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Lifestyle factors, adiposity and cardiometabolic risk factors in South Asian women living in Scotland

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Abstract

South Asians migrating to the Western world have a 3 to 5-fold higher risk of developing type 2 diabetes and double the risk of cardiovascular disease (CVD) than the background population of White European descent, without exhibiting a proportional higher prevalence of conventional cardiometabolic risk factors. Notably, women of South Asian descent are more likely to be diagnosed with type 2 diabetes as they grow older compared with South Asian men and, in addition, they have lost the cardio-protective effects of being females. Despite South Asian women in Western countries being a high risk group for developing future type 2 diabetes and CVD, they have been largely overlooked. The aims of this thesis were to compare lifestyle factors, body composition and cardiometabolic risk factors in healthy South Asian and European women who reside in Scotland, to examine whether ethnicity modifies the associations between modifiable environmental factors and cardiometabolic risks and to assess whether vascular reactivity is altered by ethnicity or other conventional and novel CVD risks.

I conducted a cross-sectional study and recruited 92 women of South Asian and 87 women of White European descent without diagnosed diabetes or CVD. Women on hormone replacement therapy or hormonal contraceptives were excluded too. Age and body mass index (BMI) did not differ between the two ethnic groups. Physical activity was assessed and with self-reported questionnaires and objectively with the use of accelerometers. Cardiorespiratory fitness was quantified with the predicted maximal oxygen uptake (VO$_2$ max) during a submaximal test (Chester step test). Body composition was assessed with skinfolds measured at seven body sites, five body circumferences, measurement of abdominal subcutaneous (SAT) and visceral adipose tissue (VAT) with the use of magnetic resonance imaging (MRI) and liver fat with the use MR spectroscopy. Dietary density was assessed with food frequency questionnaires. Vascular response was assessed by measuring the response to acetylcholine and sodium nitroprusside with the use of Laser Doppler Imaging with Iontophoresis (LDI-ION) and the response to shear stress with the use of Peripheral Arterial Tonometry (EndoPAT).

The South Asian women exhibited a metabolic profile consistent with the insulin resistant phenotype, characterised by greater levels of fasting insulin, lower levels of high density lipoprotein (HDL) and higher levels of triglycerides (TG) compared with their European counterparts. In addition, the South Asians had greater levels of glycated haemoglobin (HbA1c) for any given level of fasting glucose. The South Asian women engaged less time
weekly with moderate to vigorous physical activity (MVPA) and had lower levels of cardiorespiratory fitness for any given level of physical activity than the women of White descent. In addition, they accumulated more fat centrally for any given BMI. Notably, the South Asians had equivalent SAT with the European women but greater VAT and hepatic fat for any given BMI. Dietary density did not differ among the groups.

Increasing central adiposity had the largest effect on insulin resistance in both ethic groups compared with physical inactivity or decreased cardiorespiratory fitness. Interestingly, ethnicity modified the association between central adiposity and insulin resistance index with a similar increase in central adiposity having a substantially larger effect on insulin resistance index in the South Asian women than in the Europeans. I subsequently examined whether ethnic specific thresholds are required for lifestyle modifications and demonstrated that South Asian women need to engage with MVPA for around 195 min.week\(^{-1}\) in order to equate their cardiometabolic risk with that of the Europeans exercising 150 min.week\(^{-1}\). In addition, lower thresholds of abdominal adiposity and BMI should apply for the South Asians compared with the conventional thresholds.

Although the South Asians displayed an adverse metabolic profile, vascular reactivity measured with both methods did not differ among the two groups. An additional finding was that menopausal women with hot flushing of both ethnic groups showed a paradoxical vascular profile with enhanced skin perfusion (measured with LDI-ION) but decreased reactive hyperaemia index (measured with EndoPAT) compared with asymptomatic menopausal women. The latter association was independent of conventional CVD risk factors.

To conclude, South Asian women without overt disease who live in Scotland display an adverse metabolic profile with steeper associations between lifestyle risk factors and adverse cardiometabolic outcomes compared with their White counterparts. Further work in exploring ethnic specific thresholds in lifestyle interventions or in disease diagnosis is warranted.
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I would like to dedicate this thesis to my parents!
Author’s Declaration

The contents of this thesis have not been submitted elsewhere for any other degree, diploma or professional qualification.

This thesis has been composed by me, and I have been responsible for patient recruitment, tissue collection, laboratory studies and analysis unless otherwise acknowledged.

Stamatina Iliodromiti, November 2014.
### Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ach</td>
<td>Acetylcholine</td>
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<td>AI</td>
<td>Augmentation Index</td>
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<tr>
<td>ALSPAC</td>
<td>Avon Longitudinal Study of Parents and Children</td>
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<td>ALT</td>
<td>Alanine Aminotransferase</td>
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<td>Apo</td>
<td>Apolipoprotein</td>
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<td>Aspartate Aminotransferase</td>
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<td>ATP III</td>
<td>Adult Treatment Panel III</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<td>BHF</td>
<td>British Heart Foundation</td>
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<td>BIA</td>
<td>Bioelectrical impedance analysis</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>Blood pressure</td>
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<tr>
<td>CD</td>
<td>Compact disc</td>
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<tr>
<td>CE</td>
<td>Cholesteryl ester</td>
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<td>CETP:</td>
<td>Cholesteryl ester transfer protein</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CT</td>
<td>Computer tomography</td>
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<td>CVD</td>
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<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
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<tr>
<td>DEXA</td>
<td>Dual Energy X-Ray Absorptiometry</td>
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<td>DLW</td>
<td>Doubly labelled water</td>
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<tr>
<td>DPP</td>
<td>Diabetes Prevention Programme</td>
</tr>
<tr>
<td>E</td>
<td>Europeans</td>
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<tr>
<td>ECG</td>
<td>Electrocardiograph</td>
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<td>ELISA</td>
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<td>ENPP1</td>
<td>Ectoenzyme nucleotide polypeptide</td>
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<td>Free fatty acids</td>
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<td>Flow mediation dilatation</td>
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<td>Follicle stimulating hormone</td>
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<td>GWAS</td>
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<td>HAPO</td>
<td>Hyperglycaemia and Adverse Pregnancy Outcome</td>
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<td>HbA1c</td>
<td>Glycated Haemoglobin</td>
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<td>High Density Lipoprotein</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostatic model of assessment of insulin resistance</td>
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<td>High-performance liquid chromatography</td>
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<td>HRT</td>
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<td>IDF</td>
<td>International diabetes federation</td>
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<td>IGT</td>
<td>Impaired glucose tolerance</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>Iontophoresis</td>
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<td>International Physical Activity Questionnaire</td>
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<td>IRS</td>
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<td>Low Density lipoprotein</td>
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<td>L-NMMA</td>
<td>NG-monomethyl-L-arginine</td>
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<td>LPL</td>
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<td>MET</td>
<td>Metabolic Equivalent of Task</td>
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<td>MetR</td>
<td>Metabolic risk</td>
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MetS  Metabolic syndrome
MI    Myocardial infarction
MRI   Magnetic Resonance Imaging
MRS   Magnetic resonance spectroscopy
MVPA  Moderate to vigorous physical activity
NAFLD Non-alcoholic fatty liver disease
NCD   Non-communicable disease
NCEP  National Cholesterol Educational Program
NCEP-ATPIII  National Cholesterol Education Program Adult treatment Panel III
NEFA  Non-esterified fatty acids
NHS   National Health Service
NICE  National Institute for Health and Care Excellence
NO    Nitric oxide
OGTT  Oral glucose tolerance test
OR    Odds ratio
PAR   Population attributable risk
PAT   Peripheral Arterial Tone
Pc    Concordance correlation coefficient
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<td>PCOS</td>
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<td>PPAR</td>
<td>Peroxisome proliferator activator</td>
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<td>PU</td>
<td>Perfusion Units</td>
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<td>PUFA</td>
<td>Polyunsaturated fat</td>
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<td>PWA</td>
<td>Pulse wave analysis</td>
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<td>r</td>
<td>correlation coefficient</td>
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<td>RHI</td>
<td>Reactive Hyperaemia Index</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>ROC</td>
<td>Receiver operator curve</td>
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<td>SA</td>
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<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
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<td>SBP</td>
<td>Systolic Blood Pressure</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>Sex hormone binding globulin</td>
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<td>SIMD</td>
<td>Scottish Index of Multiple Deprivation</td>
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<td>SNP</td>
<td>Single-nucleotide polymorphisms</td>
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<td>SNP</td>
<td>Sodium nitroprusside</td>
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<td>SWAN</td>
<td>The Study of Women's Health Across the Nation</td>
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<td>Acronym</td>
<td>Full Form</td>
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<td>TC</td>
<td>Total cholesterol</td>
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<td>TNF</td>
<td>Tumour necrosis factor</td>
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<td>UK</td>
<td>United Kingdom</td>
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<tr>
<td>USA</td>
<td>United States of America</td>
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<td>VAT</td>
<td>Visceral adipose tissue</td>
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<td>VLDL</td>
<td>Very low density lipoprotein</td>
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<td>VMS</td>
<td>Vasomotor symptoms</td>
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<td>VO₂ max</td>
<td>Maximal oxygen uptake</td>
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<tr>
<td>WC</td>
<td>Waist circumference</td>
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<td>WHI</td>
<td>Women’s Health Initiative</td>
</tr>
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<td>WHO</td>
<td>World Health Organisation</td>
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<td>WHR</td>
<td>Waist-to-hip ratio</td>
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1 INTRODUCTION

1.1 Epidemiology

1.1.1 Epidemiology of cardiovascular disease and type 2 diabetes

Cardiovascular disease (CVD), encompassing coronary heart disease (CHD) and cerebrovascular events, is the leading cause of mortality worldwide being responsible for 3 out of every 10 deaths (1). Statistics are consistent across middle and high income countries and there also is a noticeable shift in the disease burden in the developing countries where non communicable diseases (NCD) - principally CVD, diabetes, cancers and respiratory disease – are now a major cause of mortality accounting for 26 million deaths in 2011 (1). NCD deaths are predicted to increase by 15% globally by 2020, with the greatest increase of 20% projected for South/East Asia and Africa (2). Type 2 diabetes is not only one of the dominant causes of deaths globally causing additional 1.3 million deaths per annum, it is also a risk factor, along with impaired glucose tolerance, doubling the risk of developing future CVD (3, 4) and contributing further to the global mortality and morbidity.

International figures of the morbidity attributed to CVD and diabetes are less reliable compared with the mortality statistics because of the lack of established registries in developing countries. The national statistics in England suggested that around 14% of the population were diagnosed with CVD in 2011 (5). The same report suggested a 2.5-fold increase in the prevalence of diagnosed diabetes since 1994, with a quoted prevalence of 7% and 4.9% among adult men and women respectively in 2011 (5). In Scotland, the reported prevalence of diabetes in both sexes in 2012 was 4.9% with 90% of cases being type 2 diabetes (6). Interestingly, the Scottish survey reported that South Asians who live in Scotland have a greater age specific incidence of type 2 diabetes. The absolute value was not quoted though because of a suboptimal 77.5% completeness of reporting ethnicity in the registered diabetic population. Three hundred forty seven (95% confidence intervals (CI): 314, 382) million adults worldwide were estimated to have been diagnosed with diabetes in 2008, 40% out of whom lived in India and China (7). Since 1980, almost 194 million new cases of diabetes, the vast majority being type 2, have been diagnosed and around 70% of them have been attributed to the population growth and ageing population. However, the remaining 30% increase in age-specific incidence of diabetes is possibly related with modifiable life style factors (7).
The obesity epidemic may have contributed to the above trend as there is a parallel increase in the trends of obesity and type 2 diabetes. In 1995, there was an estimate of 200 million obese adults worldwide with the number increasing geometrically to over 300 million in 2000 and 500 million in 2008; that 300 million out of those were women suggests that there is a sex specific gradient in obesity prevalence (8). In Scotland, national data suggest that there is a significant increase in the proportion of obese adults from 17.2% in 1995 to 26.1% in 2012, with a higher prevalence of obesity among women; however, a breakdown of the figures per ethnic group is lacking (9). The World Health Organisation (WHO) estimated that 58% of cases of type 2 diabetes and 21% of CHD globally are attributable to increasing body mass index (BMI) (10). However, some regions with the highest increase in fasting blood glucose (FBG) had the lowest increase in body mass index (BMI) over the last 30 years, i.e. men from South Asia had the second smallest increase in BMI but the sixth highest increase in average FBG and South Asian women had the fourth from the bottom increase in BMI but the sixth highest rise in FBG among 21 ethnic groups since 1980 (11).

1.1.2 Ethnic differences in cardiovascular disease, type 2 diabetes and obesity

It is well established that the disease burden varies across ethnic groups and the ethnic disparity persists or inflates when ethnic groups migrate away from their native countries, suggesting an interaction between genetic and environmental factors. In particular, groups of West African, South Asian and Hispanic descent are at increased risk of CVD and type 2 diabetes compared with populations of White European descent (12, 13); however the prevalence of metabolic risk factors for these conditions vary substantially between the groups. Asians (mainly South Asians and Chinese) and Black Africans or Caribbeans constitute the vast majority of non-White ethnic minorities in the United Kingdom (UK) (14).

Black Africans in Western world have a 2.21 (1.77 to 2.76) higher incidence rate of stroke compared with the background population after adjustment for known confounders such as age, sex and social class (15). They also exhibit a higher prevalence of hypertension (in particular women) which increases with ageing (16) and are almost 2-fold more likely to have been diagnosed with diabetes compared with the native White population (17). In addition, there is an increasing trend in obesity among both rural and urban African populations (18). However, black Africans and Caribbean Africans living in the UK have a
favourable lipoprotein profile at a greater BMI compared with the background White population (19) and enjoy significant protection from coronary heart disease (CHD) morbidity (20) and mortality (21).

In China the prevalence of diabetes has almost increased fourfold over the last 15 years with 9.7% (95% CI: 9.2-10.1) of the adult population diagnosed with type 2 diabetes and 15.5% (95% CI: 14.9–16.1) with prediabetes in 2008 compared with 2.5% and 3.4% of the population respectively in 1994 (22). Hence, it was estimated that 92 million Chinese adults had diabetes and 148 million were prediabetic in 2008. These figures suggest that China competes with India in becoming the global epicentre of diabetes epidemic (23). Obesity rate has increased in China over the last decades too but to a lesser extent thus it does not directly explain the disproportional increase in the prevalence of diabetes (24). A similar trend to obesity has been observed in the prevalence of hypertension and CVD, which have increased from 14.4‰ in 1991 to 18.8‰ in 2002 and from 31.4‰ to 50.0‰ respectively (24). Despite the increasing trends in metabolic conditions in East Asia, when Chinese migrate to the western world they still display a lower incidence of newly diagnosed diabetes and associated co-morbidities across all ages compared with other Asian or White descent groups (25). However, recent estimates derived from a large primary care dataset suggest that Chinese women in the UK have a hazard ratio for developing type 2 diabetes within 10 years of 1.96 (95% CI: 1.39-2.78) (adjusted for age, BMI, smoking status and family history) compared with the White population (26).

South Asians, an ethnic term referring mainly to people of Indian, Pakistani, Sri Lankan or Bangladeshi origin, develop CHD around 10 years earlier and at a lower BMI than ethnic groups of White descent (27). Conventional risk factors such as hypertension, diabetes, smoking or diet did not account for their excess premature risk over other groups but Indians did display an unfavourable lipid profile with an elevated Apolipoprotein (Apo)-B/ApoA-1 ratio at a younger age (27). In the UK, Indian and Pakistani men have almost 30% higher rate of CHD (28) and 50% higher mortality from CHD compared with white Europeans (29). In addition, South Asian women do not exhibit the protective effects of being female on CVD risk to the extent observed in other ethnic groups and exhibit similar rates to those observed in South Asian men (30).

In regards to diabetes prevalence on the Indian subcontinent, it has increased substantially from 3% in 1970s to 12% in 2000 (31), with marked differences in the prevalence of type 2 diabetes between rural and urban settings (9.2% in rural versus 18.6% in urban South India.
The projected estimates suggest that India would be the leading country in the absolute number of diabetes cases by 2030 (23). When South Asians migrate to the UK, they have a 3- to 6-fold greater risk of being diagnosed with type 2 diabetes compared with the White background population but similar rates of type 1 diabetes based on national statistics in 2004 (28). Estimates suggest that compared with the White population, the adjusted hazard ratio for 10-year risk for type 2 diabetes is 4.07 (95% CI: 3.24-5.11) for Bangladeshi women, 4.53 (3.67-5.59) for Bangladeshi men, 2.15 (1.84-2.52) for Pakistani women, and 2.54 (2.20-2.93) for Pakistani men, 1.71 (1.49-1.97) for Indian women and 1.93 (1.70-2.19) for Indian men (26). The same study which reviewed the records of 2,540,753 patients aged 25-79 years showed that South Asians were on the top places of risk tables quantifying crude and age-stratified rates of type 2 diabetes followed by the Caribbeans, Black Africans and Chinese whereas on the bottom were those of White European descent (26). The trend did not differ among sexes but, notably, none of the South Asian groups displayed greater prevalence of risk factors (namely smoking, BMI, family history of diabetes or hypertension) compared with the other ethnic groups.

Migration and acculturation have been blamed for the excess risk of the immigrant populations, which can be partly attributed to obesity. More interestingly, there seems to be an ethnic specific element on the associations of obesity with cardiometabolic outcomes. South Asians develop obesity related co-morbidities at a lower BMI and have higher levels of body fat for any given value of BMI compared with their White counterparts (33-36). This prompted the debate as to whether the BMI cut-off points should be lowered for Asian populations (37, 38). Despite that the formal response by WHO was that BMI cut-offs could not be altered because the lack of consistency in the associations between BMI and disease burden among Asian subgroups (39), several studies have been published thereafter reinforcing that South Asians develop the same metabolic risk with the Europeans at a lower BMI and waist circumference (WC) (40-42). A Consensus Statement from India for diagnosis of obesity recommended the cut-offs of 23-24.9 kg.m\(^{-2}\) for defining overweight and ≥ 25 kg.m\(^{-2}\) for defining obesity among South Asians living in the Indian subcontinent (43). Similarly, the National Institute for Health and Care Excellence (NICE) in the UK recommended new BMI thresholds for interventions to prevent type 2 diabetes among ethnic minority groups; specifically suggested the cut-offs of 23 kg.m\(^{-2}\) and 27.5 kg.m\(^{-2}\) for indicating increased and high risk respectively of developing type 2 diabetes among South Asians (44). Notably, a recent study evaluating 490,288 UK Biobank participants and their risk of developing diabetes type 2, recommended thresholds of 22 and 26 kg.m\(^{-2}\) for defining overweight and obesity respectively for South Asians.
living in the UK (45). In addition, ethnic specific criteria for WC have been proposed by various research groups recommending lower thresholds for South Asians (ranging from 67 to 86.6 cm for women and 73 to 99.3 cm for men depending on the endpoint used to estimate risk equivalence with the subjects of White European descent) (41, 45-47). It is evident that studies that assessed the relationship of WC with diabetes prevalence rather than with summary scores of metabolic risks recommended lower thresholds (45, 47).

Differences in the environmental factors associated with migration do not explain the whole story given that some immigrant populations (i.e. Chinese men and women in Canada compared) have maintained a low prevalence of diabetes compared with indigenous populations (i.e. background White population) (25). In addition, although Westernisation can account for the increased prevalence of type 2 diabetes in ethnic minority groups, it is unclear how it could account for the excess risk of the migrant compared with the indigenous populations. Given that South Asians in the UK have a disproportional high risk of developing type 2 diabetes (26) and premature CVD (48) compared with the background population of White European descent and that it is the largest non European group living in the UK accounting for over 5% of the general population in England, Wales (14) and 3% in Scotland (49), it provides a great model to study the interface of modifiable life style factors with metabolic risk factors in individuals who have not yet developed overt disease.

1.1.3 Sex specific differences in cardiovascular disease and type 2 diabetes in the South Asians

As it was discussed earlier, there is a sex specific element in the prevalence of type 2 diabetes and CVD in South Asians which becomes more evident with ageing. The INTERHEART study, a large case control multiethnic study looking at the risk factors contributing to the first episode of myocardial infarction (MI), confirmed that women experience their primary MI on average 9 years later than men worldwide (27). Although the same risk factors attributed to MI in both sexes, the earlier onset of MI in men was largely explained by higher prevalence of risk factors, including impaired lipid profile, smoking and alcohol consumption at a younger age in men (50). In addition, it was postulated that the role of endogenous oestrogens in CVD protection was possibly mediated through their beneficial effect on Apo levels and this is lost with the onset of menopause (50). This sex discrepancy in the mean age of primary MI was evident in participants living in South Asia too with women experiencing their first MI at an average
age of 60 years whereas men at an age of 52 years (27). However, when South Asians migrate to Westernised countries, the sex difference in CHD risk becomes narrower; the mortality ratios from MI and cerebrovascular events were 121 and 132 for men and 118 and 100 for women respectively of South Asian descent (51). In addition, South Asian men migrated to the UK (aged 30-65 years) have an age standardised CHD-mortality rate about 40% higher than men in the general population and for women the equivalent figure is 51% (52). More interestingly, from 1971 to 1991 the mortality rate from CHD for the whole UK population under 65 years fell by 29% for men and 17% for women, whereas in migrants of South Asian descent it dropped by 20% for men but only 7% for women (29). This suggests that health policies and lifestyle modifications aiming at CVD protection were less applicable or not equally effective in South Asian women living in the UK.

A large population based study in four Asian countries (DECODA study) showed that the age-specific prevalence of type 2 diabetes did not differ significantly among men and women from India (53). However, Indian women demonstrated higher prevalence of impaired fasting glucose (IFG) than men among all age groups and greater prevalence of impaired glucose tolerance (IGT) in the age group of 30-39 years (53). Notably, women living in the South Asian subcontinent face a disproportional access to medical services and support for diabetes and a cultural disparity towards preventative lifestyle measures compared with men; all resulting in higher rates of morbidity and mortality from diabetes among women (54). In South Asian migrants, the figures may vary according to the country of residence (55); in Netherlands, South Asian women have a similar prevalence of diagnosed diabetes than South Asian men (25.8% versus 24.8%) (56). Nevertheless, South Asian women have a 2-fold higher prevalence of diabetes compared with White Dutch women, whereas South Asian men expose roughly a 1.3-fold higher prevalence than that of White Dutch men (56). In the UK, the sex specific prevalence of type 2 diabetes differed among South Asian subgroups with Pakistani women having overall similar prevalence with that of Pakistani men (8.4% versus 7.3%), whereas Indian women demonstrate a slightly lower prevalence than that of Indian men (5.9% versus 9.2%) (28). A striking finding was that the risk of type 2 diabetes increased disproportionally in Pakistani women over the age of 55 years compared with that in Pakistani men of similar age (prevalence of 44.4% in women versus 25.3% in men). It is unclear why this is the case particularly as the figures in the general population did not follow a similar trend (28). Collectively the data support sex specific differences in the burden of both CVD and type 2 diabetes among South Asians, that South Asian women constitute a high risk group and that they have been largely overlooked with most of the ethnic specific research been focused on men because
of their cultural accessibility. Further detailed research in cardiometabolic risk factors is warranted in South Asian women.

1.2 Metabolic syndrome and South Asians

1.2.1 Defining the metabolic syndrome

The metabolic syndrome (MetS) is a cluster of risk factors that include central obesity, impaired glucose metabolism (manifesting as impaired glucose tolerance or impaired fasting glycaemia), dyslipidaemia and hypertension. MetS is not only associated with an elevated risk of type 2 diabetes but with increased risk of atherosclerosis and CVD (57). There are various definitions of MetS which all agree in principle but differ in the details and criteria, rendering a universal clinical diagnosis of MetS challenging. Table 1 shows the most commonly used definitions of MetS: those of the International Diabetes Federation (IDF), the World Health Organisation (WHO) and National Cholesterol Education Program Adult treatment Panel III (NCEP-ATPIII). A joint statement published in 2009 was an effort to harmonise the criteria of the MetS and suggest a common definition (58). It essentially retained the IDF criteria but abdominal adiposity (which was defined based on population and country specific definitions) is not anymore a prerequisite for diagnosis of MetS, but 1 of 5 criteria, so that the presence of any 3 out of 5 risk factors constitutes a diagnosis of MetS.

1.2.2 Metabolic syndrome in South Asians

National studies reporting the prevalence of the syndrome in the South Asian countries are lacking. Existing studies suggest that the prevalence of MetS among residents of the South Asian subcontinent varies according to the level of urbanisation, the classification criteria of the syndrome and the socioeconomic status of the participants; in urban India, the prevalence of MetS ranges from 18% to 46.5% with small sex-driven variations, whereas the overall prevalence of MetS according to the NCEP-ATPIII criteria has been reported as 9.2% among 640 participants living in urban slums (59). A single study carried out in urban Pakistan (Karachi) among 867 participants reported a striking prevalence of 49% according to the NCEP-ATPIII criteria and 34.8% based on the IDF classification (60). A rising trend in the frequency of the manifestations of MetS has been shown in rural India too (61), where the reported prevalence of MetS according to the NCEP-ATPIII criteria in a single study of 4535 participants was 26.9% among men and 18.4% among women,
whereas it did increase to 32.5 and 23.9% in men and women respectively when ethnic specific criteria in the classification of MetS were used (62).

The impact of migration on the prevalence of MetS is obscure given that the absolute percentage of the reported prevalence of MetS among South Asians residing in Westernised countries does not differ substantially from that in Indian subcontinent and can range from 20 to 49% (19, 63-66). However, the striking finding is that South Asians in Western countries have a higher prevalence of MetS compared with other ethnic groups; for example the prevalence of MetS was 28.8% among migrant South Asians living in Singapore compared with 24.2% in Malays and 14.8% in Chinese residing in the same area (63). Whether this ethnic discrepancy is a result of delayed adaptation of South Asians either in their home countries or in migrant countries to Westernised lifestyle or a consequence of higher clustering of risk factors is still unclear.

Given the large variation in the prevalence of MetS according to the classification system used and the current evidence which suggests that the definition of MetS should include ethnic specific criteria (59) in order to minimise underestimation of the prevalence of the condition across different groups (67, 68), the use of MetS as a primary outcome of ethnic specific research has inherent limitations. That the diagnosis of MetS constitutes a categorical variable limits further the use of this variable as a primary outcome. On the contrary, the manifestations of metabolic syndrome are continuous variables and can be examined as independent risk factors contributing to the background risk of an individual to develop type 2 diabetes and CVD.

<table>
<thead>
<tr>
<th>IDF</th>
<th>WHO</th>
<th>NCEP-ATPIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central obesity <strong>plus any two</strong> of the following</td>
<td>Insulin resistance <strong>plus any two</strong> of the following</td>
<td>Three out of five</td>
</tr>
<tr>
<td>TG ≥ 1.7 mmol/L or on treatment</td>
<td>TG ≥ 1.7 mmol/L</td>
<td>Central obesity (waist ≥ 88 cm in women)</td>
</tr>
<tr>
<td>HDL &lt; 1.29 mmol/L (females) or on treatment</td>
<td>HDL &lt; 1 mmol/L (females)</td>
<td>HDL &lt; 1.30 mmol/L (females)</td>
</tr>
<tr>
<td>SBP ≥ 130 or DBP ≥ 85 mmHg or on treatment</td>
<td>SBP ≥ 140 or DBP ≥ 90 mmHg or on treatment</td>
<td>SBP ≥ 130 or DBP ≥ 85 mmHg</td>
</tr>
<tr>
<td>Fasting glucose ≥ 5.6 mmol/L or diagnosed type 2 diabetes</td>
<td>BMI ≥ 30 kg/m² or waist to hip ratio &gt; 0.85</td>
<td>Fasting glucose ≥ 6.1 mmol/L</td>
</tr>
<tr>
<td>Urinary Albumin excretion ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 20 mg/min or albumin : creatinine ratio ≥ 3.4 mg/mmol</td>
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1.3 Insulin resistance

Insulin resistance is one of the key features of MetS and precedes type 2 diabetes (69). Glucose homoeostasis relies on the integrity of a feedback loop between β cells and insulin sensitive tissues. Nutrient ingestion stimulates β cells to release insulin which in turn mediates the uptake of glucose, aminoacids and fatty acids by peripheral tissues such as muscles and adipose tissue. These tissues feedback to the islet cells their need for insulin. On the contrary, in insulin resistant subjects, the uptake of insulin from the muscles and other peripheral organs is decreased and, hence, the negative feedback to islet cells diminishes. In turn, β cells start producing more insulin in order to maintain physiological glucose levels in the bloodstream; however, if their capacity to compensate is limited or becomes impaired and glucose intolerance is established. In addition, insulin resistance induces lipolysis in peripheral adipose tissue contributing further to an increase in free fatty acids and enhances hepatic glucogeneogenesis contributing to glycaemia. In turn, lipotoxicity and glucotoxicity reduce further the peripheral glucose uptake and, thereby, the negative feedback to pancreas (Figure 1). Hence, insulin resistance is well established prior to development of glucose intolerance and subsequent type 2 diabetes.
Although insulin resistance refers mainly to a cumulative defect in insulin action which results in fasting hyperinsulinaemia in order to maintain euglycaemia; postprandial hyperinsulinaemia exists prior to fasting hyperinsulinaemia developing. Epidemiological data support the order of the physiological changes in glucose metabolism; data from the British Whitehall study II which included 505 type 2 diabetics suggested that insulin sensitivity was already diminished in those who went on to develop diabetes 13 years prior to the onset of the disease and the decrease became steeper around 5 years before the diagnosis (70). In reverse, insulin secretion from β cells remained unchanged during the study period but showed a substantial compensatory phase around 3-4 years before the diagnosis of type 2 diabetes and dropped rapidly at the time of the diagnosis (70).

![Figure 1. Insulin secretion and insulin action in insulin resistant (red arrows) and normal state (blue arrows). FFA: free fatty acids.](image)

**1.3.1 Assessment of insulin resistance**

The gold standard of measuring insulin resistance is generally regarded to be the euglycaemic clamp which is based on the exogenous administration of insulin by a continuous infusion while plasma glucose levels are held at basal levels with adjustable glucose infusion (71). The need for two intravenous lines, the requirement of well calibrated glucose pumps and precision in timing of serial measurements of glucose levels while the clamp is performed limit the applicability of the procedure in large studies.
Hence, alternative measures have been used in various studies as surrogate measures of insulin resistance. The intravenous glucose tolerance test (IVGTT) represents a relatively simple alternative to the clamp method providing information on both insulin sensitivity and \( \beta \)-cell function (72). It is a less technically demanding and more cost-effective procedure than the clamp rendering it, theoretically, a more appropriate method for large epidemiological studies. However, it does involve placement of an intravenous cannula and serial blood sampling for glucose and insulin measurements prior and following a bolus injection of glucose. Plasma insulin and glucose concentrations measured multiple times during the test are minimally modelled to provide information regarding glucose kinetics and determine both insulin sensitivity and glucose effectiveness indices (73). Although the values are well correlated with that measured with the clamp, the test may be labour intensive in a study where multiple measurements are planned to be taken during one single visit.

The circulating levels of insulin, both fasting and post-prandial, have been used based on the physiological response that the greater the insulin resistance the higher the plasma insulin concentration. There is a clear linear relationship between insulin sensitivity measured with the clamp with fasting insulin levels; however, fasting levels could only explain 14% of the variation of insulin sensitivity (74). In view of the linearity of the relationship between plasma insulin levels and insulin sensitivity, insulin levels are still expected to reveal associations with other metabolic factors in large scale studies but should be used with caution when absolute quantification of insulin resistance is required in studies exploring physiological pathways (72). The oral glucose tolerance test (OGTT) is the most commonly used method to evaluate whole-body glucose tolerance and there have been attempts linking insulin sensitivity with the response to OGTT. The proposed indices of insulin resistance incorporate the glucose area under the curve with the insulin area under the curve during OGTT in composite models (75, 76). Different models display different levels of correlation (ranging \( r = 0.54, 0.73 \)) (76) with the response to the euglycaemic clamp. However, OGTT is time intense and, thereby, self restricted in studies where numerous measurements of other variables are required. In reverse, the homeostatic model assessment of insulin resistance (HOMA_{IR}) utilising basal (fasting) levels of insulin and glucose demonstrate optimal level of correlation with the response to the euglycaemic clamp (\( r = 0.88 \)) and is simple in its application (77). Therefore, the HOMA_{IR} is as a surrogate of insulin resistance for all the analyses when insulin resistance is quoted in the current study.
1.3.2 Adiposity and insulin resistance

It is well recognised that insulin resistance associated with abdominal adiposity is largely attributable to the excess abdominal adipose tissue in subcutaneous but mainly intra-abdominal compartments (78). Positive energy balance leads to hypertrophic abdominal adipocytes which demonstrate impaired lipolytic state and resistance to the anti-lipolytic effect of insulin (79, 80). The surplus of non-esterified fatty acids (NEFA) through the portal vein to the liver impairs liver metabolism and results in increased hepatic glucose and hepatic insulin resistance, which in turn is associated with de novo lipogenesis and the production of triacylglycerol-abundant lipoproteins (81). Newly synthesised triacylglycerol in the liver can be oxidised, released as very low density lipoproteins (VLDL) or stored as hepatic triacylglycerol. However, oxidation is largely inhibited by malonyl-CoA which is produced during lipogenesis, and as a consequence synthesised triacylglycerol is either stored and, thereby, increases the hepatic fat content or exported, thus attributing to an increase in VLDL plasma concentration (82). Figure 2 presents graphically the association of fatty acids (derived from multiple sources) with hepatic steatosis and VLDL synthesis. The presence of positive energy balance with subsequent hypeinsulinaemia can explain the initiation of non alcoholic fatty liver (NAFLD) and, this in turn contributes further to the insulin resistant phenotype.

Although visceral adipose tissue is considered as the most pathogenic fat depot, the majority of NEFA in the portal circulation originate from the systematic circulation rather than from intra-abdominal compartment in both obese and lean subjects (83). It is evident, though, that with increasing visceral adiposity or insulinaemia the contribution of visceral fat lipolysis to hepatic NEFA delivery increases and this is more evident in women than in men (83, 84). The latter finding of the linear association of visceral originated NEFA with hyperinsulinaemia suggests that visceral fat is more resistant to the anti-lipolytic effects of insulin compared with other fat depots (84). Hence, in vivo studies support that the cumulative effect of different fat depots leads to an insulin resistant phenotype, however, VAT can potentially play a crucial role under specific circumstances. This physiological postulation has been replicated in a large epidemiological study (n = 3,001 of mainly Caucasian background) which demonstrated that both VAT and SAT, measured with computed tomography (CT), were associated with metabolic risks but VAT was correlated stronger with each metabolic risk variable (lipid profile, blood pressure, blood glucose) after adjustment for age; the correlation coefficients were greater in women than in men for all the associations (85). A multiethnic cohort further showed that VAT was positively
associated with intima-media thickness (IMT) independent of total adiposity in both sexes and that association remained robust after adjustment for ethnicity or other risk factors for atherosclerosis, whereas SAT was not associated with IMT (86).

Figure 2. Fatty acids, positive energy balance and hepatic steatosis. Different sources of fatty acids contribute to the development of liver steatosis and synthesis of VLDL contributing to the insulin resistant phenotype. VLDL: very low density lipoprotein, NEFA: non-esterified fatty acids.

There is increasing evidence suggesting that the positive energy balance is not associated with an homogeneous fat distribution pattern in all individuals and this has resulted to the postulation of “the lipid overflow ectopic fat model” (Figure 3) (78). According to this theory the ability of the body, which can be dictated by genetic or lifestyle factors (i.e. smoking, physical inactivity) (87, 88), to cope with the surplus of energy and store fat to different compartments determine an individual’s susceptibility to develop metabolic features. When the excess energy is directed to the metabolically inert SAT, adipose tissue is expanded via hyperplasia and, up to a specific level (that can vary among individuals), the subject displays a normal metabolic profile. On the contrary, when SAT is dysfunctional (hypertrophic), absent or insulin resistant, then energy excess is deposited in viscera and other non-adipose tissues such as the liver, the pancreas, the heart or the skeletal muscles, a phenomenon described as ectopic fat deposition (78). There is now a
well established positive association, yet to clarify whether is causal or not, between ectopic fat deposition and insulin resistance (89).

Adipose tissue is not only involved in storage and mobilisation of lipids but is an endocrine organ releasing various cytokines, among which some have pro-inflammatory action such as interleukin (IL)-6 and tumour necrosis factor (TNF)-α and their expression is associated with obesity and insulin resistance (90, 91). It is unclear whether this relationship is causal and what the direction of potential causality is, but summary evidence suggests that obesity stimulates de novo recruitment and activation of macrophages (M1) into adipose tissue which activate inflammatory signalling pathways and these induce the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and resistin. In turn, these mediators act on adipocytes to induce an insulin-resistant state. Hence, a positive feedback loop is established that further augments inflammation response and insulin resistance (92). The association of adiposity with inflammation processes has been shown in population level by several studies demonstrating an association between visceral adiposity and elevated plasma C-reactive protein (CRP), a conventional plasma inflammatory marker (93-97). On the contrary, under lean conditions adipocytes secrete factors, such as IL-13, that promote alternative activation of macrophages (M2), which in turn secrete anti-inflammatory mediators, such as IL-10, which are potentially associated with insulin-sensitising processes (92, 98). Overall the data support inflammation pathways mediating the impact of obesity on insulin resistance and other metabolic consequences.
1.3.3 Insulin resistance-driven dyslipidaemia

It has been discussed above that NEFA flux to the liver increases the production and release of Apo-B abundant VLDL from the liver to the circulation (99). The impact of insulin on this process is biphasic and is modulated by the overall body insulin sensitivity. In an insulin resistant state, hyperinsulinaemia increases the flux of NEFA to the liver and induces hepatic triglyceride synthesis. In addition, peripheral insulin resistance decreases the concentration of lipoprotein lipase (LPL) contributing further to hypertriglyceridaemia. In reverse, under physiological conditions insulin hampers the production of VLDL by mainly inducing the degradation of Apo B. In addition, insulin has lipogenic action in the liver, hence promotes hepatic liponeogenesis by increasing the transcription and enzyme activity of genes that are related with triglyceride biosynthesis (99).

An additional lipid profile impairment associated with the MetS is low serum levels of HDL, which is an independent risk factor to raised LDL or triglycerides of CHD (100, 101). However, a recent meta-analysis demonstrated that there was no association between
iatrogenic induced increase in HDL levels and the risk of CHD events, CHD related mortality and overall mortality, partly because there are limited treatment options that would substantially increase the levels of HDL (102). Apo-A-I is the main element (around 70%) of the apolipoprotein content of HDL particles and is largely secreted from intestine and liver. It has been shown that individuals with high levels of TG have increased catabolic rates of Apo-A-I without an overall alteration in Apo-A-I production, which results in decreased circulating levels of Apo-A-I and, consequently, of HDL. Hence, there is an indirect effect of insulin resistance on HDL levels via hypertriglyceridaemia (103). Potential biological mechanisms that lead to an increased catabolic rate of Apo-A-I and HDL in an insulin resistant state is the reduction in the activity of lipoprotein lipase or a shift in the lipid core content by decreasing the ratio of cholesteryl-ester to triglyceride content. The former mechanism impairs the maturation process of HDL particles whereas the latter transforms the HDL particles to small and dense particles, which can be cleared rapidly from the circulation (103). Similarly to the changes in HDL particles, the composition of LDL particles changes to a similar way and the majority of individuals with hypertriglyceridaemia (TG > 2 mmol/L) display a predominance of small dense LDL molecules that are atherogenic (103). Therefore, hypertriglyceridemia, low levels of HDL, and qualitative changes in LDL particles constitute the typical dyslipidemia of insulin resistant state. There is evidence suggesting that a high TG to HDL ratio may be the single most characteristic feature of the insulin resistant syndrome that can adversely predict long-term manifestations (104). Figure 4 presents graphically the association of insulin resistance with dyslipideamia.
Chapter 1

1.3.4 Insulin resistance and vascular implications

The association between insulin resistance and hypertension has been established for over a decade (105). Exogenous insulin when administered intravenously during the euglycaemic hyperinsulinaemic clamp acts as a vasodilator by stimulating the release of nitric oxide (NO) from the endothelium (106). However, in an insulin resistant state the bioavailability of NO can be impaired and the vasodilator action of insulin can be lost (107). In addition, elevation of plasma insulin levels by infusing exogenous insulin has demonstrated its anti-natriuretic action with a decrease in the clearance of sodium through the kidneys (108). There is also evidence that hyperinsulinaemia in insulin resistant state can cause secondary elevation in blood pressure (BP) through the latter mechanism of increased proximal sodium re-absorption and, thereby, amplify its metabolic consequences (109). Although the association of hyperinsulinaemia with hypertension through the latter mechanism was evident in subjects of white European descent, it was not observed in South Asian and African subjects (109). An additional mechanism, which links obesity and insulin resistance with impaired vascular function, is through the action of NEFA; NEFA flux induces oxidative stress and pro-inflammatory mechanisms which impair the...
macrovascular function as assessed with post occlusion vasodilatation of the brachial artery (110). Hence, obesity and increase in NEFA play a role in the vascular function impairment associated with insulin resistance (110).

Although hypertension may be linked with insulin resistance, hypertension *per se* is an established independent risk factor of CHD and the major risk factor of stroke (27, 111). Hypertension, along with hyperlipidaemia, obesity and insulin resistance, contribute to the activation of M1 macrophages in the vascular wall, initiation of pro-inflammatory pathways and release of pro-inflammatory cytokines which in turn attract more immune cells and maintain a vicious circle which leads to a chronic vascular inflammation and formation of atherosclerosis (98). That the atherosclerotic plaques in vascular bed associated with clinical adverse events (stroke or CHD) are affluent in lipids and macrophages supports the key role of chronic inflammation in plaques that can potentially rupture (“unstable” plaques) (112). Whereas, lifestyle modifications (i.e. weight reduction) and treatment options (BP lowering and lipid lowering regimes) stabilise the plaques by altering their biological content (smaller lipid pool, fewer inflammatory cells, thicker fibrous cap) and thereby, contribute to the prevention from CVD events (112). Hence, the atherosclerosis model provides a biological linkage between hypertension and CVD.

1.3.5 Insulin resistance and its manifestations in South Asians

Several studies have shown that insulin resistance, assessed mainly by HOMA<sub>IR</sub> or plasma insulin levels, is more prevalent in the South Asians and occurs at a younger age (48, 113, 114). The Southall study in 1991 was the first large cross-sectional study that demonstrated that Indians and Pakistanis living in the UK had higher prevalence of diabetes (19% versus 4%) compared with the European group and greater fasting and post-prandial plasma insulin levels when exclusively non diabetics were included in the analysis. This ethnic specific discrepancy was evident in both sexes. However, South Asian women had greater BMI than their European counterparts, which may have inflated the difference in insulin levels (115). Notably, Indian men living in North America had greater insulin resistance than White men despite similar levels of total body fatness and truncal adiposity suggesting that ethnic specific differences in adiposity do not account for the magnitude of the ethnic specific discrepancy in insulin resistance (35). Similarly, a cross-sectional study between South Asian, White European and Afro-Caribbean men living in the UK demonstrated that South Asians and Afro-Caribbeans were more insulin resistant that their White counterparts with South Asians displaying additional risk factors with an adverse
lipid profile. The poor metabolic profile of the South Asians was not explained by ethnic differences in body composition, dietary fat intake or impaired metabolism of NEFA (116). Data in South Asian women are scanty and have resulted from a small number of female participants (117, 118) or have derived from epidemiological reports that highlight their excess risk of insulin resistance but have not looked at underlying risk factors in depth (119). Hence, further research is required in risk factors associated with greater insulin resistance in South Asian women.

In addition, dyslipidaemia is more prevalent among South Asians. In the INTERHEART study, native South Asians with a history of MI were more likely to have an elevated Apo-B100 (dominant apolipoprotein of the LDL particle) to Apo-A-I ratio compared with individuals from other ethnic backgrounds (43.8% versus 31.8%) (27). The lipid profile becomes more atherogenic when individuals migrate away from the Indian subcontinent as they display higher levels of total cholesterol, Apo-B and triglycerides and lower levels of HDL cholesterol compared with their siblings or other matched contemporaries living in India (120, 121). Both studies attributed the differences in metabolic profile in greater BMI and excess of fat-abundant diet among migrant Indians; however, other risk factors such as physical inactivity of adiposity were not contemplated. Subsequent studies reported that the atherogenic lipid profile consisting of lower HDL cholesterol and higher triglycerides was more prevalent among South Asians compared with individuals of White descent independent of obesity (65, 122, 123). Notably these ethnic discrepancies in the lipid profile were not always accompanied by higher total cholesterol in the South Asians (124), suggesting that the dyslipidaemic profile in the South Asians was mainly driven by the insulin resistance phenotype. Hence, using total cholesterol levels in risk prediction models would underestimate the CVD risk in the South Asian population.

There is evidence suggesting that regular physical activity improves the lipid profile by increasing HDL cholesterol levels and decreasing triglyceride levels (125-127); however, it is unclear whether these associations are modified by ethnicity given that South Asians are disproportionately predisposed to develop an adverse lipid profile compared with groups of White European background. There are limited data looking at the association of lipid profile with physical activity among South Asians and suggest that those who self report regular exercising have higher levels of HDL cholesterol than those who do not report any physical activity independent to BMI (128). However, the findings have inherent limitations because of the lack of sex stratification, potential inaccuracies in the levels of
self reported physical activity and using physical activity variable as a dichotomous rather than a continuous variable.

Despite the association of hypertension with insulin resistance, data do not support that the prevalence of hypertension is higher among South Asians. In the INTERHEART study, hypertension increased the odds of CHD by 2 to 3-fold in both sexes and this association was robust among all ethnic groups (27). However, the prevalence of hypertension among cases with CHD and controls was not substantially different in the South Asians compared with that in subjects from Western Europe (17.8% versus 20.5% respectively), hence it cannot have accounted for the excess CHD risk of the South Asians (27). In addition, summary data from cross sectional studies disputed the postulation that the prevalence of hypertension is higher among South Asian groups, and, indeed, the majority of the pooled studies showed that South Asians had lower or at least similar systolic BP with their comparators and similar or marginally higher diastolic BP compared with participants of White descent (129). Although there was great heterogeneity in the findings among different Asian subgroups, study populations and methods of measuring BP, none of the studies supported that hypertension was more prevalent among South Asians which may contribute to their excess CVD risk (129). These findings were replicated in subsequent studies too (20, 66, 130, 131). In addition, there is limited evidence whether South Asians who are known to have higher CVD risk at a younger age, demonstrate early vascular changes at micro or macro-vascular level before overt atherosclerosis is developed. This will be assessed in this study with specific focus on the function in different vascular beds in women of different ages and the association of vascular function with established and novel sex specific risk factors.

1.3.6 Summary

South Asians constitute a high risk group, even before they develop overt type 2 diabetes and CVD, by displaying a more insulin resistant phenotype with higher fasting and postprandial insulin levels, an impaired lipid profile with low levels of HDL, higher levels of triglycerides and greater levels of adiposity for any given BMI than individuals of White descent. On the contrary, other conventional cardiometabolic risk factors such as hypertension, smoking or alcohol consumption are less prevalent among the South Asians than in their Europeans comparators. Data regarding the clustering of the above risk factors in healthy South Asian women residing in the UK before they experience the onset of overt disease are scanty and I will aim to elaborate this in this study. In addition, it is unclear
how ethnicity interacts with environmental factors in women leading to their excess cardiometabolic risk.

I will discuss below what are the existing theories suggesting possible mechanisms associated with greater insulin resistance in South Asians and I will also explain why I chose to study women.

1.4 Potential mechanisms contributing to increased insulin resistance in South Asians

The mechanisms underlying differences in the metabolism of South Asians leading to increased insulin resistance and risk of diabetes and CHD have not been fully elucidated. Different hypotheses attempting to explain the predisposition of individuals to develop diabetes and their application into the South Asians are discussed below.

1.4.1 Developmental hypotheses

The thrifty genotype hypothesis was developed by Neel over 50 years ago (132) as a potential explanation to predisposition to diabetes. According to this hypothesis, individuals or specific groups have developed adaptive traits that allowed them to survive under prolonged periods of “famine”, but became disadvantageous in the modern era when lifestyle changed and the same individuals became exposed to “continuous feasting”. Hence, the so-called “thrifty genes” may have provided survival advantage to specific groups when food was not readily available by allowing them to store fat in specific body compartments and convert it to low released energy under famine conditions. Whereas, when the same groups were exposed to nutrient rich environments, the effect of the same genes became detrimental and contributed to increasing adiposity and, to a population level, to the obesity and diabetes epidemic. Although, from an evolutionary perspective, the theory provides a conceptual explanation to the increased rates of diabetes worldwide, it does not adequately explain why South Asians have a higher predisposition to insulin resistance and type 2 diabetes compared with other ethnic groups, as there is lack of evidence that they were exposed to more prolonged starvation condition compared with other groups.

The thrifty genotype was subsequently complemented by the thrifty phenotype hypothesis (133), as an attempt to explain the mismatch between intrauterine and later life environments. The key feature of the theory is that early nutritional state (fetal and
neonatal), which is largely associated with maternal nutrition, is decisive of adult phenotype and disease development (134). Intrauterine malnutrition as a consequence of limited maternal access to nutrients induces metabolic adaptations and changes in organ structures to facilitate postnatal survival in a thrifty environment. According to the hypothesis, the poorly nourished mother provides to the fetus a forecast of the postnatal environment that it will be born into and, hence, the fetus develops adaptive mechanisms in order to compensate for the postnatal nutrient deficiency (135). These mechanisms may include increased liver size, enhanced hepatic glyconeogenesis, rapid release of fatty acids from abdominal adipose compartments and increased uptake of glucose by adipose tissue. The above adaptations along with poor development of pancreatic β cell mass and subsequent decrease in insulin secretion would enhance energy turn-over in individuals who would continue to be poorly nourished postnatally. On the contrary, overnutrition in childhood and decreased energy expenditure in an individual who has already been “programmed” in utero to adapt to thrifty postnatal conditions would lead to obesity and insulin resistance (133, 136, 137). This hypothesis was developed based on the strong associations between low birth weight and increased risk of glucose intolerance or type 2 diabetes (138, 139) and was further complemented by the link between low infant weight and adult risk of glucose intolerance (140, 141). Recent experiments in rats have elucidated potential mechanisms with intrauterine growth restriction associated with changes in fat distribution, adipocyte size, lipid metabolism and glucose metabolic pathways in response to a high fat diet in the postnatal period (142).

A complementary theory is the “fetal insulin hypothesis” (143). Central to this is that fetal genetic factors regulate fetal insulin secretion or fetal tissue sensitivity to insulin and, thereby, play a key role in insulin-mediated fetal growth (143). These genetic factors interact with the effects of maternal malnutrition and have a synergistic impact on fetal growth. Subsequently, a small neonate, resulted from a combination of genetic factors and intrauterine exposures, demonstrates increased susceptibility to type 2 diabetes when is exposed to overnutrition later on in life.

Low birth or early life weight is more prevalent among children of South Asian origin compared with other ethnic groups (144, 145), and low birth weight among Indian neonates is associated with impaired glucose tolerance at the age of four (146), accelerated weight gain in childhood and increased risk of type 2 diabetes and glucose intolerance in adulthood (145). However, the existence of the above associations do not equate causality, so it cannot be postulated that in utero undernutrition attributed to maternal malnutrition
explains fully the susceptibility of South Asians to insulin resistance and type 2 diabetes. Especially when the evidence is contentious and data suggest that among individuals born in India who had higher ponderal index at birth (birthweight length$^{-3}$) and born to heavier mothers had higher prevalence of type 2 diabetes (147) which is conceptually contradictory to the “thrifty” phenotype hypothesis. Hence, this indicates that maternal obesity, glucose intolerance during pregnancy and fetal macrosomia play a key role in subsequent burden of insulin impairment in South Asians.

1.4.2 In utero exposure

The “thrifty” phenotype which links fetal undernourishment with later life disease has been complemented by other hypotheses suggesting that different types of stress in utero can affect disease prevalence in adulthood. The fuel-mediated hypothesis was initially proposed by Pedersen in the 1950s and suggested that maternal hyperglycaemia in gestational diabetes induces fetal modifications which can lead to fetal macrosomia and later life obesity and type 2 diabetes (148). This hypothesis is still relevant 60 years later and has been broadened by evidence suggesting that intrauterine exposure to excess glucose or other nutrients, such as free fatty acids, amino acids or ketone bodies increases the risk of fetal macrosomia (149, 150). The concept of fetal overnutrition does not apply only in diabetic mothers but to obese and overweight mothers too. Recent evidence from the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study replicated the Pedersen theory in contemporary populations (n = 23,316 pregnant women of a multi-ethnic background) and suggested that maternal obesity and hyperglycaemia are independent risk factors of macrosomia or neonatal obesity (151, 152). The evidence regarding long-term outcomes and fetal overnutrition, albeit limited due to the inherent difficulties of long follow-up studies, is well supported. Initial studies in Pima Indians, an ethnic group with disproportionally high prevalence of type 2 diabetes, showed that offspring of diabetic mothers were heavier during childhood and up to the age of 19 years than offspring of normoglycaemic mothers (153). This finding was replicated in a multiethnic study which showed that offspring exposed to gestational diabetes in utero had increased BMI with greater central adiposity at the age of 6 to 13 years (154). Another study showed that offspring of mothers with type 1 diabetes, an additional model of fetal overnutrition, are more likely to be overweight or obese at the age of 7 compared with offspring of normoglycaemic mothers (155). Similarly, gestational glycosuria and maternal diabetes (existing or gestational) are associated with higher offspring BMI and greater offspring fat mass (measured with DEXA) at the age of 9 and the age of 15 years (156,
In addition, data suggested that children or young adults diagnosed with type 2 diabetes had around a 7-10 higher chance of being born to diabetic mothers than normoglycaemic ones. Potential physiological mechanisms that may explain the association of fetal overnutrition with macrosomia and insulin resistance in later life is the stimulation of the fetal pancreas to secrete more insulin by the excess maternal fuels (i.e. glucose) that pass the placental barrier or dysregulation of the adipoinsular axis.

South Asian babies born in India have greater adiposity, assessed by skinfold thicknesses or cord-blood leptin, for a given birthweight, than White European babies born in Western countries (158-160). This finding has been replicated among babies conceived and born in the UK to parents of Pakistani origin compared to those born to White British parents (161). Greater gestational hyperglycaemia and higher prevalence of gestational diabetes, both evident among South Asian women (162-164), are plausible exposures that can stimulate fetal insulin secretion, fat accretion and macrosomia (151, 152) and subsequently, lead to longer term consequences for subjects of South Asian origin. Recent evidence supported this theory by demonstrating that Pakistani neonates have greater adiposity, as assessed with cord-leptin levels, compared with white British neonates and almost 20% of this disparity is mediated through higher fetal insulin secretion which is caused by greater fasting hyperglycaemia in Pakistani mothers (165).

Hence, the evidence for both extremes of the spectrum of either fetal undernourishment or overnutrition in combination with postnatal exposure in Westernised lifestyle supports the theory that adiposity and metabolic manifestations in later life among South Asians may be rooted in factors that “program” the developing fetus. However, there is evidence of a temporal transition from the “thrifty phenotype” to “developmental overnutrition” phenotype among South Asians as a consequence of urbanisation and Westernisation; studies have reported that term newborns of South Asian origin born in Canada, hence mothers were less likely to be undernourished, had an average birthweight of 3.2 kg which was greater from the average birthweight of neonates born in urban (2.8 kg) or rural India (2.7 kg) (166). Therefore, the interaction of environmental and lifestyle factors with ethnicity cannot be underestimated in the manifestation of cardiometabolic risk in South Asian immigrants.
1.4.3 Genetic factors

Type 2 diabetes is a multi-factorial disease but the diverse prevalence and severity of the disease among different populations, the heritability of the disease or associated disorders (167, 168) and the high concordance rates of type 2 diabetes in pairs of monozygotic twins (169) have led to the hypothesis that the onset and progression of type 2 diabetes has a strong genetic background. Monogenic forms of diabetes which are attributed to single gene mutation account for less than 5% of the cases, are predominantly inherited (unless a new spontaneous mutation), manifest at an early age and are less susceptible to environmental influences (170). In contrast, polygenic forms of type 2 diabetes involve multiple polymorphisms of several genes, are commonly the result of various interactions between genes and environmental factors and have modest phenotypic penetrance at an individual level (171).

Three main polymorphisms (UCSNP 43, -19 and -63) associated with the Calpain 10 gene were the first main genetic variants that were linked with an increased risk of type 2 diabetes (172). Studies in the South Asians showed that the same variants increase the risk of the disease among the carriers of the haplotype but the prevalence of the polymorphisms was very low in this ethnic group (173) and hence their contribution to the greater risk of type 2 diabetes is unlikely to be substantial at a population level. An additional common polymorphism associated with glucose and lipid metabolism is the Pro12Ala on the peroxisome proliferator activator gamma (PPAR gamma) gene, which has a protective effect against type 2 diabetes in White populations (174). However, the same polymorphism in South Asians is distributed equally between type 2 diabetes cases and controls without demonstrating a protective effect against the development of the disease (175). Various polymorphisms on other genes, such as insulin receptor substrates (IRS-2), adiponectin associated genes, ectoenzyme nucleotide polypeptide (ENPP1) or beta cell potassium channel gene (KCNJ11), have been shown to have a modest association with type 2 diabetes in South Asians (173). However, these findings have been hampered by small study sizes or the lack of an interethnic control arm.

The foremost limitation of the studies examining the genetics of common and multi-factorial diseases is being underpowered due to their small sample size. In recent years this constraint has been overcome with the genome wide associated studies (GWAS) and subsequent meta-analyses which have identified over 62 susceptibility loci for type 2 diabetes that can collectively contribute to around 10% of the disease risk (176-178).
However these studies have predominantly been performed in White populations of European ancestry. A genome-wide study in 5,561 cases and 14,458 controls of South Asian descent identified 6 new susceptibility loci in chromosomes 2, 3, 10, 15 and 20 which reached genome wide significance ($p < 5 \times 10^{-8}$) in the South Asian cohort, however, the presence of the variants in any of the six responsible loci only conferred modest increase in the risk of developing type 2 diabetes among the carriers (odds ratio (OR) ranging 1.05-1.20) (179). Notably, this association was not mediated through adiposity. Although the presence of the same variants in a European cohort increased the disease risk marginally, it did not reach a genome wide significance level ($p > 5 \times 10^{-8}$).

The common risk alleles that are associated with disease susceptibility in the South Asians were at the GRB14, ST6GAL1, AP3S2, HMG20A, VPS26A and HNF4A loci; the majority of those alleles with an established function are related with reduced peripheral insulin sensitivity, defective insulin action, impaired adiponectin encoding or lipase-mediated lipolysis (179). Whereas only HNF4A locus was associated with reduced pancreatic β cell function but the effect size of the associated single-nucleotide polymorphisms (SNPs) was substantially decreased. Mutations in HNF4A are also known to cause maturity onset diabetes of the young type 1 which is characterised by impaired secretion of insulin from β cells (180). Another genome wide study in 12,535 Indians revealed an additional South Asian specific locus associated with type 2 diabetes susceptibility at the 2q21 within TMEM163 which is known to be associated with fasting insulin levels and waist circumference (181).

Subsequent genome wide trans-ancestry meta-analysis which aggregated data from Europeans, South Asians, East Asians, Mexican and African Americans demonstrated around 60% concordance in common variants of risk alleles that increase the susceptibility of type 2 diabetes across ethnic groups (177). In addition, the study identified 7 new loci associated with type 2 diabetes that reached genome-wide significance across all ethnic groups as a result of increased power. Although the effects of the common variants were modest, they were homogeneous across different ethnic groups (177). Hence, genetic factors do not seem to fully explain the excess risk of type 2 diabetes in South Asians compared with other ethnic groups.

Genome wide studies have also identified genetic variants associated with obesity, a major risk factor of insulin resistance and subsequent type 2 diabetes. Common variants of the fat-mass and obesity associated gene (FTO) are associated with predisposition to greater BMI and hence higher risk of type 2 diabetes in populations of European ancestry (182).
The same variants in a relatively small case-control study of South Asians (n = 3,775) were more frequent among individuals with diagnosed type 2 diabetes but the association was not mediated through higher BMI (183).

Consequently, the genome-wide analysis has provided a better insight into the heritability of type 2 diabetes susceptibility and demonstrated that the prevalence of common susceptibility variants are largely similar across populations (178, 184), whereas small ethnic variations in allele variants may have resulted from different sample sizes of ethnic specific studies and cannot account for the contemporary increasing prevalence of type 2 diabetes in specific ethnic groups.

1.4.4 Migration and urbanisation

There is a general perception that urbanisation and westernisation as a consequence of migration lead to abundance of energy-dense and low-fibre foods and the adoption of inactive lifestyles which collectively contribute to greater mortality and morbidity from NCDs. Immigration of South Asians to various Commonwealth countries initially as labourers at plantations or industry has been longstanding but there was a notable shift in the destination country and the type of migration in the 1950’s; highly educated males, traders and businessmen started moving to the Western world, mainly the United Kingdom, United States of America (USA) and Canada (185). Cultural background of the immigrants and immigration policies in the host countries had historically rendered immigration as male-centric, followed by a secondary flow of South Asian females as spouses. Consequently, there have always been fundamental differences between male and female immigrants from ethnic minorities, with the former contributing to the workforce and integrating with the locals, whereas the latter being mainly housewives. Irrespective of their educational qualifications in their home countries, immigrant females used to socialise with women from the same ethnic background and exhibit limited integration due to language barriers. However, there is a significant shift in the role of South Asian women in Western communities over the last decades; British-born Asian women move up the professional ladder and young females move away from the Indian subcontinent in order to study, acquire skills and work in Europe and North America.

Urbanisation is a form of internal migration which could replicate the health outcomes of Westernisation in a smaller geographical scale. Studies in rural and urban India revealed that despite a progressive increase in the prevalence of metabolic manifestations over the
last years in both settings, there is still a discrepancy, albeit gradually less marked, between the two settings when the same definitions of metabolic risk factors are used (186-188). A large cross-sectional study which surveyed n=19,973 (n=10,442 had biochemical testing) Indians living in urban areas and n=1,983 residing in rural India demonstrated that among women the prevalence of BMI ≥ 25 kg.m⁻², diabetes and hypertension was 34%, 8% and 25% respectively in urban India compared with 28%, 5% and 22% in rural areas (186, 189). Notably, the rates of obesity, central adiposity, dyslipidaemia and low physical activity among women of rural areas were more prominent in those belonging at higher socioeconomic strata despite them self-reporting a healthier diet (186, 190). However, analysis of a sibling-pair study in 6,510 participants from India demonstrated that despite rural women being slimmer than intra-country migrant and urban women (BMI of 22.5 versus 25.2 and 25.9 kg.m⁻² respectively), all three groups had comparable levels of fasting insulin and insulin resistance index (191). This finding may be partially associated with similar levels of physical inactivity among the three groups (191). Therefore, prosperity, regardless of area of residence within the Indian subcontinent, along with associated obesity and sedentary lifestyle seem to have a detrimental effect in South Asian population and are suggestive of early stages of epidemiological transition in the burden of diabetes and MetS (186-188).

When South Asians migrate away from the Indian subcontinent they consistently display a higher prevalence of insulin resistance, type 2 diabetes or other cardiovascular morbidities compared with the background population or ethnic minorities living in the same region. More than 50 years ago a large series of autopsies showed that migrant Indian males in Singapore had 7-fold higher prevalence of coronary heart disease than that of Chinese men (192). The pattern of higher prevalence of cardio-metabolic risk factors among migrant South Asians is consistent in more recent studies. Asian Indians in Singapore showed an age-standardised prevalence of type 2 diabetes of 14.5% compared with 7% among Chinese and 10.7% among Malays living in the same area (193). This finding was not attributable to physical inactivity or obesity as Indians self reported greater prevalence of regular physical activity (25% versus 15.8% in Chinese and 19.3% in Malays) and Malays were more obese compared with the other ethnic groups (193). Notably, Indians had greater proportion of body fat compared with the other ethnic groups for any given BMI (34). McKeigue et al conducted the first large study in the UK that showed that South Asians living in London have greater insulin resistance and prevalence of diabetes compared with Europeans of White descent in the same region and this interethnic difference was mainly attributable to higher central adiposity (115). Similarly, Indian born
physicians living in the United States had almost 3-fold higher prevalence of angina/myocardial ischemia and 7-fold higher prevalence of type 2 diabetes than the natives despite their lower BMI (194). A similar pattern of greater proportion of electrocardiographic changes suggestive of clinical CVD and higher glucose intolerance was evident among South Asian migrants in Canada compared with natives or Chinese migrants (113).

Although the above studies suggest that South Asians living in Western countries have greater propensity to develop CVD and diabetes than other ethnic groups and, hence, should be the target for public health policies for preventative interventions, they do not confirm if migration per se attributes to their increased risk. On the contrary, studies comparing contemporary migrant populations with matched groups living in the Indian subcontinent are more likely to assess the effect of migration on metabolic risk factors. A study comparing migrants of Punjabi origin in London with their siblings in India showed that migrants had higher levels of total cholesterol, lower levels of HDL and higher fasting blood glucose than their siblings. However, it can be argued that these intra-ethnic differences may have been mediated by higher levels of obesity in the migrant population (120). Another study comparing British Gujaratis with non-migrant Gujaratis in India replicated that migrant Indians had higher prevalence of diabetes type 2, higher levels of fasting insulin, greater β cell dysfunction and more atherogenic lipid profile than their comparators in India (121), however these metabolic differences were largely attributed to obesity and excess fat intake among the Indians living in the UK. Hence, the adoption of a Westernised lifestyle as a consequence of migration or urbanisation seems to have a detrimental effect on South Asians’ health; however, it is unclear why South Asians are disproportionately more susceptible in Westernised life-style than the natives or other ethnic minorities exposed in the same behavioural risks.

1.4.5 Physical Inactivity

There is a large body of evidence suggesting that a low level of physical activity is a substantial modifiable risk factor for all cause mortality, CVD and type 2 diabetes (195-197). Individuals performing regular moderate to vigorous physical activity (MVPA) are benefited by an almost 30% decrease in mortality and morbidity from NCDs (195-198) with half of the risk reduction in type 2 diabetes mediated through weight loss and lower BMI (197). However, it is expected that the actual effect of physical activity on risk reduction is greater than these estimates because the majority of evidence has resulted from
self-reported questionnaires assessing the frequency and intensity of physical activity which tend to overestimate both variables (199, 200). Current recommendations for the public call for ≥ 30 minutes of moderate intensity exercise, such as brisk walking, at least five and preferably all days of the week (201, 202). The American Heart Association suggests that this recommendation should be the absolute minimum requirement in terms of both intensity and frequency of regular physical activity and should be extended to 60 minutes per day supplemented by other short bouts of activity in individuals with established MetS in order to reverse their increased risk (58). In addition, high levels of cardio-respiratory fitness (usually assessed by measuring or predicting the maximal oxygen uptake (VO\textsubscript{2 max}) during an incremental exercise test) which is the result of increased levels of physical activity is associated with 40-45% decrease in the risk of all cause mortality, 50-60% reduction in the risk of developing CVD and 50-70% decreased risk of type 2 diabetes (195, 196). Although the magnitude of the effect of both regular physical activity and high fitness on risk reduction is striking, these findings have mainly resulted from pooled data from populations of White descent and it is unclear whether the same risk reduction should be anticipated in other ethnic groups who have an innate susceptibility in CVD and type 2 diabetes.

As discussed above South Asians are more insulin resistant than their comparators living in the Indian subcontinent or Europeans and these discrepancies are mainly attributable to greater adiposity and increased obesity (115, 120, 121). However, South Asians residing in the Western world remain more insulin resistant that their counterparts of White descent even after adjustment or matching for various adiposity variables indicating that other factors contribute to their insulin resistance phenotype (35, 130). Differences in physical activity may contribute as migrant South Asians have been reported to be less physically active that their comparators of White descent, however the majority of these studies reported data on physical activity derived from self-report questionnaires (203-206).

Limited recent studies replicated the finding of lower levels of physical activity with the use of objective measures among South Asian men (207), pregnant women (208) or even children of South Asian origin (209) compared with their counterparts of White ancestry. However, equivalent studies of objectively measuring physical activity in (non-pregnant) South Asian women are lacking. Studies that assess the frequency and intensity of physical activity with the use of objective methods can infer the real effect size of increasing physical activity on health outcomes. Notably, the effect of physical inactivity in South Asian is larger than that in Europeans (210) and South Asian men need to perform greater levels of weekly MVPA in order to equate their metabolic risk profile with that of healthy
European men engaging with regular MVPA of 150 min.week\(^{-1}\) (42). Whether the same applies in women has yet to be examined.

In addition, cardiorespiratory fitness of South Asians has been shown to be lower than that of their European comparators (207, 211, 212). Lower fitness levels contribute independently and to a larger degree than physical activity to South Asian men having greater insulin resistance (207). Notably, while fitness increases with increasing levels of MVPA in both South Asian and European men, for any given level of objectively measured MVPA South Asians have lower levels of fitness than their counterparts (207). Data on the fitness levels of South Asian women are scanty; a small study (n = 32 women in total of either South Asian or European origin) showed that South Asian women were less fit than Europeans but the finding was limited to women aged 35-49 years (213). At present the effect size of increasing fitness on ameliorating insulin resistance in South Asian women is largely unknown.

Therefore, South Asian ethnicity does not only hamper the amount of physical activity undertaken largely because of cultural barriers especially in women, but more importantly modifies the relationships between physical activity and metabolic profile and MVPA and increasing fitness. Hence, the interaction between innate susceptibility and physical activity variables appears to be much steeper in South Asian men than in Europeans. However, research in South Asian women is still lagging behind in this field.

1.4.6 Diet

There are summary data suggesting that diet and nutrition play an important role in the risk of type 2 diabetes and insulin resistance, with a high intake of saturated fat, trans-fatty acids or food with high-glycaemic load contributing to an increased risk (214). On the contrary, a diet abundant in non-hydrogenated polyunsaturated fat (PUFA), fibre and minimally processed grains reduce the postprandial insulinaemic response and potentially the risk of insulin resistance and type 2 diabetes (214). In addition, a diet rich in vegetables and fruits is protective against MI, whereas an unhealthy diet accounts for approximately 30% of the population-attributable risk (PAR) of primary MI worldwide (215). Furthermore, greater energy intake in excess of energy expenditure leads to weight gain and obesity contributing indirectly to the risk of the development of CVD and type 2 diabetes.
Studies looking at the dietary intake of urban Indians residing in the South Asian subcontinent have reported low intake of monounsaturated fat, PUFA and fibre but high intake of carbohydrates, saturated fats and trans-fatty acids (largely because of the use of ghee) (216). In addition, only 26.5% of healthy South Asians consumed fruits and leafy vegetables daily as opposed to 45.2% of the individuals from other ethnic groups who participated in the INTERHEART study (figures were 20% versus 38.3% respectively among those who had experienced a primary MI) (217). When South Asians migrate to the Western world, their diet can be healthier than that of individuals of White descent exposing higher intake of PUFA and lower consumption of saturated fat (218, 219) or may start changing to resemble the Western diet (220). Hence, diet exclusively does not seem to explain the excess cardiometabolic risk of migrant South Asians compared with the background population. A study comparing risk factors in migrant Gujaratis in the UK and their contemporaries in rural India showed that the total energy intake was higher in the South Asian migrants than in the residents of rural India plus their dietary energy intake was largely derived from fat as opposed to carbohydrates (121). It can be postulated that migrant South Asians may experience delayed adaptation to the Western diet (because they are programmed for different background dietary needs), which may contribute to their excess risk compared with the Western populations. However, the same study demonstrated that energy expenditure in the migrant South Asians was higher than in their contemporaries in India but, notably, the latter group was found to have a negative energy balance. Since none of the participants reported any weight loss during the study period, it was questioned whether dietary intake was underreported in India (121).

Contradictory findings were reported from a study which looked at the dietary patterns among first and second generation South Asian women living in Glasgow compared with women of the background white population (221). The study demonstrated that first generation migrants have a more atherogenic diet with 42.4% of the total energy intake deriving from fat (15% of which was saturated fat) as opposed to 39.1% (13.5% of which was saturated fat) in the background population. On the contrary, the British-born South Asians displayed a dietary pattern similar to that of the background population (221).

Although dietary patterns may have an association with metabolic manifestations and disease prevalence, intervention studies, in particular in South Asians, which support a causal relationship between specific dietary habits and insulin resistance are lacking. For example, high dietary intake of PUFA through the consumption of fish or fish oil has a beneficial effect on glucose tolerance and insulin sensitivity in observational studies (222,
223), but this has been not replicated in a randomised controlled trial (219). In addition, the latter study did not demonstrate that ethnicity modifies the effect of PUFA consumption on any of the metabolic outcomes indicating that dietary modifications seem to have similar effects on both South Asians and Europeans (219). However, a recent interventional study showed that overfeeding with saturated fat induced hepatic and visceral fat storage whereas overfeeding with PUFA promoted lean mass proliferation (224). Although the latter study did not assess the direct effect of an unhealthy diet on insulin resistance, abnormal adipose tissue partitioning may mediate the impact of an unhealthy diet on insulin metabolism. To conclude, it is unclear whether and to what degree diet contributes to the insulin resistant phenotype of South Asians, however, it is important to assess dietary intake in ethnic specific research looking at the interaction of ethnicity with environmental factors. It is acknowledged though that there may be challenges in recording dietary intake, which are subject to substantial measure and recall bias.

1.4.7 Fat distribution

Increased adiposity and impaired fat partitioning in South Asians have been suggested as key elements contributing to their insulin resistant phenotype. South Asians cluster similar metabolic risk factors to the White Europeans and develop type 2 diabetes at a lower BMI and smaller waist circumference than their comparators of White descent (40, 41, 45). Adipose tissue, as it has already been discussed, plays a key role in the development of insulin resistance through various pathways; adipose tissue is now recognised that is not merely a storage depot for triglycerides but has well defined endocrine actions (78). It is also well established that different abdominal adipose tissue compartments and ectopic fat depots demonstrate different degrees of association with insulin resistance, dyslipidaemia and other manifestations of the metabolic syndrome (78, 89). For example, there is accumulating evidence suggesting that visceral adipose tissue (VAT) is more malicious metabolically and displays stronger associations with insulin resistance, fasting glycaemia, hypertension, micro-albuminuria and metabolic syndrome, than subcutaneous adipose tissue (SAT) (78, 225-227). The causal relationship of VAT with insulin resistance has been supported by an interventional study which showed that subjects undergoing gastric banding and omentectomy (resection of VAT) experienced a 2 to 3-fold greater improvement in insulin sensitivity than subjects undergoing gastric banding alone, despite both groups demonstrating similar degree of weight loss (228). On the contrary, reduction in SAT via liposuction did not have a beneficial effect on metabolic parameters (229).
Ethnic specific studies looking at the different fat distribution patterns among South Asians and Caucasian are contentious; a relatively small study by Forouhi et al demonstrated that South Asian women \((n = 28)\) had greater visceral fat area (measured by CT) than European women with similar BMI and WC, whereas there was no discrepancy in men \((93)\). A similar sized study in slimmer and younger men showed that South Asian men \((n = 29)\) had greater SAT but similar VAT with Europeans of similar BMI \((230)\). Whereas a larger study \((n = 207\) South Asians and \(n = 201\) Europeans were recruited as part of the Multicultural Community Health Assessment Trial) did not show an absolute difference in VAT or SAT (measured by CT) among women, however, South Asians had greater amount of SAT and marginally more VAT for any given amount of fat-free mass. In the same study South Asian men had greater VAT and SAT compared with the Europeans \((231)\). On the contrary, a study which assessed abdominal adiposity in younger subjects under the age of 40 with the use of MRI did not reveal a difference in VAT or SAT after adjustment for BMI among South Asians and Europeans (stratified by sex). However, South Asians had higher content of liver fat and more hypertrophic fat cells than the Europeans which both accounted, at least partially, for the excess plasma insulin levels in the South Asian group \((232)\). It is unclear whether the above discrepancies within ethnic specific studies resulted from different techniques in assessing abdominal adiposity, different patients’ characteristics or various sample sizes.

It is still ambiguous whether the excess metabolic risks in South Asians is explained by absolute differences in adiposity in various compartments compared with individuals of White descent or by a predisposition of the South Asians to store fat in more metabolically active compartments at lower levels of fatness. Therefore, in support of the latter concept, the “adipose tissue overflow theory” has been proposed \((233)\). Adipose tissue is divided to primary (superficial SAT) and secondary compartments (deep SAT, VAT and ectopic fat) with the latter being associated with insulin resistance and dyslipidaemia. With surplus of energy intake, fat starts initially accumulating in the primary compartments until they reach their uppermost storage limit, and thereafter, secondary compartments develop and start expanding and storing fat. According to the overflow theory, South Asians have smaller primary compartments with lower potentials of expansion; therefore, they start storing adipose tissue in secondary more metabolically adverse compartments at lower thresholds of fatness. This theory could explain why South Asians develop dyslipidaemia and insulin resistance at lower BMI and lower body fatness but, clearly, requires further testing and replication in healthy women before they develop overt disease.
In view of the conflicting findings in various ethnic specific studies, further research in fat distribution patterns and association with insulin resistance is required in well selected groups and adequately powered studies using optimal technology of assessing abdominal adiposity and ectopic fat.

1.5 Why study women?

It has already been discussed that South Asian women living in the UK constitute a high risk group of developing MetS and type 2 diabetes. However, they have been largely overlooked and the majority of research has been undertaken in men of South Asian origin largely because they are more integrated with the background white population and, hence, more accessible to research recruitment. Furthermore, women experience lifetime events, such as reproductive exposures, pregnancy and its complications and menopause, which are sex-specific but are known to be associated with long-term metabolic manifestations rendering the need for sex specific research even more imperative. It is discussed below how the commonest female-specific exposures are associated with metabolic parameters and long-term health.

1.5.1 Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a common endocrine disorder estimated to affect 4-18% of women of reproductive age (234, 235). PCOS can present with a variety of symptoms rendering its diagnosis challenging. Although there has been considerable debate regarding the preferred diagnostic criteria for this heterogeneous condition, the 2003 Rotterdam consensus criteria have been widely accepted (236). This requires the presence of at least 2 the following: chronic anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries on transvaginal ultrasonography.

An increased awareness of this disorder in the general population and medical communities has taken place in recent years, with greater understanding of the long-term associations of the condition, including the MetS and its associated comorbidities such as IGT and type 2 diabetes, in women with a history of PCOS (237, 238). Summary data suggest that women with PCOS have around 2.5-fold higher odds of developing IGT, 4.4-fold higher odds of developing type 2 diabetes and 2.9-fold higher odds of experiencing MetS than women without PCOS and those odds did not differ substantially after controlling for BMI (237). Therefore, women with PCOS should not only be treated for their presenting symptoms (i.e. oligomenorrhoea, infertility or hirsutism), but should also
be offered lifestyle advice and screening in order that the long term metabolic consequences be prevented or delayed.

There is ethnic variation in the prevalence of PCOS, and despite the lack of established registries that can accurately estimate the prevalence of the syndrome, data suggest that PCOS is more frequent among South Asians and its prevalence has been reported as high as 52% among South Asian immigrants in Britain (239). In contrast, a community survey in Sri Lanka demonstrated that the prevalence of PCOS was 6.3% among local women (240). Another striking finding is that there is ethnic variation in the manifestations of PCOS with South Asian living in the UK developing PCOS at a younger age, experiencing higher prevalence of oligomenorrhoea and exposing higher levels of fasting insulinaemia and lower levels of insulin sensitivity compared with BMI-matched white Europeans with PCOS (241). It is still unclear what the causal root of PCOS is and whether PCOS is a precursor of the MetS continuum, however, because of its close associations with MetS, gestational diabetes and type 2 diabetes (237, 242), the presence or history of PCOS should be queried in ethnic specific research which involves women as it may modify their cardiovascular risk profile.

1.5.2 Gestational diabetes

Pregnancy is considered a physiological hyperinsulinaemic state and when pancreatic beta cell function is insufficient to overcome the increased demands of insulin secretion gestational diabetes is developed. The prevalence of gestational diabetes is increasing worldwide (243) mirroring the increasing trends of obesity and type 2 diabetes. Notably, there is a substantial ethnic variation in the prevalence rates of gestational diabetes; an analysis of birth records from 1995 to 2003 in USA demonstrated that only 3% of non-Hispanic White women developed diabetes during pregnancy as opposed to 11.7-21.2 % (wide range due to ethnic subgroup variation) of South Asians (162). Similarly, a UK based study showed that South Asian women were around 11-times more likely to develop gestational diabetes than British women of White descent (244). In addition, ethnic variation extends to the phenotype of women with gestational diabetes with South Asians experiencing gestational diabetes at lower BMI and younger age than their comparators of White descent (245).

A history of gestational diabetes increases the risk of developing future type 2 diabetes and this risk is modified by ethnicity with South Asians exposing almost double the risk
compared with Europeans (246). Furthermore, ethnic minorities demonstrate higher recurrence rates of gestational diabetes in a subsequent pregnancy than White women (52-69% versus 30-37%) (247). Hence, women of South Asian background cluster metabolic risk factors at a young age that become apparent under the stress of pregnancy and, therefore, sex-specific research in studies looking at cardiometabolic risk factors in ethnic minorities becomes even more imperative.

1.5.3 Menopause and metabolic manifestations

Menopause is a universal and unavoidable stage in a woman’s life which, in some instances, may be associated with unpleasant symptoms such as vasomotor symptoms, mood swings and musculoskeletal aches. Since menopause coincides with ageing, it is associated with an increased risk of metabolic disorders that are more prevalent in older women; however, the causal effect of menopause per se on the manifestations of MetS is debatable. The associations of metabolic manifestations with menopause are discussed below and, therefore, the importance of recording menopausal status and symptoms in sex specific research.

1.5.3.1 Menopause and risk of type 2 diabetes

Menopause is characterised by a relative hyper-androgenic state and higher levels of endogenous androgens (higher levels of testosterone and lower levels of sex hormone binding globulin (SHBG)) (248) are considered to be associated with greater risk of type 2 diabetes in women (249). The Study of the Women’s Health Across the Nation (SWAN) followed up 949 women for 9 years (250) and showed that the incidence of MetS progressively increased during the menopausal transition and this trend commenced around 6 years prior to the last menstrual period (LMP) whereas fasting blood sugar levels showed a decreasing trend over the same period (250). The study concluded that the increase in the incidence of MetS was independent of age or other traditional CVD risks and was largely attributed to testosterone dominance. Hence, menopause may have an indirect effect on the risk of type 2 diabetes without having a direct impact on glucose levels. Similarly, the Diabetes Prevention programme (DPP), a randomised trial which assessed the role of metformin versus lifestyle modifications in preventing type 2 diabetes, concluded that, after adjustment for age, menopause does not affect the risk of developing diabetes among women with glucose intolerance (251). On the contrary, there are data supporting that the incidence of impaired glucose tolerance or type 2 diabetes increases substantially in post-menopausal women independent of age, BMI or other cardio-metabolic risk factors (252).
Notably, the same study demonstrated that in post-menopausal women that menopause occurred after the age of 49, years since last period was the only factor associated with an increase in the risk of impaired glucose tolerance but not with that of diabetes (252). Another study suggested that menopausal transition is indeed associated with a non-age driven change in insulin metabolism that is mainly characterised by a decrease in insulin secretion and a simultaneous reduction in insulin elimination. However, the net effect in circulating insulin levels is almost null since one adaptation counteracts the other (253). Therefore, it is still unclear why the risk of type 2 diabetes increases disproportionately in South Asian women around their age of menopause (28).

1.5.3.2 Menopause and dyslipidaemia

Early studies suggested that menopausal transition is associated with raised total cholesterol and low HDL (254, 255) but were cross-sectional in nature. A recent longitudinal multiethnic study followed up 1,054 women over 9 years and showed that the levels of total cholesterol, LDL and Apo B increased within a year of the LMP independent of age, ethnicity, weight, weight gain or medications (256). However, the increase in lipoprotein levels was not linear, which would be suggestive of an age-related effect, but it peaked during the early menopausal stage and thereafter it levelled off. Hence, menopausal transition rather than ageing seemed to have a direct effect on lipid levels. In contrast, the Melbourne Women’s Midlife health project followed up 150 women during the menopausal transition and demonstrated a substantial increase in high density HDL levels during the year preceding the LMP followed by a significant decline by the first year after the LMP (257). Thus, the net change in the levels of HDL was minimal over the menopausal transition. Additionally, changes in the levels of other lipoproteins over the menopausal transition were largely explained by the ageing effect, modifications in activity levels, alcohol consumption and smoking habits. A cross-sectional study in 542 healthy women in the UK aged 18-70 years showed that post-menopausal women had substantially increased concentrations of total cholesterol, LDL, HDL₃ and triglycerides but lower levels of HDL and HDL₂ cholesterol and these differences were independent of age or BMI (258). Although the findings may seem contradictory, they underpin the importance of menopausal age in later cardiovascular health. Matthews et al followed up peri-menopausal women for 20 years in relation to their risk of coronary calcifications and concluded that cardiometabolic risk factors at the age of enrolment in the study were strongly associated with future risk of coronary calcifications independent of late postmenopausal health status (259). Therefore, targeting women with lifestyle modification at an early stage of their
menopausal transition rather than when cardiometabolic risks have established is more likely to prevent the development of future overt disease (260).

### 1.5.3.3 Menopause and hypertension

The SWAN followed women longitudinally over 9 years and showed that both diastolic and systolic BP increased in a linear fashion along with advancing age and independent of the date of the LMP (256). An earlier cross sectional study, which compared post-menopausal with pre-menopausal women of similar age did not demonstrate a substantial difference in systolic or diastolic BP among the groups (254). These findings were consistent with the findings of the Melbourne Women’s Midlife health project, which despite of its small sample size, showed that the longitudinal changes in diastolic BP were independent of the menopausal transition (257). Therefore, the body of evidence suggests that menopause does not have a direct impact on BP. However, the alteration in the oestrogen to androgen ratio occurring during menopausal transition results in changes in sympathetic activity, endothelin activity and the action of angiotensin II and ω-hydroxylase, which may account for the sex-differences in BP in older subjects (261, 262).

### 1.5.3.4 Menopause and adipose tissue

Data suggest that body composition changes under the combined effect of chronological and ovarian ageing. Fat mass increased and skeletal mass decreased (measured by bioelectrical impedance analysis, BIA) over a 6-year follow up period in 543 women. These longitudinal changes were associated with both advancing age and increasing levels of FSH (ovarian ageing) (263). In addition, waist circumference, a surrogate marker of abdominal adiposity, increased proportionally with a marked peak prior to the LMP and levelled off within a year following the LMP (263). Another study in 156 middle-aged women who were followed up for four years showed that SAT accumulation was mainly explained by advancing age, whereas an increase in VAT was largely seen in women who went through menopausal transition during the follow up period (264). A cross-sectional study showed that Caucasian post-menopausal women had greater total fat mass (measured with DEXA) accumulated mainly centrally (android type) compared with pre-menopausal women. These differences were independent of BMI and age (265). Cross-sectional data from India also suggest that post-menopausal women store more abdominal fat (assessed by waist circumference) than age-matched pre-menopausal women, however, the groups were not matched for BMI and the impact of residual confounding effect on the above comparison cannot be underestimated (266).
1.5.3.5 Menopause and vasomotor symptoms and endothelial function

Vasomotor symptoms (VMS), namely hot flushes and night sweats, albeit their incidence differ among ethnic groups, affect the majority of menopausal women (267). Hot flushes tend to occur in peri-menopausal and early post-menopausal period but a third of women report symptoms that last up to 5 years after cessation of periods and in 20%, persist up to 15 years (268).

Hot flushes have been linked, in some studies, with metabolic disorders and mainly with modifications in endothelial function. The SWAN heart, an ancillary arm to the SWAN, showed that women with hot flushes have reduced flow mediated dilatation (FMD) of the brachial artery, greater calcification of the aorta (269) and greater carotid IMT compared with age matched non-flusher women (270). Another study replicated the finding that women with moderate to severe hot flushes have lower endothelial reactivity measured by FMD, however, they had similar IMT and lipid profile with the non flushers (271). On the contrary, Sassarini et al reported that menopausal women with severe hot flushes have increased endothelium dependant and independent vascular reactivity assessed by Laser Doppler Imaging with Iontophoresis (LDI-ION) but an adverse lipid profile compared with non flushers (272). Tuomikoski et al showed that women with a high flushing score had an increased vascular response to nitroglycerin assessed by the means of pulse wave analysis (PWA). However, they did not demonstrate any differences in arterial stiffness between the groups and did not examine other atherosclerotic factors (273). These discrepancies in the literature may be associated with baseline differences in the study populations, various responses in different vascular beds (macro-vascular versus micro-vascular) or methodological differences in assessing vascular reactivity. However, the concept that hot flushing may have an impact on the background vascular risk of an individual remains still relevant and unanswered. Examining the reactivity in different vascular beds between hot flushers and non-flushers may uncouple the effect of hot flushing on vascular reactivity.

1.6 Summary

In conclusion, the preceding literature review has provided evidence about the magnitude of the problem of increased insulin resistance along with other features of the MetS in South Asians and consequent morbidity and mortality from type 2 diabetes and CVD. The review, also, discussed how the MetS manifests in the South Asians and potential hypotheses that may explain the excess cardiometabolic risk in this ethnic group in comparison to groups of white descent. However, none of the hypotheses provides
adequate explanation why South Asians expose a higher risk than other immigrant groups in the Western world or the background population. In addition, South Asian women in the Western world constitute a high risk group of future type 2 diabetes and expose sex-specific characteristics which can potentially modify their background ethnic risk, but they have been largely overlooked to date.
1.7 Aims

This thesis aims to test the hypothesis that healthy South Asian women in the UK accumulate more metabolic risk factors than women of White European descent and that ethnicity modifies the associations between modifiable lifestyle factors and adiposity with insulin resistance and other metabolic manifestations. Should ethnicity change the magnitude of the effect of the above exposures on the metabolic outcomes, ethnic specific recommendations may be required at a population level.

This overall hypothesis will be examined by testing the more specific aims listed below:

- Assess whether the differences between accelerometer measured physical activity and sedentary time and self reported activity and sitting time are modified by ethnicity. (Chapter 3)

- Examine whether the strength of associations between routine metabolic risk factors and physical activity variables differ among objectively measured and self reported variables. (Chapter 3)

- Compare lifestyle factors, such as physical activity and fitness, body composition, dietary intake and metabolic biomarkers between South Asian women and women of White European descent both living in Scotland and without established cardio-metabolic disease. (Chapter 4)

- Assess how reproductive state and menopausal transition affect the above variables. (Chapter 4)

- Examine whether ethnicity is a substantial effect modifier of the associations between lifestyle factors or adiposity with insulin resistance, glycaemic indices or metabolic risk score. (Chapter 5)

- Compare vascular reactivity, which is a marker of vascular health and potential prognostic factor of future atherosclerosis, in a macro and micro-vascular level between healthy South Asian women and women of European descent both residing in the UK. (Chapter 6)
• Assess whether hot flushing in peri- and post-menopausal women affects vascular reactivity in both macro and micro-vascular levels. (Chapter 6)

• Determine the levels of weekly physical activity required to be performed by South Asian women living in the UK in order their cardiometabolic risk profile to equate with that of the women of White descent who achieve the current guideline levels of physical activity (150 min. week$^{-1}$). (Chapter 7)
2 GENERAL METHODS

2.1 Introduction

This chapter describes the methods employed in the recruitment of the study volunteers, acquisition of the measurements as dictated by the study protocol and analysis of the data. The study was conducted within the premises of the Glasgow Clinical Research Facilities at the Western Infirmary and was awarded ethics approval in January 2011 by the Ethics Committee 3 (REC No 11/WS/0119). Data collection took place from May 2012 to November 2013.

2.2 Inclusion and exclusion criteria

Recruitment targeted women aged 18 to 70 years of South Asian or white European descent. South Asians were defined when both parents were of Pakistani, Indian, Sri Lankan or Bangladeshi origin. Women on systemic hormonal contraceptives or on systemic hormone replacement therapy (HRT) or had stopped hormonal treatment less than three months prior to their appointment were excluded as it is established that hormonal regimes can alter the metabolic and lipid profile of individuals (274, 275). Women with diagnosed diabetes, history of stroke or transient ischemic accidents (TIA), or history of myocardial infarction (MI) were also excluded from the study. Women on medications knowing to alter the glucose metabolism (e.g. metformin) were excluded too. In addition, women with high likelihood of having undiagnosed type 2 diabetes (fasting plasma glucose \( \geq 7.0 \text{ mmol/l} \) or HbA1c \( \geq 48 \text{ mmol/mol (6.5\%)} \) or polycystic ovary syndrome (PCOS) were excluded.

2.3 Recruitment and selection

Eligible women were mainly recruited from Greater Glasgow and Clyde by general advertising and word of mouth. An advertisement was uploaded onto the University of Glasgow webpage and an invitation email calling for volunteers was sent to all University of Glasgow staff and students. Posters advertising the study were displayed around the University Main Campus and the Glasgow Royal Infirmary premises. Patients were, also, recruited from general Gynaecology, Menopause and the Breast Screening clinics. The study was also advertised by posters or by short presentations in religious centres (mosques, temples) across Glasgow as well as on Ramzan FM radio station. Lastly, an
advertisement for the study was included in the University for Third Age (U3A) newsletter to access potential volunteers over the age of 55 years.

The advertisement posters or emails had a brief description of the study including the eligibility criteria along with the contact details of the principal researcher (SI). Potential volunteers contacted the main researcher and provided they were fulfilling the eligibility criteria, an information leaflet with details of the study was posted or emailed to them. Women, who were willing to participate after reading the leaflet, contacted the main researcher and an appointment slot was allocated to them.

Volunteers attended for their main appointment in the Glasgow Clinical Research facility at the Western Infirmary either at 8:30 or 11:30 am after a minimum of 10-hours fast (overnight). Each appointment lasted around 3 hours. Women over 40 years of age were offered a second appointment for an MRI at a subsequent day (usually within one month) following their 1st visit. Women who failed to attend the MRI appointment were given a second appointment. Several women could not tolerate an MRI so either declined an MRI appointment or agreed on imaging but were unable to carry out the examination. The MRI assessment took place in the British Heart Foundation (BHF) Glasgow Cardiovascular Research Centre.

2.4 Consent and questionnaires

At the date of first visit, women were asked to read and sign the consent form before any test was carried out (Appendix I). A single copy was kept in their study records and a copy was offered to each participant for future reference.

Figure 5 shows a flowchart of the order of the tests that were performed during the visit.

Following consent, women completed a health screen questionnaire (Appendix II) about their current medications, past medical history, reproductive history, family history and history of possible injuries or other contraindications to regular exercising (i.e. shortness of breath or chest pain). In addition, it included demographic questions about the date of birth, marital status, address, years of formal education, profession and smoking status. Data were extracted in an excel proforma for subsequent analysis.

A food frequency questionnaire (Appendix III) which has been previously validated and used in South Asian cohorts was administered to each participant asking them to self-
report how frequently (range 0 to 7, never or less than every fortnight) they include specific food categories in their diet within a regular week and the amount of specific foods at each occasion (i.e. number of slices of bread per day). The answers from each questionnaire were inserted in a digital proforma with predefined coding for each answer and analysed with the help of computer software (Qbuilder, v2.0).

Participants were asked to self report their weekly physical activity by filling in the long form of the international physical activity questionnaire (IPAQ) (Appendix IV). This questionnaire has been validated in different countries and languages and has been shown to have an acceptable level of cultural adaptation. The English self-administered version was used in the current study and the South Asian subjects did not have difficulties in understanding the questions. The questionnaire is structured in a way that provides information about the weekly frequency of each activity and the duration at each occasion. Physical activity is considered any activity lasting longer than 10 minutes undertaken during work, household, leisure time or transportation. The long form contains a comprehensive set of four domains (work based activities, transport-related, domestic/gardening and leisure time) and asks explicitly about specific type of activities within each domain. Each activity is assigned a predefined intensity score, hence, there are different scores for walking, moderate-intensity and vigorous intensity activities within each domain. In addition to this, the questionnaire includes questions about the time spent sitting during weekdays and weekends. Analysis of the data enables the estimation of Metabolic Equivalent of Task (MET) spent per minute of each specific activity based on the intensity score assigned to each activity. The long form allows computation of activity specific along with domain specific scores as opposed to the short version of the questionnaire which is limited in providing activity specific scores and is mainly administered in large epidemiological studies.

Women aged over 40 years were asked to complete the Greene climacteric scale (Appendix V) which is a validated tool of assessing a variety of symptoms experienced by women during the climacteric period. The instrument was developed in Glasgow by Greene in 1998 (276) through factor analysis including a detailed range of climacteric symptoms, but only symptoms having had a factor loading > 0.35 were encompassed in the final version of the questionnaire. There are 21 symptoms in the scale, each of the symptoms can be rated from 0 to 3 according to its severity and are categorised in 5 main domains; vasomotor, somatic, psychological (further divided in anxiety and depression) and sexuality related symptoms.
Younger women under the age of 40 years were asked to complete a menstrual history questionnaire (Appendix VI) which asks details about the regularity of their cycles and symptoms usually related with polycystic ovary syndrome (PCOS) (such as hirsutism and acne). Hence, symptoms recorded in the questionnaire along with biochemical hyperandrogenism, can be suggestive of PCOS (236). Given the established association of PCOS with insulin resistance and type 2 diabetes (237), diagnosis of PCOS can be a potential confounder or effect modifier of the associations between risk factors and insulin resistance. Hence, women with diagnosed or expected PCOS were excluded from the analysis.

Lastly, women over 40 years of age who were identified as being menopausal (amenorrhea ≥ 1 year) or late peri-menopausal (intervals of amenorrhea ≥ 60 days and follicle-stimulating hormone (FSH) ≥ 10 IU/L) (277) were given a hot flush diary at the end of their appointment to record the frequency and severity of hot flushes and night sweats for seven days following their appointment. Participants were provided with a stamped envelope to post the diary to the main researcher after completing a week of recording. Post menopausal women denying any hot flushes at the time of the study were also asked whether they had had a history of hot flushing or been using systematic HRT during the transitional period.
2.5 Anthropometry and body composition

2.5.1 Height

Height was measured using a stadiometer (Invicta Plastics Ltd, Leicester, UK) with volunteers being barefoot, having their feet joint and their heels, buttocks and upper part of
back positioned against a fixed surface and touching the scale. The head was positioned at
the Frankfort plane which ensures that the triagon of the ear is at the same height with the
orbitale of the eye and, hence, the vertex was the highest point of the head. The volunteer
was instructed to take a deep breath in and the height was measured at the end of the
exhalation by the researcher lowering a moveable headboard on the top of the subject’s
head and applying gentle down-ward pressure allowing hair compression. Simultaneously,
gentle lift was applied by the researcher on the mastoid processes of the volunteer and
measurement was recorded to the nearest cm. The coefficient of variation for all sites was
under 5%.

2.5.2 Weight

Body mass was measured using a digital scale (Tanita BC418MA) which is approved for
medical and research purposes. Subjects were weighted wearing lightweight clothing,
usually hospital gowns, without shoes, having both feet flat and arms at lateral position
resting. Measurement was made to the nearest 0.1 kg. Body mass index (BMI) was
calculated as:

\[ \text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2} \]

2.5.3 Blood Pressure

Blood pressure was measured using an Omron automated monitor. Participants were seated
with their arm resting on a table. Legs were uncrossed and the participant was relaxed and
not talking. The participant was rested for at least 5 min prior to the blood pressure
measurement. The average of two measurements was used for the analysis.

2.5.4 Skinfold thickness

All skinfold measurements were made based on the International Society for the
Advancement of Kinanthropometry (ISAK) standards by a single researcher (principal
researcher) after appropriate training by an ISAK certified technician. Skinfold thickness
was obtained in duplicate from seven body sites (biceps, triceps, subscapular, suprailliac,
supraspinale, frontal thigh, medial calf) on the right body side with the use of a Harpenden
skinfold calliper (Baty International, West Sussex, UK) calibrated against international
standards. All measurements were performed by the investigator in a private lockable area
with the volunteers wearing only their underwear and a female chaperone (research nurse
Each skinfold site was located after identification of the correct anatomical landmarks and the skin was marked with a fine pen to avoid variation between repeated measurements. The skin including the subcutaneous adipose tissue was grasped gently at the marked sign with the thumb and index finger of the researcher and the calliper, held by the other hand of the researcher, positioned around 1 cm away from the edge of the fold while the researcher was holding the fold. Measurement was made 2 seconds after the full pressure of the calliper was applied. Once a complete set of measurements was obtained from all skinfold sites a second full set of measurements was repeated to avoid variation due to adipose tissue compressibility from successive measurements at the same site. Measurements were made to the nearest 0.1 mm and the mean of the two measurements was used for the analysis. The coefficient of variation for all sites was under 5%.

Each skinfold site was located as explained below:

2.5.4.1 Biceps

The site was located at the mid-point between the acromiale (superior and lateral aspect of the acromion) and the radiale (proximal and lateral border of the radius head) in the midline of the anterior surface of the biceps muscle. The site was identified with the subject having a natural position with both shoulder relaxed, arms hanging by the sides and the palm of the hand facing forward during the thickness measurement.

2.5.4.2 Triceps

This anatomical landmark was identified similarly to the biceps skinfold site (above) but it was marked on the midline over the triceps muscle (diametrical to the biceps skinfold).

2.5.4.3 Subscapular

The subscapular landmark was identified at the lower angle of the scapula and the skinfold was measured following the natural fold of the skin around 2 cm away from the landmark when the subject has assumed a relaxed standing position with the hands hanging by both sides.
2.5.4.4 Suprailiac

The site of this skinfold was directly on the top of the lateral aspect of the iliac tubercle (ilio-cristale landmark) identified with the subject having the left arm hanging by the side and the right arm close to her trunk.

2.5.4.5 Supraspinale

This skinfold site was located at the intersection of the horizontal line at the level of ilio-cristale and the line joining the right armpit with the iliospinale landmark. The iliospinale mark was located at the inferior part of the tip of the anterior superior iliac spine.

2.5.4.6 Medial calf

This skinfold site was located at the most medial aspect of the calf at the level of its maximum diameter.

2.5.4.7 Frontal thigh

This site was located with the volunteer sitting on a chair, having both feet fixed on the floor and the right knee bent at a right angle. It was at the mid-point of the distance between the inguinal fold and the superior margin of the patella.

2.5.5 Body circumferences

The girths at five body sites (waist, hip, mid-thigh, mid-calf, upper arm) were measured by the same researcher with the use of a flexible steel tape (Lufkin W606PM) to the nearest 0.1 cm. The measurements were undertaken in a lockable area with the subjects wearing only underwear or light clothing (tight shorts and vest), being barefoot and standing distributing their weight evenly on both feet. The mean of two sets of measurements was used for each site. If there was over 0.5 cm difference among the two measurements, a third one was recorded. The coefficient of variation for all sites was under 5%.

2.5.5.1 Waist circumference (WC)

The waist was measured at the level of the narrowest point between the lower costal rib and the iliac crest with the subject having their legs slightly apart and the tape being parallel to the floor. If there was no obvious narrowing the measurement was taken at the mid-point between the two landmarks.
2.5.5.2 Hip circumference

The hip was measured at the level of the greatest diameter of the buttocks with the subject’s heels being attached.

2.5.5.3 Mid-thigh circumference

It was measured at the mid-point of the distance between the superior tip of the greater trochanter of the femur and the superior point of the lateral aspect of the tibial head.

2.5.5.4 Mid-calf circumference

It was measured at the level of the mid-calf skinfold site corresponding to the maximum diameter of the calf.

2.5.5.5 Upper arm circumference

This body circumference corresponds to the girth of the upper arm at the level of the upper arm skinfolds (biceps and triceps).

2.5.6 Abdominal Adiposity

Abdominal adipose tissue was measured with magnetic resonance imaging (MRI) by using a whole body scanner (Magnetom, Siemens) operating at 3.0 Tesla with a dedicated transmit/receive coil positioned on the subject’s anterior abdominal wall. The subject laid flat on a moveable inclined board wearing surgical scrubs, barefoot and after having removed any jewellery or other metallic accessories. Initially plane localiser images were acquired followed by breath-hold axial T1-weighted images at the level of L3-L5. The T1 of the fat is relatively short, so by implementing the T1-weighted sequence, strong tissue contrast was achieved and the bright fat (subcutaneous and visceral) could be discriminated and delineated by signal intensity threshold from neighbouring darker structures.

Acquisition of the data was obtained by two trained radiologists (Dr Stuart Ballantyne and Dr Jonathan Platt) with a use of the sliceOmatic software (version 4.3, TomoVision, Visual Imaging Inc., Canada). When both examiners reviewed the same images independently, the interobserver variation was estimated 2.5% for VAT and 0.4% for SAT. Hence, the method is highly reproducible between different assessors. The software performs an automatic segmentation of fat depots based on the signal intensity cut-offs, but the operator
still needs to overlook the segmentation process to avoid incorrect segmentations due to image artefacts or subjects’ variation in the signal intensity. The operator has control over a sliding threshold and an interface that gives real time visual feedback on the performance of the threshold. Figure 6 shows the sequential steps of identifying SAT and VAT on an abdominal MRI. The surface results are displayed in pixels and mm$^2$ for each compartment measured.

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**Figure 6.** Abdominal MRI images and segmentation with the use of the SliceOmatic software.  
A: T1 weighted abdominal MR image at the level of L3-L5.  
B: Automatic segmentation of fat depots on the same MR image based on the signal intensity with the use of the SliceOmatic software.  
C: Automatic segmentation and identification of subcutaneous abdominal fat (red colour) by the operator.  
D: Subcutaneous abdominal fat coloured in red.  
E: Automatic segmentation and identification of subcutaneous fat (red) and visceral fat (blue).  
F: Visceral abdominal fat coloured in blue.
2.5.7 Hepatic fat

The deposition of fat in liver was measured during the MRI test using the technique of single-voxel magnetic resonance spectroscopy (MRS). Hydrogen proton MRS is a powerful technique that has been used to noninvasively evaluate tissue metabolism. A breath-hold image at the level of liver was obtained and a single voxel (a volume element) was positioned by the radiographer performing the test over the right lobe of the liver avoiding any obvious blood vessels. MRS does not provide anatomical information but yields a detailed spectrum of the chemical components within one voxel. Its function is based on the differences in the frequencies of water and fat protons; hence, the fat signal intensity against the water signal intensity of the same region can be used to quantify lipid infiltration. Hydrogen protons (from water) have a spectral peak at 4.7 ppm (parts per million) and the protons produced by the lipids (methylene) have a lower signal frequency.

Figure 7 graphically represents the outcome of a liver MRS with both water and lipid spectral peaks. By increasing the magnet field strength chemical shift occurs and the two signal frequencies separate. The area under the curve for each peak represents the amount of each species within the voxel, so the relative concentration of fat over water can be estimated. Since the area under the curve is estimated automatically there is no evidence of interobserver variation.
Figure 7. Liver MR spectroscopy with water and lipids spectral peaks within one voxel.

2.6 Fitness Test

Cardio-respiratory fitness was assessed with the Chester Step Test (278). This is a validated submaximal aerobic capacity test requiring the subject to step on and off a low plastic step (Reebok) at a rate set by a metronome beat played by a CD (fitnessASSIST, UK). The subject was wearing a transmitter (heart rate monitor) fastened tightly with an elastic band around her chest (Polar Electro Oy, Finland) for the duration of the test and the heart rate was displayed on a digital wristband (Polar Electro Oy, Finland). Prior to starting the recording the two electrodes of the transmitter were moistened with water and secured against the subject’s skin with the transmitter being at an upright position almost at the level of the lower part of the sternum. The height of the step could vary from 15 to 30 cm (incremental increase by 5 cm) depending on the age and reported physical activity of each subject. The aim was to choose a height which would enable the participant to exercise for at least 6 minutes (3 levels) and reach 80% of her maximum heart rate (220-Age in years) before the end of the test (10 minutes, level 5). As a general rule, women under the age of 40 years exercised at 25 cm (a few at 20 cm) and women over 40 years exercised at a step height of 20 cm with the exception of those over 60 years who exercised at a lower step height of 15 cm.

The subject was instructed to follow the metronome beat so that one beat corresponded to one full step. The metronome beat increased progressively every two minutes and the subject was encouraged to keep up with the rhythm and at the end of each level (every two minutes) the heart rate and the level of exertion was recorded. The test continued until the subject reached 80% her maximum heart rate or rate of perceived exertion (RPE) at a moderate to vigorous level (RPE=14). The maximum aerobic capacity (mlsO2*Kg^{-1}*min^{-1}) was predicted by plotting the recorded heart rates in a graphical datasheet (Excel) and the estimated maximum aerobic capacity was the value at x axis which corresponded to the intersection of the best fitted line connecting the plotted data-points and the horizontal line at the level of the maximum heart rate (y axis). The test-retest reliability in the assessment of the maximum aerobic capacity (VO_{2max}) among consecutive measurements in the same subjects has been quoted 0.8 ± 3.7 mlsO2*Kg^{-1}*min^{-1} (<5%) (278).
2.7 Objectively measured physical activity

Physical activity was measured subjectively with the use of the self reported long IPAQ and objectively by administering to the participants a pager-like device, named accelerometer (ActiGraph, LLC, Florida), to wear for seven, ideally, consecutive days following their baseline appointment. The subjects were instructed to wear the activity monitor fastened with an elastic band snugly around their waistline (over or under clothing) with the monitor unit positioned over the right hip bone. Participants were directed to wear the monitor all day during non-sleeping hours and only remove it when they undertook activities requiring immersion of the monitor in the water (i.e. showering, swimming). The researcher had programmed the accelerometer to start recording activity the next day following their appointment at 6 am as a default setting. The volunteers were also instructed to keep a log of the wearing time and days including the reason of taking the accelerometer off in the middle of the day. The device was pre-programmed through computer software by the researcher, so the subjects could not access the recording data or deactivate the device. The subjects were provided with a pre-paid envelope and asked to post the monitor and the log diary at the end of the recording period to the researcher.

The recording data from each device were accessed by the researcher with the use of the ActiLife software (version 6.7.3, ActiGraph, LLC, Florida) and the data for each individual were saved in excel formats. Valid measurements were considered when participants wore the device for at least 10 hours per day minimum for four days. Intervals of zero activity counts continuously for over 60 minutes or less than 60 minutes but were described as non-wear time by the participants were subtracted from the wear time. Non valid assessments were not included in the analysis. Accelerometer readings were summarised as 60-second epochs and Freedson cut-off points were used to define intensity of the activity; activity counts <100 counts.min\(^{-1}\) was considered as sedentary time, 100-1,951 counts.min\(^{-1}\) as light activity, 1,952-5,724 counts.min\(^{-1}\) as moderate activity and >5,724 counts.min\(^{-1}\) as vigorous activity (279). The Freedson cut off points are widely used in sports science and have resulted from calibration studies showing an optimal correlation (r=0.88) between activity counts and steady-state oxygen consumption during treadmill exercise (279). Activity was reported as minutes per day of sedentary, light, moderate and vigorous activity. Moderate to vigorous physical activity (MVPA) was defined as the summary minutes per day of moderate and vigorous activity. Actigraph accelerometer gives results that are highly reproducible and it overall demonstrates an intra-instrumental coefficient of variation of 4.1% and inter-instrumental coefficient of variation of 4.9% (280). In addition,
activity was reported as MET-minutes per day of moderate to vigorous activity. One MET is equivalent to the energy expenditure when resting. MET minutes of moderate to vigorous activity was estimated by multiplying minutes of moderate activity by 4 and minutes of vigorous activity by 8 for consistency with the analysis of the self reported questionnaire.

2.8 Vascular function

The endothelial function was assessed in two different vascular beds; the micro- and macro-vascular.

2.8.1 Micro-vascular bed

2.8.1.1 Laser Doppler Imaging with Iontophoresis (LDI-ION)

Cutaneous blood flow was measured with the use of the Laser Doppler Imager (LDI) (MoorLDI2, Moor Instruments Ltd) combined with Iontophoresis (ION). The Laser Doppler Imaging technique is based on the principle that light undergoes changes in the wavelength when hits moving blood cells (Doppler Effect) and the frequency and magnitude of these changes are proportional to the number and velocity of the red blood cells. The test was conducted in a quiet temperature controlled room after the subjects had acclimatized for at least 20 minutes. The room temperature was fixed at 22-23°C and the subject’s skin temperature recorded by a thermo-sensor attached on the forearm skin. The skin temperature should be over 29°C for the duration of the test. The participant sat on a semi-recumbent position on an examination couch with her right forearm facing upwards on an arm pad positioned directly perpendicular and 30 cm lower to the laser source.

Figure 8 shows the LDI machine and the set up for the measurement of vascular reactivity with the use of LDI-ION.

Two iontophoresis chambers made of Perspex (internal diameter, 22 mm; area, 3.8 cm²) with an internal platinum wire electrode were attached via double-sided adhesive labels on the volar aspect of the right forearm avoiding hair, broken skin or visible vessels. One chamber was connected with the anodal connection and the other with the cathodal connection on the battery-powered ION current controller (MIC-1e, Moor Instruments Ltd., Axminster, UK). 2.5 ml of 1% Acetylcholine (Ach) (Sigma-Aldrich Company Ltd., Dorset, UK) was placed in the anodal chamber and 2.5 ml of 1% Sodium Nitroprusside
(SNP) (Sigma-Aldrich Company Ltd., Dorset, UK) was introduced in the cathodal chamber, allowing the delivery of both agents simultaneously for the duration of current administration. Sodium chloride (0.5%) was used as the vehicle for these agents. 32-mm circular cover-slips were placed over the chambers to prevent fluid escaping. ION controller was programmed via the Moor software to deliver constant current in an incremental fashion; with the first scan performed without current, four scans at 5 µA, four at 10 µA, four at 15 µA, two at 15 µA followed by five scans without delivery of any current, giving a total charge of 8 mC (current x time = charge). Hence 20 repetitive scans were performed, each lasted 50 s. The voltage across the chambers was recorded at the beginning of each scan and skin resistance was calculated using the Ohm’s Law (resistance = voltage / current).

Skin perfusion was measured with the use of Laser Doppler Imager (Moor Instruments Ltd) equipped with a red laser (wavelength 633 nm; power 1 mW, beam diameter 1mm). The laser scanned in a raster fashion over both chambers and the backscattered light was collected by photodetectors and converted in a signal proportional to perfusion in arbitrary perfusion (flux) units (PU) and was displayed as a colour-coded image on the computer screen.

Figure 9 shows the flux images of repetitive scans in a single subject. The perfusion in each chamber for each scan was quantified with the use of the manufacturer’s image analysis software by outlining the region of interest (ROI) around the internal circumference of each chamber. Hence, the median flux value across approximately 700 measurement points was measured. Skin resistance was calculated for each scan and the area under the curve (AUC) was calculated as the summary skin resistance for each individual. Subsequently, each single response to the drugs displayed by each scan was corrected for individual’s skin resistance (by multiplying the perfusion units with the summary skin resistance for each individual) and the overall response to the drug was obtained by calculating the AUC. When measurements are corrected for skin resistance, the method is highly reproducible showing a non significant temporal variation for Ach of 3.5% and SNP of 4.7% (281). Similarly, a non significant variation of < 5% for both agents was calculated when the primary researcher performed temporal measurements in volunteers during her training period in this technique.
Figure 8. Laser Doppler Imaging with Iontophoresis (LDI-ION) typical set-up.

Figure 9. Skin perfusion (flux images) of repetitive scans in a single subject. The response to sodium nitroprusside (SNP) is shown in the right chamber and acetylcholine (ACh) in the left chamber of each scan. Flux is color-coded with lowest perfusion in dark blue (0 PU) and highest in dark red (1000 PU).
2.8.2 Macro-vascular bed

2.8.2.1 Peripheral Arterial Tonometry (PAT) recording

Peripheral arterial tone (PAT) was recorded using the EndoPAT 2000 device (Itamar Medical, Caesarea, Israel). It is a non invasive technique based on the PAT signal technology measuring the magnitude and dynamics of arterial tone changes in peripheral arterial beds following upper arm blood flow occlusion. It specifically captures beat-to-beat plethysmographic recording of the distal arterial pulse wave amplitude (PWA) detected by the use of pneumatic probes. Endo-PAT probes are attached at the distal two thirds of the index fingers of both hands and apply uniform pressure (approximately 70 mmHg) to the entire surface preventing distal venous distension and subsequent induction of arteriolar constriction reflex. The inflated probes are properly clamped at the fingers minimizing the risk of displacement during the test.

PAT recording was performed in a temperature controlled room after a period of at least 30 minutes of acclimatisation and 10 minutes following blood pressure measurement avoiding any residual vasoconstriction or dilation effect from the blood pressure cuff occlusion. Standard protocol was utilised including 10 minutes of baseline recording of PWA in both index fingers with the use of pneumatic finger probes, followed by inflation to suprasystolic level (at least 60 mmHg above systolic blood pressure but no less than 200 mmHg, if there was still PAT signal further incremental increase by 50 mmHg in the pressure but not beyond 300 mmHg) of the blood pressure cuff (Hokanson SC12, Bellevue, USA) placed on the non dominant hand of the participant for 5 minutes. Blood pressure cuff was then released and PAT recording continued for further 8 minutes. Figure 10 shows the output of PAT recordings in a subject with normal (A) and impaired (B) reactive hyperaemia.

Data were analysed using the manufacturer’s automatic software in an operator-independent manner. Reactive hyperaemia index (RHI) was calculated as the ratio of 1 min PAT signal beginning exactly 60 seconds after the release of the blood pressure cuff divided by the average PAT signal 210 seconds prior the occlusion in the occluded arm. The ratio was automatically corrected for the PAT signal of the contralateral arm to minimise the effect of potential systematic factors influencing the baseline vascular tone. Since RHI results from automated analysis, the method is highly reproducible without evidence of inter or intra-rater variation.
Figure 10. Peripheral Artery Tonometry (EndoPAT) typical recordings. On the top of each image is the control arm which corrects for systemic factors. A: a subject with normal reactive hyperaemia. B: a subject with impaired reactive hyperaemia.

2.9 Blood preparation and analysis

2.9.1 Fasting blood sampling

Blood sampling was performed after an overnight fast of at least 10 hours. Blood samples were collected by venepuncture from an antecubital vein with the use of a vacutainer blood collection set (BD Vacutainer © Push Button Blood Collection Set, BD, New Jersey) attached to a holder. Blood samples were collected in collection tubes (BD Vacutainer © Blood Collection Tubes, BD, New Jersey); one 9 ml ethylenediamine tetra-acetic acid (EDTA – lilac top), one 4 ml EDTA tube, one 6 ml lithium heparin (LiHep-green top), one 4 ml sodium citrate (blue top), two 5 ml serum separating tube (yellow top) and one 2 ml sodium fluoride tube (grey top). All blood samples were kept in 4° C after collection and were processed and spun in the laboratory within 6 hours of collection. The 4 ml EDTA tube, one 5 ml yellow top tube and the grey top tube were immediately sent off after collection to the Greater Glasgow and Clyde biochemistry laboratories for routine
measurement of glycated haemoglobin (HbA1c), reproductive hormones, urea and electrolytes, liver function tests and fasting plasma glucose.

**2.9.2 Blood preparation and storage**

**2.9.2.1 Haematocrit**

The remaining blood tubes (9 ml EDTA tube, LipHep tube, one yellow top tube and the sodium citrate tube) were turned on a roller for five minutes. Two glass capillary tubes were filled with whole blood from LipHep blood tube, were plugged with plasticine at the marked end and placed in a haematocrit centrifuge (Thermo Scientific Heraeus Pico 21). The capillary tubes were balanced in the centrifuge by placing them anti-diametrically and were spun at 13.3xg for 12 minutes. Haematocrit (Hct) was measured by placing each capillary tube on a digital reader. The mean value of the two measurements was used in the analyses.

**2.9.2.2 Rest of the samples**

The remaining blood samples were removed from the roller, were balanced in a centrifuge (Beckman GS-6KR) and spun at 3000 rpm for 15 min at 4° C. Using a fine 1 ml plastic pastette (Alpha Laboratories, Hampshire), supernatant liquid was aspirated and dispensed into plastic labelled 2 ml plastic Eppendorf tubes (Alpha Laboratories, Hampshire, UK). Eight 0.5 ml aliquots were closed with lilac lids (EDTA) (corresponding to the colour of the blood collection tube top), four 0.5 ml with blue lids (sodium citrate), four 0.5 ml with green lids (LiHep) and four 0.5 ml with red lids (serum separating). Aliquots were placed in plastic containers and stored at -80° C.

**2.9.3 Blood sample analysis**

**2.9.3.1 Screening blood tests**

All study subjects had blood tests taken at their baseline visit for the following analyses:

- Urea and electrolytes (U & Es)
- Liver function tests (LFTs)
- Glucose
• Total cholesterol, HDL-cholesterol (LDL-cholesterol – calculated using the Friedewald equation (282)), triglycerides (TG)

• Reproductive hormonal profile (FSH, luteinising hormone (LH), sex hormone binding globulin (SHBG), Oestradiol (E₂), Testosterone, Free Androgen Index (FAI))

• HbA1c

• C-reactive protein

Glucose and HbA1c were analysed as routine samples on the day of collection in one of the certified North Glasgow Biochemistry laboratories by using standard automated enzymatic and high performance liquid chromatography (HPLC) techniques. The department is fully approved by Clinical Pathology Accreditation and the laboratories participate in external quality assurance schemes. U & Es, LFTs (apart from gamma-glutamyl transpeptidase (GGT)) and reproductive hormones were analysed in one of the certified North Glasgow Biochemistry laboratories at the day of collection too. All the above analyses were carried out by personnel working in the laboratories. Lipids (total cholesterol, HDL, TG) and GGT were measured in thawed sera using automated enzymatic technique (Autoanalyser C311, Roche Hitachi) in batches at the end of the study in a certified biochemistry department within the BHF. The analyses were carried out by a senior technician (Elaine Butler) working in this lab.

2.9.3.2 Enzyme linked immunosorbent assays (ELISA)

Plasma insulin, adiponectin, leptin and pro-insulin were measured in the Biochemistry department at BHF by a senior technician (Elaine Butler) using commercially available ELISA kits. All ELISA procedures were based on a “sandwich” technique which, in brief, is described below: the wells of the plates were coated with a monoclonal antibody to the protein of interest. By adding plasma to the wells, protein was bound to the antibody whereas unbound molecules were removed by washing. Subsequently, a second antibody specific to another area of the protein was added and unbound molecules were removed by a second washing. The second antibody was linked to an enzyme which would catalyse the production of a fluorescent product. The intensity of the fluorescence was proportional to the initial amount of protein and, hence, the levels of the protein in the initial plasma sample were calculated.
Plasma insulin was measured with the commercially available kit (Mercodia Ultrasensitive, AB, Uppsala, Sweden) which has <0.01 % cross-reactivity with pro-insulin or c-peptide. Inter-assay coefficient of variation was < 4%. Plasma pro-insulin was measured using a commercially available kit (Mercodia AB, Uppsala, Sweden) which has <0.03 % cross-reactivity with insulin and <0.006 % cross-reactivity with c-peptide. Inter-assay coefficient of variation was < 5.5 %. Plasma leptin was measured with the use of a commercially available kit specific to human leptin (R&Dsystems, Minneapolis, USA) with an inter-assay coefficient of variation of < 5.5 %. Adiponectin was measured using a commercially kit (R&Dsystems, Minneapolis, USA) specific to human total adiponectin. The inter-assay coefficient of variation of the assay has been reported < 7.0 %.

2.10 Power calculation

Sample size calculation was based on differences in HbA1c, VO2 max and abdominal adiposity between the South Asians and European groups. The primary statistical analysis will use two-sample t-tests. The sample size was estimated with the aim of achieving at least 80% power at a significance level of 5% to detect between-group differences of 4 units in VO2 max and 0.37 units in HbA1c, assuming a normal distribution within groups and within-group standard deviations of 4.44 for VO2 max and 0.361 for HbA1c. The probability of either test detecting a between-group difference under the null hypothesis of no difference in either primary outcome will be maintained at 5% by adjusting the significance level for each individual test, using the Bonferroni correction, to 5%/2 = 2.5%. Under these assumptions, the number of subjects required per group will be 25 for VO2 max and 20 for HbA1c. The number of subjects per group required to achieve at least 80% power for both primary outcomes is therefore 25, assuming no dropout of subjects from the study. In addition, based on a previous study using CT scanning (93), in order to identify a 4 cm² difference in visceral fat area between South Asian and European women with 80% power at a statistical significant level; I need 41 women in each group of women over the age of 40 years. Assuming that 50% of women may deny MRI scanning, at least 62 women over the age of 40 years are required to be recruited in each ethnic group.

2.11 Statistical methods

Data were analysed using Stata (version 12.1, StataCorp, Texas, USA). Data were tested for normality using the Shapiro-Wilk normality test and transformed appropriately. Two sample unpaired t-test used for comparison of means of normally distributed data or non-
parametric tests were used when data were not normally distributed even after transformation.

Univariate and multivariate regression analyses between outcomes and exposures were performed with adjustment for confounders for the latter analyses. When ethnicity was a significant effect modifier of the above relationships, stratified analysis for ethnicity was performed. When ethnicity was not a significant effect modifier, regression analyses were performed on the combined group to maximise statistical power.

Statistical significance was accepted at the $p < 0.05$ level.

### 2.12 Conclusion

All the above measurements, data collection, data processing and analyses were performed by me (the principal researcher). The only exceptions are the biomarkers analyses which, depending on the nature of the biomarker, either measured in one of the North Glasgow Biochemistry laboratories or by Elaine Butler, a senior technician at the BHF. In addition, measurement of VAT and SAT by processing the MR images was performed by Dr Stuart Ballantyne (Radiologist Consultant) and Dr Jonathan Platt (senior Radiologist trainee).
3 OBJECTIVE VERSUS SELF REPORTED PHYSICAL ACTIVITY AND SEDENTARY TIME: EFFECT OF ETHNICITY AND AGE ON MEASUREMENT METHODS

3.1 Introduction

There is cumulative evidence of epidemiological data suggesting that regular physical activity of moderate to high intensity reduces the risk of future diabetes, coronary heart disease and all cause mortality (195-197). Equally, there are data highlighting that the time spent sitting daily (sedentary time) is associated with the risk of glycaemia, cardiovascular events and deaths of all causes (283-285). There are two commonly used methods of quantifying physical activity and sedentary time; self reporting and objectively measured. Self reported time refers to the use of validated questionnaires containing questions about the time an individual engaging with activities in different domains (e.g. occupational, recreational, household etc.) or the time spent sitting (e.g. watching television or sitting in the office). Each activity has been pre-assigned a degree of intensity specified at the time of the development of the questionnaire irrespective of the peak performance of individuals. Objectively measured physical activity includes the use of automatic mechanic sensors (i.e. accelerometers or pedometers), which prevent intentional interference by individuals in order to modify the results, and are designed to store and report units of physical activity (e.g. bouts, counts or steps etc.).

Self-reporting questionnaires are an inexpensive and readily available method of assessing activity and sedentary time being especially beneficial in large scale studies. Participants are more likely to comply with this method and negligible missing data, that could reduce the power of the study, are expected, especially when the questionnaires are filled in during study visits. There are numerous validated questionnaires that implement different techniques/questions to acquire data and have been used over the last decades (286). Although they have acceptable reliability, their validity and sensitivity is relatively limited since the answers are likely to be influenced by recall bias and subjects’ misconceptions about the type, frequency and intensity of physical activity (200, 287). Questions may also differ according to the type of questionnaire which may limit the generalisability of the study findings. A widely used recall questionnaire is the International Physical Activity Questionnaire (IPAQ) which comes in a long and a short form and has been interpreted in
a variety of languages and used in different countries (288). It was developed by the International Consensus Group in 1998-1999 and designed as a standardised and culturally adaptable instrument of measuring physical activity among diverse populations ranging from 15-69 years of age. Its long form provides a detailed assessment of activity in four domains (workplace, transport, household and leisure) and the time spent sitting during weekdays and weekends (288). Each domain is further divided in accurate descriptive questions about possible activities within each context. The subjects are asked with the use of open ended questions to report the frequency and duration of any potential activity lasting over 10 minutes. It is a simple questionnaire to follow with analytical and self-explanatory questions and, although it performs at least as well as other questionnaires in validation studies (287), the IPAQ reported physical activity has a modest correlation with objectively measured physical activity (correlation coefficient (r) ranging from 0.15 to 0.55 for adults ) measured by pedometers or accelerometers (287). Interestingly, the correlation between sitting time estimated by IPAQ and accelerometer derived sedentary time was weak to moderate (correlation coefficient < 0.3) and studies looking at specific ethnic groups failed to reveal a significant association (i.e. r = -0.08 for Black Africans (289) or r = -0.17 for Latin Americans (290)). Given that there is a lack of a questionnaire performing well across all domains with adequate indices for both reliability and validity and since IPAQ has been used as a tool of assessing habitual activity in ethnic specific studies including South Asians (291-293), it was considered an appropriate tool of self reported activity for our study.

Accelerometers are believed to provide an accurate estimate of daily activity and sedentary time provided that they are worn properly for a minimum of 4 days. Although, they have been criticised that they yield a degree of misclassification related to their inability to record e.g. arm movement and, thus, resistance exercise (287, 294), more advanced monitors allow recording in 3 levels minimising the chance of reporting errors.

National guidelines have been developed suggesting that a minimum of 150 min of moderate physical activity or 75 min of vigorous activity is required weekly to modify the background risk of an individual of CVD and diabetes (195). These recommendations have mainly resulted by meta-analysing epidemiological data with the majority of them assessing self reported physical activity among participants of White European descent. Although we acknowledge that current recommendations and meta-analyses can only combine already existing, instead of ideal, data, the conclusions may introduce bias and the real dose response effect of physical activity may be masked. Firstly, the recommendations
may not be applicable, without appropriate adjustment, in populations of different ethnic backgrounds (42) and, secondly, self reporting physical activity may diverge significantly from that objectively measured, resulting in over or under estimation of the strength of the relationship between physical activity and health benefits (199). The latter concern is even greater if we consider that the degree of misclassification of physical activity or sitting time can be subject to ethnic, age or sex variation i.e. individuals of specific ethnic backgrounds or gender or age groups may be more likely to unintentionally misreport the amount of time spent working out or sitting influenced by social pressure or cultural beliefs (287). Therefore, the degree of misreporting physical activity with the use of subjective tools may be modified by ethnicity or age and, despite IPAQ having been used in studies investigating activity trends among South Asian women (283, 292), the validity of this tool has not been tested for this high risk population.

The aim of the present study was to assess whether the differences between accelerometer measured physical activity and sedentary time and self reported activity and sitting time obtained from IPAQ are influenced by ethnicity and age and whether the strength of association between routine metabolic risk factors differ among objectively measured and self reported data. I hypothesise that self reported physical activity overestimates physical activity independent of age or ethnicity.
3.2 Methods

3.2.1 Participants

Participants were recruited by advertising in the University of Glasgow website, in the University of the Third Age (U3A) Newsletter and via posters in clinical settings (general practices, hospitals) and religious centres (Muslim centres and Hindi temples). Detailed description of recruitment process has been included in chapter 2.

3.2.2 Ethics

The study was granted ethics permission by the West of Scotland Ethics Committee. All the participants have given written consent prior their inclusion in the study.

3.2.3 Study Design

This was a cross-sectional study comparing self reported physical activity and objectively measured physical activity among women of South Asian and European descent. Each participant was asked to wear an accelerometer for seven consecutive days starting from the day following the study appointment. Participants were also invited to fill in a 7 day physical activity recall questionnaire (long form IPAQ). Language interpretation of the questionnaire was not warranted for this study since all participants could read and understand English. There is no formal culturally adapted version of the IPAQ to South Asians and, thereby, examining whether ethnicity modifies the accuracy of self reported physical activity would indicate whether this would be necessary.

3.2.4 Objectively Assessed Physical Activity

Participants wore accelerometers (GT3X or Actitrainer; Actigraph LLC, Pensacola, FL, USA) around their waists (monitoring device on the right side) secured with a belt for seven consecutive days. They were asked to wear them at all times apart from when sleeping, showering/bathing or swimming. Accelerometers were initialised to start logging physical activity automatically from the next day after the study appointment at 6 am as a default time. Valid measurements were considered when participants wore the device for at least 10 hours per day for a minimum of four days. Non valid assessments were not included in the analysis. Non-wear time was defined as intervals of at least 60 minutes of zero activity counts. Participants were asked to keep a diary of wear and non-wear time which was posted along with the accelerometer to the researcher. Intervals of zero activity
counts lasting less than 60 minutes which were described as non-wear time by the participants were subtracted from the wear time too.

Accelerometer readings were summarised as 60-second epochs and Freedson-cut off points were used to define intensity of the activity; activity counts $<100$ counts.min$^{-1}$ was considered as sedentary time, 100-1,951 counts.min$^{-1}$ as light activity, 1,952-5,724 counts.min$^{-1}$ as moderate activity and $>5,724$ counts.min$^{-1}$ as vigorous activity (279). The Freedson cut off points are widely used in sports science and have resulted from calibration studies showing an optimal correlation ($r=0.88$) between activity counts and steady-state oxygen consumption during treadmill exercise (279). Activity was reported as minutes per day of sedentary, light, moderate and vigorous activity and was adjusted for wear time.

Moderate to vigorous physical activity (MVPA) was defined as the summary minutes per day of moderate and vigorous activity. In addition, activity was reported as MET-minutes per day of moderate to vigorous activity. One MET is equivalent to the energy expenditure when resting. MET minutes of moderate to vigorous physical activity was estimated by multiplying minutes of moderate activity by 4 and minutes of vigorous activity by 8 (minutes of moderate activity x 4+ minutes of vigorous activity x 8) for consistency with the analysis of the self reported questionnaire.

### 3.2.5 Subjectively Assessed Physical Activity

The English version of the long form of the International Physical Activity Questionnaire (IPAQ) was administered to each participant. This is a 7 day recall questionnaire which asks questions about the duration and frequency of job related, leisure time and household physical activity including walking. It also questions about the time spent sitting in average during the weekdays and weekend.

Data were analysed in line with the IPAQ recommendations and were expressed as sitting time in minutes per day (sitting time during the weekdays + sitting time during the weekend and the sum divided by 7), moderate activity in minutes per day and vigorous activity in minutes per day. Moderate activity time was considered as the summation of the time spent doing moderate activity at work, the time spent doing moderate activity during leisure time, the time spent doing housework inside the house or gardening and the time spent cycling. Although, walking is considered as moderate activity by MET value, the IPAQ analysis protocol assesses walking as a separate activity. For consistency with the accelerometer analysis, walking time was included in the total moderate time. However, in
line with IPAQ protocol, walking was assigned an intensity of 3.3 METs, the rest of moderate activity an intensity of 4.0 METs with exclusions the vigorous activity in the garden which was given an intensity of 5.5 METs, cycling an intensity of 6 METs and housework inside the house an intensity of 3. Vigorous activity was considered the time doing vigorous activity at the workplace and during leisure time and was assigned a MET intensity of 8.

IPAQ analysis protocol has some rules how to truncate (re-code) data that are unlikely to present realistic amount of physical activity in order to minimise the chance of misclassification. The truncation process requires that the variables total walking, total moderate activity and total vigorous activity are calculated and if any of the summed behaviours exceeds 3 hours per day (or 21 hours per week) should be truncated to 3 hours (or 21 hours per week). The truncation rule becomes more complicated when calculating METs of moderate activity because different domains of moderate activity are assigned different METs intensity varying from 3 to 6. In this case, moderate activity assigned the lowest METs intensity (i.e. housework inside the house) is truncated prior to the moderate activity assigned the highest METs intensity (i.e. cycling and gardening), however, the total moderate activity should not exceed 21 hours per week, assuming that people are less likely to overestimate moderate activity of higher intensity compared with moderate activity of lower intensity.

3.2.6 Physical, biochemical and demographic measurements

Detailed description of the methods used for the measurement of anthropometric variables and quantification of biochemical factors is included in chapter 2.

3.2.7 Metabolic risk score

The clustered Cardiometabolic Risk Score was used for expressing an individual’s global metabolic risk in order to provide a summary continuous estimate by combining different variables contributing to the diagnosis of metabolic syndrome. It was calculated by using the equation Risk score= -zHDL + zInsulin + zGlucose + zTriglycerides+ (zBMI + zWC)/2+ (zSBP + zDBP)/2 (295, 296). Z score is the standard score which refers to the number of standard deviations (SD) that an observation differs from the mean value. A variety of methods, including principal component, Z scores or percentile rankings have been used in literature to combine the components of metabolic syndrome in a continuous risk score (295-298). Although this approach enables more flexibility in the analysis of a
Chapter 3

continuous rather than a dichotomous variable, some limitations should be taken into account; first, there is no consensus on the definition and variables in calculating the metabolic risk score. Second, variables are assumed to have equal weight in their contribution to the global CVD risk. In addition, the components of metabolic syndrome have partially overlapping mechanisms of pathogenic actions; therefore their total combined effect may be less than the expected additive effect of the individual factors. However, since the ultimate net value of the risk score and its association with the diagnosis of metabolic syndrome are not of interest in this study, it is justified to use the continuous risk score to cluster the cardiometabolic risk factors together.

3.2.8 Data Analysis

Data were analysed using the STATA (version 12.1, Statacorp, TX, USA) statistical package. Continuous variables were summarised by mean (standard deviation, SD) or median (interquartile range, IQR) unless stated otherwise. Data were transformed to normally distributed values by using the transformation equation with the lowest chi-square (testing for normality) assessed with the ladder of powers. Comparison of continuous summary variables among groups was done with the use of non paired t-test (if raw or transformed data had a Gaussian distribution) or Kruskal-Wallis test for not normally distributed variables. Chi-square test was used for comparing categorical variables. The bias and variability between the two methods of measuring physical activity at each level of intensity and sedentary/sitting time was calculated using the limits of agreement method and the Bradley-Blackwood test which examines the null hypothesis of equal means and variances among the two measurement methods (299). The relationships between two different ways of measuring physical activity and sedentary/sitting time were assessed using the Spearman correlation (r) and concordance correlation coefficient (Pc). Concordance coefficient is a composite way of expressing correlation and agreement between the two measurements methods by quantifying the deviance of the best fit line from the line $y = x$. The above analysis was stratified per ethnicity and, subsequently, per age group (below and above the median age) to determine whether age or ethnicity affects the strength of the correlation.

The magnitude of the effect of each activity variable on a metabolic risk was estimated by the beta ($b$) coefficient (standardised coefficient) which expresses how many standard deviations a dependent variable (metabolic risk score) changes for each standard deviation increase in the predictor variable (activity/sedentary time variables) in a multivariate linear
regression model adjusting for confounders (age and ethnicity). Prior fitting a multivariate regression model, the interactions between age and ethnicity with the exposure variable was assessed and in the presence of interaction a stratified analysis (age was stratified below and above median) was performed. Activity exposures (independent variables) were transformed to normally distributed equivalent variables prior fitting a linear regression model. Statistical significance is considered at $p<0.05$. 
3.3 Results

3.3.1 Participants

The baseline characteristics of the participants are shown in Table 2. 87 women of European origin and 92 women of South Asian origin participated in this study. Age, BMI, blood pressure (BP) and socioeconomic status determined by the Scottish Index of Multiple Deprivation (SIMD) ranking did not differ among the two ethnic groups. The South Asians had similar fasting plasma glucose (FPG) with the Europeans (4.9 versus 4.8, \( p = 0.34 \)), however, they were more insulin resistant (assessed by the HOMA\(_{IR}\): 1.94 versus 1.51, \( p = 0.002 \)). Total cholesterol and LDL did not differ among the two ethnic groups but the South Asian women had lower HDL (1.45 versus 1.73, \( p < 0.001 \)). BMI, hip and waist circumferences were similar in both groups, but South Asian had greater waist to hip ratio (WHR) (0.82 versus 0.78, \( p = 0.012 \)). All women (n = 179) filled in the long form of the IPAQ form with 153 out of them (85.5%) returning the accelerometer with valid data. Women who had valid accelerometer data (described as completers) and those who either failed to return or wear the accelerometer for the required valid duration (described as non completers) did not have significant differences in their baseline characteristics or metabolic risk factors (Table 3), minimising the probability of introducing a systematic bias by the non completers.
<table>
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</tr>
<tr>
<td>Systolic Blood Pressure (SBP) (mm Hg)</td>
<td>122.0 (108.0 - 135.0)</td>
<td>121.0 (112.0 - 134.0)</td>
<td>0.68</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (DBP) (mm Hg)</td>
<td>76.1 (11.0)</td>
<td>77.7 (9.4)</td>
<td>0.32</td>
</tr>
<tr>
<td>Glucose (mmol.l(^{-1}))</td>
<td>4.9 (4.5 - 5.2)</td>
<td>4.8 (4.5 - 5.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>Insulin (pmol. l(^{-1}))</td>
<td>9.1 (5.8 - 14.0)</td>
<td>7.0 (4.9 - 9.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>HOMA(_{IR})</td>
<td>1.9 (1.2 - 3.0)</td>
<td>1.5 (1.0 - 2.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Total Cholesterol (mmol.l(^{-1}))</td>
<td>5.2 (1.0)</td>
<td>5.3 (0.9)</td>
<td>0.19</td>
</tr>
<tr>
<td>HDL (mmol.l(^{-1}))</td>
<td>1.5 (1.2 - 1.7)</td>
<td>1.7 (1.5 - 2.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL (mmol.l(^{-1}))</td>
<td>3.0 (2.5 - 3.5)</td>
<td>3.1 (2.4 - 3.7)</td>
<td>0.97</td>
</tr>
<tr>
<td>Triglycerides (mmol.l(^{-1}))</td>
<td>1.0 (0.7 - 1.6)</td>
<td>0.8 (0.7 - 1.2)</td>
<td>0.059</td>
</tr>
<tr>
<td>BMI (kg.m(^{-2}))</td>
<td>25.1 (22.1 - 29.0)</td>
<td>25.5 (23.0 - 27.9)</td>
<td>0.86</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>78.9 (71.1 - 87.5)</td>
<td>76.5 (71.8 - 82.5)</td>
<td>0.11</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>96.1 (92.3 - 102.2)</td>
<td>97.5 (94.3 - 102.0)</td>
<td>0.16</td>
</tr>
<tr>
<td>Waist to Hip Ratio (WHR)</td>
<td>0.82 (0.76 - 0.88)</td>
<td>0.78 (0.74 - 0.84)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of the participants. Data are expressed as median (25th-75th quartiles) when they are not normally distributed or mean (standard deviation) when they are normally distributed. HDL: high density lipoprotein, LDL: low density lipoprotein. *P value resulted from Kruskal-Wallis test; ∞ p value resulted from non paired t-test of normally distributed values after normalisation; § p value resulted from non paired t-test.
<table>
<thead>
<tr>
<th></th>
<th>Completers</th>
<th>Non Completers</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>153 (85.48%)</td>
<td>26 (14.52%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.5 (38.8 - 56.1)</td>
<td>51.2 (36.2 - 60.4)</td>
<td>0.78*</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>122.0 (110.0-134.0)</td>
<td>118.5 (109.0-136.0)</td>
<td>0.76∞</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>76.68 (10.3)</td>
<td>78.00 (10.56)</td>
<td>0.55§</td>
</tr>
<tr>
<td>Glucose (mmol.l$^{-1}$)</td>
<td>4.80 (4.50-5.20)</td>
<td>4.90 (4.60-5.10)</td>
<td>0.77∞</td>
</tr>
<tr>
<td>Insulin (pmol.l$^{-1}$)</td>
<td>7.77 (5.31-10.90)</td>
<td>7.69 (4.81-10.85)</td>
<td>0.80∞</td>
</tr>
<tr>
<td>HOMA$_{IR}$</td>
<td>1.67 (1.11-2.50)</td>
<td>1.64 (1.09-2.41)</td>
<td>0.76∞</td>
</tr>
<tr>
<td>Total Cholesterol (mmol.l$^{-1}$)</td>
<td>5.22 (0.89)</td>
<td>5.43 (1.09)</td>
<td>0.28§</td>
</tr>
<tr>
<td>HDL (mmol.l$^{-1}$)</td>
<td>1.59 (1.30-1.89)</td>
<td>1.48 (1.35-1.82)</td>
<td>0.92∞</td>
</tr>
<tr>
<td>LDL (mmol.l$^{-1}$)</td>
<td>3.05 (2.48-3.54)</td>
<td>3.18 (2.48-3.76)</td>
<td>0.19∞</td>
</tr>
<tr>
<td>Triglycerides (mmol.l$^{-1}$)</td>
<td>0.94 (0.71-1.34)</td>
<td>0.94 (0.65-1.58)</td>
<td>0.80∞</td>
</tr>
<tr>
<td>BMI (kg.m$^{-2}$)</td>
<td>25.46 (22.71-28.17)</td>
<td>25.10 (23.39-29.51)</td>
<td>0.90∞</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>77.40 (71.20-84.45)</td>
<td>78.65 (72.30-86.40)</td>
<td>0.41∞</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>97.40 (93.20-102.00)</td>
<td>96.25 (93.45-102.00)</td>
<td>0.95∞</td>
</tr>
<tr>
<td>Waist to Hip Ratio (WHR)</td>
<td>0.79 (0.75-0.86)</td>
<td>0.82 (0.76-0.86)</td>
<td>0.31∞</td>
</tr>
</tbody>
</table>

Table 3. Characteristics of the participants with valid accelerometer data (completers) and non valid accelerometer data (non completers). Data are expressed as median (25th-75th quartiles) when they are not normally distributed or mean (standard deviation) when they are normally distributed. HDL: high density lipoprotein, LDL: low density lipoprotein, DBP: diastolic blood pressure, SBP: systolic blood pressure. *P value resulted from Kruskal Wallis test; ∞ p value resulted from non paired t-test of normally distributed values after normalisation; § p value resulted from non paired t-test.

3.3.2 Agreement and correlation between accelerometer derived and IPAQ reported activity measures per ethnic group.

Volunteers of both ethnic groups had a wide range of physical activity and sedentary behaviour levels (Table 4). In both groups, IPAQ underestimated sedentary behaviour by an average of ~ 43% in South Asians and ~ 32% in Europeans (p < 0.001). The Spearman’s correlation and concordance correlation coefficient demonstrated positive associations (confidence intervals did not cross zero) between IPAQ reported sitting time and accelerometer recorded sedentary time in both ethnic groups but the strength of the correlations were moderate.

Agreement between IPAQ and accelerometer reported moderate to vigorous activity was poor with not being significant among the Europeans (Pc = 0.02, 95% CI: -0.05 to 0.08, r = 0.15, 95 % CI: -0.07 to 0.36) (Figure 15). IPAQ reported MVPA was almost 4-fold higher among the South Asians and 3-fold higher among the Europeans compared with the
accelerometer derived data (Table 4). I further investigated the relationship between the two methods of measuring physical activity by looking at the agreement between the two methods in the assessment of vigorous activity separately from assessing moderate intensity activity (Figure 13; Figure 14). In both groups, the agreement in the assessment of vigorous activity was stronger with women being more likely to misclassify moderate activity \((r = 0.28\) for moderate versus \(r = 0.54\) for vigorous among the South Asians, \(r = 0.20\) versus \(r = 0.43\) respectively for the Europeans) (Table 4). Spearman’s correlation revealed stronger correlations compared with the concordance correlation coefficient for each activity or sedentary domain. In total, the associations between the two methods among the South Asian women were marginally stronger compared with the Europeans in both sedentary and activity domains (Pc 0.19 versus 0.12 and 0.08 versus 0.02 respectively) but without the ethnic differences in the performance of each method reaching statistical significance (overlapping confidence intervals of Pc) (Table 4).

The 95% limits of agreement were wide for all domains of sedentary behaviour and physical activity, independent of ethnicity, suggesting great variability in the performance of IPAQ compared with the more accurate accelerometer recordings (Figure 12; Figure 16). However, there is no apparent trend among those over-reporting physical activity and under-reporting sitting time (i.e. there is no suggestion that those having lower levels of objectively measured physical activity tend to over-report physical activity and vice versa). Table 5 demonstrates the quartiles of sitting/sedentary time (in min.day\(^{-1}\)) and MVPA (in min.day\(^{-1}\)) and (MET.min.day\(^{-1}\)) for both methods of measurement. Interestingly, summary estimates (without stratifying by ethnicity) do not have overlapping ranges of each quartile for IPAQ and accelerometer derived values of physical activity and sedentary behaviour, suggesting that the underestimation of sedentary time and overestimation of MVPA with the IPAQ questionnaire is consistent across the distribution of physical activity/sedentary time measures.
### Table 4. Accelerometer derived and IPAQ indices of physical activity and sedentary behaviour in the South Asians and the Europeans.

<table>
<thead>
<tr>
<th>Activity Level</th>
<th>South Asians</th>
<th>Europeans</th>
<th>South Asians</th>
<th>Europeans</th>
<th>South Asians</th>
<th>Europeans</th>
<th>South Asians</th>
<th>Europeans</th>
<th>South Asians</th>
<th>Europeans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary vs Sitting</td>
<td>495.83 (85.24)</td>
<td>281.31 (161.78)</td>
<td>214.52 (-50.89 to 479.93)</td>
<td>&lt;0.0001</td>
<td>0.64 (0.48 to 0.76)</td>
<td>0.19 (0.11 to 0.27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate vs Moderate</td>
<td>26.59 (19.53)</td>
<td>111.14 (85.29)</td>
<td>-84.55 (-244.04 to 74.94)</td>
<td>&lt;0.0001</td>
<td>0.28 (0.05 to 0.48)</td>
<td>0.07 (0.02 to 0.12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vigorous vs Vigorous</td>
<td>2.11 (5.04)</td>
<td>6.44 (15.27)</td>
<td>-4.33 (-29.75 to 21.08)</td>
<td>&lt;0.0001</td>
<td>0.54 (0.35 to 0.69)</td>
<td>0.33 (0.22 to 0.43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVPA vs MVPA</td>
<td>28.69 (22.23)</td>
<td>117.57 (89.27)</td>
<td>-88.89 (-255.16 to 77.38)</td>
<td>&lt;0.0001</td>
<td>0.32 (0.09 to 0.51)</td>
<td>0.08 (0.02 to 0.13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVPA.MET vs MVPA.MET</td>
<td>48.93 (25.61)</td>
<td>132.79 (75.96)</td>
<td>-83.87 (-237.85 to 70.12)</td>
<td>&lt;0.0001</td>
<td>0.15 (-0.07 to 0.36)</td>
<td>0.02 (-0.05 to 0.08)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVPA.MET vs MVPA.MET</td>
<td>119.89 (103.15)</td>
<td>423.94 (339.07)</td>
<td>-304.05 (-922.12 to 314.02)</td>
<td>&lt;0.0001</td>
<td>0.34 (0.12 to 0.53)</td>
<td>0.12 (0.05 to 0.19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVPA.MET vs MVPA.MET</td>
<td>215.65 (115.61)</td>
<td>541.28 (354.24)</td>
<td>-325.63 (-1042.17 to 390.91)</td>
<td>&lt;0.0001</td>
<td>0.17 (-0.05 to 0.38)</td>
<td>0.02 (-0.05 to 0.10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 11. Relationships between accelerometer derived sedentary time and IPAQ reported sitting time in the South Asians (left) and the Europeans (right). Solid line represents the linear regression line and dotted line represents the line of equality $y = x$. Spearman’s ($r$) and concordance ($P_c$) correlation coefficients are shown along with the regression equations.

Figure 12. Limits of agreement between accelerometer measured sedentary time and IPAQ reported sitting time in the South Asians (left) and the Europeans (right). Solid line represents the mean difference between accelerometer derived minus IPAQ reported values. Dotted lines represent the 95% confidence intervals.
Figure 13. Relationships between accelerometer derived moderate activity time and IPAQ reported moderate activity time in the South Asians (left) and the Europeans (right). Solid line represents the linear regression line and dotted line represents the line of equality $y = x$. Spearman’s ($r$) and concordance ($P_c$) correlation coefficients are shown along with the regression equations.

Figure 14. Relationships between accelerometer derived vigorous activity time and IPAQ reported vigorous activity time in the South Asians (left) and the Europeans (right). Solid line represents the linear regression line and dotted line represents the line of equality $y = x$. Spearman’s ($r$) and concordance ($P_c$) correlation coefficients are shown along with the regression equations.
Figure 15. Relationships between accelerometer derived moderate to vigorous activity time and IPAQ reported moderate to vigorous activity time in the South Asians (left) and the Europeans (right). Solid line represents the linear regression line and dotted line represents the line of equality $y = x$. Spearman’s ($r$) and concordance ($P_c$) correlation coefficients are shown along with the regression equations.

Figure 16. Limits of agreement between accelerometer measured moderate to vigorous time (MVPA) and IPAQ reported moderate to vigorous (MVPA) time in the South Asians (left) and the Europeans (right). Solid line represents the mean difference between accelerometer derived minus IPAQ reported values. Dotted lines represent the 95% confidence intervals.
Table 5. Quartile ranges for accelerometer derived and IPAQ estimates of physical activity and sedentary behaviour.

<table>
<thead>
<tr>
<th>Activity Measure</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting/sedentary time (min.day(^{-1}))</td>
<td>Accelerometer</td>
<td>&lt; 461.23</td>
<td>461.23 to 510.60</td>
<td>&gt; 556.17</td>
</tr>
<tr>
<td></td>
<td>IPAQ</td>
<td>&lt; 205.71</td>
<td>205.71 to 300.0</td>
<td>&gt; 411.43</td>
</tr>
<tr>
<td>MVPA (min.day(^{-1}))</td>
<td>Accelerometer</td>
<td>&lt; 18.62</td>
<td>18.62 to 28.71</td>
<td>&gt; 54.65</td>
</tr>
<tr>
<td></td>
<td>IPAQ</td>
<td>&lt; 60.71</td>
<td>60.71 to 102.87</td>
<td>&gt; 173.57</td>
</tr>
<tr>
<td>MVPA (MET.min.day(^{-1}))</td>
<td>Accelerometer</td>
<td>&lt; 72.91</td>
<td>72.91 to 157.24</td>
<td>&gt; 247.94</td>
</tr>
<tr>
<td></td>
<td>IPAQ</td>
<td>&lt; 219.64</td>
<td>219.64 to 403.72</td>
<td>&gt; 660.00</td>
</tr>
</tbody>
</table>

3.3.3 Agreement and correlation between accelerometer derived and IPAQ reported activity measures stratified by age

We explored the correlation and agreement among the self reported IPAQ and the accelerometer derived activity measures stratified by age (below and above median, median = 50.5 years). There was no interaction with ethnicity for either group (MVPA: p = 0.149 for those below the median age, p = 0.08 for those above the median age, sitting/sedentary behaviour: p = 0.069 for those below the median age, p = 0.913 for those above the median age), hence, stratification per ethnicity was not performed. In both age groups, IPAQ underestimated the time spent by individuals sitting and overestimated the time spent performing moderate to vigorous activity compared with accelerometer measures (Figure 17; Figure 18). Correlation and agreement between the different measures of sitting/sedentary time did not differ significantly among the two groups; although the mean correlation (r) and agreement (Pc) was higher in the younger group (Pc = 0.26 (95% CI: 0.17 to 0.35), r = 0.64 (95% CI: 0.48 to 0.76) versus Pc = 0.08 (95% CI: 0.02 to 0.15), r = 0.32 (95% CI: 0.10 to 0.51), the 95% confidence intervals overlapped. Similarly, in the assessment of MVPA, age did not have a significant impact on the performance of IPAQ against the accelerometers with both Spearman correlation and concordance correlation coefficients having overlapping 95% confidence intervals in both age groups (Pc = 0.05 (95% CI: -0.02 to 0.12), r = 0.28 (95% CI: 0.06 to 0.47) versus Pc = 0.07(0.02 to 0.13),
\( r = 0.34 \) (95% CI: 0.13 to 0.53); however, the concordance correlation did not reveal a significant correlation (\( p=0.19 \)) in the younger group limiting the interpretation of the mean value of the concordance coefficient in this group.

**Figure 17.** Relationships between accelerometer derived sedentary time and IPAQ reported sitting time in young (under median age) and older (over median age) participants. Solid line represents the linear regression line and dotted line represents the line of equality \( y = x \). Spearman’s (\( r \)) and concordance (\( P_c \)) correlation coefficients are shown along with the regression equations.

**Figure 18.** Relationships between accelerometer derived moderate to vigorous physical activity (MVPA) time and IPAQ reported moderate to vigorous physical activity (MVPA) time in young (under median age) and older (over median age) participants. Solid line represents the linear regression line and dotted line represents the line of equality \( y = x \). Spearman’s (\( r \)) and concordance (\( P_c \)) correlation coefficients are shown along with the regression equations.
3.3.4 Strength of relationship between IPAQ reported and accelerometer derived activity measures and metabolic risk score

Table 6 shows the beta (standardised) coefficient for the change in the metabolic risk score associated with a SD change in the independent variable (square root transformed for the IPAQ and accelerometer activity measures (MVPA) and the IPAQ sitting time and raw data for the accelerometer recorded sedentary time). There was a significant decrease in the metabolic risk score by 0.19 SD for every SD increase in the MVPA measured with the use of accelerometers (p = 0.031, after adjusting for age and ethnicity). The relationship was attenuated after including in the model the time spent sitting as a confounding factor reaching only borderline significance (p = 0.054). Sedentary time recorded by accelerometers, even though it had an adverse effect on the direction of the metabolic risk score; the magnitude of the effect was not significant after adjusting for confounding factors (age, ethnicity +/- MVPA). Notably, IPAQ reported activity and sitting time measures did not reveal significant associations with metabolic risk score, indicating the inferiority of self reported questionnaires in capturing the real dose response effect of habitual behaviour in metabolic risk profile. The role of ethnicity as an effect modifier was assessed prior to including it in the model as a confounder but there was no significant interaction of the independent variable with ethnicity for either model. In addition, Table 6 includes the p values for possible interactions between the IPAQ and accelerometer derived measures for each domain suggesting that lack of significant interaction between the two methods of measurement for both activity and sedentary domains. Figure 19 graphically demonstrates the adjusted (for age and ethnicity) protective effect of accelerometer recorded MVPA (solid line) against the metabolic risk (the higher the MVPA the lower the risk) in comparison with the insignificant effect of MVPA reported with the use of IPAQ (dotted line). Similarly, the adjusted regression line for accelerometer recorded sedentary time is almost unchanged across the distribution of sedentary time without demonstrating an association with the metabolic risk score. The regression line of IPAQ sitting time shows a paradoxical decline with increasing sitting time, however, it is not significant.
Table 6. Standardised (beta) coefficient for relationship between accelerometer derived and IPAQ reported indices and metabolic risk score. Beta and p value result from a model adjusted for age and ethnicity. Beta* and p* result from a model adjusted for age, ethnicity and MVPA (when sitting/sedentary is the independent variable) or age, ethnicity and sitting/sedentary time (when MVPA is the independent variable). P-interaction corresponds to p value assessing the interaction between accelerometer derived sedentary time and IPAQ reported sitting time (1) or between accelerometer derived MVPA and IPAQ reported MVPA (2).

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>p-value</th>
<th>Beta*</th>
<th>p-value*</th>
<th>p-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerometer Sedentary</td>
<td>0.06</td>
<td>0.372</td>
<td>0.18</td>
<td>0.811</td>
<td>0.107 (1)</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPAQ Sitting Time</td>
<td>-0.02</td>
<td>0.730</td>
<td>-0.02</td>
<td>0.784</td>
<td></td>
</tr>
<tr>
<td>Accelerometer MVPA</td>
<td>-0.19</td>
<td>0.031</td>
<td>-0.16</td>
<td>0.054</td>
<td>0.215 (2)</td>
</tr>
<tr>
<td>IPAQ MVPA</td>
<td>0.02</td>
<td>0.742</td>
<td>0.02</td>
<td>0.801</td>
<td></td>
</tr>
</tbody>
</table>

Figure 19. Relationships between moderate to vigorous physical activity (MVPA, square root transformed) and metabolic risk score (left) and sedentary/sitting time and metabolic risk score (right). Solid line on each plot represents the linear regression line for accelerometer derived indices; dotted line represents the linear regression line for IPAQ indices. Data are adjusted for age and ethnicity. Solid black dots represent accelerometer data-points and transparent circles represent IPAQ data-points.
3.4 Discussion

The main findings of this study were: 1. IPAQ led to significant over-reporting of physical activity and under-reporting of sedentary time compared with accelerometer derived measures and these trends were evident independent of age or ethnicity. Spearman’s and concordance correlation coefficients were greater for sedentary time compared with physical activity in both ethnic groups although the difference failed to reach statistical significance (overlapping confidence intervals). 2. Participants, irrespective of ethnicity, were more likely to misreport moderate activity compared to vigorous activity resulting in an overall weak cumulative correlation between self reported MVPA and recorded one. 3. There was a significant inverse association between accelerometer measured activity and metabolic risk score after adjusting for ethnicity and age, in contrast, IPAQ activity indices failed to reveal an association indicating that IPAQ has limitation in capturing the real duration of daily activity with the potential risk of masking the magnitude of the effect of physical activity in health outcomes.

IPAQ has been used as a method of assessing habitual activity in South Asians living in the Indian subcontinent or in Western countries in previous studies (283, 292, 293); however, its validity in this population has not been adequately examined, especially for women of South Asian origin migrating to the Western world. A smaller study including 52 Indian Asian (without specifying gender distribution) living in Singapore showed a negative correlation between IPAQ and accelerometer measured moderate activity (r = -0.17) and modest correlation for vigorous domains (r = 0.48). However, the performance of IPAQ for Indians did not differ to that of another questionnaire (Singapore Prospective Study Programme physical activity questionnaire, SP2PAQ) for the same ethnic group despite the latter being more reliable for other Asian subgroups (Chinese, Malay) (291). We demonstrated that the long form of IPAQ does not under-perform among South Asian women living in the Scotland in comparison with the reference population of White descent and its validity against objective measures is comparable with the previously published summary estimates (summary correlation coefficient ranging 0.27 to 0.49 across all physical activity domains (300)). Interestingly, in our study the correlation for the moderate and consequently MVPA between IPAQ and accelerometer was not significant for Europeans which may have resulted from their high awareness of the health benefits of systematic exercise and their desire to report frequency and duration of activity close to the national recommendations rather to their true average frequency of exercising (301). Yates et al revealed stronger association between self reported walking and glucose control for
South Asians with impaired glucose metabolism compared with Caucasians (210), in our study ethnicity did not modify the poor relationship between self reported activity and metabolic risk, but there was a trend, albeit not significant, of higher correlation coefficients for all domains of activity and sitting time with objective measures for South Asian women compared with European counterparts.

Notably, spearman’s correlation coefficient was higher for vigorous compared with moderate activity, a finding evident in previous studies, possibly related with the fact that vigorous activity tends to be more structured and can be recalled easier compared with the time spent performing lighter activity or walking which both accumulate during the day and can be overlooked (302). We acknowledge, though, in our study the overall correlation of IPAQ reported activity with objectively measured physical activity is moderate to weak ($r < 0.7$ for all domains) across the age range of both ethnic groups suggesting that the IPAQ has inherent limitations in assessing physical activity or sedentary time which may attenuate when IPAQ is used in larger scale epidemiological and population studies in order to assess the relationship of physical activity with metabolic outcomes (292). Hence, IPAQ may be valid for ranking individuals’ behaviours but not for quantifying activity duration. Therefore, caution is warranted with the use of IPAQ in future studies investigating the optimal frequency and duration of physical activity in order a clinically relevant shift in metabolic risk of individuals to be achieved. Undoubtedly, there are convincing data demonstrating favourable effects of self reported activity on all cause mortality, morbidity, diabetes type 2 and cardiovascular disease at a population level (303), hence, increasing sample size seem to improve precision but it is unlikely to overcome the limitations of the tool related with accuracy.

It can be assumed that the lack of association between self reported physical activity and metabolic risk score may be questionnaire specific and other questionnaires or versions of the same questionnaire may perform better. There is cumulative evidence that the long version of IPAQ performs equally well compared with other established questionnaires yielding a reliability score of around 0.8 and a validity score around 0.3 when it is compared with motion sensors (287). Similarly, it has been demonstrated that the performance of IPAQ is not modified by version (short versus long) or reference period (last 7 days versus usual week) (300). There is some data suggesting that interviewer-administered questionnaires have greater correlation coefficients than self reported for all domains of physical activity with the exception of moderate activity (300), the main domain where IPAQ performed poorly in our study. Therefore, I don’t anticipate that
swapping mode of administration would have affected the correlation indices between the two methods and indeed may have introduced systematic variability in the responses influenced by the social desirability effect. Interviewer administered questionnaires may have a role in ethnicity specific research when there is a great chance of low literacy or misinterpretation of the questions because of cultural or religious differences, however, in our study there was no evidence of ethnic related trend in the performance of the questionnaire which could have been reversed by interviewing the volunteers.

IPAQ, similarly to other validated questionnaires of assessing habitual activity, has promising reliability scores (expressing its ability to yield the same results when administered to the same individual on a second occasion) (287, 300), but its validity (against a gold standard technique of measuring physical activity) varies from modest to weak, often influenced by the lack of a widely accepted “gold standard” in measuring physical activity and the use of proxy measures (i.e. cardiorespiratory fitness, indirect calorimetry) in examining the reliance of the questionnaire. It has been suggested that doubly labelled water (DLW) may be the absolute criterion against which new activity questionnaires must be validated (300). DLW is the gold standard in the measurement of total daily energy expenditure and thereby of physical activity (304). However, given that energy expenditure is not only affected by the amount of daily activity but by the basal metabolic rate and thermal effect of food processing too, it is questionable whether it should be used in assessing the validity of questionnaires aiming to quantify activity time rather than ranking levels of activity. The same group suggested that motion sensors such as accelerometers and pedometers are the second best tools that can be used in validation studies (300).

Our study demonstrated that the limits of agreement between the two methods were wide and the concordance coefficients (testing the equality of the two tools for the same domains) were systematically lower than the equivalent correlation coefficients suggesting that the two instruments of assessing physical activity should not be used interchangeably. This can be explained in part because the two methods are not meant to measure exactly the same activity indices. Sitting time is not identical to sedentary time (i.e. time spent sitting down versus time < 100 accelerometer counts.min\(^{-1}\)) expecting to have an impact on the level of concordance coefficient between the two methods. Similarly, IPAQ describes activities performed for a minimum of 10 minutes and assumes specific degree of intensity for each activity without considering inter-individual variation in the degree of intensity, whereas accelerometers are designed to measure any form of physical movement.
Freedson’s count cut-points which correspond to different levels of activity intensity assessed by accelerometry have been developed under controlled laboratory conditions (279), hence, a shift in the cut-points cannot be excluded when they are applied to a field situation or to a different study sample. Therefore, the accelerometry intensity cut-points may not be identical to the MET cut-points assigned to each IPAQ domain resulting in a different classification of the intensity of similar activities by each method. In addition, the accelerometer does not count water related activity nor records precisely cycling and weight resistance activities. However, in our study only 5 women logged in their diaries or self reported one of the above activities, rendering unlikely the small measure bias of the accelerometers in these cases to be the cause of the large deviation between the two methods.

The strength of our study is the robust methodology and the use of a widely acceptable objective tool of measuring activity domains. We presented limits of agreement instead of solely correlation coefficients, which have been mainly used in literature to date, giving a more accurate insight into the extent to which the questionnaires over or under estimate the actual levels of physical activity rather than only assessing the strength of the linear relationship between the two methods. We acknowledge that the interpretation of the results is limited by the fact that the questionnaire was administered during the baseline appointment, a week in advance of the week that the accelerometer was worn. However, the participants were asked to consider their responses based on a usual week pattern and there is evidence that the reference period does not introduce significant variance in the responses (300).

3.5 Conclusion

I conclude that IPAQ, similar to other self reported methods, is subject to cognitive biases and social desirability limitations resulting in a lower convergent validity when it is compared with objective methods. However, the two methods (IPAQ versus accelerometry) cannot be used interchangeably. In summary, this study confirms my initial hypothesis that self reported physical activity overestimates actual physical activity and this is independent of ethnicity or age. Hence, objectively measured physical activity is encouraged in studies aiming to capture the real dose-response benefit of physical activity on cardiometabolic outcomes. Therefore, objectively measured physical activity is used for all analyses in the remaining chapters.
4 LIFESTYLE FACTORS, METABOLIC BIOMARKERS, ADIPOSITY AND CARDIORESPIRATORY FITNESS IN SOUTH ASIAN AND EUROPEAN WOMEN LIVING IN SCOTLAND

4.1 Introduction

The burden of diabetes and cardiovascular disease (CVD) is increasing worldwide with some ethnic groups, such as South Asians, witnessing a disproportional rise compared with others (305). The prevalence of type 2 diabetes has doubled in India during the last three decades (306) with estimates projecting that India will be the leading country in the prevalence of diabetes with 87 millions diagnosed cases by 2030 (an average 75 % rise since 2010) (23). In addition, South Asians living in the Indian subcontinent develop coronary heart disease (CHD) 10 years earlier than other ethnic groups (27). Urbanisation has allied to an unfavourable metabolic phenotype within this population; Mahadik et al reported 35.2% prevalence of metabolic syndrome diagnosed according to the Adult Treatment Panel III (ATP III) criteria among 267 urban Indians compared with 20.6% among 804 Indians living in rural regions, and, interestingly, those without established metabolic syndrome living in urban regions were more insulin resistant than their healthy counterparts in rural areas (61). Hence, urbanisation resulting in a rapid shift in the prevalence of conventional cardiovascular risk factors, such as obesity, hypertension, smoking and hyperlipidaemia contributed, at least partially, to the rise in the metabolic risk among South Asians living in the Indian subcontinent.

A substantially pronounced effect of urbanisation on metabolic risk of South Asians is exhibited when South Asians migrate away from the Indian subcontinent to the Western world. South Asians in the UK have a 3 to 5-fold risk of developing type 2 diabetes at a younger age and a lower BMI than their counterparts of White European descent (28, 115) and 30-100% higher rate of CHD and mortality from CVD than that of European comparators (21, 29, 307). In addition, non-diabetic South Asians exhibit higher fasting glycaemic indices than Europeans (41, 207). Conventional cardiovascular factors do not account for the magnitude of the inter-ethnic differences in the burden of non-communicable diseases; smoking is less prevalent among South Asians (207), apart from data on Bangladeshi men suggesting the opposite (36), and stratification per smoking status did not attenuate the ethnic differences on the incidence of ECG changes among
young South Asian and European men (48). Dietary intake did not differ largely among South Asians and White Europeans and, indeed, South Asians consumed larger quantities of cardio-protective polyunsaturated fats (218). In addition, the prevalence of hypertension and hyperlipidaemia does not differ among healthy South Asian men compared with their European counterparts (207) and the magnitude of the effect of both risk factors on the incidence of CHD does not alter after stratification per ethnicity (66).

It has been suggested that insulin resistance associated with central adiposity is the key link between South Asians and their greater risk of diabetes and CVD compared with the background population (115), however insulin resistance has been observed among South Asian men in the absence of excessive body fat or abdominal obesity compared with white Europeans (113). In addition, the greater incidence of CHD in non-diabetic South Asian men compared with Europeans (hazard ratio 1.58 (95% CI 1.06-2.36) persisted after adjustment for insulin resistance along with other conventional risk factors (i.e. age, smoking, blood pressure and hyperlipidaemia) (66). Hence, there is cumulative evidence suggesting that traditional risk factors do not explain the magnitude of the ethnic differences in the metabolic risk and ethnic stratified cut off points on the conventional risk factors should be developed.

A striking observation is that South Asian women migrating to the western countries exhibit a disproportional higher prevalence of type 2 diabetes compared with women from the background population or South Asian men of similar age. A Finnish population based survey demonstrated that the cumulative prevalence of diabetes in South Asian women is almost 10-fold higher than that in Western women and almost two fold greater than that of South Asian men (308), with the prevalence increasing disproportionally in South Asian women than men over the age of 40 and more profoundly over the age of 50. A similar trend has been observed in Pakistani women living in the UK who exhibit a higher frequency of diabetes over the age of 55 compared with Pakistani men (28). The disproportional rise in diabetes prevalence in middle-aged South Asians compared with women of white European descent raises the question whether menopause, which coincides around that time in women’s life, has a diverse impact on the metabolic risk factors of different ethnic groups.

Another notable observation is that the sex specific differences in the rate of diabetes or impaired glucose tolerance are not evident among South Asians living in the Indian subcontinent (53), indicating that migration has a greater impact on the metabolic risk of
South Asian women than on that of men. Given that both men and women migrating to the UK increase their BMI and dietary intake to the same extent compared with their contemporaries in rural India (121), westernised diet and weight gain do not explain the excess risk of developing diabetes of South Asian mid-life women. In addition, the sex-mediated protection against CHD seems to subside in South Asian women living in Western countries with studies suggest that the mortality from CHD does not differ between women and men of South Asian origin (21, 29). However, most of the ethnic specific research to date has been undertaken in men, and South Asian women, despite being a high risk group, have been largely overlooked. The sex specific differences in risk factors predisposing to diabetes and CVD among South Asians warrant detailed investigation, especially when limited data in South Asian women suggest that conventional risk factors, such as smoking, hypertriglyceridemia or abdominal adiposity measured by waist to hip ratio are less prevalent among South Asian women than men (115).

The need for sex and ethnic specific research is further fuelled by acknowledging that modifiable risk factors such as adiposity (117, 309, 310), diminished cardiorespiratory fitness (311) and low physical activity (309, 312) have been linked with insulin resistance, glycaemia and subsequent risk of diabetes and CVD. However, baseline data in South Asian women on the above risk factors are sparse rendering the implementation of targeted preventive policies unfeasible.

The aim of this chapter is to describe the demographic characteristics, body composition phenotype, physical activity, predicted maximal oxygen uptake, dietary intake and metabolic biomarkers of non diabetic South Asian women living in Scotland compared with white European counterparts living in the same region. In addition, this chapter will examine how the above variables change in each ethnic group across the reproductive trajectory. I hypothesise that South Asian women have an adverse lifestyle and cardiometabolic risk profile compared with the Europeans. My second hypothesis is that the impact of menopause on metabolic or lifestyle risk factors is independent of ethnicity.
4.2 Methods

4.2.1 Recruitment

Participants were recruited via general advertising and word of mouth as outlined in the methods chapter 2. Participants without diagnosed diabetes but with a high likelihood of having diabetes after their assessment visit (HbA1c ≥ 6.5% or fasting plasma glucose ≥ 7 mmol.l\(^{-1}\). were excluded from the analysis (313).

4.2.2 Ethics

The study was granted ethics permission by the West of Scotland Ethics Committee 3. All the participants gave written consent prior their inclusion in the study.

4.2.3 Study Protocol

All women underwent the assessments outlined in chapter 2.

4.2.3.1 Demographic characteristics

Participants provided information about their country of origin (applicable for those of South Asian origin), religion (applicable for those of South Asian origin), years of formal education (counting from primary school), current medications, family history, smoking and weekly alcohol consumption by filling a screening questionnaire (Appendix). Menopause status was defined by their menstrual history, plus the levels of follicle stimulating hormone (FSH) when required. Postcode was used to rank the location of residence based on the Scottish Index of Multiple Deprivation (SIMD) 2012 ranking. The SIMD ranking ranks small areas from most deprived (ranked 1) to least deprived (ranked 5) and was used as a proxy of socio-economic status of the volunteers.

4.2.3.2 Evaluation of dietary intake

All subjects completed a seven day food frequency questionnaire (Appendix) and the nutritional analysis was conducted with the help of Tinuviel Software (QBuilder version 2). Total energy consumed per day (expressed in calories per day) was calculated for each participant. Percentage of daily energy provided by each of the main macronutrients was calculated by dividing the energy provided by each specific source (4 calories per gram of carbohydrates, 9 calories per gram of fat and 4 calories per gram of protein) by the total
energy consumed. Subsequently, the proportion of energy provided by different types of fat (saturated, monounsaturated and polyunsaturated) and carbohydrates (sugar and starch) was calculated.

### 4.2.3.3 Evaluation of habitual activity and cardiorespiratory fitness

Participants wore accelerometers (GT3X or Actitrainer; Actigraph LLC, Pensacola, FL, USA) for seven consecutive days. They were asked to wear them at all times apart from when sleeping, showering/bathing or swimming. In addition, they self reported physical activity by filling the long form of the validated International Physical Activity Questionnaire (IPAQ).

Cardiorespiratory fitness was assessed by predicting the maximal oxygen uptake (VO2 max) with the use of the Chester step test as outlined in the methodology chapter. Resting heart rate was measured after volunteers lying flat without moving or talking for 20 minutes at the end of peripheral arterial tone assessment (Endo-PAT).

### 4.2.3.4 Evaluation of body composition phenotype

All volunteers had had their weight and height measured as described in the methods chapter. BMI was calculated by the equation: \( \text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2} \). In addition waist and hip circumferences were measured for each participant in duplicate to the nearest 0.1 cm. The mean of two measurements was used for the analysis. Waist to hip ratio (WHR) was calculated by dividing waist (measured in cm) by hip circumference (measured in cm).

Women over the age of 40 had additional detailed assessment of fat distribution including skinfold thickness at seven sites (biceps, triceps, subscapular, suprailiac, supraspinale, thigh, calf) and body circumferences at three additional sites (mid-arm, mid-thigh and mid-calf). In addition, they were offered magnetic resonance imaging (MRI) assessment of central adiposity (subcutaneous and visceral fat at the level of L3-L4) and liver fat accretion by the use of magnetic resonance spectroscopy (MRS). Based on skinfold measurement, central adiposity was defined as the summation of subcapular, suprailiac and suprapinale skinfolds; arm adiposity as the summation of bicep and tricep skinfolds; and lower body adiposity as the summation of calf and thigh skinfolds.
4.2.3.5 Metabolic biomarkers

Blood samples were centrifuged within four hours following venepuncture. All plasma and serum samples were stored at -80 ºC in labelled 0.5 ml Eppendorf tubes (Alpha Laboratories, Hampshire, UK). Plasma glucose, glycosylated haemoglobin (HbA1c) and serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, sex hormone binding globulin (SHBG) and testosterone were measured at the day of venepuncture by colleagues working within NHS Greater Glasgow and Clyde biochemistry laboratories. High density lipoprotein (HDL), total Cholesterol, triglycerides (TG) and gamma-glutamyl transpeptidase (GGT) were measured in stored plasma by colleagues with the use of Autoanalyser (C311, Roche Hitachi). Insulin, leptin, pro-insulin and adiponectin were measured in stored plasma by colleagues with the use of commercially available ELISA kits. Low-density lipoprotein (LDL) levels were calculated using the Friedwald equation (282). Cholesterol ratio was defined as the ratio of total cholesterol divided by HDL levels. Free androgen index (FAI) was defined as the ratio of total testosterone levels divided by SHBG levels. The homeostatic model assessment of insulin resistance (HOMA\textsubscript{IR}) was calculated by the equation $\text{HOMA}_{\text{IR}} = (\text{Glucose} \times \text{Insulin}) / 22.5$ (77).

Blood pressure was measured with an automated blood pressure monitor. The mean of two readings at least one minute apart was recorded.

4.2.4 Statistical Analysis

Values are presented as mean (standard deviation, SD) and ranges unless otherwise stated. Normality of the variables was tested with the Shapiro-Wilk test and visual inspection of the distribution using the probability distribution plot. Not normally distributed variables were log transformed. Unpaired t-test of raw or log transformed data was used for comparing continuous variables among different groups. Chi square test was used for comparing categorical variables among different groups and Fisher’s exact test when n < 5.
4.3 Results

4.3.1 Participants

179 women fulfilling the inclusion criteria were recruited in the study; 92 were South Asians and 87 were of White European descent. One participant of South Asian origin was found to have HbA1c of 7.6%, deeming her at high risk of undiagnosed type 2 diabetes, and, therefore, was excluded from the analyses. The majority of South Asians were of Pakistani origin and Muslims (64.8% and 65.9% respectively) (Table 7).

4.3.2 Baseline characteristics

Age and BMI did not differ among the women of South Asian origin compared with the women of White European descent (mean age 47.3 versus 47.9 years, p = 0.77, mean BMI 26.1 versus 25.9 kg.m\(^{-2}\), p = 0.89). Table 7 demonstrates that the SIMD ranking based on the postcode of residence did not differ among the two ethnic groups (p = 0.73). However, the volunteers of White European descent reported more years of formal education (16.3 versus 14.8 years, p = 0.009), the credibility of which as a measure of education may be limited because of the diverse educational systems and requirements in different countries. A greater proportion of the women of South Asian origin were married (73.6 % versus 62.1 %) and none of them disclosed being in a relationship compared with 12.6 % of women of white descent (p = 0.005). Smoking was more prevalent among the Europeans (27.6% current or ex smokers versus 3.3% among South Asians, p < 0.0001). The proportion of women taking regularly antihypertensive treatment or thyroxine did not differ among the two ethnic groups; however, more South Asians were on lipid lowering tablets and Vitamin D supplements (6.6 % and 13.5 % versus none and 2.3 %, p = 0.03 and p = 0.006 respectively). Notably, a proportion of Europeans admitted to the researcher during their visit that their General Practitioner had advised them to commence statin treatment but the individuals had declined. Hence, the discrepancy in statin treatment may reflect different degree of uptake of treatment among the two groups rather than different background risks. Interestingly, 61.5 % of the South Asian volunteers had at least a first degree relative diagnosed with type 2 diabetes (parents or siblings) compared with only 19.5 % of the Europeans (<0.0001). On the contrary, the family history of cardiovascular events did not differ among the two ethnic groups. The possibility of recall bias that may have contributed to the latter finding cannot be disputed. However, the majority of participants would be expected to remember if their parents had had a heart attack or stroke in the past.
<table>
<thead>
<tr>
<th>N</th>
<th>South Asians</th>
<th>Europeans</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin (South Asians, n, %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pakistan</td>
<td>59 (64.8 %)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>29 (31.9 %)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>1 (1.1 %)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Bangladesh</td>
<td>2 (2.2 %)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Religion (South Asians, n, %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Islam</td>
<td>60 (65.9 %)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hinduism</td>
<td>25 (27.5 %)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Christianity</td>
<td>2 (2.2 %)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Not disclosed</td>
<td>4 (4.4 %)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SIMD quintile (n, %)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (most deprived)</td>
<td>12 (13.3 %)</td>
<td>9 (10.5 %)</td>
<td>0.73</td>
</tr>
<tr>
<td>2</td>
<td>11 (12.2 %)</td>
<td>13 (15.1 %)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14 (15.6 %)</td>
<td>19 (20.1 %)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>24 (26.7 %)</td>
<td>19 (22.1 %)</td>
<td></td>
</tr>
<tr>
<td>5 (least deprived)</td>
<td>29 (32.2 %)</td>
<td>26 (30.2 %)</td>
<td></td>
</tr>
<tr>
<td>Formal Education (years)(mean, SD)</td>
<td></td>
<td></td>
<td>0.009</td>
</tr>
<tr>
<td>Married</td>
<td>14.8 (3.7)</td>
<td>16.3 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>67 (73.6 %)</td>
<td>54 (62.1 %)</td>
<td>0.005</td>
</tr>
<tr>
<td>Separated/divorced</td>
<td>17 (18.7 %)</td>
<td>16 (18.4 %)</td>
<td></td>
</tr>
<tr>
<td>Widow</td>
<td>5 (5.5 %)</td>
<td>4 (4.6 %)</td>
<td></td>
</tr>
<tr>
<td>In a relationship</td>
<td>2 (2.2 %)</td>
<td>2 (2.3 %)</td>
<td></td>
</tr>
<tr>
<td>Menopause state (n, %)</td>
<td></td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>42 (46.1 %)</td>
<td>39 (44.8 %)</td>
<td></td>
</tr>
<tr>
<td>Peri-menopausal</td>
<td>10 (11.0 %)</td>
<td>14 (16.1 %)</td>
<td></td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>39 (42.9 %)</td>
<td>34 (39.1 %)</td>
<td></td>
</tr>
<tr>
<td>Smoking (n, %)</td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Non smokers</td>
<td>88 (96.7 %)</td>
<td>63 (72.4 %)</td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>2 (2.2 %)</td>
<td>9 (10.3 %)</td>
<td></td>
</tr>
<tr>
<td>Ex smokers</td>
<td>1 (1.1 %)</td>
<td>15 (17.3 %)</td>
<td></td>
</tr>
<tr>
<td>Alcohol (units.week(^{-1})) (mean, SD)</td>
<td></td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>0.6 (2.7)</td>
<td>6.5 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Thyroxine</td>
<td>8 (9.0 %)</td>
<td>7 (8.1 %)</td>
<td>0.82</td>
</tr>
<tr>
<td>Vitamin D supplements</td>
<td>5 (5.6 %)</td>
<td>5 (5.8 %)</td>
<td>0.97</td>
</tr>
<tr>
<td>Statins</td>
<td>12 (13.5 %)</td>
<td>2 (2.3 %)</td>
<td>0.006</td>
</tr>
<tr>
<td>Family History (n, %)</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>56 (61.5 %)</td>
<td>17 (19.5 %)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>35 (38.5 %)</td>
<td>70 (80.5 %)</td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>Yes</td>
<td>40 (44.0 %)</td>
<td>33 (37.9 %)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>51 (56.0 %)</td>
<td>54 (62.1 %)</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>Yes</td>
<td>18 (19.6 %)</td>
<td>22 (25.3 %)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>74 (80.4 %)</td>
<td>65 (74.7 %)</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Demographic characteristics of the South Asian compared with the European volunteers. * (n = 2 missing values for SIMD ranking, SD: standard deviation, CHD: coronary heart disease, SIMD: Scottish Index of Multiple Deprivation).

### 4.3.3 Dietary Intake

Table 8 demonstrates that the energy consumed daily did not differ among the volunteers of different ethnic groups. Although, the South Asians seemed to consume larger amount of starch daily (absolute amount in grams per day: 104.5 versus 82.9, p < 0.0001), the
distribution of macronutrients daily, and hence, the proportion of energy provided by each macronutrient did not differ among the two groups.

### Table 8. Daily dietary energy intake and diet macronutrients composition in the South Asian compared with the European volunteers. (SD: standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>South Asians</th>
<th>Europeans</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kcal.day⁻¹)</strong></td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1527 ± 487 (620 – 2,981)</td>
<td>1556 ± 345 (522 – 2,665)</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Protein (%)</strong></td>
<td>18.7 ± 3.1 (12.0 - 27.6)</td>
<td>21.1 ± 3.2 (12.6 - 27.5)</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>Total fat (%)</strong></td>
<td>29.3 ± 5.3 (10.9 - 40.1)</td>
<td>29.2 ± 5.0 (19.9 - 43.8)</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Saturated fat (%)</strong></td>
<td>11.1 ± 2.7 (4.2 - 17.7)</td>
<td>12.1 ± 2.8 (6.9 - 22.3)</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Monounsaturated fat (%)</strong></td>
<td>10.4 ± 2.3 (2.8 - 15.7)</td>
<td>10.3 ± 2.0 (6.7 - 15.7)</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Polyunsaturated fat (%)</strong></td>
<td>5.1 ± 1.2 (1.9 - 7.9)</td>
<td>4.2 ± 0.9 (2.9 - 7.2)</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Carbohydrates (%)</strong></td>
<td>55.1 ± 6.6 (38.3 - 78.6)</td>
<td>47.4 ± 6.6 (31.6 - 66.4)</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Sugar (%)</strong></td>
<td>24.9 ± 5.9 (15.1 - 43.2)</td>
<td>24.2 ± 4.8 (14.5 - 42.1)</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Starch (%)</strong></td>
<td>29.8 ± 5.9 (12.5 - 46.2)</td>
<td>22.9 ± 5.5 (8.8 - 35.3)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

4.3.4 Physical Activity and cardiorespiratory fitness

The number of valid days the accelerometers were worn did not differ among the two ethnic groups (6.5 for the South Asians versus 6.6 for the Europeans, p = 0.26), however, the Europeans wore the accelerometers for longer time per day (858.3 minutes.day⁻¹ versus 832.9 minutes.day⁻¹, p = 0.04). Therefore, the indices of physical activity were adjusted for the total wearing time daily by making the assumption that the distribution of various intensity activities across the non-wearing time was similar to that observed during the wearing time. This was done so as to ensure that any ethnic specific differences in the physical activity indices were not driven by differences in the total wearing time of the accelerometer but reflected actual differences in the time engaged with physical activity. Table 9 demonstrates that the South Asian women engaged with MVPA for fewer minutes daily than the Europeans of similar age. Interestingly, sedentary time did not differ among the two groups but the South Asians engaged longer with light activities rather than with
moderate and vigorous ones. Self reported questionnaires, despite physical activity was over-reported by both groups, showed a similar trend in MVPA which was mainly attributable to the lower levels of walking among the South Asian women (Table 10). However, they failed to reveal an inter-ethnic difference in moderate or vigorous intensity activities separately.

<table>
<thead>
<tr>
<th></th>
<th>South Asians</th>
<th>Europeans</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>72</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Sedentary time (min.day⁻¹)</td>
<td>495.9 ± 85.8</td>
<td>514.6 ± 60.3</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>(276.0 - 690.0)</td>
<td>(361.5 - 673.2)</td>
<td></td>
</tr>
<tr>
<td>Light time (min.day⁻¹)</td>
<td>321.3 ± 85.8</td>
<td>282.6 ± 59.6</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(153.1 - 505.7)</td>
<td>(140.7 - 433.1)</td>
<td></td>
</tr>
<tr>
<td>Moderate time (min.day⁻¹)</td>
<td>26.9 ± 19.5</td>
<td>43.7 ± 23.0</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>(0.4 - 84.0)</td>
<td>(4.2 - 133.7)</td>
<td></td>
</tr>
<tr>
<td>Vigorous time (min.day⁻¹)</td>
<td>2.1 ± 5.1</td>
<td>5.1 ± 7.6</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(0 - 21.6)</td>
<td>(0 - 30.1)</td>
<td></td>
</tr>
<tr>
<td>MVPA (min.day⁻¹)</td>
<td>29.0 ± 22.2</td>
<td>48.9 ± 25.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>(0.4 - 85.6)</td>
<td>(4.2 - 141.1)</td>
<td></td>
</tr>
<tr>
<td>MVPA.MET (MET. min.day⁻¹)</td>
<td>121.3 ± 103.1</td>
<td>215.6 ± 115.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>(0 - 396.6)</td>
<td>(16.7 - 561.8)</td>
<td></td>
</tr>
<tr>
<td>VO₂ max (mls.kg⁻¹.min⁻¹)</td>
<td>32.2 ± 5.6</td>
<td>34.4 ± 7.3</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>(19.7 - 45.0)</td>
<td>(21.0 - 70.0)</td>
<td></td>
</tr>
<tr>
<td>Resting HR (beat.min⁻¹)</td>
<td>64.0 ± 8.5</td>
<td>62.2 ± 8.5</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>(42.0 - 100.0)</td>
<td>(43.0 - 81.0)</td>
<td></td>
</tr>
</tbody>
</table>

Table 9. Objectively measured physical activity, maximal oxygen uptake and resting heart rate in the South Asian and the European volunteers. * N=173 for VO₂ max N=130 for resting HR. (SD: standard deviation, VO₂ max: maximal oxygen uptake, HR: heart rate, MVPA: moderate to vigorous physical activity, MVPA.MET: moderate to vigorous physical activity expressed in metabolic equivalents (MET)).
Table 10. Self reported physical activity using the long version of International Physical Activity Questionnaire (IPAQ) in the South Asian and the European participants. *Walking time is presented separately and not included in moderate time but is included in total MVPA as it is considered a moderate intensity activity. (SD: standard deviation, MVPA: moderate to vigorous physical activity, MVPA.MET: moderate to vigorous physical activity expressed in metabolic equivalents (MET)).

### 4.3.5 Body composition

The South Asian women were lighter but shorter with greater waist to hip ratio, larger amount of subcutaneous truncal fat defined by the summation of central skinfolds and thinner calves than their European counterparts of similar age and BMI, in keeping with an android fat distribution phenotype. Table 11 and Table 12 present a detailed description of the body composition in the two groups.
Table 11. Body composition characteristics of the South Asian and the European participants aging from 18 to 70 years.

<table>
<thead>
<tr>
<th></th>
<th>South Asians</th>
<th>Europeans</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>91</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.3 ± 14.6 (18.4 - 70.8)</td>
<td>47.9 ± 13.7 (19.8 - 69.7)</td>
<td>0.77</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.0 ± 11.4 (48.0 - 97.4)</td>
<td>69.1 ± 12.2 (45.9 - 121.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.58 ± 0.06 (1.47 - 1.74)</td>
<td>1.64 ± 0.06 (1.48 - 1.79)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>26.1 ± 4.9 (18.0 - 42.7)</td>
<td>25.9 ± 4.5 (19.1 - 41.2)</td>
<td>0.87</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>80.4 ± 11.9 (61.0 - 112.0)</td>
<td>78.0 ± 10.1 (57.4 - 116.0)</td>
<td>0.17</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>98.0 ± 8.4 (84.0 - 127.2)</td>
<td>98.6 ± 7.6 (82.6 - 127.0)</td>
<td>0.56</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.82 ± 0.08 (0.66 - 1.04)</td>
<td>0.79 ± 0.07 (0.66 - 1.00)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

N = 44 South Asians and n = 55 Europeans had MRI measurement of visceral, subcutaneous and liver fat (Table 12). The women with MRI measurements had similar BMI (27.3 ± 5.2 kg.m⁻² for the South Asians versus 27.1 ± 4.5 kg.m⁻² for the Europeans, p = 0.88), waist circumference (84.1 ± 11.8 cm for the South Asians versus 81.8 ± 9.9 cm for the Europeans, p = 0.30) and subcutaneous fat, but the South Asians had substantially larger amount of fat deposited in visceral area (Table 12). The WHR for those South Asians with MRI measurements (n = 44) was greater than that of the Europeans underwent MRI assessment (n = 55) (0.85 ± 0.07 for the South Asians versus 0.81 ± 0.06 for the Europeans, p = 0.02).
Table 12. Body composition characteristics of the middle aged South Asian and European volunteers (over the age of 40 years). (SD: standard deviation) * Central adiposity is the summation of subscapular, suprailliac and suprapinale skinfolds, arm adiposity is the summation of biceps and triceps skinfolds and lower body adiposity is the summation of calf and thigh skinfolds. (Visceral, subcutaneous and liver fat refer to n = 44 for the South Asians and n = 55 for the Europeans, the rest variables were measured to n = 68 for the South Asians and n = 64 for the Europeans).
4.3.6 Metabolic Biomarkers

Table 13 demonstrates the metabolic biomarkers in both ethnic groups. The South Asians despite not being hyperglycaemic compared with the Europeans; they have higher levels of insulin, HOMA<sub>IR</sub>, HbA1c and pro-insulin at fasting state than the Europeans. However the pro-insulin to insulin ratio did not differ among the groups (1.49 ± 0.8 for the South Asians versus 1.28 ± 0.52 for the Europeans, p = 0.13). In addition, the South Asians show an insulin resistance-driven lipid pattern with lower levels of HDL cholesterol and higher levels of plasma triglycerides without having raised total cholesterol or LDL levels. Levels of SHBG in the South Asians are around one third lower than that of the Europeans; with the South Asians having lower levels of total testosterone too. The South Asians display increased levels of CRP and leptin and decreased levels of adiponectin compared with the European volunteers.
<table>
<thead>
<tr>
<th>Glycaemia markers</th>
<th>South Asians</th>
<th>Europeans</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol.l⁻¹)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>0.24</td>
</tr>
<tr>
<td>HbA1c (mmol.mol⁻¹)</td>
<td>36.4 ± 4.9 (19.0 - 47.0)</td>
<td>33.4 ± 3.5 (27.0 - 43.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.48 ± 0.45 (3.88 - 6.45)</td>
<td>5.20 ± 0.31 (4.62 - 6.08)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Insulin (IU.ml⁻¹)</td>
<td>9.8 ± 5.0 (1.7 - 20.0)</td>
<td>7.5 ± 3.6 (1.9 - 20.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>2.2 ± 1.3 (0.5 - 5.5)</td>
<td>1.6 ± 0.9 (0.4 - 4.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>Pro-insulin (pmol.l⁻¹)</td>
<td>13.5 ± 10.2 (4.0 - 63.9)</td>
<td>9.1 ± 4.9 (4.1 - 31.3)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Adipose tissue derived hormones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng.ml⁻¹)</td>
<td>31.3 ± 23.5 (4.5 - 113.0)</td>
<td>23.1 ± 20.2 (4.0 - 122.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Adiponectin (ng.ml⁻¹)</td>
<td>13.2 ± 6.1 (3.2 - 29.5)</td>
<td>16.7 ± 6.2 (4.1 - 29.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol.l⁻¹)</td>
<td>Mean ± SD (Range)</td>
<td>Mean ± SD (Range)</td>
<td>0.22</td>
</tr>
<tr>
<td>HDL (mmol.l⁻¹)</td>
<td>1.5 ± 0.4 (0.9 - 3.7)</td>
<td>1.8 ± 0.4 (0.8 - 3.2)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LDL (mmol.l⁻¹)</td>
<td>3.1 ± 0.8 (1.5 - 5.5)</td>
<td>3.1 ± 0.9 (1.0 - 6.2)</td>
<td>0.88</td>
</tr>
<tr>
<td>TG (mmol.l⁻¹)</td>
<td>1.2 ± 0.7 (0.3 - 4.2)</td>
<td>1.0 ± 0.7 (0.4 - 4.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Cholesterol Ratio</td>
<td>3.7 ± 1.3 (1.9 - 7.5)</td>
<td>3.3 ± 1.2 (1.4 - 8.3)</td>
<td>0.009</td>
</tr>
<tr>
<td>Liver Profile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (IU.l⁻¹)</td>
<td>20.1 ± 5.3 (10.0 - 37.0)</td>
<td>20.4 ± 4.9 (11.0 - 39.0)</td>
<td>0.59</td>
</tr>
<tr>
<td>ALT (IU.l⁻¹)</td>
<td>20.0 ± 9.8 (7.0 - 73.0)</td>
<td>20.5 ± 12.7 (6.0 - 93.0)</td>
<td>0.87</td>
</tr>
<tr>
<td>GGT (IU.l⁻¹)</td>
<td>18.1 ± 14.9 (3.0 - 80.0)</td>
<td>18.3 ± 17.4 (6.0 - 93.0)</td>
<td>0.89</td>
</tr>
<tr>
<td>Albumin (g. l⁻¹)</td>
<td>39.1 ± 3.3 (20.0 - 46.0)</td>
<td>40.9 ± 2.6 (31.0 - 49.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Androgen markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHBG (nmol.l⁻¹)</td>
<td>52.4 ± 28.7 (1.9 - 160.0)</td>
<td>71.0 ± 28.5 (16.1 - 151.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Testosterone (nmol.l⁻¹)</td>
<td>1.3 ± 0.6 (0.4 - 2.9)</td>
<td>1.6 ± 0.6 (0.4 - 2.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>FAI</td>
<td>3.0 ± 2.2 (0.1 - 10.8)</td>
<td>2.6 ± 1.9 (0.6 - 12.9)</td>
<td>0.28</td>
</tr>
<tr>
<td>Inflammation marker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg.l⁻¹)</td>
<td>3.1 ± 3.5 (0.3 - 22.0)</td>
<td>1.8 ± 3.1 (0.2 - 22.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122.2 ± 16.5 (93.0 - 163.0)</td>
<td>123.6 ± 16.8 (86.0 - 167.0)</td>
<td>0.56</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.1 ± 11.1 (49.0 - 98.0)</td>
<td>77.7 ± 9.4 (54.0 - 105.0)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Table 13. Metabolic biomarkers in the South Asians compared with the Europeans. (FAI: Free androgen index, SBP: systolic blood pressure, DBP: diastolic blood pressure, SHBG: Sex hormone binding globulin).
4.3.7 Body composition, lifestyle factors and metabolic markers across the reproductive trajectory in the South Asians and the Europeans

There were 39 premenopausal South Asians and 34 Europeans; 10 perimenopausal South Asians and 14 Europeans; and 42 postmenopausal South Asians and 39 Europeans in our cohort. The distribution of age was similar among the two ethnic groups in each reproductive category. Premenopausal status was defined when women reported regular menses and blood results revealed FSH $\leq 10$ IU.l$^{-1}$ (when measured up to day 5 of the cycle). Perimenopausal status was defined when women had irregular/infrequent menses (significant change in the menstrual frequency over the last year) and/or menopausal symptoms but the time since their last menstrual period was under a year and FSH $> 10$ IU.l$^{-1}$. Postmenopausal status was defined when women reported over a year since their last menstrual period. Women with a history of hysterectomy were classified as postmenopausal when FSH $\geq 40$ IU.l$^{-1}$ or when combined with a bilateral oophorectomy.

4.3.7.1 Body composition

Table 14 shows the mean values in body composition variables (BMI, waist circumference, hip circumference, waist to hip ratio) across the reproductive categories in both the South Asian and the European volunteers. All variables increase to an almost similar degree in both ethnic groups (p for interaction of menopause with ethnicity $> 0.05$ for each association), hence ethnicity does not modify the degree of association between menopausal state and body fatness variables. Figure 20 demonstrates the mean values and 95 % CI along with menopausal state.
Figure 20. BMI, waist circumference (top panel), waist to hip ratio and hip circumference (bottom panel) in relation to menopausal state in the South Asian and the European volunteers. (Bars express mean values +/- 95% confidence intervals).

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Premenopausal</th>
<th>Perimenopausal</th>
<th>Postmenopausal</th>
<th>p for trend</th>
<th>p for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europeans</td>
<td>34</td>
<td>14</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Asians</td>
<td>39</td>
<td>10</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europeans</td>
<td>34.3 (10.4)</td>
<td>51.4 (3.4)</td>
<td>58.5 (6.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Asians</td>
<td>33.7 (10.3)</td>
<td>48.8 (3.8)</td>
<td>59.5 (6.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
<td>0.98</td>
</tr>
<tr>
<td>Europeans</td>
<td>23.5 (2.8)</td>
<td>25.8 (3.7)</td>
<td>28.0 (4.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Asians</td>
<td>23.8 (3.5)</td>
<td>26.0 (5.7)</td>
<td>28.1 (5.0)</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Waist (cm)</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
<td>0.82</td>
</tr>
<tr>
<td>Europeans</td>
<td>71.7 (5.9)</td>
<td>78.8 (7.5)</td>
<td>83.1 (10.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Asians</td>
<td>73.4 (8.3)</td>
<td>81.0 (13.4)</td>
<td>86.7 (11.0)</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Hips (cm)</td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
<td>0.70</td>
</tr>
<tr>
<td>Europeans</td>
<td>95.7 (6.3)</td>
<td>98.7 (5.7)</td>
<td>101.1 (8.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Asians</td>
<td>96.3 (7.0)</td>
<td>96.8 (8.6)</td>
<td>99.9 (9.3)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
<td>0.25</td>
</tr>
<tr>
<td>Europeans</td>
<td>0.75 (0.05)</td>
<td>0.80 (0.05)</td>
<td>0.82 (0.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Asians</td>
<td>0.76 (0.06)</td>
<td>0.83 (0.09)</td>
<td>0.87 (0.06)</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Table 14. Age and body composition characteristics across reproductive stages in both the South Asian and the European volunteers. P for trend suggests if there is evidence of a trend in each variable across the different reproductive stages. P for interaction suggests if there is evidence of an interaction between ethnicity and menopausal state. P values resulted from regression models including ethnicity, menopausal state and the interaction term as independent variables. Values are expressed in mean (Standard deviation, SD).
4.3.7.2 Diet, physical activity and fitness

Table 15 and Figure 21 demonstrate the changes in objectively measured physical activity variables, fitness and dietary energy intake across reproductive stages in the South Asians and the Europeans. Ethnicity does not modify the relationship between menopausal state and lifestyle factors (p for interaction > 0.05). Interestingly, MVPA (and hence energy expenditure) decreases progressively with menopausal transition but energy intake remains unaltered.

Table 15. Physical activity variables, fitness and daily energy intake across reproductive stages in both the South Asian and the European volunteers. P for trend suggests if there is evidence of a trend in each variable across the different reproductive stages. P for interaction suggests if there is evidence of an interaction between ethnicity and menopausal state. P for interaction resulted from regression models including ethnicity, menopausal state and the interaction term as independent variables. Values are expressed in mean (SD) unless otherwise stated. (MVPA: moderate to vigorous physical activity, VO\textsubscript{2}\text{max}: maximal oxygen uptake).
Figure 21. Moderate to vigorous physical activity (MVPA), sedentary time (top panel), maximal oxygen uptake (VO₂ max) and dietary energy intake (bottom panel) in relation to menopausal state in both the South Asian and the European volunteers. (Bars express mean values +/- 95% confidence intervals).

4.3.7.3 Metabolic biomarkers

Table 16 and Figure 22 demonstrate the levels of metabolic biomarkers across the different reproductive stages. Most of the metabolic variables (apart from HDL) change significantly with reproductive ageing. Interestingly, ethnicity modifies the relationship between menopausal state and increasing levels of HbA1c with the South Asians having a substantially larger increase in HbA1c with menopausal transition compared with the Europeans (beta coefficient for menopausal state 0.31 (95% CI:0.23, 0.39) for the South Asians versus 0.17 (95% CI:0.10, 0.23) for the Europeans).
Table 16. Metabolic biomarkers across reproductive stages in the South Asian and the European volunteers. P for trend suggests if there is evidence of a trend in each variable across the different reproductive stages. P for interaction suggests if there is evidence of an interaction between ethnicity and menopausal state. P for interaction resulted from a regression model including ethnicity, menopausal state and the interaction term as independent variables. Values are expressed in mean (SD) unless otherwise stated. (FPG: fasting plasma glucose, HOMA\textsubscript{IR}: insulin resistance index, BP: blood pressure).

<table>
<thead>
<tr>
<th></th>
<th>Ethnicity</th>
<th>Premenopausal</th>
<th>Perimenopausal</th>
<th>Postmenopausal</th>
<th>p for trend</th>
<th>p for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin (IU.ml\textsuperscript{-1})</strong></td>
<td>Europeans</td>
<td>6.8 (2.5)</td>
<td>6.2 (2.2)</td>
<td>8.6 (4.5)</td>
<td>0.16</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>South Asians</td>
<td>8.5 (4.6)</td>
<td>9.3 (5.7)</td>
<td>11.1 (4.9)</td>
<td><strong>0.02</strong></td>
<td></td>
</tr>
<tr>
<td><strong>FPG (mmol.l\textsuperscript{-1})</strong></td>
<td>Europeans</td>
<td>4.6 (0.4)</td>
<td>4.8 (0.2)</td>
<td>5.1 (0.5)</td>
<td><strong>0.005</strong></td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>South Asians</td>
<td>4.8 (0.5)</td>
<td>4.9 (0.6)</td>
<td>5.2 (0.7)</td>
<td>&lt; <strong>0.0001</strong></td>
<td></td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>Europeans</td>
<td>5.0 (0.3)</td>
<td>5.2 (0.3)</td>
<td>5.4 (0.3)</td>
<td>&lt; <strong>0.0001</strong></td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>South Asians</td>
<td>5.2 (0.3)</td>
<td>5.5 (0.4)</td>
<td>5.8 (0.3)</td>
<td>&lt; <strong>0.0001</strong></td>
<td></td>
</tr>
<tr>
<td><strong>HOMA\textsubscript{IR}</strong></td>
<td>Europeans</td>
<td>1.4 (0.5)</td>
<td>1.3 (0.5)</td>
<td>2.0 (1.1)</td>
<td><strong>0.03</strong></td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>South Asians</td>
<td>1.8 (1.1)</td>
<td>2.1 (1.5)</td>
<td>2.7 (1.3)</td>
<td><strong>0.004</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol.l\textsuperscript{-1})</strong></td>
<td>Europeans</td>
<td>4.7 (0.6)</td>
<td>5.3 (0.6)</td>
<td>5.9 (0.8)</td>
<td>&lt; <strong>0.0001</strong></td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>South Asians</td>
<td>4.8 (1.0)</td>
<td>4.9 (0.8)</td>
<td>5.6 (1.0)</td>
<td>&lt; <strong>0.0001</strong></td>
<td></td>
</tr>
<tr>
<td><strong>HDL (mmol.l\textsuperscript{-1})</strong></td>
<td>Europeans</td>
<td>1.8 (0.4)</td>
<td>1.9 (0.5)</td>
<td>1.7 (0.4)</td>
<td>0.25</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>South Asians</td>
<td>1.5 (0.4)</td>
<td>1.4 (0.28)</td>
<td>1.5 (0.5)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td><strong>Triglycerides (mmol.l\textsuperscript{-1})</strong></td>
<td>Europeans</td>
<td>0.8 (0.3)</td>
<td>0.9 (0.3)</td>
<td>1.3 (0.9)</td>
<td><strong>0.001</strong></td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>South Asians</td>
<td>0.9 (0.5)</td>
<td>1.2 (0.6)</td>
<td>1.5 (0.8)</td>
<td>&lt; <strong>0.0001</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>Europeans</td>
<td>114.8 (9.9)</td>
<td>122.1 (14.8)</td>
<td>131.8 (18.4)</td>
<td>&lt; <strong>0.0001</strong></td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>South Asians</td>
<td>110.4 (11.0)</td>
<td>120.5 (11.8)</td>
<td>133.5 (13.8)</td>
<td>&lt; <strong>0.0001</strong></td>
<td></td>
</tr>
</tbody>
</table>
Figure 22. Insulin resistance, insulin levels (top panel), HbA1c and fasting plasma glucose levels (second panel), total cholesterol and HDL levels (third panel), triglyceride levels and systolic blood pressure (bottom panel) in relation to menopausal state. (Bars express mean values +/- 95% confidence intervals, BP: blood pressure, HDL: high density lipoprotein).
4.4 Summary of key result findings

4.4.1 Lifestyle factors

- Daily energy intake and diet density did not differ among the South Asians and the Europeans.

- The South Asians engaged less time daily with the beneficial MVPA and more time with light intensity activities (measured objectively) compared with the Europeans.

- The South Asians had lower levels of physical fitness compared with their European comparators.

4.4.2 Body composition

- The South Asian women were shorter and lighter but had greater central adiposity (measured by waist to hip ratio or central skinfolds) than the Europeans for a given BMI.

- MRI measurement of central adiposity demonstrated that the South Asians had similar adiposity stored at subcutaneous compartments but greater adiposity in deep or ectopic compartments (visceral tissues and liver) compared with the Europeans of similar BMI and age.

4.4.3 Metabolic Biomarkers

- The healthy non diabetic South Asians had greater fasting insulin, pro-insulin and HbA1c levels compared with the European women. They were more insulin resistant for a given level of fasting glycaemia.

- The South Asians demonstrated decreased levels of adiponectin and increased levels of leptin and CRP.

- The South Asians showed an atherogenic lipid profile with lower levels of HDL cholesterol and higher levels of triglycerides than the Europeans.

- The South Asians had lower SHBG and testosterone levels.
Blood pressure, fasting glycaemia, LDL and total cholesterol levels did not differ among the two ethnic groups.

### 4.4.4 Metabolic risk factors across reproductive stages

- Physical activity and fitness (energy expenditure) diminish with reproductive ageing in both ethnic groups but dietary intake remains unchanged.

- Central adiposity and BMI increase in both ethnic groups along with menopausal transition.

- Metabolic biomarkers deteriorate along with reproductive ageing to a similar degree in both ethnic groups, with the exception of HbA1c which increases to a substantially larger extent in the South Asians compared with the Europeans.
4.5 Discussion

This chapter describes the baseline metabolic risk factors, lifestyle characteristics and adiposity distribution of the study volunteers of South Asian origin living in Scotland in comparison with the European counterparts of similar BMI and age. The volunteers of South Asian origin exhibit greater fasting insulinaemia without associated increased glycaemia, have lower levels of serum HDL and higher levels of triglycerides, accumulate more fat centrally and ectopic tissues for a given BMI, engage less frequently with moderate and vigorous intensity activities and have decreased oxidative capacity during submaximal exercising compared with the volunteers of White European descent. Interestingly, for a given BMI and amount of subcutaneous fat (measured by MRI) South Asians accumulate more fat intra-abdominally and in ectopic tissues (liver). The above differences are independent of dietary intake with the South Asians in our study having adopted a similar diet density to the European counterparts. Metabolic and lifestyle risk factors deteriorate across the reproductive stages, but the degree of change is similar in both ethnic groups (with the exception of HbA1c).

4.5.1 Body composition

The detailed body composition phenotype suggests that South Asian women, despite their smaller body size, store more fat centrally resulting in a greater waist to hip ratio, central skinfolds and intraabdominal fat but have slimmer calves compared with the European volunteers of similar BMI. An early study suggested that South Asian women living in London had greater waist to hip ratio, subcuticular and suprailliac skinfolds, which both are considered central skinfolds, compared with Europeans living in the same region. However, European women in the latter study had lower average BMI than South Asians which may have contributed to the lower central adiposity in this ethnic group (115). A subsequent study confirmed that lean South Asian women had a larger amount of intraabdominal fat, not directly associated with the waist circumference, and total body fat compared with BMI matched Europeans, suggesting that South Asian women for a given BMI have greater localised and total adiposity predisposing them to insulin resistance and an adverse metabolic profile (93). Further studies confirmed the above observations and suggested ethnic specific cut off points for BMI and waist circumference, and indeed lower thresholds for South Asians, in order to identify individuals from different ethnic groups with increased metabolic risk (40, 41, 314).
A comparable study to ours in men observed a similar excess of an average of 0.3 units in the waist to hip ratio in South Asian men compared with Europeans (207). However, Ghouri et al demonstrated excess subcutaneous fat (measured by skinfolds) for all upper body, truncal and lower body skinfold measurements in South Asian men as opposed to the current study which showed a marked difference only in truncal skinfolds among women (207). By comparing the two cohorts (current and (207)), although the mean BMI does not differ among men and women, I observed that women of both ethnic groups have substantially higher levels of regional adiposity measured by skinfold thickness compared with men. This observation is in line with the findings of a previous study comparing South Asian men and women (without an interethnic comparator group) indicating that South Asian women had 1.5 to 2-fold greater localised (measured by skinfolds) and total body fat (measured by Dual Energy X-Ray Absorptiometry (DEXA) scanner) than South Asian men of similar BMI (315). I am not aware of a previous study showing smaller calves, but similar thighs, in South Asian women compared with Europeans, but this can be an additional feature suggesting a phenotype closer to the android type of obesity in South Asians.

The use of optimal imaging techniques allows us to visualise and directly measure adiposity in different central compartments. Visceral fat and fat accretion in other ectopic tissues (i.e. liver, intramuscular) are considered more metabolically active than superficial fat predisposing to CHD and type 2 diabetes (316). The data in South Asians and adipose tissue compartmentalisation are limited and seem conflicting; Forouhi et al firstly measured visceral fat with the use of CT in overweight middle aged women of South Asian descent (n = 28) and showed that they had greater visceral fat which was associated with increased CRP levels compared with the Europeans (93), however they did not measure fat storage in other body parts. Subsequent studies in young slim South Asian men (n = 29) (230) and young overweight men and women (n = 32 men and n = 24 women) (232) did not demonstrate ethnic differences in intra-abdominal fat or liver fat accumulation measured with the use of MRI. Interestingly, the European participants had greater subcutaneous adiposity but the South Asians had greater adipocyte hypertrophy possibly accounting for the excess insulin resistance seen in the young South Asians (230, 232). A larger study assessing central adiposity in overweight middle aged men and women (n = 103 South Asian women, mean age 45 years) with the use of CT enabled better visualisation of the fascia superficialis (compared with the use of MRI) in the abdomen and hence subsequent classification of subcutaneous fat in deep and superficial compartments (231). The latter study demonstrated that the South Asians, despite having comparable
absolute values of superficial and visceral fat to the Europeans of similar age and BMI, have greater superficial, deep subcutaneous and visceral fat for a given amount of fat free mass compared with the Europeans (231). The current MRI data add to the interpretation of the existing apparently conflicting literature; I assessed with MRI imaging slightly older women (mean age 54.8 years) and demonstrated that the South Asians had greater visceral and liver fat accretion but similar subcutaneous fat storage compared with the women of White descent of similar age and BMI. Hence, my study supports that there is evidence of ethnic differences in body fat accumulation which only become apparent with increasing BMI or/and with advancing age, suggesting an evolutionary aspect in the theory of fat compartmentalisation. This should be substantiated in prospective studies examining the impact of ethnicity on fat distribution following weight gain or advancing age.

4.5.2 Physical Activity and fitness

The South Asian women in the current cohort demonstrated lower levels of MVPA compared with European volunteers; a finding which is consistent with suggestions from self reported data (206), but the current study is the first to confirm this by using robust objective methodology. Sedentary time did not differ between the two ethnic groups, but the South Asians engaged more time with light activities and less time with moderate intensity activities compared with the Europeans. Childcare and light housework are likely to be the most frequent activities of South Asians, considering that the majority of South Asian volunteers were not employed outside the home, an activity pattern observed in previous studies too (317, 318). In contrast, self reported data showed that moderate activity was equal between the two ethnic groups, suggesting that South Asians are more likely to perceive light activities as moderate intensity exercise and overestimate the amount of beneficial MVPA they perform daily. The diverse perception of South Asians towards regular exercising needs to be addressed when interventional studies with an activity arm targeting this ethnic group are planned (319).

Furthermore, the predicted maximal oxygen uptake during exercising was marginally lower in the women of South Asian origin participating in our study compared with that of the European volunteers. The inverse association between fitness and insulin resistance is well described (311) and further analysis is warranted in our study to reveal potential inter-ethnic discrepancies in the magnitude of the association. Previous studies in men showed substantially diminished cardio-respiratory levels in South Asians compared to Europeans, which accounted for most of the excess in insulin resistance in South Asian men (207,
In South Asian women, data on fitness are limited with a small study (in total n = 32 women of both ethnic groups) demonstrating that South Asian women aged 35-49 years had in average 5 mls.kg\(^{-1}\).min\(^{-1}\) lower oxygen uptake during maximal exercising compared with Europeans of similar age (213), but Europeans were substantially slimmer too which may have confounded the extent of the difference.

### 4.5.3 Biomarkers

Mean serum insulin levels were around 1.3 times higher in the fasting state in the South Asian volunteers than in the Europeans in keeping with the early findings of the Southall study which demonstrated a similar relationship in both men and women (115), or more recent studies including South Asian women (n = 28) (93). Notably, fasting plasma glucose levels did not differ among the two ethnic groups, in contrast to previous studies in men (115, 207), suggesting that for a given level of plasma glucose, South Asian women secrete higher levels of insulin than Europeans possibly related to the fact that South Asians exhibit substantial resistance to insulin-stimulated glucose uptake (320). HOMA\(_{IR}\), a measure of insulin resistance, was impaired in the South Asian women of my study too. In addition, HbA1c was greater in the South Asians, a relationship demonstrated in an earlier study in men (207), but the value of HbA1c has been questioned recently as a marker of glycaemia in inter-ethnic comparisons with studies suggesting that there is an ethnic predisposition to higher HbA1c among South Asians with normal (321) or impaired glucose tolerance test (322).

The pattern of lower levels of HDL cholesterol and higher levels of plasma triglycerides, observed previously in men of South Asian origin (115, 323) and reproduced in a smaller study including women (n = 9 South Asian women) (320), is evident in our study too. This lipid profile is more likely to be linked with the insulin resistance phenotype (324). Although, cross sectional studies cannot postulate a causal relationship between lipid profile and insulin resistance, it has been shown that hepatic secretion of triglycerides and subsequent plasma triglycerides concentrations are directly related with the degree of hyperinsulinaemia (325) and possibly mediated through the increased release of non-esterified fatty acids (NEFA) from intra-abdominal fat cells (326). Hyperinsulinaemia, also, stimulates the catabolic rate of HDL resulting in decreasing levels of serum HDL cholesterol (327).
A striking finding of our study is that the levels of SHBG are substantially decreased in the serum of South Asian women compared with that of Europeans. The principal function of SHBG is considered to be that of a transport protein for sex steroids, however, recent studies suggest an inverse association between SHBG and glycaemia (measured by HbA1c) (328), liver fat accretion (329) or risk of type 2 diabetes (249). A meta-analysis including data from 10 prospective studies demonstrated that women with serum SHBG greater than 60 nmol/L had an 80% reduction in the risk of type 2 diabetes compared with women with lower values (249). Although the existence and direction of causality between SHBG levels and insulin resistance has yet to be revealed, SHBG may play a role as a potential predictor of type 2 diabetes (330), a role reinforced further by its minimal cyclical or fasting state variation. I am not aware of a study measuring SHBG levels in women of South Asian origin, but it has been shown that the levels of serum SHBG are not affected by menopausal status (329, 331) or ethnicity (comparing African American with Caucasians) (329), rendering its use as a potential biomarker of type 2 diabetes or metabolic syndrome across different age and ethnic groups more favorable. Notably, serum testosterone levels were lower in the South Asian volunteers, a pattern which is in contrast with previous data suggesting that increased levels of bioavailable testosterone along with decreased levels of serum SHBG in women were independent predictors of the metabolic syndrome, truncal adiposity measured by waist circumference and visceral fat accumulation (250, 332). Absolute levels of serum testosterone are known to decline with aging in women (333), however, the aging effect is expected to have negligible impact on the inter-ethnic difference of testosterone levels in our study as the average age did not differ between the two groups.

Moreover, the women of South Asian origin who participated in the current study had substantially diverse levels of adipocyte-derived and pro-inflammatory hormones compared with their Europeans comparators; adiponectin levels were lower and leptin and CRP levels were higher in the South Asians. Adiponectin, an adipocyte derived hormone, is shown to have an inverse association with BMI, intra-abdominal fat, atherogenic lipid profile (low HDL, high triglycerides) and hyperglycaemia in middle aged Caucasian women (334), suggesting that the link between visceral fat and insulin resistance syndrome is partially mediated via adiponectin. In addition, there is a large body of evidence suggesting that decreased adiponectin levels are associated with future incidence of type 2 diabetes (335, 336). Similarly, leptin is also secreted by adipocytes and is strongly associated with fasting insulin levels independent of BMI or truncal adiposity (measured by waist to hip ratio) (337). In addition, leptin is associated with fasting glycaemia, plasma
cholesterol, plasma triglycerides and risk of CHD, with the latter association persisting after adjustment for BMI (338). Data on these adipose metabolites in South Asian women, who are known to be more insulin resistant and to store more fat than their Europeans counterparts, are scanty. A small study comparing 28 South Asian women with 51 Caucasian women exhibited lower levels of serum adiponectin and increased levels of serum leptin which were consistent with greater insulin resistance in South Asians, however the South Asians were significantly heavier in that cohort (124). Another study comparing 24 younger and slimmer South Asian women with White Europeans demonstrated a similar pattern in adiponectin levels to ours (232). Similarly, a population based study demonstrated that Indian women had lower levels of adiponectin and were more insulin resistant compared with Chinese and Malays living in Singapore, an association potentially confounded by the greater BMI and excess truncal fat observed in South Asians (339). Notably, in the same study adiponectin levels were inversely associated with BMI and insulin resistance in all ethnic groups, but ethnicity modified significantly the angle of these relationships (339). A cross-sectional study comparing young slim Indian men with Caucasians matched for total body fat showed that Asians had lower levels of adiponectin and higher levels of leptin, both were associated with insulin resistance and the associations persisted after adjustment for truncal adiposity and waist circumference (130). Hence, the authors concluded that impaired adipose tissue metabolism, rather than greater regional adiposity, in South Asians could be a biological explanation of their findings (130), however, they did not study the effect of altered fat compartmentalisation on the levels of adipokines among the two ethnic groups. In addition, it has been shown that the levels of CRP, a marker of low grade chronic inflammation, are correlated with the levels of leptin (338), the biological plausibility of which is linked with the fact that both leptin and cytokines (such as IL-6 (340)) promoting hepatic CRP production are secreted by adipocytes. The increased CRP levels among the South Asian women in the current study accompanied the increased levels of leptin. This finding is in line with previous data suggesting that South Asian middle aged women had higher levels of CRP compared with Europeans which were strongly associated with visceral fat and waist girth but not with total adiposity and BMI (93).

Women of South Asian origin in our study exhibited lower mean levels of serum albumin without the levels of the rest liver markers (i.e. ALT, AST, GGT) being impaired, suggesting that there is a potential difference among the two ethnic groups in the excretion of albumin through the kidneys rather than in the production of albumin through the liver. We are not aware of a similar trend being evident in previous inter-ethnic studies involving
healthy volunteers, however, it has been demonstrated that central adiposity, assessed with an increased waist to height ratio, is associated with raised albumin to creatinine ratio in urine across different ethnic groups and this relationship is independent of pre-existing co-morbidities such as diabetes (341).

4.5.4 Changes with menopausal transition

Weight gain and increasing central adiposity along with ovarian ageing and menopausal transition has been shown previously not only in women from westernised countries (263, 264) but in women residing in the Indian subcontinent (266), however, the existence of a causal relationship between fatness and menopause has yet to be confirmed and it is still unclear whether the ageing effect confounds the above relationship. Although my study is limited by its design and cross-sectional nature to answer this, it showed that increasing BMI and waist circumference concurs with reproductive ageing to a similar degree in both the South Asians and the Europeans. There are different theories explaining the tendency of aging women to gain weight and store more fat; decreasing physical activity is evident in older women in our study and others (342), potentially contributing to weight gain (343). Sex hormones modulate energy intake and expenditure, hence the rapid oestrogenic decrease with menopausal transition could account for attenuated resting metabolic rate and increase in appetite in menopausal women (344). The menopausal women in the current cohort had comparable energy intake to the younger ones, however, when this is accompanied with decreased activity, and thereby lower energy expenditure, it is expected to lead to weight gain. In addition, oestrogen depletion inhibits the activity of lipoprotein lipase in femoral adipocytes and lipolysis in abdominal adipose tissue, predisposing to accumulation of abdominal fat (android-apple shaped) as opposed to gluteo-femoral adipose tissue (gynoid-pear shaped) (345) which is in line with the finding of increasing WHR and waist circumference in the peri and post-menopausal women.

Levels of cardiorespiratory fitness and physical activity declined substantially with the transition from pre-menopause to peri-menopause but tapered off thereafter. This is in line with the findings of a large study modelling the age-associated longitudinal trends in cardiorespiratory fitness and leisure-time physical activity in 1467 women aged 30-79 years (346). This leads to the postulation that the changes in physical activity patterns may not only reflect the impact of ageing or menopause but the concomitant effect of life events e.g. the birth of a grandchild that may contribute to a smaller net decrease in physical activity with menopausal transition (347).
An interesting finding of this study was that menopausal transition had a much greater impact on the increasing HbA1c levels in the South Asians than in the Europeans (significant ethnicity*menopause interaction), which has not been shown, to my knowledge, before. However, this study was limited by its design to eliminate the effect of ageing on the above relationship. The rest of the biomarkers deteriorated along with reproductive ageing to the same degree in both ethnic groups.

4.5.5 Strengths and weaknesses

The strengths of the current study yield in the use of objective methodology in assessing physical activity and the use of optimal imaging techniques (MRI, liver spectroscopy) for measuring central adiposity in a relatively large cohort of South Asian and European women well phenotyped for baseline characteristics. I acknowledge that the use of a submaximal test for assessing cardio-respiratory fitness may have influenced the findings but it has been shown that the Chester step test correlates well with tests measuring directly the maximal oxygen uptake (278). In addition, insulin resistance in the current study was assessed with the use of the HOMA\textsubscript{IR} model rather than by using the gold-standard euglycaemic hyperinsulinaemic clamp, however, HOMA\textsubscript{IR} is a widely acceptable model which correlates well with clamp-derived measures of insulin resistance (77). Assessment of diet with the use of self-reported food frequency questionnaires has limitations as the responses are subject to recall bias and desirability effect; however, there is no clear evidence suggesting the superiority of 24 hour recall diaries over the food frequency questionnaires (348). Lastly, the assessment of metabolic risks along with reproductive stages is limited by the cross-sectional nature of our data so longitudinal inference would be inappropriate. However, the data were analysed in that way to examine whether ethnicity modifies the relationship between reproductive ageing (without being able to discriminate it from chronological ageing) and metabolic risks.

4.6 Conclusion

I conclude that South Asian women in the current study have an adverse metabolic profile with greater glycaemic indices, central adiposity, ectopic fat accretion, increased adipokines and atherogenic lipid profile than their European counterparts. In regards to modifiable lifestyle factors, South Asians spent less time performing moderate to vigorous activities and exhibit lower levels of cardio-respiratory fitness. The extent of the effect of both physical activity and fitness on the excess metabolic risk of South Asians will be
investigated in the following chapter. However, it is noteworthy that other conventional risk factors known to affect the metabolic risk of individuals do not differ, or indeed are more favourable in South Asians; smoking and alcohol consumption were less prevalent among the South Asians compared with the Europeans. Socioeconomic status did not differ between the two groups with the exception of South Asians reporting less years of formal education, a discrepancy partially mirroring the different educational systems in different countries. Both groups in our study consumed similar density diets which, initially, seems to conflict the findings of a previous multi-ethnic study conducted in Glasgow between 1995-1996 suggesting that first generation South Asians displayed a more atherogenic diet with greater proportion of total and saturated fat compared with the general population (221). However, this finding may be in agreement with the evolutionary theory in diet proposed by the authors. In the following chapter, I will subsequently investigate how modifiable risk factors such as adiposity and low levels of physical activity interrelate with the impaired insulin metabolism and excess metabolic risk in the South Asian women compared with the Europeans.
ASSOCIATIONS OF LIFESTYLE FACTORS AND ADIPOSITY WITH INSULIN RESISTANCE, METABOLIC RISK AND GLYCAEMIA INDICES

5.1 Introduction

South Asians living in the UK exhibit 3 to 5-fold higher risk of developing type 2 diabetes (28, 115) and 2-fold higher risk of cardiovascular disease (CVD) compared with their European counterparts (21). Although conventional risk factors increase the background risk of different ethnic groups, they do not account for the excess risk of South Asians compared with the background population of white European descent (36, 48). South Asians in the UK show a predisposition in clustering of risk factors, which independently and collectively contribute to developing type 2 diabetes and CVD (19). A multi-ethnic UK based study with 4,860 participants showed there is significant interaction with ethnicity and sex in the prevalence of metabolic syndrome and the association of metabolic risk factors with coronary heart disease (CHD) (defined by major electrocardiograph (ECG) changes), with South Asian women exhibiting an age-standardised prevalence of metabolic syndrome defined by the National Cholesterol educational Program (NCEP) criteria of 31.8 % compared to 28.8 % in South Asian men or 14.4 % in European women (19). Hence, South Asian women seem to have more risk factors rendering them more susceptible to type 2 diabetes and CVD, but they have been largely overlooked to date.

There is accumulating evidence, resulting largely from studies in white participants, suggesting that increasing BMI and truncal adiposity are associated with glycaemia, insulin resistance (310, 349) and the risk of developing diabetes (350, 351). The causal relationship between adiposity and insulin resistance is supported by interventional studies suggesting that even a 7 % weight loss can ameliorate the lipid and glycaemic indices of non diabetic obese individuals (352). Women are more susceptible to the metabolic consequences of increasing central adiposity with 11 cm increase in waist circumference resulting in an almost 2.5-fold increase in their background risk of developing diabetes compared with 1.95-fold in men (350). In addition, weekly engagement with physical activity, increases insulin sensitivity in non diabetic subjects (353, 354), decreases insulin resistance (355) and attenuates an individual’s risk of developing diabetes (309). Moderate activity is protective against the risk of developing diabetes in men and, to a lesser degree, in women (13% versus 7% risk reduction respectively) with the sex difference disappearing after adjustment for central adiposity (309). Furthermore, increasing
cardiorespiratory fitness is linearly associated with increasing insulin sensitivity (211) whereas low fitness levels increase an individual’s risk of developing future diabetes by almost 2-fold (311) and decreasing cardiorespiratory fitness driven by advancing age results in an atherogenic lipid profile characterised by low HDL levels (310).

Therefore, the above associations of adiposity and modifiable lifestyle factors with insulin resistance, glycaemia and conventional metabolic biomarkers (such as lipids) are established but have not been extensively examined in ethnic and sex minorities known to be at excess risk of disease burden, such as in South Asian women living in the UK. It was demonstrated in chapter 4 that the women of South Asian origin, albeit similar age, BMI and dietary intake to the European volunteers, accumulated more fat centrally, were less active spending less time daily on moderate to vigorous physical activity (MVPA), were less fit and exhibited a metabolic profile consistent with greater insulin resistance characterised by higher fasting insulin, lower HDL levels, higher triglycerides levels and higher glycated haemoglobin (HbA1c) compared with the Europeans counterparts. Fasting glucose concentration did not differ, though, among the groups. Hence, the South Asian volunteers in the current study displayed an adiposity phenotype and lifestyle profile more compatible with insulin resistance; however, it is unclear whether adiposity and lifestyle factors have a similar effect on glycaemia, insulin resistance and metabolic risk in both ethnic groups or whether ethnicity modifies the magnitude of the effect of the above modifiable factors on metabolic indices.

The aim of this chapter is to examine the associations of adiposity and modifiable lifestyle factors with fasting glycaemia, HbA1c, insulin resistance (assessed by HOMA\textsubscript{IR}) and metabolic risk in a non diabetic relatively healthy cohort of women and test whether South Asian ethnic background alters these associations. A secondary objective of this chapter is to investigate the relationship between HbA1c and fasting glycaemia in both ethnic groups. I hypothesise that the impact of lifestyle factors and adiposity on glycaemia and insulin resistance indices is greater in the South Asian women than in the Europeans.
5.2 Methods

5.2.1 Participants and recruitment

All participants were recruited to this study via general advertisement and word of mouth. South Asian (defined as having both parents of Indian, Pakistani, Bangladeshi or Sri Lankan origin) and European women (having both parents of white European origin) aged 18-70 completed years, without coronary heart disease (CHD, symptoms of angina, previously diagnosed myocardial infarction or ischemic heart disease), cerebrovascular disease (previously diagnosed stroke or transient ischemic event), diagnosed diabetes or diagnosed polycystic ovary syndrome (PCOS) were recruited to undertake a number of investigations as described below and in the general methodology chapter 2. Women who, following the assessment, were found to have high HbA1c or fasting plasma glucose (FPG) (HbA1c ≥ 6.5 % or FPG ≥ 7.0 mmol.l⁻¹) were excluded from the analysis and were referred to their general practitioner for further investigations to rule out diabetes (313). The study was granted Ethics permission by the West of Scotland Ethics Research Committee 3.

Participant’s health history, including smoking status, alcohol consumption, current medications, years of education and menstrual history were determined by questionnaires. Dietary intake was self-reported by the participants filling a 120-item food frequency questionnaire. Socioeconomic status was determined by the Scottish Index of Multiple Deprivation (SIMD) 2012 score which uses the postcode of residence to categorise people living in Scotland in quintiles, with 1 representing the most deprived areas and 5 the most affluent ones.

5.2.2 Anthropometry

Height, weight, waist and hip circumference were measured by the principal researcher using international standard guidelines (detailed description in chapter 2). Volunteers over the age of 40 had a detailed assessment of body fat distribution accomplished by measuring skinfold thickness at seven body sites (biceps, triceps, subscapular, suprailiac, supraspinale, thigh and calf) and additional circumferences at three sites (mid-upper arm, mid-thigh and mid-calf) by the same investigator. They were also offered magnetic resonance imaging (MRI) assessment of truncal adiposity (subcutaneous and visceral fat at level L3-L4) and MR spectroscopy of the liver (detailed description in chapter 2). Body mass index (BMI) was defined by the equation BMI = weight (kg) / height (m)². Upper
arm adiposity was defined as the summation of biceps and triceps skinfolds, lower body adiposity was defined as the summation of thigh and calf skinfolds and central adiposity as the summation of suprailiac, supraspinale and subscapular skinfolds.

### 5.2.3 Physical Activity

All participants wore accelerometers (GT3X or ActiTrainer; Actigraph LLC, Pensacola, FL, USA) around their waist during non sleeping time (except when showering, bathing or swimming) for seven consecutive days. Vertical readings were summarised in 60 s epochs and Freedson’s cut points were applied to define different intensity activities (279). Sedentary time was defined as the time with activity readings $\leq 100$ count.min$^{-1}$. Non wear time was determined by no activity readings for $\geq 60$ min or a shorter period of no activity readings as described by the participants’ log diaries. Valid days were considered when they were at least 10 hours of daily wear time. Data from participants with at least four valid days of recordings were included in the analysis.

In addition, self reported activity was recorded by using the long version of IPAQ. However, following the analysis in chapter 3 which showed large discrepancy between self reported and objectively measured activity, self reported physical activity will not be used as a predictor variable of insulin resistance, glycaemia or metabolic score in this chapter.

### 5.2.4 Cardiorespiratory fitness

All participants underwent assessment of their cardiopulmonary fitness with the use of the Chester Step test. This is a submaximal test predicting maximal oxygen uptake (VO$_2$ max). Volunteers were asked to step on and off (step height choice varied from 15 to 25 cm based on their age and physical activity history) at a pace defined by an incremental tempo on a compact disc (CD). The initial step rate was 15 steps per minute and every 2 minutes the pace increased by 5 steps and the heart rate was recorded. Subjects were asked to stop the test when they reached 80% of their estimated maximum heart rate (based on the equation 220-age (years)) or when self rated intensity of the exercise exceeded moderately hard (RPE of 14 on Borg’s scale) (provided that they had reached 80% of their estimated maximum heart rate). VO$_2$ max was predicted by using the “line of best fit” in linear graph extrapolation joining the recorded heart rates at the end of each 2-min period and the age estimated maximum heart rate. Data from participants reaching at least 75% of their age estimated maximum heart rate and completing at least three levels of stepping rate (at least 6 minutes in total) were included in the analysis.
5.2.5 Blood biochemistry, blood pressure and metabolic risk score

Blood samples were obtained after an overnight fast of 10-12 h. Glucose and HbA1c were measured at the day of collection in a National Health Service (NHS) Biochemistry Laboratory within Greater Glasgow and Clyde using standardised automated enzymatic (glucose) and high performance liquid chromatography (HPLC) (HbA1c) methods. Cholesterol, HDL and triglycerides were measured in stored plasma with the use of an Autoanalyser (C311, Roche Hitachi). LDL levels were calculated using the Friedwald equation (282). Insulin was measured in stored plasma with the use of commercially available ELISA. The homeostatic model assessment of insulin resistance (HOMAIR) was calculated by the equation HOMAIR = (Glucose x Insulin) / 22.5 (77).

The metabolic risk score was calculated based on a modification of the equation suggested previously in the literature (295, 296). This score is a continuous variable and has been suggested as a means of estimating an individual’s clustered cardiometabolic risk by summing the standard scores (z-scores) variables constituting the definition of the metabolic syndrome:

\[
\text{Metabolic Risk (MetR) score} = -zHDL + zInsulin + zGlucose + zTriglycerides + (zBMI + zWC) / 2 + [z(Systolic BP) + z(Diastolic BP)] / 2
\]

Z-score of each variable corresponds to the standardised value of the variable to its mean (i.e. expresses the difference from the mean of the original variable in number of standard deviations (SD)). Hence, each variable was rescaled to have a mean of zero and a standard deviation of one.

The above equation of metabolic risk score was slightly modified for the purpose of the analyses conducted in this chapter; to ensure that any potential association between metabolic risk score and adiposity/body composition variables was not a result of including the adiposity variables (BMI and WC) in the equation estimating the dependent variable (MetR score), the adiposity/body composition variables (BMI and WC) were removed from the equation. Hence, the modified version of the MetR score used in this chapter is described by the equation: MetR score=-zHDL + zInsulin + zGlucose + zTriglycerides + [z(Systolic BP) + z(Diastolic BP)] / 2.
5.2.6 Data analysis

Summary statistics for all variables for both South Asians and Europeans are presented in Chapter 4. Independent variables were grouped according to body composition variables including BMI, waist circumference (WC), waist-to-hip ratio (WHR), central adiposity, upper arm adiposity and lower adiposity. Physical activity variables included moderate to vigorous activity per day, daily light activity and daily sedentary time. Predicted VO$_2$ max was considered as the fitness variable. Each variable was standardised (by subtracting the mean and dividing by the SD of each variable). Outcomes were insulin resistance defined by the HOMA$_{IR}$, fasting plasma glucose, HbA1c and the modified MetR score as described above. Insulin, HOMA$_{IR}$ and fasting plasma glucose were log transformed (natural logarithm) for the regression models. Linear regression models assessing the associations between each outcome and predictor variable for both ethnic group and the same models including the interaction variable between ethnicity and the independent variable were compared. The significance of interaction terms was assessed with the likelihood ratio test. If the models including the interaction term had a better fit compared with the models without, analysis was stratified per ethnic group; otherwise, when ethnicity did not modify the association between dependent and independent variables, summary measures were presented for both ethnic groups adjusted for ethnicity.

The effect of other potential covariates (age, SIMD, smoking) was assessed. Alcohol was not included as a confounder, firstly, because of the risk of introducing recording errors (problem of misreporting alcohol consumption) and, secondly, because of the u-shaped relationship with the risk of developing diabetes (protective effect when in moderation and a risk factor when in excess) (356). In addition, years of education, although they differed between the two ethnic groups, may not be a valid proxy of socio-economic status in women from South Asia, as the majority of them were housewives and socio-economic status is mainly determined by their partners’ education who are the primary workers in Asian households. Hence, duration of education was not used as a potential confounder as it may inappropriately dilute the effect of ethnicity.

In addition, the correlation between anthropometric measures was estimated by calculating the correlation coefficient (r) and 95% confidence interval. The association between fasting glycaemia and HbA1c was examined with regression models without and with adjustment for confounding covariates. Stata (version 12.1, StataCorp LP, Texas, USA)
was used for statistical analysis. The level of $p < 0.05$ was considered as statistical significant.
5.3 Results

5.3.1 Descriptive statistics

Our final dataset, after excluding women who were likely to have undiagnosed diabetes, included 91 South Asian women and 87 Europeans. The demographic and metabolic characteristics are described in detail in chapter 4. Briefly, there were no differences between groups in BMI and age, but the South Asian volunteers were shorter with greater truncal adiposity than the Europeans (assessed by waist-to-hip ratio and central skinfolds) and ectopic fat infiltration. In addition, the South Asians were less active and less fit. Socioeconomic status defined by SIMD did not differ between the two groups, but the Europeans were more likely to smoke or drink alcohol and the South Asians were more likely to have a first degree relative diagnosed with diabetes. Energy intake and dietary density did not differ between the two groups. The South Asians had greater levels of fasting insulin, HOMAIR and HbA1c, triglycerides and lower levels of HDL compared with the Europeans. Fasting blood glucose levels, systolic and diastolic blood pressure (BP) did not differ between the two groups.

5.3.2 Ethnicity as an effect modifier of the associations between anthropometry, physical activity and fitness variables with insulin resistance, glycaemia and metabolic risk score

The associations between HOMAIR and each independent variable adjusted for age was not modified by ethnicity, apart from the association with central skinfolds. In addition, ethnicity was a significant effect modifier of the association of central adiposity with MetR score:

- 10 mm increase in central adiposity (defined by central skinfolds) was associated with 0.24 (95 % CI: 0.17, 0.30, p < 0.0001) units increase in HOMAIR in the South Asians and 0.12 (95 % CI: 0.06, 0.18, p < 0.0001) units increase in the Europeans (p = 0.004 for interaction with ethnicity).

- 10 mm increase in central adiposity was associated with 1.26 (95 % CI: 0.87, 1.66, p < 0.0001) points increase in the MetR score in the South Asians and 0.69 (95 % CI: 0.31, 1.07, p = 0.001) points increase in the Europeans, p = 0.03 for interaction with ethnicity.
The associations of BMI, waist circumference, waist-to-hip ratio and central adiposity (defined by central skinfolds) with HbA1c were modified significantly by ethnicity:

- 10 units increase in BMI was associated with 0.26 % (95 % CI: 0.10, 0.42, p = 0.002) increase in HbA1c in the South Asians and but did not have a significant association with HbA1c (0.08, 95 % CI: -0.07, 0.23, p = 0.31) in the Europeans, p = 0.01 for interaction with ethnicity.

- 10 mm increase in waist circumference was associated with 0.16 % (95 % CI: 0.09, 0.23, p < 0.0001) increase in HbA1c in the South Asians but had no significant association with HbA1c in the Europeans (0.05, 95% CI:-0.02, 0.12, p=0.17), p = 0.001 for interaction with ethnicity.

- 10 mm increase in central adiposity was associated with 0.13 % (95 % CI: 0.08, 0.18, p < 0.0001) increase in HbA1c in the South Asians but did not have a significant association with HbA1c levels in the Europeans (0.03, 95 % CI:-0.01, 0.07, p = 0.17), p < 0.0001 for interaction with ethnicity.

- 0.1 increase in WHR was associated with 0.29 % (95 % CI: 0.21, 0.38, p < 0.0001) increase in HbA1c levels in the South Asians and 0.13 % (95 % CI: 0.02, 0.23, p = 0.02) increase in the Europeans, p = 0.002 for interaction with ethnicity.

- In the relationship between MVPA and HbA1c levels, ethnicity was a significant effect modifier (p = 0.02 for interaction). However, MVPA did not have a significant effect on HbA1c levels in either group (-0.01, 95 % CI: -0.06, 0.04, p = 0.65 in the South Asians) and (0.02, 95 % CI: -0.006, 0.052, p = 0.12 in the Europeans) puzzling the interpretation of the ethnicity interaction in this association.

5.3.3 Associations between anthropometric, physical activity and fitness variables with insulin resistance and glycaemia

Table 17 shows the relative effects of anthropometric, physical activity and fitness variables on HOMA_{IR}, fasting glucose concentration and HbA1c. When there was no significant interaction between ethnicity and the independent variable, summary measures are presented for both ethnic groups. Otherwise, in the presence of a significant interaction with ethnicity, the relative effects were stratified by ethnic group. In addition, the effect of
other potential confounding covariates on $\text{HOMA}_{\text{IR}}$, fasting glucose and HbA1c is demonstrated on the top of the table. Smoking (current smokers versus non smokers) or SIMD (most affluent versus most deprived areas) in our study are not risk factors of the outcomes of interest, thereby, they have not been included as confounders in subsequent multivariate models. On the contrary, age, along with ethnicity, was associated with all three outcomes.

$\text{HOMA}_{\text{IR}}$ and fasting glucose increased with increasing BMI (by one SD) in both groups by an average of 33 % and 4 % in the South Asians and the Europeans respectively. One SD increase in BMI was associated with 7 % increase on HbA1c in the South Asians as opposed to only 3 % in the Europeans. In addition, increasing waist circumference and waist-to-hip ratio increased $\text{HOMA}_{\text{IR}}$ and fasting glucose concentration to a similar extent in both ethnic groups but each variable had a larger effect in HbA1c in the South Asians compared to the Europeans. Increasing arm and leg adiposity was associated with a significant increase in insulin resistance and glycaemic indices in both groups. Increasing central adiposity had a substantially larger effect on insulin resistance and HbA1c in the South Asians rather than in the Europeans (57 % and 7 % versus 26 % and 3 % respectively). $\text{HOMA}_{\text{IR}}$, glucose concentration and HbA1c decreased with increasing VO$_2$ max to a similar extent in both groups. Similarly, $\text{HOMA}_{\text{IR}}$ and fasting glucose decreased with increasing time of moderate to vigorous activity in both groups, but increasing MVPA (one SD of MVPA) decreased HbA1c (by 6 %) only in the South Asians and not in the Europeans. When MVPA was categorised in quintiles, increasing MVPA from the lowest category to the highest quintile had almost similar inverse association with $\text{HOMA}_{\text{IR}}$, fasting glucose and HbA1c to the absolute levels of MVPA.

The associations of the predictor variables with the outcomes of interest after adjustment for age (along with ethnicity when ethnicity was not an effect modifier of the relationship) are displayed in Table 18. The summary effect or ethnic specific effect in the South Asians of anthropometry variables on $\text{HOMA}_{\text{IR}}$, glucose and HbA1c attenuated minimally or remained unchanged after adjustment for age. On the contrary, the ethnic stratified effect of increasing BMI, waist circumference or central adiposity on HbA1c disappeared after controlling for age. The association between VO$_2$ max and $\text{HOMA}_{\text{IR}}$ remained after adjusting for age with one SD increase in VO$_2$ max associated in an average 11% decrease in insulin resistance. Interestingly, the association of $\text{HOMA}_{\text{IR}}$ with MVPA quintiles persisted after including age in the model but disappeared when MVPA was integrated as a continuous variable, possibly because of the large proportion of women with minimal or
even nil time spent performing MVPA. Figure 23 presents the age and ethnic adjusted increase in HOMA_{IR} (log-transformed) and fasting glucose concentration (log-transformed) with increasing BMI, waist circumference and waist-to-hip ratio. In addition, Figure 25 demonstrates the summary age and ethnic adjusted increase in HbA1c, log-transformed HOMA_{IR} and fasting glucose with increasing arm and leg adiposity. Figure 27 shows the decrease in log-transformed HOMA_{IR} with increasing VO_{2 max} for both ethnic groups after adjustment for age and ethnicity. On the contrary, Figure 24 and Figure 26 show the ethnic stratified increase (after adjustment for age) in HbA1c with increasing BMI, waist circumference, waist-to-hip ratio and central skinfolds and in HOMA_{IR} (log-transformed) with increasing central adiposity.
<table>
<thead>
<tr>
<th>Variables</th>
<th>HOMA_{IR}</th>
<th>Fasting Glucose (mmol.l^{-1})</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative effect estimate (95% CI)</td>
<td>p value</td>
<td>Relative effect estimate (95% CI)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td>1.29 (1.09, 1.52)</td>
<td>0.003</td>
<td>1.02 (0.99, 1.05)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.17 (1.08, 1.27)</td>
<td>&lt; 0.0001</td>
<td>1.04 (1.03, 1.06)</td>
</tr>
<tr>
<td>Smoking*</td>
<td>1.14 (0.76, 1.71)</td>
<td>0.51</td>
<td>1.00 (0.94, 1.06)</td>
</tr>
<tr>
<td>SIMD*</td>
<td>0.97 (0.91, 1.03)</td>
<td>0.32</td>
<td>0.99 (0.98, 1.01)</td>
</tr>
<tr>
<td><strong>Anthropometric variables:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg.m^{-2})</td>
<td>1.33 (1.23, 1.43)</td>
<td>&lt; 0.0001</td>
<td>1.04 (1.02, 1.06)</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>1.39 (1.29, 1.50)</td>
<td>&lt; 0.0001</td>
<td>1.05 (1.03, 1.07)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>1.39 (1.30, 1.49)</td>
<td>&lt; 0.0001</td>
<td>1.06 (1.04, 1.07)</td>
</tr>
<tr>
<td>Central Adiposity (mm)*</td>
<td>SA: 1.57 (1.37, 1.81)</td>
<td>&lt; 0.0001</td>
<td>1.05 (1.03, 1.07)</td>
</tr>
<tr>
<td>Arm (mm)*</td>
<td>1.33 (1.23, 1.44)</td>
<td>&lt; 0.0001</td>
<td>1.05 (1.03, 1.07)</td>
</tr>
<tr>
<td>Lower Body (mm)*</td>
<td>1.31 (1.21, 1.42)</td>
<td>&lt; 0.0001</td>
<td>1.04 (1.02, 1.06)</td>
</tr>
<tr>
<td><strong>Physical activity variables:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVPA (min.day^{-1})</td>
<td>0.85 (0.77, 0.95)</td>
<td>0.003</td>
<td>0.97 (0.96, 0.99)</td>
</tr>
<tr>
<td>MVPA quintile*</td>
<td>0.88 (0.82, 0.95)</td>
<td>0.001</td>
<td>0.98 (0.7, 0.99)</td>
</tr>
<tr>
<td>Light (min.day^{-1})</td>
<td>1.04 (0.94, 1.14)</td>
<td>0.44</td>
<td>1.02 (0.99, 1.04)</td>
</tr>
<tr>
<td>Sedentary (min.day^{-1})</td>
<td>1.01 (0.92, 1.11)</td>
<td>0.82</td>
<td>0.99 (0.97, 1.01)</td>
</tr>
<tr>
<td>Fitness Variable</td>
<td>VO_{2} max (ml.Kg^{-1}.min^{-1})</td>
<td>0.84 (0.77, 0.92)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Table 17. Relative effect (ratios of geometric means) of predictor variables on insulin resistance index (HOMA_{IR}), fasting glucose and HbA1c levels. The estimates represent the ratio of change of the dependent variable for one standard deviation (SD) increase of a continuous predictor variable or change from baseline category for a categorical variable. Summary relative effects are adjusted for ethnicity (when there was no significant interaction with ethnicity) or stratified by ethnic group (when there was a significant interaction with ethnicity). *For categorical variables the estimates represent the ratio of change in the dependent variable for South Asians compared with Europeans (ethnicity), for non smokers compared with smokers (smoking), for most affluent regions compared with least affluent areas (SIMD ranking), for increase from low to high quintile in MVPA. ¥ Central adiposity is the summation of suprailliac, supraspinale and subscupular skinfolds, arm adiposity is the summation of biceps and triceps skinfolds and lower body is the summation of calf and thigh skinfolds. (SA: South Asians, E: Europeans, MVPA: moderate to vigorous physical activity).
<table>
<thead>
<tr>
<th>Anthropometric variables</th>
<th>HOMA&lt;sub&gt;IR&lt;/sub&gt;</th>
<th>Fasting Glucose (mmol.l&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg.m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1.30 (1.20, 1.40)</td>
<td>0.009</td>
<td>0.004</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>1.40 (1.29, 1.52)</td>
<td>&lt; 0.0001</td>
<td>0.007</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>1.42 (1.31, 1.53)</td>
<td>0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Central Adiposity&lt;sup&gt;y&lt;/sup&gt; (mm)</td>
<td>SA: 1.56 (1.36, 1.79)</td>
<td>&lt; 0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>E: 1.25 (1.14, 1.38)</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Arm &lt;sup&gt;y&lt;/sup&gt; (mm)</td>
<td>1.32 (1.22, 1.42)</td>
<td>0.0001</td>
<td>0.01</td>
</tr>
<tr>
<td>Lower Body&lt;sup&gt;y&lt;/sup&gt; (mm)</td>
<td>1.29 (1.19, 1.41)</td>
<td>&lt; 0.0001</td>
<td>0.05</td>
</tr>
<tr>
<td>Physical activity variables</td>
<td>MVPA (min.day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.92 (0.82, 1.02)</td>
<td>0.52</td>
</tr>
<tr>
<td>MVPA quintile</td>
<td>0.92 (0.85, 0.99)</td>
<td>0.04</td>
<td>0.53</td>
</tr>
<tr>
<td>Light (min.day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.98 (0.89, 1.08)</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Sedentary (min.day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.04 (0.95, 1.14)</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Fitness Variable</td>
<td>VO&lt;sub&gt;2&lt;/sub&gt; max (ml.Kg&lt;sup&gt;-1&lt;/sup&gt;.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.89 (0.81, 0.98)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 18. Relative effect (ratios of geometric means) adjusted for age of predictor variables on insulin resistance index (HOMA<sub>IR</sub>), fasting glucose and HbA1c levels. The estimates represent the ratio of change of the dependent variable for one standard deviation (SD) increase of a continuous predictor variable or change from baseline category for a categorical variable. Summary relative effects are adjusted for age and ethnicity (when there was no significant interaction with ethnicity) or stratified by ethnic group and adjusted for age (when there was a significant interaction with ethnicity). For the categorical variable MVPA quintile the relative effect represents the ratio of change in the dependent variables for increase from low to high quintile in MVPA.  
<sup>y</sup> Central adiposity is the summation of suprailiac, supraspinale and subscupular skinfolds, arm adiposity is the summation biceps and triceps skinfolds and lower body is the summation of calf and thigh skinfolds. (SA: South Asians, E: Europeans, MVPA: moderate to vigorous physical activity).
Figure 23. Summary associations of insulin resistance (HOMA\textsubscript{IR}) with BMI, waist circumference and waist-to-hip ratio (left panel) and fasting glucose levels with BMI, waist circumference and waist-to-hip ratio (right panel) in both ethnic groups. The fitted lines are adjusted for age and ethnicity and r-square corresponds to the adjusted for age and ethnicity model for each association.
Figure 24. Associations of HbA1c levels with BMI (top panel), waist circumference (middle panel) and waist-to-hip ratio (bottom panel) in the Europeans (E) and the South Asians (SA). The fitted lines are adjusted for age and r-square corresponds to the adjusted for age model for each association stratified by ethnicity.
Figure 25. Summary associations of arm adiposity with insulin resistance (HOMA$_{IR}$), glucose levels, HbA1c levels (left panel) and lower body adiposity with insulin resistance (HOMA$_{IR}$), glucose levels and HbA1c levels (right panel) in both ethnic groups. The fitted lines are adjusted for age and ethnicity and r-square corresponds to the adjusted for age and ethnicity model for each association. Arm adiposity is defined by the summation of biceps and triceps skinfolds and lower body adiposity by the summation of thigh and calf skinfolds.
Figure 26. Associations of central adiposity with insulin resistance (HOMA$_{IR}$) (left panel) and HbA1c levels (right panel) in the Europeans (E) and the South Asians (SA). The fitted lines are adjusted for age for each ethnic group and r-square corresponds to the adjusted for age model for each association stratified by ethnic group. Central adiposity is defined by the summation of suprailiac, supraspinale and subscapular skinfolds.

Figure 27. Summary association of insulin resistance (HOMA$_{IR}$) with maximal oxygen uptake (VO2 max) in both ethnic groups. The fitted line is adjusted for age and ethnicity and r-square corresponds to the adjusted for age and ethnicity model.

5.3.4 Associations between anthropometric, physical activity and fitness variables with metabolic risk score

Metabolic risk expressed as a continuous score increased with increasing BMI, waist circumference, waist-to-hip ratio, arm and leg adiposity to a similar extent in both ethnic groups. The relationships attenuated but remained significant after adjustment for age (Table 19 and Figure 28). On the contrary, increasing central adiposity resulted in significantly higher absolute increase in metabolic risk in the South Asian participants compared with the Europeans; one SD increase in central adiposity was related with an age adjusted absolute increase in metabolic score by 2.36 units in the South Asians almost double to 1.29 units increase in the Europeans (Table 19). The excess effect of central
adiposity on metabolic risk in the South Asians compared with the Europeans is graphically demonstrated in Figure 29. Metabolic score decreased with increasing VO$_2$ max and MVPA proportionally in both groups, but the protective effect of MVPA disappeared after adjustment with age. On contrary, the effect on VO$_2$ max on metabolic risk attenuated but remained substantial after adjustment for age (Table 19 and Figure 28).
### Table 19. Absolute change in metabolic risk score for one standard deviation (SD) increase of a continuous predictor variables or change from the baseline category for a categorical variable.

| Predictor Variables | Metabolic risk score<sup>a</sup> |  | Metabolic risk score<sup>b</sup> |  
|---------------------|----------------------------------|---|----------------------------------|---
|                     | Beta coefficient (95 % CI)       | p value | Beta coefficient (95 % CI)       | p value |
| Ethnicity*          | 1.62 (0.43, 2.81)                | 0.008 | -                                 |
| Age (years)         | 1.99 (1.45, 2.52)                | < 0.0001 | -                                 |
| Smoking*            | 1.80 (-0.71, 4.31)               | 0.16 | -                                 |
| SIMD*               | -0.19 (-0.62, 0.25)              | 0.40 | -                                 |
| **Anthropometric variables** |                                |    | **Anthropometric variables** |    |
| BMI (kg.m<sup>-2</sup>) | 1.83 (1.36, 2.31)             | < 0.0001 | 1.44 (0.97, 1.91) | < 0.0001 |
| Waist (cm)          | 2.26 (1.82, 2.70)                | < 0.0001 | 1.83 (1.32, 2.33) | < 0.0001 |
| Waist-to-hip ratio  | 2.26 (1.86, 2.66)                | < 0.0001 | 1.99 (1.47, 2.50) | < 0.0001 |
| **Central Adiposity (mm)<sup>y</sup>** | SA: 2.44 (1.55, 3.34) | < 0.0001 | SA: 2.36 (1.49, 3.22) | < 0.0001 |
| E: 1.51 (0.86, 2.17) | < 0.0001 | 1.29 (0.65, 1.93) | < 0.0001 |
| Arm (mm)<sup>y</sup> | 1.66 (1.15, 2.19)                | < 0.0001 | 1.52 (1.05, 2.00) | < 0.0001 |
| Lower Body (mm)<sup>y</sup> | 1.73 (1.20, 2.31)            | < 0.0001 | 1.52 (1.00, 2.08) | < 0.0001 |
| **Physical activity variables** |                                |    | **Physical activity variables** |    |
| MVPA (min.day<sup>-1</sup>) | -1.08 (-1.70, -0.45) | 0.001 | -0.34 (-0.95, 0.27) | 0.28 |
| MVPA quintile<sup>*</sup> | -0.82 (-1.22, -0.42) | < 0.0001 | -0.33 (-0.75, 0.09) | 0.12 |
| Light (min.day<sup>-1</sup>) | 0.44 (-0.27, 1.14)         | 0.22 | -0.09 (-0.79, 0.62) | 0.81 |
| Sedentary (min.day<sup>-1</sup>) | -0.10 (-0.81, 0.62) | 0.79 | 0.16 (-0.52, 0.84) | 0.64 |
| **Fitness Variables** |                                |    | **Fitness Variables** |    |
| VO<sub>2</sub> max (ml.Kg<sup>-1</sup> min<sup>-1</sup>) | -1.28 (-1.81, -0.75) | < 0.0001 | -0.58 (-1.11, -0.05) | 0.03 |

<sup>a</sup>Models are adjusted for ethnicity (except when there was a significant interaction with ethnicity, stratified analysis by ethnic group is presented).

<sup>b</sup>Models are adjusted for ethnicity and age (except when there was a significant interaction with ethnicity, stratified analysis by ethnic group is presented).

<sup>*</sup>For categorical variables beta coefficient represents the change in the dependent variable for South Asians compared with Europeans (ethnicity), for non smokers compared with smokers (smoking), for most affluent regions compared with least affluent areas (SIMD ranking), for increase from low to high quintile in MVPA.<br><br><sup>y</sup>Central adiposity is the summation of suprailiac, supraspinale and subscupular skinfolds, arm adiposity is the summation of biceps and triceps skinfolds and lower body is the summation of calf and thigh skinfolds. (SA: South Asians, E; Europeans, MVPA: moderate to vigorous physical activity).
Figure 28. Summary associations of metabolic risk score with BMI and waist circumference (top panel), waist-to-hip ratio and arm adiposity (middle panel), lower adiposity and VO2 max (bottom panel) in both ethnic groups. The fitted lines are adjusted for age and ethnicity and r-square corresponds to the model adjusted for age and ethnicity for each association.
5.3.5 Associations between subcutaneous, visceral and liver fat with insulin resistance, glycaemia and the metabolic risk score

Table 20 shows the effect size (expressed as a standardised beta coefficient) of each MRI measurement (visceral fat, subcutaneous fat and liver fat) on HOMA$_{IR}$ (log transformed values), glycaemia (log transformed values of fasting glucose), HbA1c levels and MetR score adjusted for age. The significance of the interaction term of ethnicity with each MRI measure in each model is also shown in the same table. Ethnicity is a significant effect modifier of the association between visceral fat and MetR score. Figure 30 shows the summary regression lines for both ethnic groups adjusted for age and ethnicity demonstrating the relationships between visceral fat and HOMA$_{IR}$, fasting glucose and HbA1c levels. Figure 31 demonstrates the adjusted for age regression lines describing the relationship between visceral fat and MetR score per ethnic group.
### Table 20

Effect size (standardised beta coefficient) of MRI indices on insulin resistance index (HOMA<sub>IR</sub>), fasting glucose, HbA1c levels and metabolic risk score in the South Asians (SA) and the Europeans (E). The models are adjusted for age. P-value for interaction corresponds to the significance of the interaction of the MRI variable with ethnicity in the same model.

<table>
<thead>
<tr>
<th>MRI Index</th>
<th>SA beta-coefficient</th>
<th>SA p-value</th>
<th>p for interaction</th>
<th>E beta-coefficient</th>
<th>E p-value</th>
<th>p for interaction</th>
<th>Meta-coefficient</th>
<th>Meta p-value</th>
<th>p for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous fat (cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>0.002</td>
<td>0.17</td>
<td>0.13</td>
<td>0.39</td>
<td>0.96</td>
<td>0.18</td>
<td>0.14</td>
<td>0.28</td>
</tr>
<tr>
<td>Visceral fat (cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>0.58</td>
<td>&lt;0.0001</td>
<td>0.28</td>
<td>0.20</td>
<td>0.23</td>
<td>0.32</td>
<td>0.34</td>
<td>&lt;0.0001</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>0.65</td>
<td>&lt;0.0001</td>
<td>0.48</td>
<td>&lt;0.0001</td>
<td>0.22</td>
<td>0.1</td>
<td>0.22</td>
<td>0.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Liver fat (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.43</td>
<td>0.004</td>
<td>0.69</td>
<td>0.26</td>
<td>0.09</td>
<td>0.85</td>
<td>0.34</td>
<td>&lt;0.0001</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>0.36</td>
<td>0.006</td>
<td>0.32</td>
<td>0.01</td>
<td>0.15</td>
<td>0.23</td>
<td>0.47</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The values in the table represent the effect size (standardised beta coefficient) and p-values for the interaction of the MRI variable with ethnicity. The models are adjusted for age.
Figure 30. Association of visceral fat with insulin resistance (HOMA$_{IR}$) (top panel), glucose levels (middle panel) and HbA1c levels (bottom panel) in both ethnic groups. Fitted lines are adjusted for age and ethnicity. R-square corresponds to the adjusted for age and ethnicity model for each association.
5.3.6 Correlations between anthropometry measures

Central adiposity measured by central skinfolds had a strong correlation with arm adiposity in both the South Asians \((r = 0.73, 95\% \text{ CI}: 0.59, 0.82)\) and the Europeans \((r = 0.82, 95\% \text{ CI}: 0.71, 0.88)\) and similarly with lower (leg) adiposity in both the South Asian \((r = 0.70, 95\% \text{ CI}: 0.56, 0.81)\) and the European volunteers \((r = 0.82, 95\% \text{ CI}: 0.72, 0.89)\). Confidence intervals were overlapping, hence, the summary correlation for both groups between central adiposity and arm adiposity was \(0.76 (95\% \text{ CI}: 0.68, 0.83)\) and between central adiposity and lower body adiposity was \(0.74 (95\% \text{ CI}: 0.65, 0.81)\). The correlation of BMI with central adiposity was stronger in the Europeans \((r = 0.84, 95\% \text{ CI}: 0.75, 0.90)\) than in the South Asians \((r = 0.70, 95\% \text{ CI}: 0.56, 0.81)\), \(p = 0.008\) for the inter-ethnic difference in correlation coefficients. However, in both ethnic groups waist-to-hip ratio correlated to a similar degree with the sum of the central skinfolds \((r = 0.60, 95\% \text{ CI}: 0.43, 0.74 \text{ in the South Asians versus } r = 0.62, 95\% \text{ CI}: 0.44, 0.75 \text{ in the Europeans})\). The correlation of waist circumference with central adiposity was optimal in both groups; \(r = 0.74 (95\% \text{ CI}: 0.61, 0.83)\) in the South Asians and \(r = 0.84 (95\% \text{ CI}: 0.75, 0.90)\) in the Europeans, but stronger in the Europeans \((p = 0.005)\).

In regards to MRI measures, the associations with BMI are shown in Figure 32; for any given BMI, the South Asians had greater visceral and liver fat compared with the Europeans.
Figure 32. Associations between BMI and subcutaneous fat (top panel), visceral fat (middle panel) and liver fat (bottom panel) assessed with MRI and spectroscopy.

The correlation between subcutaneous fat measured from MRI images and the sum of central skinfolds was 0.71 (95 % CI: 0.53, 0.83) for the South Asians and 0.76 (95 % CI: 0.62, 0.85) for the Europeans. The correlation between the sum of central skinfolds and visceral fat was stronger in the Europeans ($r = 0.75$, 95 % CI: 0.61, 0.85) compared with the South Asians ($r = 0.45$, 95 % CI: 0.18, 0.66), $p = 0.0003$ for the ethnic difference in
correlation coefficients. The correlation between waist circumference and visceral fat was equally strong in both ethnic groups; \( r = 0.81 \) (95 % CI: 0.68, 0.89) in the South Asians and \( r = 0.85 \) (95 % CI: 0.75, 0.91) in the Europeans, whereas the correlation between visceral fat and WHR was modest in both ethnic groups: \( r = 0.69 \) (95 % CI: 0.50, 0.82) in the South Asians and \( r= 0.77 \) (95 % CI: 0.64, 0.86) in the Europeans.

5.3.7 Correlations between fasting glucose and HbA1c

The association of fasting glucose with HbA1c is modified by ethnicity in our cohort (p = 0.04). For a given glucose concentration, HbA1c is substantially higher in the South Asians than in the Europeans after adjustment for age and BMI or for age, BMI and central adiposity as it is illustrated in Figure 33.
Figure 33. Associations of HbA1c with fasting glucose concentration (log-transformed) in the Europeans (E) and the South Asians (SA). Fitted lines are adjusted for age and BMI (top panel) and age, BMI and central adiposity (bottom panel). R-Square corresponds to the adjusted model for each ethnic group.
5.4 Summary of key result findings

- Increasing BMI and central adiposity were associated with greater metabolic risk score and insulin resistance in both the Europeans and the South Asians.

- Increasing central skinfolds had a substantially larger impact on insulin resistance in the South Asian compared with the European women.

- The South Asian women had greater visceral and liver fat for any given BMI or amount of subcutaneous fat compared with the Europeans.

- The effect size of central adiposity on insulin resistance in the South Asians was greater than that of VO\textsubscript{2} max or MVPA.

- Waist circumference had a stronger correlation with the amount of visceral fat in both ethnic groups compared with the sum of central skinfolds or the WHR.

- The South Asians had greater HbA1c for any given level of fasting glycaemia independently of BMI, central adiposity or age.
5.5 Discussion

It was demonstrated in this chapter that total body size (expressed by BMI) and truncal adiposity (assessed by waist circumference, waist to hip ratio, central skinfolds and MRI measures) were associated with metabolic risk and insulin resistance in both ethnic groups, but interestingly, increasing central skinfolds had a substantially larger effect on insulin resistance in the South Asian compared with the European women and this disparity remained after adjustment for age. In regards to HbA1c, BMI and central adiposity had a disproportional impact on its levels between the two ethnic groups with the South Asians exhibiting higher levels of HbA1c for a given BMI or central adiposity than the Europeans. Notably, in Europeans the association between central adiposity and HbA1c disappeared after adjustment with age, suggesting that the effect of increasing adiposity on HbA1c in the Europeans is possibly mediated through the adverse effect of aging. In contrast, the association remained marked in the South Asians. In both groups, increasing fitness had an inverse association with insulin resistance and metabolic score which remained significant after adjustment for age. On the contrary, the protective effect of MVPA as a continuous measure on metabolic indices faded after including age as a covariate, possibly because its effect size was diluted by the large proportion of participants with almost negligible time engaged with MVPA. An additional finding was that for any given BMI or amount of subcutaneous adiposity measured on MRI, the South Asians had greater visceral and liver fat compared with the Europeans. A secondary finding of this study was that the South Asians had higher HbA1c levels which were not accompanied by elevated fasting glucose levels and this ethnic specific discrepancy in HbA1c was independent of body size (measured by BMI), central skinfolds or advancing age.

The observation that for a given BMI, the South Asians had higher percentages of total and abdominal fat has been shown before (34, 315, 357) with studies suggesting ethnic specific cut points in BMI and waist circumference (40, 41). The striking finding of this study, though, was that similar increase in central adiposity (measured by central skinfolds) had almost 2-fold greater adverse effect on insulin resistance index (HOMA$_{IR}$) and metabolic score in the South Asian than in the European women. This finding is consistent with the adipose tissue overflow theory (233) suggesting that increasing caloric intake without accompanied increasing energy expenditure leads to fat deposition predominantly in subcutaneous region. This process is facilitated by enlargement of the existing adipocytes and adipogenesis (358). Both processes are associated with a cascade of inflammatory...
pathways characterised by macrophage infiltration, release of pro-inflammatory interleukins and tumour necrosis factor alpha (TNF-α) and matrix remodelling mediated via matrix metalloproteinase-9 (359). The above mechanisms continue until inflammatory mediators halt the recruitment of new adipocytes, when subcutaneous tissue stops acting as storage tissue and positive energy balance leads to an overflow of fatty acids to deeper adipose compartments (i.e. visceral) or ectopic tissues (i.e. hepatic). The ‘tipping’ point though when subcutaneous tissue would reach its maximum storage capacity is unknown for each individual and depends on genetic and environmental factors. Hence, South Asians are considered to have lower capacity of storing fat subcutaneously and, consequently, they reach their ‘tipping’ point and start accumulating fat in deeper compartments or ectopic tissues earlier than their counterparts of White descent (233). Visceral fat is considered more metabolically active, characterised by increased levels of lipogenesis and lipolysis, than superficial subcutaneous tissue and, thereby, contributing largely to insulin resistance, dyslipidaemia and decreased insulin sensitivity (33, 360).

According to the portal hypothesis, free fatty acids produced from lipolysis of intraabdominal adipose tissue reach the liver via the portal circulation leading to gluconeogenesis, hepatic insulin resistance, increased release of triglycerides locally and inhibition of muscle glucose transport peripherally (361, 362). In addition, liver fat accretion increase hepatic insulin resistance and glucose release from the liver. Therefore, the adverse metabolic profile of South Asian women could be mediated by higher levels of visceral/hepatic fat for a given level of BMI and subcutaneous central adiposity, which is evident in the current cohort too. Notably, when I looked at the relationship of visceral fat with the metabolic risk score, increasing visceral fat has a higher impact on the metabolic risk score in the Europeans than in the South Asians. Hence the adverse metabolic profile seen in the South Asians is not a result of greater effect size of the visceral fat compared with that in the Europeans but with the fact that they start accumulating visceral fat at a lower level of subcutaneous adiposity.

Interestingly, the effect of increasing waist circumference and waist to hip ratio on insulin resistance and metabolic risk did not differ between the ethnic groups, despite both indices being used as alternative methods of assessing truncal adiposity in women (363) and could be expected to be interchangeable with central skinfolds. In addition, central skinfolds had a poorer correlation with the metabolically adverse visceral fat in the South Asians than in the Europeans, whereas waist circumference showed an optimal association with visceral fat in both ethnic groups. Hence, although the use of central skinfolds seems to be a more precise and direct measure of subcutaneous fat in ethnic specific research, a finding
consistent with a previous study in Indian men (364), waist circumference seems to be a more accurate proxy of visceral fat which is the most metabolically active adipose tissue compartment. In addition, the association of increasing peripheral skinfolds with adverse metabolic profile is likely to be mediated via the strong correlation between arm and leg skinfolds with central adiposity.

The observation that greater anthropometric indices are associated with increased insulin resistance and metabolic risk in South Asians has already been acknowledged (207), however, the finding that body composition and predominantly central skinfolds had the largest effect on insulin resistance and metabolic risk in comparison with other objectively measured lifestyle factors in healthy South Asian women, to my knowledge, is novel. This disagrees to an extent with the findings of Ghouri et al in men suggesting that lower cardiorespiratory fitness was the key factor explaining the majority of insulin resistance and glycaemia excess in South Asian men compared with Europeans (207). Indeed, increasing fitness ameliorated insulin resistance and metabolic risk in the current cohort, in keeping with previous studies (311), but this occurred across both ethnic groups without observing an ethnic specific gradient in the relationship. This sex difference in the magnitude of the effect of established risk factors between our study and Ghouri et al.(207) may suggest different baseline metabolic phenotypes between sexes, and thereby, a modified effect of preventative exposures on the outcomes. This is further supported by the sex difference in fasting glucose levels, with South Asian men having elevated fasting glucose and insulin compared with the European men (207) whereas the South Asian women displayed similar fasting glucose but greater fasting insulin in comparison to the European women, possibly reflecting underlying sex specific defects in different levels of glucose homeostasis. It cannot be excluded, though, that the sex difference in the magnitude of the effect of cardio-respiratory fitness may partially arise from the different methods used to measure oxygen uptake; the current study applied a submaximal protocol predicting the VO\textsubscript{2} max as opposed to the study in men which used a treadmill test measuring directly the oxygen uptake at maximal effort (207). I acknowledge that the latter is perceived as the golden standard technique in measuring VO\textsubscript{2} max, but the former is a well validated method (278) culturally acceptable by the women of this cohort.

An interesting finding of the current study was the ethnic difference in HbA1c for a given level of fasting glycaemia. HbA1c is a measure of circulating glucose levels, influenced by both fasting and postprandial state (365), which tracks glycaemia over the last 4-6 weeks prior to the test. It is unclear whether this disparity between the two groups resulted from
ethnic differences in post-prandial glycaemia or ethnic differences in the affinity of haemoglobin to glucose molecules (non enzymatic glycosylation). Although post-prandial glucose was not measured in the current cohort of women, the former assumption is supported by studies showing that South Asians have higher post-prandial but similar fasting glucose compared with the European comparators (320). In healthy individuals glucose uptake during fasting state occurs predominantly in the brain, blood cells and splanchnic tissues and is largely non-insulin dependent, hence, circulating levels of glucose during fast are mainly directed by tissue demands rather than by insulin levels. On the contrary, postprandial glucose homeostasis is mainly regulated by suppressive mechanisms of endogenous glucose release and hepatic and post-hepatic glucose uptake where elevated insulin plays a major regulatory role (366). Hence, greater HbA1c in the South Asians, probably mirroring higher post-prandial levels, may indicate greater hepatic and peripheral insulin resistance, which is in keeping with the higher levels of insulin observed but equal proinsulin/insulin ratio between the two groups (eliminating the possibility of β cell dysfunction). Therefore, I postulate that higher HbA1c levels among the South Asians indicate an additional metabolic disorder in this group rather than solely an ethnic specific characteristic, in contrast to what has been concluded previously (321). This is further supported by the finding that lifestyle factors (such as fitness) and adiposity, known to affect peripheral insulin resistance, had a significant impact on HbA1c levels in the South Asian volunteers.

The hypothesis that ethnic minorities have altered levels of HbA1c has been tested before. A large epidemiological study suggested that non-diabetic non-Hispanic Blacks, an ethnic minority with higher prevalence of diabetes compared with populations of White descent (20), had consistently higher levels of HbA1c without associated hyperglycaemia which was robust after adjustment for demographic characteristics, BMI and clinical history (hypertension, hypercholesterolemia, history of CVD) (367). Given the accumulating evidence suggesting that HbA1c values in non-diabetic individuals are associated with greater incidence of type 2 diabetes and CVD (3, 368, 369), ethnic predisposition to higher HbA1c fuelled by lifestyle factors and adiposity may be an additional risk factor of future disease burden. In addition, this ethnic disparity in HbA1c levels has potential implications for screening and diagnosing diabetes in ethnic minorities. The current guidance suggesting that an abnormal HbA1c level should be followed up by a fasting glucose test or OGTT (313) would result in a large proportion of false negative results if the former approach is followed in South Asian women. Thereby, the present data imply that ethnic specific cut points in diagnosing diabetes or pre-diabetic status may be required.
The strength of the study is the detailed metabolic phenotype and life style profile of a relatively large sample of women from an ethnic group which has been largely overlooked to date. The use of objectively measured physical activity is novel in this population of women and strengthens the validity of the findings. The use of robust statistical analysis to reveal associations between exposures and outcomes and examine interaction terms was appropriate. I acknowledge that the cross-sectional nature of the study precludes me to draw conclusions about causality. It is, also, appreciated that associations, which were not modified by ethnicity in this study, may reveal a significant interaction with ethnicity in a larger study because the current study was not powered to detect significant interaction terms in multivariate models. In addition, as it was discussed before, the use of the Chester step test to predict VO$_2$ max may have introduced measurement errors in fitness levels, but given that there is no evidence suggesting a dose pattern in the variation between measured VO$_2$ max and predicted VO$_2$ max (278), it is anticipated that any potential errors were universal across all the volunteers.

### 5.6 Conclusion

I conclude that central adiposity had the greatest impact on insulin resistance and metabolic risk in South Asian women compared with other risk factors. This is probably related with their limited capacity to store fat in subcutaneous compartments, so they start accumulating fat in deeper or ectopic depots, which are known to be more metabolically active, at an earlier stage and at a lower level of adiposity. Increasing VO$_2$ max and MVPA counteract elevated insulin resistance and high metabolic score in South Asian women but do not completely eliminate the effect of increasing central adiposity.
Chapter 6

6 A STUDY OF VASCULAR FUNCTION IN WOMEN IN RELATION TO ETHNICITY AND HOT FLUSHING

6.1 Introduction

South Asians have a higher risk of early onset cardiovascular disease (CVD) and greater mortality attributed to coronary heart disease (CHD) compared with populations of White descent (21, 29) without, though, exhibiting a higher prevalence of conventional risk factors (27, 66), such as smoking, hypertension or hypercholesterolemia, which could account for their excess risk. Increased prevalence of insulin resistance in South Asians contributes partly to their CVD risk (48), but it is unclear whether this is mediated via the atherogenic pattern accompanying insulin resistance (370) and subsequent vascular changes. Studies have shown that insulin has a direct vasodilating effect on peripheral vasculature mediated through the release of nitric oxide (NO) from endothelial cells, which is reversed when NG-monomethyl-L-arginine (L-NMMA), an inhibitor of the endothelium-derived NO, is administered (106, 371). Healthy South Asian men have reduced insulin-mediated vasodilatation of the brachial artery, measured by changes in brachial artery diameter from baseline to hyperisulinaemic state during the euglycaemic clamp technique, the magnitude of which was inversely associated with the severity of insulin resistance (372). In addition, when South Asians progress into developing diabetes they are more susceptible compared with individuals without diabetes of White descent to micro-vascular end organ complications manifesting as nephropathy with associated microalbuminuria (373, 374) or retinopathy (375, 376), which are not fully explained by longer duration of diabetes (376) or suboptimal glyceamic control (377).

Causality and direction of the relationship of impaired micro-vascular function with CVD has yet to be confirmed but there is an established association between them (378, 379) and growing evidence suggests that poor endothelial function is possibly the precursor of overt atherosclerosis (380-383). Given the greater mortality of South Asians from CHD (21), premature impaired micro-vascular function before they develop established disease can be a potential pathway linking greater insulin resistance and higher prevalence of CVD in South Asians and earlier detection of these changes can lead to more effective interventions. However, studies assessing the micro-vascular function of healthy South Asians are limited.
A study comparing 26 middle-aged healthy South Asian men with 18 White Europeans showed that South Asians have impaired macro-vascular function measured by flow mediated dilatation (FMD) of the brachial artery which persists after adjustment for lipid profile, glycaemia or insulin resistance (384). In contrast, another study which included 25 South Asian men did not demonstrate reduced reactive hyperaemia (measured by FMD) or impaired endothelium independent vasodilatation of the brachial artery (response to nitroglycerin) in South Asians compared with men of white descent (372). The disparity between the two studies was possibly attributable to the different metabolic profiles of the study subjects. It is unclear whether impaired micro-vascular function precedes macro-vascular disease in South Asians; a study comparing micro-vascular reactivity with the use of Laser Doppler fluximetry in response to ischemia and heating between South Asians and Europeans did not reveal an ethnic specific difference in hyperaemia indices, but subjects with diabetes or on lipid lowering treatment were included in the analysis, which may have diluted the effect of ethnicity (385). Therefore, further investigation of micro-vascular reactivity, assessing both NO-mediated vasodilatation and reactive hyperaemia, is warranted in healthy South Asians deemed at high risk of developing future CVD.

There are data, albeit conflicting, suggesting that vascular reactivity in women is influenced, in part by traditional CVD risks, such as advancing age, hypertension, obesity, diabetes, smoking, lipid lowering and antihypertensive treatment and also by vasomotor symptoms (VMS) triggered by menopause. Secondary analysis of Women’s Health Initiative (WHI) showed that VMS was a significant effect modifier of the relationship between hormonal replacement treatment (HRT) and CVD demonstrating that cardiovascular events were more frequent among symptomatic older women being on HRT rather than in women on HRT without VMS (386). A subgroup analysis of the study of Women’s Health Across the Nation (SWAN) showed that women with hot flushes had impaired macro-vascular function measured by FMD and greater aortic calcification compared with asymptomatic women (269), however, higher prevalence of obesity and hypercholesterolemia among symptomatic women may have confounded the effect of hot flushing on vascular health. Another study showed that the severity of hot flushing and menopausal transition were the main determinants of reduced macro-vascular function (measured by FMD of the brachial artery) among middle aged Caucasian women (271). However, the same study showed that carotid intima media thickness (IMT) was not enlarged in flushers compared with non flushers or non menopausal women (271). On the contrary, another study demonstrated that women with hot flushes did not exhibit impaired endothelial function or greater arterial stiffness (measured by pulse wave analysis (PWA))
but greater responsiveness to nitroglycerin (an endothelium independent vasodilator) compared with non flushers, suggesting that symptomatic menopausal women have a protective homeostatic vascular mechanism (273). Further work from our unit demonstrated that women with hot flushing displayed a paradoxical enhanced micro-vascular perfusion (measured by Laser Doppler Imaging (LDI)) in response to both endothelium and non-endothelium dependent stimuli but an adverse lipid profile compared with menopausal women who did not flush (387).

The conflicting evidence in regards to metabolic profile, vascular function and the role of hot flushing may be attributable to different measures of vascular function used in different studies or different metabolic characteristics of the study participants, but it could, also, support the concept that the physiological significance of flushing-mediated and atheroprotective micro-vascular reactivity are different. Hence, examining different vascular beds in the same cohort of menopausal women is anticipated to elaborate the association of hot flushing with vascular health and CVD risk. Determining whether hot flushes mark underlying vascular changes instead of functional adaptation to thermal effect, more aggressive CVD risk reduction policies could be employed in women with hot flushes.

The pathogenesis of hot flushes is not entirely clear but there is evidence suggesting a combination of central and peripheral mechanisms. Core body temperature is regulated between an upper threshold for sweating and a lower threshold for shivering with the heat dissipation responses in hot flushing occurring when the core body temperature crosses the upper threshold of the thermoneutral zone. In symptomatic women, central noradrenergic pathways are activated which narrows the thermoneutral zone. Hence, women with hot flushes start perceiving heat or start losing heat through sweating to achieve thermoregulation in response to smaller fluctuation in core temperature (388). The peripheral vascular response to an increase in core body temperature is mediated via the sympathetic, cholinergic neurons which stimulate the production of sweat that cools by evaporation and vasodilatation. Therefore, heat is lost to the environment and core temperature returns to its baseline levels.

The aim of this chapter is dual; initially, the investigation of vascular reactivity between non diabetic South Asian women and European counterparts along with the effect of conventional risk factors and modifiable life-style parameters on vascular function in both groups. Secondly, to examine the association of hot flushing with vascular reactivity in two
different vascular beds in order to uncouple the relationship between hot flushing and CVD profile. The first hypothesis of this chapter is that the South Asians have decreased endothelial function than the Europeans. The second hypothesis is that women with hot flushing have altered vascular reactivity than asymptomatic women.
6.2 Methods

6.2.1 Recruitment

Women of South Asian origin and white European descent aged 18-70 years and living in Scotland were recruited. Women with diagnosed diabetes, history of myocardial infarction (MI), stroke or transient ischemic attack (TIA), peripheral vascular dysfunction (i.e. Raynaud’s disease), on systematic HRT or hormonal contraceptives were excluded from the study. In addition, women who were likely to have undiagnosed diabetes (313) or were on antihypertensive treatment or lipid lowering treatment were excluded from the analysis.

The majority of the subjects were recruited by general advertisement or word of mouth. Detailed description of the recruitment methods is given in the main methods chapter.

6.2.2 Ethics

The study was granted ethics approval by the West of Scotland Ethics committee 3.

6.2.3 Study protocol

Eligible subjects attended the Clinical Research Facility at the Western Infirmary in Glasgow for the study visit after an overnight 10-12 hour fast (water only permitted). Participants were asked to avoid physical activity, smoking or caffeinated beverages at the day of the visit. All volunteers signed a consent form before their participation in the study. Baseline information about their origin, marital status, years of education, general health, medical, reproductive and family history, smoking status, alcohol consumption and drug treatment were self-reported. All women underwent assessment of micro-vascular function with the use of Laser Doppler Imaging with Iontophoresis (LDI-ION) after acclimatisation for 20 minutes in a temperature controlled room. Middle-aged women (over 40 years of age) underwent additional assessment of vascular function with the use of peripheral arterial tonometry (PAT) as described below. The two tests were performed at least 30 minutes apart.

Diet, physical activity and fitness were measured as described in chapter 2.
6.2.4 Measurement of biomarkers and anthropometrics

Blood samples were obtained after an overnight fast of 10-12 h. Glucose and HbA1c were measured at the day of collection in a National Health Service (NHS) Biochemistry Laboratory within Greater Glasgow and Clyde using standardised automated enzymatic (glucose) and high performance liquid chromatography (HPLC) (HbA1c) methods. Samples were spun in the centrifuge at 3000 rpm, at 4°C for 15 minutes. Serum was stored at -80°C until the day of the analysis. Cholesterol, HDL and triglycerides were measured in stored plasma with the use of Autoanalyser (C311, Roche Hitachi). LDL levels were calculated using the Friedwald equation (282). Insulin was measured in stored plasma with the use of commercially available ELISA. The homeostatic model assessment of insulin resistance (HOMA\textsubscript{IR}) was calculated by the equation HOMA\textsubscript{IR}= (Glucose x Insulin)/22.5 (77). Blood pressure was measured with an automated blood pressure monitor. The mean of two readings at least one minute apart was recorded. Blood pressure was measured in the dominant hand of the subjects who underwent subsequent PAT assessment and at least 20 min prior to PAT recording.

Weight, height, waist circumference (WC) and hips were measured with standardised techniques in all participants. Waist-to-hip ratio (WHR) was calculated by the ratio of waist circumference divided by the circumference of the hips (both measured in cm). BMI was calculated by dividing the weight (measured in kg) by the square of height (measured in metres). Middle aged women underwent additional body composition measurements including skinfolds at seven sites (biceps, triceps, subscapular, suprailiac, supraspinale, thigh and calf) and upper arm, mid-thigh and mid-calf circumferences.

6.2.5 Laser Doppler Imaging with Iontophoresis (LDI-ION)

All participants underwent LDI-ION in a temperature controlled room (23±1°C) and were allowed to acclimatise for at least 20 min before the test. This is a non invasive technique of measuring skin perfusion. Laser Doppler Imaging is based on the Doppler shift caused by moving red blood cells in the underlying tissues. Iontophoresis (ION) allows for the transdermal delivery of the vasodilator agents acetylcholine (Ach) and sodium nitroprusside (SNP) under the effect of applied current. Ach binds to the muscarinic receptors of the endothelial cells of the small resistance vessels at the dermis level resulting to subsequent generation of NO. Therefore, Ach is considered the “endothelium dependent” vasodilator. SNP, a NO donor, tests the integrity of vascular smooth muscles and, thereby, SNP is considered the “endothelium independent” vasodilator. NO has a
paracrine action on vascular smooth muscles causing vasodilatation via activation of the cGMP pathway. The method has been described in details in chapter 2.

The voltage across the chambers was recorded at the beginning of each scan and skin resistance was calculated using the Ohm’s Law (resistance= voltage / current). Skin perfusion was measured with the use of Laser Doppler Imager (Moor Instruments Ltd) equipped with a red laser (wavelength 633 nm; power 1 mW, beam diameter 1mm). The laser scanned in a raster fashion over both chambers and the backscattered light was collected by photodetectors and converted in a signal proportional to perfusion in arbitrary perfusion (flux) units (PU) and was displayed as a colour-coded image on the computer screen. The perfusion in each chamber for each scan was quantified with the use of the manufacturer’s image analysis software by outlining the region of interest (ROI) around the internal circumference of each chamber. Hence, the median flux value across approximately 700 measurement points was measured. Skin resistance was calculated for each scan and the area under the curve (AUC) was calculated as the summary skin resistance for each individual. Subsequently, each single response to the drugs displayed by each scan was corrected for individual’s skin resistance (by multiplying the perfusion units with the summary skin resistance for each individual) and the overall response to the drug was obtained by calculating the AUC.

6.2.6 Peripheral Arterial Tonometry (PAT) recording

Peripheral arterial tone (PAT) was recorded using the EndoPAT 2000 device (Itamar Medical, Caesarea, Israel). It is a non invasive technique based on the PAT signal technology measuring the magnitude and dynamics of arterial tone changes in peripheral arterial beds following upper arm blood flow occlusion. It specifically captures beat-to-beat plethysmographic recording of the distal arterial pulse wave amplitude (PWA) detected by the use of pneumatic probes. The method has been described in details in chapter 2.

6.2.7 Hot flushing recording

Participants were given a flush diary and asked to record any episode of hot flushing over one week period following their appointment. Filled diaries were posted back to me in a pre-paid envelope. In addition, the participants ranked the presence and severity of hot flushes and night sweats by filling the Greene climacteric scale where “0” indicates the absence of symptoms, “1” minor symptoms, “2” mild symptoms and “3” severe/extreme
symptoms. They were also questioned during their appointment whether they experienced VMS during the menopausal transition (in the absence of current symptoms) or had ever taken HRT.

Menopausal and peri-menopausal women who reported at least 20 flushes per week or ranked the severity of their flushing symptoms as “3” were considered as severe flushers. Women who had fewer recorded episodes of flushing or ranked their flushing symptoms as “1” or “2” were considered mild/moderate hot flushers. Women who did not record hot flushes in the diary, inclusively ranked their hot flushes as “0” in the Greene scale and did not recall any hot flushing during the menopausal transition were considered as non flushers.

### 6.2.8 Statistical Analysis

Continuous variables are presented as mean ± SD (range). Non-paired t-test was used for comparison of normally distributed or log (natural log)-transformed continuous variables among groups. Non parametric Mann-Whitney U test was used for comparison of variables which did not have a Gaussian distribution after transformation. Chi-squared test was used for comparing the distribution of categorical variables among groups. Two-way analysis of variance (ANOVA) was used to examine the effect of two independent variables with multiple observations on a continuous variable. Multivariate regression models were used to examine the relationship between variables with adjustment for confounders. P<0.05 was considered as the level of statistical significance. Stata/SE 12.1 (StataCorp LP, College Station, Texas, USA) was used for statistical analysis.
6.3 Results

6.3.1 Skin perfusion in the South Asian and European women

After excluding women with potential undiagnosed diabetes, on antihypertensive, lipid lowering treatment or invalid measures of skin perfusion, 74 women of South Asian origin and 79 women of Europeans origin were included in this analysis. Baseline characteristics and metabolic biomarkers of the two groups are shown in Table 21. The pattern of the inter-ethnic differences is similar to that observed in the total cohort (chapter 4) showing that the South Asians have greater WHR, fasting insulin levels, HbA1c and triglyceride levels and lower HDL levels compared with the Europeans of similar BMI and age.
<table>
<thead>
<tr>
<th></th>
<th>South Asians</th>
<th>Europeans</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>74</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>45.4 ± 14.4 (18.4-69.5)</td>
<td>47.7 ± 13.8 (19.8-69.7)</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>BMI (kg.m(^{-2}))</strong></td>
<td>25.6 ± 4.5 (18.0-42.7)</td>
<td>25.7 ± 4.5 (19.1-41.2)</td>
<td>0.82</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>79.1 ± 10.7 (61.0-111.6)</td>
<td>77.2 ± 10.0 (57.4-116.0)</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td>0.81 ± 0.8 (0.66-1.02)</td>
<td>0.78 ± 0.66 (0.66-1.00)</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>120.4 ± 16.3 (93.0-161.0)</td>
<td>121.6 ± 15.7 (86.0-167.0)</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>75.5 ± 10.9 (49.0-97.0)</td>
<td>77.0 ± 9.0 (54.0-93.0)</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Fasting glucose (mmol.l(^{-1}))</strong></td>
<td>4.9 ± 0.6 (3.9-6.8)</td>
<td>4.8 ± 0.5 (4.1-6.5)</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>HbA1c (mmol.mol(^{-1}))</strong></td>
<td>5.5 ± 0.5 (3.9-6.4)</td>
<td>5.2 ± 0.3 (4.6-6.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Insulin (IU.ml(^{-1}))</strong></td>
<td>9.4 ± 4.8 (1.7-20.0)</td>
<td>7.1 ± 3.1 (1.9-20.0)</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>Cholesterol (mmol.l(^{-1}))</strong></td>
<td>5.2 ± 1.0 (3.1-7.4)</td>
<td>5.3 ± 0.9 (3.7-8.0)</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>HDL (mmol.l(^{-1}))</strong></td>
<td>1.5 ± 0.5 (0.9-3.7)</td>
<td>1.8 ± 0.4 (0.9-3.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>LDL (mmol.l(^{-1}))</strong></td>
<td>3.1 ± 0.9 (1.5-5.5)</td>
<td>3.1 ± 0.9 (1.0-6.2)</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol.l(^{-1}))</strong></td>
<td>1.2 ± 0.7 (0.3-4.2)</td>
<td>0.9 ± 0.4 (0.4-2.4)</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td><strong>Ach response (AUC)</strong> (PUxMΩ)</td>
<td>13,516 ± 8,560 (1,505-52,788)</td>
<td>13,719 ± 8,317 (1,814-45,588)</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>SNP response (AUC)</strong> (PUxMΩ)</td>
<td>12,347 ± 6,834 (1,170-44,329)</td>
<td>13,092 ± 6,530 (2,006-29,585)</td>
<td>0.38</td>
</tr>
<tr>
<td><strong>Skin resistance (AUC) (MΩ)</strong></td>
<td>7.1 ± 2.8 (1.5-14.0)</td>
<td>6.0 ± 2.2 (1.8-11.7)</td>
<td><strong>0.008</strong></td>
</tr>
</tbody>
</table>

**Table 21. Baseline characteristics and metabolic biomarkers of the South Asian and European participants who underwent Laser Doppler Imaging with Iontophoresis (LDI-ION). Values are expressed in mean ± standard deviation (SD) (range) and p-values resulted from non paired t-test of raw or log-transformed values. Response to Ach and SNP are corrected for skin resistance. (AUC: area under the curve, SNP: sodium nitroprusside, Ach: Acetylcholine, BP: blood pressure, WHR: waist to hip ratio).**

Micro-vascular skin response to Ach and SNP was enhanced in the Europeans compared with the South Asians (p=0.001 and p<0.001 retrospectively, two-way ANOVA on log-transformed values) as it shown in Figure 34. The South Asians had greater skin resistance compared with the Europeans (7.1 ± 2.8 MΩ versus 6.0 ± 2.2 MΩ, p=0.008) which may affect the delivery of drugs during iontophoresis (281). Hence, when the skin perfusion was corrected for skin resistance, the micro-vascular response did not differ between the
two groups (p=0.87 for Ach and p= 0.18 for SNP, two-way ANOVA on log-transformed values) (Figure 35).

Figure 34. Skin perfusion with increasing charge for acetylcholine (Ach) and sodium nitroprusside (SNP) in the South Asian compared with the European participants. P=0.001 for Ach and p<0.0001 for SNP (two-way ANOVA on log-tranformed values). Data are mean ± standard error (SEM).

Figure 35. Skin perfusion (corrected for skin resistance) with increasing charge for acetylcholine (Ach) and sodium nitroprusside (SNP) in the South Asian compared with the European participants. P=0.87 for Ach and p=0.18 for SNP (two-way ANOVA on log-tranformed values). Data are mean ± standard error (SEM).

6.3.2 Comparison of micro-vascular reactivity measured by LDI-ION and Endo-PAT in the South Asian and Europeans women

In women over the age of 40 years vascular reactivity was assessed with the use of the EndoPAT measuring reactive hyperaemia post-occlusion and with the use of LDI measuring the response to Ach and SNP delivered by the use of ION. Table 2 shows the baseline characteristics and metabolic biomarkers of the two ethnic groups who underwent both methods (excluding women with undiagnosed diabetes, on anti-hypertensive or lipid lowering treatment or invalid measures of either vascular test).
Table 22. Baseline characteristics and metabolic biomarkers of the South Asian and European participants who underwent Laser Doppler Imaging with Iontophoresis (LDI-ION) and Endo-PAT. Central adiposity refers to the summation of central skinfolds (supra-iliac, supraspinale, subscupular). Values are expressed in mean ± standard deviation (SD) (range) and p-values resulted from non paired t-test of raw or log-transformed values. Response to Ach and SNP are corrected for skin resistance. (AUC: area under the curve, SNP: sodium nitroprusside, Ach: Acetylcholine, BP: blood pressure, WHR: waist to hip ratio).

<table>
<thead>
<tr>
<th></th>
<th>South Asians</th>
<th>Europeans</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>57</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.2 ± 7.7 (40.1-69.5)</td>
<td>54.6 ± 6.9 (40.2-69.7)</td>
<td>0.26</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>26.8 ± 4.6 (19.9-42.7)</td>
<td>26.6 ± 4.8 (19.1-41.2)</td>
<td>0.73</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82.8 ± 10.7 (62.8-111.6)</td>
<td>80.0 ± 10.1 (62.0-116.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>Waist-to-hip ratio (WHR)</td>
<td>0.84 ± 0.07 (0.66-1.02)</td>
<td>0.80 ± 0.06 (0.71-1.00)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Central Adiposity (mm)</td>
<td>71.5 ± 15.9 (37.6-107.0)</td>
<td>62.6 ± 20.1 (23.4-104.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>125.3 ± 15.7 (97.0-161.0)</td>
<td>124.8 ± 16.7 (86.0-167.0)</td>
<td>0.88</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>78.1 ± 11.1 (49.0-97.0)</td>
<td>78.0 ± 9.2 (54.0-93.0)</td>
<td>0.95</td>
</tr>
<tr>
<td>Fasting glucose (mmol.¹⁻¹)</td>
<td>5.1 ± 0.6 (4.1-6.8)</td>
<td>4.9 ± 0.5 (4.2-6.5)</td>
<td>0.18</td>
</tr>
<tr>
<td>HbA1c (mmol.mol⁻¹)</td>
<td>5.6 ± 0.4 (3.9-6.4)</td>
<td>5.2 ± 0.3 (4.6-6.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin (IU.ml⁻¹)</td>
<td>10.2 ± 5.0 (1.7-20.0)</td>
<td>7.3 ± 3.3 (2.5-20.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Cholesterol (mmol.¹⁻¹)</td>
<td>5.4 ± 0.9 (3.4-7.3)</td>
<td>5.6 ± 0.8 (4.3-8.0)</td>
<td>0.33</td>
</tr>
<tr>
<td>HDL (mmol.¹⁻¹)</td>
<td>1.4 ± 0.5 (0.9-3.7)</td>
<td>1.8 ± 0.4 (0.9-3.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL (mmol.¹⁻¹)</td>
<td>3.4 ± 0.7 (1.9-4.9)</td>
<td>3.3 ± 0.9 (1.0-6.2)</td>
<td>0.69</td>
</tr>
<tr>
<td>Triglycerides (mmol.¹⁻¹)</td>
<td>1.3 ± 0.7 (0.5-4.2)</td>
<td>1.0 ± 0.4 (0.5-2.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Ach response (AUC) (PUxMΩ)</td>
<td>13,321 ± 8,344 (3,108-52,788)</td>
<td>14,163 ± 7,984 (1,814-45,588)</td>
<td>0.43</td>
</tr>
<tr>
<td>SNP response (AUC) (PUxMΩ)</td>
<td>12,504 ± 7,110 (11,197-3,092)</td>
<td>13,667 ± 6,268 (12,719-2,006)</td>
<td>0.13</td>
</tr>
<tr>
<td>Skin resistance (AUC) (MΩ)</td>
<td>7.3 ± 2.7 (3.4-14.0)</td>
<td>6.3 ± 2.3 (1.8-11.7)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Figure 36 shows that micro-vascular skin perfusion in response to Ach and SNP with increasing current was enhanced in the Europeans compared with the South Asians (p<0.0001 for both Ach and SNP, two-way ANOVA on log transformed values of...
perfusion), however when the skin response was corrected for skin resistance the response to both agents did not differ between the two ethnic groups (p=0.42 for Ach, p=0.09 for SNP, two-way ANOVA on log transformed values of perfusion) (Figure 37).

Figure 36. Skin perfusion with increasing charge for acetylcholine (Ach) and sodium nitroprusside (SNP) in the South Asian compared with the European participants over the age of 40 years. P<0.0001 for both Ach and SNP (two-way ANOVA on log-transformed values). Data are mean ± standard error (SEM).

Figure 37. Skin perfusion (corrected for skin resistance) with increasing charge for acetylcholine (Ach) and sodium nitroprusside (SNP) in the South Asian compared with the European participants over the age of 40 years. P=0.42 for Ach and p=0.09 for SNP (two-way ANOVA on log-transformed values). Data are mean ± standard error (SEM).
Chapter 6

Figure 38: Reactive hyperaemia index measured by Endo-PAT in the South Asian and European participants over the age of 40 years. P values resulted from Mann-Whitney U test. Data are values and median ± interquartile range.

Figure 38 shows the values, median and interquartile range (IQR) of the reactive hyperaemia index (RHI) measured post occlusion with the use of Endo-PAT. RHI did not differ significantly among the South Asians and the Europeans (2.34 (IQR: 2.0, 2.68) versus 2.51 (IQR: 2.11, 2.89), p=0.17).

RHI did not correlate with the response to Ach and SNP in either the South Asian or the European women (r= 0.02, 95% CI: -0.17, 0.20, r= 0.06, 95% CI: -0.13, 0.24 respectively), suggesting that the two methods measure vascular response in different vascular beds. The response to Ach was highly correlated with the response to SNP measured by LDI-ION (r=0.83, 95% CI: 0.77, 0.87).

In regards to the correlations of the vascular measurements and metabolic risk factors or modifiable lifestyle factors; RHI in both groups was correlated with the fasting levels of HDL cholesterol (r=0.21, 95% CI: 0.02, 0.38), fasting levels of insulin (r= -0.19, 95% CI: -0.36, -0.002) and the amount of moderate to vigorous physical activity performed weekly (r= 0.21, 95% CI: 0.01, 0.40). The response to Ach was correlated with total cholesterol levels (r=-0.25, 95% CI: -0.39, -0.09), triglycerides levels (r=-0.19, 95% CI: -0.34, -0.03), LDL levels (r= -0.24, 95% CI: -0.38, -0.08) and the central adiposity defined by central skinfolds (r= -0.19, 95% CI: -0.37, -0.004). The response to SNP was correlated with LDL cholesterol levels (r=-0.17, 95% CI:-0.32, -0.008).
6.3.3 Skin perfusion and hot flushing

Among the above women with valid measurements for EndoPAT and LDI-ION those who were menopausal or late perimenopausal were selected. Out of those; 28 women (12 South Asians, 16 Europeans) did not report any hot flushes, 35 (14 South Asians, 21 Europeans) reported mild to moderate hot flushes and 31 (17 South Asians, 14 Europeans) reported severe hot flushes, 12 women did not have hot flushes at the time of the study but admitted hot flushing problems during the menopausal transition, hence they were not included in any of the above categories and were excluded from the analysis. After excluding women with likely undiagnosed diabetes and women on antihypertensive or lipid lowering treatment (n=15 in total), 23 women (7 South Asians and 16 Europeans) without hot flushes (non flushers), 32 women (12 South Asians and 20 Europeans) with moderate/mild hot flushes and 24 women (14 South Asians and 10 Europeans) with severe hot flushes were included in the analysis. Since, the vascular reactivity did not differ between the ethnic groups; the South Asians and Europeans were pooled together according to their flushing status. In addition, women with moderate/mild flushing and severe flushing were pooled under the same category of women with hot flushes (flushers) for the analyses unless otherwise stated.

Table 23 and Table 24 show the demographic characteristics and metabolic markers of women with hot flushes compared with women without. The ethnic distribution and the proportion of women having a hysterectomy did not differ significantly among the two flushing categories (p=0.19 and p=0.89), but years since last menstrual period (LMP) differed significantly among the groups with symptomatic women reporting less years since LMP. However, only n= 51 (35% missing values) gave information about the LMP and the reliability of the information is questionable as it can be affected by recall bias (i.e. symptomatic women are more likely to record precisely the years since LMP). Hence, the use of this variable in further analysis is limited. Age, BMI and concentration of lipids or levels of adipokines did not differ across the groups, but there was borderline evidence suggesting that women reporting hot flushes had greater central adiposity than non flushers (p=0.06 for WHR and p=0.08 for the summation of central skinfolds). Notably, women reporting hot flushes had higher fasting insulin and were more insulin resistant (assessed by the HOMAIR) compared with non flushers.
## Table 23. Demographic characteristics and metabolic markers of women with hot flushes (flushers) compared with women without hot flushes (non flushers). Central adiposity corresponds to the summation of central skinfolds (supra-iliac, supraspinale, subscupular). Values are expressed as mean ± standard deviation (SD) (range) unless otherwise stated and p-values resulted from non-paired t-test of raw or log-transformed values. (WHR: waist to hip ratio, BP: blood pressure).

<table>
<thead>
<tr>
<th></th>
<th>Non flushers</th>
<th>Flushers</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Asians (n, %)</td>
<td>7 (30.4%)</td>
<td>26 (46.4%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Parity (n, %)</td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>0</td>
<td>2 (8.7%)</td>
<td>10 (17.9%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5 (21.7%)</td>
<td>7 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>( \geq 2 )</td>
<td>16 (69.6%)</td>
<td>39 (69.6%)</td>
<td></td>
</tr>
<tr>
<td>Hysterectomy (n, %)</td>
<td>3 (13.0%)</td>
<td>8 (14.3%)</td>
<td>0.89</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.0 ± 6.9 (45.1-67.9)</td>
<td>55.2 ± 5.6 (45.8-69.7)</td>
<td>0.29</td>
</tr>
<tr>
<td>Last menstrual period (years) median (range)</td>
<td>11 (3-17)</td>
<td>2.25 (0.3-18)</td>
<td>0.0002</td>
</tr>
<tr>
<td>BMI (kg.m(^{-2}))</td>
<td>27.5 ± 6.5 (19.4-42.7)</td>
<td>27.4 ± 4.3 (19.4-39.3)</td>
<td>0.82</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>81.5 ± 13.3 (64.8-116.0)</td>
<td>83.4 ± 10.1 (67.0-111.6)</td>
<td>0.39</td>
</tr>
<tr>
<td>WHR</td>
<td>0.81 ± 0.06 (0.72-1.00)</td>
<td>0.84 ± 0.07 (0.71-1.02)</td>
<td>0.06</td>
</tr>
<tr>
<td>Central Adiposity (mm)</td>
<td>64.2 ± 23.3 (24.1-104.9)</td>
<td>72.2 ± 16.14 (27.2-107.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>125.0 ± 18.1 (86.0-160.0)</td>
<td>128.4 ± 14.5 (97.0-167.0)</td>
<td>0.38</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>76.6 ± 11.4 (49.0-91.0)</td>
<td>80.5 ± 9.3 (59.0-97.0)</td>
<td>0.12</td>
</tr>
<tr>
<td>N</td>
<td>Non flushers</td>
<td>Flushers</td>
<td>p-value</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>HbA1c (mmol.mol⁻¹)</td>
<td>5.5 ± 0.4 (4.9-6.4)</td>
<td>5.5 ± 0.4 (4.7-6.3)</td>
<td>0.77</td>
</tr>
<tr>
<td>Insulin (IU.ml⁻¹)</td>
<td>7.2 ± 4.5 (2.9-20.0)</td>
<td>9.2 ± 4.5 (2.5-20.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>1.6 ± 1.2 (0.6-5.5)</td>
<td>2.1 ± 1.2 (0.6-5.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>Cholesterol (mmol.l⁻¹)</td>
<td>5.5 ± 0.9 (4.1-7.8)</td>
<td>5.6 ± 0.8 (3.4-8.0)</td>
<td>0.61</td>
</tr>
<tr>
<td>HDL (mmol.l⁻¹)</td>
<td>1.7 ± 0.4 (0.9-2.5)</td>
<td>1.6 ± 0.4 (0.9-3.2)</td>
<td>0.26</td>
</tr>
<tr>
<td>LDL (mmol.l⁻¹)</td>
<td>3.4 ± 0.8 (2.2-5.4)</td>
<td>3.5 ± 0.8 (1.0-6.2)</td>
<td>0.76</td>
</tr>
<tr>
<td>Triglycerides (mmol.l⁻¹)</td>
<td>1.0 ± 0.3 (0.6-1.9)</td>
<td>1.2 ± 0.6 (0.6-4.2)</td>
<td>0.17</td>
</tr>
<tr>
<td>FSH (IU. l⁻¹)</td>
<td>55.3 ± 28.6 (11.6-101.0)</td>
<td>64.2 ± 24.7 (8.1-101.0)</td>
<td>0.20</td>
</tr>
<tr>
<td>Testosterone (nmol.l⁻¹)</td>
<td>1.5 ± 0.6 (0.7-2.6)</td>
<td>1.4 ± 0.6 (0.4-2.9)</td>
<td>0.38</td>
</tr>
<tr>
<td>FAI</td>
<td>3.2 ± 2.5 (0.9-10.0)</td>
<td>2.9 ± 1.9 (0.6-10.8)</td>
<td>0.94</td>
</tr>
<tr>
<td>SHBG (nmol.l⁻¹)</td>
<td>63.1 ± 29.2 (18.0-114.0)</td>
<td>56.4 ± 24.5 (13.0-138.0)</td>
<td>0.30</td>
</tr>
<tr>
<td>Adiponectin (ng.ml⁻¹)</td>
<td>16.4 ± 7.2 (4.1-28.7)</td>
<td>16.0 ± 6.6 (3.2-29.6)</td>
<td>0.88</td>
</tr>
<tr>
<td>Leptin (ng.ml⁻¹)</td>
<td>33.2 ± 33.1 (4.0-122.5)</td>
<td>31.8 ± 22.1 (4.9-113.0)</td>
<td>0.39</td>
</tr>
<tr>
<td>CRP (mg.l⁻¹)</td>
<td>2.4 ± 3.4 (0.2-15.0)</td>
<td>2.9 ± 3.4 (0.3-22.0)</td>
<td>0.13</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>39.8 ± 3.5 (32.0-45.5)</td>
<td>39.8 ± 4.0 (27.5-45.5)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Table 24. Serum biomarkers of women with hot flushes (flushers) compared with women without (non flushers). Values are expressed as mean ± standard deviation (SD) (range) and p-values resulted from non-paired t-test of raw or log-transformed values. (FAI: free androgen index, SHBG: sex hormone binding globulin).

Figure 39 shows that women reporting hot flushes had enhanced vascular response following administration of either Ach or SNP (values corrected for skin resistance, p=0.0008 for Ach and p=0.0001 for SNP, two-way ANOVA on log transformed values). When the micro-vascular response to ION was examined separately in women with severe hot flushes (severe flushers), mild/moderate hot flushes (moderate/mild flushers) and with women without hot flushes (non flushers), there was evidence of a dose response effect of hot flushing on micro-vascular perfusion for both Ach and SNP with increasing flushing
resulting in enhanced vascular reactivity, albeit not all within groups differences reached statistical significance (Figure 40).

Figure 39. Skin perfusion (corrected for skin resistance) with increasing charge for acetylcholine (Ach) and sodium nitroprusside (SNP) in women with hot flushes (flushers) compared to women without (non flushers). P=0.0008 for Ach and p=0.0001 for SNP (two-way ANOVA on log-tranformed values). Data are mean ± standard error (SEM).

Figure 40. Skin perfusion (corrected for skin perfusion) with increasing charge for acetylcholine (Ach) and sodium nitroprusside (SNP) in women with severe or mild/moderate hot flushes compared with women without hot flushes. Response to Ach; severe flushers compared with non flushers p<0.0001, severe flushers compared with moderate flushers p=0.01, moderate flushers versus non flushers p=0.27. Response to SNP; severe flushers compared with non flushers p<0.0001, severe flushers compared with moderate flushers p=0.21, moderate flushers versus non flushers p=0.02 (two-way ANOVA with Bonferroni correction). Data are mean ± standard error (SEM).
Figure 41. Reactive hyperaemia index measured by Endo-PAT in women with hot flushes (flushers) and women without hot flushes (non flushers). P values resulted from the Mann-Whitney test. Data are values and median ± interquartile range.

Figure 41 shows that women with hot flushes have decreased RHI measured with the use of Endo-PAT compared with women without hot flushes (2.46 (IQR: 1.94, 2.66) versus 2.74 (IQR: 2.25, 3.04), p=0.03). The difference in RHI among the two groups remained significant after adjustment for insulin levels and ethnicity (p=0.02). There was no strong evidence of a linear trend between increasing hot flushing and RHI (p=0.52) when moderate flushers examined separately from severe hot flushers. There was no correlation between RHI and response to ACH or SNP (r=0.02, 95% CI: -0.17, 0.20, and r=0.06, 95% CI: -0.13, 0.24 respectively), suggesting that the two techniques assess different beds of vascular reactivity.

Table 25 displays the standardised beta coefficients and p-values resulted from multivariate models having as dependent variable the RHI or response to Ach or response to SNP and as independent variables the variables included in the table with additional adjustment for ethnicity and socioeconomic status (by using the SIMD ranking). The beta coefficients for continuous variables correspond to the degree of change in the dependent variable for one SD increase in each independent variable. The beta coefficient for hot flushing (dichotomous variable) corresponds to the change in the dependent variable for hot flushers compared with the referent group (non flushers). Hence, hot flushing is associated with decreased RHI and increased response to Ach and SNP measured by the LDI-ION independently of conventional metabolic risk factors. In addition, RHI was associated with Systolic BP, time engaged with MVPA and fitness levels. On the contrary, none of the traditional CVD risk was associated with the response measured with LDI-ION when hot
flushing was included in the model. When years since LMP are included in the multivariate analysis as a confounder (data not shown in details), hot flushing is not significantly associated with response to Ach (p=0.08) or RHI (p=0.5) but is still associated with the response to SNP (p=0.03). Whether this is a result of prolonged oestrogen insufficiency in endothelial function (longer LMP as proxy) or reduced statistical power (over 1/3 of missing values) cannot be clarified with the existing data.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Reactive Hyperemia Index (RHI)</th>
<th>Response to Acetylcholine (Ach)</th>
<th>Response to sodium nitroprusside (SNP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>beta coefficient</td>
<td>p-value</td>
<td>beta coefficient</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.17</td>
<td>0.43</td>
<td>-35.6</td>
</tr>
<tr>
<td>BMI (kg.m(^{-2}))</td>
<td>0.18</td>
<td>0.18</td>
<td>1727</td>
</tr>
<tr>
<td>Central Adiposity (mm)</td>
<td>0.06</td>
<td>0.65</td>
<td>-1335</td>
</tr>
<tr>
<td>Hot flushing</td>
<td>-0.39</td>
<td>0.04</td>
<td>4077</td>
</tr>
<tr>
<td>MVPA (min.day(^{-1}))</td>
<td>0.26</td>
<td>0.01</td>
<td>848</td>
</tr>
<tr>
<td>VO(<em>2)(</em>{\text{max}}) (ml.kg(^{-1}).min(^{-1}))</td>
<td>0.20</td>
<td>0.05</td>
<td>807</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.19</td>
<td>0.04</td>
<td>-693</td>
</tr>
<tr>
<td>Insulin (IU.ml(^{-1}))</td>
<td>-0.17</td>
<td>0.17</td>
<td>1173</td>
</tr>
<tr>
<td>HDL (mmol.l(^{-1}))</td>
<td>0.11</td>
<td>0.27</td>
<td>-1189</td>
</tr>
<tr>
<td>Triglycerides (mmol.l(^{-1}))</td>
<td>-0.06</td>
<td>0.66</td>
<td>-990</td>
</tr>
<tr>
<td>Total cholesterol (mmol.l(^{-1}))</td>
<td>0.06</td>
<td>0.63</td>
<td>-1673</td>
</tr>
</tbody>
</table>

Table 25. Associations of metabolic biomarkers and life style factor with RHI, response to Ach and response to SNP. Beta coefficients represent the change in the dependent variable (RHI, response to Ach or response to SNP) for one SD increase in each continuous variable. The beta coefficients for hot flushing correspond to the change in the dependent variable (top row) for hot flushers (compared to non flushers). The beta coefficients and p-values resulted from multivariate models adjusted for all the above co-variates plus ethnicity and socioeconomic status (SIMD ranking). (MVPA: moderate to vigorous physical activity, VO\(_2\)\(_{\text{max}}\): maximal oxygen uptake, BP: blood pressure).
6.4 Summary of key result findings

6.4.1 Micro-vascular perfusion (measured with the use of LDI-ION) and ethnicity

- Electrical skin resistance was greater in the South Asians compared with the Europeans which accounted for the ethnic differences in the vascular response to both SNP and Ach measured with used of LDI-ION.

- After correction for skin resistance, the healthy South Asian and European volunteers demonstrated similar vascular response to both chemical agents.

- The response to Ach was correlated with total cholesterol levels, triglyceride levels and central adiposity defined by the summation of central skinfolds.

- The response to SNP was correlated with LDL cholesterol levels.

6.4.2 Vascular response (measured with the use of Endo-PAT) and ethnicity

- RHI measured with the use of Endo-PAT did not differ among the healthy South Asian and European volunteers.

- RHI was correlated with HDL cholesterol levels, insulin levels and time engaged with MVPA.

6.4.3 Vascular response and hot flushing

- Menopausal women with hot flushes had enhanced response to both Ach and SNP compared with women without hot flushes.

- Hot flushing was the only factor associated with the response to Ach and SNP, whereas conventional metabolic variables had no association after including hot flushing in the regression model.

- Menopausal women with hot flushes had decreased RHI compared with non flushers.
• Hot flushing was independently associated with decreased RHI beyond conventional CVD risk variables. MVPA, systolic BP and fitness levels were additional variables associated with RHI in menopausal women.
6.5 Discussion

This chapter demonstrated that vascular reactivity measured by two different methods, either by the means of LDI-ION and response to Ach and SNP or by RHI following blood flow occlusion, did not differ among healthy South Asian and European women without established CVD. Interestingly, the South Asians had greater electric skin resistance compared with the Europeans, which influences the drug delivery through the skin under the application of low electric current. Another notable finding was that menopausal women complaining of hot flushing displayed paradoxical greater micro-vascular perfusion in response to chemical vasodilators (Ach, SNP) but lower RHI and greater insulin resistance compared with women without hot flushes.

There are limited studies looking at inter-ethnic differences in micro-vascular level and the results are greatly influenced by the characteristics of the study sample or the methods of assessment of vascular function. A study comparing South Asians, White Europeans and Caribbean Africans with diagnosed heart failure showed that South Asians had decreased response to ION-delivered Ach measured by Laser Doppler Flowmetry which was robust after adjustment for confounders suggesting that ethnicity significantly influenced the micro-vascular response among patients with established CVD (389). However, this study did not account for potential interethnic discrepancies in electrical skin resistance which may have affected the drug delivery with the use of ION (390). Given that the impaired macro-vascular function observed in South Asians was mainly mediated via the higher prevalence of co-morbidities and attenuated after adjustment for covariates, it is likely that the difference in micro-vascular function was attributable to diverse skin properties rather than accelerated disease progress (389). In keeping with this, another study did not reveal ethnic-related differences in micro-vascular function in either healthy subjects or subjects with diagnosed CHD by assessing the response to heating and ischemia which both are not expected to be influenced by electric skin resistance (385). Hence, skin resistance seems to be a determinant of micro-vascular response to ION between different ethnic groups and the current study is the largest, to my knowledge, providing strong evidence supporting this by quantifying the skin resistance among the two groups and displaying the micro-vascular response in both groups before and after correction for skin resistance. A recent smaller study (n=33) using a similar protocol compared young Black Africans with White Europeans living in South Africa and demonstrated substantial ethnic difference in skin resistance which affected the delivery and response to both Ach and SNP (391). Whether skin pigmentation, distribution of sweat ducts, density of hair follicles or other skin
elements determine low and high resistance pathways among different ethnic groups and subsequent drug delivery during ION is unknown (390). However, herein it is shown that skin resistance is an important but previously overlooked factor that influences vascular reactivity in response to ION and drug delivery in ethnic specific research and needs to be accounted for in future studies. An additional benefit of correction for skin resistance is the elimination of the inter-subject variability in skin response which has been demonstrated previously in a study assessing subjects of White descent (390).

The participants in the current study were relatively healthy without diagnosed co-morbidities in either ethnic group. Micro-vascular function assessed by the response to vasodilator agents is impaired in diabetes (392), rheumatoid arthritis (393), cardiac syndrome X (394), proteinuric glomerulonephritis (395), and hypertension (396) all of which are associated with increased incidence of CVD. The cutaneous dysfunction observed in these morbidities is possibly related with the positive association demonstrated between peripheral perfusion and macro-vascular response (397) or coronary function (398). However, the value of micro-vascular response to Ach and SNP in predicting future CVD in an entirely healthy population has yet to be confirmed. Hence, the lack of micro-vascular dysfunction in the South Asian participants compared with the Europeans, despite the former having greater metabolic risk profile, does not reject the possibility of future earlier impaired vascular response in the South Asians when they progress to a pre-diabetic status (397). It is, also, possibly related to the fact that both groups were free of overt vascular disease and those with diagnosed hypertension were excluded from the analysis. Similarly, the induced reactive hyperaemia measured with the use of Endo-PAT, despite underpinning different vascular mechanisms in response to blood flow occlusion compared with the response to Ach and SNP, did not differ among the two ethnic groups either. PAT has been suggested as a non invasive method of identifying symptomatic patients with positive angiographic findings (399) or at risk of future cardiac death and myocardial infarction (400) and it has a positive correlation with FMD in patients complaining of chest pain (401); however there is no evidence validating it as a screening tool in predicting future cardiovascular events in healthy individuals. A community study in n=1843 subjects with low prevalence of CVD, who had concurrent FMD and EndoPAT testing, failed to show an association between the two methods with the authors concluding that digital dysfunction may not be evident at very early stages of vascular dysfunction (402). In the same study, low digital RHI was associated with the presence of diabetes, higher BMI, higher cholesterol and paradoxically lower systolic blood pressure (402), factors which were not divergent between the two ethnic groups of my study.
The effect of hot flushing on vascular response is complex; the current study showed that women with hot flushing had greater responsiveness to both endothelium dependent and independent agents delivered by means of ION and, interestingly, in a dose response fashion i.e. the greater the frequency of hot flushing the larger the effect on cutaneous response. This finding is in line with a previous study using the same methodology of measuring endothelial function in a different cohort of women consisting mainly of lean women of White European descent (272). SNP dissociates to NO which activates guanylate cyclase in vascular smooth muscle and facilitates the production of intracellular cGMP. In turn, cGMP triggers calcium movement in to endoplasmatic reticulum and reduces its bioavailability and binding capacity with calmodulin, hence, vascular smooth muscle relaxes causing subsequent vasodilatation. On the contrary, ION delivery of Ach stimulates NO production by the endothelial cells which subsequently acts on smooth muscle cells to cause vasodilatation. However, there is contradictory evidence suggesting that exogenous Ach induced vasodilatation is not entirely NO-mediated; prostanoid-dependent and independent pathways play a key role (403) and a shift towards the predominance of cyclooxygenase (COX) related products favouring vasoconstriction occurs with advancing age (403). Hence, the vascular response to exogenous Ach may not reflect NO release and endothelial integrity, but it may be the net effect of various pathways with a predominance of non NO-related pathways at the age of menopause. This could explain the reason of the absence of an association between the response to Ach and SNP and conventional metabolic factors in menopausal women in the current study.

Enhanced micro-vascular response seems to suggest a healthier vascular phenotype (397, 404, 405), but it was not clear in the index study whether the increased vascular response in flushing women measured by LDI-ION was purely a result of increased vasomotor smooth muscle response or greater bioavailability of NO and thereby healthier endothelial cells. The former was supported by the finding that the existence of hot flushing had a positive association with the response to SNP along with the response to Ach in the multivariate model with adjustment for confounders. In addition, the latter assumption was less likely given that women with hot flushing did not demonstrate a healthier metabolic phenotype, but indeed displayed greater insulin resistance. Tuomikoski et al demonstrated greater responsiveness to nitroglycerin (endothelium independent agent) measured by the means of pulse wave analysis (PWA), but not in salbutamol (endothelium dependent drug) in women with hot flushes compared with women without hot flushes when the two agents were given sequentially and not concurrently (273). In the same study, women with hot flushes had prolonged onset of the reflected wave (dependent on pulse wave velocity) and
the first systolic peak (dependent on the ventricular ejection) under the effect of nitroglycerin compared with their baseline response, whereas women without hot flushes did not show any differences in PWA in response to nitroglycerin compared to their baseline response (273), which may indicate that women with hot flushes have an activated sympathetic tone. Therefore, it cannot be excluded that women with hot flushes have a more sensitive vasculature to vasodilator agents triggered by an activated autonomic system.

The pathogenesis of hot flushing is still unknown but there is evidence suggesting that both central and peripheral mechanisms are involved (406). Initial studies suggested that the peripheral blood flow was altered (407) in women complaining of hot flushing with selective vasodilatation of the forearm which attenuated, though, following anoxic stimuli (408). It has, also, been suggested that women with hot flushing have a narrower thermoregulatory zone with a small increase in core body temperature resulting in a higher perception of heat (388). Given that central norepinephrine release is greater in post-menopausal women during a hot flushing episode (409), the authors suggested that central norepinephrine acts on the pre-optic hypothalamus and causes narrowing of the thermoregulatory zone in menopausal symptomatic women (388). Hence, when body temperature rises above a threshold (which is lower in symptomatic than in asymptomatic women) afferent pathways to the thalamus are activated which subsequently lead to heat dissipation responses, such as vasodilatation of the dermis vessels, in order the body to exhalate heat loss and resume baseline core temperature. The micro-vasculature of the dermis captured by LDI is expected to contribute to thermoregulation mechanisms but it is unclear whether the larger response to chemical agents observed in women with hot flushing was superimposed by greater vasodilatation and need to lose heat.

The evidence about the physiology of the hyperaemic response to shear stress is conflicting with some studies suggesting that is mediated to an extent (around 50% when is measured by the means of EndoPAT) by the bioavailability of NO (410) and an array of other substances such as prostaglandins, adenosine, hydrogen peroxide (411) and other studies suggesting that there is no involvement of the NO pathway in hyperaemic response (412, 413). The finding that RHI was not correlated with the response to Ach or SNP suggests that different pathways are activated in response to each stimulus. In addition, RHI is influenced by autonomic sympathetic action and increasing muscle sympathetic activity measured by microneurography had an inverse correlation with RHI \( r = -0.8, \ p = 0.005 \) in a small study \( n=10 \) of healthy individuals (414). The same study suggested that
increased physical activity was associated with decreased sympathetic activation and thereby higher RHI (414). The current study substantiated in a larger cohort that objectively measured physical activity had a positive association with RHI and it cannot be excluded that this was mediated through down-regulation of the sympathetic system. Ageing is associated with increased sympathetic activity and there is evidence suggesting that in symptomatic menopausal women sympathetic tone dominates over parasympathetic (415, 416). Activated sympathetic tone causes peripheral vasoconstriction which may contribute to the reduced reactive hyperaemia induced following blood occlusion in the women with hot flushes compared with non flushers in this study. Whether reduced RHI is an adverse prognostic factor of future risk of atherosclerosis or an outcome of altered functional vasculature in healthy women with hot flushes is unclear and the former could be answered in prospective cohort studies assessing the progress from the exposure to the outcome in a chronological order. However, it cannot be ignored that the women with hot flushes were more insulin resistant (expressed by higher HOMAIR) and displayed marginally greater central adiposity compared with non flushers which was not driven by greater BMI. These findings are in line with previous studies showing that VMS are associated with greater HOMA index in a dose response fashion (i.e. evidence of a positive trend between increased frequency of VMS and higher HOMA) independently of BMI, age, ethnicity or modifiable life style factors (417). Although, my study cannot reveal the temporal and causal nature of the association of higher HOMAIR, impaired RHI and existence of hot flushing, it should be interpreted in the context of a large body of literature connecting VMS with an adverse metabolic and vascular profile; expressed by impaired endothelial function (271), elevated aortic calcification (269), elevated intima media thickness (270) or higher levels of pro-thrombotic factors (418). Pooling the conclusions of the above studies and suggesting a clear link between VMS and CVD may not be appropriate because of the different study designs and subjects’ characteristics, however, the existing evidence supports adopting a holistic approach in treating symptomatic menopausal women.

There are limited data about the prevalence of hot flushing in different ethnic groups. Although the majority of menopausal women (almost 80%) of White descent experience VMS (267), the pooled prevalence of hot flushing in South Asians varies from 14 to 42% with marked differences between rural and urban populations (267). In addition, a study conducted in South India reported that the most frequent menopausal symptoms were joint pains, backache, sleep disturbance and memory weakness rather than hot flushing (419). However, Indians who migrated to the UK reported similar frequency of VMS to the
background White population and substantially higher compared with women living in Delhi (420). Similarly, the SWAN study suggested that African women living in Northern America experience hot flushing to the same extend with White women living in the same urban areas (421). Although the prevalence of hot flushing was not the primary outcome of the current study, this work confirmed the above observations that the existence of hot flushing did not differ among the two ethnic groups living in urban regions in Scotland. Hence, South Asians are equally likely to experience hot flushing compared with the background White population. Thus medical practitioners should be questioning menopausal symptoms in midlife women of different ethnic backgrounds.

This study used a dual method of assessing vascular function in midlife women from two ethnic groups trying to uncouple different vascular mechanisms possibly influenced by ethnicity. In addition, this is the first study, to my knowledge, recognising that the existence of hot flushing has a direct effect on RHI, measured with the use of Endo-PAT, which was independent of conventional vascular risk factors. Hence, menopausal hot flushing should be considered as an additional confounding factor in the relationship of RHI with CVD in future population studies investigating the prognostic value of RHI in detecting subclinical vascular disease.

However, it is acknowledged that this study has limitations; the use of diaries for recording the number of hot flushes eliminates recall bias but is subjected to reporting bias potentially influenced by cultural factors or engagement with other activities. Hence, by recognising this limitation, number of hot flushing episodes was not considered as a continuous variable but was categorised and the majority of the analyses were performed by using hot flushing as a dichotomous variable (flushers versus non flushers). Undoubtedly, sternal skin conductance monitoring would have been an objective method of recording the intensity and frequency of hot flushing episodes but could have amplified the risk of low compliance. I appreciate that questioning women without current hot flushes about their past experience of hot flushing is subjected to recall bias too, but it is unlikely that previous significant hot flushing affecting their quality of life could have been overlooked.

6.6 Conclusion

In conclusion, this chapter showed that vascular response to ION-delivered vasodilator agents or shear stress did not differ in the cohort of healthy South Asian and European
women and despite of the South Asians having features of insulin resistance, there were not early signs of impaired vasculature function in this group. Hot flushing influenced both the response to ION and shear stress independently of conventional vascular factors, but, notably, in an opposite direction suggesting that different complex pathways dominate in each response.
7 DEFINING OBESITY AND PHYSICAL ACTIVITY
CUT-OFF POINTS FOR MIGRANT SOUTH ASIAN WOMEN

7.1 Introduction

There is growing evidence suggesting that migrant South Asians develop type 2 diabetes and CVD almost 10 years younger than the background population of white descent and at a lower BMI (27, 48, 115, 422). In chapter 4, it was shown that healthy South Asian women accumulated a greater cluster of metabolic risk factors (higher fasting insulin, lower HDL cholesterol and higher triglycerides) which could potentially predispose them at a higher risk of type 2 diabetes and other metabolic consequences compared with the Europeans of similar BMI and age. The insulin resistant phenotype in the South Asian participants was mainly associated with greater central adiposity for a given BMI and waist circumference. Hence, a metabolically impaired body distribution with increased fat deposition centrally in the South Asians compared with the Europeans for a given BMI render the current obesity cut off points unreliable for this ethnic minority.

BMI and waist circumference can be readily measured and elevated values of these markers can be observed years before the metabolic consequences are evident. On contrary, direct measurement of central adiposity requires expertise and certain infrastructure which can be unfeasible in clinical settings. Hence, BMI and waist circumference can be used as surrogate markers of body composition, with some evidence suggesting the superiority of waist circumference over BMI as an indicator of associated metabolic risk (423, 424). The universal classifications of BMI are overweight (≥ 25 kg.m\(^{-2}\)) and obese (≥ 30 kg.m\(^{-2}\)). These cut points have mainly resulted from studies on subjects of White Europeans descent to correspond to risk thresholds for future disease and mortality (38), but there is ongoing debate whether they apply in ethnic minorities. In 2000, WHO recognised the need for ethnic specific cut points for body composition recommending ≥ 23 kg.m\(^{-2}\) for defining overweight and ≥ 25 kg.m\(^{-2}\) for defining obesity in Asian populations (37). However, in 2004 the suggestions were disputed by WHO and were not adopted into international guidelines mainly because of the large variability in BMI points across different Asian ethnic subgroups (425). Thereafter, studies have suggested various BMI cut points for South Asians living in the Indian subcontinent (43) and in West world (40, 41, 45) by utilising different statistical techniques and different endpoints to define risk and derive cut points, but all agreed that the conventional BMI
classifications underestimate substantially the metabolic risk in the South Asians. A position statement about diagnosing obesity in the British South Asians by the South Asian health foundation (SAHF) urged the need for ethnic specific BMI classifications (with 27.5 kg.m\(^{-2}\) as the recommended cut point for defining obesity in healthy individuals) but still suggested adherence to the WHO guidelines (426). Recently, the National Institute for Health and Care Excellence (NICE) adopted ethnic specific points in the definition of overweight and obesity suggesting that the risk of developing diabetes of a South Asian with a BMI of 24 kg.m\(^{-2}\) equates to the risk of a European with a BMI of 30 kg.m\(^{-2}\) (44).

Similarly, ethnic specific criteria for waist circumference have been explored by studies and the body of evidence suggests lower cut-offs for South Asians compared with comparators of White descent (41, 45). A joint scientific statement introduced the concept of using ethnic specific thresholds for abdominal obesity in clinical practice but the recommendations were quite conservative with the level of waist circumference ≥ 80 cm for South Asian women as opposed to ≥ 80 cm or ≥ 88 cm (variation based on different stratification of risk by different organisations) for Caucasian women being used for defining abdominal obesity (58). A consensus statement from India recommended a two-tier approach with waist circumference for women ≥ 72 cm alerting increased cardiometabolic risk and ≥ 80 cm requiring medical attention (43). NICE guidelines have adopted the threshold of 80 cm to define abdominal obesity for South Asian women (44). However, a recent study in almost half a million participants of the UK Biobank cohort demonstrated that South Asian women living in the UK with waist circumference greater than 70 cm have similar risk of developing diabetes with Caucasian women with a waist circumference over 88 cm (45). Therefore, revisiting the current guidelines and adopting a universal approach in defining total and abdominal adiposity with ethnic and sex specific thresholds is still warranted.

While it is now generally accepted that different thresholds for BMI and waist circumference should apply according to ethnicity and sex, the universal guidelines for regular physical activity are not ethnicity specific. The minimum recommended MVPA time of 150 min.week\(^{-1}\) in order an individual to minimise the background risk of type 2 diabetes, CVD or all-cause mortality have largely resulted from cumulative work on populations of White European descent and have been extrapolated in other populations without further testing (195). South Asians are a group at high risk of developing diabetes and CVD compared with their White comparators; hence efficient lifestyle modifications in the form of increasing physical activity would have greater significance in reversing or
modifying the background risk in this population. There is evidence suggesting that South Asians are less active (when activity was measured with objective measures) than Europeans (207, 210) and this finding was replicated in the women who participated in the current study (Chapter 4). In addition, South Asian men for any given level of weekly physical activity have lower levels of cardio-respiratory fitness (207) and have reduced ability of oxidising fat during exercise (211). Therefore, the above evidence indicates that ethnicity may not only influence behaviour towards regular exercising but may modify the dose-response relationship between physical activity and cardiometabolic factors. That would warrant ethnicity tailored recommendations for the appropriate levels of MVPA for South Asians. Celis-Morales et al tested this concept in a cohort of middle aged healthy South Asian men (n=75) and demonstrated that South Asians need to exercise around 266 min.week\(^{-1}\) (in bouts of \(\geq\) 10 minutes) in order to equate their overall cardiometabolic risk with that of their European comparators (n=83) who exercise 150 min.week\(^{-1}\) (42). However, this has not been tested in South Asian women who are at higher risk of developing type 2 diabetes over the age of 55 years than men and are known to exercise even less than men (chapter 4, (207)).

The primary aim of this chapter is to determine the levels of weekly physical activity required to be performed by South Asian women living in the UK in order their cardiometabolic risk profile to equate with that of the women of White descent who achieve the current guideline level of physical activity. In order to examine this the recommended time for Europeans is defined as either 150 min.week\(^{-1}\) of MVPA measured in absolute minutes or in bouts of at least 10 minutes duration (202, 427). In addition, in order to provide external validation of our data and methodology, we have also tested the levels of BMI and waist circumference for the South Asian women which predispose to the same cardiometabolic risk profile to that of the Europeans with a BMI of 25 kg.m\(^{-2}\) (overweight) or 30 kg.m\(^{-2}\) (obese) and waist circumference of 80 cm (increased risk) or 88 cm (high risk) respectively. I hypothesise that South Asian women need to exercise longer or have lower levels of adiposity than the Europeans in order to equate their cardiometabolic profile with that of European women of similar age and BMI.
7.2 Methods

7.2.1 Ethical Approval

The study was awarded ethical approval by the West of Scotland research ethics committee 3 (REC 3). All participants gave written informed consent prior to their inclusion in the study.

7.2.2 Participants

South Asian (defined as having both parents of Indian, Pakistani, Bangladeshi or Sri Lankan origin) and White European women who lived in Scotland were included in the study. Women with history of CHD, cerebrovascular disease or diabetes were excluded from the study. Women with high likelihood of undiagnosed type 2 diabetes (n = 1) were excluded from the analysis. All participants aged 18-70 years and did not use systemic HRT or hormonal contraceptives for at least three months prior their inclusion in the study. The majority of the participants were included via general advertising and word of mouth.

7.2.3 Anthropometry

Height, weight and waist circumference were measured by the principal researcher using the ISAK guidelines. Each variable was measured in duplicate and the mean of two measurements was used in the analyses. Waist circumference was measured at the midpoint between the lower costal margin and iliac crest to the nearest 0.1 cm. BMI was defined as the ratio of weight measured in kg divided by the square height measured in metres.

7.2.4 Measurement of physical activity

Participants wore accelerometers (Actigraph G3TX+ or Actitrainer, ActiGraph LLC, FL, USA) at all times except when showering, swimming and sleeping for seven consecutive days. Vertical axis accelerometer counts were summarised into 60-second epochs and activity intensity domains were classified based on the Freedson cut-points (279). Valid data were considered when participants wore the accelerometers for at least 4 days for a minimum of 10 hours each day. Non-wear time was defined as the intervals of at least 60 min of zero activity. Moderate to vigorous physical activity (MVPA) performed weekly was defined as the summation of the time spent performing activity of moderate, hard and very hard intensity (based on the Freedson criteria) weekly (279). MVPA was used as the
activity variable in the data analysis to estimate ethnic specific cut points. In addition, current guidelines state that physical activity should be performed in bouts of at least 10 min duration (201, 202, 427). Therefore, physical activity of at least moderate intensity undertaken in bouts of 10 min duration with an allowance within a bout for interruption up to 2 min below the moderate intensity threshold (428) was calculated. This was the second activity variable (exposure) which was used in the regression models (as an additional analysis instead of absolute MVPA).

7.2.5 Biochemical markers and blood pressure measurements

Venous blood samples were collected after an overnight fast of 10-12 h. Glucose and HbA1c were analysed as routine samples on the day of collection in one of the certified NHS Biochemistry laboratories within Greater Glasgow and Clyde by using standard automated enzymatic and HPLC techniques. Centrifuged serum and plasma were stored at -80°C for subsequent analysis. Lipids (total cholesterol, HDL, TG) were measured in thawed sera using automated enzymatic technique at the end of the study. LDL levels were calculated with the use of the Friedwald equation (282). Insulin was measured in stored plasma by using a commercially available ELISA (Mercodia AB, Uppsala, Sweden) at the end of the study.

Blood pressure was measured on the non-dominant arm in duplicate by using an automated blood pressure monitor (Omron HEM705 CP, Milton Keynes UK). The mean of the two measurements was used in the analyses.

7.2.6 Statistical analysis

Statistical analysis was performed using STATA package (version 12.1, StataCorp LP, USA). Using a similar approach described previously (40, 41, 207), factor analysis was using to summarise metabolic risk variables into a single variable for each group of metabolic risk factors. The glycaemia factor combined fasting glucose and HbA1c; the insulin resistance factor combined fasting insulin levels, HDL cholesterol and TG; the blood pressure factor summarised systolic and diastolic BP; the lipid factor combined total cholesterol, HDL and TG levels. In addition, an overall cardiometabolic factor combining all the above biomarkers was derived. Additional analysis was performed by excluding HbA1c from the summary cardiometabolic factor as there are studies suggesting that the ethnic specific differences in HbA1c are independent of glycaemia (321, 322).
Regression models were fitted with each factor as dependent variable and either MVPA, MVPA in bouts of 10 minutes waist circumference or BMI as the independent variable. In addition, all models contained the interaction of ethnicity with the independent variable. The models containing MVPA as a prognostic factor were adjusted for age, daily wearing time of accelerometers and valid days of recordings because European women worn the accelerometers longer. Similar analysis was performed for MVPA of 150 min for the Europeans. The predicted values for each factor were calculated for values of BMI of 25 and 30 kg.m$^{-2}$ for the Europeans. Subsequently, the values of BMI which gave the same predicted values for the above factors for the South Asians were determined. 95% CI were estimated from the 95% confidence bands from the predicted values. The process was repeated for using waist circumference of 80 and 88 cm as “increased risk” and abdominal obesity respectively for the white Europeans.
7.3 Results

Detailed descriptive data for the South Asian and European women participated in this study have been given in Chapter 4. In the analysis for MVPA cut-off points n = 72 South Asian and n = 82 European women with valid accelerometer data were included. In the analyses determining waist circumference and BMI cut-off points n = 91 South Asians and n = 87 Europeans were included. In summary, the South Asian and European female participants did not differ in age and BMI but the South Asians had higher HbA1c and fasting insulin levels. The rest of the metabolic biomarkers were similar among the groups.

7.3.1 Factor analysis

Figure 42 shows the relationship between measured variables and underlying latent factors including the factor loading for each variable. Relationships between variables and latent factors are only shown for rotated loading factors > 0.32 (hence selected variable explains at least 10% variance in the latent factor).

![Diagram](image)

Figure 42. Relationship between measured variables and summary factors. Numbers in arrows show rotated factor loadings.
7.3.2 MVPA cut off points

Table 26 shows the equivalent thresholds in South Asian women for MVPA of 150 min.week\(^{-1}\) in the Europeans. The point estimates are significantly higher than 150 min.week\(^{-1}\) for all the factors in South Asians with the exception of the blood pressure factor; for the glycaemia factor (343 (95% CI: 277 to 468) min.week\(^{-1}\)), the insulin resistance factor (285 (95% CI: 235 to 326) min.week\(^{-1}\)), the lipid factor (192 (95% CI: 150 to 232) min.week\(^{-1}\)) and the overall cardiometabolic risk factors (95% CI: 194 (161 to 229) min.week\(^{-1}\)). However, it was significantly lower for the blood pressure factor (67 (95% CI: 27 to 96) min.week\(^{-1}\)). Removing HbA1c from the overall cardiometabolic risk factor reduced the equivalent point estimate marginally but the 95% confidence intervals crossed the cut point of 150 min.week\(^{-1}\) (190 (95% CI: 138 to 229) min.week\(^{-1}\)). Figure 43 displays graphically how the above thresholds were calculated. In addition, from the above regression models, ethnicity explained a small proportion of the variance of the overall cardiometabolic risk factor, lipid factor and blood pressure factor (3.4%, 2.7% and <1% respectively) but a higher proportion of the insulin and glycaemia factor (8.3 % and 6% respectively). In contrast, MVPA (absolute minutes) explained substantially greater proportion of the variance in all the factors compared with ethnicity; 19% of the variance of the overall cardiometabolic risk factor, 17.5% of the insulin factor, 13.3% of the lipid factor, 8.7% of the glycaemia factor and 7.9% of the blood pressure factor.

When physical activity was recorded in minutes of MVPA performed in bouts of at least 10 minutes duration (Table 27, Figure 44), the point estimates of equivalent physical activity in the South Asian women were higher for the majority of the factors with greater confidence intervals than that estimated when MVPA was included in the regression models as absolute minutes of physical activity. However, some point estimates (i.e. for insulin resistance and glycaemia factors) were not calculable in this dataset as were extending beyond the maximum value of MVPA in bouts of min.week\(^{-1}\) performed by the South Asians in this cohort (> 407 min.week\(^{-1}\)). Notably, MVPA measured in 10-minute bouts explained a lesser degree of variance of the summary factors than that explained by MVPA when measured in absolute minutes; 9.3% of the variance of the overall cardiometabolic risk factor, 7.7% of the insulin factor, 7.3% of the lipid factor, 4.3% of the glycaemia factor and 3.2% of the blood pressure factor.
## Chapter 7

### Table 26.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Equivalent MVPA values (min.week(^1))</th>
<th>Europeans (n = 82)</th>
<th>South Asians (mean, 95% CI) (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycaemia factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Insulin resistance factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lipid factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Blood pressure factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Overall cardiometabolic risk factor</td>
<td></td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Overall cardiometabolic risk factor (incl HbA1c)</td>
<td></td>
<td>150</td>
</tr>
</tbody>
</table>

Table 26. Derived values for moderate to vigorous physical activity (MVPA) in the South Asian women equivalent to MVPA of 150 min.week\(^1\) in the White European women for glycaemia, insulin resistance, lipid, blood pressure and overall cardiometabolic risk factors. Values for the South Asians are mean (95% CI). The glycaemia factor includes fasting glucose and HbA1c; the insulin resistance factor includes insulin, HDL cholesterol and triglycerides; the lipid factor includes total cholesterol, HDL cholesterol and triglycerides; the blood pressure factor includes systolic and diastolic blood pressure; and the overall cardiometabolic risk factor includes glucose, insulin, total cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure and +/- HbA1c. Models are adjusted for daily wearing time of accelerometer, valid days of recorded accelerometer data and age.
Figure 43. Relationship between the glycaemia and insulin resistance (top panel), lipid and blood pressure (middle panel) and overall cardiometabolic factor (including and excluding HbA1c) (bottom panel) and moderate to vigorous physical activity (MVPA) in the South Asian and the European women. Dashed blue lines represent the 95% CI bands around the regression line for the South Asians. Dotted blue lines represent the mean MVPA for the South Asians equivalent to MVPA of 150 min.week$^{-1}$ for the Europeans (dotted red lines).
Factors | Equivalent 10 min-bouts of MVPA values (min.week⁻¹)
---|---
| Europeans (n = 82) | South Asians (mean, 95% CI) (n = 72) |
Glycaemia factor | 150 | >407 (239.5, >407) |
Insulin resistance factor | 150 | >407 (296.3, >407) |
Lipid factor | 150 | 221.4 (157.0, 386.2) |
Blood pressure factor | 150 | 31.5 (0, 81.5) |
Overall cardiometabolic risk factor | 150 | 182.4 (123.0, 330.7) |
Overall cardiometabolic risk factor (incl HbA1c) | 150 | 220.4 (151.5, 405.7) |

Table 27. Derived values for MVPA performed in bouts of at least 10 minutes duration in the South Asian women equivalent to MVPA of 150 min.week⁻¹ in the White European women for glycaemia, insulin resistance, lipid, blood pressure and overall cardiometabolic risk factors. Values for the South Asians are mean (95% CI). The glycaemia factor includes fasting glucose and HbA1c; the insulin resistance factor includes insulin, HDL cholesterol and triglycerides; the lipid factor includes total cholesterol, HDL cholesterol and triglycerides; the blood pressure factor includes systolic and diastolic blood pressure; and the overall cardiometabolic risk factor includes glucose, insulin, total cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure and +/- HbA1c. Models are adjusted for daily wearing time of accelerometer, valid days of recorded accelerometer data and age.
Figure 44. Relationship between the glycaemia and insulin resistance (top panel), lipid and blood pressure (middle panel) and overall cardiometabolic factor (including and excluding HbA1c) (bottom panel) and moderate to vigorous physical activity (MVPA) measured in 10-minute bouts in the South Asian and the European women. Dashed blue lines represent the 95% CI bands around the regression line for the South Asians. Dotted blue lines represent the mean MVPA for the South Asians equivalent to MVPA of 150 min.week⁻¹ for the Europeans (dotted red lines).

7.3.3 BMI cut off points

Table 28 demonstrates the cut-off points for BMI in the South Asian women that equate to conventional BMI points of 25 and 30 kg.m⁻² in the Europeans for various clusters of metabolic factors. Figure 45 demonstrates how these point estimates were calculated.
<table>
<thead>
<tr>
<th>Factors</th>
<th>Overweight cut-off points</th>
<th>Obesity cut-off points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Europeans (n = 87)</td>
<td>South Asians (mean, 95% CI) (n = 91)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycaemia factor</td>
<td>25</td>
<td>21.3 (20.0, 22.3)</td>
</tr>
<tr>
<td>Insulin resistance factor</td>
<td>25</td>
<td>20.8 (20.5, 21.1)</td>
</tr>
<tr>
<td>Lipid factor</td>
<td>25</td>
<td>22.2 (21.2, 23.1)</td>
</tr>
<tr>
<td>Blood pressure factor</td>
<td>25</td>
<td>27.0 (26.3, 27.9)</td>
</tr>
<tr>
<td>Overall cardiometabolic risk factor</td>
<td>25</td>
<td>23.8 (23.1, 24.5)</td>
</tr>
<tr>
<td>Overall cardiometabolic risk factor (incl HbA1c)</td>
<td>25</td>
<td>23.2 (22.4, 23.9)</td>
</tr>
</tbody>
</table>

Table 28. Derived values for BMI in South Asian women equivalent to BMI of 25 and 30 kg.m\(^{-2}\) in the White European women for glycaemia, insulin resistance, lipid, blood pressure and overall cardiometabolic risk factors. Values for the South Asians are mean (95% CI). The glycaemia factor includes fasting glucose and HbA1c; the insulin resistance factor includes insulin, HDL cholesterol and triglycerides; the lipid factor includes total cholesterol, HDL cholesterol and triglycerides; the blood pressure factor includes systolic and diastolic blood pressure; and the overall cardiometabolic risk factor includes glucose, insulin, total cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure +/- HbA1c. The models are adjusted for age.
Figure 45. Relationship between the glycaemia and insulin resistance (top panel), lipid and blood pressure (middle panel) and overall cardiometabolic factors (including and excluding HbA1c) (bottom panel) and BMI in the South Asian and the European women. Dashed blue lines represent the 95% CI bands around the regression line for South Asians. Dotted blue lines represent the mean BMI for the South Asians equivalent to BMI of 25 kg.m$^{-2}$ and BMI of 30 kg.m$^{-2}$ for the Europeans. Dotted red lines represent the conventional BMI cut points of 25 and 30 kg.m$^{-2}$ with the associated risk factor values for the Europeans.

7.3.4 Waist circumference cut off points

Table 29 displays the equivalent thresholds of waist circumference in the South Asian women that equate to the similar metabolic risk to that of waist circumference of 80 and 88 cm in European women. Figure 46 demonstrates how these point estimates were calculated.
### Table 29.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Equivalent waist circumference values (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Europeans (n = 87)</td>
</tr>
<tr>
<td>Glycaemia factor</td>
<td>80 73.5 (72.1, 74.8)</td>
</tr>
<tr>
<td>Insulin resistance factor</td>
<td>80 71.6 (66.1, 75.3)</td>
</tr>
<tr>
<td>Lipid factor</td>
<td>80 76.7 (75.5, 77.9)</td>
</tr>
<tr>
<td>Blood pressure factor</td>
<td>80 84.7 (83.1, 86.3)</td>
</tr>
<tr>
<td>Overall cardiometabolic risk factor</td>
<td>80 78.9 (77.8, 79.8)</td>
</tr>
<tr>
<td>Overall cardiometabolic risk factor (incl HbA1c)</td>
<td>80 77.2 (76.2, 78.1)</td>
</tr>
</tbody>
</table>

- Table 29. Derived values for waist circumference in South Asian women equivalent to waist circumference of 80 and 88 cm respectively in the white European women for glycaemia, insulin resistance, lipid, blood pressure and overall cardiometabolic risk factors. Values for the South Asians are mean (95% CI). The glycaemia factor includes fasting glucose and HbA1c; the insulin resistance factor includes insulin, HDL cholesterol and triglycerides; the lipid factor includes total cholesterol, HDL cholesterol and triglycerides; the blood pressure factor includes systolic and diastolic blood pressure; and the overall cardiometabolic risk factor includes glucose, insulin, total cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure and +/- HbA1c.
Figure 46. Relationship between the glycaemia and insulin resistance (top panel), lipid and blood pressure (middle panel) and overall cardiometabolic factors (including and excluding HbA1c) (bottom panel) and waist circumference in the South Asian and the European women. Dashed blue lines represent the 95% CI bands around the regression line for the South Asians. Dotted blue lines represent the mean waist circumference for the South Asians equivalent to waist circumference of 80 cm and 88 cm for the Europeans. Dotted red lines represent the conventional waist circumference cut points of 80 and 88 cm with the associated risk factor values for the Europeans.
7.4 Summary of key result findings

* South Asian women need to perform in average 194 min.week\(^{-1}\) of MVPA or 220 min.week\(^{-1}\) of MVPA performed in at least 10 minute-bouts, in order to display the same cardiovascular risk with that of European women undertaken 150 min.week\(^{-1}\) of MVPA or MVPA in bouts respectively.

* The required time of physical activity that should be performed by South Asians vary according to the endpoint and the outcome variable used. In order to equate their insulin resistance of glycaemia risk phenotype with the Europeans, they are required to exercise longer than the above point estimates.

* It was replicated that the thresholds of BMI and waist circumference for healthy women of South Asian origin living in the UK should be lower than the conventional cut-off points.
7.5 Discussion

The study demonstrates, for the first time, that South Asian women, proportionately to South Asian men (42), need to undertake greater levels of physical activity in order to achieve an equivalent cardiometabolic risk profile, and in particular in relation to insulin resistance and lipid profile risk factors, to that of women of White descent performing 150 min.week$^{-1}$ of MVPA. Although our findings are not directly comparable to that of men (42), because the latter analysis referred to physical activity measured in 10-minute bouts whereas in the index study the thresholds of physical activity measured in 10-minute bouts were not applicable for all the metabolic factors (i.e. insulin resistant and glycaemia), our study added to the concept that physical activity guidelines should be ethnic and potentially gender specific. This is analogous to the concurrent recognition that the thresholds of overall and central obesity should differ across ethnic groups and genders (44). In addition, to add validity to our findings and our methodology, our additional analyses replicated that lower thresholds of BMI and waist circumference are required in South Asian women in order for their cardiometabolic profile to equate with that of Europeans at conventional obesity cut-offs (40, 41).

Sedentary lifestyle and low levels of physical activity are major contemporary public health concerns and have been recognised as modifiable attitudes that can alter an individual’s risk of developing type 2 diabetes and metabolic syndrome (44, 57). Hence, guidelines suggesting a minimum of 150 min.week$^{-1}$ of MVPA have been developed because they set an achievable goal that can have a measurable effect on the background risk of type 2 diabetes, CVD and overall mortality (195-197). Given that the data summarised in order to develop the current guidelines have resulted largely from populations of White European descent, that people of White descent comprise only around 15% of the world’s population and that ethnicity modifies the interaction between environmental factors and disease risk (429, 430), it is warranted that the dose-response effect between physical activity and health status in non-white populations is explored.

Our findings suggest that South Asian women in order to modify their insulin resistant phenotype are required to undertake in average 285 minutes.week$^{-1}$ of MVPA. When all metabolic risk factors (including blood pressure which was not shown to be higher in the South Asian) were included in the equation, an average of 194 minutes.week$^{-1}$ of MVPA was required. When physical activity was summarised in 10-minutes bouts, 220 minutes.week$^{-1}$ in average was estimated as the optimal duration of physical activity that
offers similar overall cardiometabolic profile in the South Asian women to that of the Europeans that perform 150 min.week\(^{-1}\) of MVPA in bouts of 10 min duration, in line with contemporary guidelines (201, 202, 427). South Asian women not only display a more sedentary lifestyle than the Europeans (chapter 4) and carry excess metabolic risks (chapter 4), they also require greater duration of weekly physical activity. Therefore, South Asian women constitute a high-risk group that are required to commit to greater lifestyle modifications than women of White descent. Hence, public health campaigns should approach methodically and consistently this ethnic groups and clear guidelines with easy messages targeting this population should be developed. However, it is anticipated that it will be a clear challenge to increase physical activity levels in a group like this with excess burden of needing to be active for longer duration weekly but exhibit a cultural aversion towards leisure time physical activity (431, 432). In addition, the innate low fitness levels of South Asians (chapter 4, (207, 211)) may hamper further the attempts of promoting longer physical activity in this population, as less fit individuals choose to be more inactive as they find physical activity more difficult.

Although the concept of increased requirements of levels of physical activity for South Asian women is in line with that for South Asian men (42), our point estimate of 220 minutes.week\(^{-1}\) for equating the overall cardiometabolic profile with that of the European women is lower than the equivalent for men of 266 minutes.week\(^{-1}\) (42). This gender discrepancy may be driven by the fact that women in our cohort were less active than men (207), hence small increases in physical activity patterns have greater impact on the metabolic markers of inactive women (433) or physical activity potentially provide a greater degree of protection in women than men (434). This is in agreement with our finding that MVPA measured in absolute duration rather than in 10-minute bouts explained greater proportion of the variance in metabolic parameters. Hence, in sedentary women any pattern of increased physical activity may have a benefit in improving cardiovascular markers rather than aiming for more demanding and less easily achievable patterns in bouts of continuous exercising. Another contributing factor to this gender difference may be that the cohort of the South Asian women was relatively healthier than that of men including women under the age of 40 years without demonstrating significant glycaemia, hence they require lower levels of physical activity compared with men in order to equate their risk with that of women of White descent. The findings of this study should be replicated in larger and more heterogeneous cohorts in order to be granted validity and could be extrapolated to other groups too.
A recent study in Sri Lankan women demonstrated women who self-reported less than 2,640 MET-minutes per week of MVPA (around 650 minutes or less of moderate intensity activity of MVPA depending on the proportion of moderate to vigorous time) are more likely to be dysglycaemic compared to women who self-reported weekly activity greater than this threshold (sensitivity 84% and specificity 85%) (292). Although the latter study supports the concept of ethnic tailored requirements in the duration of physical activity for South Asian women, it is acknowledged that the use of self-report questionnaires overestimates the amount of exercise undertaken (chapter 3, (199)) and limits the analysis exploring the actual dose-response relationship between physical activity and development of disease. A consensus report developed in India underpins the idea that South Indians (without sex stratification though) need to perform 30 min of moderate intensity physical activity, 15 min of work-related activity and 15 min of weight-bearing exercises daily and the total summary exceeds the current recommendations of 150 min.week\(^{-1}\) (43), however it is unclear how these recommendations were developed and what is the degree of evidence behind them.

In order to internally validate the methodology in defining the equivalent thresholds for physical activity in the South Asian women to that in the Europeans, a similar stepped approach was followed for cut-offs of BMI and abdominal adiposity (WC). Although our findings replicate the concept that lower thresholds of BMI and WC are required in women of South Asian descent, our point estimates are marginally higher than that calculated in previous studies (40, 41, 45). That can be related with the fact that our cohort consisted of essentially healthy women without diabetes or established cardiovascular disease, hence the South Asians did not differ in the prevalence of the disease burden (as no disease was established) compared with the Europeans. On the contrary, previous epidemiological studies examining the same hypothesis, either had diabetes as endpoint instead of metabolic risk factors, so there was a background difference in the prevalence of the disease among South Asians and Europeans (45), included participants with established diabetes or impaired glucose tolerance the prevalence of which differed among the ethnic groups (41) or there was no sex stratification (40). In addition, the parameters included in the factor analysis differed slightly between the studies of Razak (40), Gray (41) and the current analysis. Therefore, it is anticipated that applying the same methodology in defining thresholds of physical activity in higher risk groups of South Asian women will result in greater amount of required weekly time of physical activity in order to equate their risk with that of the Europeans.
A key strength of this study is that physical activity was measured with the use of an objective measure which is essential in quantifying the real dose-response relationship between physical activity and cardio-metabolic risk. In contrast, self reported physical activity has been shown to overestimate activity levels (chapter 3, (199)) and mask the magnitude of the association between activity and risk. It is acknowledged that accelerometers, albeit they are perceived the gold standard of objective measures, fail to capture activity associated with weight-bearing, cycling or swimming. However, since the participants in the current study kept a log of their activities and a minor proportion of the participants preformed any of the above activities, it is not anticipated that this limitation of the accelerometers has introduced a substantial measure bias in the analysis. In addition, the factor analysis strategy used in this study is an established methodology that simultaneously combines the varying markers used to define risk, is less dependent on the population distribution of a variable and takes in to consideration the continuous nature of a variable without dichotomizing it. In contrast, alternative methods of receiver-operating characteristic curves (ROC) and logistic regression have shortcomings; both are restricted to study dichotomous outcomes and ROC analysis is subjected to the distribution of a variable in the study population. However, it is acknowledged that the study does have some limitations; its cross-sectional nature and modest sample size cannot confer that the ethnic variation in these cardio-metabolic markers lead to a proportional ethnic variation in the future incidence of clinical events such as type 2 diabetes and CVD. Therefore, confirmation of the findings with an interventional study examining the impact of the recommended levels of physical activity in the cardiometabolic risk factors of South Asian women is warranted. In addition, the analysis could not identify cut points corresponding to the levels of physical activity measured in 10-minute bouts in relation to insulin resistance and glycaemia because of insufficient sample of participants undertaking high levels of activity in bouts. Thus, our findings need to be replicated in larger prospective studies with greater distribution in the levels of physical activity which would have disease incidence as end-points, in order definitive recommendations regarding ethnic tailored thresholds can be incorporated in guidelines.

7.6 Conclusion

Cut points for recommended physical activity in relation to the cardio-metabolic risk profile are substantially higher for South Asian women than the conventional recommended values. Further interventional studies are warranted to assess whether South
Asian women who undertake physical activity in excess of the point estimates have a decreased risk of developing type 2 diabetes and CVD.
8 GENERAL DISCUSSION

8.1 Recruitment of subjects in an ethnic and sex specific study

South Asians, defined as the ethnic group originating mainly from India, Pakistan, Sri Lanka or Bangladesh, constitute the largest non-White ethnic group in England and Scotland. Given that the prevalence of type 2 diabetes and CVD among migrant South Asians is substantially higher compared with that in the background White population or other ethnic minorities (21, 28, 36), South Asians should be considered as a high risk group for chronic disease. This has implications for healthcare provision and allocation of health services in the UK. It is established that prevention of chronic diseases is more beneficial at an individual level and more cost effective at a population level than treatment and, therefore, public health campaigns and preventive policies should target this high-risk ethnic group. Notably, South Asian women living in Western countries have a higher prevalence of type 2 diabetes as they grow older compared with South Asian men or White women of equivalent age (28), adding a sex gradient in the ethnic specific problem of disease burden. This group is largely under-investigated and it is still unclear why this sex and ethnic specific discrepancy in the development of type 2 diabetes exists. In order for preventive methods to be efficient they should be targeted and focused on modification of lifestyle factors that are known to have an impact on the metabolic profile of individuals. However, the distribution of established modifiable lifestyle factors in healthy South Asian women living in the UK is unknown as well as whether ethnicity modifies the association with insulin resistance and other metabolic risk factors in this group. The work presented in this thesis aimed to tackle these questions by recruiting and performing a series of tests in healthy South Asian and European women living in Scotland aged from 18 to 70 years.

This work has not only given me a great insight into cardiometabolic risks and their association with lifestyle factors, in particular in women who present unique characteristics because of their reproductive trajectories, but has also familiarised me with the specific characteristics of research in ethnic minorities. Ethnic specific research has inherent difficulties largely because of limited accessibility to potential participants and constrains in building a relationship of trust in order to convince them to participate. Ethnic specific research becomes even more complicated when the potential volunteers are not integrated with the indigenous population of the city in which they live and cultural and/ or language barriers exist between them and the researchers, especially when the researchers are also
from a different ethnic background. In this study, the majority of the South Asian women (especially those over the age of 40 years) migrated to the UK following their spouses at a relatively young age (in their twenties) and hence have been in the UK for over 20 years with some of them up to 50 years. However, it was striking that the majority of them were housewives, were not totally integrated with the indigenous population, were socialising exclusively with women of the same ethnic background and loyally practicing their own cultural and religious traditions. This minimal integration and independence of South Asian women had implications in the practicalities of conducting the study i.e. arrangement of transport was required for the majority of the South Asian participants. An interesting observation was that there were intra-ethnic cultural differences among women of South Asian background; although the South Asian participants were largely of Pakistani origin and practising Islam, a small portion were of Indian origin. The latter participants were mainly active or retired professionals, had a higher degree and exhibited a more sociable lifestyle. These demographic discrepancies may reinforce the concept that South Asians should not be regarded as a homogenous group; however, such analysis was not undertaken in the current study due to the lack of power.

In ethnic specific research it should be anticipated that cultural or religious attitudes of the participants may dictate the form of tests that are feasible to be performed, as some methods may not be perceived acceptable. For instance, in the current study we had to amend the method of measuring VO$_2$max, since the gold standard treadmill test at maximal effort requires two researchers and women of South Asian origin were not willing to exercise in the presence of a male technician. Therefore, this was replaced by the Chester step test that can be accomplished by one researcher. Similarly, the majority of South Asian volunteers had been reassured that the main researcher undertaking the measurements and potential chaperones were females before they agreed on participating in the study. Despite the specific features discussed above regarding ethnic and sex specific research that only became apparent whilst setting up the study, the South Asian volunteers were very willing to participate, gave very positive feedback and contributed to the positive advertisement of the study through word of mouth. The majority of them enjoyed the study visit and found it as a day away from their household activities. On the contrary, recruiting healthy volunteers of White European decent of similar BMI and age with the South Asian women was more challenging than it was initially anticipated. Time constraints, use of hormonal regimes and professional commitments contributed to the slow recruitment of the European participants.
8.2 Metabolic phenotype

The South Asian women in the current study, in line with previous studies (93, 115), demonstrated an insulin resistant phenotype with greater HOMA\textsubscript{IR}, lower levels of HDL, greater levels of TG compared with the Europeans of similar age and BMI. However, these differences were not accompanied by higher levels of total cholesterol or LDL. Fasting glucose levels did not differ among the two ethnic groups but, notably, HbA1c levels were greater in the South Asians than in the Europeans, adding evidence to the concept that greater post-prandial rather than fasting glucose in the South Asians may contributed to the ethnic discrepancy in HbA1c levels. Although previous studies have suggested an inherent ethnic disparity in HbA1c levels in South Asians without mirroring greater insulin resistance (321, 322), the current study demonstrated that for any given level of fasting glycaemia, HbA1c was substantially greater in the South Asians compared with the Europeans and lifestyle factors, such as adiposity and fitness, had a greater effect on HbA1c levels in the South Asians than in the Europeans. Hence, I postulate that greater HbA1c levels in the South Asian women of this cohort was not an ethnic specific characteristic, but rather an additional adverse metabolic biomarker. This may have implications on the current guidelines of diagnosing diabetes based on HbA1c levels and ethnic specific thresholds may be required.

In regard to the remaining metabolic risk manifestations, both groups had similar systolic and diastolic BP, whereas smoking was more prevalent in the Europeans than in the South Asians, although the number of current smokers was relatively small in both groups. In addition, abdominal adiposity measured by means of waist circumference did not differ between the groups, although the South Asians had greater WHR and central skinfolds. Additional biomarker analysis showed that the South Asian women have lower levels of adiponectin and SHBG. Both biomarkers have been linked with the development of type 2 diabetes and have been proposed as potential optimal biomarkers in the prediction of future diabetes (335, 435). It has yet to be examined though whether the relationship with future disease is causal or this pattern in both biomarkers is consistent with the multiple manifestations of insulin resistant phenotype.

Therefore, the South Asian women in this study had impaired glucose metabolism and a lipid profile consistent with a more insulin resistant phenotype than the European women without associated excess of other cardiometabolic risk factors. It should be examined in
subsequent longitudinal studies whether greater insulin resistance mediates the association of lifestyle factors with type 2 diabetes and to what extent ethnicity modifies this pathway.

8.3 Diet

Diet is considered as a modifiable lifestyle factor that has been linked with the development of chronic diseases. Dietary data of migrant South Asians have been conflicting (36, 221), which may be attributed to different ways of recording diet habits, sampling discrepancies or diet evolution as a result of increasing years of immigration. The food frequency questionnaire (FFQ) used in the current study was a validated tool that has been previously used in research involving South Asians and includes common Asian food, which should minimise the impact of both culture and recall bias on the recording of dietary data. The current study suggested that the dietary energy density did not differ between the two ethnic groups; hence, the dominance of insulin resistant phenotype among the South Asians was not directly associated with greater imbalance in the distribution of macronutrients in this ethnic group.

8.4 Adiposity and body composition

The South Asian volunteers accumulated more adipose tissue centrally, measured by central skinfolds and WHR, for a given BMI compared with the Europeans. Notably, both ethnic groups had similar amount of SAT measured with the means of MRI but the South Asian women, for a given amount of abdominal subcutaneous fat, had greater storage of fat in intraabdominal and ectopic depots (liver). Both VAT and liver fat were associated with the summary measure of metabolic risk factors (MetR score), insulin resistance index, glycaemia and levels of HbA1c whereas subcutaneous fat was more metabolically inert and although it was associated with insulin resistance and MetR score, its effect size was smaller than that of visceral and liver fat on the same dependent variables. Interestingly, the effect size of increasing visceral fat on MetR score was greater in the Europeans than the South Asians reinforcing the hypothesis that adipose tissue in the South Asians is not more metabolically harmful than in the Europeans but the South Asians start accumulating fat in secondary, more metabolically active, compartments at a lower threshold of adiposity. This is consistent with the “adipose overflow theory” which supports that South Asians have limited capacity to store fat subcutaneously (233), which is possibly related with similar underlying environmental and genetic factors that contribute to constitutional smaller neonates in the same ethnic group, and hence weight gain and increasing adiposity
results in fat accretion in deeper tissues. Since this genetic predisposition of South Asians cannot be reversed or modified, at least not without long-lasting evolutionary mechanisms as a response to adaptation to Westernised environment, lower thresholds compared with the conventional thresholds of central and overall obesity have been proposed for South Asians in order to equate their metabolic risk with that of Europeans.

It is established that lifestyle factors and adiposity have a complementary effect on the cardiometabolic risk of individuals but exploring which of them has a greater impact would tailor more efficient interventions and preventive policies. In the current study it was demonstrated that central adiposity has the largest effect size on insulin resistance and the MetR score compared with other adiposity markers, sedentary time, physical inactivity or low fitness levels in the women of both ethnic groups. Notably, the effect size of increasing central adiposity was substantially larger in the South Asians compared with that in the Europeans, which pinpoints the need for targeted and high intensity interventions in this group.

Central adiposity can be measured with different methods ranging from the readily available waist circumference to the most sophisticated methods of imaging with the use of MRI. However, in regards to risk stratification and implementation of preventive policies at a population level, the most accurate method of measuring central adiposity, which requires specialised infrastructure, is unlikely to be feasible. Hence, identification of the most accurate and valid proxy of central adiposity that can be applied in different ethnic groups is of major public health significance. In this study, by comparing different measures of adiposity, waist circumference was the single most accurate indicator that correlated strongly with central skinfolds and MRI measures in both ethnic groups. Therefore, in large-scale epidemiological studies waist circumference is an acceptable method of assessing central adiposity and in clinical settings, routine measurement of waist circumference will enable identification of high risk individuals and facilitate early interventions, especially when ethnic specific thresholds are used.

8.5 Physical Activity and fitness

It is well established that physical activity and fitness reduce the background risk of an individual for type 2 diabetes, CVD and all cause mortality. In addition, there is a dose response effect in the relationship between physical activity and cardiometabolic risk and current guidance suggests a minimum amount of weekly physical activity as the trade off
between clinically quantifiable risk reduction and feasibility of recommendations (195).
However, the dose response effect has largely been inferred from epidemiological studies which recorded the frequency and intensity of physical activity with the use of self reported questionnaires mainly in populations of White descent. I showed in this study that self reported physical activity, in particular that of moderate intensity, is largely overestimated and sitting time is underestimated compared with objectively measured data and these discrepancies seem to be surprisingly more evident in the women of European descent rather than in the South Asians. Therefore, self reported physical activity may deflate the real dose response effect and the current recommendations of desirable levels of weekly physical activity may fail to have the expected beneficial effect on disease prevention.

This study was the first to confirm using objective measures that South Asian women living in the UK are less active than their comparators of white Europeans descent and this is independent of age. Similarly, oxidative capacity during exercising is decreased in the South Asians. As expected increasing levels of MVPA and fitness have a protective effect on insulin resistance but the effect size is similar in both ethnic groups. However, for a given level of MVPA, the South Asian women have consistently lower levels of fitness, which suggests an innate defect in this ethnic group in oxidation during physical activity. Given this physiological disparity, the concept of ethnic and sex specific thresholds of weekly physical activity was examined and I showed that South Asian women need to exercise around 195 min.week$^{-1}$ at a moderate to vigorous level in order to equate their cardiometabolic risk with that of Europeans exercising 150 min.week$^{-1}$. This needs to be replicated in interventional studies or tested in epidemiological studies having as endpoints the development of type 2 diabetes and CVD.

### 8.6 The role of ethnicity

This work demonstrated that ethnicity per se modifies the associations of adiposity with cardiometabolic outcomes. Therefore, South Asian women do not only exhibit an adverse cardiometabolic profile with greater central adiposity, lower levels of objectively measured physical activity and decreased cardiorespiratory fitness, but more interestingly, a given amount of increasing adiposity has a substantially greater impact on insulin resistance and other glycaemic and metabolic factors in the South Asians than in their White comparators. Whether ethnicity modifies the associations of other lifestyle factors (e.g. physical activity and fitness) with cardiometabolic outcomes has yet to be confirmed, but I acknowledge
that my study was not powered to detect if ethnicity is a significant effect modifier of the above associations. It rather only became apparent for adiposity since it had a greater effect size than physical activity or fitness on the cardiometabolic outcomes. This finding adds support for the concept that ethnicity does not only have an impact on the approach of individuals towards the uptake of specific lifestyle behaviours, but modifies, due to potentially genetic and developmental characteristics of individual ethnic groups, their susceptibility for developing future cardiometabolic diseases. Therefore, there are inherent differences in the South Asian women that render them prone to insulin resistance and thereby to type 2 diabetes and CVD compared with women of White descent. The concept of ethnic susceptibility adds a third variable; ethnicity, on the equation of environmental exposures with cardiometabolic outcomes and sets the scene for ethnic tailored recommendations regarding lifestyle modifications or ethnic specific thresholds for risk stratification or disease diagnosis. Although there are some initial steps towards ethnic specific cut-offs for obesity and central adiposity (measured by waist circumference) there is clear evidence that this should be extended to other lifestyle factors, such as physical activity or diet, or to the thresholds of biomarkers for disease prognosis and diagnosis.

An additional unexpected finding was that vascular reactivity, measured at both micro and macro-vascular level, did not differ substantially between the two ethnic groups despite the South Asian women exhibiting a greater cluster of cardiometabolic risk factors than the Europeans. This finding suggests that healthy women of South Asian origin without overt disease do not demonstrate greater vascular changes than their European comparators and, therefore, lifestyle interventions aiming to ameliorate their cardiometabolic profile would be more meaningful at this, or even an earlier stage, when vascular impairment has not yet established. I acknowledge that both methods used, LDI-ION and EndoPAT, are experimental techniques for measuring vascular function and are not fully applicable into clinical practise, therefore, it can be argued that the use of a different measure, such as IMT, may have identified earlier vascular changes in the South Asians than in the Europeans. However, unpublished data in men (Nazim Ghouri MD thesis University of Glasgow 2013) demonstrated that there is no substantial difference in IMT between healthy South Asians and Europeans, despite the former exposing a more insulin resistant phenotype than the latter.
8.7 Menopause and hot flushing

Cardiometabolic biomarkers, adiposity and lifestyle attitudes deteriorate across the reproductive stages (from pre to post-menopausal state) and these changes occur to a similar extent in both ethnic groups. Therefore, attitudes and attributes associated with menopause do not seem to differ substantially between the two ethnic groups. I acknowledge that the cross-sectional nature of my data does not allow full separation of the effect of menopausal transition from the ageing effect, but provides evidence that the menopause along with ageing have a similar impact on both groups. An additional observation, albeit not the primary aim of this work, was that the age and experience of menopause did not differ substantially between the South Asians and the Europeans living in Scotland. There are anecdotal or scanty research data that suggest that South Asians experience menopause at a younger age than Caucasians and is mainly associated with musculoskeletal symptoms rather than with VMS (267). In the current study, these findings were not replicated and the South Asians went through the menopausal transition at a similar age and were equally likely to experience hot flushing as the Europeans. However, the Europeans were more likely to seek medical treatment for hot flushing rather than the South Asians. Recall bias may have influenced these findings but this would be expected to be equivalent in both ethnic groups.

In addition, hot flushing was shown to be an independent factor associated with decreased post occlusion vasodilatation measured with the use of EndoPAT. This finding adds further evidence to the literature suggesting that hot flushing may be an additional to the conventional risk factors of CVD (269, 271, 386). Therefore, knowledge of hot flushing may enable early identification of women at high risk of future CVD and facilitate early interventions in order to delay or cease this trajectory. In the current study it cannot be postulated that decreased RHI in hot flushers would lead to greater CVD events in the future and this should be examined in longitudinal cohorts where hot flushing would be the exposure, vascular reactivity the mediation factor and incidence of CVD the outcome.

8.8 Future research plans

The findings of this thesis enable further hypotheses generation and some of the research questions that would be interesting to be examined by using different research methodologies to validate my conclusions have already been discussed earlier in this section.
As a natural continuation of this work I would like to explore whether altered adipose tissue distribution or compartmentalisation in South Asian women has a causal effect on their greater insulin resistance and whether weight loss can reverse this. I demonstrated that central adiposity has a substantially larger impact on the cardiometabolic risk factors in the South Asians than in the Europeans however whether this reflects a mere association or a causal effect has yet to be examined. Causal inference is challenging in biomedical research and interventional studies, by providing the temporal order of the exposures and outcomes, are considered the gold standard to reveal a causal pathway. I propose an interventional study where healthy women of South Asian and white European descent without overt disease would be asked to increase their daily energy intake by 550 calories (consumption of two additional energy bars per day on top of their normal diet), so they would be expected to gain around 1 kg per two weeks. This part of the intervention will last for around 10 weeks. In turn, the participants would be asked to follow a hypocaloric diet for 20 weeks aiming to lose the weight they gained during phase one of the intervention plus 7% of their initial weight. Abdominal MRI and spectroscopy along with baseline cardiometabolic biomarkers will be performed at the time of recruitment, at the end of phase one and at the end of phase two of the intervention. In order to detect a 7% weight reduction from baseline to the end of phase 2 with 80% power, we would need to recruit 50 participants in each ethnic group, and assuming a 20% loss in follow up, 60 participants would be required in each group. By conducting this two-phase intervention, we would be able to assess whether weight gain has a similar impact on fat distribution, abdominal adipose tissue compartmentalisation and insulin resistance in both ethnic groups and whether weight loss can reverse these effects. It will also allow us to assess whether ethnicity modifies the relationship between weight gain or weight loss and fat distribution and insulin resistance. If the latter were confirmed, ethnic specific guidelines for weight loss would be warranted.

An additional finding that I would like to explore further is whether hot flushing is an independent risk factor of future CVD. Given that the pathogenesis of hot flushing has not been fully elucidated and it is still unclear why only a proportion of women develop hot flushing during the menopausal transition, it is worth exploring whether hot flushing is interlinked with other reproductive indicators which become evident over a woman’s life-course (i.e. early menarche, pregnancy complications such as pre-eclampsia or irregular cycles etc), so common causal pathways may explain them. In addition, investigating whether women with hot flushes are more prone to future CVD independent of conventional risk factors is of significant clinical importance. Hot flushing is one of the
commonest reasons that middle-aged women seek medical advice and I, as a gynaecologist, come across with many women looking for symptomatic relief for hot flushes. Knowledge that this large group of women are at high risk of CVD would enable us to adapt a holistic approach. I will address the above questions by analysing data from large cohorts (i.e. UK Biobank, Avon Longitudinal Study of Parents and Children (ALSPAC)), which contain complementary data in terms of phenotype and sample size.

8.9 Conclusions

The work for this thesis extensively phenotyped a group of South Asian women who live in Scotland, a minority group that has been largely overlooked to date despite being at high risk of type 2 diabetes and CVD. The results confirmed the findings from previous studies reporting that healthy South Asian women exhibit greater insulin resistance. I also demonstrated that South Asian women store greater central adiposity, mainly in deep and ectopic depots, show decreased physical activity indices and have lower cardiorespiratory fitness than their comparators of White descent of similar age and BMI. However, this thesis added the novel concept that mere differences in the above lifestyle factors and adiposity do not explain the magnitude of the excess cardiometabolic risk in the South Asians but ethnicity modifies the gradient of associations between conventional risks and cardiometabolic outcomes. This finding sets the foundation for further work in exploring ethnic specific thresholds in lifestyle interventions or even in disease diagnosis.
List of References


36. NICE. Assessing body mass index and waist circumference thresholds for intervening to prevent ill health and premature death among adults from black, Asian and other minority ethnic groups in the UK. NICE, 2013.


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Appendix I: Consent Form

Volunteer Identification Number:

CONSENT FORM

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I confirm that I have read and understood the information sheet dated ……………….. for the above study and have had the opportunity to ask questions.</td>
<td>Please Initial Box</td>
</tr>
<tr>
<td>2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, without my medical care or legal rights being affected.</td>
<td></td>
</tr>
<tr>
<td>3. I agree to take part in the above study.</td>
<td></td>
</tr>
<tr>
<td>4. I agree to my GP being informed of my participation in the study.</td>
<td></td>
</tr>
<tr>
<td>5. I agree to being notified of an undiagnosed condition which could give cause for concern from the tests undertaken for the purpose of the study.</td>
<td></td>
</tr>
<tr>
<td>6. I agree to my GP being notified of an undiagnosed condition which could give cause for concern from the tests undertaken for the purpose of the study.</td>
<td></td>
</tr>
<tr>
<td>7. I agree to my samples being used for future research into the prevention and treatment of diabetes and heart disease. This may involve analysis of genes associated with these diseases.</td>
<td></td>
</tr>
<tr>
<td>8. I agree to being notified of an undiagnosed condition which could give cause for concern from future research.</td>
<td></td>
</tr>
<tr>
<td>9. I agree to my GP being notified of an undiagnosed condition which could give cause for concern from future research.</td>
<td></td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of participant</td>
<td>Date</td>
</tr>
<tr>
<td>Person Taking Consent</td>
<td>Date</td>
</tr>
</tbody>
</table>

(one copy for researcher, one for participant) Version 3- 29/2/2012
Appendix II: Health screen questionnaire

Participant’s study number: ……………………..

Name: …………………………………………………….

Date of Birth: …………..

Address: ……………………………………………………………………………………………

Postcode: …………………

Both parents of South Asian origin (Indian, Pakistani, Bangladeshi or Sri Lankan) yes [ ] no [ ]

Years in the UK ………………………………………………………………………

Both parents of European origin yes [ ] no [ ]

Religion …………………………………………………………………………………

How many years of formal education have you had? [ ]

Highest Degree/Qualification …………………………………..


What is your profession? ……………………………………………………………

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

At present, do you have any health problem for which you are:

(a) on medication, prescribed or otherwise yes [ ] no [ ]

(b) attending your general practitioner yes [ ] no [ ]

(c) on a hospital waiting list yes [ ] no [ ]

In the past two years, have you had any illness which required you to:

(a) consult your GP yes [ ] no [ ]

(b) attend a hospital outpatient department yes [ ] no [ ]

(c) be admitted to hospital yes [ ] no [ ]

3. Have you ever had any of the following symptoms to a significant degree at rest or during exercise? That is, have you had to consult a physician relating to any of the following?

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Breathlessness</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>(b) Chest Pain</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>(c) Dizzy spells/Fainting</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>(d) Diabetes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>(e) Palpitations</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>(f) Tightness in chest, jaw or armyes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>(g) Other*</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

*(Please specify)

4. Do you have/or have had any muscle or joint injury which could affect your safety in performing exercise (e.g. cycling or running), strength testing or strength training?

<table>
<thead>
<tr>
<th>Injury Type</th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Convulsions/epilepsy</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>(b) Asthma</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

5. Have you ever had any of the following:

(a) Convulsions/epilepsy yes [ ] no [ ]

(b) Asthma yes [ ] no [ ]
<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A blood disorder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestive problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hearing problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disturbance of balance/co-ordination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numbness in hands or feet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disturbance of vision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney or liver problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart problems including murmurs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any other health problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>An allergy to soya protein or eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any other family illnesses</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. Have any of your family (parents, grandparents, brothers, sisters, children, aunts, uncles, cousins) ever had any of the following: (if yes please give details below including age of first diagnosis if known)
   (a) Any heart problems                       |     |    |
   (b) Diabetes                                  |     |    |
       If yes, do either or both of your parents have diabetes? 1) mother 2) father 3) both 4) neither
   (c) Stroke                                    |     |    |
   (d) Any other family illnesses                 |     |    |

7. Do you currently smoke                       |     |    |
   If yes, how many < 10 per day                |     |    |
   10-20 per day                                |     |    |
   >20 per day                                  |     |    |
   If no, have you ever smoked                   |     |    |
   If so, for how long did you smoke and when did you stop? ..............................................

8. How many units of alcohol do you typically drink in a week? ..........................

If YES to any question, please describe briefly, including listing of current medication (e.g. to confirm whether problem was short-lived, insignificant or well controlled.) (Use a separate sheet if necessary)

Reproductive History

9. Have you ever been pregnant?                  | Yes | No |
   If yes, how many times?                      |     |
   If yes, how many full term pregnancies did you have? |     |
   Did you have high blood pressure (preeclampsia) during them? Yes | No |

10. When was your last menstrual period?         |     |
    If you still have your periods, how many periods did you have over the last year? |     |
    If you still have your periods, what contraception do you use? |     |

11. Did you have a hysterectomy?                 | Yes | No |
    If yes, year of hysterectomy                 |     |

12. Between the ages of 16 and 40, about how long had been your average menstrual cycle (time from first day of one period to the first day of the next period)? (select ONE only)
    <25 d
25-34 d
35-60 d
Totally variable

13. **During your menstruating years** (not including during pregnancy), had you ever had:
   a. tendency to grow dark, coarse hair (eg upper lip, chin, breast, abdomen etc?)
      Yes [ ]   No [ ]
   b. troublesome with acne    Yes [ ]   No [ ]

**Name and address of GP**
..........................................................................................................................
..........................................................................................................................

Signature:..................................................  Date:.....................
## Appendix III: Food Frequency Questionnaire

### Bread

**How often do you eat the following breads and how many slice**

1. White or high fibre white  
   7 6 5 4 3 2 1 F R  
   No. slices or rolls per day?  
   ...........................................
   What size of slice do you have?
   1. Large
   2. Small

2. Brown or wheatgerm  
   7 6 5 4 3 2 1 F R  
   No. slices or rolls per day?  
   ...........................................
   What size of slice do you have?
   1. Large
   2. Small

3. Wholemeal/chapatis  
   7 6 5 4 3 2 1 F R  
   No. slices or rolls per day?  
   ...........................................
   What size of slice do you have?
   1. Large
   2. Small
   3. Chapatis

4. Bread rolls/crumpets  
   7 6 5 4 3 2 1 F R  
   No. slices or rolls per day?  
   ...........................................
   What size of rolls/crumpets do you have?
   1. Large
   2. Small
   3. Crumpets

5. Crispbread, Ryvita or cream crackers  
   7 6 5 4 3 2 1 F R  
   No. of slices or rolls per day?  
   ...........................................

6. Jam, marmalade or honey on bread?  
   7 6 5 4 3 2 1 F R

### Breakfast Cereals

**How often do you eat the following cereals?**

7. How often do you eat the following cereals?

1. Cornflakes  
   7 6 5 4 3 2 1 F R

2. Sugar Puffs, Special K, Ricicles, Rice Krispies etc  
   7 6 5 4 3 2 1 F R

3. Muesli, Fruit n' Fibre or Cheerios  
   7 6 5 4 3 2 1 F R

4. Weetabix, Wheat Flakes or Shredded Wheat  
   7 6 5 4 3 2 1 F R

5. Bran Flakes or Sultana Bran  
   7 6 5 4 3 2 1 F R

6. Porridge or Ready Brek  
   7 6 5 4 3 2 1 F R

7. All Bran  
   7 6 5 4 3 2 1 F R

8. Other cereal  
   7 6 5 4 3 2 1 F R

8. How many teaspoons of sugar/honey do you add?  
   .........................

9. How often do you have wheat bran?  
   7 6 5 4 3 2 1 F R
# Meats

How often do you have the following meats? Include all form in stews, casseroles, lasagne, curry etc.

<table>
<thead>
<tr>
<th>Meat Type</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. Beef (including beefburgers)</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>11. Lamb</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>12. Pork</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>13. Bacon</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>14. Ham</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>15. Chicken or other poultry</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>16. Canned meat (e.g. corned beef), pate or meat spread</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>17. Sausages</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
</tbody>
</table>

What type of sausages do you have?

1. Pork
2. Beef
3. Pork and Beef
4. Turkey
5. Low Fat

<table>
<thead>
<tr>
<th>Sausage Type</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>18. Meat pie/pastie/SAUSAGE ROLL/SAMOSA - shop bought</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>19. Meat pie/pastie/SAUSAGE ROLL/SAMOSA - home made</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>20. Liver/kidney/heart</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>21. Do you usually eat the fat on the meat?</td>
<td>Yes / No</td>
</tr>
</tbody>
</table>

# Fish

How often do you eat the following fish?

<table>
<thead>
<tr>
<th>Fish Type</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>22. White fish (cod/haddock/plaice/fish fingers/fish cakes)</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>23. Kipper/herring/mackerel/trout (including canned)</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>24. Pilchards/sardines/salmon (including canned)</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>25. Tuna (including canned)</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
</tbody>
</table>

# Vegetables & Savoury Dishes

How often do you have the following vegetables or dishes?

<table>
<thead>
<tr>
<th>Vegetable Type</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>26. Potatoes - boiled or mashed</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>27. Potatoes - jacket</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>28. Chips - shop bought, 'oven/microwave chips' or hash browns</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>29. Chips - homecooked</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>30. Potatoes - roast</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>31. Peas</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>32. Other green vegetables, salads or tomatoes</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>33. Carrots</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>34. Parsnips, swedes, turnips or sweetcorn</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>35. Baked beans</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>36. Butter beans, broad beans or red kidney beans</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>37. Lentils, chick peas or dahl</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>38. Onions (cooked/raw/pickles)</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>39. Spaghetti, other pasta or noodles</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
<tr>
<td>40. Rice (NOT pudding rice)</td>
<td>7 6 5 4 3 2 1 F R</td>
</tr>
</tbody>
</table>
41. Quiche
42. Pizza
43. Vegetable pie/pasty/samosa

**Biscuits, Cakes & Puddings**

**How often do you eat the following items?**

44. Digestive biscuits/plain biscuits
45. Other sweet biscuits
46. Chocolate, e.g. Galaxy, Mars Bar, Twix, KitKat
47. Sweets, e.g. fruit gums, pastilles, mints
48. Crisps/savoury snacks, e.g. Quavers, tortilla chips
49. Nuts
50. Ice cream, iced dessert, fool, mousse or trifle
51. Low fat yogurt
52. Low calorie yogurt, e.g. Shape
53. Other yogurt/fromage frais, e.g. thick & creamy
54. Fruitcake/sponge cake/sponge pudding - shop bought
55. Fruitcake/sponge cake/sponge pudding – homemade
56. Fruit tart/jam tart/doughnut/Danish pastry - shop bought
57. Fruit tart/jam tart - home made
58. Milk pudding e.g. rice/tapioca/macaroni

What type of milk do you use for milk pudding?
1. Ordinary/whole
2. Semi-skimmed
3. Skimmed
4. Canned milk pudding - ordinary
5. Canned milk pudding - low fat

**Fruit**

59. How often do you have fruit canned in syrup?
60. How often do you have fruit canned in juice?
61. How many apples do you eat per week?
62. How many pears do you have per week?
63. How many oranges/tangerines/satsumas/clementines/grapefruit do you have per week?
64. How many bananas do you have per week?

**Eggs & Milk Products**

65. How many eggs do you usually eat per week?
66. What type of milk do you use?
1. Whole milk
2. Semi-Skimmed
3. Skimmed
4. More than one type
Roughly how much milk do you drink in a day in tea/coffee/milky drinks/cereals??

1. None
2. Half a pint or less
3. Between half a pint and one pint
4. One pint or more

67. How much cream do you use per week? ................. grams

68. How much cheese (excluding cottage cheese) do you usually eat per week? ................. grams

69. Cottage cheese? 7 6 5 4 3 2 1 F R

Fats

70. What do you usually spread on bread?
1. Butter
2. Polyunsaturated margarine/spread
3. Other soft marg/spread (tub) (not olive spread)
4. Hard Margarine (block)
5. Low Fat Spread - polyunsaturated
6. Low Fat Spread - other
7. Lard, dripping, solid vegetable oil
8. Very low fat spread (25% fat)
9. Olive Oil Spread

How much butter/margarine/spread do you usually eat per week ............... g

71. How often do you have food that is fried? 7 6 5 4 3 2 1 F R

What types & BRANDS of fat do you have per day?
1. Olive Oil
2. Corn Oil
3. Sunflower Oil
4. Poly Fat
5. Cooking Fat
6. Vegetable Oil

Drinks

72. How many cups of tea do you have per day? .................
73. How many teaspoons of sugar/honey per cup? .................

74. How many cups of coffee do you have per day? .................
75. How many teaspoons of sugar/honey per cup? .................

76. How often do you have fruit juice/squash/fizzy drinks (NOT low calorie)? 7 6 5 4 3 2 1 F R

Which of these do you usually have?
1. Natural Juice
2. Squash  
3. Fizzy Drink  
4. More Than One

77. How often do you have the following drinks of alcohol

<table>
<thead>
<tr>
<th>Drink</th>
<th>Amount per occasion</th>
<th>7</th>
<th>6</th>
<th>5</th>
<th>4</th>
<th>3</th>
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<th>1</th>
<th>F</th>
<th>R</th>
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<tbody>
<tr>
<td>Beer/lager/stout/cider</td>
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<td>6</td>
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<td>4</td>
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<td>Sherry/port/vermouth</td>
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<tr>
<td>Spirits/liqueurs</td>
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</tbody>
</table>

Additional Questions

How often do you have..

77. Dishes made with TVP (soya mince) or Quorn? 7 6 5 4 3 2 1 F R
78. Vegetarian sausages/vegetarian burgers? 7 6 5 4 3 2 1 F R
79. Are there any foods that you eat regularly, but which are not recorded in the questionnaire? Yes / No

80. Please state each food and how often you usually eat it

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Thanks for your time!
Appendix IV: International Physical Activity Questionnaire (IPAQ long form)

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
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
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.
Appendix V: Greene Climacteric Scale

THE GREENE
CLIMACTERIC SCALE

NAME: .................................................. DATE: ..............

NUMBER: ..............................................

Please indicate the extent to which you are bothered at the moment by any of these symptoms by placing a tick in the appropriate box.

<table>
<thead>
<tr>
<th>SYMPTOMS</th>
<th>Not at all</th>
<th>A little</th>
<th>Quite a bit</th>
<th>Extremely</th>
<th>Score (0-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Heart beating quickly or strongly</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2. Feeling tense or nervous</td>
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<td>3. Difficulty in sleeping</td>
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<td>4. Excitable</td>
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<td>5. Attacks of panic</td>
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<td>6. Difficulty in concentrating</td>
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<td>7. Feeling tired or lacking in energy</td>
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<td>8. Loss of interest in most things</td>
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<td>9. Feeling unhappy or depressed</td>
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<td>10. Crying spells</td>
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<td>11. Irritability</td>
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<td>12. Feeling dizzy or faint</td>
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<td>13. Pressure or tightness in head or body</td>
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<tr>
<td>14. Parts of body feel numb or tingling</td>
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<td>15. Headaches</td>
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<td>16. Muscle and joint pains</td>
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<td>17. Loss of feeling in hands or feet</td>
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<tr>
<td>18. Breathing difficulties</td>
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<td>19. Hot flashes</td>
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<td>20. Sweating at night</td>
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<td>21. Loss of interest in sex</td>
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P (1-11) = \underline{\hspace{2cm}} \quad A (1-6) = \underline{\hspace{2cm}}
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S (12-18) = \underline{\hspace{2cm}} \quad D (7-11) = \underline{\hspace{2cm}}
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V (19-20) = \underline{\hspace{2cm}} \quad S (21) = \underline{\hspace{2cm}}
\]
Appendix VI: Menstrual History Questionnaire

1. At what age did your periods start (age of menarche)? ……………
2. When was your Last Menstrual Period (first day of your last period)? ……………
3. Are your periods heavy? (circle appropriate) YES / NO
4. How many days do your periods last? ………
5. What contraceptive method do you use (if any)? ……………………………
6. Have you ever been pregnant? (circle appropriate) YES / NO
   IF YES How many times? ……..
   IF YES what was the outcome of each pregnancy? ………………………………………

7. Please answer this question, NOT INCLUDING any time spent pregnant, receiving birth control pills or injections, after menopause, or after having both ovaries or the uterus surgically removed. Between the ages of 16 and 40, about how long has been your average menstrual cycle (time from first day of one period to the first day of the next period)? (select ONE only)
   - <25 d
   - 25-34 d
   - 35-60 d
   - Totally variable

8. Indicate what is the shortest and what is the longest length of your cycle? (only applicable if you still have periods, time from first day of one period to the first day of the next period) ……………… days

9. During your menstruating years (not including during pregnancy), have you ever had tendency to grow dark, coarse hair (eg upper lip, chin, breast, abdomen etc?) YES/NO
   IF YES Specify body sites: …………………………………………………………………

10. During your menstruating years have you ever had troublesome with acne? YES/NO

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