)	59.6 (8.8)	68.5 (7.5)	-8.9 (-14.9, -2.8) [b]	1.09
Fat mass (kg)	17.6 (8.0)	17.5 (10.7)	0.1 (-7.0, 7.1)	0.01
Body fat (%)	22.4 (8.3)	19.3 (8.3)	3.2 (-3.0, 9.4)	0.37
Waist Circumference (cm)	84.8 (9.8)	87.6 (11.5)	-2.8 (-10.8, 5.1)	0.26
Resting sBP (mmHg)	116 (12)	125 (8)	-9 (-16, -1) [b]	0.90
Resting dBP (mmHg)	77 (9)	75 (7)	2 (-4, 8)	0.26
$\dot{V}O_2$ peak ($L \cdot min^{-1}$)	3.10 (0.61)	4.12 (0.59)	-1.02 (-1.47, -0.57) [b]	1.70
$\dot{V}O_2$ peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	41 (7)	49 (9)	-8 (-14, -2) [b]	0.99

All values are mean (SD). Data were analysed using linear mixed models with group (South Asian vs. white European) included as a fixed factor.

sBP, systolic blood pressure; *dBP*, diastolic blood pressure; *$\dot{V}O_2$ peak*, peak oxygen uptake

[a] 95% confidence interval for the mean absolute difference between the groups. [b] Main effect of group ($P < 0.05$).

Table 5.2. Self-reported habitual physical activity levels and sitting time in South Asian and white European men.

	South Asians (n=15)	White Europeans (n=15)	White Europeans vs. South Asians Mean difference (95% CI[a])	Effect size	*P* value
Walking (met·min^{-1}·day^{-1})	90 (51, 158)	214 (120, 376)	-44% (-73, 16%)	0.54	0.116
Total MVPA (met·min^{-1}·day^{-1})	184 (93, 357)	489 (249, 951)	-63% (-86, 1%)	0.78	0.051
Total PA (met·min^{-1}·day^{-1})	395 (279, 558)	770 (545, 1090)	-40 (-62, -5%) [b]	0.78	0.030
Total sitting time (min·day^{-1})	331 (264, 413)	265 (212, 332)	34% (-2, 84%)	0.72	0.064
Awake time (min·day^{-1})	589 (509, 681)	680 (588, 786)	-13% (-30, 6%)	0.52	0.165

All values are geometric mean (95% confidence interval). Statistical analyses are based on log-transformed data.

Data were analysed using linear mixed models with group (South Asian vs. white European) included as a fixed factor. Models for walking, total MVPA, total PA and total sitting included awake time as a covariate.

MVPA moderate-to-vigorous physical activity; *PA* physical activity.

[a] 95% confidence interval for the ratio difference of geometric means between the groups.

[b] Main effect of group ($P < 0.05$).

5.3.3 Exercise responses

Exercise net energy expenditure (ES = 1.54, $P < 0.001$), total fat oxidation (ES = 0.93, $P = 0.018$), total carbohydrate oxidation (ES = 1.07, $P = 0.007$) and absolute exercise work rate (ES = 1.69, $P < 0.001$) were lower in South Asian than white European men (Table 5.3). All other exercise responses were similar between ethnic groups (all $P \geq 0.128$) (Table 5.3).

5.3.4 *Ad libitum* energy and macronutrient intakes

Energy and macronutrient intakes during the ad libitum meal are shown in Table 5.4. Main effects of group revealed lower absolute energy intake (ES = 1.03, $P = 0.003$), relative energy intake (ES = 0.65, $P = 0.017$), carbohydrate intake (ES = 0.83, $P = 0.015$) and fat intake (ES = 1.02, $P = 0.003$) in South Asian compared with white European men. A main effect of trial for relative energy intake revealed exercise was lower than control (ES = 1.37, $P < 0.001$). A group-by-trial interaction for carbohydrate intake ($P = 0.014$) revealed lower intake in South Asians (mean difference (95% CI) -20 (-49, 10) g, ES = 0.38, $P = 0.184$) and higher intake in white Europeans (mean difference (95% CI) 34 (5, 64) g, ES = 0.50, $P = 0.025$) after exercise. No other main effects or group-by-trial interactions were identified for ad libitum energy and macronutrient intakes (all $P \geq 0.063$).

Table 5.3. Cycling exercise responses in South Asian and white European men.

	South Asians (n=15)	White Europeans (n=15)	White Europeans vs. South Asians Mean difference (95% CI[a])	Effect size
Heart rate (beats·min^{-1})	159 (12)	154 (11)	5 (-4, 13)	0.43
RPE (6-20)	13.7 (2.1)	13.9 (1.9)	-0.3 (-1.8, 1.2)	0.20
Respiratory exchange ratio	0.94 (0.02)	0.92 (0.04)	0.02 (-0.01, 0.04)	0.63
Exercise intensity (% of $\dot{V}O_2$ peak)	67 (4)	69 (5)	-3 (-6, 1)	0.56
Work rate (Watts)	115 (21)	161 (32)	-46 (-66, -25) [b]	1.69
Work rate (% max power)	59 (6)	58 (7)	2 (-3, 6)	0.23
Total fat oxidation (g)	13.7 (7.5)	21.9 (9.9)	-8.2 (-14.9, -1.5) [b]	0.93
Total carbohydrate oxidation (g)	106.7 (19.2)	136.5 (34.6)	-29.8 (-50.9, -8.7) [b]	1.07
Fat oxidation (% total energy expenditure)	20 (9)	22 (10)	-2 (-9, 5)	0.21
Carbohydrate oxidation (% total energy expenditure)	73 (8)	69 (10)	4 (-3, 11)	0.44
Net energy expenditure (kJ)	2475 (469)	3324 (624)	-849 (-1268, - 430) [b]	1.54

All values are mean (SD). Data were analysed using linear mixed models with group (South Asian vs. white European) included as a fixed factor.

RPE rating of perceived exertion; *$\dot{V}O_2$ max* maximum oxygen uptake.

[a] 95% confidence interval for the mean absolute difference between the groups.

[b] Main effect of group ($P < 0.05$).

Table 5.4. *Ad libitum* energy and macronutrient intakes in South Asian and white European men.

	South Asians (n=15)		**White Europeans (n=15)**		**White Europeans vs. South Asians Mean difference (95% CI[a])**	**Control vs. Exercise Mean difference (95% CI[a])**
	Control	Exercise	Control	Exercise		
Absolute Energy intake (kJ)	5902 (1564)	5773 (1244)	7441 (1901)	8010 (2478)	-1888 (-3088, -687) [b]	220 (-474, 914)
Relative Energy intake (kJ)	5902 (1564)	3298 (1103)	7441 (1901)	4686 (2485)	-1464 (-2649, -278) [b]	-2679 (-3369, -1989) [c]
Carbohydrate intake (g)	166.4 (55.3)	146.8 (47)	190.7 (54.2)	224.8 (80.7)	-51.1 (-91.4, -10.8) [b,d]	7.2 (-13.7, 28.1) [d]
Fat intake (g)	58.9 (15.5)	60.2 (14.2)	83.5 (30.5)	82.3 (28.1)	-23.3 (-37.8, -8.8) [b]	0.1 (-9.5, 9.6)
Protein intake (g)	53.7 (18.8)	62.7 (35.1)	65.9 (20.3)	68.7 (20.5)	-9.1 (-24.1, 5.8)	5.9 (-4.8, 16.6)

All values are mean (SD). Data were analysed using linear mixed models with group (South Asian vs. white European) and trial (exercise vs. control) included as fixed factors.

[a] 95% confidence interval for the mean absolute difference between the groups or trials.

[b] Main effect of group ($P < 0.05$)

[c] Main effect of trial ($P < 0.001$)

[d] Group-by trial interaction ($P = 0.014$)

5.3.5 Appetite perceptions

Fasting overall appetite ratings at baseline were similar across groups and trials (main effect group $P = 0.156$; main effect trial $P = 0.871$; group-by-trial interaction $P = 0.323$) (Table 5.5). Linear mixed models for overall appetite identified a main effect of time ($P < 0.001$), group-by-time interaction ($P = 0.013$) and trial-by-time interaction ($P = 0.014$), but not a main effect of group (mean difference (95% CI) -2 (-7, 4) mm, ES = 0.07, $P = 0.521$) or trial (mean difference (95% CI) -1 (-2, 1) mm, ES = 0.02, $P = 0.590$) (Figure 5.2). Post hoc analysis of the group-by-time interaction revealed lower overall appetite in South Asian than white European participants at 6.5 h (mean difference (95% CI) -8 (-18, -0.1) mm, ES = 0.58, $P = 0.047$). Post hoc analysis of the trial-by-time interaction revealed that overall appetite ratings were lower at 4.5 h (mean difference (95% CI) -11 (-18, -3) mm, ES = 0.51, $P = 0.004$) and higher at 6.5 h (mean difference (95% CI) 8 (1, 15) mm, ES = 0.53, $P = 0.024$) in the exercise than in the control trial. Time-averaged total area under the curve for overall appetite ratings were similar across groups and trials (main effect group $P = 0.483$; main effect trial $P = 0.637$; group-by-trial interaction $P = 0.452$) (Table 5.6).

Table 5.5. Fasting overall appetite, appetite-related hormone and glucose concentrations in South Asian and white European men.

	South Asians (n=15)		White Europeans (n=15)		White Europeans vs. South Asians Mean difference (95% CI[a])	Control vs. Exercise Mean difference (95% CI[a])
	Control	Exercise	Control	Exercise		
Overall appetite (mm)	82 (13)	79 (18)	73 (14)	76 (12)	6 (-3, 16)	-1 (-6, 5)
Acylated ghrelin ($pg \cdot mL^{-1}$)	38.6 (28.9, 51.5)	34.8 (26.1, 46.5)	52.2 (39.1, 69.7)	42.7 (32.0, 57.0)	-22% (-48, 16%)	-14% (-22, -5%) [c]
Total peptide YY ($pg \cdot mL^{-1}$)	90.3 (74.9, 108.9)	94.0 (77.9, 113.3)	88.5 (73.4, 106.7)	88.9 (73.8, 107.2)	4% (-19, 33%)	2% (-8, 14%)
Insulin ($\mu U \cdot L^{-1}$)	6.5 (5.0, 8.4)	8.0 (6.1, 10.3)	4.8 (3.7, 6.3)	4.2 (3.3, 5.5)	59% (12, 125%) [b,d]	4% (-11, 21%) [d]
Glucose ($mmol \cdot L^{-1}$)	5.5 (5.3, 5.8)	5.8 (5.5, 6.1)	5.6 (5.4, 5.9)	5.8 (5.5, 6.0)	0% (-6, 6%)	4% (0, 7%) [c]

Values for overall appetite ratings are mean (SD). Values for appetite-related hormones and glucose are geometric mean (95% confidence interval) and statistical analyses are based on log-transformed data.

Data were analysed using linear mixed models with group (South Asian vs white European) and trial (exercise vs control) included as fixed factors.

[a] Normally distributed data: 95% confidence interval for the mean absolute difference between the groups or trials; and log transformed data: 95% confidence interval for the ratio difference of geometric means between the groups or trials.

[b] Main effect of group ($P < 0.05$).

[c] Main effect of trial ($P < 0.05$).

[d] Group-by trial interaction ($P = 0.030$).

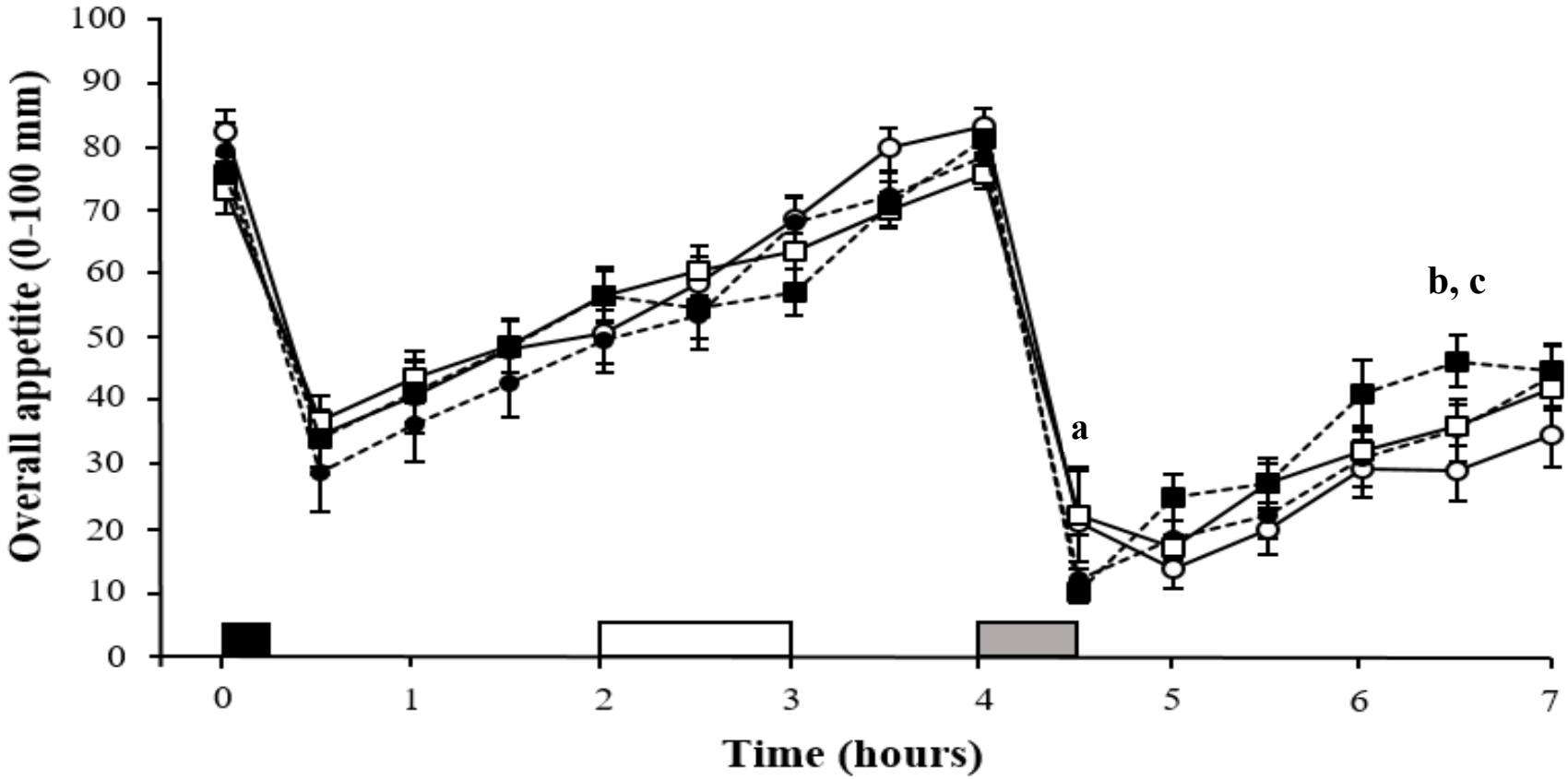

Figure 5.2. Overall appetite perceptions in South Asian (n=15) and white European (n=15) men during the control (South Asian -○-; white European -□-) and exercise (South Asian -●-; white European -■-) trials. Values are mean (s.e.m.). Black rectangle indicates standardised breakfast, open rectangle indicates exercise, and grey rectangle indicates *ad libitum* buffet meal. [a]Lower in exercise than control trial (trial-by-time interaction, $P = 0.004$), [b]Higher in exercise than control trial (trial-by-time interaction, $P = 0.024$), [c]Lower in South Asian than white European men (group-by-time interaction, $P = 0.047$).

Table 5.6. Time averaged total area under the curve for overall appetite, appetite-related hormone and glucose concentrations in South Asian and white European men.

	South Asians (n=15)		**White Europeans (n=15)**		**White Europeans vs. South Asians**	**Control vs. Exercise**
	Control	Exercise	Control	Exercise	**Mean difference (95% CI[a])**	**Mean difference (95% CI[a])**
Overall appetite	22.8 (4.5)	21.9 (3.9)	23.2 (4.3)	23.4 (4.6)	-0.9 (-3.8, 1.9)	-0.4 (-1.8, 1.2)
Acylated ghrelin (pg·mL^{-1} h)	-5.26 (7.70)	-4.27 (5.59)	-5.67 (5.60)	-3.52 (5.70)	-0.17 (-4.40, 4.06)	1.57 (-0.35, 3.50)
Total peptide YY (pg·mL^{-1} h)	7.80 (6.93)	9.79 (9.58)	6.11 (13.14)	9.80 (8.13)	0.83 (-4.77, 6.44)	2.84 (-1.87, 7.54)
Insulin (μU·L^{-1} h)	15.57 (8.19)	15.98 (12.85)	5.81 (2.76)	7.56 (3.95)	9.10 (3.70, 14.50)[b]	1.08 (-1.47, 3.63)
Glucose (mmol·L^{-1} h)	0.27 (0.23)	0.28 (0.28)	0.04 (0.31)	0.19 (0.25)	0.15 (0.02, 0.29)[b]	0.08 (-0.07, 0.23)

All values are mean (SD). Data were analysed using linear mixed models. Data for appetite-related hormone and glucose are reported as delta values.

[a] 95% confidence interval for the mean absolute difference between the groups or trials.

[b] Main effect of group ($P < 0.05$).

5.3.6 Plasma concentrations of appetite-related hormones and glucose

5.3.6.1 Acylated ghrelin

Fasting acylated ghrelin concentrations were not significantly different between groups (ES = 0.46; P = 0.211) but were lower in the exercise than the control trial (ES = 0.27, P = 0.006) (Table 5.5). Delta acylated ghrelin concentrations are displayed in Figure 5.3 and linear mixed models identified a main effect of trial (P = 0.006), time (P < 0.001) and group-by-time interaction (P < 0.001). Delta acylated ghrelin concentrations were similar between groups (mean difference (95% CI) 0.1 (-9.3, 9.4) pg mL^{-1}, ES = 0.01; P = 0.986) (Figure 5.3) whereas absolute acylated ghrelin concentrations were meaningfully, albeit not significantly, lower in South Asian than white European men (mean difference (95% CI) -25.5 (-46.3, 3.4) pg mL^{-1}, ES = 0.54; P = 0.076) (Table 5.7). The main effect of trial revealed higher delta acylated ghrelin in the exercise than in the control trial (mean difference (95% CI) 3.6 (1.1, 6.2) pg mL^{-1}, ES = 0.18, P = 0.006) (Figure 5.3), whereas absolute acylated ghrelin levels were lower in the exercise than in the control trial (main effect of trial; mean difference (95% CI) -10.1 (-14.6, 5.3) pg mL^{-1}, ES = 0.19, P < 0.001) (Table 5.7). Post hoc analysis of the group-by-time interaction revealed lower delta acylated ghrelin in the South Asian compared with the white European men at 4 h (mean difference (95% CI) -18.6 (-29.7, -7.4) pg mL^{-1}, ES = 0.84; P = 0.001). Likewise, a group-by-time interaction was identified for absolute acylated ghrelin concentrations (P = 0.006), with South Asian revealing lower acylated ghrelin at 3 h and 4 h than white European men (all ES ≥ 0.75, P ≤ 0.038) (Table 5.7). Time-averaged tAUC for delta acylated ghrelin was similar across groups and trials (main effect group ES = 0.03, P = 0.934; main effect trial ES = 0.26, P = 0.105; group-by-trial interaction P = 0.539) (Table 5.6).

5.3.6.2 Total PYY

Total PYY concentrations at baseline were similar across trials and groups (main effect group

$P = 0.760$, main effect trial $P = 0.681$, group-by-trial interaction $P = 0.749$) (Table 5.5). Linear mixed models for delta total PYY identified a main effect of trial ($P = 0.012$), time ($P < 0.001$) but not group (mean difference (95% CI) 2.3 (-10.5, 15.1) pg mL-1, ES = 0.07, $P = 0.716$) or any interaction effects ($P \geq 0.317$) (Figure 5.3). The main effect of trial revealed higher delta total PYY concentrations in the exercise than in the control trial (mean difference (95% CI) 6.2 (1.4, 11.0) pg mL-1, ES = 0.19, $P = 0.012$). Absolute concentrations of total PYY were higher in the exercise than in the control trial (main effect of trial; mean difference (95% CI) 5.0 (1.5, 8.7) pg mL^{-1}, ES = 0.14, $P = 0.004$) but similar between groups (ES = 0.09, $P = 0.756$) (Table 5.7). Time-averaged tAUC for delta total PYY was similar across groups and trials (main effect group ES = 0.09, $P = 0.763$; main effect trial ES = 0.29, $P = 0.227$; group-by-trial interaction $P = 0.714$) (Table 5.6).

5.3.6.3 Insulin

Fasting insulin concentrations were similar between trials (ES = 0.07, $P = 0.614$) but were higher in South Asian than white European men (ES = 0.92, $P = 0.011$) (Table 5.5). A group-by-trial interaction ($P = 0.030$) for fasting insulin revealed higher concentrations in South Asians (ratio difference (95% CI) 23 (-1, 52)%, ES = 0.40, $P = 0.058$) and lower values in white Europeans (ratio difference (95% CI) -12 (-29, 9)%, ES = 0.26 , $P = 0.220$) (Table 5.5) in the exercise compared with the control trial. Linear mixed models for delta insulin identified a main effect of group ($P = 0.003$), time ($P < 0.001$), and group-by-time interaction ($P < 0.001$) but were similar between trials (mean difference (95% CI) 2.6 (-2.1, 7.4) μU L^{-1}, ES = 0.07; $P = 0.276$) (Figure 5.3). The main effect of group revealed higher insulin concentrations in South Asian than white European men (mean difference (95% CI) 19.8 (7.4, 32.1) μU L^{-1}, ES = 0.54, $P = 0.003$) (Figure 5.3). Post hoc analysis of the group-by-time interaction revealed higher insulin concentrations in the South Asian than white European participants at 0.75 h, 2 h, 4.75 h and 7 h (all ES ≥ 0.57, $P \leq 0.011$). Absolute concentrations of insulin were also higher in South Asians (main effect of group; mean difference (95% CI) 96.6 (39.7, 177.7) μU L^{-1}, ES =

0.63, $P < 0.001$) (Table 5.7). Particularly, South Asians exhibited greater absolute concentrations of insulin at 0h, 0.75h, 2 h, 3h, 4h, 4.75 h and 7 h (group-by-time interaction; all ES ≥ 0.65, $P \leq 0.038$) (Table 5.7). Time averaged tAUC for delta insulin was higher in South Asian than white European men (ES = 1.16, $P = 0.002$) but similar between trials (ES = 0.12, $P = 0.393$) (Table 5.6).

5.3.6.4 Glucose

Fasting glucose concentrations were similar between groups (ES = 0.05; $P = 0.869$) but were higher in the exercise than in the control trial (ES = 0.38, $P = 0.043$) (Table 5.5). Linear mixed models for delta glucose identified a main effect of group ($P = 0.027$), trial ($P = 0.052$), time ($P < 0.001$), group-by-time ($P = 0.008$), and trial-by-time ($P = 0.011$) interactions (Figure 5.3). Delta glucose concentrations were higher in South Asian than white European men (mean difference (95% CI) 0.35 (0.04, 0.65) mmol L^{-1}, ES = 0.27, $P = 0.027$) and higher in the exercise trial than the control trial (mean difference (95% CI) 0.19 (-0.001, 0.38) mmol L^{-1}, ES = 0.15, $P = 0.052$) (Figure 5.3). Post hoc analysis of the group-by-time interaction revealed higher delta glucose in South Asians than white Europeans at 0.75 h and 2 h (both ES ≥ 0.72, $P = 0.001$) whereas post hoc analysis of the trial-by-time interaction revealed higher delta glucose in the exercise than in the control trial at 3 h and 4.75 h (both ES ≥ 0.60, $P \leq 0.039$). Absolute glucose concentrations were also higher in the South Asian than white European men at 0.75h and 2h (group-by-time interaction; all ES ≥ 0.67, $P \leq 0.008$), and in the exercise than in the control trial (mean difference (95% CI) 6.6 (3.7, 9.5) mmol L^{-1}, ES = 0.31, $P < 0.001$), particularly at 3h, 4.75h and 7h (trial-by-time interaction; all ES ≥ 0.55, $P \leq 0.038$) (Table 5.7). Time-averaged tAUC for delta glucose was higher in South Asian than white European men (ES = 0.55, $P = 0.024$) but similar between trials (ES = 0.28, $P = 0.297$) (Table 5.6).

5.3.7 Correlations

Age was positively associated with fasting insulin in South Asian (r = 0.520, $P = 0.047$) and with time averaged tAUC for insulin in white European men (r = 0.714, $P = 0.003$) (Table 5.7). There were no other significant correlations between the various predictors and baseline or exercise-induced changes in tAUC values for acylated ghrelin and total PYY ($P \geq 0.089$). Total carbohydrate oxidation during exercise was positively associated with absolute energy intake at the subsequent buffet meal in South Asian (r = 0.722, $P = 0.002$) but not in white European men (r = 0.240, $P = 0.409$).

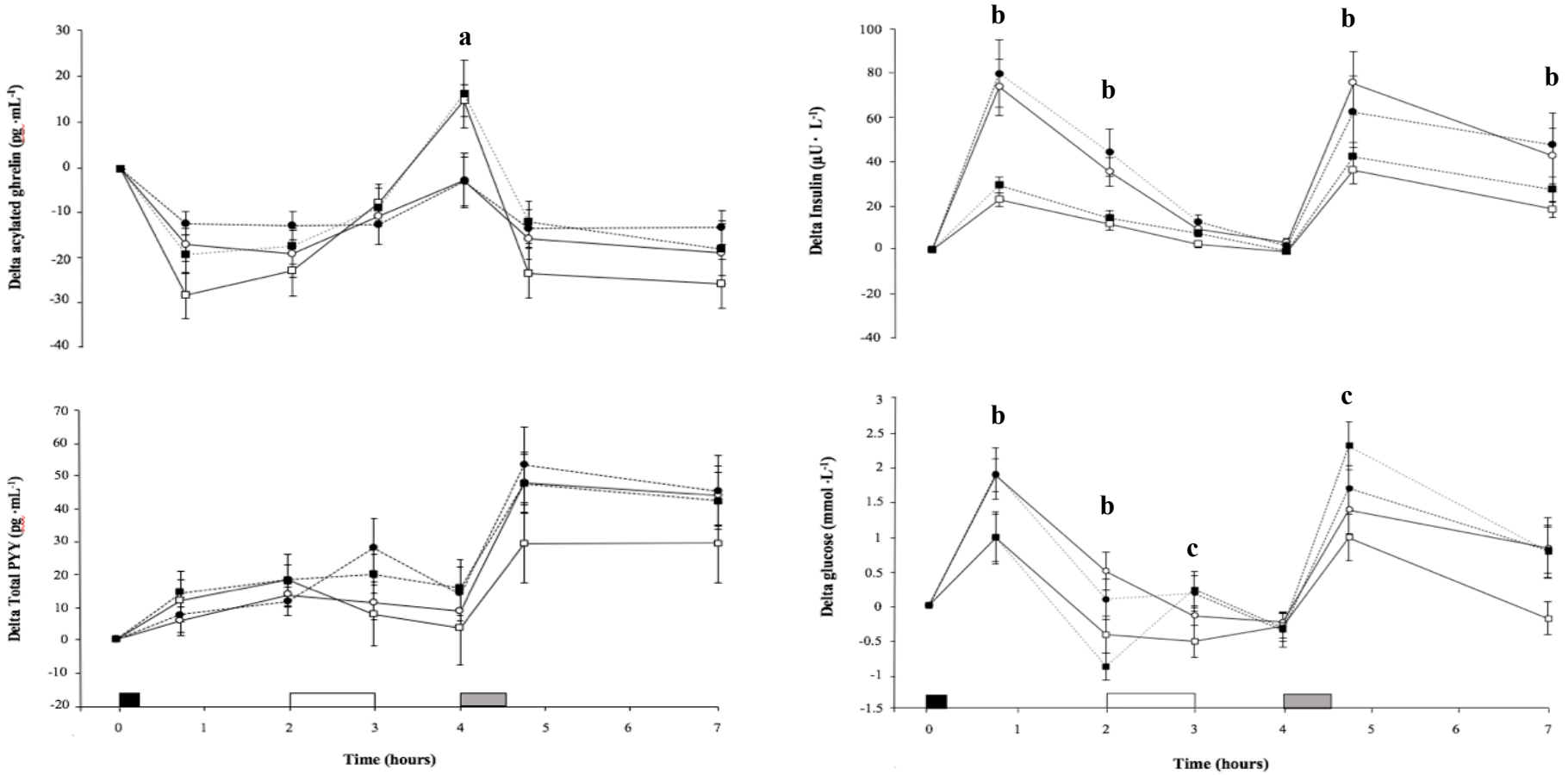

Figure 5.3. Plasma concentrations of (a) delta acylated ghrelin (top panel), (b) delta total PYY (bottom panel), (c) delta insulin (top panel) and (d) delta glucose (bottom panel) in South Asian (n=15) and white European (n=15) men during the control (South Asian -○-; white European -□-) and exercise (South Asian -●-; white European -■-) trials. Values are mean (s.e.m.). Black rectangle indicates standardized breakfast, open rectangle indicates exercise, and grey rectangle indicates *ad libitum* buffet meal. [a]Lower in South Asian than white European men (group-by-time interaction, $P = 0.001$), [b]Higher in South Asian than white European men (group-by-time interaction, all $P \leq 0.011$), [c]Higher in exercise than control trial (trial-by-time interaction, $P \leq 0.039$).

Table 5.7: Plasma Absolute concentrations of appetite-related hormone and glucose in South Asian and white European men during the control and exercise trial.

	South Asians (n=15)		**White Europeans (n=15)**		**White Europeans vs. South Asians Mean difference (95% CI[a])**	**Control vs. Exercise Mean difference (95% CI[a])**
	Control	Exercise	Control	Exercise		
Acylated ghrelin ($pg \cdot mL^{-1}$)	28.9 (22.9,36.6)	26.4 (20.9. 33.4)	39.4 (31.2, 49.8)	34.9 (27.6, 44.2)	-25.5% (-46.3, 3.4%)[d]	-10.1% (-14.6, -5.3%)[c]
Total peptide YY ($pg \cdot mL^{-1}$)	107.6 (93.1, 124.2)	115.4 (99.9, 133.2)	106.5 (92.2, 123.0)	109.5 (94.8, 126.5)	3.1% (-15.6, 26.1%)	5.0% (1.5, 8.7%)[c]
Insulin ($\mu U \cdot L^{-1}$)	23.5 (18.3, 30.3)	25.2 (19.6, 32.4)	11.6 (9.1, 14.9)	13.2 (10.3, 16.9)	96.6% (39.7, 177.0%)[b,d]	10.2% (0.2, 21.2%)[c]
Glucose ($mmol \cdot L^{-1}$)	6.0 (5.7, 6.4)	6.3 (6.0, 6.7)	5.6 (5.3, 5.9)	6.1 (5.7, 6.4)	5.8% (-1.6, 13.8%)[d]	6.6% (3.7, 9.5%)[c,e]

All values are geometric mean (95% confidence interval) and statistical analyses are based on log-transformed data.

Data were analysed using linear mixed models with group (South Asian vs white European), trial (exercise vs control) and time (7 h trial) included as fixed factors.

[a]95% confidence interval for the ratio difference of geometric means between the groups or trials.

[b]Main effect of group ($P < 0.05$).

[c]Main effect of trial ($P < 0.05$).

[d]Group-by time interaction ($P < 0.05$).

[e]Trial-by time interaction ($P < 0.05$).

Table 5.8. Ethnicity-specific Spearman's correlation coefficients between the various predictors and appetite-related hormones.

	Fasting acylated ghrelin		**Delta AUC acylated ghrelin**		**Fasting total PYY**		**Delta AUC total PYY**		**Fasting insulin**		**Delta AUC insulin**	
	South Asian	**White European**	**South Asian**	**White European**	**South Asian**	**White European**	**South Asian**	**White European**	**South Asian**	**White European**	**South Asian**	**White European**
Age	0.21	0.19	-0.061	-0.19	-0.14	0.22	-0.45	-0.24	**0.52**[a]	0.04	0.22	0.03
Body fat	-0.27	0.27	-0.025	0.375	0.25	0.24	-0.28	0.27	0.19	0.30	0.26	**0.71**[a]
$\dot{V}O_2$ max	0.05	0.01	-0.11	-0.24	-0.05	0.01	0.38	0.08	-0.20	-0.02	0.14	0.11
Baseline appetite	0.41	-0.45	0.36	0.36	-0.01	-0.31	0.06	0.21	-0.26	-0.14	-0.01	0.05
AUC appetite	-0.03	0.34	-0.12	-0.29	0.23	0.17	0.18	-0.32	-0.41	0.25	-0.28	0.26

AUC, area under the curve*; $\dot{V}O_2$ max,* maximum oxygen uptake; *MVPA* moderate-to-vigorous physical activity

[a] $P < 0.05$

5.4 Discussion

The primary finding of this study was that appetite perceptions, energy intake and appetite-related hormones in response to acute exercise were similar between South Asians and white European men. However, subtle differences in appetite-measures between the groups were detected with South Asian men exhibiting lower: *(1)* appetite perceptions 2 hours after the buffet meal; *(2)* delta acylated ghrelin concentrations at 4 h; and *(3)* carbohydrate ingestion at the buffet in response to exercise. Furthermore, 60 min of moderate-to-vigorous intensity cycling increased concentrations of delta acylated ghrelin and total PYY and induced subtle changes in appetite perceptions such as transient suppression in appetite perceptions at 4.5 h and stimulation at 6.5 h, without provoking energy compensation in South Asian and white European men. Overall, these findings demonstrate that exercise induces an energy deficit irrespective of ethnicity and suggest similar exercise-induced responses in appetite perceptions and appetite-related hormones between South Asian and white European men.

To the authors' knowledge, the present study is the first to compare acute exercise effects on appetite responses between healthy South Asian and white European men. Our results demonstrate that subjective feelings of appetite were similar between the ethnic groups in response to 60 min moderate-to-vigorous cycling. However, a subtle difference in appetite perceptions between ethnicities was detected at 2 h after the *ad libitum* buffet meal with South Asians exhibiting lower appetite ratings than white European men across both trials. Although this represents a subtle difference in appetite feelings between groups, this difference may be the result of the greater appetite feelings in white European participants due to their greater net energy expenditure during exercise. Compared with the control trial, subtle differences in appetite perceptions including a transient appetite

suppression at 4.5 h (1.5 h post-exercise) and stimulation at 6.5 h (3.5 h post-exercise) were observed with exercise in both ethnic groups. Existing evidence suggests transient appetite suppressions during and immediately after moderate-to-high intensity exercise, which typically returns to resting control values within 30 to 60 min of exercise cessation (Broom et al. 2007; Becker et al. 2012; Martins et al. 2007; Douglas et al. 2017). In most of these studies, however, exercise was performed following an overnight fast whereas studies investigating appetite feelings in response to postprandial exercise revealed a prolonged and more substantial appetite suppression after exercise compared with the fasted exercise and control trial (Deighton et al. 2012; Cheng et al. 2009). In this regard, Cheng et al. (2009) demonstrated that cycling for 50 min at 60% of $\dot{V}O_2$ max 2 h after a standardised meal suppressed appetite feelings for a longer period of time than performing the same exercise after an overnight fast. Likewise, Deighton et al. (2012) demonstrated that running for 60 min at 70% of $\dot{V}O_2$ max approximately 2 h after a standardised breakfast also prolonged appetite suppression than performing the exercise trial after an overnight fast. Therefore, it is plausible that the transient appetite suppression at 4.5 h (1.5 h post-exercise) in the exercise than control trial in our study reflects the prolonged appetite suppression effects induced by exercise performed postprandially. In contrast, the transient appetite stimulation at 6.5 h (3.5 h post-exercise), may relate to the extra energy expended during the exercise.

The present study investigated differences in circulating acylated ghrelin in South Asian and white European individuals in response to exercise. Although the mechanisms underlying appetite responses to exercise are still unclear, previous studies suggest that suppression of circulating acylated ghrelin concentrations during exercise, with perturbations typically returning to control values within 30 min, may contribute to the acute exercise-induced appetite suppression (Deighton and Stensel, 2014). In the present

study, however, delta acylated ghrelin was not decreased during or immediately after exercise which may be explained by the timing of exercise after the standardised breakfast given that at 2 h acylated ghrelin values were still lower than baseline and this may have influenced the response of this gut hormone during exercise. Furthermore, the present study revealed greater delta acylated ghrelin concentrations in the exercise compared with the control trial which differs from previous studies reporting lower or no change in absolute or delta acylated ghrelin concentrations during or shortly after exercise (Broom et al. 2017; Wasse et al. 2013; Deighton et al. 2013a). This disparity, however, is most likely induced by the elevated fasting (baseline) acylated ghrelin concentrations in the control than in the exercise trial, and by the method used to represent the data (delta change instead of absolute concentrations). Indeed, normalising the acylated ghrelin values across the 7 h for the baseline levels (delta) resulted in a downwards shift of the control values, particularly after both meals, which accentuated the difference in delta acylated ghrelin across 7 h between trials. In support of this, the examination of the absolute data revealed lower absolute acylated ghrelin concentrations over the 7 h trial in the exercise than in the control trial, which seems to reflect the lower fasting (baseline) acylated ghrelin in the exercise than in the control trial. In this regard, although individual variability in fasting acylated ghrelin concentrations has been previously reported (Larson-Meyer et al. 2012), it remains unknown how baseline concentrations were different between trials.

The present study also examined differences in acylated ghrelin between South Asian and white European men. Preliminary evidence from our laboratory reported lower fasting acylated ghrelin concentrations in South Asian than white European men at rest (Study 1, Chapter 3), whereas in the present study fasting values were not statistically different between groups, although values were visibly lower in South Asian than white

Europeans. In our previous research South Asians exhibited an elevated adiposity than white European men and the ethnic group difference in fasting acylated ghrelin levels was mitigated after controlling for body fat percentage. Conversely, in the present study body fat percentage did not differ significantly between groups which may explain why fasting acylated ghrelin levels were not statistically lower in South Asian compared to white European participants. Delta acylated ghrelin concentrations were similar between ethnic groups suggesting no differences in this gut peptide across both trials between South Asian and white European men. However, it is possible that these data also result from the use of delta change to represent the data as the absolute data revealed a meaningfully, albeit not significant, lower acylated ghrelin concentrations in South Asian than white European men across both trials. The different results of the absolute data compared with the delta values, however, may reflect the tendency for resting acylated ghrelin concentration to be lower in South Asians than white Europeans at baseline (fasting), which influenced the absolute concentrations throughout the trials. Indeed, normalising the acylated ghrelin values throughout for the baseline (delta), shifted the curves down for the white European, due to their higher fasting values, more than for the South Asian group. Furthermore, the grater meal induced suppression particularly after breakfast (0.75 h) was cancelled out by the greater elevation before the buffet meal (4 h) in white European men, resulting in similar mean delta acylated ghrelin concentrations across the 7 h trial between groups, thus no main effect of ethnicity for delta values. The lower concentration of delta acylated ghrelin in South Asian men observed before the *ad libitum* buffet meal, in line with the absolute data, represents an important finding of the present study. Based on previous studies, insulin resistance and compensatory hyperinsulinemia are shown to be inversely associated with acylated ghrelin concentration, independently of BMI and adiposity (Flanagan et al. 2003; McLaughlin et

al. 2004; Becker et al. 2012; Hazell et al. 2015). In this study, South Asians exhibited higher fasting insulin and elevated postprandial glucose and insulin concentrations compared with BMI-matched white European men, which are indicative of a greater degree of insulin resistance. Therefore, it is plausible that the different levels of acylated ghrelin before the buffet meal in this study reflects the insulin resistance phenotype in South Asians (Sattar and Gill, 2015). Furthermore, the inversely association between insulin and acylated ghrelin concentrations irrespective of adiposity, would support the trend of lower fasting acylated ghrelin in South Asian than white European men with similar body fat percentage observed in the present study.

It is well-established that South Asian individuals are more insulin resistant than white Europeans which has been linked to their greater percent body fat (Sattar and Gill, 2015; Gholap et al. 2011). However, it has been suggested that the greater insulin resistance in South Asians is only partly explained by differences in adiposity with studies showing greater fasting or postprandial insulin in South Asians even after adjustment for adiposity (Ghouri et al. 2013; Sattar and Gill, 2015; Chandalia et al. 1999). This is in agreement with our findings where South Asians exhibited higher fasting insulin and elevated glucose and insulin post-prandial despite having similar levels of body fat than white European men. There is evidence that exercise-related factors such as low levels of physical activity and cardiorespiratory fitness and reduced capacity for fat oxidation during exercise are key features of the insulin resistance phenotype in South Asians (Afaq et al. 2019; Biddle et al. 2019; Ghouri et al. 2013; Yates et al. 2018; Hall et al. 2010). Specifically, Hall and colleagues (2010) demonstrated positive associations of cardiorespiratory fitness and fat oxidation during submaximal exercise with insulin sensitivity index in South Asian men (Hall et al. 2010). Additionally, in the same study the lower insulin sensitivity index in South Asians than white European participants was

abolished after adjusting for cardiorespiratory fitness and fat oxidation during exercise, although the mechanisms explaining the lower capacity for fat oxidation in South Asians and how this relates to their insulin resistance remain unclear. Regardless of the mechanisms, the reduced cardiorespiratory fitness and fat oxidation during exercise in South Asians in this study supports previous work regarding the differential cardiorespiratory fitness and capacity for fat oxidation during exercise between South Asians and white European men.

Concentrations of fasting total PYY and across the 7 h trial were similar between South Asian and white European men supporting our previous findings (Benedetti et al. 2019). Furthermore, in agreement with previous investigations, delta PYY concentrations were greater in the exercise than in the control trial (Martins et al. 2007; Kawano et al. 2013; Douglas et al. 2017), over the 7 h trial. In this study the greater delta total PYY in the exercise compared with the control trial appears to be driven by the increase of total PYY after the exercise bout and the *ad libitum* buffet meal, although we did not identify any exercise by time effect. Considering previous studies reporting increase in total PYY concentrations concomitantly with a reduction in hunger (Douglas et al. 2017), it is possible that the increase in total PYY concentrations contributed to the appetite suppression observed in this study immediately after the buffet meal, although perturbations in PYY appear to not explain the transient appetite stimulation at 6.5 h, since PYY did not change at this time point. However, it warrants mention that other satiating hormones as well as physiological factors not examined in the present study, mediate exercise-induced appetite responses (Hussain and Bloom, 2013) which may contribute to explain the transient appetite changes observed in the present study.

An important consideration in the context of the benefits of physical activity for the management of weight and fat loss concerns subsequent energy intake response. For weight/fat loss to occur, a sustained negative energy balance is required, and the majority of previous evidence has demonstrated no energy compensation in response to an acute bout of exercise during the following meal (Deighton et al. 2014; Alajmi et al. 2015; Douglas et al. 2017). In agreement with this, in the present study South Asian and white European participants did not exhibit exercise-induced changes in absolute energy intake at the *ad libitum* meal, whereas relative energy intake was lower after exercise. Taken together, these data suggest that exercise induces a short-term energy deficit in South Asian and white European men. However, our finding that carbohydrate intake after exercise was reduced in South Asian men and increased in white European men is intriguing. Although this difference may be merely related to the lower net energy expenditure over the 60 min cycling in South Asian than white European men, previous evidence demonstrated a positive association between carbohydrate oxidation during exercise and energy intake at a test meal provided 60 min after a bout of cycling performed at 70% of maximum heart rate (Hopkins et al. 2014). In agreement with these findings, we identified a strong positive association, in South Asian but not in white European men, between total carbohydrate oxidation during exercise and absolute energy intake at subsequent the buffet meal. Therefore, the lower carbohydrate oxidation in South Asian men during exercise may be speculatively linked with lower glycogen depletion which elicited a lower compensatory drive to ingest food and restore glycogen stores at the buffet meal. The present study also revealed lower absolute energy intake across both trials in South Asian than white European men which may be explained by the ethnic differences in fat free mass. Specifically, in the present study South Asian exhibited a lower fat free mass (also known as lean body mass) than white European men

which is in agreement with previous data (Hall. et al. 2010; Ghouri et al. 2013; Sattar and Gill; 2015). Furthermore, several studies have shown that fat free mass is an important determinant of resting metabolic rate, daily energy expenditure and day-to-day food intake (Blundell et al. 2011; Weise et al. 2013; Hopkins et al. 2017). In this regard, Weise et al. (2013) demonstrated that *ad libitum* energy intake (measured objectively in a laboratory environment) were positively associated with fat free mass in lean and obese individuals. These data were in line with those by Blundell et al. (2011), who reported that self-selected meal size and total daily energy intake were positively correlated with lean body mass in overweight and obese individuals. Thus, it is possible that the lower absolute energy intake in South Asian men in our study may reflect their lower fat free mass which induced a lower drive to ingest food at the buffet meal than white European counterpart.

Although this research provides novel findings on the effects of exercise on appetite regulation and food intake in healthy South Asian men compared with white European men, a limitation of this study is that acylated ghrelin and total PYY were the only gut hormones related to appetite examined. Thus, additional gut hormones should be considered for further research comparing exercise-induced appetite responses in South Asian and white European individuals. Secondly, the timing of the exercise 2 h after the standardised breakfast may have influenced acylated ghrelin responses as the concentrations of this hormone was still lower than the fasting values at the start of exercise. Lastly, the population sample was mostly limited to South Asian men originating from India and Pakistan, therefore, further investigations are required in other South Asian groups (e.g., Bangladeshis, Sri Lankan, Nepalese and Bhutanese) and in South Asian and white European women.

In conclusion, overall a single bout of acute moderate-to-vigorous intensity exercise induced similar appetite responses between South Asian and white European men although some subtle differences were observed where South Asians exhibited lower appetite feelings 2 h after the buffet meal and lower levels of delta acylated ghrelin before the buffet meal compared with white European men. Furthermore, exercise did not provoke any compensatory changes in energy intake in both groups on the day of exercise. These findings provide evidence that acute exercise induces a short-term energy deficit irrespective of ethnicity and suggest similar exercise-induced responses in appetite perceptions and appetite-related hormones between South Asian and white European men.

Chapter 6 – General discussion

This final chapter aims to integrate and discuss the main findings of the studies presented in this thesis. Previous research suggests that South Asians have an elevated risk of CVD and T2D than white European individuals, which has been associated to the higher prevalence of different risk factors including greater adiposity, insulin resistance or dyslipidaemia (Gholap et al. 2011; Khunti et al. 2013). However, these traditional risk factors have been reported to not fully explain the elevated risk of CVD and T2D in South Asian compared with white European individuals (Forouhi et al. 2006). Therefore, this thesis investigated ethnic differences in traditional and unconventional CVD and T2D risk markers such as CRP, IL-6, leptin, FFAs as well as appetite-related hormones in relation to physical activity/cardiorespiratory fitness and compared appetite measures in response to acute exercise in South Asian and white European men.

6.1 Acylated ghrelin and total PYY

Ethnic differences in the short-term regulation of appetite and energy intake may underlie the well-established elevated adiposity and associated in South Asian than white European men. In this regard, the first Study 1 (Chapter 3) revealed lower fasting acylated ghrelin concentrations in the South Asian compared with the white European group, which represents a novel finding of this study. Although the reason for this finding is unclear, it may be linked to the ethnic group differences in adiposity observed. In this regard, previous research has demonstrated that individuals with obesity exhibit lower circulating concentrations of fasting ghrelin compared with lean individuals (Le Roux et al. 2005). Although our findings only revealed a large inverse correlation between body fat percentage and acylated ghrelin in the white European men, the ethnic group

difference in fasting acylated ghrelin concentrations was mitigated after controlling for body fat percentage. Therefore, it was suggested that the lower acylated ghrelin concentration in the South Asian men may be associated to the higher body fat levels observed in this group. The findings from Study 3 (Chapter 5) appear to support these results, where concentrations of fasting acylated ghrelin were not statistically different between South Asian and white European men, with both groups exhibiting similar percentage of body fat. Thus, it is plausible that ethnic differences in fasting concentrations of this gut hormone observed in Study 1 (Chapter 3) may reflect the differences in adiposity between ethnic groups. This thesis also examined ethnic differences in plasma total PYY between South Asian and white European men. Despite the between-group differences in acylated ghrelin in Study 1 (Chapter 3), the same study did not reveal significant differences in fasting total PYY and this finding was supported in Study 3 (Chapter 5), suggesting no ethnic-differences in concentrations of this gut peptide.

However, whereas the mechanisms explaining the fluctuation of ghrelin concentrations related to changes in body weight/fat remain unclear, there is evidence showing that decreased ghrelin levels observed in obesity may be related more to insulin resistance than to higher BMI or fat mass (Flanagan et al. 2003; McLaughlin et al. 2004). In this regard, McLaughlin and colleagues (2004) demonstrated lower ghrelin concentrations in the obese insulin-resistant group compared with the obese insulin-sensitive group (McLaughlin et al. 2004). Additionally, the same study exhibited negative correlations of ghrelin with insulin resistance and insulin concentrations, with these data suggesting that insulin resistance and hyperinsulinemia may be associated with ghrelin suppression independently of adiposity (McLaughlin et al. 2004). In line with this, Study 1 (Chapter

3) revealed elevated fasting insulin in South Asian than white European participants, which may have contributed to the lower acylated ghrelin in the South Asian group.

6.2 Glucose and insulin

Study 1 (Chapter 3) also revealed higher glucose and insulin OGTT concentrations in South Asian than white European men, supporting previous research (Gholap et al. 2011; Tziomalos et al. 2008; Peters et al 2013). These differences are indicative of a greater degree of insulin resistance and were further supported by the higher HOMA-IR and lower insulin sensitivity index observed in Study 1 (Chapter 3) in the South Asian participants. One factor suggested to contribute to the excess insulin resistance in South Asian individuals represents their greater total/abdominal adiposity and lower lean mass compared with white Europeans (Sattar and Gill, 2015). In support of this, the findings of Study 1 (Chapter 3), revealed higher levels of body fat and lower lean body mass for a given BMI compared with white European individuals (Lear et al. 2007). However, the greater insulin resistance in South Asians may persist after adjustment for total and abdominal adiposity (Sattar and Gill, 2015), as shown in study 1 (Chapter 3) where insulin sensitivity index and insulin OGTT remained significantly higher in South Asian than white European participants after controlling for body fat percentage. This was also demonstrated in Study 3 (Chapter 5) where South Asians exhibited markedly higher fasting insulin and elevated glucose and insulin post-prandial concentrations despite having similar levels of body fat than white European men.

6.3 Leptin

Discriminating only for total and abdominal adipose tissue may hide other factors intrinsic of the adipose tissue in South Asian individuals. Particularly, previous evidence suggests greater fat accumulation in the deep subcutaneous adipose tissue (DSAT) and

visceral adipose tissue (VAT) in South Asians, which has been linked with insulin resistance and associated adverse metabolic effects (Sniderman et al. 2007). Furthermore, South Asians exhibit dysfunctional adipocytes characterised by defect in adipose tissue cells maturation and hypertrophic adipocytes compared with white Europeans, which has been associated with greater risk of insulin resistance and T2D (Sniderman et al. 2007; Bakker at al. 2013; Sattar and Gill, 2015). Dysfunctional adipocytes have also been related to greater levels of plasma leptin in South Asians than Caucasians, independent of total and abdominal adiposity (Bakker et al. 2013; Abate et al. 2004; Chandalia et al. 2007). In support of this, in Study 1 (Chapter 3) we demonstrated substantial elevated fasting concentrations of plasma leptin in South Asian compared with white European men, with the between-group difference in leptin level diminishing, but not eliminated completely, after controlling for body fat percentage. Additionally, according to previous evidence, hyperinsulinemia may induce an increase in leptin levels suggesting that insulin resistance and higher concentrations of insulin may have a role in promoting hyperleptinemia (Wang et al. 1999). While the mechanisms explaining the link between hyperleptinemia and insulin resistance is not completely understood, it is plausible that the elevated insulin concentrations observed In Study 1 (Chapter 3) may have contributed to the higher leptin concentrations in the South Asian compared with the white European group. Consequently, it is possible that irregularities in adipose tissue metabolism concomitant with insulin resistance may exacerbate the CVD and T2D risk in South Asians.

6.4 Free fatty acids

Dysfunctions in adipose tissue metabolism have been also associated with elevated concentrations of FFAs in South Asian compared with white European individuals,

which has been proposed to be an important link between adipose tissue and skeletal muscle/liver insulin resistance in the South Asian population (Abate et al. 2004; Chandalia et al. 2007). Particularly, high levels of circulating FFAs may cause a chronic supply of lipids to skeletal muscle leading to an accumulation of intramuscular triglyceride and fatty acids metabolites, which inhibit insulin action (Hall et al. 2008). Nonetheless, not all FFAs contribute equally to the development of insulin resistance (Rasic-Milutinovic et al. 2012) and metabolomics analytical methods represent the most advanced and frequently used techniques for the identification and quantification of individual metabolites such as FFAs in human plasma (Patti et al. 2012). Although the initial results based on the GC-MS method were unsatisfactory, probably due to the inadequacy of the capillary column used in our experiments, we could separate the majority of the South Asian from most of the white European men based on the LC-MS method. To the author's knowledge, only one previous study examined ethnic differences in individual FFAs concentrations (Ralston et al. 2013). However, the study from Ralston and co-workers focused mainly on ethnic-specific associations between individual plasma FFAs and markers of insulin resistance, without explicitly examining ethnic differences in the baseline concentrations of FFAs (Ralston et al. 2013). Study 2 (Chapter 4) investigated ethnic differences in the FFAs metabolic profile with LC-MS and revealed higher concentrations of five FFAs (laurate, myristate, palmitate, γ-linolenic and linoleate) in the South Asian group. Given that insulin resistant individuals typically exhibit higher levels of SFAs and low concentrations of PUFAs such as linoleic acid, γ-linolenic acid or EPA and DHA (Warensjö et al. 2005), it was surprising that baseline concentrations of γ-linolenic and linoleic acid were higher in the South Asian group, who exhibited greater levels of fasting insulin and elevated glucose and insulin OGTT than white European participants. However, our findings appear to support the data from

Ralston and colleagues (2013) where elevated γ-linolenic and linoleic acid were observed in the South Asian compared with the Caucasian group. Study 2 (Chapter 4) also demonstrated positive associations between AUC glucose and four FFAs such as palmitate, oleate, eicosatrienoic and docosahexenoic acid in South Asian participants. The association between AUC glucose and oleate, eicosatrienoic and docosahexenoic acid appears to be in contrast with previous epidemiological evidence reporting positive effects of these unsaturated fatty acids on insulin sensitivity (Warensjö et al. 2005). Conversely, the association between palmitate and AUC glucose confirms previous studies reporting saturated fatty acids-induced insulin resistance, although this evidence is not related directly to South Asian populations (Ebbeson et al. 2010). The present study also revealed a strong correlation of FFAs with body fat percentage in the South Asian group and between FFAs and physical activity in the white European group. Whereas the associations of FFAs with body fat percentage in South Asians may simply reflect their greater body fat percentage, ethnic differences in factors involved in myocellular lipid mobilisation may underlie these responses. South Asians exhibit higher perilipin 5 (PLIN-5), a lipid droplet-associated protein in skeletal muscle, in response to a 5-day high fat diet compared with Caucasian males (Gemmink et al. 2017). Perilipin 5 plays a key role in the activation of the adipose triacylglycerol lipase (ATGL), which is involved in the intracellular triglyceride mobilisation (Schreiber et al. 2018). Thus, it may be possible that in our study ethnic differences in ATGL/PLIN-5-induced lipolysis may have contributed to the higher levels of FFAs linked to body fat in the South Asian group. Conversely, the associations between FFAs and total step counts in the white European group only seems to suggest that fatty acid metabolism is less responsive to physical activity in South Asian than white European men. These data may reflect the fact that the FFAs release is also promoted by the hormone sensitive lipase (HSL), which responds to

corticosteroids and catecholamine release (Schreiber et al. 2018). Thus, ethnic differences in levels of hormones controlling HSL activation may have influenced these results.

Although the current study provided evidence that levels of circulating FFAs are different between South Asians and white European men, the potentially confounding effects of body fat percentage may have accentuated the differences in FFAs between ethnic groups. Thus, it may be beneficial for future research to investigate FFAs metabolic profile in South Asian and white European individuals matched by body fat percentage. Furthermore, investigating FFAs in response to exercise and/or food intake and explore how these responses correlate with cardiometabolic risk markers may elucidate the role of exercise and energy intake in modulating individual plasma FFAs concentrations and in optimising health outcomes in South Asians.

6.5 Inflammatory and lipid markers

The present thesis also measured circulating concentrations of fasting IL-6 and CRP which represent key indicators of chronic low-grade inflammation and have been implicated in explaining the excess CHD risk in South Asian individuals (Tziomalos et al. 2008). Particularly, in Study 1 (Chapter) we demonstrated elevated fasting IL-6 and CRP concentration in South Asian compared with white European men which supports several previous studies (Anand et al. 2004; Arjunan et al. 2015; Bastard et al. 1999), although this finding is not universal (Peters et al. 2013). The divergent inflammatory profiles between ethnicities in the present study were diminished after controlling for body fat percentage supporting previous findings (Chambers et al. 2001; Arjunan et al. 2015) although South Asian individuals have shown to exhibit higher CRP concentrations than Caucasians despite similar levels of body fat (Chandalia et al. 2003).

Thus, future work is required to determine the independent contribution of ethnicity and adiposity on inflammatory markers in South Asians. Consistent with previous studies (Arjunan et al. 2013; Anand et al. 2004; Anand et al. 2000), in Study 1 (Chapter 3) we also demonstrated a more unfavourable fasted lipid profile in South Asians compared with the white European men including lower concentrations of HDL-C coupled with an elevated TC/HDL-C ratio and higher TAG concentration. These findings highlight that South Asian men exhibit an adverse inflammatory and lipid marker profile, which may contribute to the their heightened cardiometabolic health risk compared with individuals of white European descent.

6.6 Physical activity and cardiorespiratory fitness

Several studies conducted in UK South Asians reveal lower levels of physical activity than their white European counterparts, which is likely to contribute to the excess T2D and CHD risk in this population (Fischbacher et al. 2004; Williams et al. 2011a; Williams et al. 2011b). The existing evidence on habitual physical activity levels in South Asians has largely been gleaned from self-report questionnaires which carries a limitation due to issues of participant recall bias (Yates et al. 2015; Williams et al 2011a), but data using accelerometry are emerging (Celis-Morales et al. 2013; Afaq et al. 2019; Ghouri et al. 2013; Iliodromiti et al. 2016). In Study 1 (Chapter 3), we reported similar physical activity levels between South Asian and white European men using an accelerometery device, which represents a strength of this study. Although these findings appear to contradict the aforementioned studies, the South Asian participants accumulated less total activity (CPM) and fewer steps, and stark differences in CVD and T2D risk markers were still apparent between the ethnic groups. This is supported by previous studies suggesting that South Asian individuals are more insulin resistant than white European

individuals even after adjustment for habitual physical activity levels (Ghouri et al. 2013).

A contributing factor that may relate to ethnic differences in cardiometabolic risk is the association between physical activity and cardiorespiratory fitness. Increasing evidence suggests that South Asians have lower cardiovascular fitness levels compared white European people, which has been suggested to be a key feature of the insulin resistance phenotype in this ethnic group independent of physical activity levels (Hall et al. 2010; Ghouri et al. 2010; Arjunan et al. 2013; Arjunan et al. 2015). In support of this, despite the similar levels of objectively-measured physical activity between the groups, in Study 1 (Chapter 3) we demonstrated markedly lower cardiorespiratory fitness in South Asian compared with white European participants. Similar data were observed in study 3 (Chapter 5) with South Asians exhibiting lower cardiorespiratory fitness than white European participants despite similar self-reported habitual physical activity levels and sitting time between ethnic groups. However, the reasons explaining the lower cardiorespiratory fitness in South Asians are not completely understood. In addition, it has been demonstrated that low cardiorespiratory fitness was the strongest predictor of the excess insulin resistance seen in UK South Asian compared with white European men (Ghouri et al. 2013), although the findings reported in Study 1 (Chapter 3) only revealed a positive association between $\dot{V}O_2$ max and insulin sensitivity index in the white European men. Low skeletal muscle fat oxidation may also exacerbate insulin resistance in South Asian individuals. Indeed, there is evidence demonstrating positive associations of cardiorespiratory fitness – an index of oxidative capacity at the whole-body level – and fat oxidation during submaximal exercise with insulin sensitivity index in South Asian men (Hall et al. 2010). In the same study the lower insulin sensitivity index in South Asian than white European participants was abolished after adjusting for

cardiorespiratory fitness and fat oxidation during exercise. Low lipid oxidation in skeletal muscle may lead to an accumulation of intramuscular lipids, which inhibit insulin action (Hall et al. 2008). Although the mechanisms explaining the lower capacity for fat oxidation in South Asians and how this relates to their insulin resistance remain unclear, the reduced cardiorespiratory fitness and fat oxidation during exercise observed in South Asians in Study 3 (Chapter 5) supports the findings from Hall et al. (2010). Given the importance of physical activity as a method of enhancing cardiovascular fitness levels, our findings add further weight to the proposition that there may be a requirement for South Asians to engage in greater physical activity levels than white Europeans to optimise health outcomes.

6.7 Exercise and appetite

Physical activity has been also proposed as an effective strategy in the management of obesity and weight control with a growing interest by the research community in examining the underlying mechanisms linking physical activity, appetite and weight management. A plethora of studies have investigated the appetite and energy intake responses during and after acute bouts of continuous aerobic exercise (50-70% $\dot{V}O_2$ max, 30-90 min). In this regard, the majority of these studies have shown a transient suppression of appetite perceptions during exercise, known as 'exercise-induced anorexia', with appetite feelings typically return to resting control values within 30-60 min of exercise termination (Deighton and Stensel, 2014). Furthermore, single sessions of exercise have consistently shown to reduce orexigenic hormone acylated ghrelin concentrations and increase levels of anorexigenic appetite-related hormones such as total PYY, without stimulating subsequent changes in absolute energy intakes (Shubert et al. 2013). However, it remains unknown to date how differences in individual ethnicity

background modulate appetite perceptions, energy intake and appetite-related hormones in response to exercise, which seems relevant and may extend findings from Study 1, since lower levels of fasting acylated ghrelin were found at rest (Chapter 3). In study 3 (Chapter 5), we demonstrated similar appetite perceptions, energy intake and appetite-related hormones in response to acute exercise between South Asians and white European men, although subtle differences in appetite-measures between the groups were detected. Particularly, South Asians exhibited lower appetite ratings than white European men 2 h after the *ad libitum* buffet meal across both trials. Although this represents a subtle difference in appetite feelings between groups, this difference may be driven by the greater appetite feelings in white European participants due to their greater net energy expenditure during exercise. Study 3 (Chapter 5) also identified lower concentrations of delta acylated ghrelin in South Asian than white European men before the *ad libitum* buffet meal (4 h), which may be related to the different concentrations in plasma insulin observed between groups. However, no significant main effect of group for delta acylated ghrelin concentrations was observed. It is possible that these results are influenced by the use of delta change to represent the ghrelin concentration as the absolute data revealed a significantly lower mean acylated ghrelin concentration across both trials in South Asian than white European men. This may provide some support to the data in Study 1 (Chapter 3) reporting lower fasting acylated ghrelin in South Asian than white European men (Study 1, Chapter 3). Furthermore, Study 3 (Chapter 5) revealed subtle changes in appetite feelings between trials including a transient suppression in appetite perceptions at 4.5 h (1.5 h post-exercise) and stimulation at 6.5 h (3.5 h post-exercise) in the exercise than control trial. While the stimulation of appetite 3.5 h post-exercise may relate to the extra energy expended during the exercise, the lower appetite perceptions 1.5 h post exercise may reflect a prolonged appetite suppression which has been previously reported

when exercise is performed after a meal (Deighton et al. 2012; Cheng et al. 2009), instead of following an overnight fast of 9 h (Broom et al. 2007; Becker et al. 2012; Martins et al. 2007; Douglas et al. 2017). In this regard, Cheng et al. (2009) demonstrated that cycling for 50 min at 60% of $\dot{V}O_2$ max 2 h after a standardised meal suppressed appetite feelings for a longer period of time than performing the same exercise after an overnight fast of 9 h. Likewise, Deighton et al. (2012) demonstrated that running for 60 min at 70% of $\dot{V}O_2$ max approximately 2 h after a standardised breakfast also prolonged appetite suppression than performing the exercise trial after an overnight fast of 10 h. Thus, these data suggest that manipulation of the exercise protocols in appetite studies may represent a potential avenue for future investigations. Differences in appetite-related hormones between trials were also observed including increased concentrations of delta acylated ghrelin and total PYY in the exercise compared with the control trial. Whereas the greater total PYY concentrations in the exercise trial is in agreement with the absolute total PYY concentrations and with previous investigations (Martins et al. 2007; Kawano et al. 2013; Douglas et al. 2017), the elevated delta acylated ghrelin concentrations in the exercise trial differed from the absolute acylated ghrelin concentrations and from previous studies reporting lower or no change in absolute or delta acylated ghrelin concentrations during or shortly after exercise (Broom et al. 2017; Wasse et al. 2013; Deighton et al. 2013a). However, as reported in Chapter 5.4, this finding may reflect the differences in fasting acylated ghrelin concentrations between trials, but also the method used to express acylated ghrelin (delta change instead of absolute concentrations) as the absolute acylated ghrelin concentrations were greater in the exercise than in the control trial. However, the greater elevation in acylated ghrelin, delta and absolute concentrations, in white European than South Asian men before the *ad libitum* buffet meal, represented an important finding of the present study. Despite the mechanisms explaining the lower pre-

prandial acylated ghrelin concentrations in South Asians than white European men observed in this study remains unclear, these data provide additional evidence regarding the differential acylated ghrelin concentrations between South Asian and white European men. Despite subtle differences in appetite perceptions between trials and an increase in appetite-related hormones in the exercise trial, South Asian and white European participants did not exhibit exercise-induced changes in absolute energy intake at the *ad libitum* meal, which suggests that exercise induces a short-term energy deficit irrespective of ethnicity. The lower absolute energy intake across both trials in South Asian than white European men was speculatively linked with the lower fat free mass in South Asian than white European men, which is in agreement with previous data. Previous studies have shown that fat free mass is lower in South Asian than white Europeans (Hall. et al. 2010; Ghouri et al. 2013; Sattar and Gill; 2015) and that it is an important determinant of resting metabolic rate, daily energy expenditure and day-to-day food intake (Blundell et al. 2011; Weise et al. 2013; Hopkins et al. 2017). Thus, it is possible that the lower absolute energy intake in South Asian men in our study may reflect their lower fat free mass which induced a lower drive to ingest food at the buffet meal than white European counterpart.

6.8 General conclusions

Taken together, the findings of the present thesis suggest lower levels of acylated ghrelin and an adverse CVD and T2D risk marker profile in South Asian compared with white European men including higher concentrations of insulin, TAG, leptin and CRP, and lower HDL-C. Although objectively assessed and self-reported physical activity levels and sedentary time were similar between the ethnic groups, the lower cardiorespiratory fitness and fat oxidation in the South Asian men may contribute to the heightened cardio-

metabolic heath risk in this population. Levels of acylated ghrelin appear to be lower in South Asian men, but further work is required to determine the independent contribution of ethnicity and adiposity on this gut hormone. The current thesis also provides evidence that levels of circulating FFAs are different between South Asian and white European men, which may contribute to the elevated CVD and T2D risk in South Asians. Finally, we provided evidence that 60 min moderate-to-vigorous cycling induces a short-term energy deficit irrespective of ethnicity and suggests similar exercise-induced responses in appetite perceptions and appetite-related hormones between South Asian and white European men. These findings may represent a valid strategy of exercise to induce weight/fat loss and optimise health outcomes in South Asians.

In summary, compared with white European, South Asian men revealed:

- lower circulating acylated ghrelin but higher leptin concentrations;
- an adverse inflammatory and metabolic risk profile including elevated concentrations of fasting insulin, TAG, CRP, and lower HDL-C;
- a different FFA metabolic profile including greater levels of laurate, myristate, palmitate, γ-linolenate and linoleate and positive associations between individual FFA concentrations and markers of cardiometabolic risk;
- lower levels of total physical activity and cardiorespiratory fitness;
- similar exercise-induced responses in appetite perceptions, energy deficit and appetite-related hormones.

6.9 Limitations and future directions

The studies conducted in this thesis have a few limitations. The number of participants in the studies was small, and was mostly limited to South Asian men, but not women,

originating from India. Additionally, the potentially confounding effects of body fat percentage may have accentuated the differences in FFAs between ethnic groups. Lastly, acylated ghrelin and total PYY were the only gut hormones with appetite-regulating capabilities examined in response to exercise, and the timing of the exercise 2 h after the standardised breakfast may have influenced the changes in acylated ghrelin in response to exercise, as the concentrations of this hormones were still lower than the fasting values at the start of exercise. Therefore, below are a few suggestions for further research:

- An examination of chronic effects of exercise on appetite measures and CVD and T2D risk markers based on longitudinal studies.
- An examination of unconventional parameters for CVD and T2D risk with a larger sample size and in additional South Asian groups (e.g., Bangladeshis, Sri Lankan and Bhutanese) and in South Asian women.
- An investigation into unconventional parameters for CVD and T2D risk, such as FFAs in South Asian and white European individuals matched by body fat percentage but also in response to exercise/food intake to clarify the role of adiposity, energy intake and exercise in modulating individual plasma FFAs concentrations between South Asian and white European individuals.
- Finally, from a mechanistic point of view, an investigation into additional appetite-related gut hormones such as GLP-1 or PP.

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APPENDIX A

Informed Consent Form

Title of the study:

Ethics Code:

Statement by participant

I ..... give my consent to the research procedures that are outlined above, the aim, procedures and possible consequences of which have been outlined to me

- I confirm that I have read and understood the information sheet/letter of invitation for this study. I have been informed of the purpose, risks, and benefits of taking part.

Study title:

- I understand what my involvement will entail and any questions have been answered to my satisfaction.
- I understand that my participation is entirely voluntary, and that I can withdraw at any time without prejudice.
- I understand that all information obtained will be confidential.
- I consent the Cheek Buccal Swab sample collection for genotyping analysis[1]
- I understand that although the genotyping analysis that will be performed will not give me significant information about the risk of disease, I would like to be informed about the results anyway
- I agree that research data gathered for the study may be published provided that I cannot be identified as a participant.
- Contact information has been provided should I (a) wish to seek further information from the investigator at any time for purposes of clarification (b) wish to make a complaint.

[1]*Genotyping is the process of determining differences in a specific sequence of the DNA, known as Single Nucleotide Polymorphisms (SNPs) associated with Type 2 diabetes (T2D).*

Participant Signature: ..... Date: .....

Participant Name: ……...

Participant ID: …….

Statement by investigator

- I have explained this project and the implications of participation in it to this participant without bias and I believe that the consent is informed and that he/she understands the implications of participation.

Researcher Signature: ..... Date: ……..

Researcher Name: ……..

APPENDIX B

Health Screening questionnaire

Participants number/code: ……

Date of birth: ……

Occupation…….

Blood Pressure……..

Pulse (HR) ……..

Height…….

Weight……

Waist circumference …….

1. General health screening questionnaire

Do you consider yourself to be healthy?	Yes / No
Are you vegetarian (no meat or fish)	Yes / No
Do you eat a special diet?	Yes / No If yes, specify
Do you exercise regularly?	Yes / No If yes, answer questions on next page
Have you been sick within the past 4 weeks?	Yes / No
If yes, when Describe illness	

Do you have high blood pressure?	Yes / No
Are you exposed to any hazardous chemicals in your job?	Yes / No
If yes, what?	
Do you use tobacco?	Yes / No If yes, answer questions on next page
Do you drink alcoholic beverages?	Yes / No If yes, answer questions on next page
Are you currently under a doctor's care?	Yes / No
If yes, why?	
Have you been hospitalised in the last 6 months?	Yes / No
If yes, why and when?	
Are there any inherited health problems in your family?	Yes / No If yes, describe
Have you taken aspirin or any pain relievers in the past 4 weeks?	Yes / No If yes, what and when?
Are you taking any prescribed medication? Including diet pills, antacids / stomach medicine, cold or allergy medicine	Yes / No
If yes, what?	
Do you take vitamin supplements or herbal remedies?	Yes / No
If yes, what?	

2. Lifestyle screening questionnaire

Alcohol

1: Have you had a drink in the last 48 hours Y/N

If yes, what ……..

2: How much do you drink in a typical week?

Nothing …….

Beer …….pints litres

Cider …….pints litres

Wine …….glasses (assume 6 glasses per standard sized bottle

Spirits ……..bottles

For how many years has this been typical…....

Smoking

1: How many cigarette pack years have you smoked? .....

A pack year is 20 cigarettes per day for one year

2: If you roll your cigarettes how many ounces per week do you smoke? .....

3: If you smoke cigars and pipes, how many days per week do you smoke .....

How many years have you smoked for? .....

Exercise

1: How many days in the past week have you performed physical activity where your heart beats faster and your breathing is harder than normal for 30 minutes or more? (In 3 ten minutes bouts or one 30 minute bout).

2: How many days in a typical week have you performed activity such as this?

3. Ethnicity monitoring questionnaire

A : White	
British	
Irish	
Any other White background	(please write in)
B : Mixed	
White and Black Caribbean	
White and Black African	
White and Asian	
Any other mixed background	(please write in)
C : Asian or Asian British	
Indian	
Pakistani	
Bangladeshi	
Any other Asian background	(please write in)
D : Black or Black British	
Caribbean	
African	
Any other Black background	(please write in)
E : Chinese or other ethnic group	
Chinese	
Any other (please write in)	
Not stated	
Not stated	

Time of sample collection ……

APPENDIX C

Pre Screening for Blood Sampling

This form must be completed prior to any work involving blood sampling

Title of the study:
Ethics Code:

- ☐ Fingertip capillary blood
- ☐ Earlobe capillary blood
- ☐ Venous whole blood

- Please indicate the trained phlebotomist who will be performing the venepuncture: .....

- ☐ Lactate (Biosen/Lactate Pro)
- ☐ Glucose (Biosen /Accutrend)
- ☐ HCt measurement (Hawksley capillary tubes and centrifuge)
- ☐ Hemoglobin (HemoCue)
- ☐ Cholesterol
- ☐ Other chemistries
- ☐ Any other; please specify .....

Please answer the following questions:	**YES**	**NO**	
1. Are you suffering from any known active, serious infection?	☐	☐	
2. Have you had jaundice within the previous year?	☐	☐	
3. Have you ever had any form of hepatitis?	☐	☐	
4. Have you any reason to think you may be HIV positive?		☐	☐
5. Have you ever been involved in intravenous drug use?		☐	☐
6. Are you a haemophiliac?		☐	☐
7. Is there any other reason you are aware of why taking blood might be hazardous to your health?	☐	☐	
8. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?	☐	☐	

I have been fully informed of and understand:

- **The procedure for the sampling and analysis of blood for the above**
- **The possible risks of contamination to myself and participants**
- **The benefit of being inoculated against Hepatitis B**

I agree to undertake all necessary health and safety procedures and precautions during blood sampling to avoid contamination and accept that I will be excluded from the laboratory should I neglect to demonstrate sufficient care and responsibility.

I have read and understood the University guidelines on the management of needlestick injuries and am aware of what to do in the event of such an accident.

Signed: ..... Date: .....
Name: .....
Signed by supervising member of staff: …….....

APPENDIX D

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	**1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	**2. Do you feel pain in your chest when you do physical activity?**
☐	☐	**3. In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	**5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	**7. Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

continued on other side...

APPENDINX E

Meals form

Project title:
Ethics code:
Date:
Participant ID:

Standardised breakfast

You will be provided with a standardised breakfast upon arrival to the laboratory consisting of:

- Sandwich with cheese, mayo, tuna or Ham
- 1 Chocolate muffin
- 1 glass orange juice

Ad libitum buffet meal

You will be given also 30 minutes to access to a buffet meal made of the following items: Orange juice, semi skimmed milk, Granola, Oats, Corn Flakes, white bread, brown bread, butter, margarine, mayonnaise, cheese, ham, tuna, salted crisps, chocolate bars, cereals bar, cookies, muffin, apple, oranges and bananas.

I confirm acceptance of the food listed above and I have informed the investigators about any allergy/intolerance.

Name and Surname:
Participant's signature:

Name of Researcher: Simone Benedetti
Email: k1442446@kingston.ac.uk
Tel: 020 8417 2476

APPENDINX F

FOOD DIARY

An example of how you might write your food diary *before* you have made any changes to what you eat. Can you pick out which things on this list you might cut down on and which you could eat more of?

Day & Date	Time	Food Description	Amount
Monday 24/11/05	8.30 am	White toast, butter & jam Boiled egg Coffee & 2 sugars	2 pieces 1 2 cups
	10.30am	Coffee & 2 sugars KitKat	1 cup 1 whole bar
	12.30am	Cheese & salad sandwich, mayonnaise & butter Apple Orange juice Bag of Maltesers	(2 rounds of white bread) Small carton Whole bag
	3pm	Tea & one sugar Scone, jam and cream Grapes	1 cup 1 scone 5 grapes
	5.30pm	Crisps	3 crisps
	6.30pm	Roast beef, Yorkshire pudding, roast potatoes, boiled peas, boiled carrots & gravy Rhubarb crumble and custard Wine	1 large plate + small seconds One bowl Half a bottle
	8.30pm	Hot chocolate + 2 sugars	1 cup

DAY 1

Day & Date	Time	Food Description & Preparation	Amount
Day: ..... Date: ___/___/____			

DAY 2

Day & Date	Time	Food Description & Preparation	Amount
Day: …… Date: ___/___/___			

DAY 3

Day & Date	Time	Food Description & Preparation	Amount
Day: …… Date: ___/___/___			

APPENDINX G

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

APPENDINX H

Visual Analogue Scale

Time Point: **Time:** **Temp:** **Humidty:**

Subject Number: _____ Trial_____ Date: _____

Please indicate how hungry you are now by circling a relevant number

Not Hungry Fairly Hungry Hungry Very Hungry

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Place a mark on the horizontal lines below after considering the following questions:

How hungry do you feel?

I am not hungry at all — I have never been more hungry

How satisfied do you feel?

I am completely empty — I cannot eat another bite

How full do you feel?

Not at all full — Totally full

How much do you think you can eat?

Nothing at all — A lot