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# **The cardiometabolic phenotype of UK South Asian men**

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## Abstract

Migrant South Asian populations in Europe, North America the Westernised countries have a greater cardiovascular disease (CVD) risk than their respective indigenous populations. Both overall and premature CVD morbidity and mortality is significantly higher in migrant South Asians than in white populations in the UK and globally. Despite this, the role of ultrasound as a screening tool for CVD risk assessment in South Asians has not been studied extensively. Data also suggest that increased susceptibility to the adverse effects of insulin resistance and type 2 diabetes mellitus (T2DM) may contribute to the increased CVD risk.

South Asians living in the United Kingdom also have a 3-5 fold increased prevalence of T2DM, developing the disease around a decade earlier and at a lower body mass index (BMI) compared to white Europeans. Furthermore, non-diabetic South Asians have higher fasting glycaemia and are more insulin resistant than Europeans. Liver fat is also associated with insulin resistance and T2DM risk and is considered to play a causal role in diabetes. Limited data suggest that South Asians have higher liver fat content than age- and BMI-matched Europeans, but it is not currently clear whether this contributes to the observed ethnic difference in insulin resistance.

The first aim was to determine the extent to which increased insulin resistance and fasting glycaemia in South Asian, compared to white European men, living in the UK, was due to lower cardiorespiratory fitness (maximal oxygen uptake [ $VO_{2max}$ ]) and physical activity. The second aim was to determine whether South Asians have increased liver fat compared to Europeans and the extent to which any such differences can explain the increased insulin resistance observed between these groups. The final aim was to determine whether South Asians had a difference in carotid intima-media thickness (cIMT) or carotid plaque presence compared to Europeans; and if so, whether any measured risk factors (if any) could account for any such observed differences in cIMT and/or carotid plaque disease.

100 SA and 100 age and BMI-matched European men without diagnosed diabetes, aged 40-70 years, had fasting blood taken for glucose concentration, insulin, plus

other risk factors, and underwent 2-dimensional carotid ultrasound for measurement of intima-media thickness and carotid plaque analysis, assessment of physical activity (using accelerometry),  $VO_{2max}$ , body size and composition, and demographic and other lifestyle factors. For addressing the first aim of this thesis, 13 South Asian and 1 European man had  $HbA_{1c}$  levels  $>6.5\%$  indicating potential undiagnosed diabetes and were excluded from the analyses relating. Linear regression models were used to determine the extent to which body size and composition, fitness and physical activity variables explained differences in insulin resistance (assessed by Homeostasis Assessment Model of Insulin Resistance,  $HOMA_{IR}$ ) and fasting glucose between SA and Europeans. For the second aim, 28 South Asian and 24 European participants were chosen at random (but matched for age) within 4 months of their original main study visit to undergo magnetic resonance spectroscopy for quantification of liver fat.

$HOMA_{IR}$  and fasting glucose were 67% ( $p<0.001$ ) and 3% ( $p<0.018$ ) higher, respectively, in South Asians than Europeans. Lower  $VO_{2max}$ , lower physical activity and greater total adiposity in SA individually explained 68% (95% confidence interval [CI], 45-91%), 29% (95% CI, 11-46%) and 52% (95% CI, 30-80%), respectively, and together explained 83% (95% CI, 50-119%) (all  $p<0.001$ ), of the ethnic difference in  $HOMA_{IR}$ . Lower  $VO_{2max}$  and greater total adiposity respectively explained 61% (95% CI, 9-111%) and 39% (95% CI; 9-76%) (combined effect 63% (95% CI 8-115%); all  $p<0.05$ ) of the ethnic difference in fasting glucose. Unadjusted mean liver fat content did not differ significantly between South Asians compared to Europeans (5.28 (standard deviation [SD], 2.11)% vs 5.41 (SD,2.35)%,  $p=0.913$ ), but following adjustment for alcohol consumption was significantly lower in South Asians than Europeans (5.30 (SD, 2.10)% vs 9.03 (SD, 2.22)%  $p=0.017$ ). Adjustment for alcohol-adjusted liver fat did not attenuate the difference in  $HOMA_{IR}$  between ethnic groups. There were no significant differences in unadjusted or age-adjusted in mean cIMT between South Asians and Europeans. There was an increased odds ratio for the presence of plaque disease in South Asians compared to Europeans, however this was not significant (OR 1.57, 95% CI 0.89-2.77,  $p=0.13$ ).

Lower cardiorespiratory fitness is a key factor associated with the excess insulin resistance and fasting glycaemia in middle-aged South Asian compared to

European men living in the UK. Also, whilst clear associations between liver fat and insulin resistance were observed in South Asians and Europeans, these results challenge the notion that excess liver fat *per se* explains the greater insulin resistance observed in South Asians. Finally, cIMT is similar between South Asian and European men and there is also currently no clear evidence for more carotid plaques in South Asian compared to European men living in the UK. This important negative finding highlights the need for further studies on carotid plaque or research in alternative screening methods for CVD which are more sensitive in identifying subclinical CVD.

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## Declaration

I declare that I am the author of this thesis, and that no part of it has been reported in another thesis. I was involved in the work described in this thesis from the earliest planning stages, and contributed to overall design of the CURVES study, in collaboration with my supervisors and the MRI team as acknowledged earlier. I was responsible for tailoring the protocol for carotid ultrasound analysis and also arranged the repertoire of blood samples to be collected and the protocol for specimen collection, processing and storage.

I was responsible for recruitment and vetting of volunteers as well as booking of visits. All the carotid ultrasound scans were performed by me.

Whenever and wherever third party involvement in data collection and analysis has occurred, such activity has been appropriately mentioned and credited in the Methods chapter and/or Acknowledgements section.

Statistical analysis was in some cases carried out by me, with more complex analyses being performed by colleagues in the Robertson Centre for Biostatistics after discussion with myself and my supervisors regarding the statistical approach to be taken and again has been appropriately mentioned and credited in this thesis.

The work reported in this thesis is entirely my own.

## Publications, posters and presentations containing work undertaken in this thesis

- Celis-Morales CA, **Ghouri N**, Bailey MES, Sattar N, Gill JMR ‘Should Physical Activity Recommendations Be Ethnicity-Specific? Evidence from a Cross-Sectional Study of South Asian and European Men’. *PLoS ONE*. 2013; 8(12): e82568. doi:10.1371/journal.pone.0082568. PMID: 24349313.
- **Ghouri N**, Purves D, McConnachie A, Wilson J, Gill J and Sattar N. Lower cardiorespiratory fitness contributes to increased insulin resistance and fasting glycaemia in middle-aged South Asian compared to European men living in the United Kingdom. *Diabetologia*. 2013; 52(10):2238-49. PMID: 23811809.
- Low cardiorespiratory fitness contributes to increased HbA1c concentrations in South Asian compared to European men’. **Poster at 2012 Diabetes UK Annual Professional Conference.**
- Low cardiorespiratory fitness contributes to increased HbA1c concentrations in South Asian compared to European men. **Oral Presentation at Scottish Society for Experimental Medicine**, hosted at the University of Glasgow on 25/11/11.

## Publications related to the work in this thesis

- Gill JMR, Celis-Morales CA, **Ghuri N**. 'Physical activity, ethnicity and cardio-metabolic health: does one size fit all?' *Atherosclerosis*. 2014; 232(2):319-333.
- **Ghuri N**, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology*. 2010; 52(3):1156-61.
- **Ghuri N**, Preiss D, Sattar N. **Correspondence** on Liver enzymes, nonalcoholic fatty liver disease and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology*. 2011; 53(5):1172.

## List of abbreviations

<i>Abbreviation</i>	<i>Definition</i>
ABV	actual body volume
ADP	air displacement pletismography
ALT	alanine aminotransferase
ASE	American Society of Echocardiography
AST	aspartate aminotransferase
BHF	British Heart Foundation
BMI	body mass index
CHD	coronary heart disease
CI	confidence interval
cIMT	carotid intima-media thickness
CRP	C-reactive protein
CURVES	Carotid Ultrasound and Risk of Vascular disease in Europeans and South Asians study
CV	coefficient of variation
CVD	cardiovascular disease
DM	diabetes mellitus
FECO <sub>2</sub>	percentage fraction of expired carbon dioxide
FEO <sub>2</sub>	percentage fraction of expired oxygen
FRS	Framingham Risk Score
GCP	Good Clinical Practice
GGT	$\gamma$ -glutamyltransferase
GP	General Practitioner
GCRC	Glasgow Cardiovascular Research Centre
GCRF	Glasgow Clinical Research Facility
HbA1c	glycated haemoglobin A1c
HOMA <sub>IR</sub>	Homeostasis Assessment Model of Insulin Resistance
HR	hazard ratio
HtR <sub>max</sub>	maximum heart rate
IHD	ischaemic heart disease
IL-6	interleukin 6
IMT	intima-media thickness
IQR	inter-quartile range
LCCA	left common carotid artery
LD	least deprived
LFTs	liver function tests
MD	most deprived
METS	metabolic equivalents
MI	myocardial infarction
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MVPA	moderate-to-vigorous physical activity
NAFLD	nonalcoholic fatty liver disease
OR	odds ratio
PWV	Pulse wave velocity
RCCA	right common carotid artery
RER	respiratory exchange ratio
RR	relative risk
SAA	surface area artefact

SD	standard deviation
SES	socio-economic status
SIMD	Scottish Index of Multiple Deprivation
SMR	standardised mortality ratio
STPD	standard room temperature and pressure
T2DM	type 2 diabetes mellitus
TGV	thoracic gas volume
TV	tidal volume
VCO <sub>2</sub>	carbon dioxide production
VIL	volunteer information leaflet
VO <sub>2</sub>	oxygen uptake
VO <sub>2max</sub>	maximum oxygen uptake during exercise
VO <sub>2pred</sub>	predicted oxygen uptake
WHR	waist-hip ratio
2D	two-dimensional
3D	three-dimensional

## Chapter 1

### Introduction and Literature Review

#### 1.1 Cardiometabolic disease and associated risk factors

Cardiovascular disease (CVD) due to coronary heart disease (CHD) and cerebrovascular disease, accounted for nearly 180,000 of the 561,666 deaths in the UK in 2010, making it the second highest cause of death behind cancer (1) and is the leading cause of death in western Europe and North America. Whilst age-standardised deaths from CVD are falling, absolute deaths due to CVD have risen between 1990 and 2010 - in 2010 CVD accounted for 25 % of global deaths, having accounted for 20% in 1990 (2). Further, recent large scale longitudinal data (>800,000 people) largely from Europe and North America examining the association of diabetes mellitus (DM) with CVD mortality showed that after adjustment for age, sex, smoking status, and body mass index (BMI), the hazard ratio (HR) among persons with DM as compared with persons without DM was 2.32 (95% confidence interval [CI], 2.11-2.56) for death from CVD (3). Further, middle-aged adults with DM but without known vascular disease died about 6 years younger than people without DM - around 58% of this survival difference at 50 years of age can be attributed to excess vascular deaths (3).

Following the very first publication from the Framingham Study over 60 years ago (4), prevention of CVD (primary or secondary) has focused on the management of established modifiable risk factors. These traditional or established risk factors include blood cholesterol, tobacco smoking, hypertension, diabetes mellitus (DM) and more recently, reduced physical activity (5). Interest has grown in additional 'novel' risk factors, such as insulin resistance and cardiorespiratory fitness (6). It is also well recognised that the risk of morbidity and mortality from CVD is influenced by ethnicity.

#### 1.2 South Asians and risk of cardiometabolic disease

People of South Asian origin (people from India, Pakistan, Bangladesh and Sri Lanka) were one of the first ethnic groups recognised to have increased CHD

risk. One of the first studies published on CHD and South Asians (using autopsy data), showed that migrant Asian Indian males in Singapore had seven times the CHD prevalence of Chinese men (7). Subsequently, this increased risk has been confirmed by a number of reports indicating that migrant South Asian populations in the UK and North America and elsewhere in the world have a greater CHD risk than their respective indigenous populations (8). The global INTERHEART study, found that myocardial infarction (MI) occurred on average 10 years earlier in South Asians than in white populations based on case-control data from 52 countries (9). Furthermore, the age-standardised CHD mortality in South Asians living in the UK has been around 50% higher than the indigenous white population (10). More recently the age-standardised mortality of migrant South Asians in the UK shows persistence of increased mortality from ischaemic heart disease (IHD), with Indian men having a standardised mortality ratio (SMR) increased by 31%, Pakistani men by 62% and Bangladeshi men by 75% (Table 1.1) (11). Similarly, the same study showed that deaths from cerebrovascular disease remain in excess for migrant South Asian men. Also, CHD mortality in young migrant South Asian men and women (aged <40) is over double that of Europeans (12;13).

**Table 1.1 Deaths from IHD in the UK for men 2001-2003.**

<b>Country</b>	<b>Number of IHD Deaths</b>	<b>SMR (95% CI)</b>
England and Wales	149,950	96 (96-97)
Scotland	3813	104 (100-107)
India	2528	131 (126-137)
Pakistan	1044	162 (152-172)
Bangladesh	409	175 (158-193)

The increased CHD burden in the migrant South Asian population is not entirely explained by traditional CVD risk factors, such as cholesterol, blood pressure and smoking (14-17). The largest dataset comparing the traditional risk factors

in South Asian compared to European men comes from the 2004 health survey for England (18). Data from the survey confirmed that with the exception of the increased prevalence of DM and increased prevalence of smoking (Bangladeshi men only), there were little differences in traditional risk factors between South Asians and the general population (Table 1.2). A systematic review comparing blood pressure between South Asians, even indicated that blood pressure levels may be lower in Pakistanis and Bangladeshis compared to Europeans living in the UK (19).

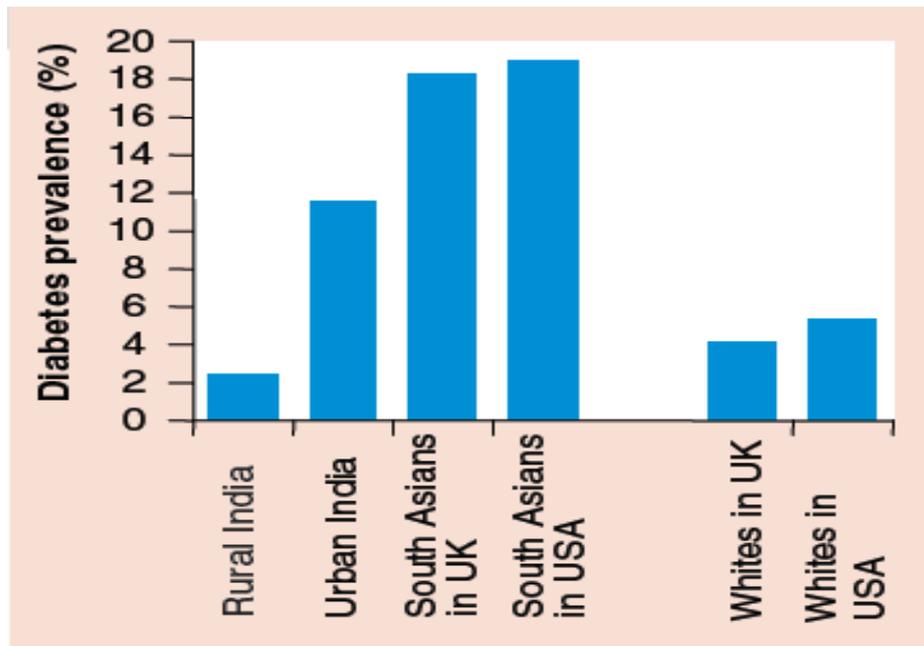
**Table 1.2 Differences in CVD risk factors between South Asian men and the general population living in the UK. (*Health Survey for England 2004 (18)*)**

	Indians	Pakistanis	Bangladeshis	General Population
Current smoking (%)	20	29	40	24
Cholesterol	5.3	5.3	5.3	5.5
Systolic blood Pressure (mmHg)	127	124	121	130
Diabetes (%) *	9.8	17.9	19.0	3.8

\* Self-reported history

The data in Table 1.2 show similar total cholesterol values in South Asian and European men, with similar data reported the Southall study in 1991 and in more recently in patients with diabetes living in Scotland (20). Although LDL levels are similar, it has been found that South Asians have smaller and more dense LDL cholesterol particles, which are considered to be more atherogenic (21;22). Further, HDL cholesterol, which is known to be predictive against CVD (23) was lower in the South Asian group in the Southall study. Further, HDL cholesterol may be less protective in South Asian, as particle size (which is positively associated with reduced CVD) is previously been found to be smaller in South Asians (22).

The Southall study was the first large cross-sectional study to compare measured metabolic data from Indian and Pakistani subjects with that from age and BMI-matched European subjects (24). This showed that migrant South Asians have a higher prevalence of DM (19% versus 4%), and this figure is similar for South Asians living in the USA (Figure 1.1) (24;25). More recent data from Scotland showed that the prevalence of DM in South Asians has little changed, with the prevalence remaining 3-4 times higher compared to Europeans (20). In terms of undiagnosed type 2 diabetes mellitus (T2DM) in South Asians in the UK, data suggest that this might be as high as 20% in adults aged 20-75, with a further 28% having impaired glucose tolerance (26). This figure for hidden T2DM is 14 times higher than the suggested 1 in 70 prevalence for the general UK population (27). Additionally, South Asians living in Scotland are diagnosed with diabetes more than a decade earlier compared to Europeans (45.9 years v 57.3 years) (28). Similar findings have been demonstrated in the 20-year follow-up data from the Southall cohort (as well as subjects in the Brent study) (29). The follow-up data (collected until 2011) showed that incident T2DM in South Asians was 33% - nearly 2.5 times higher than that of Europeans (14%) over the 20 years. Further, the average age of diagnosis was 66-67 years of age in Europeans, but 5 years earlier in South Asians.



**Figure 1.1 Prevalence of DM in South Asians**

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Previous prospective (~16yr) UK follow-up data from Southall & Brent studies also suggest that South Asians are more sensitive to the adverse CVD complications from T2DM (14). The data described an age-adjusted HR for male CHD mortality for South Asians of 1.64 (95% CI 1.24-2.16), compared to Europeans, increasing to 2.14 (95% CI, 1.56-2.94) after adjustment for smoking and cholesterol. Further analysis using multivariable models that adjusted for conventional risk factors and diabetes and/or impaired glucose regulation, features of insulin resistance, or the metabolic syndrome showed that CHD mortality remained significantly higher in South Asian men (HR 1.6-1.9). The South Asian diabetic subgroup suffered nearly half of all the South Asian CHD deaths, but only 13% of European CHD deaths occurred in the European diabetics. Further, in the South Asian group, diabetes was associated with nearly threefold higher mortality risk compared with South Asians without diabetes at baseline, yet in Europeans, the excess mortality associated with diabetes was only 1.5-fold.

To summarise, whilst South Asians have more atherogenic lipid profiles and a higher prevalence of diabetes associated with poorer glycaemic control, differences in traditional cardiovascular risk factors still fail to fully explain the increased CVD risk in South Asians. There has been suggestion that quantifying the increased risk can be achieved by adding 10 years to the age of the person when using the Framingham Risk Score (FRS) (30), whilst newer UK-based risk factor scoring engines now factor in ethnicity when predicting future CHD risk (31). Regardless of what method is used for scoring CHD or CVD risk, it is clear the reasons for ethnic disparities remain unanswered, and the traditional cardiovascular risk factors of blood pressure, smoking, blood cholesterol and DM fall short in fully accounting for this increased CVD risk. Thus pre-clinical disease states such as insulin resistance and the role of novel cardiometabolic risk factors including cardiorespiratory fitness, physical activity and liver fat as potentially mediating as well as independent factors deserve exploration in South Asians given the size of this high-risk ethnic group locally and worldwide. Further, in parallel to this exploration, the role and evaluation of various pre-existing ultrasound-based screening methods for subclinical CVD is of merit if one is to maximise screening potential in South Asians who experience premature cardiometabolic disease.

Sections 1.4 to 1.8 will discuss some of these potentially relevant and more novel risk factors particularly those which have little or no published data on South Asians, including cardiorespiratory fitness, objectively-measured physical activity and liver fat measurement. Finally section 1.9 will discuss the role of ultrasound-based carotid plaque scoring as a potentially more sensitive screening tool for CVD in South Asians.

### **1.3 Insulin resistance**

#### **1.3.1 Insulin resistance and cardiometabolic risk**

Insulin resistance can be defined as ‘the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population’ (32). In other words an

individual with insulin resistance requires more insulin to have the same effect on controlling blood glucose, or the body cannot produce enough insulin to control blood sugar, leading to impaired glucose regulation and ultimately T2DM (33).

Briefly, insulin resistance is strongly associated with an increased adiposity state such as obesity. The connection between obesity and increased levels of circulating insulin was first described in the 1960s (34) with the research showing that, in systemic insulin resistance, there was compensatory activity by insulin-secreting beta cells. It is also recognised that obese individuals have increased circulating levels of free fatty acids and this is associated with insulin resistance and dysglycaemia. Finally it is known that obese normoglycaemic individuals have increased beta-cell function (34;35). Thus obesity-induced glucose intolerance appears to reflect failure of beta cell activity (36).

Several mechanisms have been suggested to explain why obesity causes insulin resistance and subsequently T2DM. These include increased production of adipokines/cytokines which contribute to insulin resistance (37); ectopic fat deposition (mainly in the liver but also in skeletal muscle), and the resulting effect on glucose/insulin homeostasis (described in more detail in section 1.7.1) (38); and impaired mitochondrial activity (39). Impaired mitochondrial activity (reflected by reduced mitochondrial mass and/or function) could be one of many important pathologies connecting obesity to diabetes, both by decreasing insulin sensitivity and by compromising beta-cell function. Though it is recognised that impaired mitochondrial activity is not a consistent finding as demonstrated in a small cohort of Indian men with diabetes compared to Indian men without diabetes (13 in each group) (40)

Insulin resistance is adversely associated with a number of traditional CVD risk factors (41). Longitudinal data also supports the role of the severity of insulin resistance with CVD risk in subjects with pre-existing T2DM (42). Longitudinal data directly linking Insulin resistance to incident CVD has been demonstrated in Italians from the Bruneck study, which followed up 839 subjects without CVD at baseline for 15 years (43). Results showed a significant association between increased insulin resistance at baseline and incident CVD even in subjects

without diabetes after adjustment for traditional as well as several novel cardiovascular risk factors (HR 2.2, 95% CI, 1.3 -3.7,  $p < 0.05$ ).

### **1.3.2 Insulin resistance in South Asians**

Several studies in addition to the Southall study (which demonstrated increased fasting and 2-hour post 75g glucose load insulin levels in South Asians) have confirmed that South Asians are more insulin resistant (determined by HOMA-I; hyperinsulinaemic euglycaemic clamp; insulin sensitivity index; and fasting and 2-hour post 75g glucose load insulin levels respectively) than their European counterparts (14;44-46). Further, studies have shown that even after adjustment for BMI, waist-hip ratio (WHR), and skin fold thickness, insulin levels (both fasting and post glucose-load) remain significantly higher in South Asians (45-47), thus greater insulin resistance is not fully explained by excess adiposity. Despite the increased prevalence of insulin resistance in South Asians, follow-up data from the Southall and Brent Studies, showed that the increased CVD mortality observed in the South Asians was little influenced after adjustment for insulin resistance (as determined by HOMA-IR) (14).

## **1.4 Adiposity**

### **1.4.1 Adiposity and cardiometabolic risk**

Worldwide the prevalence of DM in 2010 was estimated at 285 million people (48). In 2012, the UK prevalence of DM was estimated to be 4.6% - giving three million people a diagnosis of diabetes in the UK (49). Around 90% of adults with DM have T2DM (49). Both the prevalence and incidence of T2DM have been increasing in the UK. The prevalence has increased from 2.8% in 1996 to 4.5% in 2011 (49;50). The growing incidence of DM is nearly all T2DM in aetiology - the incidence of T2DM increased from 2.60/1000 person-years in 1996 to 4.31/1000 person-years in 2005, whilst the incidence of type 1 diabetes remained relatively constant (50). This growing incidence and prevalence of T2DM being linked to the increasing prevalence of obesity - the proportion of individuals newly

diagnosed with T2DM who were obese increased from 46% to 56% over the 10-year period.

This adverse metabolic effect of obesity is expected, as it is recognised that weight gain is an important proven risk factor in developing T2DM (51-54). For example, a 5-year follow-up of over 50,000 healthy men aged 40-75 years, found that men with a BMI >35 were at 42-fold higher risk of developing T2DM than men with a BMI <23 (52). Moreover, the landmark data from Hillier and colleagues illustrated how severity of obesity lowered the age of onset of T2DM. Defining early onset of T2DM as a diagnosis before the age of 45, they calculated the mean BMI in this group to be 39, however the usual group (age >45), had a mean BMI of 33 (55). There was also a significant ( $p<0.0001$ ) inverse linear relationship between BMI and age at diagnosis of T2DM (Table 1.3). Research is also confirming the belief that with growing numbers of younger people becoming obese, the number of younger adults being diagnosed with T2DM is also on the rise (56). Thus obesity is a key, if not *the* main driver for the growing (and premature) prevalence of T2DM.

**Table 1.3 Relationship between age of diagnosis of T2DM and BMI.**

Age at Diagnosis T2DM	BMI
<30	38.3
31-35	37.1
36-40	38.3
41-45	36.3
46-50	35.9
51-55	35
56-60	33.9
61-65	31.7
66-70	31.1
>70	28.8

The relationship between obesity or increased BMI and CVD/CHD is not as clear as the relationship obesity has with T2DM. It is well-known that an obese state is associated with higher blood pressure, higher non-HDL cholesterol, lower HDL cholesterol and a higher prevalence of diabetes (57). However studies have argued that these are the mediators by which BMI is thought to affect cardiovascular mortality (58;59). Nonetheless, equally contemporary prospective data including a meta-analysis showed a 40% increase in CHD mortality for every 5Kg/m<sup>2</sup> increase in BMI above 25Kg/m<sup>2</sup>, confounding factors such as traditional risk CVD risk factors, medication and intentional weight loss due to disease were not adjusted for (57;60). One recent prospective study assessed the independent association of obesity separately for fatal or nonfatal CHD over a 15-year period by accounting for traditional risk factors and deprivation in a cohort of over 6,000 previously healthy middle-aged men living in the west of Scotland (West of Scotland Coronary Prevention Study, WOSCOPS) (61). A minimally adjusted model (age, sex, treatment with statins) and a maximally adjusted model (including known CVD risk factors and deprivation) were generated, using BMI 25-27.4 kg/m<sup>2</sup> as the reference BMI. The risk of fatal CHD events was increased in obese men (BMI 30.0-39.9 kg/m<sup>2</sup>) in both the minimally adjusted model (HR=1.75, 95% CI, 1.12, 2.74) and the maximally adjusted model (HR=1.60, 95% CI, 1.02, 2.53).

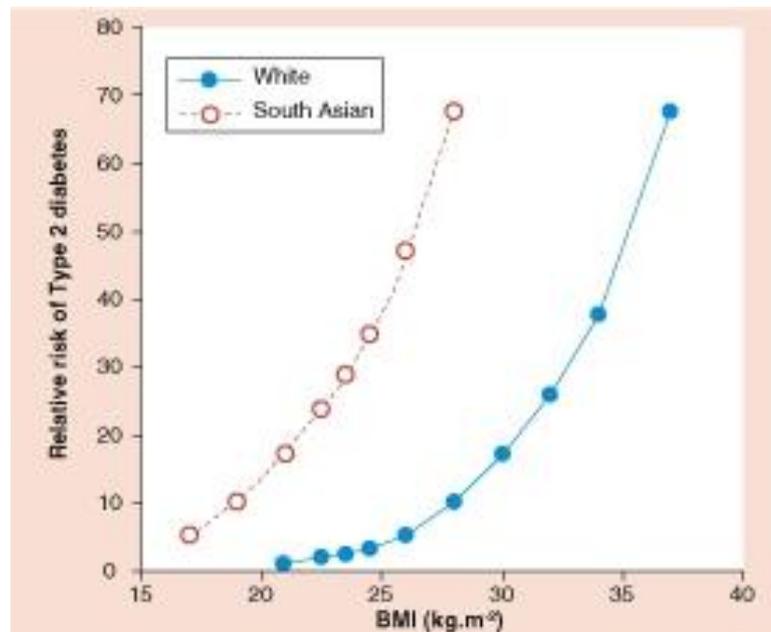
A possible additional pathological effect of obesity, beyond its association with traditional factors, is that of inflammation. Adipose tissue is considered an endocrine organ that releases proinflammatory cytokines (62), rendering obesity a low-grade inflammatory state (63;64). Recent data have suggested that in established CVD, markers of inflammation—namely C-reactive protein (CRP) and interleukin 6 (IL-6) - are more strongly associated with fatal than non-fatal events (65). Therefore if inflammation increases the risk of CHD mortality, the same might be hypothesised about the role of adiposity - a source of circulating inflammatory markers.

### 1.4.2 Adiposity in South Asians

Much of the aforementioned data in relation to obesity and risk of T2DM comes from populations predominantly of European extraction. Yet much of the projected increased global prevalence of T2DM is attributable to Asia, namely the Indian subcontinent, China and the Middle East (48). The prevalence of diabetes is expected to rise 54% between 2010 and 2030, however the prevalence is expected to rise by 72% in South Asian countries. Conversely, the prevalence in Europe is only expected to rise by 20%. India, China and Pakistan will provide 63 million of the 145 million extra people who will have diabetes by 2030, with India alone contributing 37 million people.

In light of these epidemiological data, is it possible that obesity, or indeed weight gain in general has a stronger association with onset of T2DM in such high risk populations? As described earlier adiposity does not fully explain the increased insulin resistance seen in South Asians. A recent review plotted the relative risk of T2DM against BMI for people of South Asians and European origin (Figure 1.2) (25). The graph illustrates clearly that the BMI threshold associated with the exponential increase in diabetes risk occurs at markedly lower BMI range (18.5-25 at 22-23 for South Asians), whereas the increase in Europeans occurs at a BMI in excess of 30.

Similarly, a landmark study from Canada looking at a multiethnic population (South Asian, Chinese, Aboriginal and Europeans) calculated ethnic-specific BMIs for an equivalent risk of impaired glucose control akin to risk of diabetes (66). They calculated the risk for a European with a BMI of 30, and subsequently calculated the equivalent BMI that would give the same risk in the other ethnic groups. The equivalent BMIs in the South Asian, Chinese and Aboriginal populations were 21.0, 20.6 and 21.8 respectively. More recently a similar larger study performed in the UK comparing migrant South Asians with the indigenous white European population (ADDITION-Leicester cohort), calculated risk equivalent BMIs for T2DM of 22.6 for men and 21.5 for women (67).



**Figure 1.2 Association of BMI with risk of T2DM in South Asians and Europeans**

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One possible explanation is that the current BMI categories for weight are not as useful or sensitive in other ethnicity groups in relation to metabolic risk. For example, though South Asians have similar absolute waist circumferences compared to BMI-matched Europeans (68;69), South Asians have an increased WHR (24), thus indicating that South Asians have more adiposity concentrated centrally. Increased WHR is recognised as a stronger predictor of risk of T2DM compared to BMI in Asians (70). Similarly, BMI-matched South Asians have more body fat compared to Europeans (44;47;71). Also, for a given BMI, South Asians have significantly lower muscle mass (44;47), with increased abdominal adiposity (44), larger adipocyte size (44;68) and greater amounts of adipose tissue in the deep subcutaneous compartment than Europeans (72), although interestingly increased visceral fat is not a consistent finding (44;46). Thus relative adiposity as opposed to BMI appears more influential for T2DM risk in South Asians compared to Europeans.

The greater adverse effects of adiposity observed in South Asians have led to recent consensus statements, including the recommendation that the threshold

for obesity should be lowered to BMI 25 kg.m<sup>-2</sup> (normal 18-22.9 kg.m<sup>-2</sup>, overweight 23-24.9 kg.m<sup>-2</sup>) in South Asian populations (73-75) . Similarly, findings from the ADDITION cohort from Leicester UK, suggested South Asian-specific waist circumferences (a surrogate for WHR) for equivalent glycaemic and lipid factors compared to Europeans for the central obesity cut off points - South Asian women and men required waist circumferences of 69 and 84 cm respectively for the glycaemic factor, and 74 and 91 cm respectively for the lipid factor. This in contrast is much lower than the all-encompassing 88/102 cm for European women/men. Further, prospective data from Mauritius and AusDiab surveys indicated that South Asian women and men required waist circumferences of 70/80 cm for diabetes equivalent risk to Europoid women and men with waist 88/102 cm (76). Accordingly, the waist circumference threshold criterion for diagnosis of the metabolic syndrome lower is South Asian (80/90 cm) than in European (88/102 cm), women and men respectively (77).

## **1.5 Cardiorespiratory fitness**

### **1.5.1 Cardiorespiratory fitness and cardiometabolic risk**

Exercise tests can provide a number of indices of *in vivo* skeletal muscle and whole body oxidative capacity. It has been reported that maximal oxygen uptake, or VO<sub>2max</sub> (i.e. a measure of whole-body oxidative capacity, which correlates strongly with skeletal muscle oxidative enzyme activities in heterogeneous populations (78;79)) is a strong predictor of whole-body insulin sensitivity in Europeans independent of visceral adiposity in both higher-risk as well as normal subjects (79-82). Moreover epidemiological data report a 2.6-fold increased risk of incident T2DM, independent of BMI, in men with low compared to high cardiorespiratory fitness levels (83).

Data from a large meta-analysis which aimed to define quantitative relationships between cardiorespiratory fitness and CHD events, CVD events, or all-cause mortality in healthy men and women showed that compared with participants with high cardiorespiratory fitness, those with low cardiorespiratory fitness had a relative risk (RR) for CHD/CVD events of 1.56 (95% CI, 1.39-1.75; p<0.001),

adjusting for heterogeneity of study design. Compared with participants with intermediate cardiorespiratory fitness, those with low cardiorespiratory fitness had a RR for CHD/CVD events of 1.47 (95% CI, 1.35-1.61;  $p < 0.001$ ), adjusting for heterogeneity of study design (84). Further a more recent study attempted to quantify the role of cardiorespiratory fitness in improving risk classification for CVD in previously healthy adults. Data from a study following up more than 66,000 subjects for a median of 16 years showed that a single measurement of fitness significantly improves classification of both short-term (10-year) and long-term (25-year) risk for CVD mortality when added to traditional risk factors (85).

### 1.5.2 Cardiorespiratory fitness in South Asians

Limited data are available comparing cardiorespiratory fitness levels between men of South Asian and of European descent.  $VO_{2max}$  values in sedentary South Asians are typically around 10-15% lower than comparable sedentary European groups (45;86;87). Interestingly, the relatives of patients with T2DM, who, like South Asians, have increased insulin resistance (88;89) and risk of T2DM (90;91), also have similarly lower values for  $VO_{2max}$  (~10-15% lower) than age, BMI and body-fat matched controls (81;82). However more recent data from a local study ( $n = 39$ , South Asians=20, Europeans =19) of young men indicated that the difference in  $VO_{2max}$  values was far greater (in excess of 20%) between South Asians and White Europeans even after adjustment of fat mass ( $p < 0.0005$ ), (47). The study also showed that adjustment for  $VO_{2max}$  attenuated the difference in insulin sensitivity between young South Asian and European men. **Thus it is important to determine if difference in cardiorespiratory fitness persists into later life when the risk of T2DM is greater and whether it is as influential in attenuating the unfavourable metabolic profile in South Asians.**

## 1.6 Physical activity

### 1.6.1 Definition and measurement of physical activity

Physical activity can be defined as *'any bodily movement produced by skeletal muscle that requires energy expenditure'* (92). The American College of Sports Medicine recommends moderate-intensity aerobic (endurance) physical activity for a minimum of 30 minutes for five days every week or vigorous-intensity aerobic exercise for 20 minutes for three days each week (93). Similarly, guidelines relating to physical activity to avoid weight gain for adults are 30 minutes of moderate intensity activity everyday (94). Moderate intensity aerobic activity is equivalent to a brisk walk which accelerates the heart rate, whereas vigorous-intensity exercise is equivalent to jogging and causes rapid breathing and a substantial increase in heart rate (93). In UK the weekly physical activity recommendations are for adults to undertake a minimum of 150 minutes of moderate intensity activity in sessions lasting at least 10 minutes or more (95). Additionally, similar benefits can be achieved through 75 minutes of vigorous intensity activity spread over the week (95). Physical activity targets can be met by combining intensities and types of activity as well as performing daily activities including washing dishes, house chores and walking to and from the car (93).

Physical activity can be measured subjectively by a physical activity diary or derived from validated questionnaire-based data which allow energy expenditure to be estimated. Such methods are cost effective and allow data collation from large study groups; however accuracy and validity of data are debatable in comparison to objective measurements of physical activity (96-98). Limitations include understanding of terminology e.g. 'moderate' and 'vigorous' exercise which can be interpreted differently by individuals completing the questionnaire (99). Accuracy in recall and honesty could lead to response bias in quantifying activity undertaken are other factors influencing validity of results. Finally repetition of the questionnaire by the same individual may not reproduce similar results especially if repeated after a period of time during which the individual has modified his/her physical activity. Further, it has been shown in one study that energy expenditure calculated from three of four frequently used physical

activity questionnaires (using data obtained on duration, intensity of each reported activity [in metabolic equivalents, METs] over the reporting period to measure the average activity level in METs/min), overestimated objective measurement of energy expenditure (100).

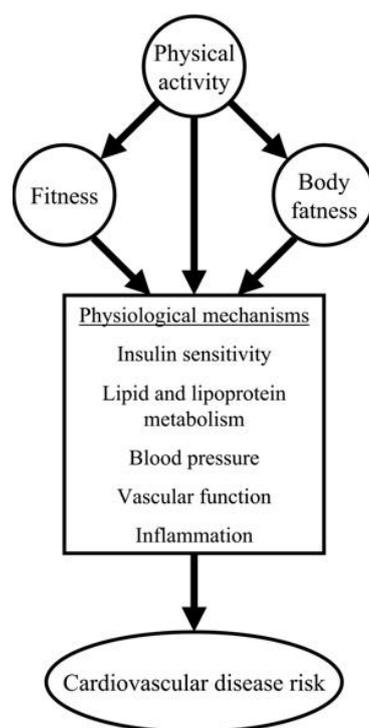
Alternatives to written records of activity include objective measurement of physical activity such use of accelerometry, which is considered as a practical non-invasive and reliable way to measure physical activity (101;102). This method involves subjects wearing the accelerometer usually by attaching it to their waist - this has to be worn at all times while awake. Accelerometers measure body movements in terms of acceleration, which can then be used to estimate the intensity of physical activity over time (103). Thus accelerometers provide information on the frequency, duration and intensity of physical activity (102). An advantage is that data can be obtained any time of the day either during the day or night depending on individual's activity patterns and activity intensity (including estimates of energy expenditure). Finally novel data comparing accelerometers to validated questionnaire-based data showed that the questionnaire-based data overestimated moderate-to vigorous physical activity (MVPA) activity by 250% compared to accelerometer data (397.8 MET.min.day<sup>-1</sup> v 155.9 MET.min.day<sup>-1</sup>) (104).

### **1.6.2 Physical activity and cardiometabolic risk**

It is recognised that reduced physical activity is associated with increased insulin resistance and an increased risk of T2DM (105;106). Data from a longitudinal cohort study found that regular physical activity was associated with a reduced risk of T2DM (107). In the Nurses Health Study it was seen that vigorous exercise per week was associated with a 33% reduction in risk of T2DM in women in comparison to no exercise performed (108). Evidence from cohort studies suggests that those who are more active reduce the risk of T2DM even if they are at a high risk of T2DM e.g. in obese individuals (53). Finally the results from the Finnish Diabetes Prevention Programme demonstrated that those subjects that underwent lifestyle intervention (individualized counselling aimed at reducing weight, total intake of fat, and intake of saturated fat and increasing intake of

fibre and to undertake physical activity - 30 mins moderate activity daily), their cumulative incidence of diabetes after four years was 11% (95% CI, 6-15%) in the intervention group and 23% (95% CI, 17-29%) in the control group. During the trial, the risk of T2DM was reduced by 58% ( $p < 0.001$ ) in the intervention group - this reduction in the incidence of T2DM was directly associated with changes in lifestyle (109).

Physical activity favourably influences CVD risk factors including reducing blood pressure, increasing HDL cholesterol and reducing body fat (94;110;111). However, even after controlling for these factors, physical activity has still been shown to be an independent risk factor for CVD (112), indicating that, as well as modifying the above risk factors, physical activity may influence CVD risk through other non-classical mechanisms (Figure 1.3).



**Figure 1.3 Mechanisms by which physical activity influence CVD risk**

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Despite the above studies showing the influence of physical activity on cardiometabolic risk, misclassification arising from the use of questionnaires to assess physical activity can substantially underestimate the true relationship

between activity and risk of cardiometabolic disease risk and should be borne in mind when interpreting such data (104;114).

### 1.6.3 Physical activity in South Asians

There is established evidence that physical activity levels are much lower in South Asians living in the UK than any other population (99;115-117). A detailed review on physical activity focused on studies that included the amount of time spent undertaking physical activity, or measured physical fitness or calculated energy expenditure of South Asians living in the UK (7 studies in adults, 5 in youths) (99). The review consistently demonstrated that South Asians reported lower levels of physical activity in comparison to Europeans or the general population. Further, across three South Asian subgroups (Bangladeshis, Indians and Pakistanis) it was also found that women had low physical activity levels in comparison to men and older people also had a low physical activity level.

Similar observations have been found by Williams and colleagues who reviewed physical activity data in South Asians from The Health Survey for England 2004 (118). The study reported adherence to physical activity recommendations, with only 11% of Bangladeshi and 14% Pakistani women achieving adequate physical activity levels. Also South Asians were ~33% less active than Europeans on a weekly basis- the total MET-min/week in South Asians being 973 MET-min compared to 1465 MET-min in Europeans, ( $p < 0.001$ ). The study also showed that South Asians were 60% less likely to comply with the current governmental guidelines on physical activity recommendations (OR 0.41, 95% CI, 0.38-0.45). Interestingly South Asians born in the UK reported higher levels of physical activity (by ~50%) than those born elsewhere ( $p < 0.001$ ). Recent data from Leicester, UK, once again demonstrated that South Asians were more likely fall below the minimum physical activity recommendations for health compared to Europeans (age-adjusted odds ratio [OR] for South Asian men = 2.35, 95% CI, 1.89-2.93, age adjusted OR for South Asian women = 2.25, 95% CI, 1.81-2.80) (117), indicating that little has changed over the past few years.

Several reasons have been suggested for reduced physical activity in South Asians compared to Europeans. One reason could be that South Asians engage in a restricted range of leisure activities in comparison to Europeans thus limiting options for expending energy (119). Another possibility could be that South Asian women could face barriers to participating in physical activity fitness, cultural norms and social expectations (120). Finally one cannot overlook any exacerbations of the above described limitations of questionnaire-based data in South Asians due to cultural interpretation of the terminology used (99).

**Ultimately there is a need for objective measurement of physical activity in South Asians to obtain a more accurate picture of how this compares to the indigenous European population and whether this may influence differences in cardiometabolic risk between the two ethnic groups.**

#### **1.6.4 Sedentary time and cardiometabolic risk**

Whilst the terms ‘reduced physical activity’ and ‘physical inactivity’ are used interchangeably, the two terms are not synonymous. ‘Sedentary’ can be defined as time ‘spent sitting down’ (121); whereas ‘physical activity’ refers to body movement (99), which as described above can be of varying intensities. It has been suggested that sedentary time is associated with an increased risk of T2DM which influences vascular and metabolic risk factors independent of time spent undertaking any form of physical activity (122). A recent meta-analysis of cross-sectional and prospective data of nearly 800,000 individuals showed that sedentary time is associated with an increased risk of DM, CVD and cardiovascular and all-cause mortality; with the strength of the association is most consistent for DM (123).

Further analysis from the Health Survey for England 2004 data showed that physical inactivity explained >20% of the excess CHD mortality observed in the South Asians, even after adjustment for potential confounding variables (socioeconomic position, smoking, diabetes and existing CVD) (116). More recently, cross-sectional data from Gill and colleagues found that for South Asians living in UK, time spent sitting was significantly associated with increased 2-h post glucose load plasma glucose concentrations (121). These data indicate

that sedentary time appears to influence cardiometabolic risk independently of less intensive physical activity, although once again one cannot overlook the subjective element of recording sedentary time.

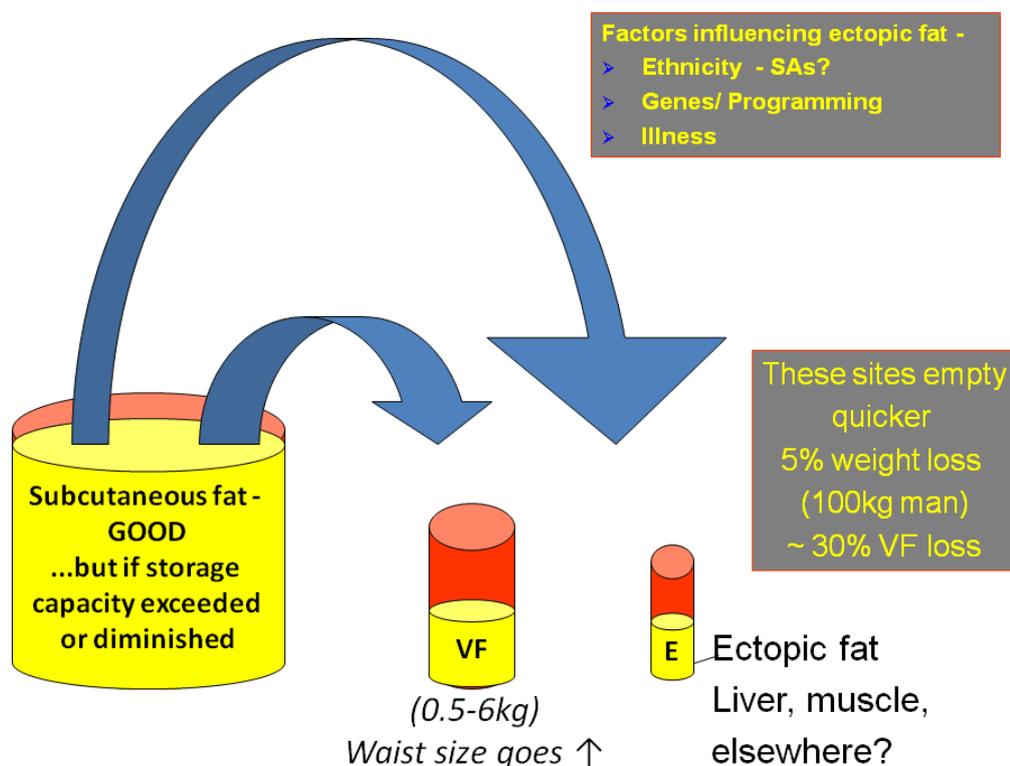
## **1.7 Liver fat**

### **1.7.1 Association of liver fat with obesity and pathogenesis of dysglycaemia**

Ectopic fat deposition in the liver is also a pathological process which contributes to insulin resistance, with on-going deposition exacerbating the insulin resistant state (124). It is not surprising therefore that liver fat has a strong association with obesity and T2DM. Obesity is a common feature of people with non-alcoholic fatty liver disease (NAFLD) - a condition when a person has more than 5% fat in their liver (125). Up to 93% of people with NAFLD are thought to be obese (126). Likewise, 75 % of people with obesity have NAFLD, compared to a prevalence of 16% in people with a normal BMI (127).

The most compelling data describing the associations between NAFLD, obesity/adiposity and T2DM comes from pilot data from bariatric surgery outcomes and reduced-energy diets in obese patients with T2DM. By applying the concept of ‘tracing the reverse route from cure to cause’ in T2DM pathogenesis (128), it can be alluded to that if a reduction in adiposity, (particularly liver fat content), is associated with a significant improvement in glycaemia in T2DM (129-131), then the presence of liver fat in particular is likely to contribute to the development of T2DM. One study clearly demonstrated that in a group of obese subjects with T2DM, weight loss from a strict moderately hypocaloric very-low-fat diet followed by a period of weight stabilization, resulted in 8% weight loss over a mean of 7 weeks, which was associated with an 81% reduction in liver fat; with the reduction in overall body adiposity only falling 1.9% over the same period (132). Mean fasting plasma glucose improved from 8.8 mmol/l to 6.4 mmol/l in these subjects. Therefore in an obese state, it appears that the relatively small quantity of fat deposited in the liver has a disproportionate role in glucose homeostasis. Thus given the current body of evidence, one can conclude that for many individuals, obesity drives insulin resistance, increases

the chances of ectopic fat deposition, chiefly in the liver which appears have a significant influence on the progression of insulin resistance which culminates in the onset of T2DM (Figure 1.4).



**Figure 1.4 Fat deposition in obese individuals**

VF: visceral fat; EF: ectopic fat

### 1.7.2 Liver fat and cardiometabolic risk

Plentiful data link liver enzymes (alanine aminotransferase [ALT] and  $\gamma$ -glutamyltransferase [GGT]) to incident T2DM. A recent meta-analysis showed that one logged unit higher ALT was associated with a HR of 3.05 (95% CI, 2.59-3.59,  $I^2 = 26\%$ ) and one logged unit higher GGT with a HR of 2.56 (95% CI, 2.31-2.84,  $I^2 = 32\%$ ) in univariate age-adjusted analyses for the development of T2DM (133). In the model adjusted for major risk factors for DM, one unit higher logged ALT gave a HR for diabetes of 1.85 (95% CI, 1.57-2.18,  $I^2 = 19\%$ ), and the HR per one unit higher logged GGT was 1.92 (95% CI 1.66-2.21,  $I^2 = 55\%$ ). However whilst there was adjustment for common DM risk factors for all studies

(age, sex, BMI/waist circumference, smoking) included in the meta-analysis, other variables including physical activity, family history of DM, cholesterol, insulin sensitivity and fasting plasma glucose were not consistently adjusted for. In the same meta-analysis, data on ultrasound-diagnosed NAFLD as a determinant of incident T2DM were examined in 3 Asian studies. The pooled RRs comparing mild vs. no NAFLD for incident T2DM was 2.52 (95% CI, 1.07-5.96), but there was evidence of considerable heterogeneity between studies ( $I^2=90\%$ ).

Whilst the risk of T2DM in NAFLD is clearly elevated, data linking liver fat with risk of incident CVD are less convincing (134). There is a robust association between higher GGT levels (135), even within the normal range and incident CVD events (136); and this association is influenced by age, with the association being much stronger in younger adults (137). However, evidence to suggest that GGT can add incremental information in CVD risk prediction is limited (138). By contrast, current data suggest ALT levels are not significantly associated with CVD risk (139). With regard to image-reported NAFLD, the current evidence base is inconsistent and, due to study design, often unable to fully consider confounding or mediation by established cardiovascular risk factors (134). Even where adjustments have been made, they have often included weak variables such as metabolic syndrome, rather than the full range of continuous established CVD risk factors used in clinical practice. Thus currently, a diagnosis of NAFLD (or increases in liver enzymes) is in itself currently controversial with opinion divided on this matter (134;140).

### **1.7.3 Liver fat in South Asians**

Limited comparative data on liver fat in South Asians exist in current medical literature. One comparative study (using magnetic resonance spectroscopy [MRS] to quantify liver fat between South Asians and Europeans indicate that South Asian men had nearly triple the hepatic triglyceride content) compared to Europeans after adjustment for age and BMI (1.94% v 0.75%,  $p<0.001$ ) (141). Further, the increased hepatic triglyceride content was associated with the increased prevalence of insulin resistance in the South Asian group. However both groups were poorly matched for age and body composition.

A more recent study comparing MRS-quantified liver fat between South Asians and Europeans living in Canada showed that healthy South Asians had significantly more liver fat 9.9% v 2.2% p <0.05) (68). However on gender sub-analysis, the 32 South Asian men (who were of similar BMI) who despite having double the liver fat content (10.8% v 4.7%) compared to the 21 European men, this difference was not statistically significant. This may be due to the small numbers in each group, as the study was not powered for this outcome measure. Further, both these studies did not accurately record or adjust results for alcohol consumption. **Thus more research is needed in this area to determine whether South Asians indeed have more liver fat compared to Europeans, accounting for the confounding effect of alcohol and whether any observed difference influence cardiometabolic differences observed between these two ethnic groups.**

## **1.8 Carotid Ultrasound markers of CVD risk**

### **1.8.1 Screening for CVD**

In 1991 Dauz and Braunwald (142), introduced the concept of the CVD continuum, and highlighted the need for the identification and early detection of factors that predispose to the development and progression of coronary artery disease, shifting from the focus of managing overt disease. It is recognised that the pathogenesis of atherosclerosis involves the interaction between lipid and the inflammatory process, with the initiating step in atherosclerosis thought to be an inflammatory insult that precedes clinical manifestation by a number of years (143), rather than primary abnormalities in plasma lipid (144;145). Medium and large-sized arteries are affected first. The earliest visible lesion of atherosclerosis is the fatty streak, which is due to an accumulation of lipid-laden foam cells (lipid-rich macrophages) in the intimal layer of the artery (146). With time, the fatty streak can regress, but more often progresses into a fibrous plaque (atheroma), the defining lesion of established atherosclerosis. Figure 1.5 summarises the macroscopic changes that occur in atherosclerosis over time.

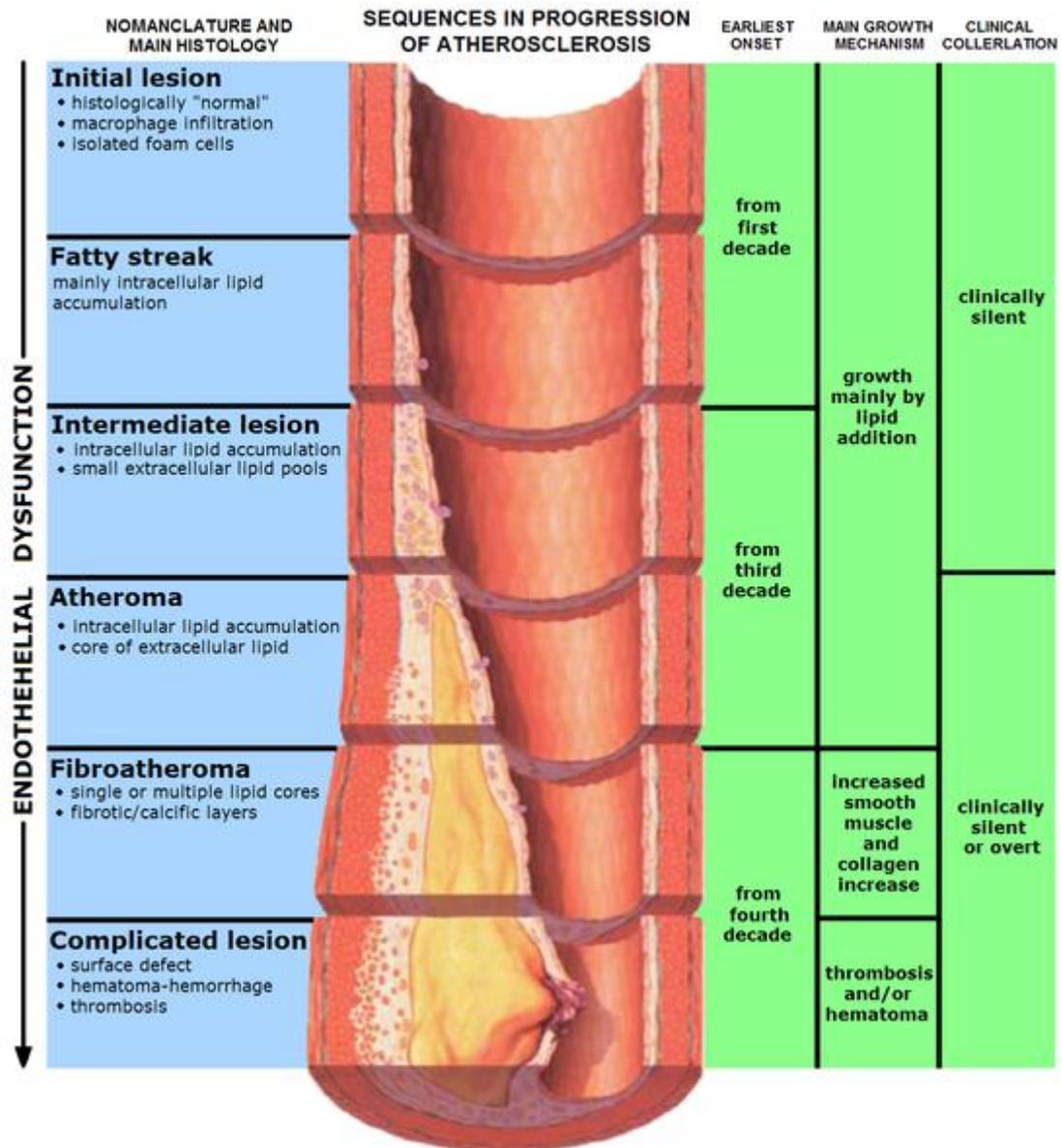
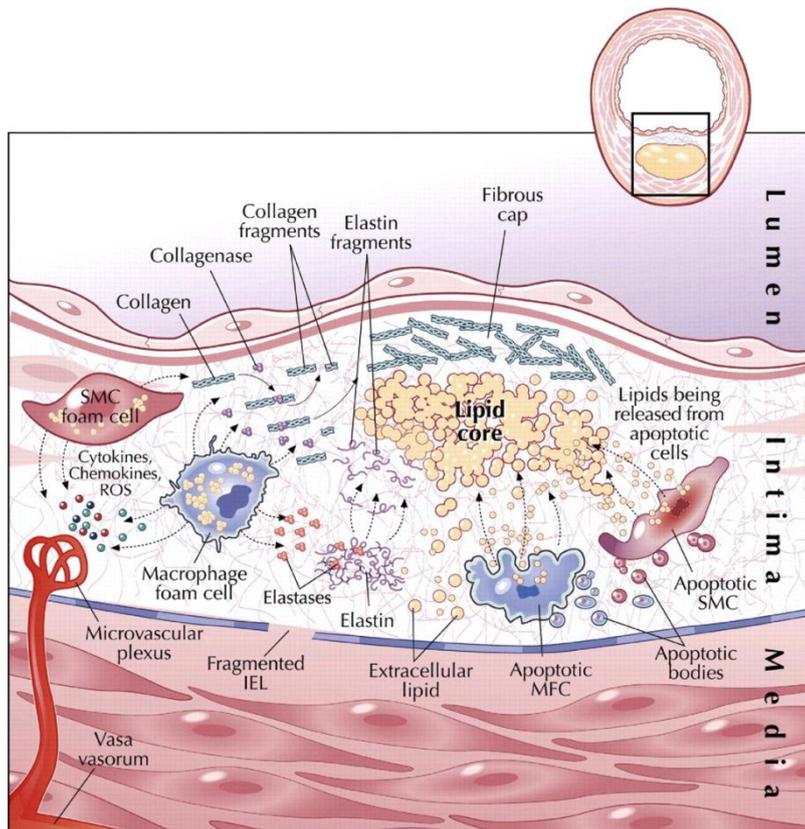


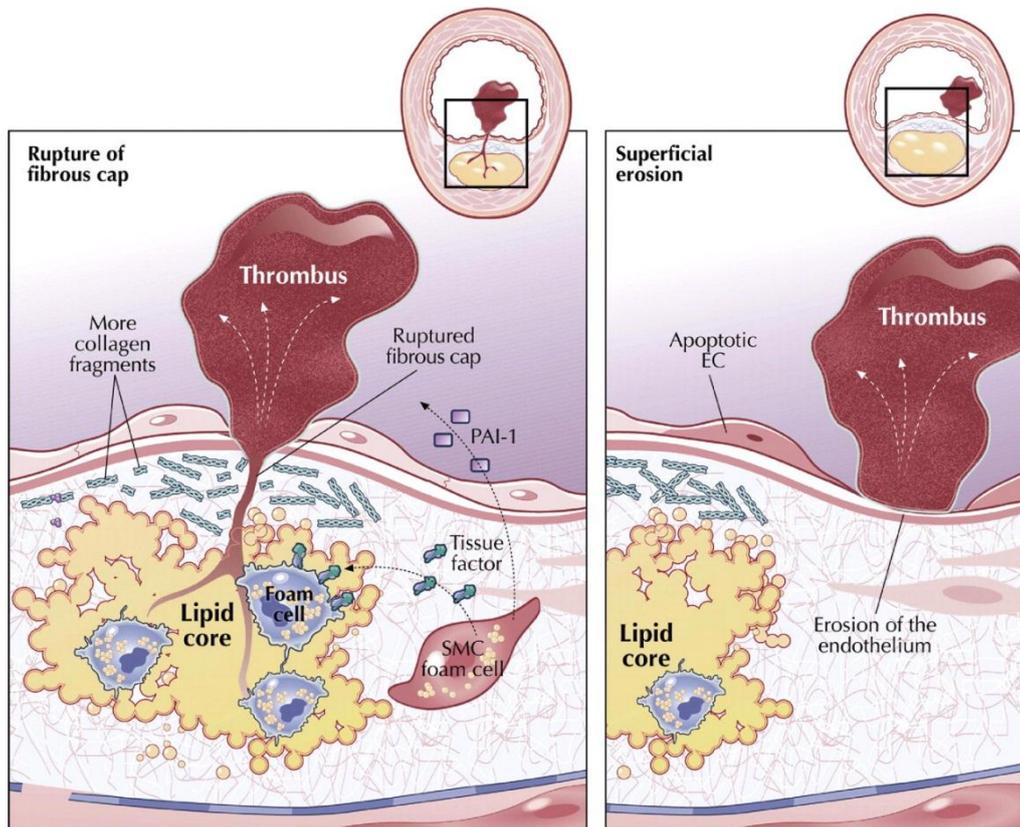
Figure 1.5 Summary of the macroscopic changes in atherosclerosis over time (147)

Over time the atherogenic lesions grow resulting in clinically silent disease manifesting either in a gradual process e.g. stable angina, or more acutely such as a fatal MI as a result of the lipid-rich plaque becoming unstable, fissuring or rupturing, resulting in the overlying artery thrombosing and occluding. Rupture of the plaque can be due to direct physical damage as a result of endovascular procedures, hemodynamic stress due to traditional risk factors e.g. hypertension, use of tobacco products, high blood cholesterol levels, or enzymes released from platelets and leukocytes (Figure 1.6A and 1.6B) (148;149).



**Figure 1.6A Mature atherosclerotic plaque and intralésional components**  
*Reproduced from (149) with permission of Elsevier Ltd*

SMC - smooth muscle cell; HDL - high-density lipoprotein ECM - extracellular matrix, IEL - internal elastic lamina; MFC - macrophage foam cell, ROS - reactive oxygen species



**Figure 1.6B Thrombotic complications of atherosclerosis**

*Reproduced from (149) with permission of Elsevier Ltd*

EC - endothelial cell; PAI-1 - plasminogen activator inhibitor-1

Despite the established knowledge of the pathophysiology underlying atherosclerotic vascular disease, the challenge of identifying asymptomatic individuals at moderate or higher risk of short-term future CVD events remains elusive. Numerous algorithms exist incorporating multiple risk factors in an attempt to improve risk prediction. Furthermore, despite the aetiological relevance of traditional risk factors in atherosclerosis, these CVD risk factors have a varied performance in predicting which asymptomatic subjects will go on to develop CVD (150;151). For example, one study showed the poor screening performance of blood pressure is reflected by the fact that individuals in the top 10% of the distribution of systolic blood pressure experienced only 21% of all IHD events and 28% of all strokes at a given age (150). Using several cardiovascular risk factors in addition to blood pressure did not improve the screening performance of blood pressure. Among persons in a specified age group, the 5% at highest risk experienced 17% of all heart disease deaths with risk computation

based on blood pressure alone, 22% when based on blood pressure and cholesterol in combination, and only 28% using blood pressure, cholesterol, smoking and three other cardiovascular risk factors all in combination. Newer algorithms incorporating novel factors such as social deprivation and glycated haemoglobin A1c (HbA1c) continue to be developed with an attempt to better predict future vascular events in individuals (151). Proponents of those in favour of such tools indicate that their under-usage rather than their limitation may be one of the reasons why individuals miss out on primary prevention (151).

### **1.8.2 Carotid intima-media thickness and prediction CVD**

As described, atherosclerosis reflects an interactive process between plasma lipid (predominantly LDL) and the inflammatory cascade, with the process predominantly affecting the intima, and tending to involve the media only once advanced stages are reached. Given that this process can be subclinical for a variable, substantial period of time, one might conceive that screening for asymptomatic vascular disease, particularly in high risk groups, such as South Asians would be beneficial.

The general consensus historically is that noninvasive screening is only beneficial in patients without existing atherosclerotic disease who are classed as intermediate risk for having a primary cardiovascular event (e.g. one major risk factor, FRS 6-20%, family history of first degree relative with premature CVD) (152;153). This has been upheld in the most recent American Heart Association guidelines (154). Although subsequent to this one systematic review suggests the evidence remains inconclusive (155) and even more recently there has been further evidence and opinion suggesting that high risk patients (e.g. those with 3 or more major risk factors) should only get screening if there is a possibility that it down classifies a patient (i.e. reclassifying a patient to a lower-risk category) and as a result a patient is not subjected to potentially unnecessary preventative medical therapy (153;156).

With regard to screening modalities, much discussion has focussed on the role of carotid ultrasound scanning (152). A carotid ultrasound scan (USS) can be utilised

in one, two or three dimensions. One-dimensional (1D) carotid USS by using B-mode ultrasound to quantify the degree of atherosclerosis started in the early 1980s (157). This led to the measurement of IMT (intima-media thickness), with IMT becoming a validated measure in arteries in 1986 (158). The researchers were able to identify a 'double line' image which represented echoes from the intima and adventitial boundaries. The distance between these two lines represented the thickness of the intima and media combined - when the adventitia was removed the outer echogenic line disappeared. They compared arteries with no plaque with those exhibiting plaque, and found that in order to maximise the accuracy and reduce variability in the measurement of carotid IMT (cIMT), IMT should be measured in the far wall of the common carotid artery (either maximum or mean cIMT), in a segment with no plaque. This has remained the approach in many protocols for measurement of cIMT, including prospective studies looking at cardiovascular outcomes in relation to cIMT (159-166), as well as an outcome measure in drug trials e.g. HMG CoA reductase inhibitors (statins) (167).

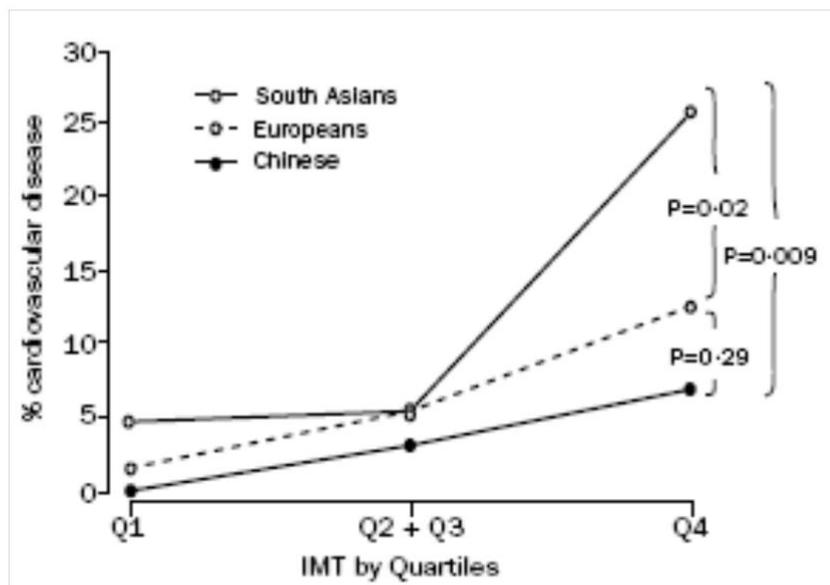
Carotid IMT is recognised as a surrogate risk factor for CHD (159-164) and stroke (161;162;164-166). In a meta-analysis of 8 studies, Lorenz and colleagues calculated an age and sex adjusted RR for MI of 1.26 (95% CI, 1.21-1.30) per 1 standard deviation (SD) difference in baseline cIMT and RR for stroke of 1.32 (95% CI, 1.27-1.38) per 1 SD difference in baseline cIMT (164). Prior to the collation of this evidence, at The American Heart Association Prevention Conference V (2000) it was already suggested that the measurement of cIMT in clinical practice adds incremental information to traditional risk factor assessment in asymptomatic people, particularly those aged >45 years (168), with subsequent statements echoing this belief (152;169). An advantage of measuring cIMT is the availability of semi-automated software to read images and measure cIMT (170). A relatively recent small study (n= 43 women) showed that the use of automated software greatly reduced the time spent measuring segments (mean 57.5s for manual, 2.5s for automated); as well as showing better variation and reproducibility coefficients (171). However a very recent meta-analysis using general population cohort data has questioned the merit of the usage of progression of cIMT in clinical trials (172). During a mean follow-up of 7 years (using 36,984 participants), whilst the mean cIMT of two ultrasounds

2-7 years apart was associated with an increased HR (1.16, 95% CI, 1.10-1.22) for combined CVD endpoints (MI, stroke or vascular death), the outcome for yearly cIMT progression was not increased (HR 0.97, 95% CI, 0.94-1.00) for the same endpoint. As a result the authors stated that no conclusion can be made on the use of cIMT progression as a surrogate in clinical trials.

### **1.8.3 Carotid intima-media thickness in South Asians**

Currently there are no published prospective longitudinal data on cIMT and cardiovascular outcomes on South Asians. Furthermore, there is only one cross-sectional study comparing cIMT between South Asians and Europeans from the same population (173). This study involved 342 South Asians, 326 Chinese and 317 European men and women in Canada consisting of both asymptomatic individuals as well as those with known CVD (prevalence 8.6%, 1.9% and 4.9%, respectively). Overall all three groups were of similar age but South Asians had the greatest prevalence of CVD (history of MI, angina, silent MI, PTCA, CABG, or stroke): South Asians 10.75, Chinese 5.4%, Europeans 2.4%,  $p < 0.001$ . However, when the three ethnic groups were compared for maximum cIMT (asymptomatic or diseased individuals, adjusted for age, sex and recruitment centre) South Asians did not have greater maximum cIMT compared to Europeans (0.72 mm v 0.75 mm,  $p < 0.001$ ), with the European subgroup having highest maximum cIMT out of the 3 ethnic groups.

However, when cardiovascular events were plotted against cIMT quartiles (Q1 lowest cIMT), for combined groups (diseased and disease-free) for each ethnic group, South Asians had an excess of CVD prevalence compared with the other groups (Figure 1.7). For example, those within the highest quartile of cIMT, the prevalence of CVD was 26% among South Asians, 13% among Europeans, and 7% among the Chinese group and the differences were significant ( $p < 0.001$ ), however it was not clear whether these were ethnic-specific quartiles or not. Thus these data may suggest that absolute cIMT values are not a sensitive marker for CVD risk in South Asians, unless ethnic-specific risk-stratified values are proposed.



**Figure 1.7 Prevalence of CVD in relation to cIMT in South Asians**  
*Reproduced from (173), with permission of Elsevier Ltd*

#### 1.8.4 Carotid plaques and prediction of CVD

It is argued that carotid plaques are pathologically more representative of atherosclerosis and that carotid plaque imaging is a potentially more predictive marker of vascular risk than cIMT. This is based on the rationale that carotid plaque and cIMT represent different underlying pathophysiological entities (174). As described earlier, early atherosclerotic plaque is focal, involving the intima, localizing to branches and bends in arteries, probably relating to disturbances of flow. However cIMT also includes (and is largely dominated by) the thickness of the inner layer (media) of the carotid artery.

As per early opinion and subsequent consensus statements, cIMT is measured in areas without plaque (175;176), or when a number of measurements from normal segments of the artery are averaged (with only a couple of slices that unintentionally intersect plaque) - the methodology adopted in most of the studies included in the meta-analysis undertaken by Lorenz and colleagues described earlier (164). Therefore what is measured is something different from

atherosclerosis, if atherosclerosis is defined as plaque. Furthermore, whilst atherosclerosis is chiefly an intimal process, it has been highlighted that IMT is approximately 80% media, and only 20% intima, therefore any measurements made, and any changes in cIMT are likely to be a reflection of changes in the media (177); thus a key determinant of cIMT is the effect of blood pressure which is known to cause medial wall hypertrophy (178). The researchers found that cIMT was only weakly associated with coronary artery disease; and others have found that the association of cIMT with coronary atherosclerosis is less strong than its association with left ventricular mass and less strong than the association between carotid plaque and coronary atherosclerosis (179;180).

Carotid plaque disease can be recorded as plaque presence/absence, or by a scoring system, counting and grouping the number of carotid plaques present, or by subjectively measuring two dimensional plaque area. Evidence from prospective studies is compelling for carotid plaque and cardiovascular risk prediction. Two-dimensional (2D) ultrasound detectable carotid plaque is currently defined as a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value, or demonstrating a thickness >1.5mm as measured from media-adventitia interface to intima-lumen interface (176).

The majority of existing evidence suggests that at worst, presence of carotid plaque is non-inferior to cIMT for predicting future CVD events, with the presence of large plaques being a stronger predictor for future CVD events. For example, data from as early as 1991, from the Kuopio Ischaemic Heart Disease Risk Factor Study (181) found plaque presence to be more strongly predictive than carotid IMT of future cardiovascular events. This prospective longitudinal study involved 1,288 men asymptomatic from ischaemic heart disease followed up for up to 2.5 years. Data linking the association between acute coronary events and both cIMT and carotid plaques showed that the presence of mean intimal-medial thickening (cIMT >1 mm) was associated with a 2.17-fold (95% CI, 0.70-6.74) HR, small carotid plaques with a 4.15-fold (95% CI, 1.51-11.47) HR, and large ("stenotic") plaques with a 6.71-fold (95% CI, 1.33-33.91) HR for MI. Data from a study of 10,000 low risk, healthy individuals in Italy supports this (182). Table 1.4 provides published data comparing cIMT and 2D carotid plaque

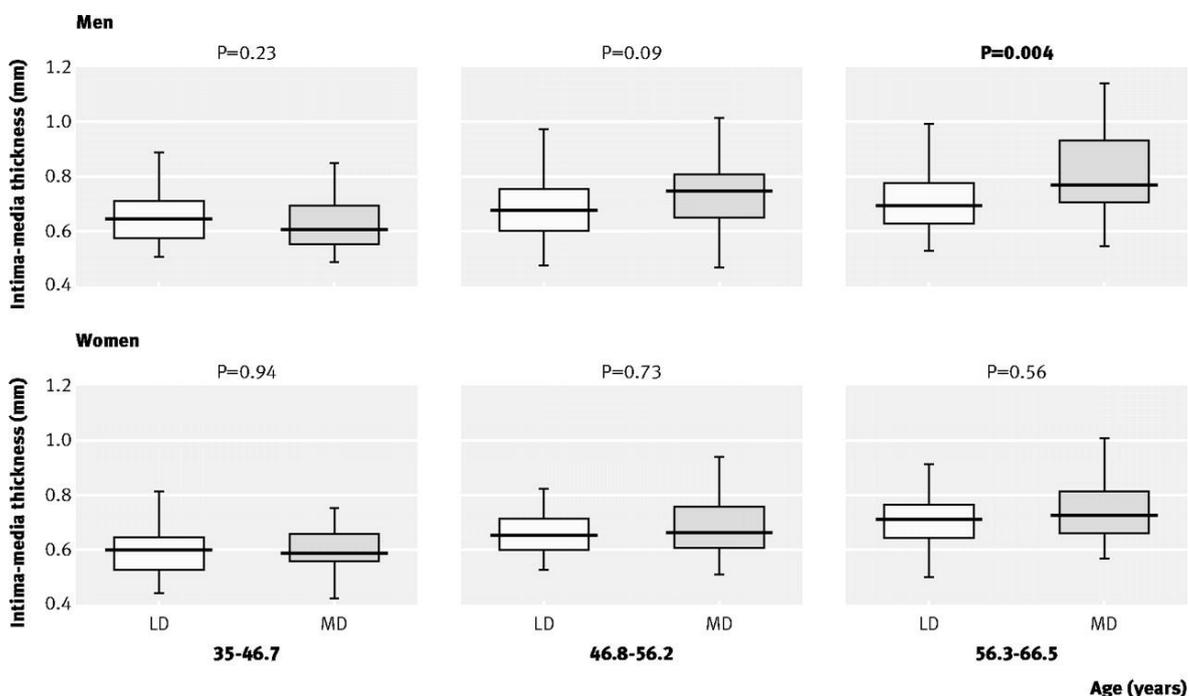
imaging and strength of prediction of cardiovascular outcomes from prospective longitudinal studies of subjects asymptomatic of CVD.

**Table 1.4 Summary of studies comparing cIMT to 2D carotid plaque imaging in relation to CVD outcomes.**

Study	Population size, breakdown and age (years)	Follow-up	Primary outcome	Risk associated with cIMT thickening	Risk associated with carotid plaque (CP)
KIHD <sup>(181)</sup>	1,288 men aged 42-60	1 month - 2.5 years	AMI	2.17-fold (95% CI, 0.70-6.74; p>0.05) HR of AMI per mm increase in cIMT	small carotid plaques 4.15-fold (95% CI, 1.51-11.47; p<0.01); large ("stenotic") plaques 6.71-fold (95% CI, 1.33-33.91; p<0.01) risk of AMI
CAFES-CAVE <sup>(182)</sup>	10,000 subjects (6055 men) aged 35-65	10 years	'Cardiovascular event'*	Event incidence 8.6% in subjects with >1mm thickness in cIMT	Event incidence 39.38% for nonstenosing plaques, 81.06% for stenosing plaques
Tromso <sup>(183)</sup>	6,226 (3208 women) aged 55-74	mean - 5.4 years median - 5.8 years	MI	Adjusted RR for MI 1.73 in men, 2.86 in women for highest v lowest quartile	Adjusted RR for MI 1.56 in men, 3.95 in women for highest tertile compared to no plaque disease
Rotterdam MI <sup>(184)</sup>	6,389 subjects (61.9% female) mean age 69.3 (SD 9.2)	7-10 years	MI	HR 1.20 per SD increase in IMT thickness; 1.95 for 'severe'** cIMT thickening	HR 1.83 for 'severe' carotid plaque disease compared to no plaque disease
Rotterdam CVA <sup>(185)</sup>	6,913 subjects (60.3% female) mean age 69.5 (SD 9.2)	Mean 6.2 years	Stroke	Stroke RR 2.23 for highest tertile vs lowest tertile	Adjusted RR for stroke rr 1.55 for highest tertile, v lowest tertile

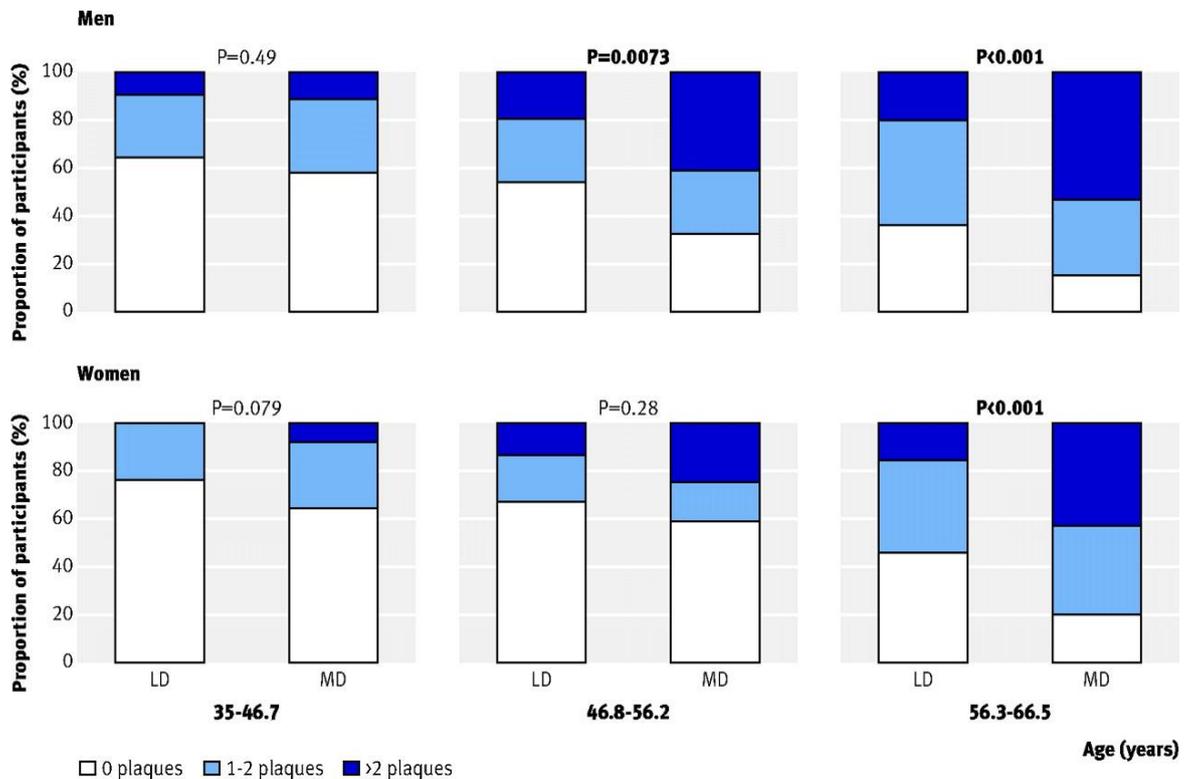
AMI - Acute myocardial infarction; mm - millimetre; \*Defined as the development of cardiovascular signs and symptoms and/or complications related to arteriosclerosis requiring hospital admission and treatment (occurrence of ECG-documented angina, non-fatal, acute MI and coronary death, and requirement for revascularisation). Silent MI noted by ECGs obtained at annual follow-up examinations were not counted as clinical events. \*\*cIMT >1.12 mm

In data from a recent local study (PsoBID study) (186), age and sex adjusted cIMT was significantly higher in participants from the most deprived (MD) areas than in those from the least deprived (LD) areas (0.70 mm (SD 0.16 mm) v 0.68 mm (SD 0.12 mm);  $p=0.015$ ). On subgroup analysis, however, this difference was only apparent in the highest age tertile in men (56.3-66.5 years) (Figure 1.8). However, the difference in unadjusted mean plaque score between participants from the most deprived and those from the least deprived areas was more striking than the difference in intima-media thickness (least deprived 1.0 (SD 1.5) v most deprived 1.7 (SD 2.0);  $P<0.0001$ ). In addition, a significant difference in plaque score was apparent in the two highest age tertiles in men (46.8-56.2 years and 56.3-66.5 years;  $P=0.0073$  and  $P<0.001$  respectively) i.e ~10 years earlier in the life course than that needed to detect measurable differences in cIMT (Figure 1.9). Neither adjustment for traditional cardiovascular risk factors nor measured ‘novel’ risk factors, either alone or in combination, abolished the area-level deprivation-based difference in plaque presence.



**Figure 1.9** Differences in cIMT between socioeconomic groups stratified by age

*Reproduced from (186) with permission of BMJ Publishing Group Ltd*



**Figure 1.9 Differences in carotid plaque presence between socioeconomic groups stratified by age**

*Reproduced from (186) with permission of BMJ Publishing Group Ltd*

Interestingly, two recent studies have incorporated both cIMT and carotid plaque presence to determine whether they enhance risk factor models for CVD prediction (156;187). The former showed that 23% of subjects would be reclassified by the addition of cIMT and carotid plaque to traditional risk factors (of which 80% receiving a downward reclassification), with plaque having a slightly better reclassification effect than cIMT when added to traditional risk factors (albeit statistically nonsignificant). The latter study which incorporated cIMT and carotid plaque (by virtue of elaborate composite measurements of cIMT) showed very modest improvements in risk reclassification. Finally a recent systematic review (studies up to September 2011) aimed to review the evidence which included studies that evaluated the added value of cIMT and/or carotid plaque scoring in the prediction of developing CVD (188). Once again a similar conclusion was made - when carotid ultrasound screening is undertaken, there is no noninferiority demonstrated for plaque over cIMT.

Finally, relatively novel validated three-dimensional (3D) ultrasound techniques exist and are demonstrated to possibly be more informative than existing 2D cIMT and plaque measures (189), which can detect earlier responses to intervention e.g. statin therapy over a six-month period(190) exist. However published long-term longitudinal data or indeed CVD outcome data based on 3D imaging of carotid arteries is lacking.

Similarly, more descriptive and detailed contemporary imaging modalities of carotid plaque are being used (191). These include radiofrequency assessment of carotid plaque and intravenous ultrasound. The former can provide information on the content of plaque, by virtue of how echo lucent a plaque is and whether echogenicity/lucency is uniform or not (Echo-lucent carotid plaques are lipid-rich and have a greater potential for clinical complications (192;193). Heterogeneous plaques have a hypochoic component and are associated with the presence of intra-plaque haemorrhage, ulceration and lipids, more likely to result in adverse events (194). Further, some data suggest that in hospitalised cardiological patients, carotid plaque presence and morphology assessed by ultrasound are independent predictors of death (195). However, such analysis of plaque requires a trained individual as semi-automated software or simple criteria (e.g. plaque presence and score) are not currently available. Intravenous ultrasound can provide information on plaque type e.g. features indicative of vulnerability and thus more prone to rupture. The obvious drawback of this modality is that it is invasive and thus not an ideal screening option. Though this can be overcome by use of CT angiography, the access to and cost associated with this modality limits its practicality.

### **1.8.5 Carotid plaques in South Asians**

The analyses of cIMT association suggest that left ventricular mass, blood pressure and age, are considered the main predictors of IMT is of major relevance in South Asians. Left ventricular mass is a reflection of high systemic arterial blood pressure (196) and a review of blood pressure levels in South Asians showed no significant difference in blood pressure levels between South Asians (as a combined entity) and Europeans (197). Furthermore, the premature

cardiovascular morbidity and mortality seen in South Asians (9;12;13), may precede cIMT progression to reach sufficient thickness to classify a South Asian as high risk and worthy of primary prevention, i.e. the window of opportunity would already be missed. **In light of all this evidence, it is unsurprising that cIMT has a low sensitivity as a measure of clinically important atherosclerotic plaques or as a predictor for vascular disease and suggests that carotid plaque may be a superior screening tool in South Asians.**

### **1.9 Endothelial function and arterial stiffness**

Endothelial dysfunction and changes in vasomotor tone are recognised preclinical stages in the pathogenesis of atherosclerosis (198). Endothelial function assessment includes measuring the response to an endothelium-dependent, nitric oxide-mediated stimulus such as acetylcholine, or reactive hyperaemia, and a direct (endothelium independent) nitrovasodilator, like sodium nitroprusside or nitroglycerin (199). Endothelial function can also be assessed non-invasively by pulse wave analysis (200). Arterial ‘stiffness’ (sometimes referred to as compliance or ‘distensibility’) has been implicated as a feature of subclinical atherosclerosis (201). Several methods exist for the determination of arterial stiffness these include established as well as newer methods for non-invasively measuring arterial ‘stiffness’ and related intermediate cardiovascular phenotypes, and are listed in the Table 1.5 (201).

**Table 1.5 - Methods for assessing arterial stiffness**

<b>Pulse wave analysis</b>	<b>Direct Assessment</b>	<b>Other Methods</b>
Augmentation index	PWV	Pulse pressure
Large artery compliance	Local compliance measures	AASI
Small artery compliance		
PT/TT ratio		

PT/TT - peak time/total time, PWV - pulse wave velocity, AASI - Ambulatory arterial stiffness index

Several of these methods used to identify asymptomatic cardiovascular phenotypes have been shown to predict cardiovascular risk. One meta-analysis suggested that central augmentation index independently predicts future CV events and all-cause mortality (202). The same meta-analysis also suggested that pulse pressure may also have predictive value. PWV has also been found to be associated with cardiovascular events even after adjustment for cardiovascular risk factors (203). Recently, a non-invasive test for endothelial function has been developed which bases on the principle of reactive hyperaemia assessed by fingertip peripheral arterial tonometry without the need for ultrasound or plethysmographic assessment of brachial artery vasodilation. The Endo-PAT2000 has been validated against conventional reactive hyperaemia-based assessment of endothelial function (204) and has been found to correlate with traditional and metabolic cardiovascular risk factors in the Framingham study (205).

There are some published data on endothelial function and arterial stiffness in South Asians. One other study used flow-mediated dilation to assess responses to insulin in a clamp study in healthy non-diabetic South Asians compared to whites (206). This study showed the percent increase in resting brachial artery diameter from before the clamp to after the clamp, was significantly reduced in the Asian Indians compared with whites. However brachial artery diameter was similar and

the percent changes in brachial artery diameter in response to both reactive hyperaemia and nitroglycerin were not significantly different before the hyperinsulinaemic clamp between the 2 groups. Several small studies have found increased arterial stiffness in young South Asians aged in their 20s to early 30s compared to matched Europeans using flow-mediated brachial artery vasodilation (207), augmentation index (208) and maximum vasodilatory response to the beta-2 agonist isoproterenol (209). Finally, noninvasive measurement of PWV has recently been found to be increased in healthy UK South Asian males compared to their European peers (210).

However the duration and preparation required to undertake assessments of endothelial function and potential invasive component make the use of such modalities are not practical when studying larger groups of subjects particularly when attending for comprehensive phenotyping. Similarly these reasons make these forms of vascular assessment impractical for use as a screening modality.

### **1.10 Summary and thesis aims**

The aim of the preceding literature review was to alert the reader to the seriousness and scale of the impact of cardiometabolic disease in South Asians living in the UK and further afield, and to highlight the research questions that this thesis is designed to aim to answer. Whilst there are emerging data on novel modifiable risk factors associated with cardiometabolic disease such as cardiorespiratory fitness and physical activity, data in studies of South Asians are either small scale (e.g. data relating to cardiorespiratory fitness), limited by quality (e.g. lack of objectively-measured physical activity) or inconclusive (e.g. role of liver fat in metabolic disease). Further, proposed methods for improved screening for CVD in South Asians (e.g. use of cIMT) lack sensitivity and this raises the question of whether alternative methods may be more sensitive in this high risk ethnic group.

The aims of this thesis therefore are to try and address several of these areas which there are few or no published data on them, with the focus on South Asian men as the population to be studied. South Asian men were chosen for several

reasons, though as discussed in this introduction, South Asian women are also at increased risk of cardiometabolic disease. These include - South Asian men being at higher absolute risk of cardiometabolic disease compared to South Asian women; the components of the study design (anthropometric measurements, fitness tests) and the fact that the author and the technician undertaking these components were both male would make South Asian women highly unlikely to participate as cultural and religious sensitivities dictate that for women in particular same gender investigators/care providers are key to participation except in the case of necessity (211); and finally it is recognised that in addition to the previous point, South Asian women are generally a very challenging subpopulation to recruit from (212), thus increasing the risk of under-recruitment and subsequent study underpowering. Thus using a study population of healthy age-matched South Asian and European men, I aimed to address the following areas:

- Present comparative data on cardiorespiratory fitness, objectively-measured physical activity and other metabolic phenotypical data in middle-aged South Asian and European men (Chapter 3).
- To determine the extent to which differences in insulin resistance and glycaemia between these ethnic groups can be explained by differences in the factors mentioned above (Chapter 3).
- To determine whether South Asians have increased liver fat compared to Europeans and the extent to which such differences contribute to the increased insulin resistance observed in South Asian men compared to European men. (Chapter 4)
- Present comparative data on cIMT and carotid plaque presence between South Asian men and European men, whether any differences in carotid plaque presence between South Asian men and European men occur at an earlier age than differences in cIMT and finally to determine what risk factors (if any) could account for any such observed differences in cIMT and/or carotid plaque disease (Chapter 5).

## Chapter 2

### General methods

#### 2.1 Introduction

This chapter describes the methods of subject recruitment and subsequent tests undertaken by the selected participants. The study was named and publicly referred to as the **Carotid Ultrasound and Risk of Vascular disease in Europeans and South Asians (CURVES)** study. Ethical Approval for this study was granted by the West of Scotland Research Ethics Service Regional Ethics Committee 1 (Reference - 09/S0703/118 and was conducted according to the principles expressed in the Declaration of Helsinki. The author was a registered medical practitioner with Advanced Life Support accreditation.

##### 2.1.1 Inclusion and exclusion criteria

Individuals were considered suitable for participation in the study if they met the following inclusion criteria and did not meet any of the exclusion criteria listed below:

##### *Inclusion criteria*

- Male aged 40-70
- South Asian (i.e. both parents from India, Pakistan, Bangladesh or Sri Lanka) or European (i.e. both parents of European origin).
- Currently residing in the UK and registered with a UK general practitioner.

##### *Exclusion criteria*

- Known CHD (symptoms of angina, previously labelled IHD, previous MI, coronary artery bypass grafting, percutaneous revascularisation)
- Previous stroke or TIA
- Known DM (Type 1 or Type 2)

- Musculoskeletal or other disorder (e.g. significant respiratory or circulatory disorder), that prevents exercise testing on treadmill
- Absolute contraindications to magnetic resonance imaging (MRI) i.e. cardiac pacemaker, aneurysm clip, artificial heart valve, ear (cochlear) implant, metal fragments in eyes, head or body or any procedure to remove metal, metallic implant)

If an individual had an underlying medical condition not listed in the exclusion criteria, but could confound results (e.g. ongoing treatment for malignancy), it was at the discretion of the author whether to enrol or exclude the individual in question.

### **2.1.2 Recruitment, screening and selection**

Participants were recruited from the West of Scotland - the majority of participants living in NHS Greater Glasgow and Clyde catchment area with a minority recruited from NHS Lanarkshire, NHS Ayrshire and NHS Lothian.

Participants were recruited via: (i) local advertising using posters displayed in prominent locations throughout the city of Glasgow, word of mouth, and (ii) by writing to potentially eligible participants identified from primary care databases - as agreed with the Scottish Primary Care Research Network, volunteering General Practitioner (GP) surgeries were visited to generate lists of potential participants from computerised data according to standard procedure. The relevant GPs vetted the lists. Practice-headed letters together with the Volunteer Information Leaflet (VIL) (Appendix A1) and an expression-of-interest return form (with prepaid envelope) were sent to suitable individuals. Interested respondents were then pursued for recruitment.

The majority of participants (95 South Asians and 93 Europeans) were recruited by method (i). For recruitment method (ii), 470 potential volunteers (220 South Asians and 250 Europeans) meeting the study inclusion criteria were identified from the databases of four primary care practices, and sent letters informing them of the study and inviting their participation. Fifteen individuals (7 South

Asians, 8 Europeans) replied positively to these invitation letters. Three of these individuals did not respond to further attempts at contact, and thus 12 participants (7 Europeans, 5 South Asians) were recruited to the study by method (ii). During the Muslim holy month of Ramadan (in which Muslims are obliged not to eat from dawn to dusk), no volunteers who were Muslim were recruited to participate or indeed in the 2-3 week period after Ramadan (as many choose to keep 6 additional voluntary fasts in the subsequent month). There were two reasons for this - firstly the nature of what the visit entailed (overnight fast, exercise treadmill test to volitional fatigue) would make participation impractical and importantly inconvenient for participants. Secondly, it has been demonstrated that fasting can influence the metabolic profile in the short-term (213) and such physiological changes would confound the results of this study.

Interested potential participants were communicated with in person, by email or by phone to discuss participation in more detail and confirm eligibility for participation. All interested individuals would receive an electronic or hard copy of the VIL. They were given the opportunity to ask questions and also meet in person if they wanted to discuss things further. Once eligibility was confirmed, participants were given an appointment to attend the British Heart Foundation (BHF) Glasgow Cardiovascular Research Centre (GCRC) for the main study visit.

As all participants were chosen locally, and were required to read the VIL (which was printed in English), the method of recruitment excluded participants who could not understand written English. It was recognised that some older South Asian men may not be able to speak good English, but were considered for inclusion if able to speak Urdu or Hindi, as the author could verbally communicate fluently in these languages

## 2.2 Study protocol

### 2.2.1 Overview of main study visit

Eligible participants attended BHF GCRC at 9am after an overnight fast ( $\geq 10$  hours, allowed to drink water only). Participants were asked to bring trainers and shorts. Informed written consent was obtained in duplicate (last page of VIL, Appendix A1), and participants were given one copy to keep. Consent was obtained by the author, who had undertaken Good Clinical Practice training. If consent was given, a letter to the participant's GP informing the GP of participation was either posted to the GP Practice or given to the subject to hand in to the GP surgery themselves. Figure 2.1 summarises the main visit which usually lasted 3-3.5 hours. All components of the visit up to and including the physical examination were undertaken in the BHF GCRC, with the remaining components undertaken at the purpose built exercise laboratory in the West Medical Building, University of Glasgow. Participants who completed the main visit received feedback on investigations undertaken (refer 2.6) and were offered £20 by the University of Glasgow towards travel/transport costs, but received no other incentive for participation.

A health questionnaire (Appendix A2) and validated dietary intake questionnaire (Appendix A3) were completed (validated in European populations). The health questionnaire enabled comprehensive data to be collected on health history (including concurrent medication being taken), family health history smoking habits and alcohol consumption. Years of full-time education was also recorded as was socio-economic status (SES) and was determined by using the Scottish Index of Multiple Deprivation (SIMD) 2006 score (an area-based measure of deprivation based on residential postcode - <http://www.scotland.gov.uk/Topics/Statistics/SIMD>), and categorised in quintiles with quintile 1 representing the most affluent, and quintile 5 representing the most deprived group. Habitual diet was assessed using a 120-item food-frequency questionnaire (214).

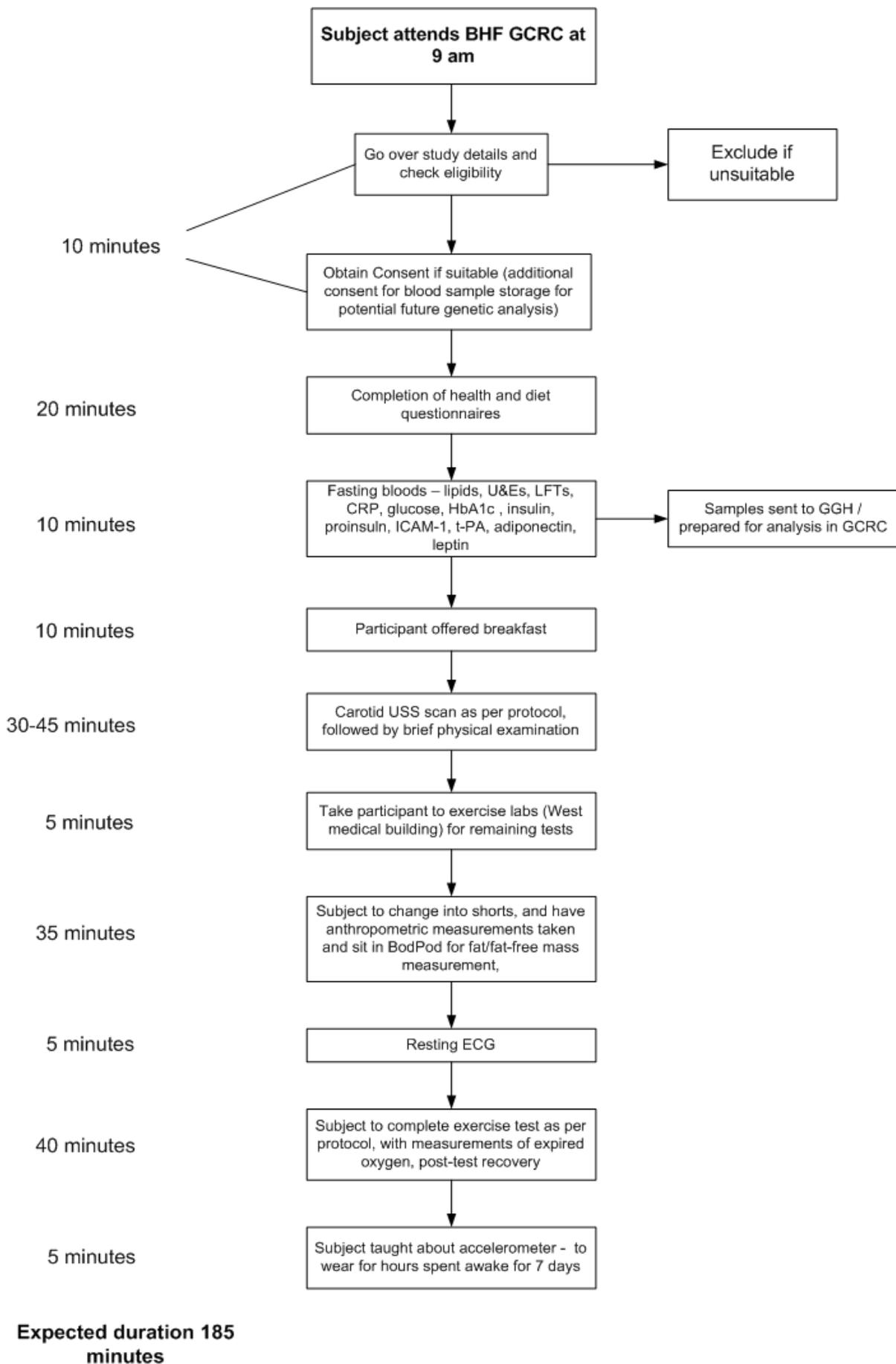


Figure 2.1 Overview of Main visit

## **2.2.2 Blood tests**

### **2.2.2.1 Routine blood tests**

Fasting venous blood samples were taken by the Glasgow Clinical Research Facility (GCRF) nursing and trained support staff by routine venepuncture. Samples were collected for urea, creatinine, electrolytes, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, liver function tests (LFTs) - ALT, aspartate aminotransferase (AST), GGT glucose, HbA1C and CRP were collected. The samples were then analysed as routine samples on the day collected in the NHS Biochemistry Laboratory at Gartnavel General Hospital, Glasgow using standard automatic enzymatic (for glucose, lipids and LFTs), immunoturidimetric (CRP) and HPLC (HbA1c methods). The laboratory participates in the U.K. National External Quality Assessment Service (UKNEQAS) scheme and all tests had inter-assay coefficients of variation (CVs) <5%.

### **2.2.2.2 Non routine blood tests**

Additional venous blood samples were obtained for non-routine blood tests to be analysed in the University of Glasgow Biochemistry Laboratory facility in the BHF GCRC and for storage for future analysis including genetic analysis which all participants consented to. One 4 ml EDTA tube was collected for insulin and C peptide measurement. The sample was spun at 4 degrees Celsius at 3000 RPM for 10 minutes the plasma split into 4 x 0.5 ml aliquots. If consent had been given, one further 9 ml EDTA tube was collected for potential future genetic analysis. The sample was spun at 4 °C at 3000 RPM for 10 minutes and one 0.5 ml aliquot consisting of the aspirated buffy coat was prepared. All sample preparation was undertaken by the GCRF nursing and trained support staff. All samples were stored in the freezers in BHF at -80 °C until the time of analysis. Measurement of insulin, proinsulin and C-peptide were undertaken using commercially available enzyme-linked immunoassays (ELISA) (Merckodia AB, Uppsala, Sweden), in a single batch at the end of the study.

## **2.2.3 Carotid Ultrasound**

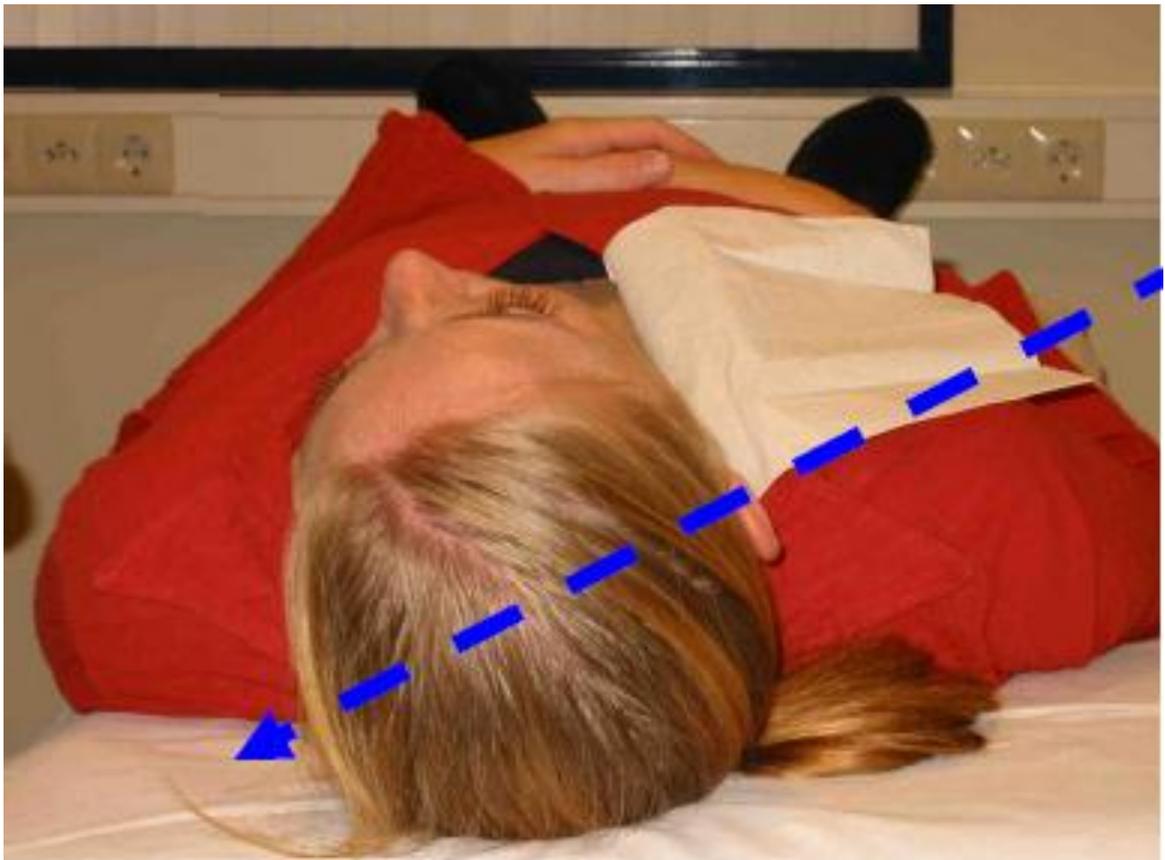
### **2.2.3.1 Training**

Prior to commencing recruitment for the study, a period of training in carotid ultrasound under the tutelage of Dr Kevin Deans (KD), Consultant Chemical Pathology and Metabolic Medicine, Aberdeen Royal Infirmary, UK, was undertaken by the author. KD was trained in cIMT scan analysis by the Department of Vascular Medicine, Academic Medical Centre, Amsterdam who met the American Society of Echocardiography (ASE) standards for USS scanning and cIMT analysis (169). Training was undertaken on a Siemens Acuson Sequoia 512 scanner with an L8 5-12 MHz linear array broadband transducer (Siemens Medical Solutions, Erlangen, Germany), in the GRFCF, in the Western Infirmary, Glasgow. Scans would be saved as a Digital Imaging and Communications in Medicine (DICOM) files to enable offline analysis. An identical model was used in the BHF GCRC for scanning the participants for the study.

### **2.2.3.2 Scanning Protocol**

All participants underwent carotid ultrasound scans in the BHF GCRC, during which both left and right carotid arteries were assessed. All participants were scanned by the author, using the same machine which was used for his training. A protocol for scanning was created (Appendix A4) and followed for all scans. This protocol was adapted from the protocol used in the PsoBid study, which included the same carotid ultrasound outcomes of interest that were measured and analysed (186). In summary, with the subject supine, the right carotid artery was scanned and then the left. B mode still images and dynamic clips were recorded from three sites in the following order: distal 2 cm of the common carotid artery, the carotid bulb, and the proximal internal carotid artery. Finally, an M mode image of wall movement in the distal 1 cm of the common carotid artery was recorded over at least two cardiac cycles to enable potential assessment of carotid artery stiffness. Doppler images of each internal carotid artery were obtained prior to the B mode still images and clips to exclude pre-existing significant stenosis. If velocity was above 1.25m/s, the participant

would not be included in the study, and appropriate clinical advice was sought from the Stroke Team at the Western Infirmary, Glasgow regarding further management. The typical position of the participant's neck is depicted in Figure 2.2. On occasion, to enhance or indeed obtain images, participants had to turn their head more to the contralateral side and thus the 45 degree angle to the horizontal was reduced slightly. The mean IMT of the right common carotid artery (RCCA ) and left common carotid artery (LCCA) was calculated to give the mean cIMT for each participant.



**Figure 2.2 Participant position for carotid USS**

*Image obtained with patient supine, neck turned from midline towards opposite side (i.e. arteries scanned from ear to ear)*

### **2.2.3.3 Carotid intima-media thickness image reproducibility data**

To ensure that images of adequate quality were being obtained, 9 healthy male volunteers aged 21-33 each had two carotid USSs 6-8 days apart. Images were

saved, with KD undertaking the cIMT measurements offline using validated semi-automated software by Siemens - Syngo US Workplace release 3.5, which includes the Arterial Health Package (AHP) (215). For each visit the mean IMT of the right common carotid artery and left common carotid artery was calculated. The software measured the mean cIMT for the 1 cm region immediately proximal to the carotid bulb for each side and the mean of these two values was subsequently calculated.

The difference between the means of each visit was then calculated, and the mean of this difference was calculated to give the mean absolute difference - a marker of quality of image reproducibility. The mean absolute difference for the 9 volunteers was 0.0357mm, falling within the ASE cut-off of 0.055mm - thus meeting the ASE standards of repeatability (169) (Table 2.1).

**Table 2.1 Carotid intima-media thickness image reproducibility**

Volunteer	RCCA1*	LCCA1*	Mean 1*	RCCA2*	LCCA2*	Mean 2*	Difference*
1	0.604	0.691	0.6475	0.55	0.558	0.554	0.0935
2	0.566	0.408	0.487	0.514	0.425	0.4695	0.0175
3	0.631	0.717	0.674	0.649	0.665	0.657	0.017
4	0.406	0.424	0.415	0.375	0.3	0.3375	0.0775
5	0.442	0.552	0.497	0.505	0.496	0.5005	0.0035
6	0.409	0.398	0.4035	0.426	0.431	0.4285	0.025
7	0.588	0.522	0.555	0.503	0.552	0.5275	0.0275
8	0.473	0.465	0.469	0.421	0.469	0.445	0.024
9	0.614	0.397	0.5055	0.488	0.452	0.47	0.0355
<b>MAD</b>							<b>0.0357</b>

*\*All values are in mm*

RCCA - right common carotid artery; LCCA - left common carotid artery; MAD - mean absolute difference

#### **2.2.3.4 Carotid intima-media thickness intra-reader and inter-reader reproducibility**

All scans were read by Greig Logan (GL), a then BSc Honours student at the University of Glasgow. GL was trained by KD in cIMT analysis using the AHP software. To ensure GL met the ASE intra-reader reproducibility measurements, 10 scans from the 18 volunteer scans (9 volunteers each having 2 scans on separate days, as described above) were randomly selected for inter-reproducibility assessment. The cIMT was measured for the LCCA and RCCA for each of the 10 scans and the mean of these two measurements was then taken. The process was repeated 10 times, with the order of subject measurement taken at random (lists generated by the author) to ensure the examiner did not gain familiarization with the scans. Thus a total of 200 measurements were taken (100 LCCA, 100 RCCA), giving 100 means. Each subject's mean absolute difference of IMT had to be  $<0.055\text{mm}$  with a CV of  $<6\%$  in order to comply with the ASE intra-reader reproducibility standards (169) and was met by GL (Appendix A5, Table 1).

For inter-reader reproducibility, the same scans were sent to an external examiner with whom the author was trained by (KD) who read the scans in the same manner as GL as described above. As per ASE standards (169), Inter-reader reproducibility was achieved if the overall means for each of the 10 scans all were had a difference of  $< 0.11\text{mm}$ . Data in Appendix A5, Table 2 confirms this criterion was met.

#### **2.2.3.5 Carotid ultrasound data analysis - cIMT measurements**

Scans were analysed using the AHP software. All scans were analysed by the same reader (GL). GL was blinded to the identities of the participants. All scans were analysed after study recruitment had been completed. The mean cIMT for each participant was calculated from their respective LCCA and RCCA cIMT measurements using the same method described in 2.2.3.3.

#### **2.2.3.6 Carotid ultrasound data analysis - carotid plaque scoring**

As detailed in the scanning protocol, images were burned to CD to enable offline analysis. Carotid plaque score was determined by KD, who was blinded to the identities of the study participants and used the AHP software to view the 6 video corresponding to the respective sites from which images and video clips were taken. All scans were analysed after study recruitment had been completed. A plaque was defined as a 'focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value, or demonstrating a thickness >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface' (176). To adjust for any unreadable video clips, the plaque count was then converted into a plaque score by dividing this value by the number of readable images present and multiplying the outcome by six (the maximum possible number of images per participant) (184).

#### **2.2.4 Blood pressure**

Blood pressure was measured on the left arm after at least 15 minutes of seated rest using an automated blood pressure monitor (Omron HEM705 CP, Omron Healthcare UK Limited, Milton Keynes, UK) which has been validated according to the European Society of Hypertension International Protocol (216). The four blood pressure measurements that were taken during the carotid ultrasound scan were used to calculate the mean systolic and diastolic blood pressure used for analysis in the study. The same sphygmomanometer was used throughout the study.

#### **2.2.5 Physical examination**

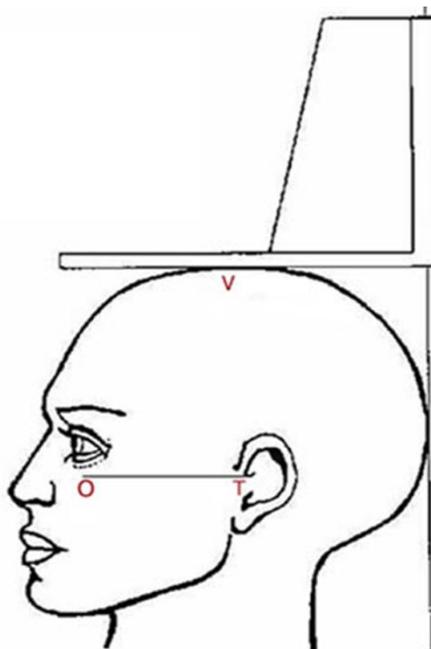
All participants underwent a basic physical examination performed by the author. Examination included assessment of the cardiovascular and respiratory systems and any other relevant system at the author's discretion.

## 2.2.6 Anthropometric measurements

### 2.2.6.1 Height

Participants had their vertical height measured barefoot using a stadiometer (Invicta Plastics Ltd, Leicester, UK). The stretch stature was adopted for all height measurements (217), to negate circadian variation in height (218).

The stretch stature is the maximum distance from the floor to the vertex of the head. The vertex is the highest point on the skull when the head is held in the Frankfort plane. To achieve the Frankfort plane, the head is orientated position in which the head when the orbitale and tragion form an invisible horizontal line with each other and this line is made perpendicular to the long axis of the body (Figure 2.3).



**Figure 2.3 Position of the head in the Frankfort Plane**

*(modified from (217))*

O- Orbitale; T - Tragion; V - Vertex

The method of adopting the stretch stature involved the subject standing with his back, buttocks and heels against a stadiometer, with the participant's feet together and flat on the floor. Either the research fellow or senior exercise laboratory technician would stand in front of the subject and orientate the head

to the Frankfort plane. His hands were far enough along the line of the participants jaw to ensure that upward pressure is transferred through the mastoid processes.

The participant was asked to take and hold a deep breath. Whilst the head was kept in the Frankfort plane, a gentle upward lift through the mastoid processes was applied. At the same time the headboard was brought firmly down on the vertex, flattening the hair as much as possible by an assistant who also ensure that the feet did not come off the ground. Measurement was made at the end of the participant's deep inward breath, recorded to the nearest 0.001 m.

#### **2.2.6.2 Body mass**

Body mass was measured with the participant wearing light clothing (e.g. shorts and t-shirt), minimal jewellery and their footwear removed. Body mass was measured using a manual balance weighing scale (Avery, Birmingham UK), with the same scale used for all participants in the study. Participants would stand with their feet flat on the balance in the anatomical position. Mass was measured to the nearest 0.05 Kg.

#### **2.2.6.3 Skinfold thickness**

Skinfold measurements for all participants were performed by the same trained individual (John Wilson, JW). The between-day CV for all skinfold measurements was <5%. Measurements were done in private. Measurements were made using Harpenden skinfold callipers (Cranlea & Company, Birmingham UK). The between-day CV for all skinfold measurements was <5%. The same callipers were used on all participants, and measurements were made to the nearest 0.1 mm. All measurements were measured on the participant's right side, and skinfold measurements were taken from the following sites:

### *Upper Body*

- Biceps
- Triceps
- Subscapular
- Iliac Crest
- Supraspinale

### *Lower Body*

- Front Thigh
- Medial Calf

All measurements were undertaken with the participant standing up with the arms hanging loosely by his side unless stated otherwise. The landmarks for each skinfold (including nomenclature) and position for measurement are described below and based on international guidelines (217).

**Biceps:** The elbow was flexed to 90° and the midpoint between the most lateral point of the acromion and inferior border of the olecranon was marked. The arm was then left to hang loosely with the arm supinated. The measurement was made in the midline of the biceps muscle at the level of the mark.

**Triceps:** This was measured on the posterior aspect of the arm in the midline at the same level of the biceps skinfold measurement.

**Subscapular:** The subscapular was first identified (the undermost tip of the inferior angle of the scapula). To facilitate the identification of the inferior edge of the scapula, the participant would slowly reach behind his back with his right arm. If the inferior angle was palpated with the arm moved, then the inferior angle would remain palpated as the hand returned back to the resting position. The skinfold was measured 2 cm at a 45° angle inferiolaterally from the subscapular.

**Iliac crest:** The participant folded his right arm across his chest AND the skinfold was measured above the iliocristale with the callipers held over a diagonal fold beginning at the anterior axillary line.

**Supraspinale:** A straight line from the anterior axillary border to the undermost part of the tip of the anterior superior iliac spine was marked. The participant then folded his right arm across his chest to allow a horizontal line to be marked from the most lateral aspect of the iliac tubercle on the iliac crest (iliocristale) towards the front of the abdomen. The point where these two lines intersected was the site of skinfold measurement with the participant's right arm hanging by his side and the callipers held at 45° to the vertical.

**Medial Calf:** The participant stood with his right foot on an anthropometric box. The skinfold was measured on the medial aspect of the calf at the level of maximum girth which was prior determined when measuring the maximum calf circumference (see later). The most medial point was located by the technician viewing the site from the front of the participant. The skinfold was measured by holding the callipers held vertically.

**Front thigh:** The participant was seated with his back upright and the knee is flexed to 90 degrees. The skinfold was measured parallel to the long axis of the thigh and marked at the midpoint between the middle of the inguinal fold (crease at the angle of the trunk and thigh) and the superior margin of the anterior surface of the patella (determined while the knee was flexed). The measurement was made in the midline of the thigh at level of the mark. On occasion if the skin of the thigh was taught, the author would hold and lift the skin a few centimetres either side of the marked point to facilitate the use of the callipers.

All skinfolds were held between the technician's thumb and index finger. The callipers were applied and the measurement was made after pressure had been applied for between two and five seconds. For all sites measured, two measurements were taken, and the mean of the two measurements was to be used for analysis. If the second measurement was not within 5% of the first measurement, a third measurement was taken. If the third measurement was

within 5% of either the first or second measurement, then the mean of the closest two measurements would be used for analysis; if this third measurement did not fall within 5% of either of the previous two measurements, then the mean of all three measurements would be used for analysis.

#### **2.2.6.4 Body circumferences**

Body circumferences were measured at five sites - mid upper arm, waist, hips, mid-thigh and maximal calf. Measurements were done in private with the participant wearing shorts only. A non-elastic tape was used and all measurements were performed by the same technician throughout the study. All measurements were undertaken with the participant standing up with the arms hanging loosely by his side unless stated otherwise. Limb girths were measured on the participant's right side. The landmarks for each skinfold and position for measurement are described below and based on international guidelines (217). All measurements were done in duplicate, and the mean was used for analysis. All measurements were taken horizontally the tape was parallel to the floor. The waist hip ratio was calculated by dividing the mean waist circumference by the mean hip circumference.

**Mid-upper arm (arm relaxed):** The girth of the arm was measured at the level of the mark used for the biceps skinfold.

**Waist:** The participant would stand in with his feet close together and his arms folded in front of his chest and hands place on the contralateral shoulder. The technician kneeled in front of the participant and the circumference was circumference was measured at the level of the narrowest point between the superior border of the iliac crest and the inferior costal margin (10<sup>th</sup> rib) at the end of a normal expiration. If there was no obvious narrowing, measurement was made at the midpoint between the above bony landmarks.

**Hips:** The participant kept his hands in the same position as for the waist measurement, and kept his feet close together. The technician kneeled by the right side of the participant. The circumference was measured at the greatest

posterior protuberance of the buttocks, at approximately the level of the pubic symphysis.

**Mid-thigh:** The participant kept his hands in the same position as for the waist measurement with his feet separated. This circumference was measured at the midpoint between the most superior point of the greater trochanter (not the most lateral) and the upper most part on the lateral border of the head of the tibia.

**Calf:** The participant stood on the anthropometric box with the technician kneeling by the right side of the participant. The maximum girth was determined by wrapping the tape round the participant's calf and measuring the girth at what appeared to be the area of maximum girth. The tape was moved up and down slightly in a series of up and down measurements to determine the position of maximum girth.

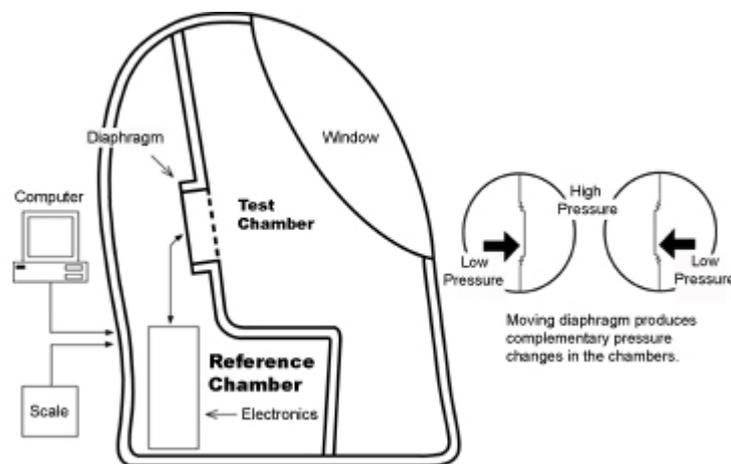
## **2.2.7 Body fat measurements using air displacement ptythesmography**

### **2.2.7.1 Principles and technique**

Total body fat content (expressed as mass in kg and percentage of total body mass) and lean body mass (expressed as mass in kg and percentage of total body mass) were measured using air displacement ptythesmography (ADP). ADP was performed using the BOD POD body composition system (Life Measurement Instruments, California, USA) according to the manufacturer's instructions and recommendations. This system comprises of the BOD POD chamber, digital Scale (accommodating body mass measurements up to 250 kg), Calibration Standards and Specially configured computer system and Windows®-based BOD POD software (219).

ADP for all participants was measured by the author. Participants would wear shorts only and be asked to remove all jewellery (including wedding bands/rings). All participants would then be asked to wear a swimming cap to trap any air pockets present in the hair (regardless of degree of scalp hair)

(220). As per product software instruction, the system would be calibrated for mass and volume measurements. This involved a volume calibration with and without a 50 litre metal cylinder. Mass calibration was done using a 20 kg weight. Required participants data was then entered onto the computer, including gender, age and height (using the height obtained from the anthropometric measurements). The participant then entered the BOD POD and sat still inside the anterior (test) chamber (450 litres), with their hands resting on their lap. The test chamber was connected to a rear measuring chamber (300 litres) via oscillating diaphragms (used to induce pressure changes in the anterior chamber (Figure 2.4). The participant would be instructed to breathe normally. The recommended procedure, consisting of two measurements of body volume (50 seconds each), was adopted and when, occasionally, body volumes differed in excess of 150ml, the system required that a third measurement be performed (221). The final result reported by the BOD POD instrumentation was the mean of the two (or the two closest) measurements. Air conditioning was switched off for the duration of the calibration and measurement process to avoid potential changes in air pressure.



**Figure 2.4 Body volume measurement using BOD POD (219)**

**Calculations of body volume:** The instrumentation measures the volume of air in the anterior chamber, using pressure changes induced by the oscillating diaphragm according to Boyle's laws (inverse relationship between pressure and volume at constant pressure and temperature (222)), and provides raw body

volume (raw BV; litres) for each participant, simply by calculating the difference between the volumes of air in this chamber, with and without the participant being present. Raw BV measurements are adversely influenced by adiabatic conditions created by the participant's presence (this warmer air, approximately 37°C, is more compressible than the ambient air), therefore the manufacturer's software applies certain corrections to the thoracic gas volume (TGV; litres) and the air in close proximity to the skin (using the surface area artefact, SAA; litres) to adjust to isothermal conditions. All calculations were performed automatically by the system software. Actual body volume (ABV) was calculated using Equation 2.1 (220;221). TGV (Equation 2.2) was predicted from functional residual capacity (FRC; litres) and tidal volume (TV; litres) (223). The system predicts FRC using a series of equations (224) with TV assumed to be constant at 1.2 litres for men (age range 15-91 years). Calculation of SAA has been described in detail by Dewit and Colleagues (220).

$$\text{ABV (litres)} = \text{raw BV} + 0.4\text{TGV} - \text{SAA}.$$

**Equation 2.1 Actual body volume (ABV)**

$$\text{TGV} = \text{FRC} + 0.5\text{TV}$$

**Equation 2.2 Thoracic gas volume (TGV)**

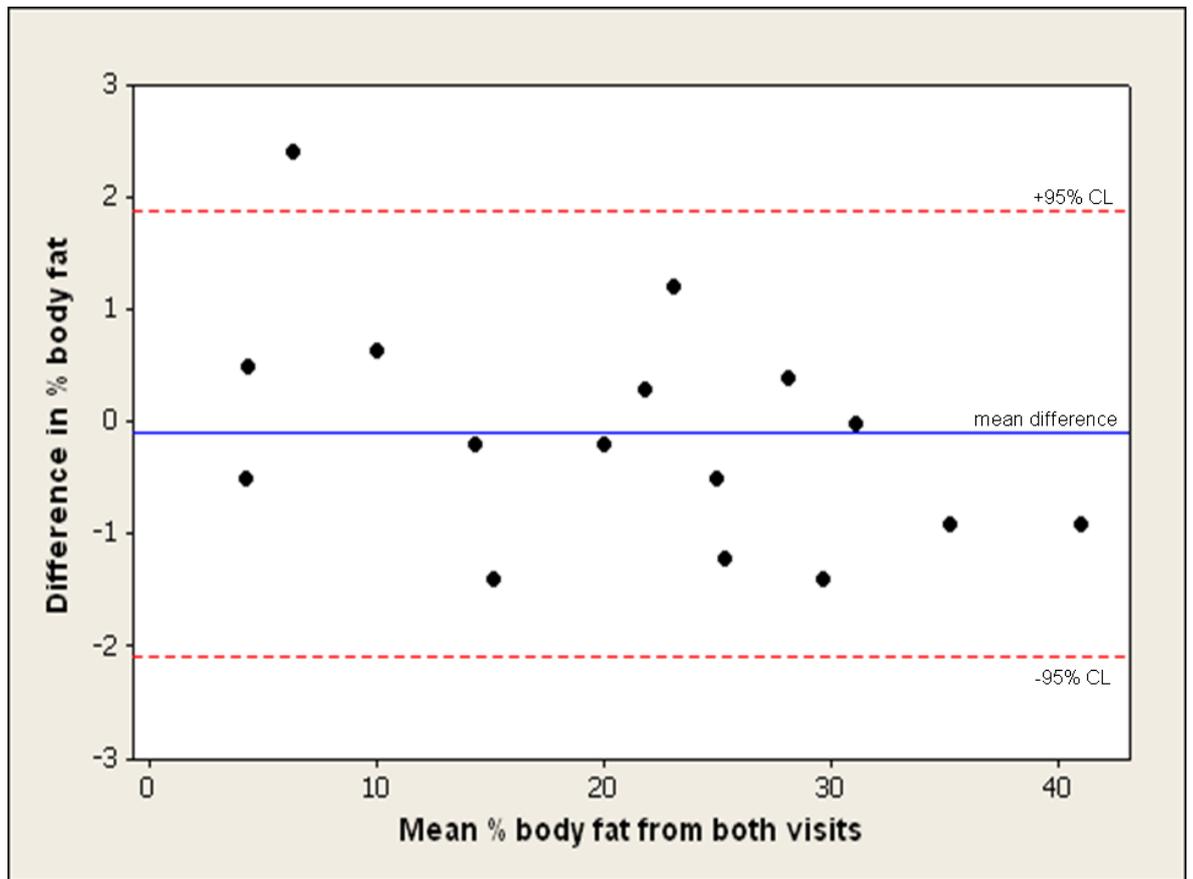
Body density was calculated as body mass/volume (kg/litre). Percentage body fat was calculated according to Equation 2.3 (225). Fat mass was calculated from percentage body fat and body mass

$$\% \text{ body fat} = (495 / \text{body density}) - 450$$

**Equation 2.3 Percentage body fat**

### 2.2.7.2 Reproducibility Data

To validate the BOD POD for reliability, 16 volunteers (2 South Asian, 1 East African and 13 European) each underwent body composition measurements by the BOD POD on two separate occasions 6-8 days apart. A Bland-Altman plot was created, by assigning the mean percentage body fat measurement for each participant as the x-axis value, and the difference between the two measurements as the ordinate (y-axis) value (Figure 2.5). 95% confidence limits were created using 1.96 standard deviations (SDs) either side of the mean difference in % body fat between visit 2 and visit 1 for the 16 participants. In addition, the within-participant CV i.e. the difference between measurements, or difference in test-retest values for the same participant expressed as a percentage was calculated as an index of reproducibility using online software (226). The CV for this sample was 8.2% i.e. a participant who had a body fat value of 30% would expect to have a retest body fat value between 27.5% and 32.5%. The within-participant SD (calculated as the SD of the difference between the 2 visits divided by  $\sqrt{2}$ ) was calculated prior to calculating the within-participant CV (227). This test-retest reliability being similar to previously published data for the BOD POD (228).



**Figure 2.5 Bland-Altman plot for reproducibility of BOD POD**

*Difference is visit 2- visit 1. CL- confidence limits*

## 2.2.8 Exercise testing

### 2.2.8.1 Protocols and selection

Hybrid continuous incremental uphill walking protocols based on the Balke (229) and Taylor (230) protocols were created to be used in the study. A total of 9 protocols were created. All protocols had a starting speed of 4.5, 5.0, or 5.5  $\text{Km.h}^{-1}$  respectively and the gradient would increment by a fixed value (2, 2.5 or 3%), starting at 0% at 2 minute intervals. Additional increments in work-rate (increments resulting in similar increments in predicted oxygen uptake,  $\text{VO}_{2\text{pred}}$ ) were calculated to be used once the maximum gradient had been exceeded (25%) in each of the protocols by increasing the speed of the treadmill. The appropriate increase in walking speed was determined using Equation 2.5 (231).  $\text{VO}_{2\text{pred}}$  determined by adding  $\text{VO}_{2\text{pred}}$  for the previous stage to the incremental

change in  $VO_{2pred}$  ( $\Delta VO_{2pred}$ ), and speeds converted from  $Km.h^{-1}$  to  $m.min^{-1}$  (Appendix A6). Appendix A7 details the 9 protocols used.

$$VO_{2pred} = 3.5 + 0.1 \times \text{speed (m.min}^{-1}) + (\text{gradient \%} \times \text{speed (m.min}^{-1}) \times 1.8)$$

### Equation 2.5

The ideal protocol is one that would result in the exercise test duration to exceed 8 minutes, but not substantially exceed 12 minutes (232). The appropriate protocol would be determined by JW for all participants, who would enquire from the participant directly about the frequency, duration, intensity and nature of regular physical activity undertaken. JW is experienced in this regard; having been in charge of a number of previous exercise test-based studies at the University of Glasgow.

The rationale behind using variations of the Balke protocol was to allow for a more gradual warm-up, with smaller increments compared to the standard Bruce protocol (233). It was considered safer and easier for unfit and older participants and also for participants not accustomed to running, given the upper age limit of the study group, with older South Asians even more likely to be unaccustomed to undertaking regular physical activity (118). Finally, by having a choice of nine different protocols, it would enable each participant to have a tailored exercise test.

The main three disadvantages of this type of protocol were: 1) the time factor for fit individuals - being an easier test, it takes much longer for fitter individuals to reach a maximum effort; and 2) with this type of test incrementing by gradient initially, it can be technically challenging for individuals who have reached the maximum gradient of the treadmill to start running at such a gradient; and 3) a participant may lose motivation to exercise to maximal effort if the test is taking too long. If factor (1) was an issue, the option to attend for an incremental running-based treadmill test was offered, using a protocol that JW has devised and used in previous studies undertaken in the exercise laboratory. If factor (2) was an issue, the participant could re-

attend for a repeat test using the same protocol or selecting a more appropriate protocol from the remaining 8 options. Factor (3) was addressed by having music and verbal engagement with the participant during the exercise test including encouragement when volitional fatigue was being reached (as described in section 2.2.8.2).

#### **2.2.8.2 Preparation and initiation**

In preparation for the test, the technician would go through the exercise test in detail with the participant. This included 1) technique of optimal walking style on the treadmill (with demonstration, and an opportunity for familiarisation on the treadmill including walking at the initial test speed); 2) safety issues, including how to terminate the test in the case of an emergency or unexpected reason to stop; 3) an explanation of how expired air would be measured (expired air from the mouth) and reassurance that despite the nose being clipped-off, the participant would not be deprived of room air; 4) explanation of the equipment being worn (headset to hold mouth piece for tubing to allow expired air collection and nose clips; and 5) how the participant should communicate with the team during the test using gesticulations as verbal communication would not be possible (3 gestures were used - one to indicate that the participant was progressing satisfactorily, the second to indicate that the participant was starting to tire, and the final gesture to indicate that the participant could only perform for a further minute or so).

JW oversaw all the exercise tests for the study and the same treadmill (Woodway PPS 5Med-1, Woodway GmbH, Weil am Rhein, Germany) was used for all the exercise tests. The author was also present for all the exercise tests and would be responsible for treadmill operation as well as ECG and heart rate monitoring (as described in section 2.2.8.3). On occasion a third person would form part of the team conducting the test and would assume responsibility for treadmill operation in such an instance.

The participant would wear the necessary equipment - headset (which held the mouth piece), nose clips (to ensure that all inspired and expired air would be

orally), ECG pads/leads and heart rate monitor ( as described in section 2.2.8.3). Prior to commencing the test, the participant would be given the opportunity to walk wearing the headset and nose clips to confirm comfort and for familiarisation. Once the participant was satisfied the test would commence after the participant had reached the initial walking speed for the test. Each team member started a stopwatch in synchronisation when the test started.

### **2.2.8.3 ECG, heart rate and participant monitoring**

Continuous ECG monitoring was performed for the duration of the test, using multiple lead monitoring (234). 12-lead ECGs were recorded at every minute throughout the test and be reviewed in real time by the author. A resting test prior test commencement was also performed and reviewed by the author to exclude any underlying pathology he deemed unsafe for the participant to undertake the exercise test. Heart rate was monitored continuously throughout the test using a short-range telemetry system. This consisted of a chest wall sensor with built-in transmitter and a watch that would receive the signal and display the heart rate (Polar s610i Heart Rate Monitor, Polar electro Oy, Kempele, Finland). The heart rate was recorded 45 seconds into every minute of the test by the treadmill operator. If the signal was lost from this monitor, the heart rate being recorded on the ECG machine would be used instead.

### **2.2.8.4 Termination of exercise test and recovery**

The exercise test would terminate once the participant had given a maximum effort, which usually coincided with the participant completing the last minute of expired air collection. The participant would subsequently straddle the running surface of the treadmill to enable the operator to reduce the speed and gradient of the treadmill to allow the participant to go back on the running surface of the treadmill and actively recover from the test. If the participant was unable to complete this last minute, they would straddle the treadmill when they wanted to terminate the test. The participant would be given verbal encouragement throughout the test, to minimise premature indication of 'one

minute left' gesture. The test could also be terminated by the participant (by straddling the treadmill) even if maximal effort was not achieved, but was experiencing chest pain, troublesome breathlessness, or pain or discomfort elsewhere that was affecting the participant's ability to continue on the treadmill.

Premature termination of the test could also occur at the request of the author if the participant indicated any of the following ECG changes were identified: 1) horizontal or downsloping ST depression or ST elevation in excess of 0.2 mV (test terminated before this in the presence of concomitant chest pain, typical of angina, associated with horizontal or downsloping ST depression >0.1 millivolts); 3) sustained ventricular tachycardia or frequent runs of non-sustained ventricular tachycardia (235). The participant would be asked to straddle the treadmill if premature termination was indicated.

The stopwatches would be stopped as soon as the participant had straddled the treadmill and the time displayed would be considered as the duration of the test for that participant (to the nearest second). The time recorded by the author would be used. If it was felt that the author's time was inaccurate the time recorded by the technician was used as a back-up.

After the speed and gradient of the treadmill had been reduced, the participant returned back onto the running/walking surface of the treadmill, the participant would walk at a reduced pace on the flat until the heart rate had fallen to around 120/beats per minute and the participant was no longer feeling exhausted.

#### **2.2.8.5 Expired air collection and analysis**

JW would start the collection of expired air when it was felt that the participant was reaching maximal effort during the exercise test. JW was experienced in recognising when a participant would be approaching maximal effort, paying particular attention to pattern of respiration and heart. Expired air was collected in 1 minute intervals until termination of the exercise test in Douglas

Bags, using the standard technique (236). This would enable minute by minute values of oxygen uptake ( $VO_2$ ), and carbon dioxide production ( $VCO_2$ ) to be calculated.

Expired air analysis for each bag of collected air was two-stage:

**Stage 1:** A flow meter extracted 500 ml of air through the sampling port of the Douglas bag at a constant flow rate. This air passed through a gas analyser (Servomex 4000 series, Servomex Group Ltd, East Sussex, UK) which measured the percentage fraction of expired oxygen ( $FEO_2$ ) and the percentage fraction of expired carbon dioxide ( $FECO_2$ ).

**Stage 2:** The remaining air was extracted by a vacuum at a constant flow rate through a dry gas meter (Harvard Apparatus Ltd, Kent, UK), which measured the volume of expired air. The dry gas meter's thermometer measured the temperature of the expired air.

Before each exercise test, the gas analyzer was calibrated using known reference gases (BOC Gases, BOC Ltd, Surrey, UK) and barometric pressure was recorded from the barometer in the exercise laboratory. All the gas measurements were corrected to standard room temperature and pressure (STPD) for a dry gas. Values of  $VO_2$  and  $VCO_2$  were calculated from expired air using the Haldane transformation of the Fick equation as shown in equations 2.6-2.8) (237).

$$VI = VE_{STPD} \times (100 - FEO_2 - FECO_2) / 79.04$$

#### Equation 2.6 Volume of Inspired air

VI - volume of inspired air;  $VE_{STPD}$  - volume of expired air corrected to standard temperature and pressure.

$$VO_2 \text{ (l/min)} = VI \times 0.2093 - (VE_{STPD} \times FEO_2 / 100)$$

#### Equation 2.7 Oxygen consumption

$$VCO_2 \text{ (l/min)} = (VE_{STPD} \times FEO_2 / 100) - VI \times 0.0003$$

### Equation 2.8 Carbon dioxide production

Achievement of  $VO_{2max}$  was assessed by confirming a minimum of two of the three following criteria: (i) respiratory exchange ratio (RER)  $\geq 1.15$ ; (ii) achievement of heart rate  $\geq 90\%$  of age predicted maximum; and (iii) plateau of  $VO_2$  (238). Respiratory exchange ratio (RER) was calculated by the formula in equation 2.9. The age predicted maximum heart rate ( $HtR_{max}$ ) was determined by the formula in equation 2.10 (239). Plateau of  $VO_2$  was determined by having a difference of  $< 2.1$  ml/kg/min in  $VO_2$  between the last 2 bags of collected expired air (230). If the criteria were fulfilled, then highest calculated value of  $VO_2$  (which may not necessarily be from the last Douglas Bag of collected expired air) would then be considered to be the participant's  $VO_2$  max

$$RER = VCO_2 / VO_2$$

### Equation 2.9 Respiratory exchange ratio (RER)

$$HtR_{max} = 220 - \text{Age}$$

### Equation 2.10 Predicted maximum heart rate

#### 2.2.9 Habitual physical activity measurements using accelerometry

Objective measurement of habitual physical activity was assessed by seven-day accelerometry (Actigraph 3TX+ or ActiTrainer, Actigraph, Florida, USA). Participants would wear the device by attaching it to their waistband of their outermost lower garment using a belt clip on their right or left side. The accelerometer display was switched off. Participants were encouraged to wear the accelerometer for all the time spent awake, only removing the accelerometer if the participant was indulging in an activity which could result in it becoming damaged (e.g. showering contact sports) or falling off (e.g.

football). Where possible, if the accelerometer had to be removed, the participant was asked to keep it in their trouser pocket for the duration of removal where possible.

Accelerometers were programmed to collect data on activity only. Vertical axis accelerometer readings were summarized in 60-second epochs. Actilife software (version 5 and subsequent updates, ActiGraph, Florida, USA) was used to programme accelerometers and download data after the accelerometer had been worn. Freedson cut-offs were used for the demarcation of intensity domains of activity (240). A template spreadsheet was created for data to be transferred on to, to enable Freedson cut-offs to be determined. A minimum of 4 valid days' data was required for analysis. A valid day was defined as having 10 or more hours of accelerometer wear. A day would commence with the first time the accelerometer was put on after waking up from overnight/daily sleep for the very first time. 'Wear' time was determined by subtracting 'nonwear' time for each subsequent 24 h thereafter. 'Nonwear' was defined by 'an interval of at least 60 consecutive minutes of zero activity intensity counts, with allowance for 1-2 min of counts between 0 and 100' (241). However to prevent over estimation of 'nonwear', participants were asked to complete a booklet detailing the daily times the accelerometer was put on and taken off, and further opportunity to detail additional times when the accelerometer was taken off and put back on. If it appeared that an interval of >60 minutes inactivity as defined above occurred whilst the participant was awake, and it was not documented that the accelerometer had been removed, then this period of time was included as 'wear' time.

#### **2.2.10 Magnetic resonance spectroscopy for quantification of liver fat**

52 participants (28 South Asians, 24 Europeans) were recruited from the CURVES study to undergo MRS and were chosen at random (but matched for age) within 4 months of their original main study visit. It was intended to recruit equal numbers of participants from each ethnic group. All participants had no known parenchymal liver disease.

MRS was performed with a 1.5T HDxt GE Signa magnetic resonance scanner (GE Healthcare, Milwaukee) using an 8-channel cardiac coil at The Beatson West of Scotland Cancer Centre, Glasgow, UK. Scott Harvey (SH), Medical Physicist, was responsible for contacting volunteers and performing the scans under the supervision of Dr John Foster (JF) Consultant Clinical Scientist and Deputy Head of MR Physics for NHS Greater Glasgow and Clyde. Consent was undertaken by trained MRI staff working in the centre and clinical supervision and review of scans was provided by Dr Stuart Ballantyne, Consultant Radiologist, Gartnavel General Hospital.

In accordance with previously published data (242), a 30 x 30 x 30mm MRS single voxel was acquired near the base of the liver (to minimise effects of respiratory motion) avoiding large vessels and at least one centimetre from the liver's edge. Two consecutive spectra were acquired - with echo times (TE) of 144 ms and 35 ms, respectively (both with repetition times (TR) of 1500 ms) - in the same location for each volunteer using the point-resolved spectroscopy (PRESS) sequence. The manufacturer's software (SAGE, GE Healthcare, Milwaukee) was used to obtain liver fat and liver water ratios, which was converted into a liver fat fraction percentage (FF) using the equation  $FF = 100 \times FA / (FA + WA)$  where FA is the area under the fat peak and WA is the area under the water peak (243). The two values obtained with the different TE were averaged to give a liver fat fraction percentage for each volunteer. Anonymised data were stored on the Computers connected to the MR scanner. All analysis was performed offline by SH under the Supervision of JF.

## **2.3 Power calculations**

### **2.3.1 Carotid plaque, insulin resistance, $VO_{2max}$ and body fat analysis**

Based on observation of carotid plaques in 40% of European men aged 40-70 (186) and assuming a 1.5 relative risk (i.e. plaques in 60% of SA men aged 40-70), then 97 men per ethnic group would be needed for 80% power to detect differences between plaques between the South Asian and European groups at a significance of  $p=0.05$ . The power calculation was checked by Professor Ian

Ford, Professor of Biostatistics, University of Glasgow, UK. Based on previously published local data (47), this number of participants would enable detection of differences  $\sim 0.4$  units,  $\sim 3$  ml kg<sup>-1</sup> min<sup>-1</sup> and  $\sim 2$  kg in HOMA<sub>IR</sub>, VO<sub>2max</sub> and fat mass, respectively, with the same power, between the South Asian and European groups. The aim was to therefore recruit 100 men from each ethnic group for the main study visit.

### **2.3.2 Differences in liver fat**

There is a relative lack of data on hepatic fat in SAs but one prior study reported a near tripling in hepatic fat (by determination of liver triglyceride levels comparing 23 SAs with 73 European men (1.94% v 0.75%,  $p < 0.001$ ) (244). Based on these data, as determined by David Purves (DP) and overseen by Dr Alex McConnachie (AM) of the Robertson Centre for Biostatistics, University of Glasgow, it was estimated that a sample size of 22 in each group would be required to show, with 90% power, a difference of 0.53 and 0.95 in the log means of insulin resistance and liver fat respectively (equivalent to a percentage difference of 68% and 158% in the means of insulin resistance and liver fat respectively), at the 5% significance level, between Asian and European men.

## **2.4 Statistical analysis**

Unless stated otherwise, all statistical analysis was performed using Minitab (version 15.1.30.0, Minitab Inc., State College, Pennsylvania) and undertaken by the author and overseen by his supervisors - where necessary, more complex analyses were have been undertaken by DP and overseen by AM with appropriate acknowledgement. The specific methods of analysis and presentation of data are described accordingly in the respective 'Data analysis' sections in chapters 3, 4 and 5.

## 2.5 Insulin resistance calculation

Insulin resistance was assessed using fasting insulin and glucose levels in the Homeostasis Assessment Model of Insulin Resistance (HOMA<sub>IR</sub>) (245). It is the most widely used method and is considered presently as the best surrogate method for insulin resistance using fasting glucose and insulin measurement (246). Insulin resistance was calculated using equation 2.7. As the laboratory assay measured in insulin in mIU/l, each result was multiplied by 6 to obtain the corresponding value in pmol/l.

$$\text{HOMA IR} = (\text{fasting glucose [mmol/l]} \times \text{fasting insulin [mIU/l]}) / 22.5$$

**Equation 2.11 HOMA<sub>IR</sub>**

## 2.6 Participant notification of results of tests

All participants were conveyed the results of their routine blood tests, blood pressure and useful anthropometric results. General lifestyle advice was given by the author relevant to the participant's results. If a participant had blood test results that were abnormal, this was discussed with the participant, and if appropriate, and consent had been given, the participant's GP was written to informing them of the participant's results with any appropriate advice or follow-up suggestions. It was left to individual GPs to take things forward with the participant in question. Those who expressed an interest to know the results of the exercise test were provided with also provided their VO<sub>2max</sub> results.

## Chapter 3

### Contribution of cardiorespiratory fitness and objectively measured physical activity to the increased insulin resistance and fasting glycaemia observed in middle-aged South Asian compared to European men living in the United Kingdom

#### 3.1 Introduction

South Asians living in the United Kingdom have a 3-5 fold increased prevalence of T2DM, and develop the disease around a decade earlier and at a lower BMI, compared to Europeans (25;28). In addition, non-diabetic South Asians exhibit higher glucose levels than Europeans (14;67). While the mechanisms for their higher risk are not fully understood, greater insulin resistance is a strong candidate (14;44;47).

Adiposity (46;80;247), cardiorespiratory fitness (80;83) and physical activity (105;113) are key factors influencing insulin resistance, glycaemia and diabetes risk. South Asians carry more body fat than Europeans and this may be distributed more centrally (24;44;248). However, it appears that South Asians remain more insulin resistant than Europeans after matching or adjustment for a range of adiposity markers (46;47). Furthermore, limited data from small studies suggests that cardiorespiratory fitness, as assessed by  $VO_{2max}$ , is ~10-20% lower in South Asians than matched Europeans (45;47;87), with previous local data (47) showing that adjustment for  $VO_{2max}$  attenuated the difference in insulin sensitivity between young South Asian and European men. In addition, habitual levels of physical activity amongst South Asians living in the UK appear lower than in Europeans (99;116-118). Low physical activity has been shown to explain part of the excess CVD risk observed in South Asians (118), and could conceivably contribute to their increased diabetes risk. However, available data on physical activity in South Asians have all been obtained from self-report questionnaires: these have limited validity compared to objective measures (96;98), which is potentially exacerbated in South Asians due cultural interpretation of the terminology used in the questionnaires (99). Misclassification arising from the use of questionnaires to assess physical activity

can substantially underestimate the true relationship between activity and risk of vascular and metabolic disease (104;114).

Thus, from the available data, the extent to which lower cardiorespiratory fitness and lower physical activity contributes to increased insulin resistance and glycaemia in South Asians is not established. Thus it was hypothesised that these factors would explain a substantial proportion of the increased insulin resistance and glycaemia observed in South Asian men living in the UK.

## **3.2 Methods**

### **3.2.1 Participants and recruitment**

All participants were recruited and screened as detailed in section 2.1. Specific inclusion and exclusion criteria are defined in section 2.1.1. All participants received payment for towards transport and received feedback as detailed in sections 2.2.1 and 2.6 respectively.

Participants' health history, including smoking status and family health history, education history, SES information and dietary information were obtained as described in section 2.2.1

### **3.2.2 Blood biochemistry and blood pressure measurements**

Venous blood samples were obtained after an overnight fast of 10-12 hours as described in section 2.2.2.1. Samples were sent for routine tests outlined in section 2.2.2.1. Plasma was stored for the subsequent measurement of insulin as described in section 2.2.2.2. Insulin resistance was assessed by  $HOMA_{IR}$  and was calculated as outlined in section 2.5.

Blood pressure was measured as described in section 2.2.4.

### **3.2.3 Anthropometry**

Anthropometric measurements were performed on all participants as detailed in section 2.2.6. Total body lean and fat mass was measured using ADP as described in section 2.2.7.

### **3.2.4 Determination of cardiorespiratory fitness**

All participants had a clinical assessment and resting ECG performed prior to undertaking the exercise test to ensure no contraindications to maximal exercise. Exercise testing was undertaken as described in section 2.2.8. Cardiorespiratory fitness was determined by measuring  $VO_{2max}$  as described in sections 2.2.8.5 and 2.2.8.6. A valid measure of  $VO_{2max}$  was achieved in 199 of the 200 participants.

### **3.2.5 Measurement of physical activity**

Objective measurement of physical activity was obtained through accelerometry as described in section 2.2.9. Valid data were obtained for 85 South Asians and 84 Europeans.

### **3.2.6 Data analysis**

The sample size for the CURVES study was determined as calculated in section 2.3.1.

Summary statistics are presented for all variables for both South Asians and Europeans. Continuous variables were compared between the ethnic groups by t-tests (or Wilcoxon rank sum test for non-normally distributed variables) and Fisher's Exact test for categorical variables.

Factor analysis was used to reduce the number of anthropometric variables into underlying latent factors. Three factors emerged relating to ‘total adiposity’ (identified by biceps, triceps, subscapular, supraspinale, suprailiac, thigh, and calf skinfolds, and fat mass), ‘body size’ (height, lean mass, and hip, mid-thigh and mid-calf circumferences), and ‘central adiposity’ (waist circumference and WHR). A summary measure was calculated to describe each factor. Each variable was standardised (by subtracting the mean and dividing by the standard deviation), summed according to the factor analysis groups and then standardised again. Initially, linear regression models, adjusted for ethnicity (i.e. nullifying the ethnicity effect), were used to assess associations between total adiposity, body size, central adiposity, physical activity, sedentary behaviour and fitness (expressed as standardised measures) on HOMA<sub>IR</sub> and fasting glucose concentration. Effects of potential confounding covariates (age, education, SES, smoking, alcohol, diabetes family history) on HOMA<sub>IR</sub> and fasting glucose concentration were also assessed. Both outcome variables were log transformed for analysis. Then, relative effects of ethnicity on HOMA<sub>IR</sub> and glucose was calculated: when it was the only predictor in the model; after separate adjustment for total adiposity, body size, central adiposity, physical activity, sedentary behaviour and fitness; and in the final model including adjustment for all of the above variables which significantly influenced the ethnicity effect. Thus, this analysis enabled determination of the extent to which differences in HOMA<sub>IR</sub> and fasting glucose concentration between South Asians and Europeans could be explained by body composition, fitness, and physical activity variables. The absolute differences in geometric means for HOMA<sub>IR</sub> and fasting glucose concentration between the groups were calculated for each model, using the mean value for continuous predictors and the reference level for any categorical variables, and 95% CIs for the difference were derived by bootstrapping. The percentage change in the relative percentile difference between South Asians and Europeans in HOMA<sub>IR</sub> and fasting glucose concentration were calculated with 95% CIs and p-values derived by bootstrapping (reductions greater than 100% describe a change in direction of the effect).

All the above analysis was undertaken by DP and AM and in consultation with the author and his supervisors.

The statistical software package R for Windows v2.14 was used for all analysis. Statistical significance was accepted at  $p < 0.05$ .

### 3.3 Results

#### 3.3.1 Demographic, metabolic and dietary variables

Thirteen South Asian men and one European man who completed the full study protocol were subsequently noted to have HbA1c concentrations  $>6.5\%$  ( $>48$  mmol/mol) and/or fasting glucose concentrations  $>7$  mmol/l, indicating possible undiagnosed diabetes. Their data were excluded from the analyses. The final data set therefore included 87 South Asian and 99 European men. Demographic and metabolic variables for the final South Asian and European cohorts are presented in Table 3.1. Baseline data for all 200 participants are presented in Table 5.1. There were no differences between groups in age or BMI, but South Asians were shorter than the Europeans. South Asians also had completed more years in education, were less likely to smoke or consume alcohol, and were more likely to have a parent or sibling with T2DM. There were no differences in SES. South Asians had significantly higher glucose, HbA1c, insulin, HOMA<sub>IR</sub>, CRP and diastolic blood pressure, and lower HDL-cholesterol, than the Europeans. Mean  $\pm$  SD reported energy intake (expressed per kg body mass) did not differ between Europeans and South Asians ( $19.9 \pm 6.0$  vs  $18.7 \pm 6.5$  kcal.kg<sup>-1</sup>.day<sup>-1</sup>,  $p = 0.21$ ). There were also no differences in reported protein, carbohydrate or fat intakes between the two groups (data not shown), but reported alcohol intake was higher in the Europeans than South Asians ( $22.1 \pm 18.3$  vs  $1.2 \pm 6.0$  g.day<sup>-1</sup>,  $p < 0.001$ ).

**Table 3.1 Demographic and metabolic variables for South Asian and European men.**

		South Asian (N = 87)	European (N = 99)	p-value
<b>Demographic and lifestyle variables</b>				
Age (years)		48.3 (6.8)	49.7 (6.8)	0.162
Body mass (kg)		82.0 (12.1)	85.7 (13.9)	0.056
Height (m)		1.74 (0.07)	1.79 (0.06)	<0.001
BMI (kg/m <sup>2</sup> )		27.1 (3.8)	26.9 (4.3)	0.700
Years in Education		15.4 (3.4)	14.4 (3.2)	0.042
SIMD Quintile	1	6 (6.9%)	7 (7.1%)	0.751
	2	10 (11.5%)	13 (13.1%)	
	3	16 (18.4%)	19 (19.2%)	
	4	23 (26.4%)	18 (18.2%)	
	5	32 (36.8%)	42 (42.4%)	
Smoking Status	never-smoker	71 (81.6%)	54 (54.5%)	<0.001
	ex-smoker	5 (5.7%)	35 (35.4%)	
	current	11 (12.6%)	10 (10.1%)	
Alcohol Consumption <sup>b</sup> (units per week)	none	81 (93.1%)	10 (10.2%)	<0.001
	≤ 20	4 (4.6%)	69 (70.4%)	
	≥ 21	2 (2.3%)	19 (19.4%)	
Parental Diabetes Status <sup>b</sup>	yes	50 (57.5%)	12 (12.2%)	<0.001
Sibling Diabetes Status <sup>b</sup>	yes	13 (14.9%)	2 (2.0%)	0.002
<b>Metabolic variables</b>				
Glucose (mmol/l)		5.3 (4.9, 5.7)	5.1 (4.8, 5.4)	0.018
HbA1c (mmol/mol) <sup>c</sup>		39.0 (37.0, 41.0)	36.0 (33.0, 38.0)	<0.001 <sup>a</sup>
HbA1c (%) <sup>c</sup>		5.7 (5.5, 5.9)	5.4 (5.2, 5.6)	<0.001 <sup>a</sup>
Insulin (pmol/l) <sup>c</sup>		88.9 (69.5, 119.5)	54.2 (36.8, 79.9)	<0.001 <sup>a</sup>
HOMA <sub>IR</sub> <sup>c</sup>		3.0 (2.4, 4.2)	1.8 (1.1, 2.6)	<0.001 <sup>a</sup>
Total Cholesterol (mmol/l)		5.4 (1.0)	5.6 (1.0)	0.107
HDL-Cholesterol (mmol/l)		1.1 (1.0, 1.3)	1.3 (1.1, 1.6)	<0.001 <sup>a</sup>
LDL-cholesterol (mmol/l)		3.5 (0.9)	3.6 (0.8)	0.458
Triacylglycerol (mmol/l)		1.4 (1.0, 2.2)	1.3 (0.9, 1.8)	0.135 <sup>a</sup>
AST (U/l)		23.3 (6.8)	25.1 (8.6)	0.100
ALT (U/l)		26.0 (21.0, 35.5)	26.0 (20.0, 35.5)	0.857 <sup>a</sup>
GGT (U/l)		30.0 (22.0, 42.5)	28.0 (20.5, 43.5)	0.572 <sup>a</sup>
CRP (nmol/l) <sup>b</sup>		17.1 (10.5, 38.1)	10.5 (5.7, 23.8)	0.003 <sup>a</sup>
Systolic blood pressure (mm Hg)		126.3 (15.4)	127.5 (12.9)	0.568
Diastolic blood pressure (mm Hg)		77.8 (9.1)	74.9 (7.2)	0.018

Values are mean (SD) for normally distributed variables with p values calculated by t-tests and median (inter-quartile range, IQR) for non-normally distributed variables with p-values calculated by Wilcoxon test<sup>a</sup>. Categorical variable p-values are calculated by Fisher's Exact test. <sup>b</sup>n = 98 for Europeans; <sup>c</sup>n = 86 for South Asians.

### 3.3.2 Body composition, fitness and physical activity variables

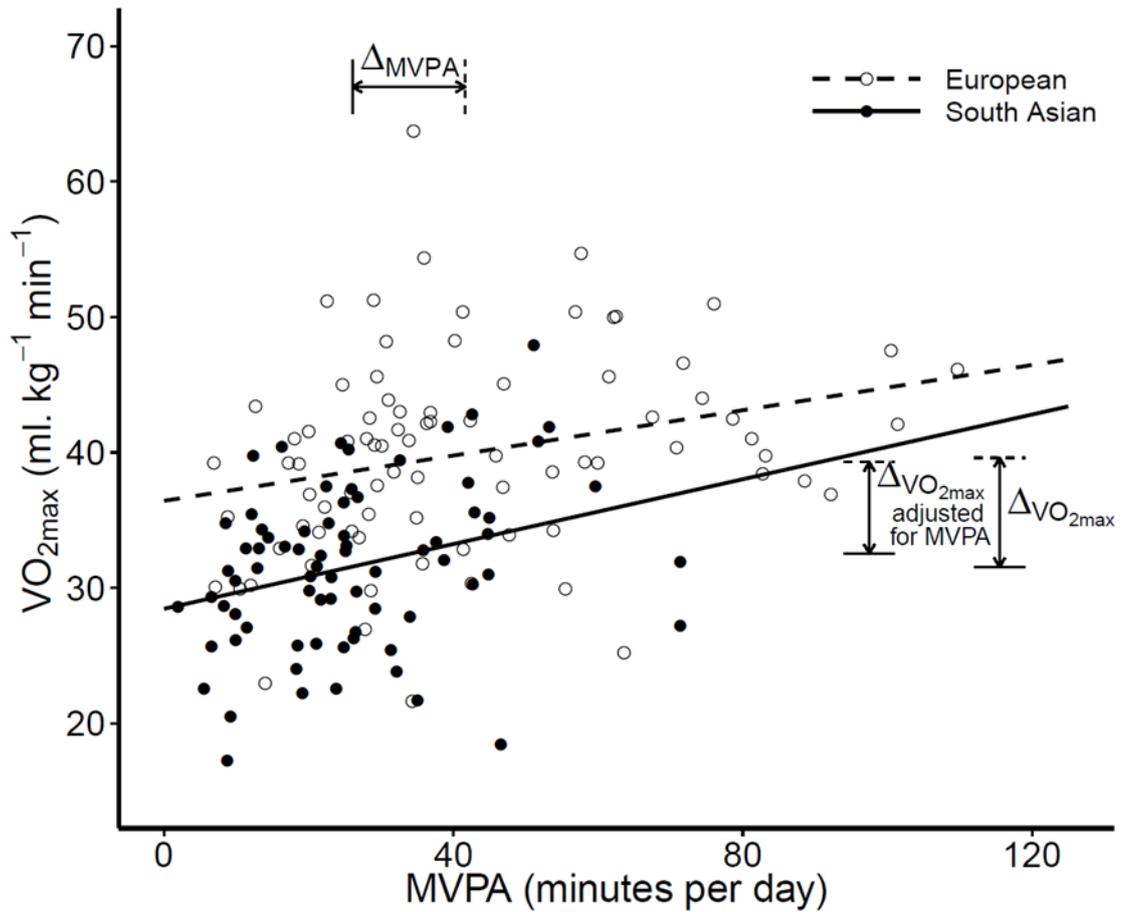
Body composition, fitness and physical activity variables are presented in Table 3.2. There were no differences in waist or hip circumferences between the South Asian and European groups, but WHR was higher in the South Asians. South Asians also had a smaller mid-thigh circumference, and higher values for all skinfold thicknesses than the Europeans. Lean mass was lower and fat mass and percentage body fat were higher in the South Asians. Total adiposity and central adiposity factors were higher and body size units lower in South Asians than Europeans. Cardiorespiratory fitness (as assessed by  $VO_{2max}$ ) was lower in South Asians than Europeans, irrespective of whether this was expressed in absolute terms (i.e. l/min) (lower on average by 24%), per kg body mass (by 21%) or per kg lean mass (by 16%).

South Asians also engaged in less moderate and vigorous physical activity than Europeans: the proportion of time spent in moderate-to-vigorous physical activity was ~32% lower in South Asians than Europeans. This equated to South Asians engaging in ~23 minutes of moderate-to-vigorous physical activity (MVPA) per day compared to ~34 minutes per day for the Europeans. There were significant correlations between  $VO_{2max}$  (in  $ml.kg^{-1}.min^{-1}$ ) and (log) MVPA in both South Asians ( $r = 0.326$ ,  $p = 0.004$ ) and Europeans ( $r = 0.326$ ,  $p = 0.003$ ). However, the lower  $VO_{2max}$  values in South Asians could not be explained by their lower levels of physical activity: South Asians'  $VO_{2max}$  values were lower than Europeans' across the range of MVPA levels, and their  $VO_{2max}$  values remained significantly lower than Europeans after adjustment for MVPA (Figure 3.1).

**Table 3.2 Body composition, fitness, and physical activity variables for South Asian and European men.**

	South Asian (N = 87)	European (N = 99)	p-value
<b>Anthropometric variables</b>			
<b>(a) Body circumferences</b>			
Waist (cm)	97.3 (10.9)	95.6 (11.2)	0.315
Hips (cm)	99.5 (6.6)	100.5 (7.1)	0.336
Waist-to-hip ratio	0.98 (0.07)	0.95 (0.06)	0.009
Mid-upper arm (cm)	34.5 (8.2)	33.4 (3.3)	0.238
Mid-thigh (cm)	52.6 (4.3)	54.0 (4.0)	0.030
Mid-calf (cm)	37.7 (3.4)	38.6 (4.1)	0.101
<b>(b) Skinfold thicknesses</b>			
Biceps (mm) <sup>e</sup>	8.0 (3.6)	6.8 (2.9)	0.015
Triceps (mm)	16.4 (5.8)	12.6 (4.3)	<0.001
Subscapular (mm) <sup>e, k</sup>	25.7 (7.3)	18.3 (7.1)	<0.001
Suprailiac (mm) <sup>g, j</sup>	23.4 (6.2)	18.2 (6.2)	<0.001
Supraspinale (mm)	18.0 (6.6)	12.6 (5.4)	<0.001
Thigh (mm) <sup>e</sup>	19.1 (7.9)	14.6 (5.7)	<0.001
Calf (mm) <sup>l</sup>	13.0 (5.4)	10.4 (4.2)	<0.001
<b>(c) Fat and lean mass</b>			
Lean mass (kg) <sup>e</sup>	57.2 (6.4)	63.3 (7.1)	<0.001
Fat mass (kg) <sup>e</sup>	24.4 (9.0)	21.8 (9.7)	0.058
Percentage body fat <sup>e</sup>	29.2 (7.4)	24.8 (7.4)	<0.001
<b>(d) Summary anthropometric factors</b>			
Total adiposity <sup>b</sup> (standardised units)	0.4 (1.0)	-0.3 (0.9)	<0.001
Body size <sup>c</sup> (standardised units)	-0.3 (0.9)	0.3 (1.0)	<0.001
Central adiposity <sup>d</sup> (standardised units)	0.1 (1.0)	-0.1 (1.0)	0.058
<b>Fitness variables</b>			
VO <sub>2 max</sub> (l.min <sup>-1</sup> ) <sup>e</sup>	2.6 (0.4)	3.4 (0.6)	<0.001
VO <sub>2 max</sub> (ml.kg <sup>-1</sup> .min <sup>-1</sup> ) <sup>e</sup>	31.5 (5.8)	39.6 (7.5)	<0.001
VO <sub>2 max</sub> (ml.kg lean mass <sup>-1</sup> .min <sup>-1</sup> ) <sup>f</sup>	44.7 (6.4)	53.0 (7.8)	<0.001
<b>Physical activity variables</b>			
Sedentary (% of wear time) <sup>h, l</sup>	66.5 (9.5)	64.2 (9.1)	0.136
Light physical activity (% of wear time) <sup>h, l</sup>	30.4 (9.2)	30.8 (8.1)	0.789
Moderate-to-vigorous physical activity (% of wear time) <sup>h, l</sup>	2.8 (1.7, 4.1)	4.1 (3.0, 6.8)	<0.001 <sup>a</sup>
Total accelerometer wear time (min.day <sup>-1</sup> ) <sup>h, l</sup>	824.5 (85.1)	863.0 (74.2)	0.003

Values are mean (SD) for normally distributed variables with p-values calculated by t-tests and median (IQR) for non-normally distributed variables with p-values calculated by Wilcoxon test<sup>a</sup>. Categorical variable p values are calculated by Fisher's Exact test. <sup>b</sup>Derived from biceps, triceps, subscapular, supraspinale, suprailiac, thigh, and calf skinfolds, and fat mass; <sup>c</sup>derived from height, lean mass, and hip, mid-thigh and mid-calf circumferences; <sup>d</sup>derived from waist circumference and waist-to-hip ratio. <sup>e</sup>n = 98, <sup>f</sup>n = 97, <sup>g</sup>n = 95 and <sup>h</sup>n = 83 for Europeans; <sup>i</sup>n = 86, <sup>j</sup>n = 84, <sup>k</sup>n = 82, <sup>l</sup>n = 75 for South Asian



**Figure 3.1 Relationship between  $VO_{2max}$  and moderate-to-vigorous physical activity in South Asian and European men.**

Solid circles and the solid line represent South Asian men with open circles and the dotted line representing European men. Solid and dotted vertical bars indicate mean MVPA levels in South Asian and European men, respectively; the horizontal arrow shows the mean difference in MVPA between ethnic groups. Solid and dotted horizontal bars, with corresponding vertical arrows indicate mean  $VO_{2max}$  values in South Asian and European men and the mean ethnic difference, both unadjusted and adjusted for MVPA.

### 3.3.3 Associations between anthropometric, physical activity and fitness variables with $HOMA_{IR}$ and fasting glucose concentration

Table 3.3 shows ethnicity-adjusted relative effects of anthropometric, physical activity and fitness variables on  $HOMA_{IR}$  and fasting glucose concentration.  $HOMA_{IR}$  increased with increasing total adiposity, body size, central adiposity

and time spent sedentary and decreased with increasing MVPA and  $VO_{2max}$ . Fasting glucose concentration increased with increasing total adiposity and decreased with increasing  $VO_{2max}$ . For these analyses  $VO_{2max}$  was expressed in  $ml.kg^{-1}.min^{-1}$ , but results were similar when  $VO_{2max}$  was expressed in  $l.min^{-1}$  or in  $ml.kg \text{ lean mass}^{-1}.min^{-1}$ . Effects of potential confounding covariates (smoking status, alcohol consumption, years in education and SES) on  $HOMA_{IR}$  and fasting glucose concentration are shown in Appendix A8.  $HOMA_{IR}$  was higher in ex-smokers (but not current smokers) than non-smokers (by 26%, 95% CI: 2%, 56%,  $p=0.03$ ). Other than this, none of measured covariates were significantly associated with  $HOMA_{IR}$  or fasting glucose concentration.

**Table 3.3 Associations between  $HOMA_{IR}$  and fasting glucose concentration with standardised anthropometric, physical activity and fitness variables.**

Predictor	$HOMA_{IR}$	Fasting glucose concentration
	Relative effect estimate (95% CI), p-value	Relative effect estimate (95% CI), p-value
<i>Anthropometric variables</i>		
Total adiposity <sup>a</sup>	1.40 (1.30, 1.50) $p<0.001$	1.02 (1.00, 1.03) $p=0.013$
Body size <sup>b</sup>	1.21 (1.12, 1.31) $p<0.001$	1.00 (0.99, 1.02) $p=0.666$
Central adiposity <sup>c</sup>	1.40 (1.31, 1.49) $p<0.001$	1.01 (1.00, 1.03) $p=0.075$
<i>Physical activity variables</i>		
Moderate-to-vigorous physical activity	0.86 (0.79, 0.94) $p=0.001$	0.99 (0.97, 1.01) $p=0.263$
Sedentary	1.10 (1.01, 1.20) $p=0.024$	1.01 (0.99, 1.02) $p=0.299$
<i>Fitness variables</i>		
$VO_{2max}$ <sup>d</sup>	0.74 (0.68, 0.80) $p<0.001$	0.98 (0.97, 1.00) $p=0.020$

Relative effects are for a 1 SD change in the predictor variable, adjusted for ethnicity. Values are presented with 95% CIs and p values. <sup>a</sup>Derived from biceps, triceps, subscapular, supraspinale, suprailiac, thigh, and calf skinfolds, and fat mass; <sup>b</sup>derived from height, lean mass, and hip, mid-thigh and mid-calf circumferences; <sup>c</sup>derived from waist circumference and waist-to-hip ratio. <sup>d</sup>expressed in  $ml.kg^{-1}.min^{-1}$ .

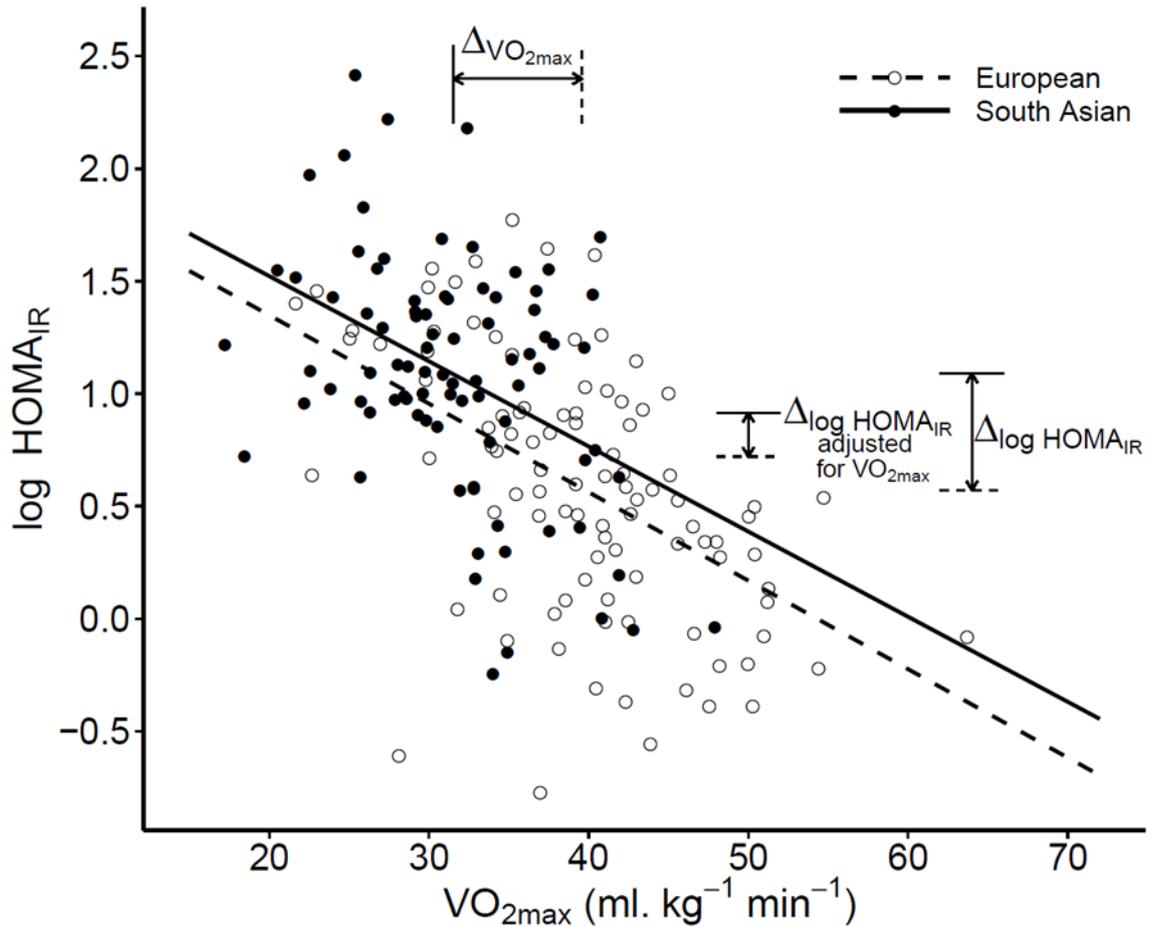
### 3.3.4 Contribution of anthropometric, physical activity and fitness variables to the difference in HOMA<sub>IR</sub> between Europeans and South Asians

Table 3.4 shows the relative effect of ethnicity, in relative and absolute terms, on HOMA<sub>IR</sub>, unadjusted and in models adjusted for anthropometric, physical activity and fitness variables. In unadjusted analysis, HOMA<sub>IR</sub> values in South Asians were 67% (95% CI, 42-96%,  $p < 0.001$ ), higher than the values observed in Europeans. Ethnicity contributed 18% of the total variance in HOMA<sub>IR</sub>. Adjusting for total adiposity attenuated the difference in HOMA<sub>IR</sub> between ethnic groups by 51.7% (95% CI, 29.5-80.3%), whereas adjusting for body size increased the difference by 28.5% (95% CI, 11.5-48.3%). Adjusting for MVPA reduced the difference in HOMA<sub>IR</sub> between South Asians and Europeans by 27.5% (95% CI, 10.7-45.9%). However, the difference in VO<sub>2max</sub> between South Asians and Europeans explained more of the difference in HOMA<sub>IR</sub> than any other variable: adjustment for VO<sub>2max</sub> attenuated the difference in HOMA<sub>IR</sub> between ethnic groups by 67.5% (95% CI, 45.1-90.9%). This is further illustrated in Figure 3.2. Adjustment for central adiposity or sedentary time did not significantly alter the ethnicity effect on HOMA<sub>IR</sub>. Simultaneously adjusting for the four variables which significantly changed the ethnicity effect (total adiposity, body size, MVPA and VO<sub>2max</sub>) attenuated the difference in HOMA<sub>IR</sub> between South Asians and Europeans by 83.4% (95% CI, 50.1-118.9%). Repeating the analyses after adjustment for smoking, alcohol consumption, education and SES produced similar findings. In these confounder-adjusted analyses, the difference in VO<sub>2max</sub> between South Asians and Europeans explained 77.8% (95% CI, 38.4-132.2%) of the ethnic difference in HOMA<sub>IR</sub>, and the contribution of total adiposity to the difference in HOMA<sub>IR</sub> between the ethnic groups was attenuated slightly to 41.3% (95% CI, 0.5-95.7%).

**Table 3.4 relative effects of ethnicity on HOMA<sub>IR</sub>, unadjusted and adjusted by anthropometric, physical activity and fitness variables.**

	Ethnicity effect estimate for HOMA <sub>IR</sub>								
	N	Unadjusted <sup>a</sup>			Adjusted <sup>b</sup>			Percentage change <sup>c</sup>	
		Relative effect estimate (95% CI)	p-value	R <sup>2</sup> <sub>adj</sub>	Relative effect estimate (95% CI)	p-value	R <sup>2</sup> <sub>adj</sub>	Percentage change estimate (95% CI)	p-value
<b><i>Anthropometric variables</i></b>									
Total adiposity <sup>d</sup>	185	1.67 (1.42, 1.96)	<0.001	0.17	1.32 (1.15, 1.53)	<0.001	0.43	-51.7 (-72.1, -34.3)	<0.001
Body size <sup>e</sup>	185	1.67 (1.42, 1.96)	<0.001	0.17	1.86 (1.59, 2.18)	<0.001	0.26	28.5 (10.4, 52.3)	<0.001
Central adiposity <sup>f</sup>	185	1.67 (1.42, 1.96)	<0.001	0.17	1.53 (1.34, 1.74)	<0.001	0.46	-21.2 (-38.1, 3.4)	0.080
<b><i>Physical activity variables</i></b>									
Moderate-to-vigorous physical activity	157	1.57 (1.32, 1.86)	<0.001	0.14	1.41 (1.18, 1.68)	<0.001	0.19	-27.5 (-49.9, -10.0)	<0.001
Sedentary	157	1.57 (1.32, 1.86)	<0.001	0.14	1.53 (1.29, 1.82)	<0.001	0.16	-6.0 (-17.9, 2.5)	0.162
<b><i>Fitness variables</i></b>									
VO <sub>2 max</sub> <sup>g</sup>	184	1.66 (1.41, 1.95)	<0.001	0.17	1.22 (1.03, 1.44)	0.022	0.35	-67.5 (-94.8, -45.0)	<0.001
<b><i>All Statistically Significant Factors</i></b>									
Total adiposity + Body size + Moderate-to-vigorous physical activity + VO <sub>2 max</sub>	156	1.56 (1.31, 1.85)	<0.001	0.14	1.09 (0.91, 1.31)	0.332	0.45	-83.4 (-120.7, -55.3)	<0.001

Estimates for the relative effect (ratios of geometric means) of ethnicity on HOMA<sub>IR</sub> and the percentage change between these effects are presented with 95% CIs and p values. Adjusted R<sup>2</sup> (R<sup>2</sup><sub>adj</sub>) for unadjusted and adjusted models also displayed. <sup>a</sup>unadjusted relative ethnicity effect (for South Asians compared to white Europeans); <sup>b</sup>ethnicity effect adjusted for predictor variables; <sup>c</sup>percentage change in unadjusted race effect estimate to adjusted model effect. <sup>d</sup>Derived from biceps, triceps, subscapular, supraspinale, suprailiac, thigh, and calf skinfolds, and fat mass; <sup>e</sup>derived from height, lean mass, and hip, mid-thigh and mid-calf circumferences; <sup>f</sup>derived from waist circumference and WHR. <sup>g</sup>expressed in ml.kg<sup>-1</sup>.min<sup>-1</sup>.



**Figure 3.2 Relationship between HOMA<sub>1R</sub> and VO<sub>2max</sub> in South Asian and European men**

Solid circles and the solid line represent South Asian men with open circles and the dotted line representing European men. Solid and dotted vertical bars indicate mean VO<sub>2max</sub> values in South Asian and European men, respectively; the horizontal arrow shows the mean difference in VO<sub>2max</sub> between ethnic groups. Solid and dotted horizontal bars, with corresponding vertical arrows indicate mean HOMA<sub>1R</sub> values in South Asian and European men and the mean ethnic difference, both unadjusted and adjusted for VO<sub>2max</sub>.

### 3.3.5 Contribution of anthropometric, physical activity and fitness variables to the difference in fasting glucose concentration between Europeans and South Asians

Table 3.5 presents similar data for the fasting glucose concentration. In unadjusted analysis, fasting glucose concentrations in South Asians were 3% (95% CI, 1-6%,  $p < 0.017$ ) higher than in Europeans. Ethnicity contributed to 3% of the variance in fasting glucose. Adjusting for total adiposity attenuated the ethnic difference in fasting glucose by 39.1% (95% CI, 9.4-76.1%). Similar to effects on  $HOMA_{IR}$ ,  $VO_{2max}$  explained more of the difference in fasting glucose concentration between South Asians and Europeans than any other variables, with adjustment for  $VO_{2max}$  attenuating the ethnic difference by 60.7% (95% CI, 9.2-111.0%). The ethnicity effect for fasting glucose concentration was not significantly influenced by body size, central adiposity, MVPA or sedentary time. Simultaneously adjusting for total adiposity and  $VO_{2max}$  attenuated the ethnic difference in fasting glucose by 63.4% (95% CI, 8.2%-114.6%). Repeating the analyses after adjustment for smoking, alcohol consumption, education and SES produced essentially similar findings, with  $VO_{2max}$  still explaining more of the difference in fasting glucose concentration between the ethnic groups than other any variable, attenuating the difference by 41.6% (95% CI, 1.7-232.0%). In these confounder-adjusted analyses no other variables significantly contributed to the ethnicity difference.

**Table 3.5: Relative and absolute effects of ethnicity on fasting glucose concentration, unadjusted and adjusted by anthropometric, physical activity and fitness variables.**

	N	Relative ethnicity effect estimate for fasting glucose concentration				Absolute difference in fasting glucose concentration between Europeans and South Asians (mmol/l)		Percentage change in ethnicity difference with adjustment <sup>c</sup>	
		Unadjusted <sup>a</sup>	p-value	Adjusted for predictor variable	p-value	Unadjusted <sup>b</sup>	Adjusted for predictor variable	Percentage change estimate	p-value
<i>Anthropometric variables</i>									
Total adiposity <sup>d</sup>	186	1.03 (1.01, 1.06)	0.017	1.02 (0.99, 1.05)	0.163	0.18 (0.03, 0.33)	0.11 (-0.05, 0.27)	-39.1 (-76.1, -9.4)	0.008
Body size <sup>e</sup>	186	1.03 (1.01, 1.06)	0.017	1.04 (1.01, 1.07)	0.016	0.18 (0.03, 0.33)	0.19 (0.03, 0.33)	5.4 (-15.9, 26.4)	0.600
Central adiposity <sup>f</sup>	186	1.03 (1.01, 1.06)	0.017	1.03 (1.00, 1.06)	0.033	0.18 (0.03, 0.33)	0.16 (0.01, 0.30)	-10.6 (-31.7, 1.8)	0.128
<i>Physical activity variables</i>									
Moderate-to-vigorous physical activity	158	1.04 (1.01, 1.07)	0.016	1.03 (1.00, 1.07)	0.063	0.20 (0.04, 0.36)	0.16 (0.00, 0.33)	-18.0 (-48.6, 10.6)	0.220
Sedentary	158	1.04 (1.01, 1.07)	0.016	1.04 (1.01, 1.07)	0.023	0.20 (0.04, 0.36)	0.19 (0.03, 0.34)	-5.2 (-19.2, 5.5)	0.406
<i>Fitness variables</i>									
VO <sub>2</sub> max <sup>g</sup>	185	1.03 (1.00, 1.06)	0.023	1.01 (0.98, 1.05)	0.430	0.17 (0.02, 0.30)	0.07 (-0.09, 0.22)	-60.7 (-111.0, -9.2)	0.028
<i>All Statistically Significant Factors</i>									
Total adiposity + VO <sub>2</sub> max	185	1.03 (1.00, 1.06)	0.023	1.01 (0.98, 1.04)	0.460	0.17 (0.02, 0.30)	0.06 (-0.09, 0.22)	-63.4 (-114.6, -8.2)	0.030

Estimates for the effect of ethnicity on fasting glucose concentration, expressed as a relative effect (ratios of geometric means); in terms of absolute difference in the geometric mean (in mmol/l); and the percentage change between these effects after adjustment are presented with 95% CIs and p values. <sup>a</sup>unadjusted relative ethnicity effect (for South Asians compared to Europeans); <sup>b</sup>unadjusted ethnicity effect in mmol/l; <sup>c</sup>percentage change in unadjusted ethnicity effect estimate to adjusted model effect. <sup>d</sup>Derived from biceps, triceps, subscapular, supraspinale, suprailiac, thigh, and calf skinfolds, and fat mass; <sup>e</sup>derived from height, lean mass, and hip, mid-thigh and mid-calf circumferences; <sup>f</sup>derived from waist circumference and waist-to-hip ratio. <sup>g</sup>expressed in ml.kg<sup>-1</sup>.min<sup>-1</sup>.

### 3.4 Discussion

The main novel finding of this chapter was that low cardiovascular fitness, as measured by gold-standard maximal incremental treadmill test, was the single most important factor associated with the increased insulin resistance and fasting glycaemia observed in middle-aged South Asian compared to European men living in the UK. Ethnicity explained 18% of the variance in HOMA<sub>IR</sub>, with HOMA<sub>IR</sub> values being 67% higher (1.2 HOMA<sub>IR</sub> units) in South Asians than Europeans. South Asians' lower VO<sub>2max</sub> levels (statistically) explained more than two-thirds of this ethnic difference (0.8 HOMA<sub>IR</sub> units). The ethnic difference in fasting glucose concentration was less pronounced. Ethnicity explained 3% of the variance in fasting glucose, and values were 3% higher (-0.2 mmol/l) in South Asians than Europeans: nevertheless over 60% of the ethnic difference in fasting glucose (-0.1 mmol/l) could be explained by VO<sub>2max</sub>. Higher total adiposity and lower MVPA in South Asians also associated with their greater HOMA<sub>IR</sub>; their increased total adiposity also associated with their higher fasting glucose values. Fitness, anthropometric and physical activity variables together explained over four-fifths of the increased insulin resistance and three-fifths of the increased glycaemia observed in South Asians.

The finding that greater total adiposity explained about half of South Asians increased insulin resistance and two-fifths of their increased fasting glycaemia compared to Europeans, is consistent with other reports (24;44;248), although interestingly South Asians smaller body size acted in the opposite direction, appearing to attenuate the difference in HOMA<sub>IR</sub> between the groups. Furthermore, these findings demonstrate, using robust objective measures, that levels of physical activity were significantly lower in South Asians than Europeans in our sample, which is consistent with the suggestions from previous self-reported data (99;116-118), and reveals for the first time that this contributes to their greater insulin resistance. However, the most striking finding from this study is the extent to which lower cardiorespiratory fitness was associated with South Asians insulin resistant phenotype. This is consistent with data from animal models which have demonstrated a causal link between low fitness and insulin resistance (249) and with epidemiological data reporting 2.6-fold increased risk of incident T2DM, independent of BMI, in men with low

compared high cardiorespiratory fitness levels (83). Importantly, the present data demonstrate that although fitness increases with increasing physical activity, South Asians' lower  $VO_{2max}$  values could not be simply explained by their lower physical activity levels. South Asians had lower fitness levels than Europeans at all levels of physical activity, and adjusting for MVPA only modestly attenuated the ethnic difference in  $VO_{2max}$  (see Figure 3.1). Interestingly, recent data from the NHANES study support the suggestion of innate differences in fitness between ethnic groups, with Ceaser and colleagues reporting systematic differences in  $VO_{2max}$  between Mexican Americans (highest), non-Hispanic whites and non-Hispanic blacks (lowest), which were independent of time spent in (self-reported) physical activity and demographic factors (250). There is a strong heritable component to  $VO_{2max}$ , with heritability estimates in this trait of up to 50% (251;252) thus, it seems likely that there will be a genetic (and/or early origins) contribution to the lower  $VO_{2max}$  values in South Asians. Our group have recently reported that South Asians have a reduced ability to oxidise fat during exercise than Europeans (47), suggesting that there may be innate differences in the physiology of South Asians compared to Europeans which may contribute to this effect. The fact that South Asians increased insulin resistance and glycaemia is strongly associated with their lower fitness levels, and that increasing physical activity is the only way to increase cardiorespiratory fitness, suggests that **South Asians need to engage in greater levels of physical activity than Europeans to achieve the same levels of cardiorespiratory fitness and minimise their metabolic risk.** This has potential implications for physical activity guidance, which, at present, do not take ethnicity into account. Recent consensus statements have recommended that the BMI threshold for obesity in South Asian populations should be lowered from 30 kg/m<sup>2</sup> to BMI 25 kg/m<sup>2</sup> (73;75), in recognition of the fact that substantially lower BMIs are needed in South Asians to confer equivalent cardiometabolic risk factor profiles to those observed in populations of white European origin (66;67). The present data suggest that differential physical activity guidance for South Asians may also be needed. Larger epidemiological studies, and intervention trials, comparing the dose-response relationship between (objectively measured) physical activity and cardio-metabolic disease risk in South Asians and Europeans are urgently needed to enable appropriate levels of physical activity to minimise risk in South Asian populations to be robustly quantified.

This study has a number of strengths. We were initially rigorous to exclude those with known diabetes and those with potential undiagnosed diabetes (remarkably 13% of South Asian men (vs. 1% of European men)) were excluded from further analysis, to prevent potential confounding. This is the largest study to compare objectively measured physical activity and cardiorespiratory fitness between South Asian and European men. Thus, differences in fitness and activity between these groups and the contribution of these factors to insulin resistance and glycaemia can be robustly assessed from the present data. Furthermore, we measured a wide range of adiposity variables and used factor analysis to derive robust summary measures of body size and composition to enable us to determine which specific features of South Asians greater adiposity contributed to their increased insulin resistance and glycaemia. The groups were well-matched for age, BMI and socioeconomic status, and although the South Asians spent more years in education, smoked less and drank less alcohol than the Europeans, adjusting for these factors did not change the key study outcomes: difference in  $VO_{2max}$  remained the most important factor associated with South Asians greater insulin resistance after adjustment for these potential confounders. However, the study does have limitations. By nature of the recruitment methods, the study cohort represents a self-selected group, and thus, a degree of caution is warranted in extrapolating the findings to the general UK South Asian population. However, as the findings with respect to fitness, physical activity, adiposity and metabolic variables in South Asians are fully consistent with the body of previously published data on this topic, this seems unlikely to have introduced substantial bias into the findings. Insulin resistance was assessed using  $HOMA_{IR}$ , as the size of this single centre study made use of the gold-standard euglycaemic hyperinsulinaemic clamp unfeasible. Similarly, glycaemia was assessed from fasting glucose concentrations: HbA1c was not chosen as a marker of long-term glycaemia in this analyses because of potential confounding from known ethnic differences in glycation rates independent of glycaemia (253). Data were not collected on the specific country of origin for South Asian (i.e. whether from India, Pakistan, Bangladesh or Sri Lanka) participants, due to questionnaire design and it is recognised that whilst collectively South Asians are at increased risk of cardiometabolic disease, the degree of increased risk factor burden may not be equivalent between the contributory backgrounds (254). Finally, the cross-sectional nature of the study,

with simultaneous assessment of exposure and outcome variables, means that it is not possible to definitively exclude reverse causality as a potential influence of the findings.

In conclusion, the present findings show that **low cardiorespiratory fitness is the single most important factor associated with the increased insulin resistance and fasting glycaemia in middle-aged South Asian**, compared to European, men living in the UK. Although they were less physically active than their European counterparts, South Asians' lower fitness levels could not be explained by their lower physical activity levels, suggesting that low fitness is an innate feature of the South Asian phenotype. Though the observational nature of this study means that conclusions cannot be drawn about causality, present data suggest that South Asians may need to engage in higher levels of physical activity than Europeans to overcome their lower innate fitness and the associated metabolic consequences. This raises the possibility that ethnicity-specific recommendations for physical activity, and thus prevention of T2DM, may be needed.

## Chapter 4

### Does liver fat contribute to the increased insulin resistance in middle-aged South Asian compared to European men living in the United Kingdom?

#### Introduction

South Asians living in the United Kingdom have a 3-5 fold increased prevalence of T2DM, developing the disease around a decade earlier and at a (BMI) compared to white Europeans (25). Furthermore, non-diabetic South Asians have higher fasting glycaemia and are more insulin resistant than Europeans (47).

Liver fat is associated with insulin resistance and T2DM risk and is considered to play a causal role in diabetes (128). Limited data suggest that South Asians have higher liver fat content than age- and BMI-matched Europeans (68;141), but it is not currently clear whether this contributes to the observed ethnic difference in insulin resistance. The purpose of this chapter was (a) to determine whether South Asian men have increased liver fat percentage compared to white European men living in the UK and (b) to determine the extent to which such differences could explain the increased insulin resistance observed in South Asian compared to European men.

#### 4.2 Methods

##### 4.2.1 Participants and recruitment

All participants (28 South Asians, 24 Europeans) were recruited from the wider CURVES study and screened as detailed in section 2.1 and were chosen at random (but matched for age) within 4 months of their original main study visit. It was intended to recruit equal numbers of participants from each ethnic group. Specific inclusion and exclusion criteria are defined in section 2.1.1. All participants received payment for towards transport and received feedback as detailed in sections 2.2.1 and 2.6 respectively.

Participants' health history, including smoking status and family health history, education history SES information and dietary information were obtained as described in section 2.2.1

#### **4.2.2 Blood biochemistry and blood pressure measurements**

Venous blood samples were obtained after an overnight fast of 10-12 hours as described in section 2.2.2.1. Samples were sent for routine tests outlined in section 2.2.2.1. Plasma was stored for the subsequent measurement of insulin as described in section 2.2.2.2. Insulin resistance was assessed by  $HOMA_{IR}$  and was calculated as outlined in section 2.5.

Blood pressure was measured as described in section 2.2.4.

#### **4.2.3 Anthropometry**

Anthropometric measurements were performed on all participants as detailed in section 2.2.6. Total body lean and fat mass was measured using ADP as described in section 2.2.7.

#### **4.2.4 Magnetic resonance spectroscopy for quantification of liver fat**

MR spectroscopy technique and subsequent quantification of liver fat were undertaken as detailed in section 2.2.10.

#### **4.2.5 Data analysis**

The sample size for this study was determined as calculated in section 2.3.2. Statistical analysis was performed using R for Windows v2.14. Values are presented as mean (SD) for normally distributed and log transformed variables with p-values calculated by t-tests and median (IQR) for non-normally distributed

variables with p-values calculated by Wilcoxon test. Linear regression modelling was undertaken to determine the extent to which differences in insulin resistance (calculated by Homeostasis Model Assessment estimated insulin resistance, HOMA<sub>IR</sub> (245)) between ethnic groups could be explained by differences in liver fat content. Outcome variables were log transformed for analysis to better meet the assumption of normally-distributed model residuals.

### 4.3 Results

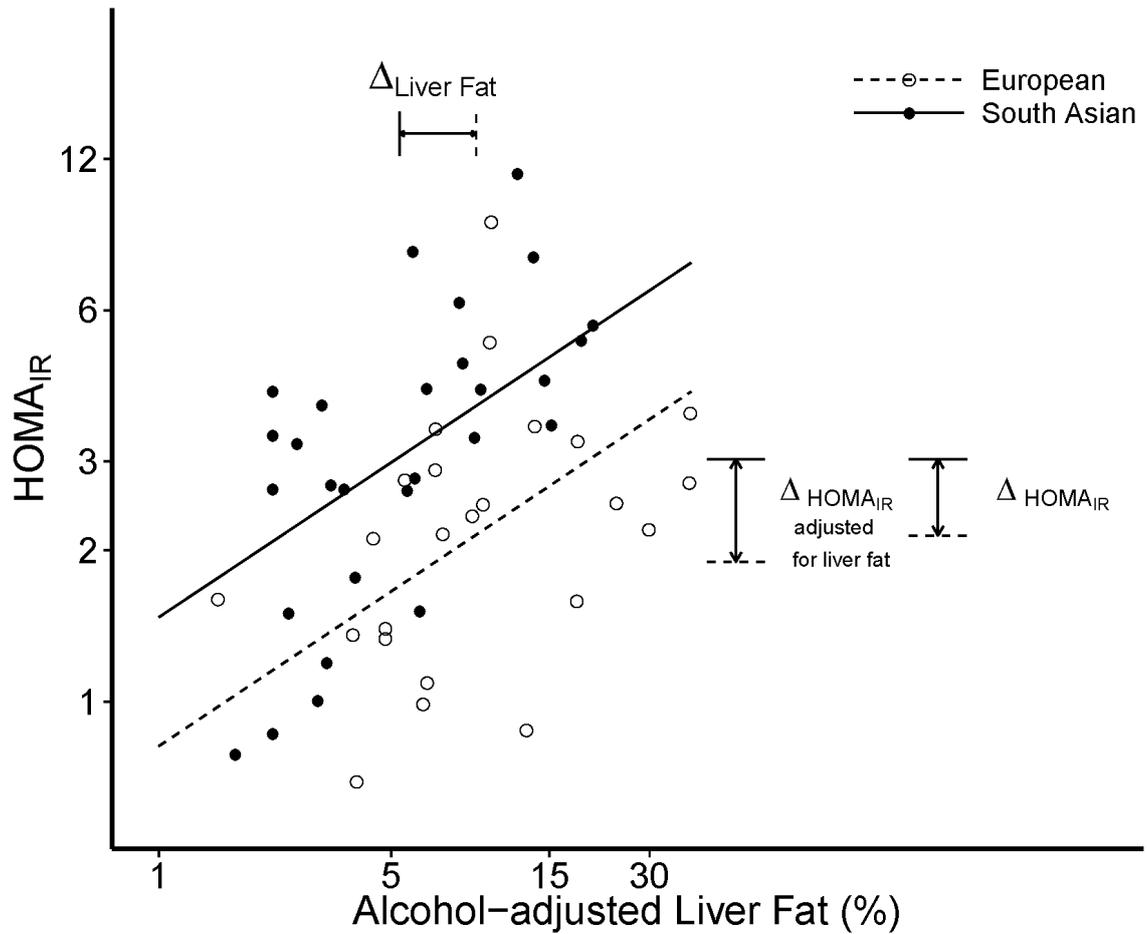
Baseline demographic, metabolic and anthropometric variables for the two ethnic groups are presented in Table 4.1. The two groups were well-matched for age and BMI. Waist circumference was numerically, but not significantly, higher in South Asians than Europeans. South Asians had higher HbA1c, fasting insulin and HOMA<sub>IR</sub> than Europeans, although fasting glucose concentrations were similar. South Asians also had a lipoprotein profile consistent with greater insulin resistance with lower HDL cholesterol concentrations, but similar LDL cholesterol concentrations. However, liver fat levels did not differ significantly between South Asians and Europeans. As reported, alcohol consumption differed markedly between South Asians than Europeans: only one Asian in the sample consumed alcohol and, in contrast, only two Europeans did not. After adjusting for alcohol consumption, to correct for potential confounding effects, the liver fat percentage in South Asians was 41% (10% to 62%; p=0.016) lower than in Europeans. Liver enzyme concentrations were similar in South Asians and Europeans.

**Table 4.1 Demographic, metabolic and anthropometric variables for South Asian and European men**

	South Asian (N=28)	European (N=24)	p-value
<b><i>Demographic, anthropometric and lifestyle variables</i></b>			
Age (years)	49.8 (8.2)	50.3 (5.9)	0.799
BMI (kg.m <sup>-2</sup> )	28.5 (4.8)	27.4 (3.2)	0.314
Waist (cm)	101.1 (12.5)	96.8 (7.3)	0.148
Waist-to-hip ratio	0.99 (0.07)	0.97 (0.05)	0.233
Percentage body fat	29.3 (8.2)	25.7 (6.7)	0.010
Alcohol Consumption (grams per day)	0.16 (0.85)	21.19 (13.25)	<0.001
<b><i>Metabolic variables</i></b>			
Glucose (mmol.l <sup>-1</sup> )	5.25 (4.90, 5.70)	5.00 (4.80, 5.55)	0.333 <sup>a</sup>
HbA1c (%)	5.60 (5.50, 5.83)	5.40 (5.28, 5.60)	0.010 <sup>a</sup>
HbA1c (mmol/mol)	38.0 (37.0, 40.3)	36.0 (33.8, 38.0)	0.010 <sup>a</sup>
Insulin (mU.l <sup>-1</sup> )	15.1 (9.7, 19.6)	10.1 (6.7, 12.3)	0.016 <sup>a</sup>
HOMA <sub>IR</sub>	3.36 (2.41, 4.44)	2.27 (1.39, 2.99)	0.027 <sup>a</sup>
Total Cholesterol (mmol.l <sup>-1</sup> )	5.33 (0.83)	5.39 (0.98)	0.80
HDL-Cholesterol (mmol.l <sup>-1</sup> )	1.11 (0.17)	1.27 (0.24)	0.010
LDL-cholesterol (mmol.l <sup>-1</sup> )	3.49 (0.71)	3.44 (0.84)	0.806
Triglycerides (mmol.l <sup>-1</sup> )	1.35 (0.98, 2.00)	1.20 (0.93, 1.68)	0.473 <sup>a</sup>
AST (U.l <sup>-1</sup> )	22.0 (19.0, 26.3)	23.5 (20.0, 28.5)	0.265 <sup>a</sup>
ALT (U.l <sup>-1</sup> )	25.5 (20.5, 36.0)	26.5 (21.8, 44.0)	0.538 <sup>a</sup>
GGT (U.l <sup>-1</sup> )	27.0 (19.0, 35.5)	28.0 (20.8, 45.3)	0.441 <sup>a</sup>
Systolic blood pressure (mm Hg)	123.9 (11.9)	129.3 (12.4)	0.111
Diastolic blood pressure (mm Hg)	76.5 (7.0)	76.8 (6.9)	0.490
<b><i>Liver fat</i></b>			
Unadjusted liver fat (%) <sup>b</sup>	5.28 (2.11)	5.41 (2.35)	0.913
Alcohol adjusted liver Fat (%) <sup>b</sup>	5.30 (2.10)	9.03 (2.22)	0.017

Values are mean (SD) for normally distributed variables with p-values calculated by t-tests and median (IQR) for non-normally distributed variables with p-values calculated by Wilcoxon test<sup>a</sup>. Geometric means presented (p-values calculated using t-tests on log means)<sup>b</sup>.

Figure 4.1 shows the relationship between alcohol-adjusted liver fat content and HOMA<sub>IR</sub> in South Asians and Europeans. There was a positive association between adjusted liver fat percentage and HOMA<sub>IR</sub> with both ethnic groups, with no significant ethnicity-liver fat interaction. Adjusted liver fat and ethnicity together explained 31% of the variance in HOMA<sub>IR</sub> (ethnicity alone explained 5.6% of the variance in HOMA<sub>IR</sub>). There was a significant offset in the regression lines: for any given alcohol-adjusted liver fat percentage HOMA<sub>IR</sub> was 80% (31% to 149%;  $p < 0.001$ ) higher in Asian men compared to Europeans. Adjustment for alcohol-adjusted liver fat percentage did not attenuate, and in fact slightly increased, the difference in HOMA<sub>IR</sub> between ethnic groups (see Figure 1).



**Figure 4.1 Relationship between HOMA<sub>IR</sub> and alcohol-adjusted liver fat in South Asian and European men**

Solid circles and the solid line represent South Asian men with open circles and the dotted line representing European men. Solid and dotted vertical bars indicate mean liver fat percentages (adjusted to zero alcohol consumption) in SA and European men, respectively; the horizontal arrow shows the mean difference in liver fat percentage between ethnic groups. Solid and dotted horizontal bars, with corresponding vertical arrows indicate mean log HOMA<sub>IR</sub> values in South Asian and European men and the mean ethnic difference, both unadjusted and adjusted for liver fat.

#### 4.4 Discussion

The main finding of this chapter is that, despite having a phenotype consistent with greater insulin resistance, middle-aged South Asian men did not have greater percentage liver fat than Europeans of similar age and BMI. These results indicate that while increasing levels of liver fat were associated with increasing insulin resistance in both South Asians and Europeans - consistent with the body of evidence showing clear associations and a potential mechanistic link between liver fat and insulin resistance (128) - South Asians were markedly more insulin resistant than Europeans for any given level of liver fat. No previous study has directly investigated whether increased liver fat levels can explain the greater insulin resistance, but previous studies have reported that South Asians have higher levels of liver fat compared to Europeans (68;141). The present data suggest that, while some South Asians may have greater levels of liver fat than Europeans, increased percentage liver fat is not a consistent feature of the South Asian phenotype. Because of clear differences in alcohol consumption between the European and South Asian groups, we adjusted our data for the potential confounding effects of alcohol on liver fat levels. However, our findings were robust to this effect: adjusting for alcohol consumption acted to reduce, rather than increase, liver fat levels in South Asians, relative to Europeans. Thus, factors other than increased liver fat *per se* must be responsible for South Asians' greater insulin resistance. Data from the previous chapter suggest that differences in cardiorespiratory fitness as well as previous local data suggesting that the capacity of skeletal muscle to oxidase fat are also likely to play a key role (47). It is also possible that differences in ectopic fat in the pancreas may contribute to greater dysglycaemia- novel data indicate that pancreatic fat is more influential than liver fat in the dysglycaemic process (255). Finally, it remains possible that a given level of liver fat may be more metabolically toxic in South Asians than Europeans, a suggestion which requires further study.

In conclusion, these data indicate that although clear associations between liver fat percentage and insulin resistance were observed in South Asians and in Europeans, increased insulin resistance in South Asians cannot be explained by a greater liver fat percentage.

## Chapter 5

### Differences in ultrasound-based measures of carotid atherosclerosis between middle-aged South Asian compared to European men living in the United Kingdom

#### 5.1 Introduction

Migrant South Asian populations in Europe, North America the Westernised countries have a greater CVD risk than their respective indigenous populations (8). Several studies have shown that both overall and premature (i.e. 10 years earlier and age <40) CHD morbidity and mortality is significantly higher in migrant South Asians than in white populations in the UK and globally (9;12;13), with there being little change over a 30-year period (11). Similarly, deaths from cerebrovascular disease remain in excess compared to White Europeans for migrant South Asian populations (11).

The increased CHD burden in migrant South Asians is not fully explained by traditional CVD risk factors, such as total cholesterol, blood pressure, smoking and diabetes (14-18), though South Asians have a more atherogenic lipoprotein profile (22;23). Prospective data from one study showed that the age-adjusted HR for male CHD mortality in South Asians to be 1.64 (95% CI, 1.24-2.16), compared with white Europeans, increasing to 2.14 (2.14 (95% CI, 1.56-2.94) after adjustment for smoking and cholesterol (14). Further analysis using multivariable models adjusting for conventional risk factors and T2DM and/or impaired glucose regulation, features of insulin resistance, or the metabolic syndrome showed that CHD mortality remained significantly higher in South Asian men (HR 1.6-1.9). In other words, current risk models do not account for the excess CVD risk in South Asians. Thus identification of other markers to identify the increased CVD risk in South Asians is a pressing clinical concern. Indeed, in recognition of the increased ethnicity-associated CVD risk, one of the latest risk scores that has been developed to estimate a patient's 10 year risk of CVD based on certain key known risk factors in England and Wales, incorporates ethnicity into the equation (256).

Carotid intima media thickness is recognised as a surrogate risk factor for coronary heart disease (159-164) and stroke (161;162;164-166). A key strength of this method is the availability of semi-automated software to read images and measure cIMT (170), making it a rapid, accurate and potentially reproducible screening modality. Currently there are no published prospective longitudinal data on cIMT and CVD outcomes on South Asians and only one cross-sectional study comparing cIMT between South Asians and Europeans from the same population (173). This study indicated that cIMT was not higher in South Asians compared with Europeans, despite South Asians having a higher number CVD events, suggesting that absolute cIMT values may not be a sensitive marker of excess CVD risk in South Asians.

Two-dimensional assessment of carotid plaque has also been used as a modality to facilitate CVD risk stratification and is also associated with future risk of MI and stroke (183-185;257) Furthermore, when incorporated into risk stratification models - as is the case for cIMT - carotid plaque presence/absence appears to provide enhanced risk classification (156;187). Thus, both cIMT and carotid plaque assessment may have a role when considering patients for preventative medical therapy (156).

In addition, one cross-sectional study which compared age-related cIMT differences, with differences in carotid plaque disease between population groups of two socioeconomic extremes, demonstrated that differences between affluent and deprived populations in plaque score were apparent earlier in the life-course (~10 years) than differences in cIMT (186). This suggests that plaque score may be a more sensitive marker of deprivation associated CVD risk than cIMT. Neither adjustment for traditional cardiovascular risk factors nor measured 'novel' risk factors, either alone or in combination, abolished the area-level deprivation-based difference in plaque presence. These intriguing data suggest the possibility that plaque score be a more sensitive measure of CVD risk than cIMT and therefore may reveal a difference in CVD risk between South Asians and Europeans not evident from cIMT measures.

There are no published data comparing carotid plaque presence between Europeans and South Asians from the same population. Thus the aims of this

chapter are to determine: (i) whether South Asians have a difference in cIMT or carotid plaque presence compared to Europeans; and if so, (ii) whether differences carotid plaque presence between South Asians and Europeans occur at an earlier age than differences in cIMT and (iii) to determine whether any measured risk factors (if any) could account for any such observed differences in cIMT and/or carotid plaque presence.

## **5.2 Methods**

### **5.2.1 Participants and recruitment**

All participants were recruited and screened as detailed in section 2.1. Specific inclusion and exclusion criteria are defined in section 2.1.1. All participants received payment for towards transport and received feedback as detailed in sections 2.2.1 and 2.6 respectively.

Participants' health history, including smoking status and family health history, education history, SES information and dietary information were obtained as described in section 2.2.1.

### **5.2.2 Carotid ultrasound scanning and analysis**

The scanning protocol used was as described in section 2.2.3.2. The author performed all the scans and met predefined reproducibility criteria for scan quality as described in section 2.2.3.3. All scans for cIMT measurements were analysed as described in sections 2.2.3.5 using software as described in section 2.2.3.3 by a trained reader (GL) as described in section 2.2.3.4

All scans for plaque scoring were analysed by KD, a trained individual (as described in section 2.2.3.1), who was blinded to the participant group, using the software described in section 2.2.3.3 to view data and was blinded to the identities of the participants. The criteria applied for plaque identification and scoring was as described in section 2.2.3.6.

### **5.2.3 Anthropometry**

Anthropometric measurements were performed on all participants as detailed in section 2.2.6. Total body lean and fat mass was measured using ADP as described in section 2.2.7.

### **5.2.4 Blood biochemistry and blood pressure measurements**

Venous blood samples were obtained after an overnight fast as described in section 2.2.2.1. Samples were sent for routine tests as detailed in section 2.2.2.1. Plasma was stored for the subsequent measurement of insulin as described in section 2.2.2.2. HOMA<sub>IR</sub> was calculated as outlined in section 2.5.

Blood pressure was measured as described in section 2.2.4.

### **5.2.5 Determination of cardiorespiratory fitness**

All participants had a clinical assessment and resting ECG performed prior to undertaking the exercise test to ensure no contraindications to maximal exercise. Exercise testing was undertaken as described in section 2.2.8. Cardiorespiratory fitness was determined by measuring  $VO_{2max}$  as described in sections 2.2.8.5 and 2.2.8.6. A valid measure of  $VO_{2max}$  was achieved in 199 of the 200 participants.

### **5.2.6 Measurement of physical activity**

Objective measurement of physical activity was obtained through accelerometry as described in section 2.2.9. Valid data were obtained for 85 South Asians and 84 Europeans.

### 5.2.7 Data analysis

The sample size for the CURVES study was determined as calculated in section 2.3.1.

Summary statistics are presented for all variables for both South Asians and Europeans. The dataset for this chapter include the full cohort (100 South Asians and 100 Europeans) recruited into the CURVES study. Continuous variables were compared between the ethnic groups by t-tests (or Wilcoxon rank sum test for non-normally distributed variables) and Chi Square test for categorical variables.

Factor analysis was used to reduce the number of anthropometric variables into underlying latent factors as described in section 3.2.6. Linear regression was used to model cIMT and logistic regression for modelling the predictors of plaque presence. Missing values were removed from the relevant analyses. The difference in the predicted probability of plaques between South Asians and Europeans was calculated across the age range with a 95% CI derived by bootstrapping. The odds of a South Asian having carotid plaque compared to Europeans are presented, with 95% CI and p-value, when ethnicity was the only predictor in the model and when adjusted for participant age together with select demographic, lifestyle and CHD risk factor variables. Logistic regression modelling and ORs are presented for adjusted and unadjusted models. A composite group of predictors was selected using backwards elimination on each subgroup, to select variables significant at the 10% level for the final model.

Statistical analyses were undertaken by DP and AM in consultation with the author and his supervisors. The statistical software package R for Windows v2.14 was used for all analysis. Statistical significance was accepted at  $p < 0.05$ .

## 5.3 Results

### 5.3.1 Demographic, metabolic and dietary variables

Baseline data for all 200 volunteers are presented in Table 5.1. There were no differences between groups in age or BMI, but South Asians were shorter than the Europeans. South Asians also had completed more years in education, were less likely to smoke or consume alcohol, and were more likely to have a parent or sibling with T2DM. There were no differences in SES. South Asians had significantly higher glucose, HbA1c, insulin, HOMA<sub>IR</sub>, CRP and diastolic blood pressure, and lower HDL-cholesterol, than the Europeans. Mean  $\pm$  SD reported energy intake (expressed per kg body mass) did not differ between the Europeans and SA ( $19.8 \pm 6.0$  vs  $18.7 \pm 6.4$  kcal.kg<sup>-1</sup>.day<sup>-1</sup>,  $p=0.21$ ). There were also no differences in reported protein, carbohydrate or fat intakes between the two groups (data not shown), but reported alcohol intake was higher in the Europeans than SA ( $21.9 \pm 18.3$  vs  $1.1 \pm 5.6$  g.day<sup>-1</sup>,  $p<0.0001$ ).

**Table 5.1 Demographic and metabolic variables for all South Asian and European men.**

		South Asian (N=100)	European (N=100)	p-value
<b>Demographic and lifestyle variables</b>				
Age (years)		49.4 (7.2)	49.7 (6.8)	0.755
Body mass (kg)		82.0 (12.1)	85.7 (13.9)	0.049
Height (m)		1.74 (0.06)	1.78 (0.06)	<0.0001
BMI (kg.m <sup>-2</sup> )		27.1 (3.9)	26.9 (4.2)	0.692
Years in Education		15.6 (3.5)	14.4 (3.2)	0.010
SIMD Quintile	1	9 (9.0%)	7 (7.0%)	0.620
	2	10 (10.0%)	14 (14.0%)	
	3	17 (17.0%)	19 (19.0%)	
	4	26 (26.0%)	18 (18.0%)	
	5	38 (38.0%)	42 (42.0%)	
Smoking Status	never-smoker	81 (81.0%)	54 (54.0%)	<0.0001
	ex-smoker	6 (6.0%)	36 (36.0%)	
	current	13 (13.0%)	10 (10.0%)	
Alcohol Consumption <sup>b</sup> (units per week)	none	93 (93.0%)	10 (10.1%)	<0.0001
	≤ 20	5 (5.0%)	70 (70.7%)	
	≥ 21	2 (2.0%)	19 (19.2%)	
Parental Diabetes Status <sup>b</sup>	yes	54 (54.0%)	13 (13.1%)	<0.0001
Sibling Diabetes Status <sup>b</sup>	yes	17 (17.0%)	2 (2.0%)	0.0004
<b>Metabolic variables</b>				
Glucose (mmol.l <sup>-1</sup> )		5.4 (5.0, 5.8)	5.1 (4.8, 5.4)	0.0005 <sup>a</sup>
HbA1c (%) <sup>c</sup>		5.7 (5.5, 6.1)	5.4 (5.2, 5.6)	<0.0001 <sup>a</sup>
HbA1c (mmol.mol <sup>-1</sup> ) <sup>b</sup>		39.0 (37.0, 43.0)	36.0 (33.0, 38.0)	<0.0001 <sup>a</sup>
Insulin (mCU.l <sup>-1</sup> ) <sup>b</sup>		14.9 (7.4)	9.2 (5.3)	<0.0001
HOMA <sub>IR</sub> <sup>b</sup>		3.3 (2.4, 4.6)	1.8 (1.1, 2.7)	<0.0001 <sup>a</sup>
Total Cholesterol (mmol.l <sup>-1</sup> )		5.3 (0.9)	5.6 (1.0)	0.053
HDL-Cholesterol (mmol.l <sup>-1</sup> )		1.1 (1.0, 1.3)	1.3 (1.0, 1.3)	<0.0001 <sup>a</sup>
LDL-cholesterol (mmol.l <sup>-1</sup> )		3.4 (0.9)	3.6 (0.8)	0.457
Triglycerides (mmol.l <sup>-1</sup> )		1.7 (1.1)	1.5 (0.9)	0.0423 <sup>a</sup>
AST (U.l <sup>-1</sup> )		23.8 (7.6)	25.1 (8.5)	0.242
ALT (U.l <sup>-1</sup> )		27.0 (22.0, 36.2)	26.0 (20.0, 35.2)	0.758 <sup>a</sup>
GGT (U.l <sup>-1</sup> )		32.5 (22.0, 47.0)	28.0 (20.8, 42.8)	0.261 <sup>a</sup>
CRP (mg.l <sup>-1</sup> ) <sup>c</sup>		1.8 (1.1, 3.8)	1.2 (0.6, 2.5)	0.0028 <sup>a</sup>
Systolic blood pressure (mm Hg)		126.8 (15.0)	127.6 (12.8)	0.686
Diastolic blood pressure (mm Hg)		78.2 (8.9)	74.9 (7.2)	0.004

Values are mean (SD) for normally distributed variables with p values calculated by t-tests and median (IQR) for non-normally distributed variables with -values calculated by Wilcoxon test<sup>a</sup>. Categorical variable p values are calculated by Fisher's Exact test. <sup>b</sup>n = 99 for South Asians and Europeans respectively.

### 5.3.2 Body composition, fitness and physical activity variables

Body composition, fitness and physical activity variables for all 200 volunteers are presented in Table 5.2. There were no differences in waist or hip circumferences between the South Asian and European groups, but WHR was higher in the South Asians. South Asians also had a smaller mid-thigh circumference, and higher values for all skinfold thicknesses than the Europeans. Lean mass was lower and fat mass and percentage body fat were higher in the South Asians. Total adiposity and central adiposity factors were higher and body size units lower in South Asians than Europeans. Cardiorespiratory fitness (as assessed by  $VO_{2max}$ ) was lower in South Asians than Europeans.

South Asians also engaged in less moderate and vigorous physical activity than Europeans: the proportion of time spent in moderate-to-vigorous physical activity was ~29% lower in South Asians than Europeans. This equated to South Asians engaging in ~22 minutes of MVPA per day compared to ~31 minutes per day for the Europeans.

**Table 5.2 Body composition, fitness, and physical activity variables for all South Asian and European men.**

	South Asian (N=100)	European (N=100)	p-value
<b>Body composition variables</b>			
<b>(a) Body circumferences</b>			
Waist (cm)	97.8 (10.9)	95.7 (11.2)	0.183
Hips (cm)	99.4 (6.6)	100.5 (7.1)	0.240
Waist-to-hip ratio	0.98 (0.07)	0.95 (0.07)	0.0009
Mid-upper arm (cm)	34.3 (7.7)	33.4 (3.3)	0.289
Mid-thigh (cm)	52.4 (4.2)	53.9 (4.0)	0.011
Mid-calf (cm)	37.6 (3.3)	38.6 (4.0)	0.063
<b>(b) Skinfold thicknesses</b>			
Biceps (mm) <sup>l</sup>	8.0 (3.7)	6.8 (2.9)	0.0120
Triceps (mm)	16.0 (5.7)	12.6 (4.2)	<0.0001
Subscapular (mm) <sup>g,1</sup>	25.5 (7.0)	18.3 (7.1)	<0.0001
Suprailiac (mm) <sup>f,1</sup>	23.2 (6.1)	18.3 (6.3)	<0.0001
Supraspinale (mm)	17.8 (6.4)	12.6 (5.4)	<0.0001
Thigh (mm) <sup>l</sup>	18.6 (7.8)	14.7 (5.7)	0.0001
Calf (mm) <sup>e</sup>	12.6 (5.2)	10.4 (4.2)	0.0011
<b>(c) Fat and lean mass<sup>l</sup></b>			
Lean mass (kg)	57.1 (6.2)	63.1 (7.2)	<0.0001
Fat mass (kg)	24.5 (9.0)	21.8 (9.7)	0.044
Percentage body fat	29.3 (7.4)	24.9 (7.4)	<0.0001
<b>(d) Summary anthropometric factors</b>			
Sum of arm (mm) <sup>p</sup>	23.9 (8.8)	19.2 (6.5)	<0.0001
Sum of central area (mm) <sup>c</sup>	65.0 (16.9)	48.8 (16.6)	<0.0001
Sum of leg (mm) <sup>d</sup>	31.2 (11.8)	25.0 (8.9)	<0.0001
<b>Fitness variables</b>			
VO <sub>2 max</sub> (l.min <sup>-1</sup> ) <sup>l</sup>	2.52 (0.43)	3.33 (0.63)	<0.0001
VO <sub>2 max</sub> (ml.kg <sup>-1</sup> .min <sup>-1</sup> ) <sup>l</sup>	31.2 (5.8)	39.4 (7.8)	<0.0001
VO <sub>2 max</sub> (ml.kg lean mass <sup>-1</sup> .min <sup>-1</sup> ) <sup>j</sup>	44.2 (6.3)	52.8 (8.0)	<0.0001
<b>Physical activity variables<sup>h,1</sup></b>			
Sedentary (% of wear time)	66.3 (10.0)	64.3 (9.0)	0.185
Light physical activity (% of wear time)	30.6 (9.6)	30.8 (8.0)	0.901
Moderate-to-vigorous physical activity (% of wear time)	2.8 (1.6, 4.2)	4.1 (3.0, 6.8)	<0.0001 <sup>a</sup>
Total accelerometer wear time (min.day <sup>-1</sup> )	813.4 (90.6)	864.0 (74.4)	0.0001

Values are mean (SD) for normally distributed variables with p-values calculated by t-tests and median (IQR) for non-normally distributed variables with p-values calculated by Wilcoxon test<sup>a</sup>. Categorical variable p-values are calculated by Fisher's Exact test. <sup>b</sup>Derived from biceps, triceps, subscapular, supraspinale, suprailiac, thigh, and calf skinfolds, and fat mass; <sup>c</sup>derived from height, lean mass, and hip, mid-thigh and mid-calf circumferences; <sup>d</sup>derived from waist circumference and waist-to-hip ratio. <sup>e</sup>n = 99, <sup>f</sup>n = 97, <sup>g</sup>n = 94, <sup>h</sup>n = 85 for South Asians; <sup>i</sup>n = 99, <sup>j</sup>n = 98, <sup>k</sup>n = 97 and <sup>l</sup>n = 84 for Europeans;

### 5.3.3 Carotid ultrasound analysis

Differences in ultrasound markers of atherosclerosis for are presented in Table 5.3. There were no significant differences in unadjusted or age-adjusted differences in mean cIMT between South Asians and Europeans. Numerically there was increased odds ratio for the presence of plaque disease in South Asians compared to Europeans, however this was not statistically significant (OR 1.57, 95% CI 0.89-2.77, p=0.13).

**Table 5.3 Differences in cIMT and plaque scores between South Asian and European men.**

	South Asian N=100	European N=100	South Asian - European (adjusted for age)	p-value
<b><i>cIMT analysis</i></b>				
Mean (SD) of cIMT	0.64 (0.16)	0.65 (0.12)	-0.01 (-0.05, 0.03)*	0.638
<b><i>Plaque Analysis</i></b>				
Median (IQR) of plaque score	0.00 (0.00, 1.00)	0.00 (0.00, 1.00)		0.194**a
0 plaques	52 (52.0%)	63 (63.0%)		
1 to 2 plaques	38 (38.0%)	29 (29.0%)		0.161**b
>2 plaques	10 (10.0%)	8 ( 8.0%)		

\*95% CI; \*\*not adjusted for age; <sup>a</sup>Wilcoxon test, <sup>b</sup>Chi-squared test for trend

Figure 5.1 shows the differences in cIMT for each ethnic group, separately split by three consecutive age decades. The expected trend of increasing mean cIMT with age was present for each ethnic group; however, there were no differences in mean cIMT between the ethnic groups in any age group. In regression analysis cIMT increased by 0.08 mm (95% CI: 0.06, 0.11; p<0.001) for a ten year increment in age, with a similar increase observed for each ethnic group (p-value for ethnicity by age interaction = 0.177). Figure 5.2 shows the carotid plaque distribution by age. There was a significant linear age-association for plaque presence in Europeans only (p=0.006).

As expected, with both ethnic groups combined, cIMT was strongly correlated with plaque presence (odds ratio (OR) 1.35 per 0.1mm increase, 95% CI, 1.01-1.67,  $p=0.005$ ), and this association remained after adjustment for age (OR 1.27 per 0.1mm increase, 95% CI, 1.01-1.60,  $p=0.042$ ); and there was no significant ethnicity interaction ( $p=0.755$ ). Both systolic and diastolic blood pressure were significantly associated (per 10mm Hg increase) with cIMT even after adjustment for age and BMI and antihypertensive treatment for the cohort as a whole (systolic blood pressure 0.03 mm increase in cIMT, 95% CI, 0.02-0.05,  $p<0.001$ ; diastolic blood pressure 0.05 increase in cIMT 95% CI, 0.03,-0.08  $p<0.001$ ) and there was no significant ethnicity interaction (systolic blood pressure  $p=0.642$ , diastolic blood pressure  $p=0.087$ ).

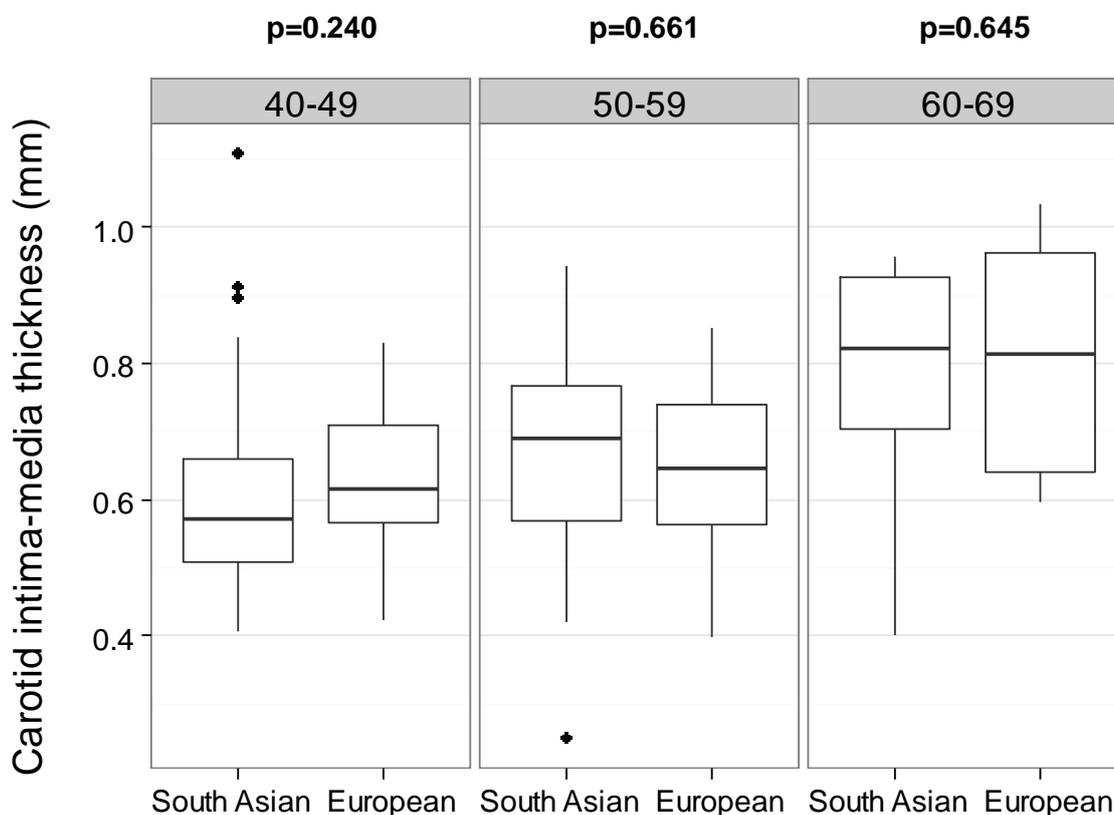
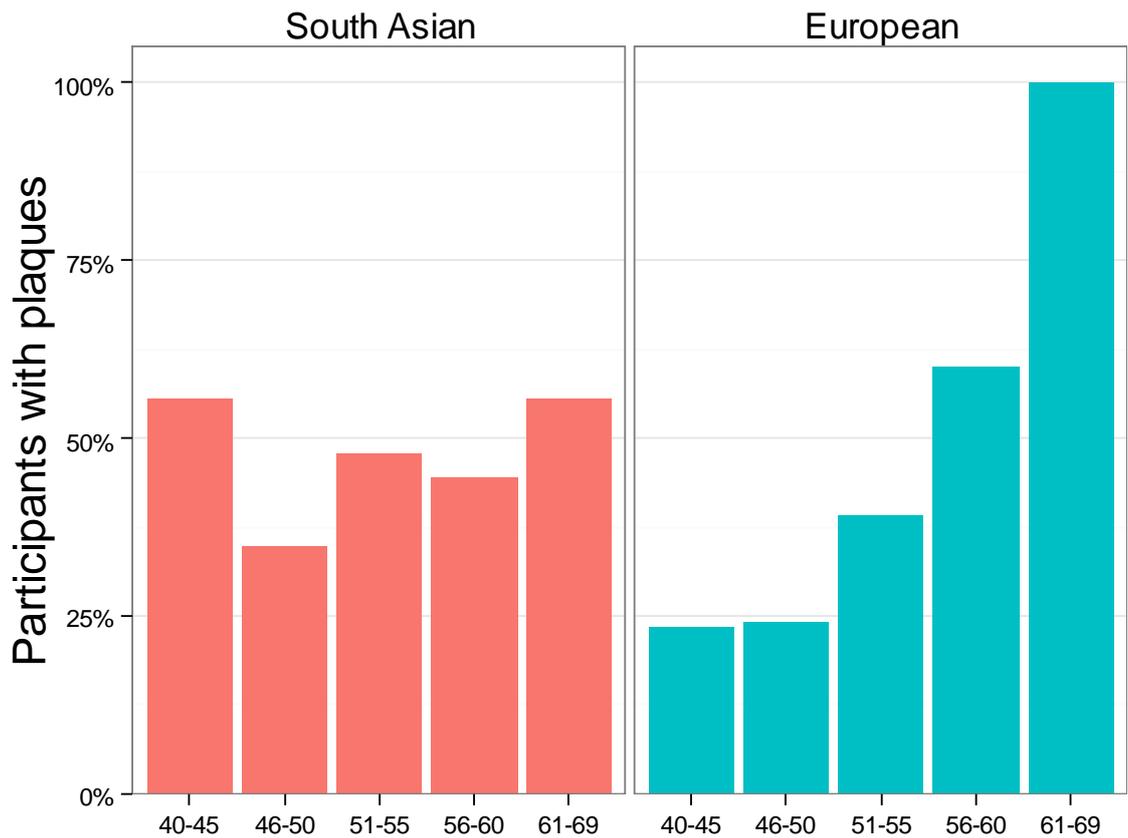


Figure 5.1 Carotid intima-media thickness by age tertile and ethnicity



**Figure 5.2 Presence of carotid plaque by ethnicity and age\***

\*Percentage of participants within each category

### 5.3.4 Univariate and multivariate analyses for plaque presence

Table 5.4 presents univariate and multivariable regression analyses showing the effects of groups of variables on the odds of plaque presence between South Asians compared to Europeans. All models are adjusted for age (quadratic, at age 50), ethnicity and their interaction. Plaque presence rather than plaque score was used as the dependent variable in these analyses because plaque score did not fit conventional distributions that might be used for regression analyses and it was decided that the binary approach to transformation would encompass most of the information in the data. The unadjusted odds for plaque presence was 57% (95% CI: -11% to 177%) higher in South Asians compared to Europeans. Further adjustment for age and ethnicity and their interaction increased the OR to 1.88 (95% CI: -17% to 327%), but this was not significant ( $p=0.130$ ). Addition of traditional CVD risk factors to the age- and ethnicity-adjusted model did not

attenuate the OR, and this remained the case even after further adjustment for fasting glucose and/or insulin resistance. The final model (which consisted of significant predictors selected from variable subgroups by backward selection) included ethnicity and age (quadratic) interaction and HDL cholesterol and attenuated the OR to 1.30 (95% CI: -33% to 327%) but this was not significant (p=0.097)

**Table 5.4 Adjusted ORs for plaque presence between South Asians and Europeans**

	Odds Ratio(95% CI)	p-value
Unadjusted	1.57 (0.89, 2.77)	0.116
Age <sup>†</sup> (quadratic) at 50 years	1.88 (0.83, 4.27)	0.130
BMI	1.89 (0.83, 4.30)	0.129
Skinfold Measures <sup>a</sup>	1.88 (0.81, 4.36)	0.138
Body size <sup>b</sup>	1.96 (0.83, 4.60)	0.120
Central fats <sup>c</sup>	1.83 (0.80, 4.19)	0.150
Combined adiposity measures <sup>d</sup>	2.09 (0.82, 5.37)	0.122
Smoking	1.95 (0.83, 4.58)	0.125
Smoking + Systolic blood pressure + cholesterol / HDL ratio	1.77 (0.74, 4.24)	0.200
Classic risk factors <sup>e</sup>	1.90 (0.76, 4.73)	0.167
Insulin resistance	1.67 (0.70, 3.96)	0.246
Classic risk factors + insulin resistance	1.77 (0.69, 4.54)	0.232
Classic risk factors + fasting glucose	1.83 (0.71, 4.71)	0.208
Classic risk factors + insulin resistance + glucose	1.76 (0.68, 4.56)	0.243
Metabolic risk factors <sup>f</sup>	1.60 (0.67, 3.80)	0.288
Novel risk factors <sup>g</sup>	1.89 (0.82, 4.35)	0.131
Moderate to vigorous physical activity	1.89 (0.75, 4.76)	0.176
VO <sub>2max</sub> <sup>*</sup>	1.83 (0.74, 4.56)	0.190
Final Model <sup>h**</sup>	1.30 (0.67, 2.53)	0.097

Table shows the relative effect of ethnicity, adjusted for age (quadratic) and their interaction, on the odds of plaques and further adjusted by the stated variables.

<sup>a</sup>Skinfold measures: Suprailiac, supraspinale, triceps, subscapular, thigh, calf, biceps and fat mass.

<sup>b</sup>Body Size: Lean mass, height, mid-thigh, mid-calf and hip circumference.

<sup>c</sup>Central Fats: Waist circumference and waist-to-hip ratio.

<sup>d</sup>Combined effect of a, b and c

<sup>e</sup>Traditional risk factors: Total cholesterol, HDL cholesterol, smoking status, systolic blood pressure and diastolic blood pressure

<sup>f</sup>Metabolic risk factors: HDL cholesterol, triglycerides and insulin

<sup>g</sup>Novel risk factors: CRP, AST, ALT and GGT

<sup>h</sup>Final model: Ethnicity and age (quadratic) interaction and HDL.

<sup>†</sup>: at age 50 years

\* ml.kg<sup>-1</sup>.min<sup>-1</sup>

\*\* Significant predictors selected from variable subgroups by backward selection.

## 5.4 Discussion

The main finding from this chapter confirmed that there was no difference in cIMT despite their being the expected differences in the usual cardiometabolic risk factors observed in middle-aged South Asian compared to European men living in the UK. The other finding from this study was that there was no significant difference in carotid plaque presence, though pre-defined subgroup analysis suggests South Asians may have more plaques at younger ages with apparently no obvious increase in prevalence in older South Asians. That noted, it could be that the results were inadvertently biased against seeing higher plaque load in older South Asians given that none of the participants could have demonstrable CVD; more South Asian men are known to have premature CVD (9), and thus proportionally more older South Asians would have been ineligible for the study. Clearly, these latter observations are speculative and hypothesis generating and larger studies are needed to examine this issue.

The finding that cIMT is not increased in South Asians is consistent with the one previous published study comparing South Asian men with those of European ancestry (173). However unlike data presented in this analysis, the earlier data combines healthy subjects with those who have CVD. Furthermore novel published data on cIMT measurements in children living in the UK showed that cIMT levels in South Asian children were similar to those of European children (258). These data in combination with this present analysis indicate that cIMT between South Asians and Europeans remains similar throughout life and hence may suggest that absolute cIMT values are not a sensitive marker for CVD risk in South Asians. Of interest, South Asians have narrower carotid artery diameter compared to Europeans, thus relative to vessel diameter, South Asians have a greater cIMT compared to Europeans (259) but the relevance of this finding is uncertain.

One possible explanation for the lack of difference in cIMT is that whilst atherosclerosis is chiefly an intimal process, It has been highlighted that IMT is approximately 80% media, and only 20% intima, therefore any measurements made, and any changes in cIMT are likely to be a reflection of changes in the media (177) thus a key determinant of cIMT is the effect of blood pressure which

is known to cause medial wall hypertrophy (178). Data from this present analysis show that South Asians have similar systolic blood pressure compared to Europeans and whilst South Asians have higher diastolic blood pressure, the mean values fall well within the normal range for healthy adults. Further, a systematic review of blood pressure in South Asians showed no significant difference in blood pressure between South Asians and Europeans (197).

The aim of this study was to investigate plaque presence/quantity and not carotid plaque morphology. Atherosclerotic plaque morphology/size has been indicated to be different between South Asians and Europeans, with South Asians having more aggressive morphology in coronary arteries for example (260;261). Thus the negative finding in this analysis should not be interpreted to indicate that South Asians are not more susceptible to atherosclerotic plaque or subsequent pathological manifestations from them.

This analysis has a number of specific strengths. To the author's knowledge, it is the first study measuring and comparing carotid plaque and cIMT between South Asian with European adults in the UK. The groups were well-matched for age, BMI and SES, and although the South Asians spent more years in education, smoked less and drank less alcohol than the Europeans, these factors were found not to significantly confound the key study outcomes. Furthermore, compared to the previous published data from Canada (which had a study population spanning a similar age-range), participants in this analysis were better matched for age and present clearer comparative data on individuals without CVD (173)

However, this study does have limitations. A degree of caution is warranted in extrapolating the findings to the general UK South Asian population. By nature of the recruitment methods, the study cohorts represented a self-selected groups owing to the exclusion criteria as mentioned earlier. This healthier South Asian cohort, may have resulted in an underpowering of the study if CVD risk was lower than in the average South Asian cohort. However, as the findings with respect to ethnic cIMT differences, fitness, physical activity, adiposity and metabolic variables in South Asians are fully consistent with the body of previously published data in this area, this suggests substantial bias is unlikely. That said, if the same exclusion criteria were to be applied, based on the data in

this study population in which South Asians had an unadjusted OR of 1.57 for plaque presence compared to Europeans, a sample size of 317 per group would be required to show a difference at 80% power at the 5% significance or 423 per group at 90% power. A more pragmatic approach if the study were to be repeated, would be to relax the inclusion criteria to include participants with known diabetes in subjects over the age of 50 given the premature onset and increased prevalence of diabetes of South Asians (25;28). An alternative to this could recruitment of the original sample size, but limit the age range from age 40-50 years, given the suggestive findings of increased plaque in this age group, the relative increased premature CVD burden in South Asian males and the clinical importance of identifying such individuals at increased. Further, the major weakness of cIMT measurement and 2 dimensional plaque scoring is that change over time is limited because plaque propagates longitudinally along the artery wall ~2.4 times quicker than it extends into the arterial lumen (262) and data suggest that South Asians may have longer atherosclerotic lesions (260;261). Validated three-dimensional (3D) ultrasound techniques exist and are demonstrated to possibly be more informative than existing 2D cIMT and plaque measures (189), which can detect earlier responses to intervention e.g. statin therapy over a six-month period(190) exist. However published long-term longitudinal data or indeed CVD outcome data based on 3D imaging of carotid arteries is lacking. Finally, the cross-sectional nature of the study, with simultaneous assessment of exposure and outcome variables, means that conclusions on causality cannot be definitively made or refuted.

In conclusion, this present study strongly reaffirms that cIMT is similar between South Asian and European men despite greater risk factors in the former group. They also suggest no overall difference in plaques in South Asians although there is a strong suggestion for greater plaques at younger ages, an observation which requires further investigation and extension to women. Of course, ideally, prospective studies linking plaques to outcomes in South Asians (particularly in younger South Asians) are need to investigate whether these measures help improve CVD risk prediction.

## Chapter 6

### General Discussion

#### 6.1 Experimental chapter summaries

The broad aim of this thesis was to further the understanding of the high risk cardiometabolic phenotype associated with South Asian men living in the UK. By undertaking this cross-sectional phenotyping study between middle-aged South Asian and European men, three interlinked aims were addressed in Chapters 3, 4 and 5 respectively.

The aims of Chapter 3 were to present comparative data on cardiorespiratory fitness, objectively-measured physical activity and other metabolic phenotypical data between South Asian and European men and to determine the extent to which differences in insulin resistance (as assessed by HOMA<sub>IR</sub>) and glycaemia between these ethnic groups can be explained by such factors. South Asians were 67% more insulin resistant than Europeans. Lower VO<sub>2max</sub>, lower physical activity and greater total adiposity in South Asian individuals explained 68%, 28% and 52%, respectively, and together explained 83% (all p<0.001), of the ethnic difference in HOMA<sub>IR</sub>. Thus lower cardiorespiratory fitness is the single most important factor explaining increased insulin resistance in middle-aged South Asian, compared to European, men living in the UK. Although they were less physically active than their European counterparts, South Asians' lower fitness levels could not be explained by their lower physical activity levels, suggesting that low fitness is an innate feature of the South Asian phenotype.

The aims of chapter 4 were to determine whether South Asian men had increased liver fat compared to white European men and the extent to which such differences can explain the increased insulin resistance observed in South Asian men. Unadjusted liver fat content did not differ significantly between both groups, but following adjustment for alcohol consumption was significantly lower in South Asians than Europeans. Adjustment for alcohol-adjusted liver fat did not attenuate the difference in HOMA<sub>IR</sub> between ethnic groups. Thus whilst clear associations between liver fat and insulin resistance were observed in South

Asians and Europeans, these results challenge the notion that excess liver fat *per se* explains the greater insulin resistance observed in South Asians.

The aims of chapter 5 were to present comparative data on cIMT and carotid plaque presence between South Asians and Europeans and to determine whether any age-related changes in differences in carotid plaque disease precede any age-related changes in differences in cIMT between ethnic groups and finally to determine what risk factors (if any) would account for any such observed differences in cIMT and/or carotid plaque presence. The analysis showed that mean cIMT was similar between South Asians and European men and there was a tendency for increased odds for the presence of plaque disease in South Asians compared to Europeans, particularly in younger South Asians.

## **6.2 Strengths of data**

The published data from this thesis represent the largest cohort of South Asians living in Scotland with this degree of cardiometabolic phenotyping for which there is a well-matched European comparator group. This study demonstrates the feasibility and potential success from recruiting from this sizeable minority ethnic population living in Scotland and moreover that good quality matching with European controls is also achievable. Further, as mentioned in Chapter 1, the published data represent the largest published study on measurements of fitness as well as the first published data on objectively measured physical activity in South Asian adult males. These large and novel data coupled with the storage of blood samples (for which ethical approval has been granted) allows for potential further metabolic and genetic analysis and phenotyping (as described later).

The results on liver fat comparisons between these two ethnic groups are important in contextualising the role of liver fat as a pathophysiological influence on the increased metabolic risk seen in South Asians. The findings in this thesis highlight that previous conclusions and hypotheses require further examination and thus should be interpreted more cautiously.

Finally the non-dissimilarity in markers of carotid artery atherosclerosis is an important negative finding as it indicates that such methods cannot be used with any degree of confidence in stratifying cardiac risk in South Asians. These findings highlight the need for further research in alternative screening methods for CVD which are more sensitive in identifying subclinical CVD.

### **6.3 Public health and clinical implications from current findings**

The demonstration that reduced cardiorespiratory fitness in South Asians compared to their European peers which simply cannot be explained by their reduced physical activity levels has important public health implications. As mentioned in section 3.4, the current physical activity guidelines are not ethnic-specific and thus may be inadequate to reduce risk of cardiometabolic disease in South Asians. Consequently increased prescribed physical activity levels may be necessary in South Asians to derive equivalent cardiometabolic benefit.

The results described in chapter 3 emphasise the importance to engaging with South Asians with lifestyle interventions even in preclinical cardiometabolic disease even from childhood as their inherent risk is excessive. Published data comparing South Asian and European Children living in the London area demonstrate that South Asian children have a higher-risk cardiometabolic phenotype (including behavioural patterns) (263-265). Thus if such behaviour is left unchanged, this may yet exacerbate cardiometabolic risk longer-term. Indeed very recent qualitative data from Scotland itself indicate that in order to get South Asian adults to undertake physical activity, then external motivators for doing so are important (266). These include undertaking physical activity as a means to an end, which included the opportunities that physical activity provided for social activity and enjoyment. The goals of weight reduction and improving mental and physical health and were also mentioned. Hence such motivational reasons need to be conveyed from a young age to maximise sustained positive lifestyle change.

The final reason as to why it is important to engage with South Asians with preclinical metabolic derangement are because when South Asians do develop

T2DM, they appear to experience suboptimal processes of care and this may explain the persisting differences in glycaemic control intermediate outcomes (20;28;267-269). Further, South Asians appear to be more sensitive to the macro and microvascular complications of T2DM (14;270), hence metabolic disease prevention is vital.

#### **6.4 Implications for future research**

Whilst the results presented in this thesis have a number of strengths as previously mentioned, the cross-sectional nature of this study means that conclusions cannot be drawn about causality. Thus to strengthen the conclusions made in Chapter 3, it would be beneficial to perform intervention trials using structured exercise programmes or measures to improve fitness and measure change in metabolic risk factors - for example prospective data in healthy and high risk European population groups for T2DM has shown that an improvement in cardiorespiratory fitness is associated with improvement in insulin resistance (80;271). Such trials are potentially achievable in South Asians living in Scotland as reflected by the recent conclusion of the Prevention of Type 2 Diabetes and Obesity in South Asians (PODOSA) trial (<http://www.podosa.org/>), which is a cluster randomized controlled trial investigating whether family-based lifestyle intervention (dietary modification and increased physical activity) can induce long-term weight loss and prevent diabetes in individuals with impaired glucose tolerance and/or impaired fasting glycaemia of Indian and Pakistani origin (121). The study screened over 1300 individuals, recruiting over 170 subjects and is the first interventional trial undertaken in South Asians in Scotland. Further, the limitations highlighted in Chapter 5 including suggestions for future study design if the study were to be repeated are potentially worth pursuing.

Another area of future research is to replicate some of the outcomes from this present study in South Asian women. South Asian women living in the UK, just like their male counterparts are at increased risk of cardiometabolic disease Table 6.1 (11). However it is recognised and discussed in published literature they are less-well studied (including those born in the UK) compared to their male counterparts (211;212). Further subtle cardiometabolic differences exist

between South Asian women and European women compared to South Asian men and European men such as the role of adiposity on interleukin-6 and differences in liver fat content (68;272). Presently a local study is on-going to investigate the association between adiposity, nutritional habits and physical activity with metabolic risk in South Asian women compared to European women. The investigator has contributed to its design as well as facilitating current recruitment of South Asian women into the study.

**Table 6.1 Deaths from IHD in the UK for women 2001-2003**

<b>Country</b>	<b>Number of IHD Deaths</b>	<b>SMR (95% CI)</b>
England and Wales	125 289	97 (96-97)
Scotland	2767	108 (105-112)
India	1672	149 (142-157)
Pakistan	454	174 (159-192)
Bangladesh	97	167 (136-204)

Of key importance is to pursue the investigation of ethnic-specific physical activity guidelines for those ethnic groups such as South Asians who are at increased risk of cardiometabolic disease. Given the recent published qualitative data from fieldwork in Scotland, engaging in physical activity for its own sake is of limited importance in South Asians (266). Thus if an evidence-base is created to demonstrate the value of increased physical activity in relation to reducing cardiometabolic disease risk, then this may act as an important goal which will change attitude and behaviour in South Asians - as tangible goals are important positive factors for this high risk ethnic group (266).

In an attempt to address this area, the author with his supervisors and colleagues have utilised this existing thesis data to determine the level of moderate physical activity required in South Asians to confer a similar cardiometabolic risk profile to that observed in Europeans undertaking the

current UK weekly recommended moderate physical activity level of 150 minutes a week (95). The results from this analysis indicate that the equivalent moderate physical activity value for the overall cardiometabolic risk factor was 266 (95% CI 185-347) min/week (273). Thus South Asian men need to engage in at least two-thirds more the amount of physical activity that is presently recommended in the UK for adults (95).

Whilst this present study's findings suggest that liver fat is not increased in South Asians compared to Europeans, all studies to date have had relatively small sample sizes. The cost of MR scanning make larger scale studies financially more challenging, but at least one such study in well-matched, well phenotyped South Asians would be beneficial in addressing this growing area of interest. Indeed coupling such imaging with an intervention-based study as described above would be an efficient method of further investigation. Further, novel data suggests that pancreatic fat rather than liver fat is more influential in the dysglycaemic process (255), and is a potential area for future research. Our team attempted to image the pancreas using MRI/MRS on the study subjects, but given the current resources, were not convinced that image quality would permit for accurate analysis.

## 6.5 Final conclusions

In conclusion, this thesis describes studies which have attempted to increase understanding of the cardiometabolic phenotype of South Asians. Novel findings from the studies suggest that increased insulin resistance in South Asians, which has a significant pathophysiological association with T2DM risk, is most strongly adversely influenced by their reduced cardiorespiratory fitness compared to their white European counterparts, with differences in physical activity not explaining the differences in cardiorespiratory fitness observed between the two groups. Similarly, data from this thesis suggest that South Asians are likely to need to engage in higher levels of physical activity than Europeans to overcome their lower innate fitness and the associated metabolic consequences. This raises the possibility that ethnic-specific recommendations for physical activity levels to promote healthy living and reduce cardiometabolic disease are needed. Further, the strong association of increased liver fat on increased insulin resistance and subsequent onset of T2DM has been questioned by this thesis. Finally this thesis suggests that existing carotid ultrasound screening modalities for subclinical CVD may lack sensitivity in South Asians. This highlights the need for further study in existing (e.g. focussing on younger South Asians, aged 40-50) and novel screening methods for CVD which may be more sensitive and thus could be utilised in this high risk ethnic group for CVD.

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## Appendix A1 - VOLUNTEER INFORMATION LEAFLET

(Version 2.1 12/5/10)

**A novel assessment of vascular disease and its association with insulin resistance, fitness and adiposity in South Asians**

**Short Title:** Carotid plaques, insulin resistance, fat and fitness in South Asians

**Lay title:** Fatty deposits in the arteries in the necks of South Asians and their relation to blood sugar control, fitness and body fat

**Study Doctor:** Dr Nazim Ghouri

**Other investigators:** Prof Naveed Sattar (Principal Investigator, University of Glasgow), Dr Jason Gill, Dr Stuart Ballantyne, Prof Ian Ford

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

### **What is the purpose of the study?**

Individuals of South Asian ancestry have higher heart disease risk but why this is so is not known. One theory suggests South Asians carry more fat but have less muscle size and this has never been explored. We also do not know how best to assess heart disease risk in South Asians. This proposed study will measure thickening of arteries in the necks of South Asians by two different methods, good measures of heart disease risk, and relate this to a detailed look at their fat and muscle levels. If we are correct, this information will help reduce heart disease risk in South Asians.

### **Why have I been chosen?**

You have been chosen because you are a healthy adult male aged between 40 and 70, with both parents of either European or South Asian (i.e. Indian, Pakistani, Bangladeshi or Sri Lankan) origin. The study will compare the two different groups to establish whether there are differences in the thickness in the arteries in the neck and if there is any connection with the body's ability to control sugar and the amount and location of body fat. We are excluding men who have angina or diabetes, or have had a previous heart attack, stroke, or mini-stroke (TIA), or have problems with their joints, muscles, limbs and breathing that would prevent them from going on a treadmill and exercising. We are also excluding anyone who is not allowed to have an MRI scan (cardiac pacemaker, aneurysm clip, artificial heart valve, ear (cochlear) implant, metal fragments in eyes, head or body or any procedure to remove metal, metallic Implant)

### **Do I have to take part?**

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

### **What is my part in the study?**

If you agree to take part in the study and meet entry requirements then your participation will consist of 1 visit lasting around 3 hours and 1 further short visit for an MRI scan of your abdomen. You will be paid £20 as a token of gratitude for your participation and we will arrange taxi journeys to and/or from the University if you require transport.

### **What do I have to do?**

If you are happy with the information provided and agree to participate, you can attend directly for the main visit. If you have any questions or wish to discuss things before agreeing, we can discuss things via telephone, email or in person before attending for the main visit.

You will be asked to report to the BHF Cardiovascular Research Centre to attend for the main visit (address at bottom of first page, map on last page). The first part of the visit will take place here. The rest of the main visit will take place in the University's purpose built exercise-testing facility in the neighbouring West Medical Building

You will be asked to attend for the main visit after an overnight fast (not having had anything to eat or drink, except water, for at least 10 hours). It may, therefore be more convenient for you to arrange visits in the morning. We ask that you wear/bring a pair of shorts and other clothes and footwear you will find comfortable exercising in.

- We will re-discuss with you the study and your involvement, and then help you complete confidential questionnaires regarding your health, family history, and diet. We will also measure your blood pressure. There will be an opportunity for you to ask questions. You will also complete a questionnaire to see if you are allowed to have an MRI scan. We will then take bloods from a vein in your forearm. We will take samples to check the function of your kidneys and liver, and measure glucose, insulin and fat levels and other markers related to cardiovascular disease. If you are agreeable, we will also store a sample of blood for any future analysis, including looking for genes associated with vascular disease and diabetes. We will take approximately 20-30 mls of blood (4-6 teaspoons) for this. This will last around 45 minutes. You will then be offered breakfast.
- The next part of this visit will involve you having an ultrasound scan of the arteries in your neck. This will take up to 45 minutes. This is a non-invasive pain-free scan and will involve some water-based jelly being put on your neck and a small probe will be placed over this area and images of your arteries will be taken and stored. These images will then be analysed later on to measure the thickness of the arteries and count any fatty deposits that may be seen.

You will then be taken over to the West Medical building to complete the last parts of the main visit. Here you will have some simple body fat distribution measurements taken, sit in a special device (BodPod) to measure your body fat levels, and will then be put on a treadmill and made to exercise (walk/run) to your capacity.

- The distribution of your body fat will be determined by measuring body girths and by using callipers to measure skin fold thickness at seven different sites on your arms, upper body and legs (a sophisticated version of "pinch an inch"). Your height, weight and waist and hip circumferences will also be recorded. You will need to wear only underclothing for these measurements which will be made in private. These measurements only take a few minutes.
- The BodPod is a computerized, egg-shaped chamber, in which the individual sits. The measurement will be done with you wearing a pair of shorts or surgical scrub trousers only (privacy provided). The measurement will take around 5 minutes, and

you will be expected to sit still for the measurement. This measurement will be able to tell us what proportion of your body is made up of fat and how much is 'fat-free'.

- The exercise treadmill test will be done to determine your fitness level i.e. how fit you are. You will have quick medical examination and a heart tracing (ECG) will be done to ensure your safe to have the test. The test will involve you initially walking on the treadmill at a fixed speed on the flat, and you will have self-adhesive electrodes attached to your chest to monitor how hard your heart is working (continuous ECGs). The speed and slope will be gradually increased at fixed time intervals. You will have to walk faster and possibly run to keep up with the speed of the treadmill, and this will get progressively harder. The test will stop if you indicate that you can no longer keep up with the treadmill, or if your ECG shows changes that require the test to be stopped. You will also be wearing a mouth piece that will have a tube, to measure the amount of oxygen and carbon dioxide you are breathing in and out. As this test involves you exercising to maximal effort, you should wearing clothing and footwear you will feel comfortable exercising in.
- You will then be asked to wear an accelerometer (pager-like machine to sit on the waist) for one week to allow objective measurement of habitual physical activity and cardiorespiratory fitness. The accelerometer will be worn for the hours spent awake for the seven day period. The accelerometer is to be returned back to the University or picked up by study doctor (if practical)

If we receive additional funding for this study, then you may be invited to attend for an MRI scan of your abdomen at Gartnavel Hospital. The scans would be arranged for a mutually convenient time.

- For the MRI scan, you will be asked to wear a gown or you can bring a tracksuit (without zips) or pyjamas to change into. The MRI staff at the hospital will double check that you are safe to have the scan and take your consent. You will be asked to lie on a padded table that will slide you into the MRI scanner. The MRI scan is non-invasive and will capture images of your abdomen, particularly your liver. This scan will be able to tell us more specifically about any fat you may have in your liver and abdomen

#### **What are the possible disadvantages and risks of taking part?**

- Blood sampling may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood.
- The exercise tests will be at a maximal level and the possibility exists that, very occasionally, certain changes may occur during or shortly after the test. They include abnormal blood pressure, fainting or a change in the normal rhythm of the heartbeat. At least two people will be present for all exercise tests (including the Study doctor) which will be undertaken in a room with all the necessary health and safety equipment.
- The scan to measure the amount of fat in you liver will involve you lying still in a relatively confined space in the MRI scanner for 20-30 minutes. The scanner can be a bit noisy and some people who suffer from claustrophobia can find the confined space a bit unbearable.
- There is a small possibility that taking part in this study will reveal a health problem that you already have such as high cholesterol, high blood pressure possible diabetes or angina. If such a problem is revealed, with your permission, we will inform your GP to ensure that you receive appropriate treatment. If necessary, a

clinical consultant physician (Professor Naveed Sattar) will supervise any follow-up care. Finally, if a condition such as diabetes is diagnosed, you may have to inform any insurance or mortgage companies you may take policies with in the future.

### **What are the possible benefits of taking part?**

You will receive a free health 'MOT' - the information gained during the study will allow us to give you detailed feedback about your blood pressure, cholesterol, blood sugar level, of "insulin resistance", any fatty/thickness changes in the arteries of your neck, about your fitness levels and about your body fat. The knowledge gained from your participation may help develop screening tests that will help predict future risk of vascular disease in groups of people. Also, the knowledge gained will help guide future research investigating how to reduce this increased risk through lifestyle changes and may also help direct the development of new and drugs to prevent and treat groups at increased risk of vascular disease.

### **What if something goes wrong?**

The chance of something going wrong is extremely small. All of the procedures involved in this study are low risk. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. The University of Glasgow has in force a Public Liability Policy and Clinical Trials Policy which provides cover for negligent harm that may arise from participation. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms may be available to you.

### **Will my taking part in this study be kept confidential?**

All information which is collected about you during the course of the research will be kept strictly confidential and only be handled by the individuals listed above or any staff that work under their supervision. Any information about you which leaves the University will have your name and address removed so that you cannot be recognised from it.

### **Who is organising and funding the research?**

This study is mainly being funded by Chest, Heart and Stroke Scotland, a charity that provides care and support throughout Scotland for people affected by Stroke, Heart disease and lung disease, as well as their families and other carers. They fund research into all aspects of prevention, diagnosis, treatment, rehabilitation and the social impact of chest, heart and stroke illness.

### **What will happen to my samples after the study has finished?**

The blood samples that you provide for this study may be useful for future research into the prevention and treatment of diabetes and heart disease; this may involve analysis of certain genes associated with these diseases. Any use of your samples in future research will require further approval from a Research Ethics Committee and samples will be analysed in such a way that the results will not be directly traceable to you. If you do not wish your samples to be used in future research, please indicate this on the consent form.

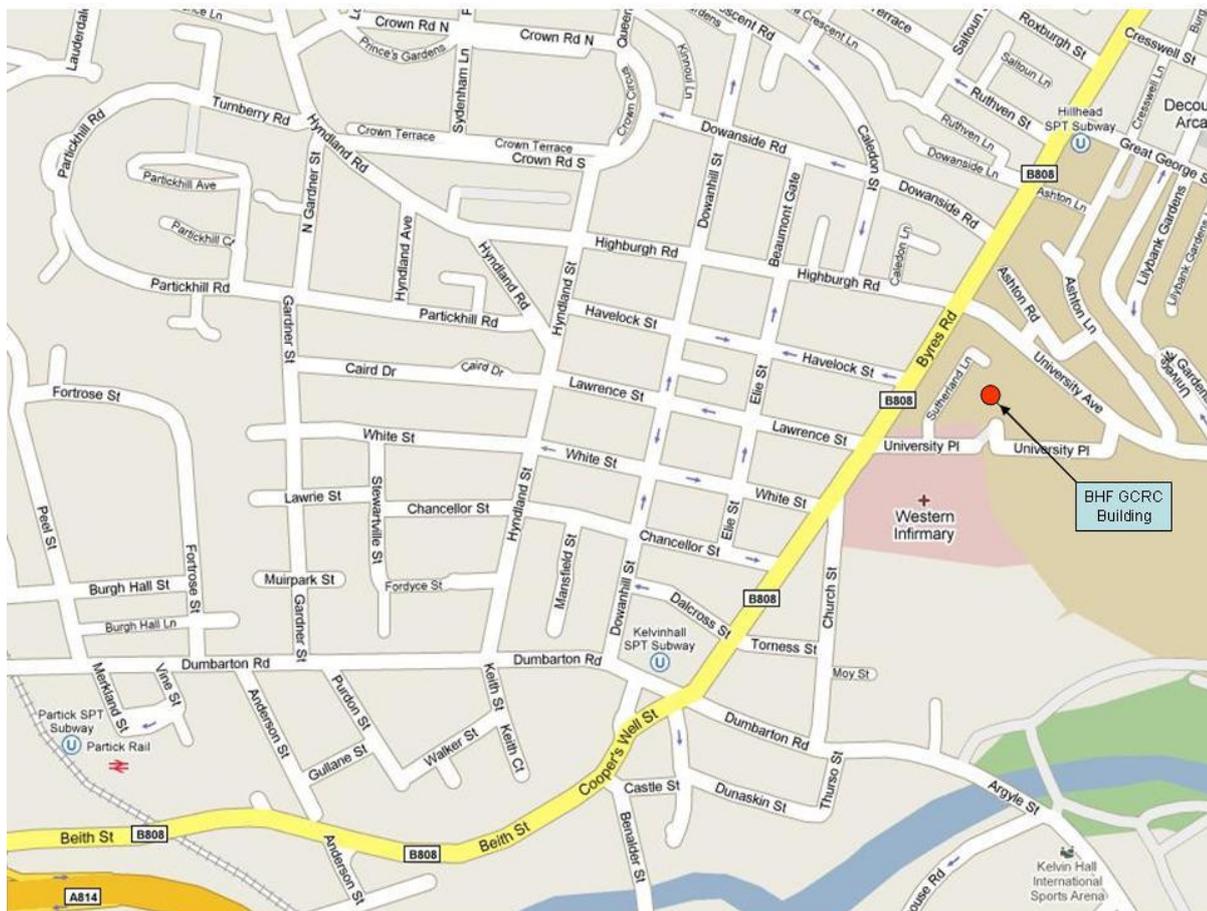
### **Who has reviewed the study?**

This study has been reviewed and approved by the West of Scotland (1) Research Ethics Committee.

### Contact for Further Information

You may ask any questions you like now or at any time about your rights as a participant in a research study or about the research study itself. Dr Nazim Ghouri can be contacted by phone on 0141 3303076 or 07740782912 or e-mail [n.ghouri@clinmed.gla.ac.uk](mailto:n.ghouri@clinmed.gla.ac.uk), to discuss things or to meet in person.

**You will be given a copy of this information sheet and a signed consent form to keep for your records.**



Volunteer Identification Number for this trial:



## CONSENT FORM

UNIVERSITY  
*of*  
GLASGOW

**Title of Project: A novel assessment of vascular disease, and its association with insulin resistance and adiposity in South Asians**

Name of Researcher: \_\_\_\_\_

### *Please initial box*

1. I confirm that I have read and understand the information sheet dated 19th January 2010 for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.
3. I agree to take part in the above study.
4. I agree to my GP being informed of my participation in the study.
5. I agree for my samples to be used for future research into the prevention and treatment of diabetes and heart disease. This may involve analysis of genes associated with these diseases. 

Yes	<input type="checkbox"/>
No	<input type="checkbox"/>

\_\_\_\_\_  
**Name of Patient      Date      Signature**

\_\_\_\_\_  
**Name of Person taking consent      Date      Signature**

(if different from researcher)

\_\_\_\_\_  
**Researcher      Date      Signature**

1 for patient; 1 for researcher.



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?  
yes [ ] no [ ]

5. **Have you ever** had any of the following:

- (a) Convulsions/epilepsy yes [ ] no [ ]
- (b) Asthma yes [ ] no [ ]
- (c) Eczema yes [ ] no [ ]
- (d) Diabetes yes [ ] no [ ]
- (e) A blood disorder yes [ ] no [ ]
- (g) Digestive problems yes [ ] no [ ]
- (h) Hearing problems yes [ ] no [ ]
- (i) Disturbance of balance/co-ordination yes [ ] no [ ]
- (j) Numbness in hands or feet yes [ ] no [ ]
- (k) Disturbance of vision yes [ ] no [ ]
- (l) Thyroid problems yes [ ] no [ ]
- (m) Kidney or liver problems yes [ ] no [ ]
- (n) Heart problems including murmurs yes [ ] no [ ]
- (o) Any other health problems yes [ ] no [ ]
- (p) An allergy to soya protein or eggs yes [ ] no [ ]

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 yes [ ] no [ ]
- (b) Diabetes yes [ ] no [ ]
- (c) Stroke yes [ ] no [ ]
- (d) Any other family illnesses yes [ ] no [ ]

7. Do you currently smoke yes [ ] no [ ]

If yes, how many < 10 per day [ ] 10-20 per day [ ] >20 per day [ ]

If no, have you ever smoked yes [ ] no [ ]

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)**

.....  
.....  
.....  
.....  
.....  
.....  
.....

Name and address of GP

.....  
.....

Signature:.....

Date:.....

**Measurements**

Blood pressure - Left arm (BEFORE MMode image RIGHT carotid).....mm Hg

Blood pressure - Left arm (AFTER MMode image RIGHT carotid).....mm Hg

Blood pressure - Left arm (BEFORE MMode image LEFT carotid).....mm Hg

Blood pressure - Left arm (AFTER MMode image LEFT carotid).....mm Hg

## Appendix A3 - Food frequency questionnaire



**BREAKFAST CEREALS**

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,<br>Coco Pops, Frosties or Crunchy Nut Cornflakes | 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 |
| Other Cereal   | 7 6 5 4 3 2 1 F R |

Please specify brand/type

.....

How many teaspoons of sugar/honey do you add?

.....

How often do you have wheat bran?

7 6 5 4 3 2 1 F R

  41-42

  43-44

 45

 46
**MEATS**

How often do you have the following meats?

Include all forms of each meat, eg use in stews, casseroles, lasagne, curry etc.

- |  |                   |
|--|-------------------|
| Beef (including beefburgers)                         | 7 6 5 4 3 2 1 F R |
| Lamb   | 7 6 5 4 3 2 1 F R |
| Pork   | 7 6 5 4 3 2 1 F R |
| Bacon  | 7 6 5 4 3 2 1 F R |
| Ham  | 7 6 5 4 3 2 1 F R |
| Chicken or other poultry                             | 7 6 5 4 3 2 1 F R |
| Canned meat (e.g., corned beef), paté or meat spread | 7 6 5 4 3 2 1 F R |
| Sausages   | 7 6 5 4 3 2 1 F R |

What type of sausages do you have?

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

Meat pie/pastie/sausage roll/samosa - shop bought

7 6 5 4 3 2 1 F R

Meat pie/pastie/sausage roll/samosa - home made

7 6 5 4 3 2 1 F R

Liver/kidney/heart

7 6 5 4 3 2 1 F R

Do you usually eat the fat on meat?

Yes / No

 47

 48

 49

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 59

**FISH****How often do you eat the following fish?**

White fish (cod/haddock/plaice/fish fingers/fish cakes)	7 6 5 4 3 2 1 F R
Kipper/herring/mackerel/trout (including canned)	7 6 5 4 3 2 1 F R
Pilchards/sardines/salmon (including canned)	7 6 5 4 3 2 1 F R
Tuna (including canned)	7 6 5 4 3 2 1 F R

<input type="checkbox"/>	60
<input type="checkbox"/>	61
<input type="checkbox"/>	62
<input type="checkbox"/>	63

**VEGETABLES & SAVOURY DISHES****How often do you have the following vegetables or dishes?**

Potatoes - boiled or mashed	7 6 5 4 3 2 1 F R
Potatoes - jacket	7 6 5 4 3 2 1 F R
Chips - shop bought, 'oven/microwave chips' or hash browns	7 6 5 4 3 2 1 F R
Chips - homecooked	7 6 5 4 3 2 1 F R
Potatoes - roast	7 6 5 4 3 2 1 F R
Peas	7 6 5 4 3 2 1 F R
Other green vegetables, salads or tomatoes	7 6 5 4 3 2 1 F R
Carrots	7 6 5 4 3 2 1 F R
Parsnips, swedes, turnips or sweetcorn	7 6 5 4 3 2 1 F R
Baked beans	7 6 5 4 3 2 1 F R
Butter beans, broad beans or red kidney beans	7 6 5 4 3 2 1 F R
Lentils, chick peas or dahl	7 6 5 4 3 2 1 F R
Onions (cooked/raw/pickled)	7 6 5 4 3 2 1 F R
Spaghetti, other pasta or noodles	7 6 5 4 3 2 1 F R
Rice (NOT pudding rice)	7 6 5 4 3 2 1 F R
Quiche	7 6 5 4 3 2 1 F R
Pizza	7 6 5 4 3 2 1 F R
Vegetable pie/pasty/samosa	7 6 5 4 3 2 1 F R

<input type="checkbox"/>	64
<input type="checkbox"/>	65
<input type="checkbox"/>	66
<input type="checkbox"/>	67
<input type="checkbox"/>	68
<input type="checkbox"/>	69
<input type="checkbox"/>	70
<input type="checkbox"/>	71
<input type="checkbox"/>	72
<input type="checkbox"/>	73
<input type="checkbox"/>	74
<input type="checkbox"/>	75
<input type="checkbox"/>	76
<input type="checkbox"/>	77
<input type="checkbox"/>	78
<input type="checkbox"/>	79
<input type="checkbox"/>	80
<input type="checkbox"/>	81

**BISCUITS, CAKES & PUDDINGS****How often do you eat the following items?**

Digestive biscuits/plain biscuits	7 6 5 4 3 2 1 F R
Other sweet biscuits	7 6 5 4 3 2 1 F R
Chocolate, e.g., Galaxy, Mars Bar, Twix, KitKat	7 6 5 4 3 2 1 F R

<input type="checkbox"/>	82
<input type="checkbox"/>	83
<input type="checkbox"/>	84

Sweets, e.g., fruit gums, pastilles, mints 7 6 5 4 3 2 1 F R

Crisps/savoury snacks, e.g., Quavers, tortilla chips 7 6 5 4 3 2 1 F R

Nuts 7 6 5 4 3 2 1 F R

Ice cream, iced dessert, fool, mousse or trifle 7 6 5 4 3 2 1 F R

Low fat yogurt 7 6 5 4 3 2 1 F R

Low calorie yogurt e.g., Shape 7 6 5 4 3 2 1 F R

Other yogurt/fromage frais, e.g., thick & creamy 7 6 5 4 3 2 1 F R

Fruitcake/sponge cake/sponge pudding - shop bought 7 6 5 4 3 2 1 F R

Fruitcake/sponge cake/sponge pudding - homemade 7 6 5 4 3 2 1 F R

Fruit tart/jam tart/doughnut/Danish pastry - shopbought 7 6 5 4 3 2 1 F R

Fruit tart/jam tart - home made 7 6 5 4 3 2 1 F R

Milk pudding e.g., rice/tapioca/macaroni 7 6 5 4 3 2 1 F R

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

85

86

87

88

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96

**FRUIT**

How often do you have fruit canned in syrup? 7 6 5 4 3 2 1 F R

How often do you have fruit canned in juice? 7 6 5 4 3 2 1 F R

How many apples do you have per week? .....

How many pears do you have per week? .....

How many oranges/tangerines/satsumas/clementines/  
grapefruit do you have per week? .....

How many bananas do you have per week? .....

98

99

100

101

102

103

**EGGS & MILK PRODUCTS**

How many eggs do you usually eat per week? .....

104-105

**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 and one pint
- 4 One pint or more

 106

**What type of milk do you have?**

- 1 Whole
- 2 Semi-skimmed
- 3 Skimmed
- 4 More than one type

 107

**How much cream do you use per week?**

(1 tablespoon=20g; small carton=150g; large carton=300g) .....

   108-110

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

   111-113

(Suggestion: divide amount bought for household by number of people in house)

**How often do you eat cottage cheese?** 7 6 5 4 3 2 1 F R

 114

**FATS**

**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
- 0 Bread eaten dry

  115-116

Brand name & description on packet/tub .....

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

   117-119

(One block or small tub = 250g. Spread on one slice of bread: Thinly=5g; Medium=8g; Thickly=13g.)

**How often do you have food that is fried?**  
(e.g., fish/onions/mushrooms/tomatoes/eggs)

7 6 5 4 3 2 1 F R

 120

**What types and BRANDS of fat do you use in cooking?**

Frying .....	solid/liquid	<input type="checkbox"/>	121
Chips .....	solid/liquid	<input type="checkbox"/>	122
Roast Potatoes .....	solid/liquid/eaten out	<input type="checkbox"/>	123
Home made cake .....		<input type="checkbox"/>	124
Home made pastry .....		<input type="checkbox"/>	125

**DRINKS**

How many cups of tea do you have per day? .....   126-127

How many teaspoons of sugar/honey per cup? .....  128

How many cups of coffee do you have per day? .....   129-130

How many teaspoons of sugar/honey per cup? .....  131

How often do you have fruit juice/squash/fizzy drinks (NOT low calorie)? 7 6 5 4 3 2 1 F R  132

Which of these do you usually have? 1 Natural Juice  
2 Squash  
3 Fizzy Drink  
4 More than one  133

How often do you have drinks containing alcohol? 7 6 5 4 3 2 1 F R  134

When you drink, how many do you have? .....

Please specify how many drinks of each type per occasion:

Beer/lager/stout/cider	Number of pints	.....	<input type="checkbox"/> <input type="checkbox"/> 135-136
Wine	Number of glasses	.....	<input type="checkbox"/> 137
Sherry/port/vermouth	Number of glasses	.....	<input type="checkbox"/> 138
Spirits/liqueurs	No. of single measures	.....	<input type="checkbox"/> 139

**HEIGHT, WEIGHT & ACTIVITY**

What is your height? ..... ft ..... ins OR .....cm

140-143

What is your weight? ..... st ..... lbs OR .....kg

144-147

How physically active is your occupation?

- 1 Not very active
- 2 Moderately active
- 3 Very active
- 4 Not working

148

How physically active is your leisure time?

- 1 Not very Active
- 2 Moderately active
- 3 Very active

149

Questions for women only..

Are you pregnant? Yes / No

150

Are you breast feeding? Yes / No

151

**ADDITIONAL QUESTIONS**

How often do you have..

Dishes made with TVP (soya mince) or Quorn? 7 6 5 4 3 2 1 F R

152

Vegetarian sausages / Vegetarian burgers? 7 6 5 4 3 2 1 F R

153

Are there any other foods that you eat regularly, but which are not recorded in the questionnaire? Yes / No

If Yes, please state each food and how often you usually eat it

Food	Frequency
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

154

155-157

158-160

Diet Code

161

# Appendix A4 - Carotid ultrasound protocol

## *Procedure*

- ❖ Switch the Acuson Sequoia on by pushing the button below the monitor on the left.

To enter participant identifiers:

- a) Press Begin/End button in top left corner of control panel.
  - b) Select “Start New Patient” on the soft key displayed on the base of the monitor.
  - c) Enter subject initials from participant list in “Patient Name” field.
  - d) Press “TAB” OR “Enter” to move to next field.
  - e) Enter SA study number in “Patient ID” field.
  - f) Leave “Study Type” as “SA study Trial”.
  - g) Leave “Exam Preset” as “SA study Trial”.
  - h) Enter date of birth. Age is calculated automatically.
  - i) Enter sex (M for Male, F for Female).
  - j) Enter sonographer ID number.
- 1) Press “Begin Imaging” on the soft key displayed on the base of the monitor.
  - 2) Ensure subject is lying reclined and comfortably cushioned on the examination couch.
  - 3) Use wedge; ear-to-ear plane at 45 degrees to the horizontal; head turned to the contralateral side of scan.
  - 4) Apply ultrasound gel to neck.
  - 5) The left button on the screen scrolls through the examinations. Press “protocol” before pressing the left button on the screen to advance to the next stage of the examination. Each stage should be completed in turn:

## *R\_ICA\_DOP: Doppler of right internal carotid artery:*

- i) Use 4 cm depth setting (default).
- ii) Identify ICA.
- iii) Press “Cursor” key to position Doppler gate in entrance of ICA.
- iv) Press “Angle” knob and adjust angle to be parallel to blood flow.
- v) Press “PW” button.
- vi) Use trackball to adjust position to optimise trace.
- vii) Press “Gain/Frz/Run” to freeze image.
- viii) Rotate “Gain/Frz/Run” to scroll through strip if necessary, to obtain best available strip.
- ix) Press “Calipers On”.
- x) Use trackball to position caliper on peak of Doppler trace.
- xi) Double check that angle is set correctly.
- xii) Press “Image Store” to store image.
- xiii) N.B. If velocity is above 1.25m/s, note this and pass information to Kevin Deans as critical action limit.
- xiv) Press “Protocol” button and use left soft key to move to the next stage of the imaging protocol.

## *RCCA\_1:*

*Identify the common carotid artery (CCA) region in RES4 (the arterial wall proximal to the dilatation), then:*

- i) Press “RES” button.
- ii) RES (Regional expansion selection) 2 (2x2cm) box pops up.

- iii) Move box to area of interest with trackball.
- iv) Press “RES” button again.
- v) Once real time image is satisfactory, press “Clip Store”.
- vi) Once clip has been stored, press “Gain/Frz/Run” to freeze image.
- vii) Rotate “Gain/Frz/Run” wheel to find optimal image where double line pattern is clearest.
- viii) To place a caliper mark:
  - (1) Press “Calipers On”
  - (2) Use trackball to move caliper to desired position.
  - (3) On CCA image, place caliper mark at start of carotid bulb.
- ix) To store image, press “IMAGE STORE”
- x) Press “RES” again to leave “RES” mode.
- xi) Press “Protocol” button and use left soft key to move to next stage of protocol.

### **RBUL\_1:**

**Identify the carotid bulb region in RES4 (the arterial wall between the dilatation and the flow divider), then image in RES2:**

- i) Follow above procedure to store clip and image.
- ii) Place two caliper marks: one at start of the dilatation and one at level of flow divider. Left click (“Select” key) to anchor caliper.
- iii) Press “Protocol” button and use left soft key to move to next stage of protocol.

### **RICA\_1:**

**Identify the right internal carotid (ICA) region in RES4 (the arterial wall distal to the flow divider) then image in RES2:**

- i) Place caliper mark at level of flow divider.
- ii) Press “Protocol” button and use left soft key to move to next stage of protocol.

### **R\_MMODE:**

- i) Take blood pressure using Omron sphygmomanometer.
- ii) Enter BP (SBP, DBP and HR) in relevant fields in laptop.
- iii) Return to right common carotid artery.
- iv) Press “RES” to magnify distal common carotid artery.
- v) Press “M-MODE”.
- vi) Use trackball to position M-line in B-mode part of the image.
- vii) When image is satisfactory, freeze image and rotate to find best image.
- viii) Store image in M-mode.
- ix) Repeat blood pressure measurement (using same arm throughout) and record in laptop.
- x) Press “Protocol” button and use left soft key to move to next stage of protocol.

Repeat above steps for imaging the left carotid arterial wall segments:

### ***L\_ICA\_DOP: Left internal carotid artery Doppler***

- i) Press “Protocol” button and use left soft key to move to next stage of protocol.

### *LCCA\_1: Left common carotid artery, longitudinal section*

- i) Place caliper mark at start of carotid bulb.
- ii) Press "Protocol" button and use left soft key to move to next stage of protocol.

### *LBUL\_1: Left carotid artery bulb, longitudinal section*

- i) Place caliper marks at start of bulb and at level of flow divider.
- ii) Press "Protocol" button and use left soft key to move to next stage of protocol.

### *LICA\_1: Left internal carotid artery, longitudinal section*

- i) Place caliper mark at level of flow divider.
- ii) Press "Protocol" button and use left soft key to move to next stage of protocol.

### **L\_MMODE:**

- i) Take blood pressure using Omron sphygmomanometer and record SBP, DBP and HR values in laptop.
- ii) Return to left common carotid artery.
- iii) Press "RES" to magnify distal common carotid artery.
- iv) Press "M-MODE".
- v) Use trackball to position M-line in B-mode part of the image.
- vi) When image is satisfactory, freeze image and rotate "Gain/Frz/Run" wheel to find best strip.
- vii) Store image in M-mode.
- viii) Repeat blood pressure measurement and record in laptop.
- ix) Press "Protocol" button and use left soft key to move to next stage of protocol.

### **To review images:**

- a) Press "REVIEW"
- b) Press "QUAD" button to alternate between viewing four images per screen or one image per screen.
- c) To delete any images, highlight image and press "DELETE".
- d) A total of 16 images should be present (4 quad pages): 6 images, 6 clips, 2 Dopplers and 2 M-modes.

### **To end examination:**

- a) Press "BEGIN/END" - returns user to demographic page.
- b) Press "START NEW PATIENT" soft key - closes examination and stores images on hard drive.
- c) Alternatively, press "STUDY/UTIL".

### **To copy studies to CD burner:**

- a) Ensure other Acuson Sequoia instrument and switching device are both switched on.
- b) Insert blank CD-R into CD burner.
- c) Press "STUDY/UTIL"
- d) Select subjects to be copied to CD. Studies which have not been copied are highlighted with an asterisk.

- e) Press “COPY to MDR”.
- f) Once studies have been copied to MDR, MDR display shows “Staging Files”.
- g) Press “Eject”.
- h) On ejecting CD, studies are burned to CD.
- i) Label CD with SA study study numbers and subject initials.

***Clean probe with Cutan wipes and T-spray cleaning solution only.***

***To switch Acuson Sequoia off, push power switch. Do not unplug the instrument until the system has completely powered down and the screen is completely dark.***

## Appendix A5 - Carotid intima-media thickness-reader validation

**Table 1 Intra-reader reproducibility**

Scan	Run (Mean of LCCA and RCCA, mm)										Mean IMT (mm)	Standard Deviation	Coefficient Variance (%)
	1	2	3	4	5	6	7	8	9	10			
1	0.548	0.535	0.521	0.562	0.5395	0.5315	0.534	0.5255	0.526	0.518	0.53405	0.013	2.3
2	0.541	0.5425	0.554	0.52	0.519	0.508	0.523	0.5225	0.5255	0.535	0.52905	0.014	2.3
3	0.4435	0.439	0.434	0.432	0.4495	0.438	0.4375	0.4415	0.434	0.379	0.4378	0.006	1.4
4	0.651	0.65	0.6435	0.656	0.6175	0.6435	0.6295	0.6545	0.639	0.6435	0.6428	0.012	1.9
5	0.385	0.3815	0.363	0.353	0.357	0.349	0.3645	0.339	0.334	0.3515	0.35775	0.016	4.6
6	0.4975	0.496	0.475	0.482	0.4865	0.488	0.476	0.4875	0.481	0.469	0.48385	0.009	1.9
7	0.4525	0.454	0.4535	0.441	0.456	0.4575	0.4535	0.438	0.4515	0.4495	0.4507	0.006	1.4
8	0.555	0.5315	0.5515	0.5345	0.5375	0.512	0.5235	0.542	0.539	0.529	0.53555	0.013	2.4
9	0.442	0.4515	0.429	0.4635	0.4755	0.45	0.455	0.433	0.4425	0.4455	0.44875	0.014	3.1
10	0.5265	0.5265	0.516	0.501	0.4945	0.509	0.52	0.491	0.4925	0.4975	0.50745	0.014	2.8

**Table 2 Inter-reader reproducibility**

Scan	Mean IMT (mm)		Difference (mm)
	Author	External	
1	0.53405	0.546	0.012
2	0.52905	0.541	0.011
3	0.4378	0.538	0.099
4	0.6428	0.721	0.078
5	0.35775	0.366	0.008
6	0.48385	0.525	0.041
7	0.4507	0.474	0.023
8	0.53555	0.532	0.004
9	0.44875	0.463	0.014
10	0.50745	0.533	0.028

## Appendix A6 - Formulae for calculating predicted oxygen uptake ( $VO_{2pred}$ ) and additional speeds for gradient plateaux

### Speed equivalents

$$\begin{aligned} 4.5 \text{ km.h}^{-1} &= 75 \text{ m.min}^{-1} \\ 5.0 \text{ km.h}^{-1} &= 83.33 \text{ m.min}^{-1} \\ 5.5 \text{ km.h}^{-1} &= 91.67 \text{ m.min}^{-1} \end{aligned}$$

Calculating predicted oxygen uptake ( $VO_{2pred}$ ) in  $\text{ml.kg}^{-1}.\text{min}^{-1}$  when gradient increasing and speed fixed

$$VO_{2pred} = 3.5 + 0.1 \times \text{speed (m.min}^{-1}) + (\text{gradient \%} \times \text{speed (m/min)} \times 1.8)$$

### *Speed 4.5 km.h<sup>-1</sup>*

$$\begin{aligned} VO_{2pred} &= 3.5 + 7.5 + (\text{gradient \%} \times 75 \times 1.8) \\ &= 11 + (\text{gradient \%} \times 135) \end{aligned}$$

### *Speed 5.0 km.h<sup>-1</sup>*

$$\begin{aligned} VO_{2pred} &= 3.5 + 8.33 + (\text{gradient\%} \times 83.33 \times 1.8) \\ &= 11.83 + (\text{gradient\%} \times 150) \end{aligned}$$

### *Speed 5.5 km.hr<sup>-1</sup>*

$$\begin{aligned} VO_{2pred} &= 3.5 + 9.17 + (\text{gradient\%} \times 91.67 \times 1.8) \\ &= 12.67 + (\text{gradient\%} \times 165) \end{aligned}$$

Calculating new speeds when maximum gradient attained

### *Starting Speed 4.5 km.hr<sup>-1</sup>*

*Gradient increment 2%*

$$\text{Incremental } \Delta VO_{2pred} = 2.7 \text{ ml.kg}^{-1}.\text{min}^{-1}$$

$$\text{New speed using max gradient of 24\% (km.hr}^{-1}) = \frac{\text{predicted O}_2 \text{ uptake} - 3.5}{8.87}$$

*Gradient increment 2.5%*

$$\text{Incremental } \Delta VO_{2pred} = 3.4 \text{ ml.kg}^{-1}.\text{min}^{-1}$$

$$\text{New speed using max gradient of 25\% (km.hr}^{-1}\text{)} = \frac{\text{predicted O2 uptake} - 3.5}{9.17}$$

*Gradient increment 3%*

$$\text{Incremental } \Delta\text{VO}_{2\text{pred}} = 4 - 4.1 \text{ ml.kg}^{-1}.\text{min}^{-1}$$

$$\text{New speed using max gradient of 24\% (km.hr}^{-1}\text{)} = \frac{\text{predicted O2 uptake} - 3.5}{8.87}$$

***Starting Speed 5.0 km.hr***<sup>-1</sup>

*Gradient increment 2%*

$$\text{Incremental } \Delta\text{VO}_{2\text{pred}} = 3 \text{ ml.kg}^{-1}.\text{min}^{-1}$$

$$\text{New speed using max gradient of 24\% (km.hr}^{-1}\text{)} = \frac{\text{predicted O2 uptake} - 3.5}{8.87}$$

*Gradient increment 2.5%*

$$\Delta\text{VO}_{2\text{pred}} = 3.7 - 3.8 \text{ ml.kg}^{-1}.\text{min}^{-1}$$

$$\text{New speed using max gradient of 25\% (km.hr}^{-1}\text{)} = \frac{\text{predicted O2 uptake} - 3.5}{9.17}$$

*Gradient increment 3%*

$$\text{Incremental } \Delta\text{VO}_{2\text{pred}} = 4.5 \text{ ml.kg}^{-1}.\text{min}^{-1}$$

$$\text{New speed using max gradient of 24\% (km.hr}^{-1}\text{)} = \frac{\text{predicted O2 uptake} - 3.5}{8.87}$$

***Starting Speed 5.5 km.hr***<sup>-1</sup>

*Gradient increment 2%*

$$\text{Incremental } \Delta\text{VO}_{2\text{pred}} = 3.3 \text{ ml.kg}^{-1}.\text{min}^{-1}$$

$$\text{New speed using max gradient of 24\% (km.hr}^{-1}\text{)} = \frac{\text{predicted O2 uptake} - 3.5}{8.87}$$

*Gradient increment 2.5%*

$$\text{Incremental } \Delta\text{VO}_{2\text{pred}} = 4.1 \text{ ml.kg}^{-1}.\text{min}^{-1}$$

$$\text{New speed using max gradient of 25\% (km.hr}^{-1}\text{)} = \frac{\text{predicted O2 uptake} - 3.5}{9.17}$$

*Gradient increment 3%*

Incremental  $\Delta\text{VO}_{2\text{pred}} = 4.9 - 5.0 \text{ ml.kg}^{-1}.\text{min}^{-1}$

New speed using max gradient of 24% ( $\text{km.hr}^{-1}$ ) =  $\frac{\text{predicted O}_2 \text{ uptake} - 3.5}{8.87}$

## Appendix A7

### Exercise test Protocols 1-9

**Protocol 1 - Predicted Oxygen Consumption ( $VO_{2pred}$ ) - 4.5 km/hr + 2% / 2min**

<i>Speed (Km.hr<sup>-1</sup>)</i>	<i>Gradient %</i>	<i>Pred O2 uptake (ml.kg<sup>-1</sup>.min<sup>-1</sup>)</i>
4.5	0	11.0
4.5	2	13.7
4.5	4	16.4
4.5	6	19.1
4.5	8	21.8
4.5	10	24.5
4.5	12	27.2
4.5	14	29.9
4.5	16	32.6
4.5	18	35.3
4.5	20	38.0
4.5	22	40.7
4.5	24	43.4
4.8	24	46.1
5.1	24	48.8

**Protocol 2 - Predicted Oxygen Consumption ( $VO_{2pred}$ ) - 4.5 km/hr + 2.5% / 2min**

<i>Speed (Km.hr<sup>-1</sup>)</i>	<i>Gradient %</i>	<i>Pred O2 uptake (ml.kg<sup>-1</sup>.min<sup>-1</sup>)</i>
4.5	0	11.0
4.5	2.5	14.4
4.5	5	17.8
4.5	7.5	21.1
4.5	10	24.5
4.5	12.5	27.9
4.5	15	31.3
4.5	17.5	34.6
4.5	20	38.0
4.5	22.5	41.4
4.5	25	44.8
4.9	25	48.2
5.2	25	51.6
5.6	25	55.0
6.0	25	58.4

**Protocol 3 - Predicted Oxygen Consumption ( $VO_{2pred}$ ) - 4.5 km/hr + 3% / 2min**

<i>Speed (Km.hr<sup>-1</sup>)</i>	<i>Gradient %</i>	<i>Pred O2 uptake (ml.kg<sup>-1</sup>.min<sup>-1</sup>)</i>
4.5	0	11.0
4.5	3	15.1
4.5	6	19.1
4.5	9	23.2
4.5	12	27.2
4.5	15	31.3
4.5	18	35.3
4.5	21	39.4
4.5	24	43.4
5.0	24	47.5
5.4	24	51.5
5.9	24	55.6
6.3	24	59.6

**Protocol 4 - Predicted Oxygen Consumption ( $VO_{2pred}$ ) - 5.0 km/hr + 2% / 2min**

<i>Speed (Km.hr<sup>-1</sup>)</i>	<i>Gradient %</i>	<i>Pred O2 uptake (ml.kg<sup>-1</sup>.min<sup>-1</sup>)</i>	
5.0	0	11.8	
5.0	2	14.8	
5.0	4	17.8	
5.0	6	20.8	
5.0	8	23.8	
5.0	10	26.8	
5.0	12	29.8	
5.0	14	32.8	
5.0	16	35.8	
5.0	18	38.8	
5.0	20	41.8	
5.0	22	44.8	
5.0	24	47.8	
5.3	24	50.8	
5.7	24	53.8	

**Protocol 5 - Predicted Oxygen Consumption ( $VO_{2pred}$ ) - 5.0 km/hr + 2.5% / 2min**

<i>Speed (Km.hr<sup>-1</sup>)</i>	<i>Gradient %</i>	<i>Pred O2 uptake (ml.kg<sup>-1</sup>.min<sup>-1</sup>)</i>	
5.0	0	11.8	
5.0	2.5	15.6	
5.0	5	19.3	
5.0	7.5	23.1	
5.0	10	26.8	
5.0	12.5	30.6	
5.0	15	34.3	
5.0	17.5	38.1	
5.0	20	41.8	
5.0	22.5	45.6	
5.0	25	49.3	
5.4	25	53.0	
5.8	25	56.7	
6.2	25	60.4	
6.6	25	64.1	

**Protocol 6 - Predicted Oxygen Consumption ( $VO_{2pred}$ ) - 5.0 km/hr + 3% / 2min**

<i>Speed (Km.hr<sup>-1</sup>)</i>	<i>Gradient %</i>	<i>Pred O2 uptake (ml.kg<sup>-1</sup>.min<sup>-1</sup>)</i>	
5.0	0	11.8	
5.0	3	16.3	
5.0	6	20.8	
5.0	9	25.3	
5.0	12	29.8	
5.0	15	34.3	
5.0	18	38.8	
5.0	21	43.3	
5.0	24	47.8	
5.5	24	52.3	
6.0	24	56.8	
6.5	24	61.3	
7.0	24	65.8	

**Protocol 7 - Predicted Oxygen Consumption ( $VO_{2pred}$ ) - 5.5 km/hr + 2% / 2min**

<i>Speed (Km.hr<sup>-1</sup>)</i>	<i>Gradient %</i>	<i>Pred O2 uptake (ml.kg<sup>-1</sup>.min<sup>-1</sup>)</i>
5.5	0	12.7
5.5	2	16.0
5.5	4	19.3
5.5	6	22.6
5.5	8	25.9
5.5	10	29.2
5.5	12	32.5
5.5	14	35.8
5.5	16	39.1
5.5	18	42.4
5.5	20	45.7
5.5	22	49.0
5.5	24	52.3
5.9	24	55.6
6.2	24	58.9
6.6	24	62.2
7.0	24	65.5

**Protocol 8 - Predicted Oxygen Consumption ( $VO_{2pred}$ ) - 5.5 km/hr + 2.5 % / 2min**

<i>Speed (Km.hr<sup>-1</sup>)</i>	<i>Gradient %</i>	<i>Pred O2 uptake (ml.kg<sup>-1</sup>.min<sup>-1</sup>)</i>
5.5	0	12.7
5.5	2.5	16.8
5.5	5	20.9
5.5	7.5	25.0
5.5	10	29.2
5.5	12.5	33.3
5.5	15	37.4
5.5	17.5	41.5
5.5	20	45.7
5.5	22.5	49.8
5.5	25	53.9
5.9	25	58.0
6.4	25	62.1
6.8	25	66.2
7.3	25	70.3

**Protocol 9 - Predicted Oxygen Consumption ( $VO_{2pred}$ ) - 5.5 km/hr + 3% / 2min**

<i>Speed (Km.hr<sup>-1</sup>)</i>	<i>Gradient %</i>	<i>Pred O2 uptake (ml.kg<sup>-1</sup>.min<sup>-1</sup>)</i>
5.5	0	12.7
5.5	3	17.6
5.5	6	22.6
5.5	9	27.5
5.5	12	32.5
5.5	15	37.4
5.5	18	42.4
5.5	21	47.3
5.5	24	52.3
6.1	24	57.2
6.6	24	62.2
7.2	24	67.1
7.7	24	72.1

**Appendix A8: Associations between HOMA<sub>IR</sub> and fasting glucose concentration with smoking status, alcohol consumption, years in education and SES.**

Predictor	HOMA <sub>IR</sub>		Fasting Glucose		
	Relative effect estimate (95% CI)	p-value	Relative effect estimate (95% CI)	p-value	
Ethnicity (European vs. South Asian)	<b>1.67 (1.42, 1.96)</b>	<b>&lt;0.001</b>	<b>1.03 (1.01, 1.06)</b>	<b>0.017</b>	
Years in Education (per one year increase)	0.98 (0.96, 1.01)	0.191	1.00 (1.00, 1.01),	0.280	
SIMD Quintile (vs. 1 <sup>st</sup> Quintile)	2	1.21 (0.82, 1.77)	0.334	1.01 (0.94, 1.08),	0.799
	3	1.22 (0.85, 1.74)	0.279	1.02 (0.96, 1.08),	0.545
	4	1.23 (0.87, 1.75)	0.238	0.98 (0.92, 1.04),	0.421
	5	1.32 (0.95, 1.84)	0.100	1.01 (0.95, 1.07),	0.752
			(0.569)		(0.322)
Smoking Status (vs. non smoker)	Ex-smoker	<b>1.26 (1.02, 1.56)</b>	<b>0.030</b>	1.03 (1.00, 1.07),	0.088
	Current	1.02 (0.79, 1.31)	0.909	1.03 (0.99, 1.08),	0.144
			(0.091)	(0.119)	
Alcohol Consumption (units per week) (vs. non-drinker)	≤20	0.92 (0.68, 1.23)	0.561	1.01 (0.96, 1.07),	0.574
	>21	0.86 (0.60, 1.22)	0.390	1.02 (0.96, 1.09),	0.476
			(0.690)	(0.769)	

Values are presented with 95% confidence intervals (95% CI) and p values, with p values for the overall effect of categorical variables given in parenthesis.