

The relationship between body fat distribution,
insulin sensitivity, and postprandial lipids in
Europeans and South Asians:

A cross-sectional study

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Dissertation for the degree of Doctor of Philosophy (PhD), 2000



Abstract

Metabolic disturbances associated with central obesity and insulin resistance might underlie the higher rates of diabetes and coronary heart disease in South Asians compared with Europeans. A cross sectional study of 135 healthy South Asians and Europeans, aged 40-55 years, was performed to test whether lower insulin sensitivity in South Asians is explained by ethnic differences in body fat pattern and to establish if there are ethnic differences in postprandial triglyceride and intramyocellular lipid (IMCL) content that are associated with insulin sensitivity.

Visceral fat area (VFA), measured by CT scan, was higher in South Asians than in Europeans in analyses adjusted for age, sex and body mass index ($p=0.001$). VFA was strongly associated with insulin sensitivity index (ISI), measured by the short insulin tolerance test, in both groups independently of total % body fat (measured by DEXA scan). In age and sex adjusted analyses ISI was $0.71\% \text{ min}^{-1}$ lower in South Asians (95% CI -1.18 to -0.25 , $p=0.003$). Adjustment for body fat pattern and triglyceride (fasting and 8 hour postprandial) reduced the ethnic difference in ISI to $-0.41\% \text{ min}^{-1}$ (95% CI -0.86 to 0.03, $p=0.066$). In both groups 8 hour postprandial triglyceride was highly correlated with ISI and VFA and the relationship of ISI to VFA was eliminated by adjusting for triglyceride. In a sub-study, mean IMCL content (measured by magnetic resonance spectroscopy) was higher in South Asians ($p=0.046$). In Europeans IMCL was correlated positively with % body fat, waist/hip ratio, VFA and negatively with ISI. In South Asians IMCL was not significantly related to ISI or obesity.

We conclude that body fat pattern and IMCL cannot account for ethnic difference in insulin sensitivity. Alterations of lipid metabolism, possibly in the postprandial period, are likely to underlie the association of central obesity with insulin resistance.

Keywords

South Asian, European, ethnic differences, central obesity, percent body fat, intramyocellular lipid, insulin sensitivity, triglyceride, and postprandial triglyceride

*This thesis is dedicated to my mother, Bharti Gandhi, and to
my father, Jagdish Gandhi*

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Acknowledgements

This project was funded by the Wellcome Trust as part of a Research Training Fellowship in Clinical Epidemiology.

The idea for the project was conceived by Paul McKeigue, my supervisor through the Training Fellowship and this project. I have enjoyed the numerous discussions we have had over the course of this project, and I have imbibed much of the enthusiasm that Paul has for this area of research.

The project could not have been completed in time without the help of Mary Rowley and Saiqa Mullick, who assisted with the fieldwork. I am indebted to Sheelagh Kerr for setting up the database, and willingly giving her help even after leaving the LSHTM. Special thanks go to Janbibi Mazar for help with booking appointments, data entry and administrative matters. I also thank Chris Brandon-Jones for general computing help coupled with good humour! Stephen Sharp's help with statistical programming was also indispensable. I enjoyed the many discussions I had with my colleague and friend Sarah Wild. A heart felt thanks to Naveed Sattar, my friend in Glasgow, for keeping me in good cheer through the writing up stage, and for patiently sending me numerous journal articles of interest, while I tried to write the PhD from home!

I would like to thank Mandeep Sidhu, Ian Godslan, David Crook, Melek Worthington, and Tony Proudler at the Wynn Division of Metabolic Medicine; Uday Bhonsle at the Medical Physics Department at Northwick Park Hospital; Dr Bill Svennson, Paul Eathorne and Tom Rabbitte at the Radiology department of Ealing Hospital; and Gabby Jenkinson, Jimmy Bell and Louise Thomas at the MRI Unit at the Hammersmith Hospital. I would also like to thank Dr Jaspal Kooner at the Department of Cardiology at Ealing Hospital for providing space for this study to take place.

This study could not have been done had it not been for the co-operation of the general practitioners in the London Borough of Ealing who helped with recruitment. Of course, the greatest gratitude goes to the participants who agreed to take part in the study, and persisted in continuing with the study despite its invasive nature involving multiple visits, and often making demands on their valuable time.

Helping all along the way in the background were also Basia Potok, and latterly Stefania Parise, who, by providing excellent care for little Roshan and Sara enabled me to escape to work with confidence.

Finally, I would like to thank my husband, Parto, without whose encouragement, help, moral support, and forbearance this thesis would have never been written.

Table of Abbreviations

Abbreviation	Full term
%fat	Percent body fat
β	Beta: regression coefficient
¹ H-MRS	Proton magnetic resonance spectroscopy
4S	Scandinavian Simvastatin Survival Study
ACE	Angiotensin converting enzyme
Acetyl-CoA	Acetyl co-enzyme A
AFCAPS/ TexCAPS	Air Force/Texas Coronary Atherosclerosis Prevention Study
ALP	Atherogenic lipoprotein phenotype
ApoB	Apolipoprotein B
ApoB-100	(Endogenous or hepatic derived) Apolipoprotein B component - 100
ApoB-48	(Exogenous or intestinal derived) Apolipoprotein B component - 48
ARIC	Atherosclerosis Risk in Communities
BIA	Bio-electrical impedance analysis
BIP	Bezafibrate Infarction Prevention trial
BMD	Bone mineral density
BMI	Body mass index
c.v.	coefficient of variation
CAD	Coronary artery disease
CARE	Cholesterol and Recurrent Events trial
CE	Cholesteryl ester
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CI	Confidence Interval
CM	Chylomicron
CRP	C-reactive protein
CT	Computed axial tomography
DART	Diet and Reinfarction Trial
DEXA	Dual-energy X-ray absorptiometry
DPP	Diabetes Prevention program
EDTA	EthyleneDiamineTetraacetic Acid
ELISA	Enzyme linked immunosorbent assay
FMD	Flow mediated dilatation

Abbreviation	Full term
FTT	Fat tolerance test
GTT	Glucose tolerance test
HDL	High density lipoprotein
HSS	Helsinki Heart Study
HU	Hounsfield Units
IDL	Intermediate density lipoprotein
IL-6	Interleukin – 6 cytokine
IMCL	intramyocellular lipid
IRAS	Insulin Resistance Atherosclerosis Study
ISI	Insulin sensitivity index
ITT	Insulin tolerance test
IV/iv	Intravenous
IVGTT	Intravenous glucose tolerance test
KBr	Potassium bromide
K_{ITT}	Rate constant for insulin tolerance test
LDL	Low density lipoprotein
LIPID	Long-term Intervention with Pravastatin in Ischaemic Disease trial
ln	Natural logarithm
Log	Logarithm
Lp(a)	Lipoprotein (a)
LPL	Lipoprotein lipase
mmHg	Millimetres of mercury
MRI	Magnetic resonance imaging
m-RNA	Messenger- Ribonucleic acid
MRS	Magnetic resonance spectroscopy
n	number
n-3 series	Alpha linolenic acid (fatty acid)
n-6 series	Linoleic acid (fatty acid)
NEFA	Non-esterified fatty acid
NIDDM	Non-insulin dependent diabetes mellitus
NMR	Nuclear magnetic resonance
NS	Not significant
OGTT	Oral glucose tolerance test
p	p value

Abbreviation	Full term
PAI-1	Plasminogen activator inhibitor-1
PCOS	Polycystic ovary syndrome
PET	Positron emission tomography
ppm	Parts per million
PUFA	Polyunsaturated fatty acid
r	Correlation coefficient (Pearson's product moment)
ROI	Region of interest
RP	Retinyl palmitate
rpm	Revolutions per minute
RR	Relative risk
SD/sd	Standard deviation
sem/SEM	Standard error of the mean
Sf	Svedberg flotation
SFA	Subcutaneous fat area (abdominal)
SITT	Short insulin tolerance test
SSPG	Steady state plasma glucose
TFA	Total fat area (abdominal)
TG	Triglyceride
TGRL	Triglyceride rich lipoproteins
TNF- α	Tumour necrosis factor – alpha
VA-HIT	Veteran Affairs High-Density Lipoprotein Cholesterol Intervention Trial
VFA	Visceral fat area (abdominal)
VLDL	Very low density lipoprotein
WHR	Waist/hip ratio
WOSCOPS	West Of Scotland Coronary Prevention Study

Overall plan of the thesis

The rates of insulin resistance, diabetes mellitus and coronary heart disease are known to be higher in people originating from India, Pakistan and Bangladesh (South Asians) than in people of European descent. This project was designed to test a set of related hypotheses about the mechanisms relating obesity (generalised obesity and central obesity) to insulin resistance and increased coronary heart disease (CHD) risk in South Asians. It is hoped that the results will guide further research on the aetiology and control of both diabetes and CHD.

This thesis is arranged in four parts. The first part deals with the background and rationale to this area of research and is presented in chapters 1, 2, 3 and 4. These deal specifically with the epidemiology of coronary heart disease in South Asians and prevalence of the insulin resistance syndrome in this group in chapter one; with the proposed role for postprandial lipids in chapter two; and with the potential role for skeletal muscle cell triglyceride stores in insulin resistance in chapter three. The aims of the study are described in chapter four.

The second part describes the methods employed to achieve the aims and objectives of this study (chapter 5). In the third part the results are presented along with relevant discussion (chapters 6 to 9). The final part of the thesis is dedicated to overall discussion, conclusions and suggestions for future research (chapters 10 and 11).

Publications arising from this study

1. Forouhi NG, Jenkinson G, Thomas EL, Mullick S, Mierisova S, Bhonsle U, McKeigue PM, and Bell JD. Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. *Diabetologia* 1999; **42**: 932-935.
2. Forouhi NG, Mullick S, Eathorne P, Kooner JS and McKeigue PM. Elevated postprandial triglyceride: ethnic and sex differences, and relation to the insulin resistance syndrome. To be submitted to *Atherosclerosis* 1999.
3. Forouhi NG, Sattar NS and McKeigue PM. Relation of C-reactive protein to cardiovascular risk factors in Europeans and South Asians. Submitted to *Arteriosclerosis, Thrombosis and Vascular Biology* 1999.

PART 1

BACKGROUND

- 1970s of the world as understood
- the Post-1945 World, the State of the World
- system was based in 1917 but it was

CHAPTER 1: Epidemiology of Coronary Heart Disease in South Asians Overseas

1.1. Use of the term “South Asian”

Different terms have been used to describe the people originating from the Indian subcontinent, which includes the countries of India, Pakistan, Bangladesh and Sri Lanka. Some of these have included “Asian”, as is in common use in the U.K., but which in the United States has been used more commonly for people originating from the Far East; and “Indian Asians” or “Asian Indians” as has been used in the published literature in the past on both sides of the Atlantic to distinguish these people from Pacific Indians and Native American Indians. Throughout this dissertation the term “South Asian” is used to denote origin from any part of the Indian subcontinent.

However, the people originating from countries as diverse as those that form part of the subcontinent are not homogeneous, and it is fair to ask the question whether they can all be studied together when considering their risk for coronary heart disease or diabetes.

1.1.1. Heterogeneity of the people constituting “South Asians”

Within India it has been claimed that there might be genetic differences in the people of north India (Aryan descent) and south India (Dravidian descent). However this has not been supported by genetic studies¹. Within each of the parts of the Indian subcontinent there is great heterogeneity in the population: there are people of diverse cultural, religious and linguistic backgrounds such as Hindu Gujaratis, Punjabi Sikhs, Muslim Bangladeshis and Muslim Pakistanis, Tamil South Indians and Sri Lankans, many of whom are Hindu, but many converted to Christianity, and to Buddhism, with widely differing dietary and lifestyle habits. Moreover, South Asians have historically emigrated since 1834 to various parts of the world as indentured labourers^{2,3}, forming sizeable communities in Mauritius, the West Indies, Natal, the Straits Settlements, Fiji and East Africa. The indentured labour system was banned in 1917 but a steady migration of professionals, traders, agricultural

and manual workers and their families has continued ever since. Migration, on peoples' own initiative, to the U.K. from the Indian subcontinent increased after 1960 and reached a peak around 1966-67⁴. There has also been a steady stream of migration to North America. It is estimated that there are some 11 million South Asians living outside of the subcontinent.

1.2. High risk for coronary heart disease among South Asians

It has long been observed in several parts of the world that mortality and morbidity from coronary heart disease (CHD) are higher in people of South Asian descent⁵. This is reflected in the higher relative risks for CHD among South Asians when compared with other ethnic groups. For example, when compared with populations known to be at low risk for CHD, such as the Chinese⁶, Melanesians⁷ and Africans⁸, the age standardised CHD death rates calculated from national mortality data have ranged between 3 to 4 times higher among immigrant South Asian men than in other groups settled in the same country. Even when compared with populations known to be at high risk for CHD, such as Europeans⁹ and South Africans¹⁰, the relative risk for CHD mortality in South Asians was higher by a magnitude of around 50%.

The high coronary mortality is seen in the heterogeneous subgroups of people within the South Asian population - thus Gujarati Hindus, Punjabi Sikhs and Muslims from Pakistan and Bangladesh all share the high CHD mortality^{11,12}. Although minor variations in heart disease risk factor profiles between Indian sub-communities have been demonstrated¹³, the overwhelming evidence points to all cultural groups in the Indian subcontinent sharing an increased risk of CHD morbidity and mortality. Thus it is reasonable when investigating CHD risk to consider individuals from different parts of the Indian subcontinent collectively, even though they may originate from a wide range of cultural backgrounds.

There are two important observations about the high rates of CHD in South Asians. Firstly, the high rates of CHD are common to South Asian populations residing around the world. This raises the point that any environmental factor that might explain the high rates of CHD in South Asians must be common to all the main ethnic groups of the Indian subcontinent, to both sexes, and persist several generations after migration. Secondly, the higher relative risk for CHD and type 2 diabetes in South Asians does not wear off with

increasing generations since migration, i.e., it continues to diverge from the rates in other ethnic groups in the same country. For example, in Singapore, a rapid transition to affluence has been shared by all three of the main ethnic groups residing there (Chinese, Malays and Indians), but the prevalence of diabetes is much higher in Indians than in Chinese¹⁴. From studies of CHD in other migrant populations (such as the Japanese in the U.S.A.) it has emerged that CHD rates in those settled overseas the longest and in their descendants converge to those in the host population¹⁵. This has formed the basis of the view that between-population differences in CHD rates are environmentally rather than genetically determined. However given the contrasting situation in South Asians where higher risk persists several (five or six) generations post-migration⁵, a genetic explanation is more likely to apply than an environmental one.

The relative risk of coronary disease in South Asian men is highest at early ages: between 1979 and 1983, CHD mortality in men aged less than 40 years born in South Asia was more than twice the U.K. national average¹⁶. In patients undergoing coronary angiography the anatomical distribution of disease does not differ between South Asians and Europeans, but the extent and severity of lesions are greater in South Asians^{17,18}. Case fatality six months after acute myocardial infarction was reported to be twice as high in South Asians compared with Europeans¹⁹, but a large part of this excess hazard was attributable to non-insulin dependent diabetes (NIDDM): adjustment for NIDDM reduced the hazard ratio from 2.02 to 1.26¹⁹.

Despite a reduction in CHD mortality in all Western European countries between 1970 and 1985, over the same period there was a 6% and 13% increase in CHD mortality for South Asian men and women respectively^{11,16,20}. By 1991, however, there was a decline in CHD mortality compared with 1970-72, among those born in the Indian sub-continent (20% for men and 7% for women)⁹. However, this decline was of a smaller magnitude than those born in England and Wales (29% for men and 17% for women)⁹.

The consistency of the high risk of CHD among South Asian people in several studies from around the world, affecting both sexes and with early onset, particularly in men, has driven research to try to identify the risk factors that contribute to this excess risk. The speculation has been that there might be a common underlying explanation for the high CHD risk.

1.2.1. Coronary heart disease in the Indian Subcontinent

There are no current population based national data on CHD incidence, prevalence or mortality in the Indian subcontinent, although studies from some towns in India exist. The largest of these was a population study in Delhi in 1985-86, of 13,723 men and women²¹. Prevalence of ECG changes was 7% in men and 4.3% in women aged 45-64 years. However the overall prevalence of Minnesota-coded major Q waves was 3.6%²². A survey of 725 adults aged over 30 years in the city of Varanasi showed a 6.5% prevalence of CHD (based on WHO chest pain questionnaire and ECG changes)²³. The prevalence of CHD in urban settings in India is reported to be higher than that among inhabitants of rural areas and is approximately equivalent to that among South Asians who have settled outside the subcontinent^{22,24-26}.

1.2.2. Coronary risk factors in South Asians

A thorough description of the prevalence of conventional risk factors such as levels of smoking, blood pressure and serum cholesterol in South Asians has been given before by McKeigue and colleagues^{5,27}. A brief summary is given here. Compared to the U.K. national average of about 30%, the prevalence of smoking in men of Gujarati Hindu and Indian or Pakistani Muslim origin is generally comparable, higher among Bangladeshi Muslims, and much lower (about 4%) in Punjabi Sikhs, whose religion prohibits them from smoking. Smoking rates are very low in all groups of South Asian women except Bangladeshis. No South Asian community studied in the U.K. has higher average plasma cholesterol levels in middle age than the national average of about 6.0 mmol/l. It has been found that compared with Europeans, average blood pressure levels are higher in Punjabi Hindus and Sikhs, similar in Gujarati Hindus and Pakistani Muslims, and lower in Bangladeshis. However, although conventional risk factors such as smoking, hypertension and hypercholesterolaemia remain highly important risk factors for CHD within each ethnic group of Europeans as well as South Asians, they fail to account for the excess of CHD rates in South Asians when compared to Europeans^{22,28,29}.

Non insulin dependent diabetes mellitus is present in about 20% of South Asian men and women aged over 40 years in the U.K., compared with about 5% of Europeans²⁹. In a case control study the proportion of all myocardial infarctions which were attributable to

NIDDM - the aetiologic fraction - was found to be 21% in South Asians versus 3% in Europeans³⁰. However, most South Asian patients with CHD are not diabetic³¹ and glucose intolerance cannot alone explain more than a small proportion of the coronary risk in South Asians³². Levels of haemostatic factors such as fibrinogen were similar to or lower in South Asians than those in Europeans^{33,34} and factor VIIc was lower in Bangladeshi than European men²⁸. Thus haemostatic factors also do not account for the excess CHD risk in South Asians.

An adverse diet among South Asians has been implicated in promoting CHD. However in studies that assessed diet similar proportion of total energy intake was derived from fat in both European and South Asian groups, and the polyunsaturated/saturated fat ratio was higher in South Asians^{33,35,36}. Psychosocial factors possibly contributing to CHD were investigated in a cross sectional study of Punjabi origin South Asians with the general population in Glasgow³⁷. A much larger proportion of South Asians reported worse socio-economic circumstances as well as stress factors such as longer working hours, low income, crowded housing, liability to attack and perceived lack of social support. The authors of the study proposed that stress and socio-economic deprivation, along with the high prevalence of diabetes was likely to explain the excess risk of CHD in South Asians. However the study was based on a small number of 173 South Asians, and no attempt was made at studying specifically the relationship between coronary risk factors and socio-economic status or CHD and socio-economic status. Additionally the participants expressed little dissatisfaction about these conditions. No clear-cut role of these stresses in promoting CHD has been found in South Asians, but it is plausible that they play some contributory role.

Based on the failure of the above risk factors to account for the excess CHD in South Asians, and on the results of cross-sectional surveys in east London²⁸ and Southall, west London²⁹, McKeigue *et al* have suggested that a pattern of physiological disturbances related to central obesity and insulin resistance underlies the high coronary risk and diabetes prevalence in South Asians.

1.3. The insulin resistance syndrome

1.3.1. Background

In 1975 in their seminal paper Miller and Miller³⁸ proposed that a reduction in plasma HDL-cholesterol concentration may accelerate the development of atherosclerosis and hence CHD, by impairing the clearance of cholesterol from the arterial wall. This was later also confirmed by findings from the Framingham study in 1977³⁹. Around the same time a predictive role for triglycerides was also shown in prospective studies⁴⁰. High levels of fasting triglyceride and low levels of plasma HDL-cholesterol have been found fairly consistently in South Asians overseas compared with other groups. This was specifically reported in South Asians in Trinidad compared with people of African origin⁴¹ and in the U.S.A. compared with European descent Americans⁴². In Fiji higher plasma triglyceride levels were shown compared to Melanesians⁴³, and in East London mean HDL-cholesterol was 0.3 mmol/l lower and plasma triglycerides after a glucose load were 50% higher among Bangladeshis than in Europeans²⁸. In fact in the latter study McKeigue *et al* identified a pattern of interrelated metabolic disturbances among South Asians of Bangladeshi descent: glucose intolerance, low plasma HDL-cholesterol, and high concentrations of insulin and triglyceride after a glucose load. In other populations these disturbances are associated with insulin resistance^{44,45}.

1.3.2. Definition of the insulin resistance syndrome

The insulin resistance syndrome (also known as the 'metabolic syndrome' or 'Syndrome X' first defined by Reaven) has been defined as a cluster of related factors that include resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinaemia, increased levels of VLDL triglycerides, decreased levels of HDL-cholesterol and hypertension⁴⁴. Insulin resistance is also associated with central obesity - a pattern of obesity in which a high proportion of body fat is deposited intra-abdominally.

The mechanisms underlying the associations of features of the insulin resistance syndrome are poorly understood. Resistance to insulin-stimulated glucose uptake is probably responsible for the glucose intolerance, hyperinsulinaemia, and hypertension. Failure of

insulin to suppress release of non-esterified fatty acids (NEFA) from adipose tissue (particularly increased intra-abdominal adipose tissue of central obesity) may cause hypertriglyceridaemia⁴⁶, low plasma concentrations of HDL-cholesterol, and changes in the composition and size of particles in the LDL fraction⁴⁷.

1.3.3. The relation of coronary risk to insulin resistance in South Asians

McKeigue and colleagues proposed in 1988 that a tendency for this insulin resistance syndrome to develop was generally present in South Asians overseas, and that this tendency might underlie the high rates of CHD and type 2 diabetes^{5,28}. They later confirmed these findings in further studies of South Asians living in north-west London, where they also found a tendency towards central obesity associated with all the above features^{22,29}. Waist-hip ratio (as an index of central obesity) was higher in South Asian than European men although the average body mass index was no higher in South Asian men than in European men²⁹. In the Southall study²² adjusting for central obesity, glucose intolerance and hyperinsulinaemia reduced the odds ratio for major Q waves on ECG from 2.4 (when adjusted for age, smoking and cholesterol) to 1.5, explaining two thirds of the excess CHD morbidity in South Asian men.

1.3.3.1. Support for the insulin resistance hypothesis in South Asians

Several lines of evidence support the insulin resistance hypothesis as the explanation for the high CHD risk in South Asians. Socio-economic status, smoking, plasma cholesterol, blood pressure, haemostatic factors and fatty acid composition of plasma lipids differ markedly between the various South Asian groups who share the high CHD risk^{28,29,33,35}. It is also important to highlight that in some South Asian groups who share high CHD risk, some of the risk factors associated with insulin resistance are not more unfavourably distributed than in Europeans. For instance, in comparison with Europeans, South Asian Muslims do not have higher systolic and diastolic blood pressures, Sikhs do not have lower HDL cholesterol, and Gujarati Hindus do not have higher fasting triglyceride levels. As high CHD risk is common to all these groups, it follows that the increased CHD risk in South Asians cannot be mediated mainly through raised blood pressure, low HDL-cholesterol or raised fasting triglyceride. However, common to all South Asian groups at

high risk of CHD are central obesity, hyperinsulinaemia, high diabetes prevalence and raised triglyceride level after a glucose load^{28,29}.

The narrowing of the sex difference in CHD mortality that occurs in South Asians after the age of 40 years parallels the changes in body fat pattern and lipoprotein pattern that occur in women around the time of the menopause. The attenuation of the sex difference in coronary risk in older South Asians is also explained by the insulin resistance syndrome, since the insulin resistance syndrome is associated with several risk factors which are normally less prevalent in women than in men. These include central obesity, raised plasma triglyceride, and lower HDL-cholesterol. A similar loss of women's immunity to CHD occurs in NIDDM⁴⁸.

Several other groups have also confirmed these findings of higher WHR, higher levels of fasting triglyceride and lower levels of HDL-cholesterol combined with higher prevalence of glucose intolerance among South Asians. These cross sectional^{49,50} and case control studies^{31,51} have supported the hypothesis that features of the insulin resistance syndrome promote CHD risk in South Asians.

Studies among young South Asian adults also suggest early onset of features of insulin resistance. Young (mean age 21 years) male offspring of South Asian patients with coronary disease were found to have significantly higher fasting insulin levels and waist-hip ratios than age matched male offspring of European patients⁵². Additionally, young relatives of South Asian patients with NIDDM compared with age matched subjects without such a family history were demonstrated to have significantly higher fasting insulin levels and poorer insulin sensitivity measured by the short insulin tolerance test⁵³. It thus appears that South Asian people have a tendency to features of the insulin resistance syndrome from an early age. The insulin resistance hypothesis provides a unifying explanation for the high rates of both diabetes and CHD in South Asians.

1.3.4. Origins of insulin resistance: genes or environment?

1.3.4.1. Genetic hypothesis

The pathophysiology and aetiology of insulin resistance and development of type 2 diabetes is not clearly understood. Neel first proposed a "thrifty genotype" hypothesis in 1962⁵⁴ to

explain the between population variation in the prevalence of diabetes which have been observed this century. He proposed that during the early stages of human evolution a series of genes were selected that gave a survival advantage to individuals who underwent cyclic changes in food availability, as would be associated with the hunter-gatherer or early agriculturist lifestyle. Expression of these genes would result in a “quick insulin trigger” (large bursts in insulin secretion) in response to hyperglycaemia, thus leading to reduced urinary caloric loss and the efficient storage of dietary calories as fat. This would provide an energy store (from breakdown of fat in adipocytes to release NEFA) to be used in times when food was more scarce and hence would be beneficial when there was a cyclical availability of food. He also proposed that such a setting would become detrimental to health in conditions of more plentiful food supply, as the quick insulin trigger would become over-stimulated, leading to beta-cell decompensation and diabetes.

This hypothesis came under question when it was reported that the high rates of diabetes (and glucose intolerance) previously recorded in the Pacific island of Nauru were decreasing, despite the recent transition from undernutrition to relative affluence⁵⁵.

1.3.4.2. Environmental hypothesis

Exactly 30 years after Neel’s hypothesis, Hales and Barker suggested a “thrifty phenotype” hypothesis⁵⁶ as an alternative to the thrifty genotype hypothesis. Their hypothesis proposes that the high rates of diabetes result from undernutrition in fetal and infant life, followed by relative over nutrition later, suggesting an environmental rather than genetic basis for the disease. They postulated that this hypothesis better explains the changes seen in Nauru. Based on the associations seen between low birth weight and subsequent development of CHD, hypertension and type 2 diabetes (or glucose intolerance), they have proposed that the peri-conception and pregnancy period has influences on foetal growth (such as foetal undernutrition) which “programme” the susceptibility to development of these disorders in later life⁵⁷⁻⁵⁹. They showed in particular that factors associated with low birth weight and with high adult BMI contribute independently to an individual’s glucose tolerance.

There has been support for this hypothesis from other groups. Recent evidence suggests particularly increased risk among small babies of women with high body mass index⁶⁰. A study of twins in Denmark⁶¹ found that in both monozygotic and dizygotic twins discordant

for type 2 diabetes, the birth weights of the diabetic twins were significantly lower than those of the non-diabetic twins. This supported the role of a non-genetic (environmental) intrauterine component, such as intrauterine environment, for the development of diabetes later in life. One study has found results conflicting with this hypothesis. This study⁶² compared 174 Russian adults exposed *in utero* to malnutrition during the 1941-42 siege of Leningrad, with controls born before the siege or outside Leningrad. Fasting and 2h glucose were not significantly higher in the group exposed to malnutrition *in utero* than in controls. However, it is uncertain whether malnutrition in this population was restricted to those classified as exposed. The Dutch famine study⁶³, in contrast, reports a higher mean 2h glucose in those exposed to famine during mid gestation or late gestation than in those not exposed, and this effect was independent of BMI. This famine of 1944-45 was sharply delimited and provides a good setting for testing the hypothesis that impaired nutrition in foetal life may cause increased risk of diabetes in adult life.

The “Barker” hypothesis has been extended to other populations at high risk for diabetes, including South Asians, Native Americans and Mexican-Americans. In a study of 1179 Pima Indians there was a U-shaped relationship between birth weight and prevalence of diabetes⁶⁴. Prevalence of diabetes was raised only in those with birth weight less than 2.5 kg or more than 4.5 kg. In a study in the south Indian city of Mysore the prevalence of diabetes was positively related to ponderal index (weight/ length³) at birth⁶⁵ (in contrast to the inverse associations seen in European populations). The authors suggested that the effects of gestational diabetes on size at birth and beta-cell function in adult life might account for this association. In a study of 4 year old children in India there was an inverse association between low birthweight (weight less than 2.4kg) and 30 minute post-load glucose and insulin levels in 201 routine deliveries⁶⁶. However no association was seen in low birthweight children looked after in a special care baby unit. Whincup and colleagues⁶⁷ have studied these relationships in children aged 10-11 years from 10 towns in the U.K., including children of South Asian origin. They found that in all children childhood obesity was a stronger determinant of insulin level and insulin resistance than size at birth (either ponderal index or birthweight). It appears that in populations at high risk for diabetes the inverse relationship between glucose intolerance and size at birth is less clear, and the relationship may be U shaped or reversed. This could be attributable to the effects of glucose intolerance during pregnancy.

1.3.4.3. Which hypothesis explains the ethnic differences in prevalence of insulin resistance and diabetes?

There arises a question as to whether impaired foetal growth can account for ethnic differences in prevalence of diabetes. In other words is the thrifty phenotype hypothesis important only in explaining the high prevalence of diabetes *within* populations or does it explain variation *between* populations as well? Although reduced foetal growth may be part of the explanation for high prevalence of diabetes in the high risk populations, the evidence that genetic factors underlie the ethnic differences in diabetes risk remains compelling. Evidence from migrant (see sections 1.1.1 and 1.2) and admixture studies suggests that differences in diabetes risk between high risk ethnic groups such as Pacific islanders and low-risk ethnic groups such as Europeans are attributable to genetic factors. In Nauruans inverse associations of diabetes with genetic markers of European admixture have been reported⁶⁸. Admixture in Nauru resulted mainly from unions between Nauruan women and European men; thus the effect was to introduce European genes but not European maternal environment. It is possible that ethnic differences in the risk of diabetes could be a consequence of ethnic differences in foetal growth which have a genetic basis.

There is yet another hypothesis called the “foetal insulin hypothesis” as an alternative explanation of the association of low birth weight with diabetes and vascular disease⁶⁹. This hypothesis has been proposed in 1999 by Hattersley and Tooke, and it proposes that the association between low birthweight and adult insulin resistance is principally genetically determined. Central to this hypothesis is the concept that insulin-mediated foetal growth will be affected by foetal genetic factors that regulate either foetal insulin secretion or the sensitivity of foetal tissues to the effects of insulin. However this hypothesis is not entirely consistent with the findings of the Danish twins study⁶¹ or the Dutch famine study⁶³. Of practical importance is the point that if ethnic differences in disease risk have a genetic basis it is possible to map the genes underlying these differences by studying populations where there has been recent admixture⁷⁰.

1.3.5. The basis of the metabolic defect in insulin resistance

Although insulin resistance is strongly associated with central obesity in South Asians as in other ethnic groups, it is not known whether the primary defect is central obesity, resistance to insulin action, or some other defect. Many studies have demonstrated that excessive accumulation of fat in the abdominal region is strongly associated with metabolic alterations such as disturbed plasma lipoprotein profile⁷¹⁻⁷³, hyperinsulinaemia, insulin resistance and glucose intolerance⁷⁴⁻⁷⁶. In the cross-sectional study in Southall, adjusting for waist-hip ratio (WHR) explained only about half of the ethnic difference in insulin levels²⁹. However, WHR is not an accurate measure of the proportion of body fat sited intra-abdominally: the correlation with CT scan measures is only about 0.55⁷⁷. To establish whether the ethnic difference in insulin resistance can be explained by central adiposity, we need to measure visceral fat distribution directly by CT scan.

If adiposity is important then there should not be a difference in insulin resistance between lean people of South Asian and European origin. In other conditions associated with insulin resistance, susceptibility to insulin resistance depends on the levels of adiposity. Thus overweight women with polycystic ovary syndrome (PCOS) are more insulin resistant than weight-matched controls, but when lean women with PCOS are compared with lean controls there is no difference in insulin sensitivity⁷⁸. In a study in Swedish men, the association between thinness at birth and raised post-load insulin levels was present only in men who were overweight⁷⁹. South Asian infants born in the U.K. are thinner at birth than native British infants, and it is possible that the tendency to insulin resistance in South Asian adults is similarly dependent on the degree of obesity. It is important to test whether in South Asians the slope of the regression line for the relation between central obesity and insulin resistance is parallel to that for Europeans, or the lines cross over with varying adiposity.

1.4. Mediation of increased coronary risk

It is not established which component of insulin resistance promotes CHD. It is possible that hyperinsulinaemia and insulin resistance increase the risk for CHD indirectly through their effects on cardiovascular risk factors, such as raised triglyceride and lowered

HDL-cholesterol levels. It is also possible that hyperinsulinaemia and insulin resistance have direct effects on the coronary artery wall and thereby promote atherosclerosis⁸⁰.

As discussed earlier (section 1.3.3.1) the effect of insulin resistance on the excess CHD risk in South Asians cannot be mediated mainly through blood pressure or HDL cholesterol since these risk factors are not unfavourably distributed in all the groups of South Asian settlers in the U.K. who share high CHD risk^{28,29}. Other disturbances associated with central obesity and insulin resistance include elevated plasminogen activator inhibitor (PAI-1)⁸¹, elevated IDL and predominance of small dense LDL^{82,83}, the last of which has been particularly speculated to be associated with the increased atherogenic risk in South Asians. However, one study⁸⁴ concluded that compared to European-descent men, South Asian men in Texas had larger LDL particles and a lower prevalence of small dense LDL in the presence of normolipidaemia. In contrast a recent study of 92 men in London has found a preponderance of lower LDL size among South Asians compared to Europeans and Afro-Caribbeans⁸⁵.

Raised triglyceride levels are seen both in obesity and insulin resistance. Coronary heart disease in South Asians is more strongly associated with raised plasma triglyceride levels than with any other metabolic measurement, but ethnic differences in fasting triglyceride levels are too small to account statistically for the excess prevalence of CHD in South Asian compared with European men²². It is possible that postprandial rather than fasting triglyceride levels could account for the excess risk of CHD in South Asians. There are no published studies that have measured postprandial lipid metabolism in South Asians. The potential role of postprandial lipids in insulin resistance and coronary heart disease is explored in chapter 2.

The mechanism of how insulin resistance comes about is also not known. One possibility is that it comes about as a result of increased stores of triglyceride in skeletal muscle cells. The evidence for this is presented in chapter 3.

1.4.1. The controversy about the role of insulin and insulin resistance in CHD

The hypothesis that insulin resistance or hyperinsulinaemia is causally related to the risk of CHD is still controversial. An extensive review of the evidence from prospective studies

for and against the association between baseline insulin level and future risk of CHD has been presented by Laakso⁸⁶. Many reject the insulin hypothesis⁸⁷⁻⁸⁹. Jarrett⁸⁷ has noted that raised fasting and post-load insulin levels not only fail to predict CHD consistently when other risk factors are taken into account, but sometimes fail to predict even in univariate analyses. However, even if insulin fails to be a risk factor in univariate analysis, it does not necessarily imply that hyperinsulinaemia cannot contribute to accelerated atherogenesis or thrombogenesis. It is still possible that the effects of insulin are mediated through its impact on other cardiovascular risk factors. The insulin resistance hypothesis does not assume that insulin only directly promotes atherogenesis. Thus it is possible that the associations between hyperinsulinaemia and CHD are mediated through disturbances of lipoprotein metabolism such as raised plasma triglycerides, low HDL-cholesterol and small dense LDL.

Nevertheless it has been observed that raised fasting or post-load insulin levels are far less consistently predictive of cardiovascular disease than would be expected if the insulin resistance hypothesis is correct. To explain this McKeigue and Davey⁹⁰ have argued that this is largely attributable to failure to control for the effects of undernutrition and weight loss resulting from poor health. Poor nutritional status is a strong predictor of mortality in the elderly, and smoking is a powerful confounder of this relationship. The strength of the co-morbidity effects is demonstrated by the observation that men who died of CHD were leaner than those who survived in some of the studies such as the Rancho Bernardo cohort⁹¹, the Gothenburg Study⁹², and in the Multiple Risk Factor Intervention Trial⁹³. Thus it is plausible that failure to control for confounding by co-morbidity is responsible for the lack of association between raised insulin levels and cardiovascular disease in several cohort studies.

McKeigue and Davey⁹⁰ have proposed that the key question for public health is not whether raised insulin levels are an independent risk factor for CHD, but whether measures to alleviate the syndrome of metabolic disturbances associated with insulin resistance and central obesity will reduce the risk of CHD.

In summary, the reasons for the relative excess of NIDDM and CHD in South Asians remain unclear but features associated with the insulin resistance syndrome remain a strong candidate for providing a unifying explanation. Thus preventative strategies to reduce the

risk of coronary heart disease and diabetes in South Asian communities may have different emphases to those designed for European groups. For instance the most important intervention in South Asians may constitute alleviation of insulin resistance. There remain many gaps in our knowledge about the relationship between body fat distribution (especially central obesity), insulin resistance and lipid metabolism. This study was designed to investigate some of these.

1.5. Measurement of insulin resistance

In contrast to clinical syndromes of insulin resistance which are rare and typified by striking clinical features such as acanthosis nigricans and ovarian hyperandrogenism in women, the insulin resistance and its related metabolic disturbances that most people get, and has been thus far referred to, develops insidiously and without symptoms. Dose-response studies point against an insulin receptor binding defect in the majority of people with insulin resistance, but suggest instead changes in post receptor signalling or intracellular response. Glucose metabolism and storage have been the focus of investigations into alterations in intracellular metabolism. Indirect calorimetry and isotopic studies have demonstrated reduced glucose oxidation⁹⁴ and diminished insulin-stimulated glycogen synthesis⁹⁵ in insulin resistant subjects at lower and higher insulin concentrations, respectively.

Insulin is important in the regulation of substrate metabolism. Insulin regulates glucose metabolism (causes glycogen synthesis, and prevents gluconeogenesis) as well as fat metabolism (anti-lipolytic effect; suppresses production of NEFA from adipocytes) and protein metabolism. Most studies quantitate insulin action in terms of its glucose regulatory effects. This is due in large part to the technical ease of measurement of glucose concentration.

Traditionally in epidemiological studies insulin resistance has been “approximated” by the surrogate measure of plasma insulin level, often in response to an oral glucose load. This provides an indirect measure of insulin action, but alterations in insulin secretion and/or clearance can alter insulin concentrations in the absence of any change in insulin action. The correlations of fasting and post-glucose insulin levels with insulin sensitivity evaluated by the euglycaemic clamp technique range at most from 0.6 to 0.7⁹⁶. Thus measurement

of plasma insulin level alone is inadequate in a study such as the current one, the primary objective of which is to compare insulin sensitivity in South Asians and Europeans, and to study its relationship specifically with obesity. We wanted to measure insulin sensitivity more accurately, and more reflective of the dynamic situation *in vivo*. The choice of methods available to do this is summarised below.

1.5.1. Clamp techniques

The euglycaemic hyperinsulinaemic clamp technique⁹⁷ is currently regarded as the “gold standard” for measuring insulin sensitivity *in vivo*, against which other methods are compared⁹⁸⁻¹⁰². It involves artificial elevation of plasma insulin using an infusion of exogenous insulin over a period of 3 to 6 hours. Plasma glucose is kept at a physiological level by infusing glucose at a rate adjusted to the prevailing insulin concentration. Samples are taken from an arterialised hand vein for the duration of the study. The rate of glucose infusion represents insulin-mediated glucose disposal assuming that the steady state insulin level is sufficient to suppress hepatic glucose production. This assumption can be tested by infusing labelled glucose and measuring the ratio of labelled to unlabelled (endogenously produced) glucose in the plasma.

A hyperglycaemic hyperinsulinaemic clamp can be used to measure acute and steady-state glucose-stimulated insulin secretion, in addition to insulin-mediated glucose disposal. Overall the clamp techniques relate net blood glucose elimination rate to the plasma insulin concentration.

However, clamp techniques are complex, prolonged, invasive and expensive, and not suitable for large numbers of subjects. In absolute terms the clamp may considerably overestimate insulin sensitivity by the presence of sustained hyperinsulinaemia and the increasing rate of glucose infusion required to maintain euglycaemia. This creates a non-physiological state, because in normal physiology there is never such sustained hyperinsulinaemia. Thus while clamp-derived measures provide an adequate reference, their use in epidemiological studies should be viewed with caution.

1.5.2. Intravenous glucose tolerance test and minimal modelling

Minimal model analysis of glucose and insulin concentration profiles during an intravenous glucose tolerance test (IVGTT) avoids the procedural difficulties of the clamp. Minimal model analysis uses mathematical analysis of the non-steady state glucose and insulin concentrations during an IVGTT to provide a measure of the constant that best relates change in glucose disposal rate to change in plasma insulin concentration. An IVGTT involves a bolus injection of glucose into an antecubital vein, and frequent sampling from the contralateral arm. The modelling technique was originally described by Bergman in 1979⁹⁹. The index of insulin sensitivity derived during minimal modelling represents the ability of insulin to enhance total net glucose disappearance from the extracellular fluid (by suppressing glucose production as well as by increasing its utilisation).

The complexity of this method lies in the computer modelling rather than in the metabolic test itself. The test is moderately invasive and time-consuming (3 hours) but does not require sophisticated equipment. One disadvantage is that intravenous administration of glucose bypasses gastrointestinally stimulated secretion of insulin via substances such as cholecystokinin-pancreozymin (CCK-PZ), and so the peak insulin secreted is less than that in response to an oral challenge. Additionally, although a physiological insulin dynamic is employed (in comparison with the clamp), the technique depends on there being a sufficient insulin response (i.e. beta cell response).

1.5.3. Insulin suppression test

In this test endogenous insulin secretion is suppressed with somatostatin (or with intravenous propranolol and adrenaline), coupled with a fixed constant glucose and insulin infusion^{103,104}. Steady-state plasma glucose (SSPG) represents an index (the higher the glucose, the greater the magnitude of insulin resistance). Although relatively simple to perform, it assesses the ability of both insulin and glucose to facilitate glucose uptake. Interpretation may be confounded by the effect of the agents used to inhibit insulin secretion on insulin action and by differences in urinary glucose excretion since plasma glucose may exceed the renal threshold in some but not all subjects during study.

1.5.4. Short insulin tolerance test

The measurement of glucose disappearance from plasma after intravenous insulin injection is the basis of the insulin tolerance test, which has been used widely to estimate *in vivo* insulin action¹⁰⁵⁻¹⁰⁸. There are however two main criticisms of the insulin tolerance test: (a) insulin induced fall in glucose results in a counter-regulatory hormonal response (which includes secretion of glucagon, catecholamines, growth hormone and cortisol), which in turn slows the glucose disappearance rate from plasma^{106,107,109}, and (b) insulin injection may induce hypoglycaemia which is both unpleasant and potentially dangerous.

Both these problems can be overcome by modification of the test. Firstly the blood glucose decline can be measured over the initial 15 minutes, before the onset of the counter-regulatory response which usually sets in about 20 minutes after insulin injection¹¹⁰⁻¹¹². Secondly, to avoid symptomatic hypoglycaemia in healthy subjects, a reduced dose of insulin bolus (0.05 units per kg) can be used^{102,113}. This is a modification of the insulin dose of 0.1 units per kg body weight which has been validated against the euglycaemic hyperinsulinaemic clamp in normal, obese and diabetic individuals^{100,101}.

With these modifications the low dose short insulin tolerance test (SITT) is derived. In this test, glucose levels are measured every 2 minutes from an arterialised hand vein after the injection of a bolus of short acting insulin, and the test terminated at 15 minutes with intravenous glucose. The rate of disappearance of glucose (K_{ITT}) is calculated as $0.693/t_{1/2}$ the glucose half life ($t_{1/2}$) being derived from the slope of the plasma glucose concentration curve from 3 to 15 minutes¹⁰¹. This K_{ITT} is equivalent to multiplying the slope (of the decline in log transformed blood glucose concentration from 3 to 15 minutes) by a -100 factor¹¹³. The glucose decline occurring in the first 15 minutes after insulin administration is the function of insulin-stimulated glucose uptake by tissues, as well as insulin-inhibited glucose output by the liver.

The advantages of the SITT include simplicity, safety and brevity. Even at the lower dose of insulin (0.05 u/kg) the intra-individual reproducibility has been shown to be good (coefficient of variation $6.9 \pm 2.6\%$ ¹⁰²), with close correlation of the SITT derived insulin sensitivity with that derived from euglycaemic clamp studies ($r=0.81$, $p<0.005$)¹⁰².

The choice of the SITT for the current study is defended in section 5.10.1.

1.6. Other proposed risk factors for CHD

A whole host of potential risk factors for CHD have been proposed over the past few years. Evidence for the possible role of some of these risk factors in explaining the higher CHD risk in South Asians is beginning to emerge.

Elevated level of lipoprotein(a) [Lp(a)] has been reported to be an independent risk factor for CHD^{114,115}. Lp(a) is an LDL-like substance containing a highly carbohydrate-rich protein, apo(a), with striking homology with plasminogen, a key protein of the coagulation process. Higher Lp(a) levels have been reported in South Asians compared with Europeans^{116,117} and compared with Malay and Chinese residents of Singapore¹¹⁸. The extent to which Lp(a) could account for the ethnic differences in CHD mortality has not as yet been examined.

Evidence for the association between concentrations of total plasma homocysteine and vascular disease has recently been reviewed¹¹⁹. It has been suggested that raised homocysteine levels in South Asians may contribute to their excess CHD risk¹²⁰.

It has been suggested that the occurrence of a procoagulant and proinflammatory state in the vasculature may be associated with increased coronary risk. A recent review presents the evidence for how triglyceride rich lipoproteins may promote atherosclerotic vascular disease by adverse effects on platelet function, coagulation and vascular inflammation¹²¹. There is also a growing body of evidence that C-reactive protein, a marker of inflammation, is associated with CHD¹²². No studies of an ethnic comparison of C-reactive protein have been reported in the literature.

Some studies have reported associations between CHD and persistent bacterial infection with *Chlamydia pneumoniae* and *Helicobacter pylori*^{123,124}, although there is still uncertainty about how far these associations can be accounted for by residual confounding by factors such as smoking and socio-economic status¹²³⁻¹²⁵. Whether these chronic infections may be associated with the higher CHD risk in South Asians is as yet unknown.

CHAPTER 2: Postprandial lipids

2.1. The association of postprandial lipids with CHD

2.1.1. Relation between fasting triglyceride concentration and CHD

A high fasting plasma triglyceride concentration has long been implicated as a risk factor for coronary heart disease (CHD). The first case-control study of this association showed elevated fasting triglyceride levels among CHD cases compared with controls¹²⁶. The earliest prospective study demonstrated an increased incidence of CHD among men with elevated triglyceride levels at baseline, compared with men with lower levels¹²⁷. However even in this early study it was speculated that the triglyceride association may not be independent of other plasma lipid levels. In the decades of research since then the independent role of triglycerides remained controversial as a number of studies found that while fasting triglyceride was univariately associated with CHD, this association did not remain statistically significant after controlling for other lipid risk factors, especially HDL-cholesterol¹²⁸. Part of the reason for this cancellation of effect of triglyceride may have been due to the fact that there is a strong inverse association between triglyceride and HDL-cholesterol levels, resulting in multi-colinearity in multivariate statistical models. However, Hokanson and Austin performed a meta-analysis of population based prospective studies of the relation between fasting triglyceride level and CHD¹²⁹. They showed that based on combined data from 17 studies, even after adjustment for HDL-cholesterol there was an independent association between fasting triglyceride and CHD incidence in men [RR 1.14, 95% CI 1.05 to 1.28] and women [RR 1.37, 95% CI 1.13 to 1.66]. These relative risks were calculated and standardised with respect to a 1 mmol/l increase in triglyceride concentration.

It has been previously shown that plasma triglyceride levels were strongly associated with prevalent myocardial infarction in South Asians, but the ethnic differences (South Asian vs. European) in all fasting lipid fractions, including triglyceride, were small and inconsistent²². Two decades ago Zilversmit hypothesised that postprandial triglyceride-rich

lipoproteins (chylomicron remnants) may be important in atherogenesis¹³⁰. Since then there have been several studies of the relation between postprandial triglyceride levels and the risk for CHD.

2.1.2. Relation between postprandial triglyceride concentration and CHD

In a case-control study of 101 men, Patsch *et al*¹³¹ showed that postprandial hypertriglyceridaemia was associated with coronary stenosis defined angiographically. In this study single postprandial triglyceride levels at 6 and 8 hours after the meal were highly discriminatory ($p < 0.001$) between cases and controls, even after adjusting for the effect of fasting triglyceride level. The triglyceride levels at 6 and 8 hour time points exhibited a greater difference between cases and control subjects than did the global magnitude of postprandial lipaemia.

Supplementation of the test meal with vitamin A and subsequent measurement of retinyl palmitate (retinyl ester) in plasma provides a reliable marker of chylomicron and chylomicron remnant metabolism, and is a more sensitive index of the nature of the particles that contain triglyceride than the measurement of triglyceride level alone¹³²⁻¹³⁵. In a smaller study of 40 normolipidaemic cases and controls, Groot *et al*¹³⁶ showed that patients with angiographic coronary artery disease showed a marked delay in the clearance of postprandial retinyl esters as well as in the normalisation of plasma triglyceride concentrations between 6 to 12 after the test meal compared with controls. The authors argued that this delayed clearance was due to a slower rate of chylomicron remnant removal.

In a further case-control study of 170 normolipidaemic people Weintraub *et al*¹³⁷ demonstrated that the area below the chylomicron remnant retinyl palmitate curve was significantly increased in the coronary artery disease (CAD) group (mean 23.4 $\mu\text{mol/l.h}$, SD 15.0) as compared with controls (mean 15.3 $\mu\text{mol/l.h}$, SD 8.9), $p < 0.001$. This was following a fat meal of 50g fat per square metre of body surface. The association of postprandial response with CAD was highly significant before ($p = 0.0001$) and after ($p = 0.001$) adjusting for differences in basal triglycerides. The authors argued that the higher level of chylomicron remnants in normolipidaemic patients with CAD may explain their susceptibility to atherosclerosis. Further, Simpson *et al*¹³⁸ showed that the magnitude of postprandial lipaemia is higher in CAD patients, irrespective of whether

hypercholesterolaemic or not. Fenofibrate was able to decrease lipidaemia in cases and controls, but did not abolish the differences between these groups.

Additional evidence for an association of postprandial lipaemia with atherosclerotic risk is provided by the offspring study of Uiterwall *et al*¹³⁹. Although 80 sons of men with severe CAD and 55 sons of control individuals had similar post absorptive concentrations of plasma triglycerides, postprandial responses to a liquid challenge meal providing 77.5 g fat per m² of body surface area differed significantly. The early rise in serum triglyceride concentrations and the maximum concentrations reached at 4-6 hours after the meal were comparable in the two groups. However, triglyceride concentrations remained significantly higher 8h (+27%), 10h (+33%) and 12h (+17%) after the meal in the sons of patients, suggesting delayed clearance compared with the sons of control individuals.

There is convincing evidence for the role of postprandial triglyceride rich lipoproteins (TGRL) in CHD risk among people of European descent^{140,141}. Whether ethnic differences in postprandial triglyceride level could account for different rates of CHD in Europeans and South Asians is not known. No one has reported on postprandial triglyceride levels in a comparative study of South Asians and Europeans, and the role of postprandial lipids in insulin resistance or risk for CHD has not been examined among South Asians. We hypothesise that South Asians are likely to have elevated levels of postprandial lipids compared with Europeans, and that ethnic differences in postprandial triglyceride level will accompany the ethnic differences in central obesity, plasma lipids and insulin resistance. We wanted to test whether ethnic differences in insulin sensitivity could be accounted for by body fat distribution (including measurement of visceral fat area directly by CT scan).

Meal tolerance tests are difficult and laborious to perform on large numbers of subjects as they involve giving a fatty meal in the fasting state, followed by a further 12 hour period of fasting, and several measurements of lipids at hourly intervals in this period. One study, however, demonstrated that postprandial measurements were more reproducible than fasting triglyceride determinations, and that reliable postprandial measurements can be implemented in epidemiologic studies by using a modified fat tolerance test¹⁴². In this study it was shown that the correlation between postprandial triglyceride levels (for a single postprandial triglyceride concentration measured at 9 hours after a fatty meal), over a wide range of levels at two visits was strong ($r=0.87$). In addition, the reliability coefficient (percentage of total variation in analyte which is attributable to between participant variation) was 85% for the postprandial triglyceride, compared with 64% for

the fasting triglyceride level¹⁴². It has also been proposed by Patsch¹³¹ and Weintraub¹³⁷ that a single 8 hour postprandial triglyceride level can discriminate well between cases of CAD on angiography and matched controls.

2.2. Relation between postprandial triglyceride concentration and insulin resistance

By the 1970's the highly significant associations between fasting triglyceride, insulin resistance and hyperinsulinaemia were defined¹⁴³. However, very little information has been available about the relationship between insulin resistance and postprandial lipaemia. The potential importance of this association is due to two reported research findings. Firstly, as discussed above it has been proposed that postprandial lipaemia might play a part in the pathogenesis of CHD. Secondly, it has been proposed that resistance to glucose disposal and/or compensatory hyperinsulinaemia may also increase the risk of CHD⁴⁴. Few studies have now addressed this issue.

Reaven's group¹⁴⁴ showed in a study of 37 non-diabetic individuals that insulin resistance [measured by the steady state plasma glucose (SSPG) in an insulin suppression test] was associated with plasma total postprandial triglyceride concentration ($r=0.66$, $p<0.001$) and retinyl palmitate ($r=0.51$, $p<0.01$). The association was also strong for postprandial triglyceride concentration and retinyl palmitate in both Sf (Svedberg flotation) >400 (chylomicrons) and Sf 20 to 400 (chylomicron remnants and VLDL) fractions. They concluded that insulin resistance plays an important role in regulating the postprandial concentration of TGRL, including those of intestinal origin. However a limitation of this study is that no adjustment was made for the fasting triglyceride level, which is known to be a strong predictor of the postprandial triglyceride response. Thus we don't know if postprandial triglyceride concentration was an independent associate of insulin resistance.

In contrast Byrne *et al*¹⁴⁵ failed to find an association between features of the insulin resistance syndrome and postprandial triglyceride concentration in a study of 57 men. Also Weintraub *et al*¹³³ did not find a significant association between fasting insulin and the postprandial triglyceride response in a study of 15 subjects. However, another group found an association between fasting insulin level and postprandial triglyceride in a study

of 113 young (mean age 26 years), healthy, normal weight men¹⁴⁶. They found that fasting and postprandial triglyceride peak values showed a bimodal frequency distribution, with a low fasting and a high fasting triglyceride group, and “normal” responders and “high” responders to the fat-load. Fasting insulin levels were significantly higher in high responders than in normal responders, whereas they did not differ between the low and high fasting triglyceride group. In a recent study in men, Couillard *et al*¹⁴⁷ showed that visceral obesity is associated with an impaired postprandial triglyceride clearance, and that visceral obesity may contribute to fasting and postprandial hypertriglyceridaemia by altering NEFA metabolism in the postprandial state. They reported that features of the insulin resistance syndrome, namely fasting hypertriglyceridaemia, hyperinsulinaemia and low HDL-cholesterol concentrations as well as increased visceral adipose tissue accumulation were all significant correlates of an impaired postprandial TGRL clearance.

A recent cross-sectional study of 26 men has shown that healthy first degree relatives of patients with type 2 diabetes are insulin resistant and have 50% higher 6-hour incremental area under the curve compared with a control group¹⁴⁸.

Although studies of relation between postprandial lipaemia and insulin resistance are few, there exist studies of the relation between postprandial lipaemia and diabetes. Postprandial lipaemia is increased in NIDDM, together with a defective removal of remnant particles^{149,150}. It is not clear what the underlying mechanism is for postprandial lipaemia in NIDDM, but it seems that there might be a defect in the removal of remnant particles. Curtin *et al*¹⁵¹ showed that NIDDM patients had an altered pattern of apoB-48 in response to an oral fat load. ApoB-48 is the structural protein of chylomicrons and is a more specific marker for remnant particles than is retinyl palmitate. It has been shown that elevated postprandial lipaemia is mainly due to an increase in apoB-100 (structural protein of endogenous, or hepatic derived VLDL-triglyceride) containing particles and only a minor part represents the apoB-48 particles¹⁴⁰. A competition between apoB-48 and apoB-100 particles for removal could explain the accumulation of apoB-100 containing particles in the postprandial state, particularly if VLDL-apoB-100 production is increased, as occurs in NIDDM.

2.2.1. Mechanism underlying the relation between insulin resistance and postprandial lipaemia

It has been long debated which comes first in the sequence of events: hyperinsulinaemia, insulin resistance or dyslipoproteinaemia. Evidence from prospective studies is sparse, but tends to support the concept that insulin resistance and compensatory hyperinsulinaemia precedes the development of hypertriglyceridaemia and an atherogenic lipoprotein phenotype^{152,153}. Another study has shown that families with endogenous hypertriglyceridaemia are at an increased risk of developing glucose intolerance and NIDDM during a 10 year follow-up period¹⁵⁴. However, no prospective data are available for postprandial triglyceride.

On the other side of the argument, there is a growing body of opinion that lipid disturbances come first, leading to development of insulin resistance and NIDDM. The evidence for a key role of continuously elevated levels of NEFA in the pathogenesis of insulin resistance and NIDDM has been reviewed in an excellent review by Boden¹⁵⁵. It is proposed that NEFA provide an important link between obesity (particularly central obesity) and insulin resistance. However Frayn¹⁵⁶ proposes that it is a bit simplistic to think of any one change as 'primary'. Rather the many associations of insulin resistance are so interrelated that disturbance to any one may bring about the whole pattern of metabolic disturbance.

The mechanism by which insulin resistance is related to postprandial lipaemia is not clearly understood. The normal effect of insulin is most pronounced in the postprandial period and is mediated both through inhibition of the intracellular hormone-sensitive lipase and through an increase in the re-esterification of fatty acids within adipose tissue¹⁵⁷. The reduced delivery of NEFA to the liver, coupled with the direct suppressive effect of insulin, leads to postprandial diminution of hepatic VLDL-triglyceride secretion under normal circumstances. Insulin also stimulates the activity of peripheral LPL, aiding the removal of TGRL from the circulation. In insulin resistance increased flux of NEFA to the liver secondary to a failure of insulin to suppress NEFA release from adipose tissue is an important factor leading to the elevation of circulating TGRL^{46,158,159}. Thus hepatic VLDL-triglyceride secretion may continue in the postprandial period, with enlargement of the circulating triglyceride pool. Furthermore, the insulin activation of adipose tissue LPL is blunted in the insulin resistance syndrome, which impedes the clearance of VLDL

particles and lowers HDL-cholesterol levels¹⁵⁶. It has been argued by Frayn¹⁶⁰ that inappropriate release of NEFA in the postprandial period is likely both to reduce the sensitivity of glucose metabolism to insulin and to accentuate postprandial lipaemia.

It has been shown that increased visceral obesity is related to insulin resistance⁷⁴⁻⁷⁶. It has also been shown that visceral obesity is related to postprandial lipaemia^{147,161,162}. Thus visceral obesity is an obvious link between insulin resistance and postprandial lipaemia.

2.3. Mechanism of association between postprandial lipaemia and CHD risk

Postprandial lipaemia could affect the atherosclerotic process directly through the postprandial lipoproteins, or indirectly through its effects on other lipoproteins such as LDL and HDL cholesterol. Other mechanisms may be involved, such as induction of platelet function that may favour thrombosis¹⁶³.

2.3.1. Direct mechanisms

The hypothesis first put forward by Zilversmit¹³⁰ focused on the atherogenic potential of chylomicron remnants of exogenous (dietary) origin. However, the “triglyceride intolerance hypothesis” formulated by Miesenböck and Patsch^{131,164} suggests that hypertriglyceridaemia is potentially atherogenic through cholesterol transferred from cholesterol-rich lipoproteins such as HDL and LDL to triglyceride-rich lipoproteins (VLDL), which owing to their enrichment with cholesteryl esters (CE), cannot be degraded properly and end up in the vessel wall. When the integrated level of TGRL's in the postprandial state is high, cholesteryl ester transfer protein (CETP) - mediated transfer of CE from HDL and LDL to TGRL's in exchange for triglycerides is extensive. Hence HDL-cholesterol levels decrease, LDL particle size is reduced, and CE-rich remnants of TGRL's are formed. Each of these consequences of postprandial triglyceride metabolism is potentially atherogenic. Rapp *et al*¹⁶⁵ isolated intact TGRL from human atherosclerotic plaque tissue. These lipoproteins resembled the TGRL encountered in plasma, except for a slightly larger particle size and enrichment in apolipoprotein E. Thus this study

demonstrated clearly that the TGRL are deposited in the atherosclerotic plaque. However the authors did not emphasise that plasma contained apolipoprotein B-48, whereas the plaques did not. From this it could be interpreted that chylomicron remnants are not directly implicated in atherogenesis. Instead, their metabolism might induce atherogenic alterations in the properties of other cholesterol containing lipoproteins. In an extensive review of the relation between triglyceride and CHD, Karpe and Hamsten¹⁶⁶ speculate that the metabolism of postprandial TGRL represents a repeated daily atherogenic influence on other lipoprotein species in the plasma, rather than inducing atherosclerosis *per se*.

2.3.1.1. Direct effects on the vascular endothelium

It has been suggested that postprandial lipaemia could directly affect the vascular endothelium. Sattar¹⁶⁷ has reviewed the accumulating evidence which suggests that TGRL may be directly damaging to the endothelium. It is suggested that TGRL can cross the endothelial barrier and enter the arterial wall, and bring about endothelial damage possibly through oxidative mechanisms. Impaired endothelial function is considered a marker of atherogenesis. Recent technology has allowed the study of flow-mediated dilatation (FMD) of the brachial artery by ultrasound as a marker of endothelial function. Thus reduced FMD is considered a marker of endothelial dysfunction.

One study showed a significant reduction in FMD in the brachial artery following a single high-fat meal in 10 normocholesterolaemic volunteers¹⁶⁸. The same group also found that pre-treatment with vitamin C and vitamin E blocked this effect, supporting the hypothesis that oxidative stress is a link between postprandial TGRL and endothelial damage¹⁶⁹. Another group¹⁷⁰ measured FMD after inducing a transient hypertriglyceridaemia by infusion of a lipid containing solution (Intralipid®). They found that FMD decreased from 7% to 1.6% in response to the intralipid infusion, showing that transient triglyceridaemia decreases vascular reactivity. It is plausible that repeated elevations of postprandial triglyceride could have more permanent effects on the endothelium.

2.3.2. Indirect mechanisms

Postprandial lipaemia could be atherogenic in an indirect way by modifying other lipoproteins important in atherosclerosis such as LDL and HDL. The magnitude of

lipaemia is positively associated with the plasma levels of apoB¹⁷¹, possibly reflecting a tendency for an individual with poor fat tolerance to overproduce apoB containing lipoproteins. A mechanism leading to loss of HDL₂ as a result of pronounced postprandial lipaemia has been demonstrated¹⁷². The levels of HDL₂ exhibit a negative association with CHD at least as powerful as all other known risk factors. Postprandial lipaemia could affect LDL in regard to its atherogenicity. LDL was the first lipoprotein species with a demonstrated role in atherosclerosis¹⁷³. Modified LDL are taken up by macrophages and endothelial cells, leading to the formation of foam cells, one of the first steps in atherosclerosis¹⁷⁴. Individuals with triglyceride intolerance and the triglyceride intolerance syndrome usually exhibit a preponderance of smaller denser LDL¹⁷⁵; also, during postprandial lipaemia, LDL are more oxidisable and can lead to CE accumulation in macrophages¹⁷⁶. Thus postprandial lipaemia can exert its atherogenic potential through its effect on LDL.

In conclusion postprandial lipaemia affects all three major lipoprotein classes, i.e. TGRL become enriched in CE, LDL size is reduced, and HDL cholesterol levels are lowered, leading to foam cell formation and atherosclerosis. Magnitude and duration of postprandial lipaemia determine how much cholesterol is diverted from LDL and HDL into TGRL through which it causes atherosclerosis.

CHAPTER 3: Intramyocellular lipid content and insulin resistance

3.1. Muscle triglyceride stores as a possible mediator of relation of obesity to impaired insulin action

3.1.1. The mechanism

Although numerous experimental studies in humans and animals have demonstrated that weight gain increases insulin resistance while weight loss ameliorates it, the mechanism of the association between obesity (including central obesity) and insulin resistance is poorly understood. Most insulin-mediated glucose disposal takes place in skeletal muscle^{177,178}, and in insulin resistant individuals there is an impairment in the ability of muscle to take up glucose and store it as glycogen^{179,180}. This is associated with impaired glycogen synthase activity in muscle¹⁸¹, but the cause of this defect is not clear.

One possible physiological mechanism for insulin resistance is the glucose-fatty acid cycle, in which glucose and non-esterified fatty acids (NEFA) compete for utilisation by muscle^{182,183}. The key points of this cycle are as follows: the increased availability of NEFA in blood produces an increase in intra-muscular acetyl-CoA and citrate content; acetyl-CoA inhibits pyruvate dehydrogenase, and this in turn reduces glucose oxidation; citrate inhibits phosphofructokinase 1 and thus glycolysis itself, eventually resulting in the impairment of glucose uptake. However, this hypothesis has come into question because many groups¹⁸⁴⁻¹⁸⁶ have not been able to reproduce the findings in rat skeletal muscle that Randle *et al* demonstrated in rat heart muscle. While the suppressive effect of NEFA on carbohydrate oxidation has been generally confirmed by others, it remains controversial whether NEFA also inhibits insulin-stimulated glucose uptake (i.e., causes peripheral insulin resistance). However, in an extensive review Boden¹⁵⁵ argues that the reason many studies failed to find inhibition of glucose uptake by NEFA is because this inhibition

develops late (after 4h of fat infusion), and insufficient time of fat plus insulin infusion has been given in these studies (about 2h).

There have been several attempts to pursue the hypothesis that insulin-stimulated glucose uptake is blocked by elevated NEFA levels in plasma¹⁷⁹. Although such an effect can be demonstrated experimentally¹⁸⁷, several lines of evidence indicate that elevated plasma NEFA levels are unlikely to be an important determinant of insulin resistance in the population. In normoglycaemic individuals steady-state measurements of resistance to insulin-mediated glucose uptake do not correlate with the plasma NEFA response to a meal¹⁸⁸. Although South Asians are on average more insulin-resistant than Europeans, fasting and post-challenge NEFA levels are no higher in South Asians than in Europeans¹⁵⁸.

In their original hypothesis, however, Randle and Hales did not suggest that insulin resistance in non-insulin dependent diabetes resulted only from elevated plasma NEFA levels. They proposed that glucose uptake was inhibited also by NEFA derived from breakdown of triglyceride stores within muscle cells¹⁸². Thus there are two potential ways in which NEFA may promote insulin resistance. Firstly it is possible that there is substrate competition between glucose and muscle-cell derived NEFA, which could cause insulin resistance. Another possibility is that NEFA induce insulin resistance by a direct impairment of glucose transport.

There is now a growing body of evidence for the latter possibility. Boden *et al*¹⁸⁹ have shown that increasing concentration of NEFA decreases glucose uptake in a dose-dependent fashion. The decrease is caused (i) mainly by a reduction in glycogen synthesis (either due to impairment of glycogen synthase activity, or due to a reduction in glucose transport/phosphorylation), and (ii) to a lesser extent by a reduction in carbohydrate oxidation. Roden *et al* have further shown that NEFA induce insulin resistance by initial inhibition of skeletal muscle glucose transport/phosphorylation^{190,191} which is then followed by a 50% reduction in both the rate of muscle glycogen synthesis and glucose oxidation¹⁹⁰. Roden *et al* conclude that in contrast to the mechanism of substrate competition between NEFA and glucose as a cause of insulin resistance, elevation of plasma NEFA causes insulin resistance by inhibition of glucose transport and/or phosphorylation, with a subsequent decrease in rates of glucose oxidation and muscle glycogen synthesis¹⁹⁰. More support for this mechanism of inhibition of glucose transport activity by elevated NEFA has come more recently from Shulman's group¹⁹². They found that rates of whole-body

glucose uptake, glucose oxidation and muscle glycogen synthesis were 50-60% lower following a lipid infusion (compared to a glycerol infusion), and were associated with a 90% decrease in the increment in intramuscular glucose-6-phosphate concentration, implying diminished glucose transport or phosphorylation activity. To distinguish between these two possibilities, intracellular glucose concentration was measured and found to be significantly lower in the lipid infusion studies, implying that glucose transport is the rate-controlling step.

3.1.2. Evidence for the role of intramyocellular lipid in insulin resistance

Recent evidence from animal studies supports the idea that triglyceride stores in skeletal muscle are an important determinant of insulin resistance. Correlations of around -0.90 were reported between insulin-stimulated glucose uptake and triglyceride levels in the hindquarter muscles of rats by Storlien *et al*¹⁹³. In comparison, the correlation between muscle triglyceride levels and plasma triglyceride was only 0.44. High fat diets increase muscle triglyceride levels in rats and also induce insulin resistance; the drug benfluorex blocks both these effects¹⁹⁴.

Evidence for the role of intramyocellular lipid (IMCL) in insulin resistance in humans is slowly accumulating. In a study of patients undergoing minor surgery, mean triglyceride levels in muscle biopsies taken from the rectus abdominis muscle were six times higher in patients with non-insulin dependent diabetes than in controls¹⁹⁵. It has been shown that IMCL content in muscle biopsies is an important source of energy within the muscle¹⁹⁶ and that increased IMCL content is associated with impaired insulin-stimulated glucose uptake in type I diabetes mellitus¹⁹⁷. Lithell *et al*¹⁹⁸ have shown that the energy supply to the muscles during heavy physical work (such as long distance skiing) is partly derived from intracellularly stored lipid. Their group, and others¹⁹⁹ have shown that slow twitch fibres (or type I fibres) have a larger content of triglyceride than fast twitch fibres (or type II fibres). Lithell *et al*¹⁹⁸ showed that during heavy exercise triglyceride stores in slow twitch fibres are decreased.

Intramyocellular lipid content measured in gastrocnemius muscle biopsy in a study of normoglycaemic women was negatively associated with glycogen synthase activation, demonstrating that increased muscle triglyceride stores are associated with decreased insulin-stimulated glycogen synthase activity²⁰⁰. In addition IMCL content was positively

associated with features of the insulin resistance syndrome (including waist to hip ratio and fasting NEFA)²⁰⁰. However in this study there was no association between insulin sensitivity measured directly by the short insulin tolerance test and IMCL content. No mention was made of the level of physical activity of the 27 participants. The authors of this study argued that one of the reasons for the lack of association between insulin sensitivity and IMCL content could be due to a poor correlation between the biopsy lipid content and the overall skeletal muscle lipid content in the subjects (which would determine insulin sensitivity). They cite in favour of this argument the fact that the triglyceride content of skeletal muscle is extremely variable both within any one muscle and between different muscles²⁰¹. It is probably related to the fact that gastrocnemius muscle does not contain as high a density of type I or slow twitch fibres as other muscles, especially the soleus.

Another study measured IMCL content in biopsy specimens of the vastus lateralis muscle of 38 non-diabetic Pima Indian men and quantified insulin sensitivity by the euglycaemic clamp²⁰². Percent body fat was measured by under water weighing. A negative relationship was found between IMCL and insulin sensitivity ($r = -0.53$, $p < 0.001$). Insulin resistance was significantly related to IMCL content independently of all measures of obesity (percent body fat, waist to thigh ratio and BMI).

3.2. Non-invasive measurement of intramyocellular lipid

Until recently the studies of this relationship were limited due to the invasive nature of muscle biopsy to measure IMCL. However a group of physicists in Tübingen, Germany reported in 1993 that muscle cell triglyceride stores can be detected *in vivo* by nuclear magnetic resonance (NMR) spectroscopy²⁰³. Using double spin echo localisation technique, they were able to identify proton NMR signals in human soleus muscle from two different compartments containing fatty acids or triglycerides of similar composition. One of these compartments corresponds to triglyceride within adipocytes and the other to triglyceride within muscle cells. Other groups have also found the same²⁰⁴ and validated the technique²⁰⁵. One group in London has also used this technique and performed reproducibility studies²⁰⁶. They reported that the intra-examination coefficient of variation for proton NMR spectroscopy measurements of IMCL in the soleus muscle was 4% and

the inter-examination coefficient of variation for repeated determinations in three different examinations was 11%²⁰⁶. This is much lower than the 24% coefficient of variability in repeated biopsies of human skeletal muscle²⁰⁷. As described above (section 3.1.2) the intracellular lipid content of biopsy specimens is determined to a large extent by fibre type (slow twitch or fast twitch)¹⁹⁸, which in turn is dependent upon the choice of muscle type for study. Use of NMR spectroscopy of soleus muscle takes away this limitation, as reflected in the lower coefficients of variation for repeat measurements.

Since this non invasive technique of NMR spectroscopy for the quantification of IMCL has become available, few studies of relation of IMCL content and insulin resistance have been reported. One recently reported by Krssak *et al*²⁰⁸ in 23 healthy people showed an inverse correlation between IMCL content and insulin sensitivity measured by the euglycaemic hyperinsulinaemic clamp ($r = -0.69$, $p = 0.002$). They also found that IMCL was not related to BMI, fasting triglyceride, NEFA, glucose or insulin. In a series of small studies McGarry's group have also reported increased IMCL content in association with insulin resistance²⁰⁹⁻²¹¹. Two very recent studies have reported increased IMCL content in non-diabetic offspring of type 2 diabetic parents^{212,213}. The number of studies in Europeans is limited, and performed in small numbers of subjects, and no such studies have been performed in South Asians.

In summary: the hypothesis that IMCL content is an important determinant of insulin resistance is attractive because it can provide an explanation for the experimental dissociation of the effects of changes in energy balance and obesity on insulin resistance. In humans a hyperinsulinaemic response to glucose challenge can be induced by hyperalimentation or reversed by calorie-restricted diets within a few days, before there has been any appreciable change in total body fat stores^{214,215}. It may even be possible to account for the relation of insulin resistance with central obesity, since NEFA derived from adipocytes drained by the portal vein would drive hepatic synthesis of very-low-density lipoprotein (VLDL) triglyceride particles⁴⁶ which deliver triglyceride to peripheral tissues.

Based on the above evidence we wanted to test three closely related hypotheses:

1. That insulin resistance is associated with high IMCL content;

2. That IMCL content is higher in South Asians than in Europeans;
3. That high IMCL content is associated with central obesity

If our main (first) hypothesis is confirmed, in future studies one can hope to define behavioural and pharmacological interventions that reduce muscle cell triglyceride stores and reverse insulin resistance.



CHAPTER 4: Hypotheses, Aims and Objectives

It is now clear that the high prevalence of diabetes mellitus in South Asian people is one manifestation of a pattern of metabolic disturbances related to central obesity and insulin resistance in this group. It is not clear whether central obesity is the primary defect in this syndrome, or whether some other more fundamental disturbance underlies resistance to insulin action, central obesity and lipid disturbances in South Asians. Although CHD risk in South Asians is strongly associated with raised plasma triglyceride levels, the differences between South Asians and Europeans in fasting plasma lipid levels are too small to account for the excess coronary risk in South Asians compared with Europeans. There remain many gaps in our knowledge about ethnic differences in the relationship between body fat distribution (especially central obesity), insulin resistance and lipid metabolism. This study was designed to investigate some of these closely related issues. Our specific hypotheses, objectives and aims were as follows:

4.1. Hypotheses:

1. That central obesity is the primary disturbance underlying the resistance to insulin action and disturbances of lipid metabolism in South Asians
2. That central obesity will account for the ethnic difference in insulin resistance
3. That disturbances of postprandial lipids, rather than fasting lipids, may mediate the excess coronary risk in South Asians compared with Europeans, and that elevated postprandial triglyceride is part of the cluster of CHD risk factors associated with the insulin resistance syndrome
4. That the relation between insulin-mediated glucose uptake and central obesity is mediated through triglyceride stores in skeletal muscle

To test these hypotheses specific objectives and aims were defined as follows:

4.2. Objectives

1. To test whether differences in body fat distribution can account statistically for the difference in insulin sensitivity between South Asians and Europeans
2. To test whether there are differences in postprandial lipid handling between South Asians and Europeans sufficient to account for the excess coronary risk in South Asians
3. To test whether muscle cell triglyceride stores can account for the relationship of insulin resistance to central obesity and ethnicity

4.3. Aims

1. To determine whether the tendency to insulin resistance in South Asians compared with Europeans is present at all levels of adiposity, or only at high levels of adiposity [i.e. are the regression lines for the association between insulin sensitivity and obesity parallel, or do they cross over with varying adiposity]
2. To test whether the ethnic difference in insulin resistance can be explained by central adiposity (when measured accurately by CT scan)
3. To test whether elevated postprandial triglyceride levels are associated with insulin sensitivity and obesity, and whether the ethnic difference in insulin sensitivity (and hence in the risk for CHD) can be accounted for by postprandial triglyceride level
4. To test whether excess triglyceride stores in skeletal muscle (measured non-invasively by nuclear magnetic resonance spectroscopy) can account for the relationships of ethnicity and central adiposity to insulin resistance
5. To test whether bio-electrical impedance analysis is a valid and reproducible epidemiological field method to measure total percent body fat in South Asians

Although not part of the original *a priori* hypotheses and objectives of this study, the opportunity to measure ethnic differences in plasma concentrations of a marker of

inflammation, C-reactive protein, became available towards the end of the study. The rationale for doing this, and the results and the relevant discussion relating to this are presented in chapter 9.

PART 2
METHODS

CHAPTER 5: Subjects and Methods

5.1. Study design and setting

The most appropriate study design to achieve the objectives of this study (section 4.2) was a cross sectional study of European and South Asian men and women. We wanted to study individuals who were young enough to not have already developed chronic illnesses of interest (CHD and diabetes), and thus an age group of 40 to 55 year old people was chosen. We also wanted to study people over a wide range of body size; thus we decided to include people with body mass index (BMI) ranging from 17 to 34 kg/m².

The London Borough of Ealing was chosen as the setting for the study for two main reasons: (i) good contact already exists with general practitioners in the area, with successful collaboration in previous studies^{22,29}, (ii) the Borough has the second largest ethnic minority population of the outer London boroughs, one in three of its residents being born outside the United Kingdom²¹⁶. In the 1991 Census, over 52,000 or 19% of the population of Ealing defined themselves as Indian, Pakistani, or Bangladeshi. This reflects immigration predominantly of Sikhs from the Indian state of Punjab, and to a lesser extent, of Gujarati Hindus and Pakistani Muslims, which occurred largely in the late 1960s and 1970s.

5.2. Sample size

The sample size was calculated on the basis that we wanted to be able to detect a difference of 0.3 %/min/kgm⁻² in the slope of the relationship of insulin sensitivity to body mass index (BMI) between South Asians and Europeans. Having 42 people of each ethnic group would have 90% power to detect such a difference at the 5% significance level. This difference in slope would mean that if at a BMI of 20 kg/m² there is no ethnic difference in insulin sensitivity, then at a BMI of 25 kg/m² there would be a mean ethnic difference in insulin sensitivity of 1.5% min⁻¹. This difference would be equivalent to the difference in

insulin sensitivity between non-diabetic and diabetic Europeans. However the target sample size was increased to 60 people of each ethnicity, to allow for (i) analyses separately by sex group if necessary, (ii) other objectives of the study (such as the postprandial lipid comparisons), (iii) tests for interactions and (iv) drop-outs from the study.

5.3. Ethical approval and consent for study

Ethical approval for the study was obtained from the local ethical committees of the London School of Hygiene & Tropical Medicine and Ealing Hospital NHS Trust. Additional ethical approval was obtained for the nuclear magnetic resonance spectroscopy (see 5.5.7 below) from the ethical committees of the London School of Hygiene & Tropical Medicine and the Hammersmith Hospital. Written informed consent was obtained from all participants for the whole study at the first visit. Further written informed consent was obtained at each of the separate visits for the short insulin tolerance test and the fat tolerance test.

5.4. Subject recruitment

We wrote to partners at six general practices in the Southall and Greenford areas of the Borough of Ealing. We sent them a full copy of the study protocol and explained the planned nature of research. We asked for their participation in the study and for their consent to download copies of their practice lists held by the Ealing and Hammersmith Health Authority and to contact eligible patients. Four of the practices gave written consent to participate. This enabled the release from the Ealing and Hammersmith Health Authority of names, sex, dates of birth, NHS numbers and addresses of all patients registered with those 4 practices. From this a list was made only of people qualifying on age criteria of between 40 to 55 years old. Permission was then gained to go through each of these names individually on the practice database (computerised in 3 practices, but done manually from GP records in 1 practice), to delete from the list any subjects who had any of the exclusion criteria, which are summarised below. With the help of the practice

manager addresses and other details were amended as appropriate. Those whom the practice manager could identify as being of other than South Asian or European ethnicity were excluded at this stage. If the practice records had a note of the patient's BMI, those falling outside the range of 17 to 34 kg/m² were also excluded.

5.4.1. Exclusion criteria for the study

Age: Age outside the range 40 to 55 years

Illness: People were excluded if there was a history of diabetes mellitus, ischaemic heart disease, epilepsy, gall bladder or liver disease, chronic severe illness, or any weight losing conditions including cancer or malabsorption. People with known psychiatric illness, alcohol abuse or drug abuse were also excluded. Any patients with known HIV positive status or Hepatitis B positive status were also excluded.

Body mass index (BMI): Persons with BMI outside the range of 17 to 34 kg/m² were excluded. This information on BMI was available from GP records in some patients, but was calculated from the height and weight information provided in the short questionnaire that was sent to all potential participants.

Drugs: People on any drugs influencing insulin resistance or glucose metabolism were excluded from the study; these included beta-blockers, ACE-inhibitors, thiazide diuretics and loop diuretics. Women who were on hormone replacement therapy or the oral contraceptive pill were excluded because of potential influence on lipids in the fat tolerance test. Lipid lowering drugs also constituted an exclusion criterion.

Pregnancy: Women were asked to state clearly if there was any chance of them being pregnant. If there was, or if they were breast-feeding, they were excluded from the study.

Ethnicity: Any persons who were of any other ethnicity except of South Asian origin or European origin were excluded from the study.

Other: People who declared that they were planning to move out of the area within the next year were also excluded, as it would be difficult to trace them, and also attendance may have become difficult for five separate visits, if they had moved outside the study area.

From June 1996, three waves of mailshots were performed. Roughly equal numbers of South Asians and Europeans were approached. Each eligible person was sent an invitation letter with a covering letter signed by their General Practitioner recommending the research project, an information sheet, and a brief reply questionnaire. The questionnaire was designed to collect basic information such as telephone number, current height and weight to calculate BMI, medical diagnoses and drug history to establish eligibility. Those who responded with a completed questionnaire were then sent a second, more detailed information sheet, to enable potential participants to decide whether they wanted to commit themselves to what was going to be quite an involved study, requiring at least 5 separate visits to the hospital. A breakdown of the response rates, eligibility and withdrawal after the second information sheet is as follows:

Total number mailed	1638
Total responded	351 (21.5%)
Ineligible	89
Withdrew	52 (after reading 2nd information sheet)
Eligible	210
In reserve	67 (as enough numbers consented to participate)
Attended for screening	143

5.5. The protocol for study visits

To complete all the tests to achieve the study objectives it was necessary for each participant to attend for five separate visits. The tests and measurements at these visits were as follows.

5.5.1. Visit one for screening health check

Prior to this visit a letter of appointment was sent to each participant detailing the need for overnight fasting for 12 hours, and to bring with them a completed self-administered questionnaire. On arrival at Ealing Hospital each person was welcomed, and the health check explained in detail, in English, Hindi or Punjabi, as appropriate. Consent was taken, fasting status confirmed, and then each person started on the same sequence of screening investigations as follows.

Blood pressure was measured twice with an automated sphygmomanometer (OMRON, U.K.) after resting for five minutes in a chair.

The participant was then sent to the “anthropometry station” where he/she was given a gown, and asked to remove clothes down to underwear. Height was measured by a stadiometer and weight on an electronic weighing scales (Soehnle). BMI was calculated as weight in kg divided by the square of the height in metres. Measurement of waist (at the midway point between iliac crest and lower end of rib cage) and hip (across the greater trochanters) circumferences were performed using a spring-loaded measuring tape.

After dressing, the participant then had measurement of bio-electrical impedance (Bodystat- single frequency BIA) to give a measure of percent body fat. For this an electrode each was placed on the right foot and wrist as per manufacturer’s instructions and a tiny 800 μ amp current passed. The participant’s height and weight were entered into the machine. A reading for impedance and percent body fat was obtained.

Lastly the subject went to the phlebotomy station where a fasting venous sample was taken for triglyceride, glucose and insulin assays, and then a 75g oral glucose load given in the form of Lucozade (donated by Smith Kline Beecham), consumed steadily over 5 minutes to perform the standard oral glucose tolerance test (OGTT)²¹⁷. At this point we reviewed the self-completed questionnaire with the participant to complete any unanswered questions or to seek clarification. Participants were then given a slip of paper detailing the time at which they should return for their 2-hour sample after emphasising the need to continue fasting and refrain from smoking. After the completion of the OGTT the participants were offered breakfast.

5.5.1.1. Referral to general practitioners

On the basis of results from screening, participants were referred to their general practitioners for further management if any of the following were identified:

- i) systolic blood pressure greater than 140 mmHg
- ii) diastolic blood pressure greater than 90 mmHg
- iii) serum fasting triglycerides greater than 2.3 mmol/l
- iv) impaired glucose tolerance (two-hour glucose > 7.8 but less than 11.1 mmol/l)
- v) diabetes mellitus (two-hour glucose > 11.1 mmol/l)

Those with fasting triglyceride level > 3.5 mmol/l were excluded from the rest of the study as performing the fat tolerance test could potentially result in acute pancreatitis in such individuals. Those with newly diagnosed diabetes were also excluded, but those with impaired glucose tolerance were requested to continue with the study.

5.5.1.2. Frequency matching on BMI

Once the information on BMI was available after the screening visit, only those participants who had BMI between 17 and 34 kg/m² were invited to continue with the rest of the study. We wanted to study people over a wide range of BMI so that the relation between obesity and insulin resistance could be assessed over a wide range. Efforts were made to achieve equal numbers of participants (in each of the 4 sex and ethnic groups) in categories of BMI < 23.0 , 23.0 to 25.9, 26.0 to 28.9 and > 29.0 .

5.5.2. Visit two for DEXA scan

The DEXA scans were performed according to standardised protocols at the Medical Physics Department of the Northwick Park Hospital. They were performed by the same operator on each occasion (Consultant Medical Physicist, Mr U. Bhonsle). The operator was not blinded to the sex or ethnicity of the participants, but the degree of automation of the scanning procedure makes operator bias unlikely. Measurements were made with a

total body scanner, Hologic QDR 4500 W (Waltham, Massachusetts, USA), and regional distribution measured using both a default option and manually determined regions. The machine uses a dual energy (100kV_p & 140kV_p) x-ray fan beam and the subject receives an entrance exposure of about 1 mR, (\equiv 8.6 μ Gy). Each whole body scan takes about 5 minutes. The subject lies supine, with their arms by their side. The whole body image is divided into 10 regions of interest (ROI): head, left and right arms, left and right legs, pelvis, lumbar spine, thoracic spine and left and right ribs. Using the ROI, fat, lean tissue and bone regions are determined, and their values calculated. By comparing these values to the spine phantom (provided by the manufacturer) a fat/lean tissue ratio is found. The masses of fat, lean tissue and bone are then calculated.

The main measurement of interest to this study was the total percent body fat, so that the measurement of the same by BIA (as a field method) could be validated in South Asians, which has not been done before. A by-product of the scan, of interest to the participants, was measurement of bone mineral density (BMD) to screen for risk of osteoporosis. Participants and their general practitioners were informed of presence of osteopaenia or osteoporosis in the lumbar and hip regions (according to standard guidelines by the WHO), so that appropriate action could be taken.

5.5.3. Visit three for CT scan

All participants were sent a detailed letter to explain what was involved in this visit, including the dose of radiation they would be exposed to. To determine the visceral adipose tissue area, a single-slice CT scan of the abdomen at the level of L4-L5 was taken. The standard protocol for CT was followed at the Radiology Department of Ealing Hospital. Abdominal fat was measured by a single slice (10 mm thickness) CT scan at the level of L4-L5 using a Toshiba X-speed scanner, with exposure time 2.7 seconds and exposure factors 160 mA and 120 kV. Participants were scanned in a supine position with arms stretched above their heads. Total fat area (TFA) of the abdomen was calculated by delineating the surface of the scan with a graph pen and computing the area in the attenuation range of -190 to -30 HU. The visceral fat area (VFA) was measured by drawing a line within the muscle wall surrounding the abdominal cavity²¹⁸. Subcutaneous fat area (SFA) was calculated as the difference between TFA and VFA.

A scannogram was first performed in each person to determine the exact positioning of the x-ray beam. All scans were performed by two designated CT radiographers.

Both the visits for scanning (DEXA and CT scan) were performed on weekends in between the visits for metabolic measurements.

5.5.4. Visit four for the short insulin tolerance test (SITT)

The subjects were asked to attend after a 12 hour overnight fast. They were asked to abstain from alcohol and smoking during the fasting period.

A cannula was inserted retrogradely into a dorsal right hand vein for venous access for test samples, and a winged butterfly inserted in the ipsilateral antecubital vein for administration of insulin and intravenous glucose. The hand was placed in a warm water bath (Grant Beckerman) maintained at 43°C to “arterialise” the venous blood. Arterialised blood was taken because of the previous finding that no significant correlation between the euglycaemic insulin clamp and SITT measured insulin sensitivity indices was found with venous blood sampling, while a close correlation was found when arterialised blood was used¹⁰⁰. It has been shown that a heated superficial hand vein can adequately “replace” the artery for the measurement of total body glucose kinetics²¹⁹. Twenty minutes later a baseline ‘arterialised’ blood sample was taken for the zero minute sample. Immediately following that a bolus of human actrapid insulin was administered intravenously into the antecubital vein at a dose of 0.05 units/kg body weight. Blood samples were then taken at 3, 5, 7, 9, 11, 13, and 15 minutes for measurement of blood glucose, and at 5 minutes for insulin measurement. The test was terminated at 15 minutes with 25 ml of 50% dextrose intravenously into the antecubital vein and the blood glucose checked again using a glucometer (Boehringer Mannheim). If glucose level was below 4.5 mmol/l oral Lucozade was offered, biscuits given and a further bolus of intravenous glucose given if necessary until the glucose level rose above 4.5 mmol/l. All participants were additionally given a full breakfast before leaving the hospital building.

None of the participants suffered a frank (biochemically confirmed) hypoglycaemic attack. In general there were no problems with the test, except in one lady who had a swollen arm as a result of inadvertent extravasation of the dextrose solution into the tissues. She recovered fully. Some participants complained of a strong sense of tingling and discomfort

in the shoulder region during the administration of the dextrose solution at the termination of the test, but this passed away in every case, within 30 minutes of the injection, with no sequelae.

5.5.5. Visit five for the fat tolerance test

The fat tolerance test was performed using the method of Patsch^{171,220}. Participants attended after an overnight fast of 12 hours. On arrival the protocol for the fat tolerance test was explained in detail and any questions answered. A fasting sample of blood was taken for baseline measurement of lipids. Then the participants drank the test meal over 5 to 15 minutes. The test meal consisted of a cream-based liquid containing 65 gram of fat per square meter of body surface area. The meal was made by the registrant prior to each visit, according to the following recipe: 350 ml of heavy whipping cream (39.5% fat), 20 g of chocolate flavoured Nesquik[®], 15 g of granulated sugar, and 12 g of instant non-fat dry skimmed milk, made up per 2 square meters of body surface area. To this was added 100,000 IU of vitamin A drops (Roche, U.K.), the rationale for which is described in section 2.1.2. This recipe provided 1400 kcal per 2 square meters of which 83% were derived from fat (138g), 14% from carbohydrate (48g) and 3% from protein (9.5g). The volume of the test meal was reduced in proportion to the participant's body surface area (using the formula: volume = 175 ml x body surface area).

The participants were then asked to consume only plain water or up to 2 cups of plain (sugarless, black) tea or coffee, refrain from any food, smoking and any vigorous exercise until their return for the postprandial sample. For most participants this was a single sample at 8 hours, but in those who consented and were able to attend twice (n=35), two postprandial samples were collected at 6 hours and 8 hours. All participants were then offered a meal in the hospital canteen.

The test meal was tolerated well. A few participants reported feeling nauseated but only one person vomited after the meal. (Some people actually enjoyed the drink and asked for the recipe!). No one reported gastrointestinal discomfort or symptoms of malabsorption. The details of the lipid analyses and the laboratory protocol for these are given below in section 5.8.

5.5.6. Total number participating after the screening visit

Of the 143 people who attended for the screening health check (visit 1) a number of people were not eligible and some withdrew from the study. The reasons for these were as follows:

Ineligibility: A total of 11 people were ineligible (TG >3.5 mmol/l in 4 European men, BMI < 17 in 1 European man, HRT use in 1 European woman, newly diagnosed diabetes on OGTT in 1 South Asian woman, diagnosis of cancer in 1 European man and “inappropriate” ethnicity - n=3).

Withdrawals: A total of 12 people withdrew from the study (3 - no reason given; 3 - moved away; 2 - lack of time; 2 - road traffic accident; 2 - felt tests too invasive).

5.5.6.1. Numbers completing each part of study

The number who participated in each of the subsequent visits is shown in Table 1 below.

Table 1: Number of people attending for various stages of the study by sex and ethnic groups.

	European		South Asian		Total
	Men	Women	Men	Women	
DEXA scan	31	30	27	31	119
CT scan	30	31	30	29	120
SITT	29	28	27	30	114
FTT	28	29	28	28	113

5.5.7. Visit six for nuclear magnetic resonance spectroscopy

This part of the study was performed in a sub-sample of 40 men only.

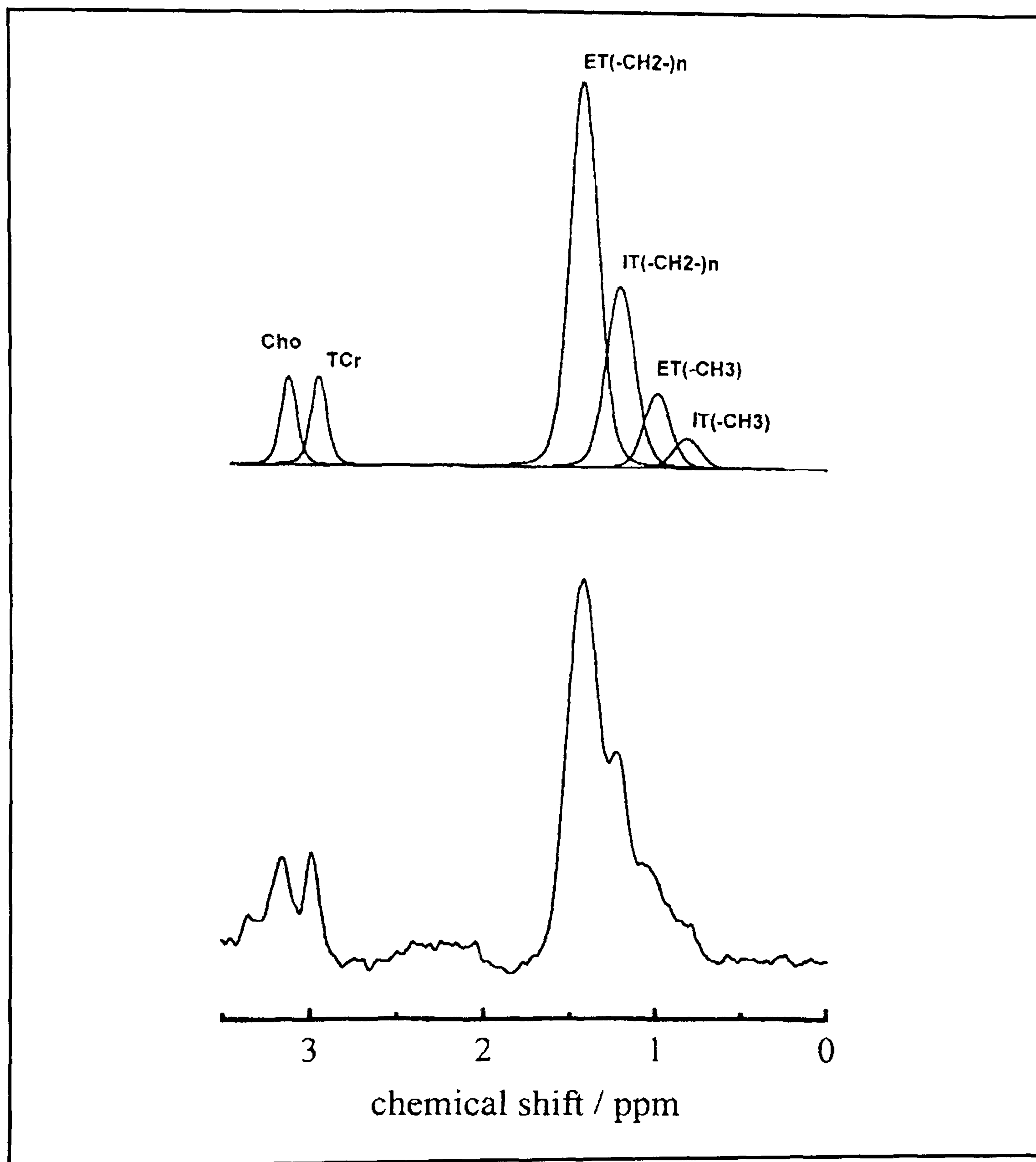
This method for measuring triglyceride stores in skeletal muscle became available during the course of the study (the technique was being developed and validated at the start of the study). 20 men of each ethnic group were randomly chosen from among the men who had

completed the rest of the study to have this measurement. Those men who had any metal implants from any source, including previous surgery were excluded, as this technique uses strong magnetic fields. Standard protocol for MRI scan in use at the Robert Steiner MRI Unit at the Hammersmith Hospital was applied. Briefly, the participant was asked to lie on a narrow couch, having removed all metal objects including jewellery, watch and rings. The region of interest was the left lower leg - in particular a region of the soleus muscle that contains no visible septa or adipose tissue. Subjects were positioned supine, with their left leg immobilised in a 30 cm diameter quadrature bird cage coil. The couch was then moved into the MRI scanner, with the head of the participant remaining outside the scanner whenever possible. The scanning was done over a period of 20 minutes.

Magnetic resonance spectroscopy data were acquired on a 1.5T Picker prototype system. Transverse T1 weighted MR images (TR 300, TE 30 ms) were acquired to determine the placement of the ^1H -MRS voxels, with a slice thickness of 10 mm, a 14 cm field of view and 128x256 data matrix. Spectra were obtained using a double spin echo- PRESS (Point RESolved Spectroscopy) sequence²⁰⁶ with TE/TR=135/1500 ms and an 8 cm³ voxel. Spectra were acquired with both the creatine signal and the water signal as an internal standard.

Spectra were analysed as previously described²⁰⁶. Briefly, the muscle spectra were analysed by VARPRO, an iterative non-linear least square fitting method operating in the time domain. The water peak was quantified by using one exponentially decaying sinusoid (corresponding to one Lorentzian line in the frequency domain), while total creatine (TCr = PCr + free Cr) and choline (Cho) resonances were modelled as two single Gaussian decaying sinusoids with equal damping factors. The (-CH₂)_n and -CH₃ resonances from intracellular and extracellular triglycerides were deconvoluted and modelled as one Gaussian decaying sinusoid each. Peak areas for each signal were obtained and lipid resonances were quantitated with reference to internal water or to total creatine after correcting for T₁ and T₂. The spectrum generates 4 lipid peaks [corresponding to -CH₃ and -CH₂ moieties in myocytes and adipocytes] - as shown in Figure 1. There is a difference in resonance frequencies of about 0.2 ppm (Larmor frequency difference 12-13 Hz at 1.5 T) between adipocyte (1.6 ppm) and intramyocellular (1.4 ppm) TG methylene proton signals. Spectral analysis then resolves the lipid peaks to give a single measurement of the myocellular triglyceride content (in mmol/kg dry weight), using creatine as an internal standard. This technique has been validated against muscle biopsy,

and reproducibility studies at the Hammersmith Hospital have shown intra and inter-examination coefficient of variation of 4 and 13% respectively (8 subjects examined 3 separate times)²⁰⁶.



Cho: Choline containing compounds; TCr: total creatine; $(-\text{CH}_2)_n$ and $-\text{CH}_3$ resonances of extramuscular (ET) and intramuscular (IT) triglyceride; ppm: parts per million

Figure 1: Typical magnetic resonance spectra of the soleus muscle

5.6. Questionnaire data

A self-administered questionnaire, sent by post, was filled out by all participants prior to attending for the first visit for the study. It included questions on personal medical history, family history, drug history, smoking and alcohol consumption, occupation, diet and exercise. At the first (screening) visit we reviewed the self-completed questionnaire with the participant to complete any unanswered questions or to seek clarification. If communication in English was a problem, clarification was provided in Hindi or Punjabi. All questionnaires were fully completed.

Occupational social class was coded as manual or non-manual based on the Registrar General's Classification. Data on physical activity and smoking were coded as follows:

5.6.1. Physical activity

- i. The subjects stated if they participated in any regular sport (% active)
- ii. How far they walked daily (< ¼ mile [1], ¼ to 1 mile [2], 1-3 miles [3], or > 3 miles [4]). From this was derived a walking score as follows: *upto 1 mile/day* [1], *1-3 miles/day* [2] or *> 3 miles/day* [3]
- iii. The number of hours per week spent in watching television (< 8 h [1], 9-15 h [2] or > 15 h [3])
- iv. The subjects self-rated the amount of exercise they took on a regular basis The latter was recoded to an activity score as follows: *much less active* [1], *somewhat less active* [2], *about as active* [3], *somewhat more active* [4], or *much more active* [5], *compared to others their age*

5.6.2. Smoking

Questions on smoking elicited a response to whether the participant was a current smoker, an *ex-smoker* or a *never smoker*. The category of *ever smoker* included ex-smokers and current smokers.

5.7. Sample collection, preparation and transport to the laboratory

The protocols for sample preparation at the specimen collection site at Ealing Hospital were as follows:

5.7.1. Screening visit

At 0 hours: Take 20 ml blood from participant into syringe, and divide it as follows:

- 10 ml into Lithium Heparinised tube, place on ice, mix, spin within 30 minutes (3000 rpm, 10-15 minutes). Then store (at -20°C) 1.0 ml aliquots of plasma in Eppendorf tubes for glucose and insulin assays (and a spare sample for backup).
- 5 ml into EDTA tube, place on ice, mix, spin within 30 minutes (3000 rpm, 10-15 minutes). Then store a 1.0 ml aliquot at -20°C for a backup sample. Leave cells and 1 mm above the buffy coat: save this in the EDTA tube itself (at -70°C) as “BUFFY COAT” for future DNA analysis.
- 5 ml into Plain tube, keep at room temp for 30 -45 minutes until clot forms, spin (3000 rpm, 10-15 minutes), spin again if supernatant not clean looking. Then store (at -20°C) 1.0 ml aliquots in Eppendorf tubes for fasting triglyceride and NEFA assays (and a spare sample for backup).

At 2 hours (post glucose load): Take 16 ml blood from patient into a syringe and divide as follows:

- 10 ml into Lithium Heparinised tube, place on ice, mix, spin within 30 minutes (3000 rpm, 10-15 minutes). Then store (at -20°C) 1.0 ml aliquots of plasma in Eppendorf tubes for glucose and insulin assays (and a spare sample for backup).
- 6 ml into Plain tube, keep at room temp for 30 -45 minutes, spin (3000 rpm, 10-15 minutes), spin again if supernatant not clean looking. Then keep 0.5 ml aliquots of serum for NEFA and a spare one for backup at -20°C.

5.7.2. Visit for the short insulin tolerance test

During this test 4 ml samples of blood were drawn from the arterialised hand vein at -6, 0, 3, 5, 7, 9, 11, 13 and 15 minutes and placed into Lithium Heparinised tubes. The same protocol was followed for sample preparation and storage as above for glucose levels at all time points, and for insulin levels at -6 and 5 minutes.

5.7.3. Visit for the fat tolerance test

- At 0 hr (fasting): 40-45 ml blood was drawn and placed into 4 EDTA (10 ml) tubes and 1 Sodium - Citrate (5 ml) tube.
- At 8 hr (postprandial): 35-40 ml blood was drawn and placed into 4 EDTA tubes.

The tubes were immediately placed on ice and spun in a centrifuge as soon as possible at 3000 rpm for 15 minutes. The supernatant was then divided into aliquots for storage or transport to the laboratory as summarised in Table 2.

Table 2: Summary of protocol for handling of samples

EDTA Aliquot size/type/n	Label/timing	transport	Storage/analysis
1 x 6ml (0h)	FRESH LIPID 0h	ice/dry ice	-70°C or analyse in batches (for VLDL)
2 x 6ml (8h)	FRESH LIPID 8h	ice	1. chylo spin* 2. freeze for VLDL
2 x 1ml (0h)	LIPID1 & 2, 0h	ice/dry ice	analyse (total & HDL cholesterol, TG, NEFA)
2 x 1ml (8h)	LIPID1 & 2, 8h	ice/dry ice	analyse (TG, NEFA)
0.5 ml x 2 preservative	ApoB48 & 100 (0h, 8h)	ice/dry ice	freeze -20/70°C
1 ml (wrapped in foil)	RP 8h	ice/dry ice	freeze -20/70°C

RP = retinyl palmitate, TG = triglyceride, VLDL = very low density lipoprotein, NEFA = non-esterified fatty acids.

* The 8-hour (postprandial) "fresh lipid" sample was subjected to ultracentrifugation on receipt at the laboratory to separate into chylomicron and non-chylomicron fractions. The protocol for this is summarised in section 5.8.6.

Extra EDTA plasma aliquots (0.5 - 1.0 ml) were frozen at -70°C for future analyses for CETP, leptin, C-reactive protein, anti-oxidants, and LDL-subfractions. Two further spare aliquots were also stored. Three aliquots of sodium-citrate plasma were also stored (at -70°C) for future analyses for coagulation factors including PAI-1.

5.8. Laboratory analyses

All laboratory analyses were performed at the Wynn Division of Metabolic Medicine (Wellington Road, St. John's Wood, London, NW8). The laboratory's own Standard Operating Procedures with good quality control were used. However there was no previously established procedure at the Wynn Laboratory for the separation of the chylomicron and non-chylomicron fractions of plasma. Hence there was no established quality control for this particular procedure. Technical problems associated with this procedure are explained in section 5.8.6. Details of the laboratory methods were as follows.

5.8.1. Glucose

Glucose was measured on samples collected in lithium-heparinised tubes. The plasma aliquots were transported to the laboratory on dry ice in batches. Prior to analysis the plasma samples were thawed and centrifuged in the lab. Using kits supplied by Boehringer Mannheim the glucose assay was performed using a Cobas Mira discrete analyser (Roche, Switzerland). Glucose was converted to gluconate and hydrogen peroxide by glucose oxidase. Peroxidase then catalysed the reaction between hydrogen peroxide and 4-aminophenazone to form a chromogen which was measured colorimetrically. The resulting absorbance was proportional to the initial glucose concentration. Internal quality was assessed using control sera and assayed chemistry controls supplied by Medical Analysis Systems, US and Randox Laboratories, (Co. Antrim, Ireland) respectively. External quality control was assessed using the Randox Laboratories system. Within- and between-assay coefficients of variation were <1% and <2.5% respectively.

5.8.2. Insulin

Plasma insulin concentration was determined by a microplate-based chemiluminometric assay specific for plasma insulin (supplied by Molecular Light Technology Research Limited, Cardiff, U.K.). Samples were kept at -20°C at Ealing Hospital and transferred in batches, on dry ice, to the laboratory, where they were stored at -70°C. Insulin concentration was measured in one batch once all the samples had been stored. The samples kept at -70°C were thawed at room temperature before centrifuging at 3000 rpm for 10 minutes. The assay demonstrated negligible (below 2%) molar cross-reactivity with intact proinsulin, split 32-33 proinsulin and des 31,32 proinsulin. No detectable cross-reactivity was observed with C-peptide at levels up to 18ng/mL. Between assay coefficient of variation was less than 7% (range 13-110 micro Units/ml).

5.8.3. Total cholesterol

This was measured with colorimetric assay using a MIRA analyser on fresh samples of EDTA plasma. The enzymatic reagent ("CHOL" from Boehringer Mannheim) cleaves cholesteryl esters to release free cholesterol, then sets up a colour-producing reaction with the free cholesterol. Standardisation was performed using Precinorm-L (human based material supplied lyophilised and stored at 2-6°C) obtained from Boehringer Mannheim. Internal quality control was performed using the HDL plus series (1, 2 and 3) obtained from Biostat. External quality control was assessed using the Randox Laboratories system (as above). Between-assay coefficient of variation was <2%.

5.8.4. HDL-cholesterol

HDL-cholesterol measurement was performed on fresh plasma samples stored at 4°C within 48 hours of being taken. HDL was separated from chylomicrons, VLDL, IDL and LDL by spinning for an hour with a manganese chloride and heparin solution. Under these conditions, the apolipoprotein B present in other lipoproteins (but not HDL cholesterol) forms complexes with mucopolysaccharides. The complexes sedimented to form a pellet, and HDL-cholesterol was measured in the supernatant using colorimetric assay and a Cobas Mira analyser. Internal quality control was performed using the HDL

plus series (Biostat). The between-assay coefficient of variation was $<5\%$; that within assays $<1\%$. External quality control was assessed using the Randox Laboratories system (Crumlin, Co. Antrim, Ireland).

5.8.5. Triglycerides

Triglyceride levels were measured using a colorimetric assay in fresh whole plasma samples kept at 4°C and transported to the lab on ice within 48 hours of collection. Triglycerides were cleaved with Triglycerides N (WAKO, Alpha Laboratories, Eastleigh, U.K.) to release glycerol and free fatty acids. The ensuing colour-producing reaction between glycerol and reagent was measured with a Cobas Mira analyser. Thus in effect it is total glycerol that is measured in this assay, rather than triglyceride. Plasma glycerol levels depend on the rate of lipolysis by hormone-sensitive lipase in fat cells and by lipoprotein lipase at the vessel wall^{221,222}. If there are differences in lipolytic activity or in the half life of glycerol between the two ethnic groups, this could affect interpretation of the triglyceride concentration. Internal quality control was assessed using the HDL plus series (Biostat). Within-assay CV was $<1\%$ and between-assay CV $<4\%$. External quality control was assessed using the Randox Laboratories system as above. Triglyceride in the non-chylomicron fraction was also measured as above, while triglyceride in the chylomicron fraction was derived from subtraction of non-chylomicron TG from whole plasma TG. The procedure for chylomicron separation was as follows.

5.8.6. Chylomicron separation

Ultracentrifugation was performed to separate the chylomicron and non-chylomicron fractions of the 8 hour (postprandial) plasma. Aliquots from each of these two fractions were then stored for future analysis of ApoB-48, ApoB-100 and retinyl palmitate.

Four ml plasma was pipetted into swing-out rotor (SW40 Ti) ultracentrifuge tubes (Polyallomer 14x95 mm centrifuge tubes obtained from Beckman). This was overlaid with 1.006 g/ml density KBr solution up to 1 cm from the top. This solution was made by adding 8.436g solid KBr (Merck, product number 10195) to 1.0 litre of distilled de-ionised water. Overlaying was best done using a fine-tip Pastette (Alpha LW4060) and letting the salt solution ooze out at the level of the meniscus, no higher and no lower. This process is

best visualised if a dark object is placed behind the tube. The tubes were balanced and placed into accurate positions on the rotor, and then spun at 18,000 rpm for 20 minutes at 4°C.

The supernatant, which forms the chylomicron fraction, (top 2.5 ml of the density layer) was taken off, mixed, and aliquotted as follows. 0.5 ml aliquots, mixed with preservative, were stored at -20°C for future analysis of ApoB-48 and ApoB-100 in the chylomicron fraction of plasma. A 1.0 ml sample was stored wrapped in foil at -70°C for future analysis of retinyl palmitate in the chylomicron fraction of plasma. One 0.5 ml aliquot was kept as a spare 8h sample of the chylomicron fraction (at -70°C).

The infranatant was made up to 10 ml with density solution 1.006 g/ml in plain 10 ml tubes (white cap). This formed the non-chylomicron fraction of plasma and was mixed and aliquotted in an identical way as for the chylomicron fraction above. In addition triglyceride level was measured in a 0.5 ml aliquot of the non-chylomicron fraction of plasma.

Quality control was established using blood from 4 healthy members of the laboratory staff 4 hours after a high fat meal at an Indian Restaurant. Forty mls of blood from each volunteer was collected into EDTA tubes and spun at 3000 rpm in a Mistral centrifuge for 15 minutes. Five ultracentrifugal spins were set up every day with 2 pooled plasma samples in each spin for three consecutive days. Chylomicrons were separated as per the procedure above and total triglyceride and non-chylomicron triglyceride concentration was measured on each of the samples. Chylomicron triglyceride level was obtained by subtracting the non-chylomicron triglyceride concentration from the total triglyceride concentration. The coefficients of variation for between assay were 1.03% for total triglyceride, 2.2% for non-chylomicron triglyceride and 1.8% for chylomicron triglyceride concentration.

There was an unforeseen problem with the chylomicron separation procedure in the laboratory. The Biochemist who had devised a written protocol for the procedure, left his post unexpectedly. Any technical problems associated with this procedure (new for this particular laboratory) were thus dealt with by non lipid-specialist laboratory staff. This remains a limitation of this part of the study.

5.8.7. Very low density lipoprotein (VLDL) triglyceride and cholesterol

Two ml plasma was transferred to an Ultra-Clear tube. The tube was then filled to within 2 cm of the top by gently overlaying the sample with d1.006 g/ml KBr solution, which was made as described above. The tubes were balanced and placed into accurate positions on the rotor, and then spun at 18,000 rpm for 20 minutes at 4°C. The supernatant, which forms the chylomicron fraction, was then removed. The tubes were topped up again with the density solution up to 2 cm below the top, and capped. They were weighed and paired within 1 gram of their weight, ready to be spun. The centrifuge tubes were placed in the rotor and spun for 18 hours at 32,000 rpm at 4°C (overnight). The next morning the ultracentrifuge was stopped and the vacuum turned off. The centrifuge tubes were carefully removed and placed in order (tubes 1-44).

Each centrifuge tube was carefully uncapped and the upper 2.0 ml containing VLDL harvested into a 2.0 ml volumetric flask, draining off the supernatant at the level of the meniscus. A set of 2.5 ml tubes numbered 1-44 were used to transfer the product. For VLDL-cholesterol measurement the HDL cholesterol assay was used [see 5.8.4]. For VLDL-triglyceride measurement the triglyceride assay was used as described above [section 5.8.5].

5.8.8. Non-esterified fatty acids (NEFA)

Non-esterified fatty acid (NEFA) concentrations were measured on serum (from visit one, on frozen samples) and EDTA plasma (from fat tolerance visit, on fresh plasma, within 48 hours of collection), using kits supplied by Alpha Laboratories (Wako NEFA-C, 994-75409). The assay was performed using a Cobas Mira discrete analyser (Roche, Switzerland) and was based on the acylation of coenzyme A by NEFA in the presence of acyl-CoA synthetase. The acyl-CoA produced is oxidised by added acyl-CoA oxidase with the generation of hydrogen peroxide. Hydrogen peroxide, in the presence of peroxidase, permits the oxidative condensation of 3-methyl-N-ethyl-(β -hydroxyethyl)-aniline with 4-aminoantipyrine to form a purple coloured adduct which can be measured colorimetrically at 550nm. The assay was standardised against the manufacturer's provided standard. Due to the lability of NEFA, there are no commercially available Quality Control materials that provide target ranges. At the Wynn Division of Metabolic

Medicine, internal quality control is monitored using three commercially available materials (Lyphocheck) and two internally prepared serum pools. Also, due to the lability of NEFA, there are no external quality assurance schemes for NEFA. The between assay coefficient of variation at the lab is $<7\%$ and the within assay coefficients of variation ($n=10$) are as follows:

Fasting pool, mean concentration 1.0 mEq/l	$<1\%$
Suppressed pool, mean concentration 0.2 mEq/l	$<5\%$

5.9. Data entry and statistical analyses

Fox-Pro and File Maker-Pro databases were used for double data entry. These data were converted into the statistical package STATA (version-5, STATACORP, Texas, USA) format using DBMSCOPY software programme. Some of the laboratory data was supplied in Microsoft Excel. This was read directly into STATA.

A programme was written in STATA to derive the insulin sensitivity index (ISI). For this the slope of the rate of fall of log glucose over 3 to 15 minutes during the short insulin tolerance test was multiplied by -100 giving the ISI in $\% \text{ min}^{-1}$ (see section 1.5.4). The graph of the relation between fall in log glucose with time is shown in Figure 2.

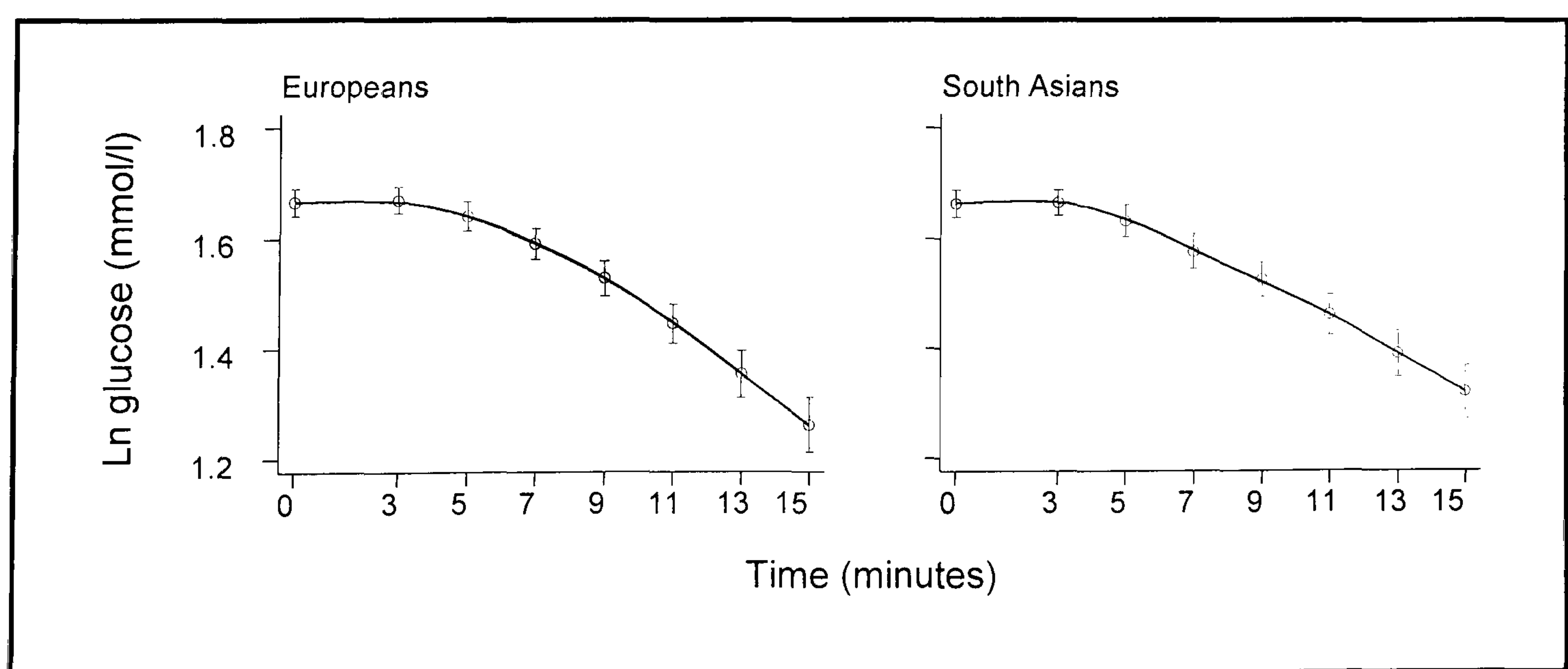


Figure 2: Graph to show the rate of fall of Ln glucose with time in Europeans and South Asians following a bolus of i.v. insulin in the short insulin tolerance test

Data were “cleaned” and any discrepancies resolved by checking against “hard-copy” records. The distribution of each continuous variable was examined for normality, and those that were skewed were log (natural) transformed to ensure normality. Tests of significance for ethnic differences in variables were based on unpaired students’ t-tests (and standard errors) of mean values, and on geometric mean and 95% confidence intervals for log transformed variables. For categorical variables tests of significance for differences between groups were based on the χ^2 statistic. Pearson’s product moment correlation coefficients were used to explore the univariate association between continuous variables. Multiple linear regression analyses were performed to examine the joint effect of more than one variable on the dependent variables. To facilitate comparison of strength of association between different explanatory variables contributing to insulin sensitivity, continuous variables were divided by their standard deviations. For each of these variables the standardised regression coefficient is the coefficient associated with increase of the variable by one standard deviation.

For testing the significance of the difference in slopes (β) between groups tests of interaction were used.

5.10. Justification for the choice of methods used in this study

In this section I have described the rationale for why particular methods were chosen in this study. The discussion focuses on the methods used for measuring (A) insulin sensitivity, (B) postprandial lipids and (C) percent body fat.

5.10.1. Measurement of insulin sensitivity

In the current study one of the primary objectives [section 4.2] was to compare insulin sensitivity in South Asians and Europeans, and to study its relationship specifically with obesity. Thus we wanted to measure insulin sensitivity accurately. A full discussion of the choice of methods available to do this has been given in section 1.5.

Practical, logistic and ethical considerations led to the short insulin tolerance test (SITT) being used as the method of choice for measuring insulin sensitivity in this study. This

choice was based on the fact that we needed to perform the test on a large number of people (n=120), and we wanted the test to be valid, reliable, safe and acceptable. The SITT fulfils all these criteria as shown in section 1.5.4. This choice of method enabled the participant to give up a maximum of one hour of their time in the morning, and continue with the rest of the day's activities unchanged (e.g. go to work). This was particularly important in this study, where we were already asking the participants to take time off work for the multiple visits involved. The SITT was also chosen because the registrant was able to perform up to four - six of these tests in a morning with minimal help (which often entailed only time keeping).

5.10.2. Measurement of postprandial lipids

The methods available to measure lipids in the postprandial state all require the administration, orally or intravenously, of a fat containing meal (amount of fat varies according to the meal chosen), before and after which blood lipids are measured - fat tolerance test. Patsch's test meal^{171,220} has been used extensively before to test the difference between cases and controls of coronary artery disease. They have specifically suggested that a single postprandial sample taken at around 6 to 9 hours^{131,142} may be just as discriminatory as the area under the curve of a full fat tolerance test with hourly blood samples. This was an important consideration in our choice of method, due to the multiple visits involved, to keep high compliance. We thus chose to measure a single postprandial sample at 8 hours after the test meal. In some participants who consented (n=35), a second sample was also taken at 6 hours postprandially.

5.10.3. Measurement of total percent body fat

A number of different methods are available for quantifying total body fat. These include underwater weighing and densitometry, deuterium dilution, ⁴⁰K counting, bio-electrical impedance analysis (BIA), and DEXA scan. The use of BIA as a fast, cheap and efficient field method has been validated^{223,224} in people of European descent, but not in South Asians. Recommendations for the use of BIA for estimating adiposity have been made by, and reviewed by Houtkooper and colleagues²²⁵. We measured percent fat by DEXA scan as the standard, against which we wanted to validate the use of BIA in South Asians.

PART 3

RESULTS

CHAPTER 6: Relation between obesity, body fat distribution, insulin sensitivity and postprandial lipids

6.1. Presentation of Results

6.1.1. Baseline characteristics

135 people (70 European and 65 South Asian) were eligible to proceed with the study (with fasting triglyceride <3.5 mmol/l and non-diabetic on OGTT).

Table 3 summarises the distribution of baseline characteristics among Europeans and South Asians. The mean age of participants was 46.9 (range: 40.2-56.4, sd: 4.7) years. The mean age was similar across all sex and ethnic groups. Prevalence of ever-smoking was much lower in South Asians compared with Europeans. There was no significant ethnic difference in blood pressure. A higher number of South Asians were in manual occupations [48 % of Europeans and 71 % of South Asians reported manual occupations] though the difference was only significant among women. Levels of physical activity were low in both groups, and especially so in South Asian women.

There was no significant ethnic difference in the reported family history of ischaemic heart disease (48% in Europeans, 52% in South Asians), hypertension (18% in Europeans, 22% in South Asians), or stroke (21% in Europeans and South Asians). There was a trend towards higher prevalence of family history of diabetes in South Asians (20.6%) compared with Europeans (10.6%; $p=0.116$). 73.5 % of European women, and 77.4 % of South Asian women were pre-menopausal. Only 4% of Europeans but 48% of South Asians described themselves as vegetarian ($p<0.001$).

Table 3: Distribution of baseline characteristics in Europeans and South Asians

	Men			Women		
	European	S. Asian	p	European	S. Asian	p
n	32	33		38	32	
Age (years)	47.3 ± 0.8	46.6 ± 0.9	NS	47.7 ± 0.8	45.7 ± 0.7	0.081
% Ever smoker	62.5	21.2	0.001	60.5	0	<0.001
% in manual occupation	57.1	71.0	NS	40.6	72.0	0.018
Mean 'activity' score (1-5)	2.28 ± 0.16	1.97 ± 0.17	NS	2.31 ± 0.15	1.75 ± 0.17	0.016
Mean walking score (1-3)	1.94 ± 0.13	1.54 ± 0.12	0.030	1.53 ± 0.09	1.47 ± 0.12	NS
% active in sport	25.0	21.2	NS	39.5	9.4	0.004
Mean TV-hours score (1-3)	2.19 ± 0.13	1.57 ± 0.13	0.001	1.97 ± 0.14	1.37 ± 0.13	0.003
Mean systolic BP (mmHg)	124.1 ± 2.8	126.8 ± 2.7	NS	112.2 ± 2.4	112.9 ± 3.2	NS
Mean diastolic BP (mmHg)	81.2 ± 2.0	84.9 ± 2.0	NS	72.5 ± 1.4	76.4 ± 1.8	0.095

Values are mean and SEM, except when percentages are given. Details of physical activity measures and smoking are given in sections 5.6.1 and 5.6.2 respectively

6.1.2. Differences in fat distribution

Although mean BMI, waist girth and WHR were similar between the ethnic groups of both sexes, South Asian women had greater total percent fat than European women (Table 4). The range of BMI for the whole group was from 17 to 34.3 kg/m². Mean VFA was generally higher in South Asians compared with Europeans, but in men this did not reach statistical significance. South Asian women had significantly greater VFA than European women [$\beta=0.36$, $se=0.10$, $p=0.001$, adjusted $R^2=25\%$]. This ethnic difference in women persisted after adjustment for SFA and percent fat [$\beta=0.21$, $se=0.10$, $p=0.047$, adjusted $R^2=42\%$]. However when further adjustment was also made for TFA, the ethnic difference in VFA was abolished [$\beta=0.003$, $se=0.04$, $p=0.928$, adjusted $R^2=93\%$]. As

expected, women of both ethnic groups had more total fat than men, but a lower degree of central obesity than men (lower waist girth, WHR, and VFA; $p < 0.001$ for sex difference in each central obesity measure in both ethnic groups).

The correlations of VFA measured by CT scan with both waist girth and WHR were high ($p < 0.0001$) in all groups (except $p = 0.003$ for association between WHR and VFA in European women) as follows. Correlation coefficients (r) between VFA and waist girth: 0.81 and 0.70 in European men and women, and 0.70 and 0.80 in South Asian men and women respectively; Correlation coefficients (r) between VFA and WHR: 0.72 and 0.51 in European men and women, and 0.69 and 0.71 in South Asian men and women respectively.

Table 4: Distribution of anthropometric variables in men and women of European and South Asian descent.

	Men			Women		
	European	S. Asian	p	European	S. Asian	p
n	32	33		38	32	
BMI (kg/m ²)	25.8 (0.7)	25.9 (0.5)	NS	25.2 (0.6)	25.8 (0.6)	NS
Waist girth (cm)	87.6 (1.9)	88.8 (1.6)	NS	77.1 (1.6)	79.3 (1.5)	NS
Waist hip ratio	0.90 (0.01)	0.92 (0.01)	NS	0.79 (0.01)	0.80 (0.01)	NS
% fat (DEXA)	22.9 (1.3)	25.7 (1.2)	NS	35.0 (1.2)	39.8 (1.0)	0.003
% fat (BIA)	21.9 (0.7)	23.3 (0.5)	NS	33.6 (0.9)	37.2 (0.9)	0.007
Visceral fat area* (cm ²)	111.5 [91 – 137]	126.1 [108- 148]	NS	65.8 [57- 76]	86.4 [73- 102]	0.019
Subcutaneous fat area (cm ²)	193.6 (15.8)	230.1 (16.2)	0.112	263.4 (19.5)	284.2 (15.8)	NS
Total fat area (cm ²)	323.2 (25.9)	366.4 (21.8)	NS	334.7 (22.5)	379.5 (21.1)	NS

*: geometric means of (backtransformed) log(natural) variables and 95% confidence intervals; otherwise means and SEM are given

6.1.3. Differences in glucose, insulin and insulin sensitivity index

The pattern of glucose and insulin concentrations was consistent with greater insulin resistance in South Asians (Table 5). Fasting insulin and 2h glucose levels were significantly higher among South Asians and there was a much exaggerated post-glucose insulin response in South Asians compared with Europeans. Three South Asian men and 5 South Asian women had glucose intolerance (2h glucose >7.8 but less than 11.1 mmol/l), while all Europeans were normoglycaemic. Mean ISI was higher among Europeans than South Asians [(mean and SEM 3.5 ± 0.2 and 2.9 ± 0.6 %/min. respectively, $p=0.010$), and ($\beta=-0.71$, 95% CI -1.18 to -0.25, $p=0.003$ for South Asians versus Europeans in age and sex adjusted regression analysis)].

Table 5: Mean level of glucose, insulin and insulin sensitivity index by sex and ethnic group

	Men			Women		
	European	S. Asian	p	European	S. Asian	p
n	32	33		38	32	
Fasting glucose (mmol/l)	5.3 ± 0.1	5.4 ± 0.1	NS	4.9 ± 0.1	5.1 ± 0.1	0.106
2 hr glucose (mmol/l)	4.9 ± 0.2	5.7 ± 0.3	0.010	4.8 ± 0.2	6.2 ± 0.3	<0.001
Fasting insulin* ($\mu\text{u/ml}$)	4.9 [4.0 - 6.1]	8.7 [6.9 - 11.1]	0.001	4.6 [4.0 - 5.3]	6.1 [4.9 - 7.6]	0.037
2 hr insulin* ($\mu\text{u/ml}$)	10.5 [7.4 - 14.8]	42.2 [27.4- 65.1]	<0.001	17.2 [13.8- 21.6]	34.8 [25.8- 47.0]	<0.001
Insulin (%/min) sensitivity index	3.3 ± 0.3 (n = 29)	2.4 ± 0.2 (n = 27)	0.024	3.7 ± 0.2 (n = 28)	3.2 ± 0.2 (n = 30)	NS

*: geometric means of (backtransformed) log(natural) variables and 95% confidence intervals; otherwise mean and SEM are given

When the sexes were analysed separately, this difference was significant only among men (Table 5). When the analysis was restricted to those with normal glucose tolerance during the OGTT the findings were unchanged. Among South Asians, women had higher ISI and lower fasting insulin and fasting glucose than men [$p=0.009$, $p=0.036$ and $p=0.019$].

respectively]. Among Europeans, fasting glucose was lower in women than men ($p < 0.001$), but there was no significant sex difference in 2h glucose, insulin or ISI.

The strength of association between ISI and other markers of insulin sensitivity was generally greater in South Asians compared with Europeans (Table 6).

Table 6: Correlation coefficients for relation between ISI and glucose and insulin levels

	Europeans		South Asians	
	r	p	r	p
Fasting glucose (mmol/l)	-0.21	NS	-0.40	0.002
2h glucose (mmol/l)	-0.18	NS	-0.35	0.008
Fasting insulin* ($\mu\text{u/ml}$)	-0.37	0.005	-0.51	<0.001
2h insulin* ($\mu\text{u/ml}$)	-0.37	0.005	-0.45	<0.001

*: log (natural transformed)

6.1.4. Differences in lipids including postprandial triglyceride levels

These results are summarised in Table 7 and Table 8. There was no significant ethnic difference in the fasting levels of total cholesterol or HDL-cholesterol, though the latter was significantly higher in South Asian women than men. Fasting triglyceride level was higher in South Asian men than European men, but not significantly so (Table 7). The 8-hour postprandial triglyceride level was higher in South Asian men than European men, with a 61% increase over the fasting level among South Asian men. In women there was no ethnic difference in the postprandial triglyceride level. At 8 hours women of both ethnic groups had a more favourable postprandial lipid profile (with lower triglyceride level) than men. The validity of the data for the proportion of triglyceride concentration at 8 hours in the chylomicron and non-chylomicron plasma fractions is uncertain, as there is an unusually high amount in the chylomicron fraction. It is possible that there were technical problems with the preparation of the plasma fractions in the laboratory as referred to in section 6.3.4.

At 6 hours postprandially there was a very exaggerated triglyceride response among South Asian men, even though the numbers of subjects studied at this time were small (Table 7). For both fasting and 2h NEFA level during the OGTT, and fasting NEFA level during the fat tolerance test, South Asian women tended to have higher NEFA levels than European women. There was no ethnic difference in levels of VLDL-triglyceride or VLDL-cholesterol but there was a significant sex difference in both ethnic groups, with lower levels in women compared with men.

Table 7: Mean fasting and postprandial triglyceride levels, and fasting total and HDL-cholesterol in South Asian and European men and women

Lipids: (mmol/l)	Men			Women			p1	p2
	European	S. Asian	p	European	S. Asian	p		
n	28	28		29	28			
Fasting Chol*	5.65 ± 0.24	5.40 ± 0.17	NS	5.24 ± 0.16	5.15 ± 0.17	NS	NS	NS
Fasting HDL-Chol	1.39 [1.25- 1.55]	1.26 [1.16- 1.38]	NS	1.56 [1.43- 1.71]	1.51 [1.40- 1.62]	NS	0.106	0.004
Fasting TG	1.58 [1.30- 1.92]	1.72 [1.45- 2.04]	NS	1.24 [1.06- 1.44]	1.27 [1.02- 1.58]	NS	0.056	0.036
8h TG	1.97 [1.56- 2.48]	2.51 [1.96- 3.21]	NS	1.45 [1.17- 1.79]	1.39 [1.06- 1.83]	NS	0.060	0.003
8h Chylo TG	1.34 [1.05- 1.71]	1.75 [1.36- 2.25]	0.137	0.97 [0.79- 1.20]	0.92 [0.70- 1.24]	NS	0.059	0.002
8h Non-chylo TG	0.62 [0.51- 0.76]	0.75 [0.59- 0.95]	NS	0.47 [0.38- 0.59]	0.48 [0.36- 0.63]	0.098	0.074	0.016
6h TG	3.27 (n=8) [2.15- 4.96]	5.71 (n=7) [4.27- 7.62]	0.057	2.58 (n=8) [1.73- 3.85]	3.17 (n=12) [1.97- 5.11]	NS	NS	0.103

Geometric means and 95% CI are given, except for total cholesterol (*) where arithmetic mean and SEM is given; exact p values are given for p<0.150

p: p value for ethnic differences amongst men and amongst women

p1: p value for sex differences in Europeans; p2: p value for sex differences in S. Asians

Table 8: Mean fasting and postprandial lipids (NEFA, VLDL-TG and VLDL-cholesterol) in South Asian and European men and women.

Lipids: (mmol/l)	Men			Women			p1	p2
	European	S. Asian	p	European	S. Asian	p		
n	28	28		29	28			
Fasting NEFA-OGTT	0.29 [0.25- 0.34]	0.34 [0.28- 0.40]	NS	0.33 [0.27- 0.40]	0.40 [0.35- 0.47]	0.098	NS	0.100
2h NEFA – OGTT	0.07 [0.04- 0.10]	0.06 [0.04- 0.09]	NS	0.03 [0.02- 0.04]	0.04 [0.03- 0.05]	0.065	0.002	0.131
Fasting NEFA – FTT	0.36 [0.29- 0.45]	0.34 [0.27- 0.42]	NS	0.35 [0.30- 0.41]	0.45 [0.38- 0.54]	0.042	NS	0.048
8h NEFA – FTT	0.77 [0.68- 0.88]	0.89 [0.75- 1.05]	NS	0.81 [0.71- 0.92]	0.89 [0.80- 0.98]	NS	NS	NS
Fasting VLDL-TG	0.49 [0.38- 0.62]	0.56 [0.44- 0.70]	NS	0.37 [0.30- 0.45]	0.36 [0.27- 0.48]	NS	0.085	0.025
8h VLDL-TG	0.43 [0.33- 0.57]	0.52 [0.40- 0.69]	NS	0.28 [0.20- 0.37]	0.23 [0.16- 0.33]	NS	0.032	0.001
Fasting VLDL-Chol	0.35 [0.27- 0.44]	0.38 [0.31- 0.47]	0.106	0.27 [0.22- 0.34]	0.24 [0.18- 0.32]	NS	0.106	0.016
8h VLDL-Chol	0.28 [0.22- 0.37]	0.30 [0.22- 0.40]	NS	0.17 [0.12- 0.24]	0.12 [0.08- 0.18]	NS	0.039	0.001

All values are geometric means and 95% CI. exact p values are given for p<0.150

p: p value for ethnic differences amongst men and amongst women

p1: p value for sex differences in Europeans

p2: p value for sex differences in S. Asians

The marked sex difference in postprandial triglyceride in South Asians (with lower level in women vs. men) persisted when adjusted for age and percent fat [standardised $\beta = -0.84$, $se = 0.31$, $p = 0.008$] or age and SFA [$\beta = -0.76$, $se = 0.39$, $p = 0.061$]. But the sex difference in 8h triglyceride level was abolished when VFA was accounted for $\beta = 0.09$, $se = 0.38$, $p = 0.813$]. The pattern was identical in Europeans, i.e., adjusting for age,

percent fat or SFA did not account for the lower 8h triglyceride level in women, but adjusting for VFA abolished this difference. When also taking the effect of fasting triglyceride into account, in South Asians the same pattern as above was true, but in Europeans the sex difference in 8h triglyceride level was no longer significant after adjusting for age and fasting triglyceride.

6.1.5. Relation between obesity and metabolic variables (glucose, insulin, ISI and triglyceride concentration)

Table 9 and Table 10 show the correlations of generalised obesity (BMI and percent fat) and abdominal obesity with ISI, insulin, glucose and triglyceride levels in men and women.

Table 9: Pearson's product moment correlation coefficients of obesity measures with metabolic variables in European and South Asian men

	BMI	% Fat	WHR	W girth	VFA	SFA	TFA
European men							
Fasting glucose	0.29	0.27	0.41 ^a	0.33 ^a	0.05	0.32	0.23
2h glucose	0.16	0.17	0.10	0.10	0.17	0.15	0.14
Fasting insulin	0.42 ^a	0.45 ^a	0.29	0.45 ^a	0.32	0.57 ^c	0.51 ^b
2h insulin	0.07	0.28	0.01	0.02	0.19	0.16	0.15
ISI	-0.38 ^a	-0.56 ^b	-0.28	-0.43 ^a	-0.50 ^b	-0.48 ^a	-0.49 ^b
Fasting TG	0.35	0.40 ^a	0.40 ^a	0.34	0.63 ^c	0.35	0.51 ^b
8h TG	0.38 ^a	0.48 ^b	0.31	0.32	0.60 ^c	0.46 ^a	0.55 ^b
S. Asian men							
Fasting glucose	0.11	-0.12	0.18	0.01	0.19	-0.13	0.01
2h glucose	0.08	0.06	0.49 ^b	0.13	0.44 ^a	-0.14	0.08
Fasting insulin	0.59 ^c	0.38	0.57 ^b	0.62 ^c	0.61 ^c	0.36	0.54 ^b
2h insulin	0.54 ^b	0.47 ^a	0.66 ^c	0.60 ^c	0.61 ^c	0.39 ^a	0.53 ^b
ISI	-0.41 ^a	-0.29	-0.55 ^b	-0.47 ^a	-0.34	-0.29	-0.37 ^a
Fasting TG	0.33	0.10	0.52 ^b	0.49 ^b	0.32	0.23	0.27
8h TG	0.53 ^a	0.45 ^a	0.69 ^c	0.61 ^c	0.49	0.46	0.51 ^b

^a p<0.05, ^b p<0.01, ^c p<0.001, Insulin, triglyceride (TG) and visceral fat area (VFA) have been log (natural) transformed. W girth: Waist girth.

Table 10: Pearson's product moment correlation coefficients of obesity measures with metabolic variables in European and South Asian women

	BMI	% Fat	WHR	W girth	VFA	SFA	TFA
European women							
Fasting glucose	0.46 ^b	0.46 ^b	0.24	0.40 ^a	0.53 ^b	0.40 ^a	0.48 ^b
2h glucose	0.33 ^a	0.18	0.55 ^c	0.44 ^b	0.22	0.15	0.18
Fasting insulin	0.61 ^b	0.55 ^b	0.44 ^a	0.64 ^c	0.65 ^c	0.50 ^b	0.58 ^c
2h insulin	0.22	0.22	0.45 ^b	0.27	0.28	0.15	0.20
ISI	0.01	0.03	-0.11	-0.11	-0.37 ^a	0.05	-0.04
Fasting TG	0.19	0.07	0.55 ^b	0.39 ^a	0.37 ^a	0.13	0.20
8h TG	0.19	0.01	0.70 ^c	0.47 ^b	0.44 ^a	0.08	0.17
S. Asian women							
Fasting glucose	0.31	0.17	0.39 ^a	0.38 ^a	0.42 ^a	0.01	0.14
2h glucose	0.13	-0.01	0.45 ^b	0.29	0.35	-0.09	0.04
Fasting insulin	0.30	0.09	0.33	0.35 ^a	0.38 ^a	0.06	0.16
2h insulin	0.19	0.06	0.18	0.16	0.27	0.06	0.11
ISI	-0.38 ^a	-0.21	-0.19	-0.30	-0.35	-0.02	-0.13
Fasting TG	0.32	0.10	0.58 ^c	0.49 ^b	0.56 ^b	0.16	0.34
8h TG	0.13	-0.06	0.42 ^a	0.26	0.40 ^a	-0.03	0.13

^a p<0.05, ^b p<0.01, ^c p<0.001; Insulin, triglyceride (TG) and visceral fat area (VFA) have been log (natural) transformed. W girth: waist girth

ISI and insulin levels were associated with all measures of obesity, and particularly with central obesity. The main departure from this pattern was that ISI in European women was not significantly associated with most measures of obesity (except with VFA). In men there was a striking ethnic difference in the strength of the association between insulin concentration and measures of central obesity: in South Asian men these correlation coefficients were generally larger than in European men. There was a strong correlation of measures of central obesity (VFA, waist girth and WHR) with fasting and 8h triglyceride level in all 4 sex/ethnic groups. In men, but not women, generalised obesity, TFA and SFA were also associated with 8h triglyceride level. The correlations between VFA and percent fat were stronger in men (r=0.79 in Europeans and 0.68 in South Asians) than in women (r=0.48 in Europeans and 0.59 in South Asians).

6.1.6. Ethnic difference in the relation between insulin sensitivity and obesity

6.1.6.1. Insulin sensitivity index as the dependent variable

6.1.6.1.1. ISI and BMI

The relationship between ISI and BMI in all participants ($n=114$) of the two ethnic groups is shown in Figure 3.

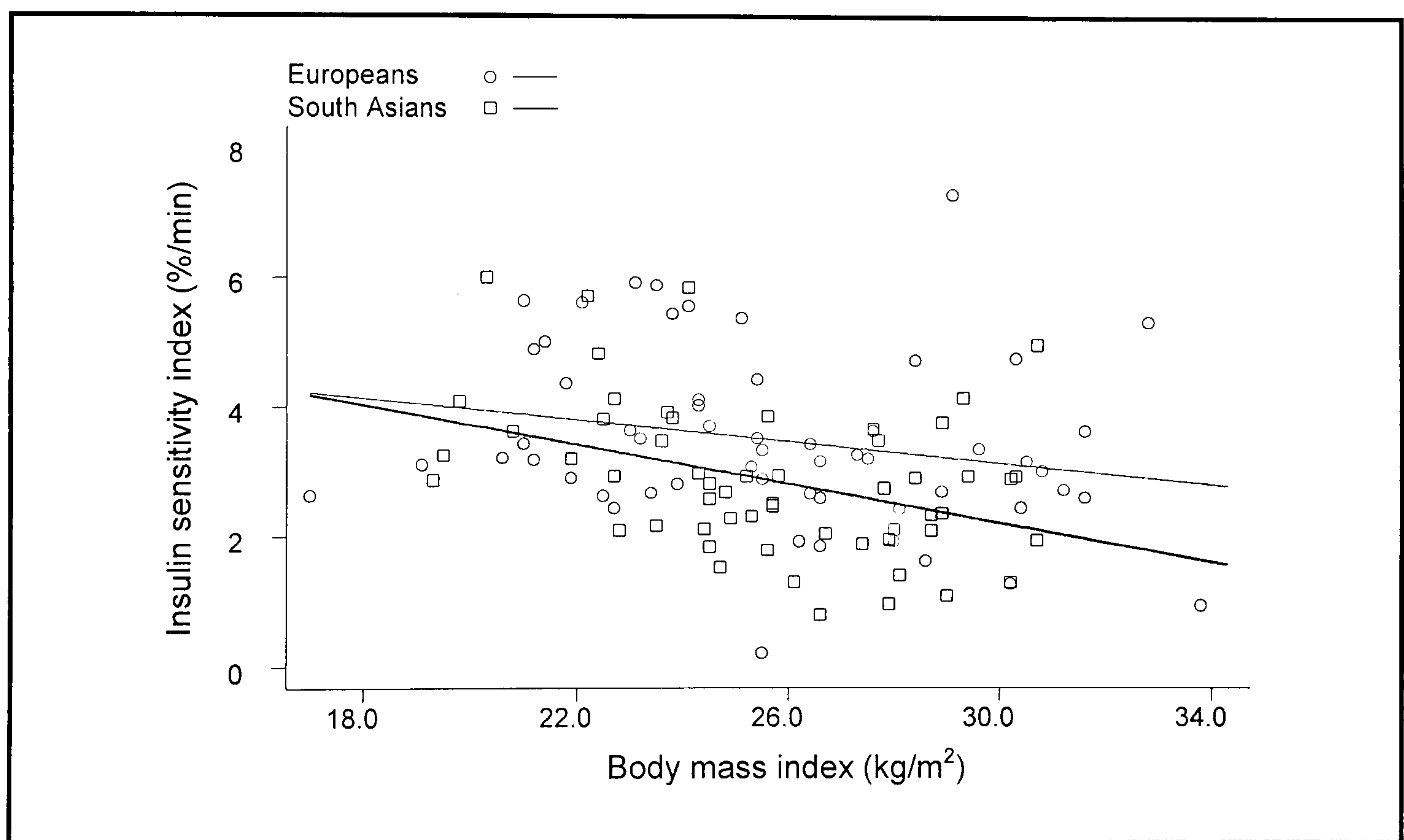


Figure 3: Relationship between insulin sensitivity index and BMI in Europeans and South Asians

The regression line for this association was steeper in South Asians [$\beta=-0.15$, $p=0.003$] than in Europeans [$\beta=-0.08$, $p=0.093$], but the slopes were not significantly different from each other [p for interaction between ethnicity and BMI=0.334]. The slope of the regression line for the association between ISI and BMI, adjusted for age and sex was -0.07 ($se=0.05$, $p=0.150$) in Europeans and -0.14 ($se=0.05$, $p=0.003$) in South Asians [also not significantly different; p for interaction =0.285]. There was no interaction

between sex and BMI, thus the results have been presented for the whole group of participants.

6.1.6.1.2. *ISI and percent fat*

There was a significant interaction between sex and percent fat [$p=0.020$], thus analyses were performed separately for men and women. Among men there was a significant association between percent fat and ISI in Europeans [$\beta=-0.11$, $p=0.003$] but not in South Asians [$\beta=-0.05$, $p=0.145$], and the slopes of the regression lines were not significantly different [p for interaction =0.201]. In women there was no significant association between percent fat and ISI.

6.1.6.1.3. *ISI and central obesity*

The relationship between ISI and VFA in all participants of the two ethnic groups is shown in Figure 4.

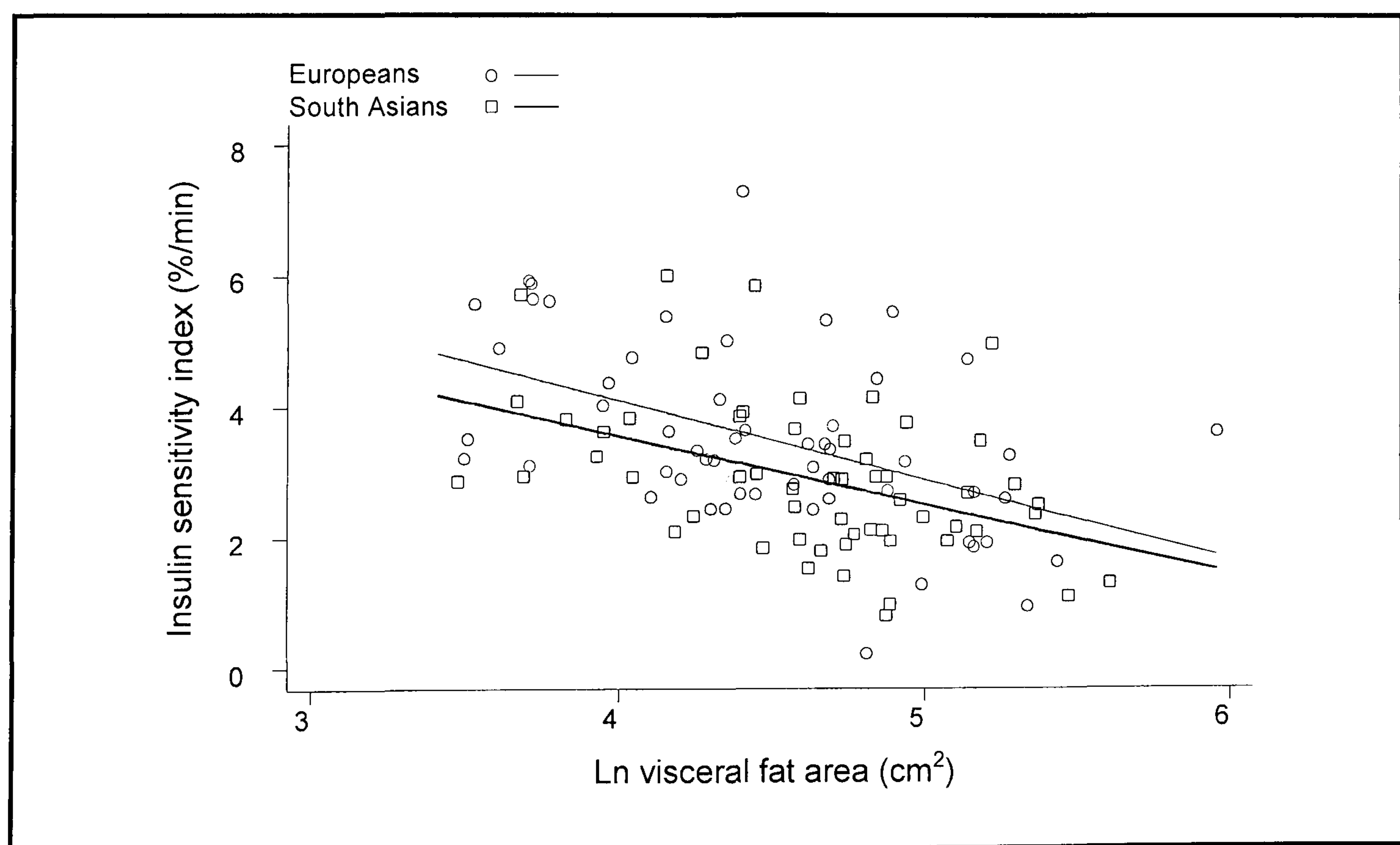


Figure 4: Relationship between insulin sensitivity index and visceral fat area in Europeans and South Asians

Although the association between ISI and VFA was strong in each group, the slopes of the regression lines were not significantly different in Europeans [$\beta=-1.21$, $p<0.001$] and South Asians [$\beta=-1.05$, $p=0.001$], with p for interaction =0.706. The results were similar in analyses adjusted for age and sex, and there was no interaction between sex and VFA [p for interaction =0.476]. The results were in the same direction when either waist girth or WHR ratio were used as markers of central obesity.

6.1.6.2. Fasting insulin level as the dependent variable

6.1.6.2.1. Fasting insulin and BMI

In regression analyses adjusted for age and sex, the association between fasting insulin and BMI was strong in both Europeans [$\beta=0.06$, $p<0.001$] and South Asians [$\beta=0.09$, $p<0.001$], but there was no significant ethnic difference [p for interaction =0.376]. There was no interaction between sex and BMI [$p=0.407$]. Figure 5 shows graphically the relation between fasting insulin and BMI in European and South Asian participants.

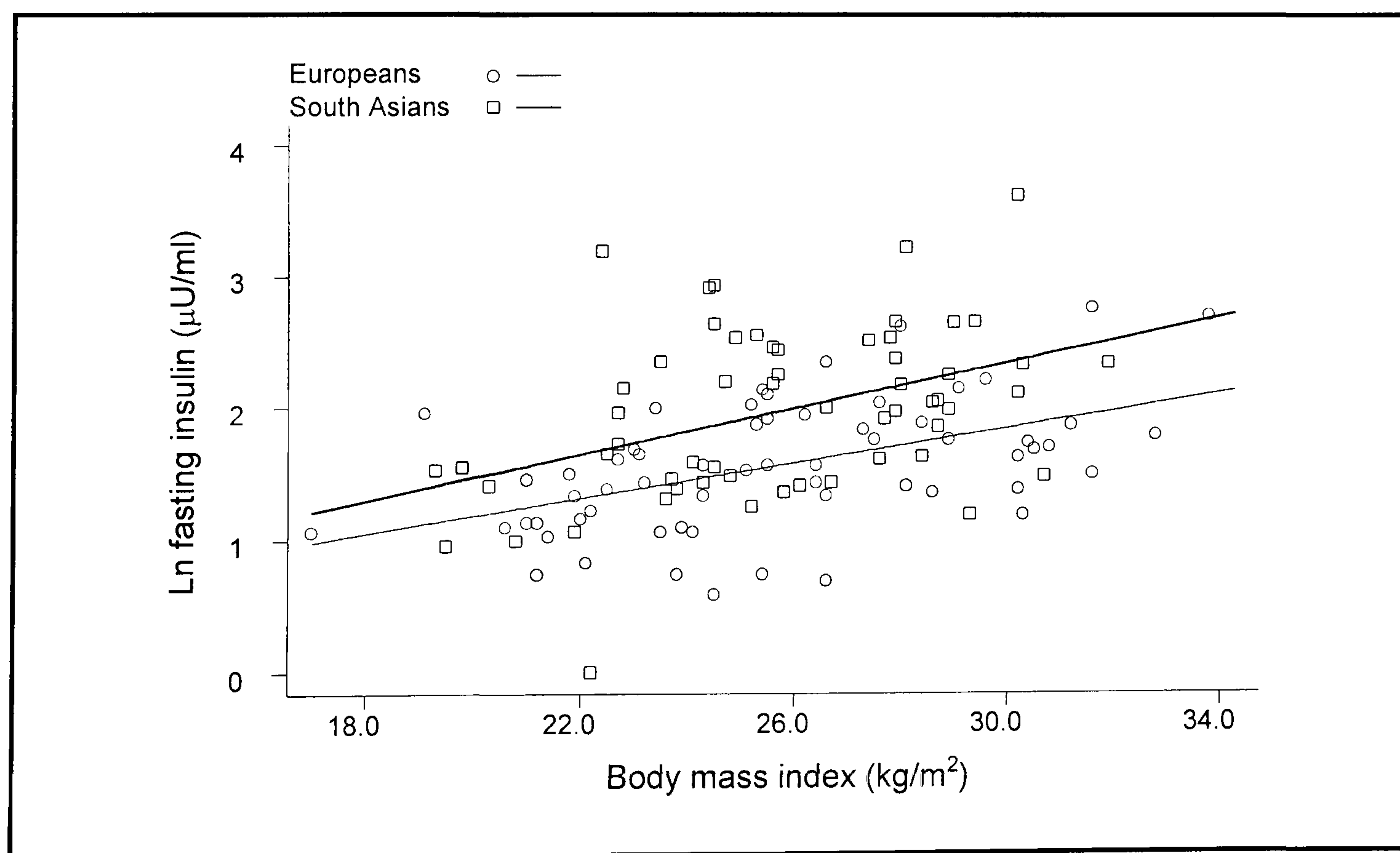


Figure 5: Relationship between fasting insulin and BMI in Europeans and South Asians

6.1.6.2.2. Fasting insulin and percent fat

There was no evidence of interaction [$p=0.267$] between sex and percent fat, so analyses are presented for the whole group combined. In regression analysis of the whole group adjusted for age and sex the association between percent fat and fasting insulin was stronger in Europeans [$\beta=0.03$, $p<0.001$] than in South Asians [$\beta=0.03$, $p=0.041$], with borderline interaction between ethnicity and percent fat [$p=0.107$]. The crude association between fasting insulin and percent fat was significant in Europeans [$\beta=0.02$, $p=0.015$] but not in South Asians [$\beta= -0.006$, $p=0.532$], and is shown in Figure 6.

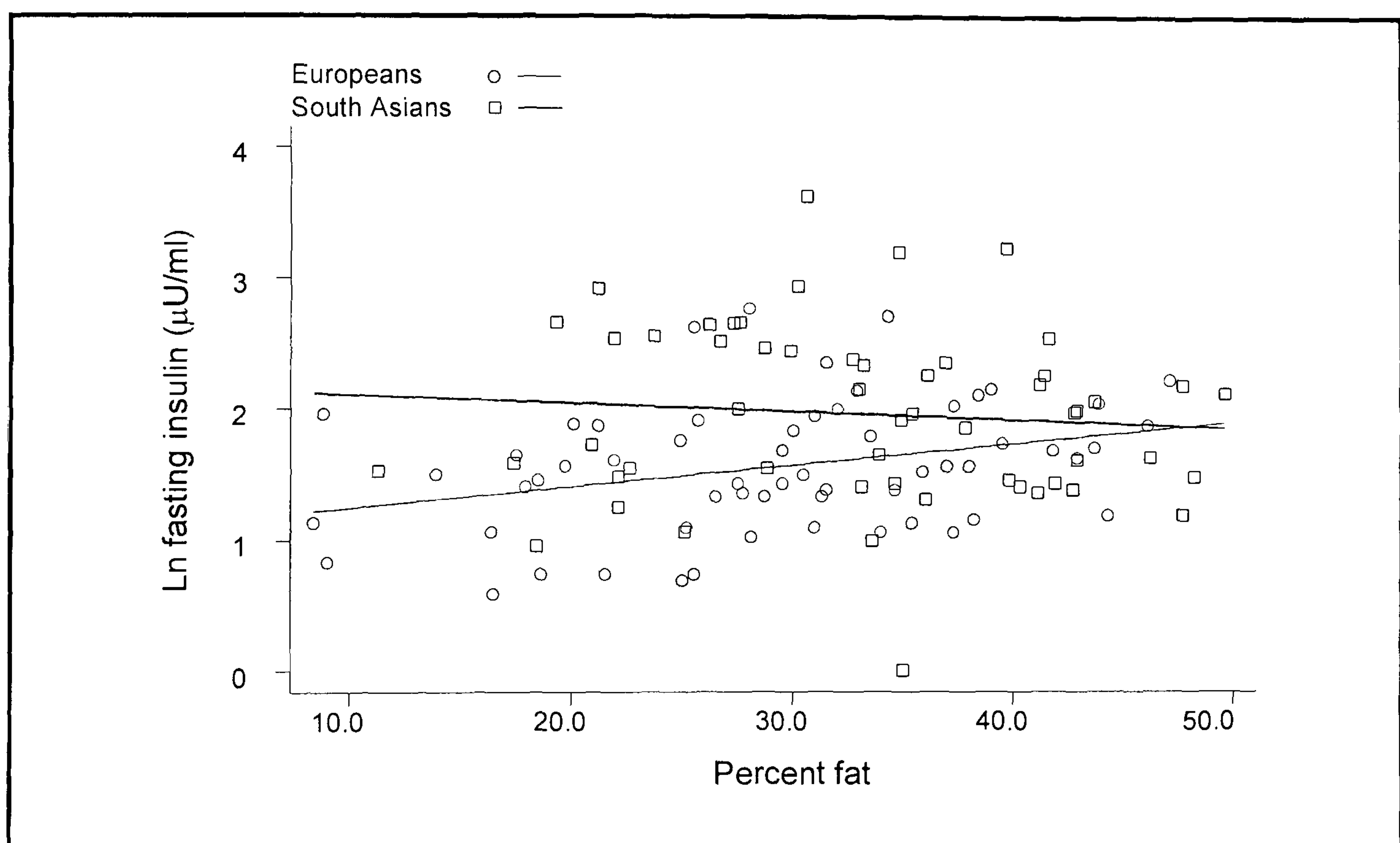


Figure 6: Relationship between fasting insulin and percent fat in Europeans and South Asians

6.1.6.2.3. Fasting insulin and central obesity

The relationship between fasting insulin and VFA in all participants of the two ethnic groups is shown in Figure 7. The association between fasting insulin and VFA was strong in each group, with a significantly steeper slope [p for interaction =0.060] in South Asians [$\beta=0.73$, $p<0.001$] than in Europeans [$\beta=0.37$, $p=0.001$]. When the analysis was repeated with additionally adjusting for age and sex [no interaction between sex and VFA,

$p=0.730$], the slopes of the regression lines were significantly different at the 5% level [p for interaction =0.045].

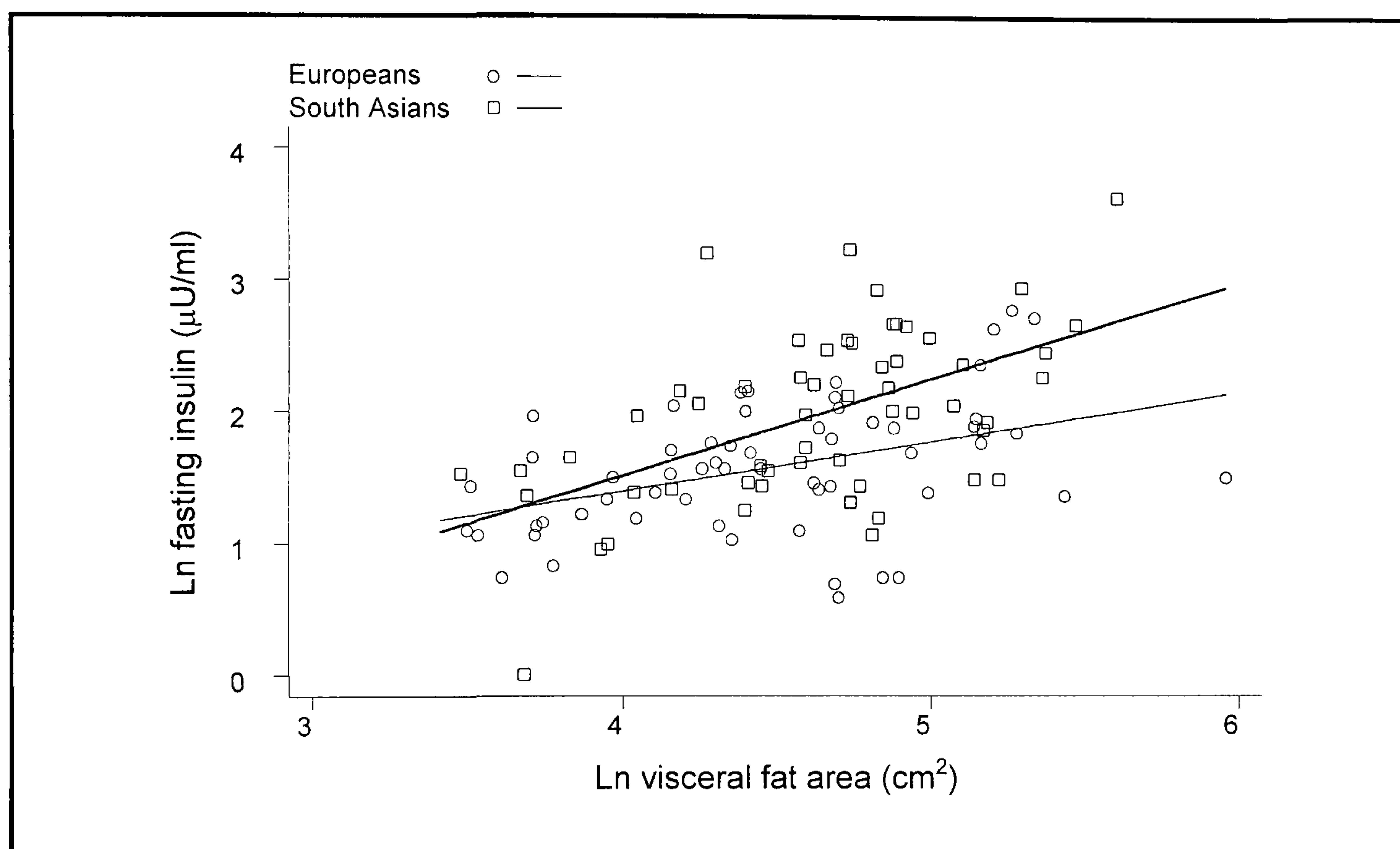


Figure 7: Relationship between fasting insulin and visceral fat area in Europeans and South Asians

The results were in the same direction when either waist girth (Figure 8) or WHR (Figure 9) were used as markers of central obesity.

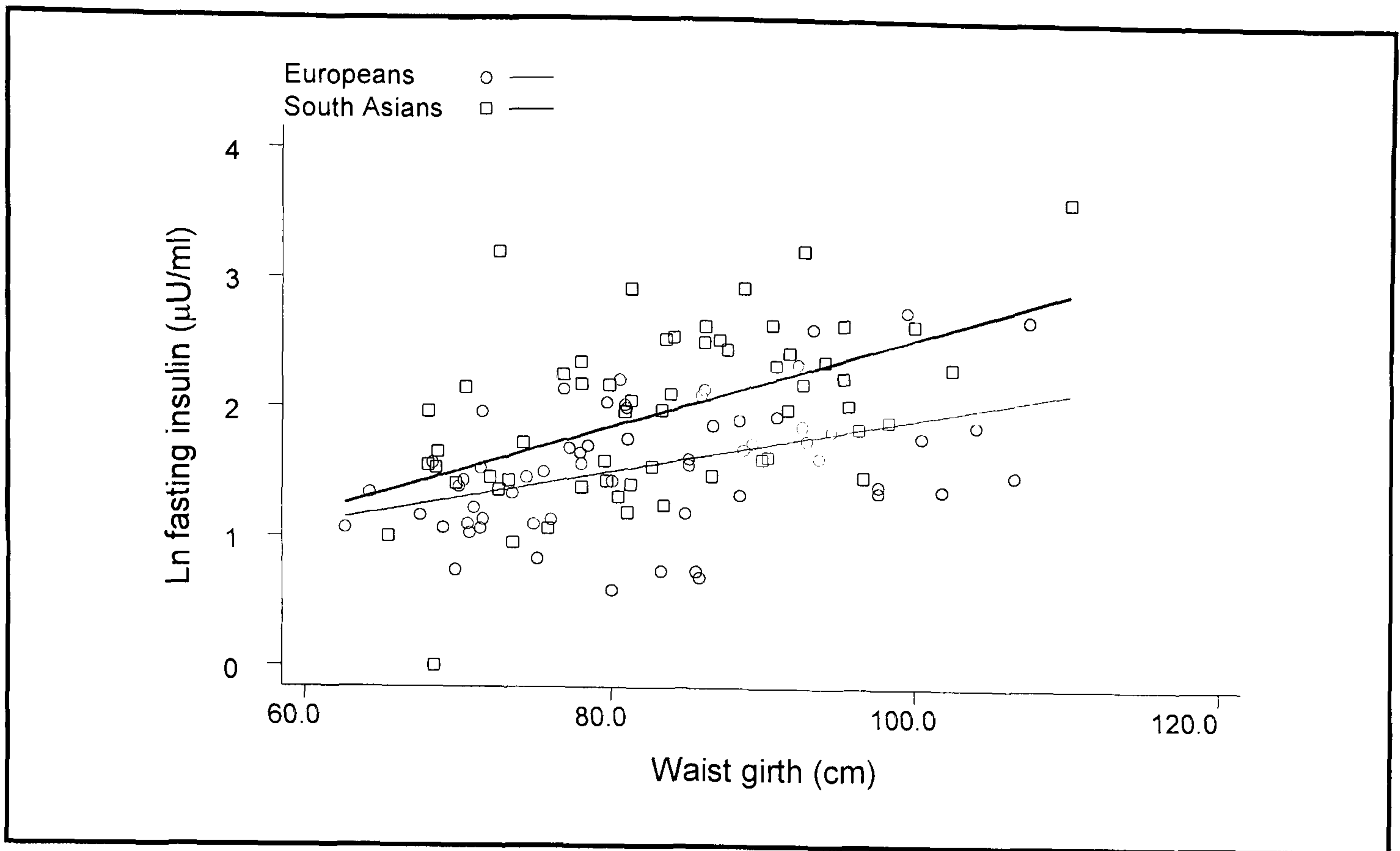


Figure 8: Relationship between fasting insulin and waist girth in Europeans and South Asians

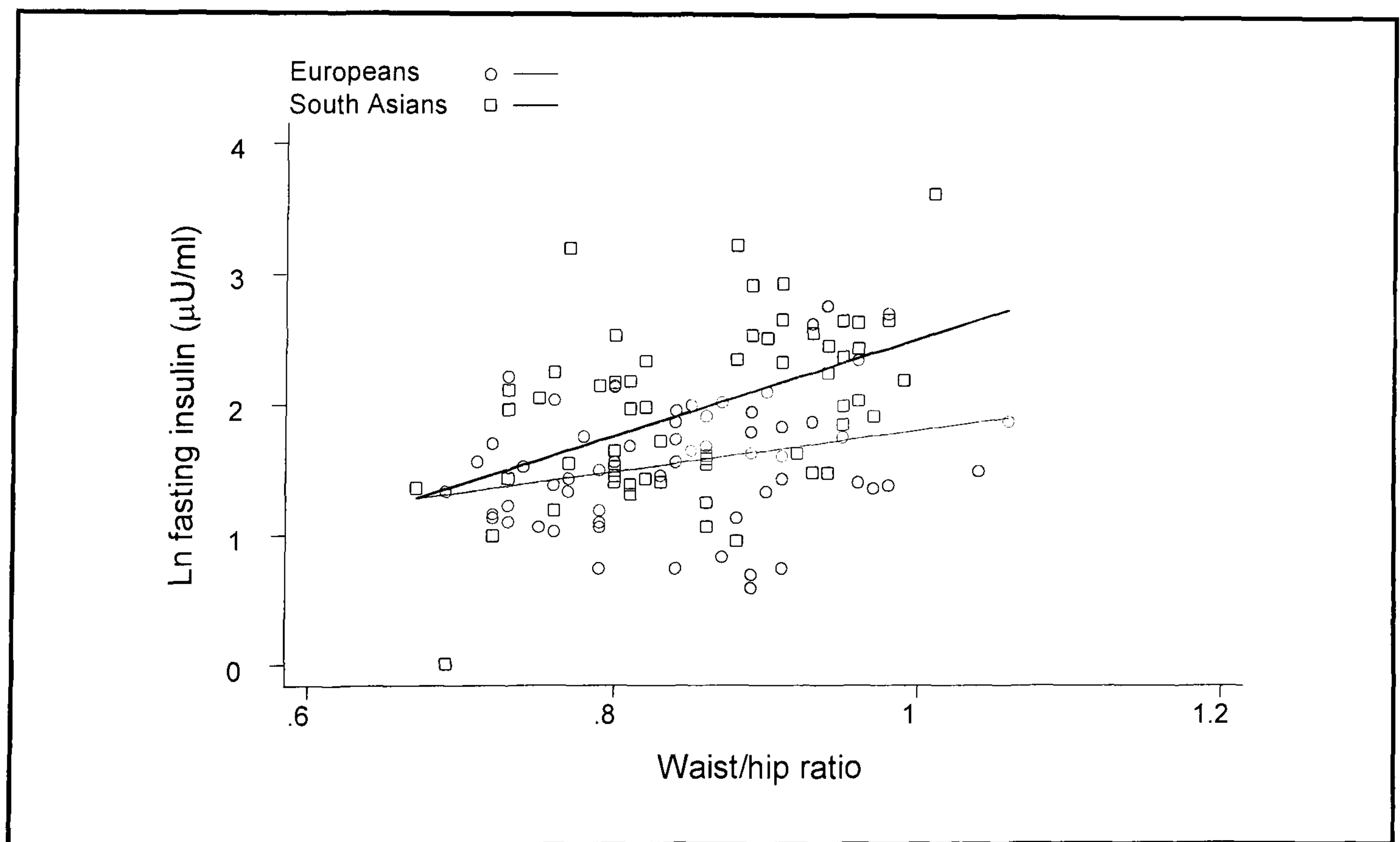


Figure 9: Relationship between fasting insulin and waist/hip ratio in Europeans and South Asians

6.1.6.3. 2h insulin level as the dependent variable

6.1.6.3.1. 2h insulin and BMI

In contrast to the lack of ethnic difference in association between BMI and fasting insulin, there was a significant ethnic difference in the association between BMI and 2h insulin. The slope of this association in the whole group was much steeper [p for interaction = 0.047] in South Asians [$\beta=0.12$, $p=0.006$] than in Europeans [$\beta=0.02$, $p=0.553$]; Figure 10. In analyses further adjusted for age and sex the results were unchanged. There was no evidence for a sex/BMI interaction [$p=0.364$].

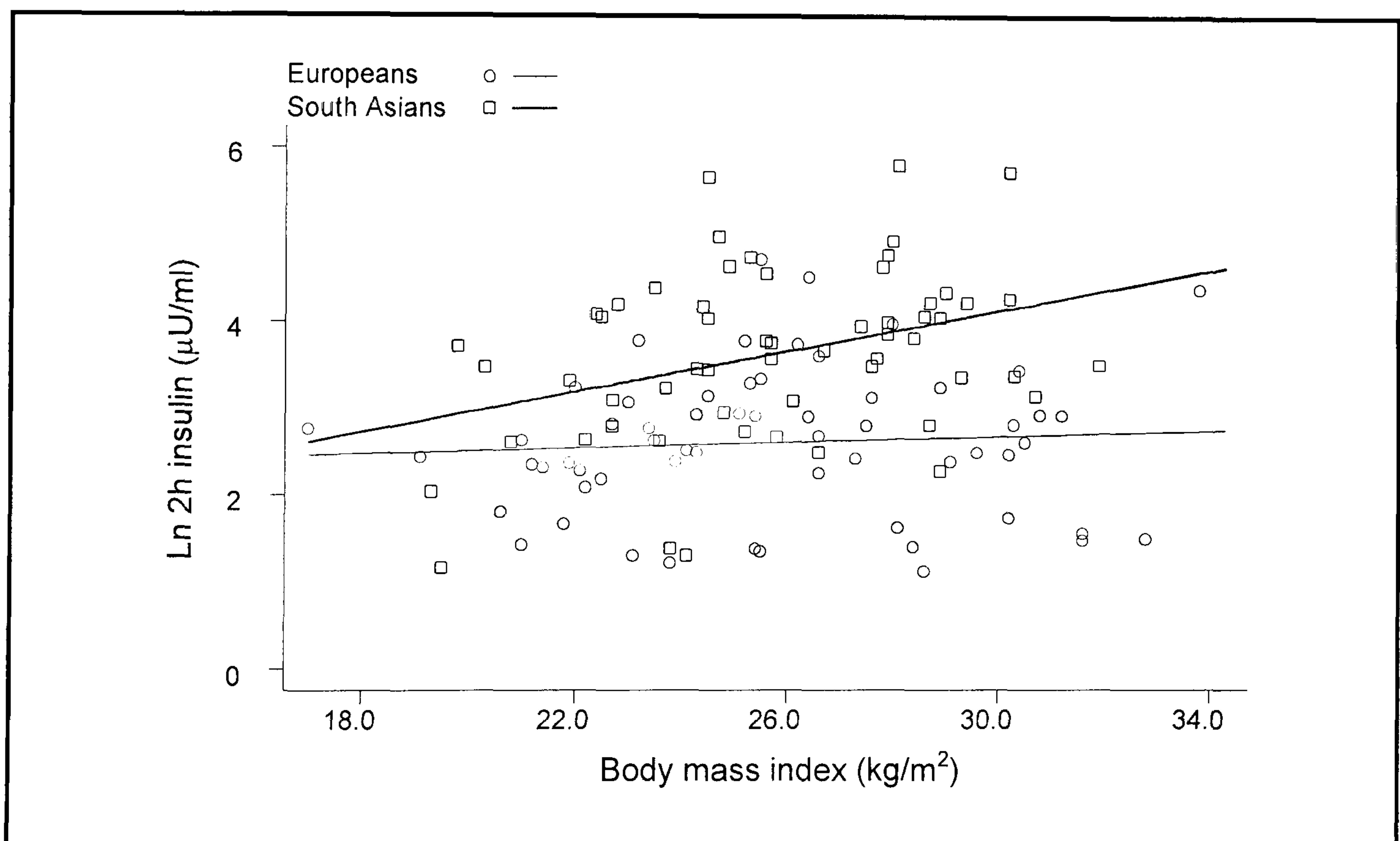


Figure 10: Relationship between 2h-insulin and BMI in Europeans and South Asians

6.1.6.3.2. 2h insulin and percent fat

There was an interaction [$p=0.052$] between sex and percent fat, so analyses were performed separately for men and women. There was no significant association between 2h insulin and percent fat except in South Asian men [$\beta=0.09$, $p=0.019$].

6.1.6.3.3. 2h insulin and central obesity

The relationship between 2h insulin and VFA in all participants is shown in Figure 11. There was no association between 2h insulin and VFA in Europeans [$\beta=0.04$, $p=0.839$], but there was a strong association in South Asians [$\beta=0.93$, $p=0.001$]; p for interaction =0.007. The results were unchanged when age and sex were added to the models [p for sex/VFA interaction =0.300]. The results were in the same direction (and same order of magnitude) when either waist girth or WHR were used as markers of central obesity (Figure 12 and Figure 13 respectively).

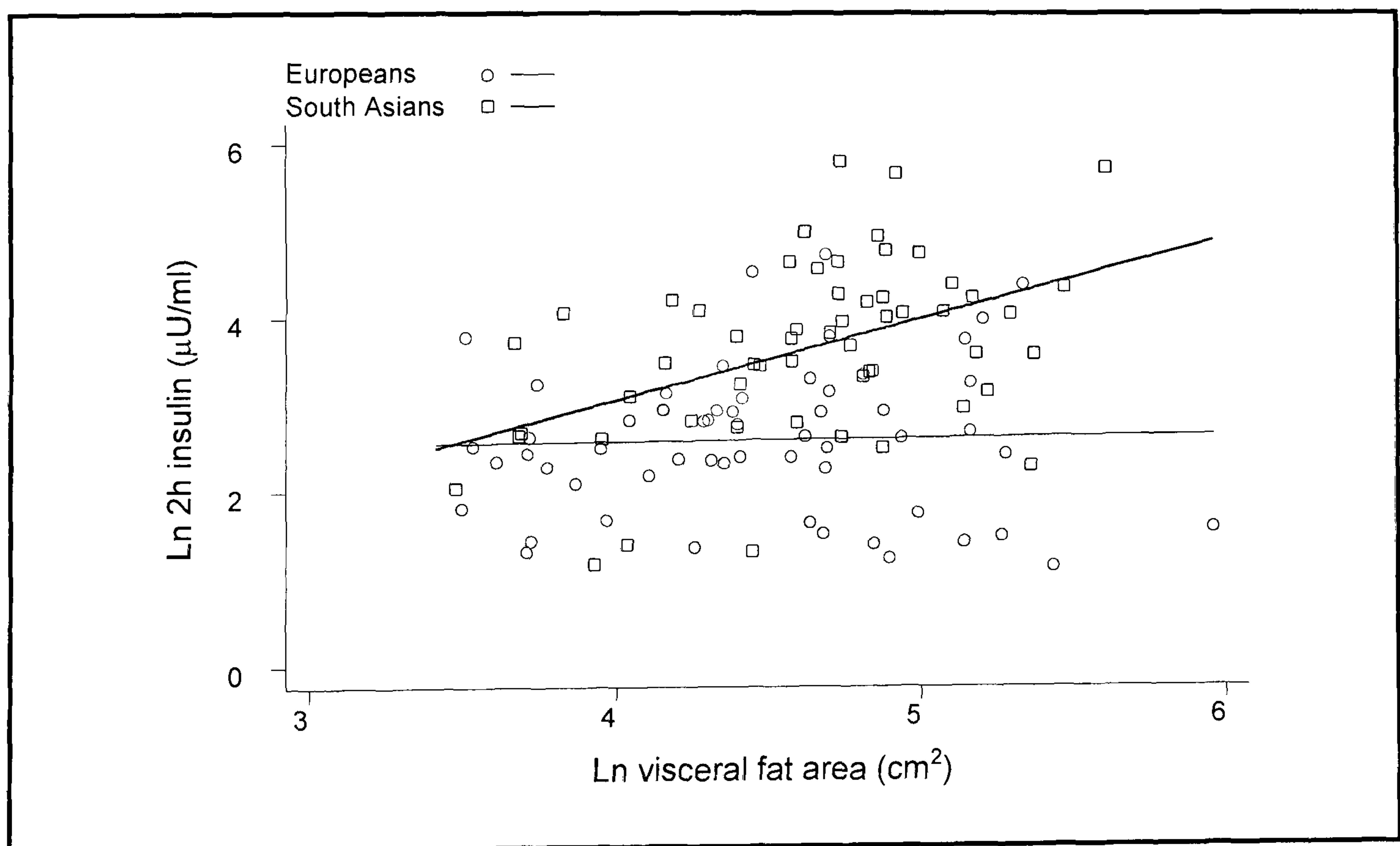


Figure 11: Relationship between 2h-insulin and visceral fat area in Europeans and South Asians

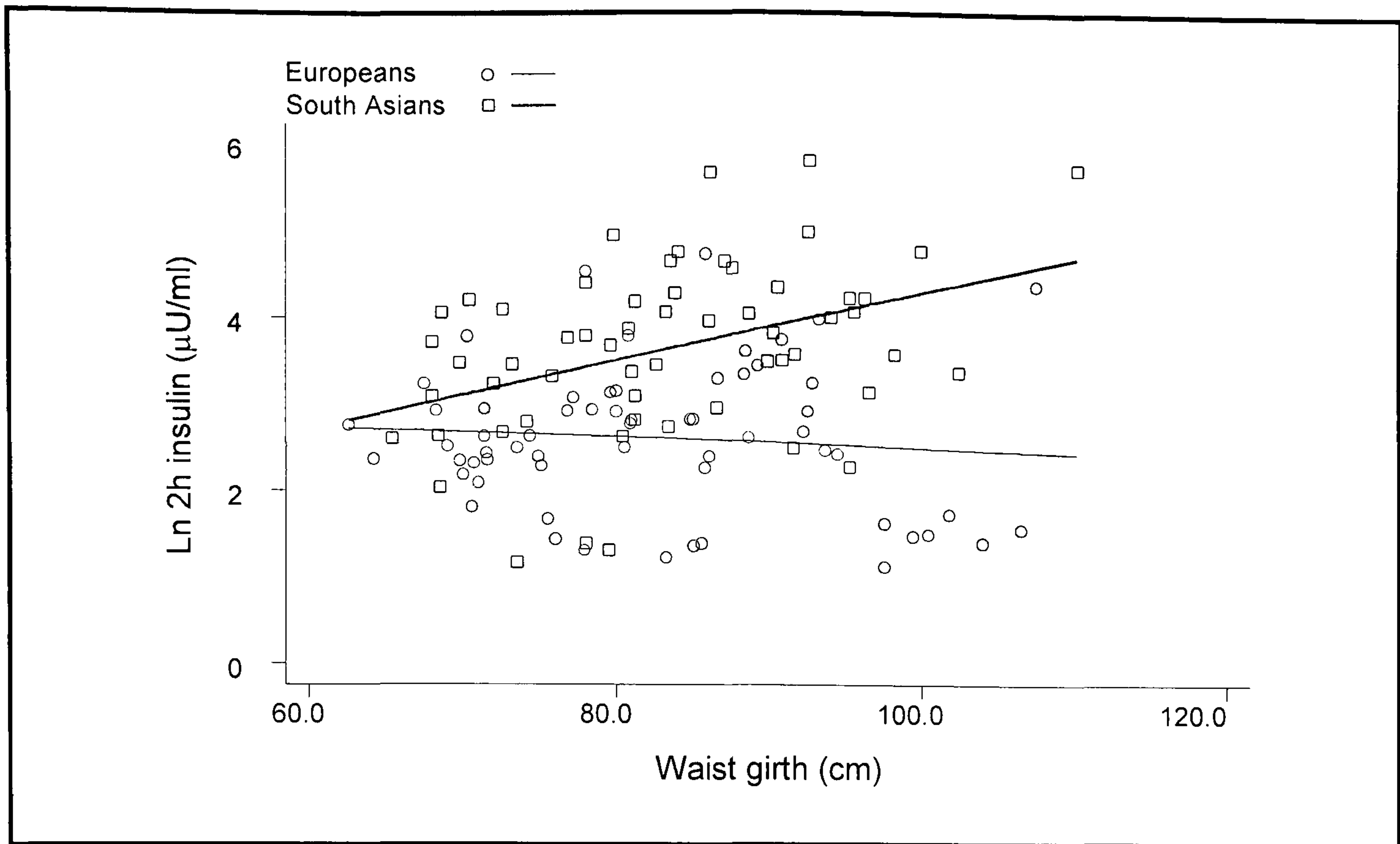


Figure 12: Relationship between 2h-insulin and waist girth in Europeans and South Asians

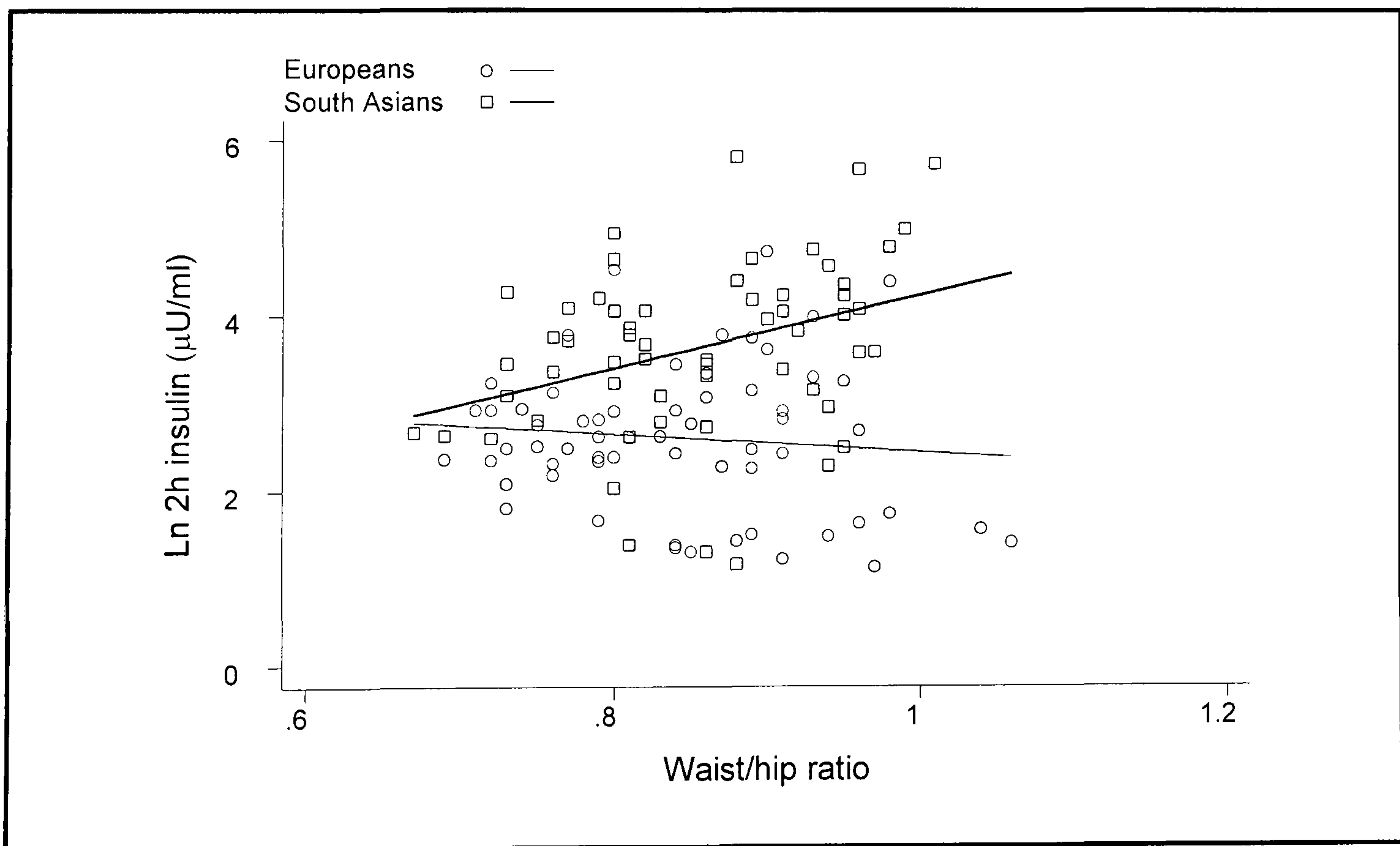


Figure 13: Relationship between 2h-insulin and waist/hip ratio in Europeans and South Asians

6.1.7. Relation between insulin sensitivity, visceral obesity, lipids and ethnic origin

There was a significant strong relationship of ISI and VFA in both ethnic groups [in regression analyses adjusted for sex and age: standardised $\beta = -0.64$, $se = 0.18$, $p = 0.001$, adjusted $R^2 = 21\%$ in Europeans; $\beta = -0.43$, $se = 0.17$, $p = 0.015$, adjusted $R^2 = 20\%$ in South Asians]. In the whole group (in analyses adjusted for age, ethnicity and sex) there persisted a significant relation between ISI and VFA after adjustment for % fat and SFA [$\beta = -0.45$, $se = 0.17$, $p = 0.009$, adjusted $R^2 = 25\%$]. However, this relationship was eliminated after additional adjustment for triglyceride level (fasting and 8h) [$\beta = 0.02$, $se = 0.19$, $p = 0.911$, adjusted $R^2 = 34\%$]. The results for the effect of VFA on ISI in each ethnic group separately with and without adjustment for overall obesity and triglyceride level, are shown in Table 11.

Table 11: Multiple linear regression with insulin sensitivity as dependent variable and visceral fat area as independent variable, adjusted for age, sex, obesity and lipids, in Europeans and South Asians.

independent variables	Europeans				S. Asians			
	β	s.e.	p	R^2 %	β	s.e.	p	R^2 %
VFA , age, sex	-0.64	0.18	0.001	20.6	-0.43	0.17	0.015	19.8
VFA, age, sex, % fat	-0.53	0.26	0.046	19.6	-0.40	0.22	0.076	18.0
VFA age, sex, % fat, SFA	-0.51	0.26	0.054	21.6	-0.41	0.22	0.076	17.7
VFA, age, sex, % fat, SFA, 0h TG	-0.11	0.33	0.743	22.4	0.002	0.25	0.993	28.9
VFA, age, sex, % fat, SFA, 8h TG	0.09	0.32	0.767	28.0	-0.04	0.23	0.854	32.5
VFA, age, sex, % fat, SFA, 0h TG, 8h TG	0.03	0.33	0.914	27.3	0.003	0.24	0.989	31.5

log (natural) transformed values of VFA and TG were used. VFA= visceral fat area, TG= triglyceride, β = standardised β , R^2 = adjusted R^2 value. The standardised beta (β) is the slope of the relationship of ISI on VFA, with the continuous variables standardised to unit standard deviation (see methods section 5.9).

In analyses adjusted for age and ethnicity, there was a significant association between SFA and ISI in men [$\beta=-0.55$, $se=0.18$, $p=0.004$] but not women [$\beta=0.04$, $se=0.16$, $p=0.786$]; p value for sex/SFA interaction =0.014. In men, when additional adjustment was made for VFA, the significance of association with SFA was lost [$\beta=-0.28$, $se=0.22$, $p=0.210$]. Thus the association between ISI and VFA was independent of SFA, but the converse was not the case.

The results were similar when fasting or 2h insulin were chosen as the marker of insulin resistance for the whole group. That is, there was a significant association between fasting insulin and VFA, and 2h insulin and VFA that was independent of % fat and SFA. This association was eliminated after additionally adjusting for triglyceride level (fasting or 8h).

6.1.8. The ethnic difference in insulin sensitivity

In standardised regression analyses adjusted for age and sex ISI was 0.71 %/min lower in South Asians than in Europeans (Table 12). The contribution of obesity and triglyceride level to the ethnic difference in ISI was examined, and is summarised in Table 12.

Adjustment for total adiposity (measured by BMI or percent fat), body fat distribution or triglyceride level did not account for more than one-third of the ethnic difference in ISI.

In a multivariate model with adjustment for age, sex, percent fat, VFA, as well as fasting and 8h triglyceride only 42% of the ethnic difference in ISI was accounted for [$\beta= -0.41$, $se=0.22$, $p=0.066$, adjusted $R^2=33\%$]. With additional adjustment for variables measuring level of physical activity, none of the reported results were significantly altered, and measures of physical activity were not significant in the model. Adding smoking status was also not significant in the model. However addition of social class (manual vs. non-manual occupation) was significant in the model, and increased the ethnic difference in ISI [$\beta=-0.63$, $se=0.24$, $p=0.009$]. Among women adjustment for menopausal status did not alter the model.

With fasting or 2 h insulin as dependent variable the results were similar; none of the measured variables accounted for more than half the ethnic difference.

Table 12: Effect of measures of obesity and body fat distribution on age and sex adjusted association between insulin sensitivity index and ethnicity.

variables adjusted for:	β	s.e.	p	R ² %
age, sex	- 0.71	0.24	0.003	11.1
age, sex, BMI	- 0.71	0.23	0.002	16.8
age, sex, %fat	- 0.49	0.24	0.045	16.0
age, sex, waist girth	- 0.66	0.22	0.004	19.8
age, sex, waist-hip ratio	- 0.62	0.23	0.008	15.7
age, sex, visceral fat area*	- 0.52	0.23	0.024	24.5
age, sex, subcutaneous fat area	- 0.66	0.24	0.007	14.6
age, sex, visceral/subcutaneous fat area ratio*	- 0.69	0.24	0.004	14.1
age, sex, total fat area	- 0.61	0.23	0.010	18.0
age, sex, fasting triglyceride*	- 0.59	0.22	0.009	23.5
age, sex, 8h postprandial triglyceride*	- 0.57	0.21	0.008	29.4

Independent variable of primary interest: ethnic group coded as ethnicity: 2=South Asian, 1=European; n=114. Dependent variable: insulin sensitivity index. *: log (natural) transformed variables; all obesity and metabolic variables are standardised continuous variables. β = standardised β , R² = adjusted R² value.

6.1.9. Relation between postprandial triglyceride concentration and insulin resistance

Univariate associations of postprandial triglyceride level and features associated with the insulin resistance syndrome are presented in Table 13. This shows that postprandial triglyceride level was associated with features of the metabolic syndrome in all 4 sex/ethnic groups. To assess whether there was an independent association of postprandial triglyceride with insulin resistance, multivariate analyses were performed for the entire group (adjusted for age, sex and ethnic group) with and without adjustment for fasting triglyceride level. The results are presented in Table 14 and show that by and large postprandial triglyceride concentration had an independent association with features of the insulin resistance syndrome.

Table 13: Pearson's product moment correlation coefficients (r) for the relation between 8h triglyceride level and metabolic and obesity features of the insulin resistance syndrome

	Men		Women	
	European	S. Asian	European	S. Asian
ISI (%/min)	-0.29	-0.64 ^b	-0.42 ^d	-0.45 ^d
Fasting insulin* (μu/ml)	0.45 ^d	0.64 ^b	0.51 ^c	0.62 ^b
2h insulin* (μu/ml)	0.31	0.75 ^a	0.61 ^b	0.42 ^d
Fasting glucose (mmol/l)	-0.05	-0.04	0.09	0.47 ^d
2h glucose (mmol/l)	0.35 ^e	0.36 ^d	0.22	0.41 ^d
HDL-cholesterol* (mmol/l)	-0.44 ^d	-0.76 ^a	-0.53 ^c	-0.32 ^c
Fasting NEFA* (mmol/l)	0.11	0.03	0.07	0.49 ^c
8h NEFA* (mmol/l)	0.35 ^e	0.38 ^d	0.19	0.66 ^b
Fasting VLDL-TG* (mmol/l)	0.78 ^a	0.61 ^b	0.73 ^a	0.80 ^a
8h VLDL-TG* (mmol/l)	0.87 ^a	0.80 ^a	0.90 ^a	0.93 ^a
Fasting VLDL-Chol* (mmol/l)	0.71 ^a	0.60 ^b	0.72 ^a	0.77 ^a
8h VLDL-Chol* (mmol/l)	0.73 ^a	0.66 ^a	0.85 ^a	0.87 ^a
Fasting triglyceride* (mmol/l)	0.77 ^a	0.76 ^a	0.81 ^a	0.91 ^a
VFA* (cm ²)	0.60 ^b	0.49 ^c	0.44 ^d	0.40 ^d
SFA (cm ²)	0.46 ^d	0.46 ^d	0.08	-0.03
Waist girth (cm)	0.32 ^e	0.61 ^b	0.47 ^c	0.26
Waist-hip ratio	0.30	0.69 ^b	0.70 ^a	0.42 ^d
BMI (kg/m ²)	0.38 ^d	0.53 ^c	0.19	0.13
Percent fat (%)	0.47 ^d	0.45 ^d	0.01	-0.06

*These variables were log (natural) transformed; a=p<0.0001; b=p<0.001; c=p<0.01; d=p<0.05; e=p<0.1

Table 14: Multivariate analyses for the relation between 8h triglyceride level (dependent variable) and features of the insulin resistance syndrome, adjusted for sex, ethnicity, and age, with and without adjustment for fasting triglyceride level

independent variables: age, sex, ethnic group +	No adjustment for fasting TG				Adjustment for fasting TG			
	β	se	p	%R ²	β	se	p	% R ²
ISI (%/min)	-0.31	0.06	<0.001	25.9	-0.12	0.04	0.004	70.3
Fasting insulin* (μ u/ml)	0.37	0.05	<0.001	36.2	0.16	0.04	<0.001	71.8
2h insulin* (μ u/ml)	0.37	0.06	<0.001	31.4	0.18	0.04	<0.001	72.9
2h glucose (mmol/l)	0.24	0.06	<0.001	18.4	0.10	0.04	0.010	71.6
HDL-Chol* (mmol/l)	-0.35	0.06	<0.001	31.8	-0.16	0.04	<0.001	74.1
0h NEFA* (mmol/l)	0.11	0.06	0.086	10.4	-0.06	0.04	0.092	70.6
8h NEFA* (mmol/l)	0.25	0.06	<0.001	21.5	0.10	0.04	0.005	71.9
0h VLDL-TG* (mmol/l)	0.50	0.04	<0.001	58.3	0.03	0.08	0.709	69.8
8h VLDL-TG* (mmol/l)	0.61	0.03	<0.001	78.8	0.43	0.05	<0.001	81.7
0h VLDL-Chol* (mmol/l)	0.47	0.05	<0.001	53.5	0.05	0.06	0.458	70.0
8h VLDL-Chol* (mmol/l)	0.55	0.04	<0.001	64.8	0.26	0.05	<0.001	74.8
Visceral fat area* (cm ²)	0.36	0.06	<0.001	28.4	0.07	0.05	0.118	70.8
SFA (cm ²)	0.16	0.07	0.018	12.3	0.04	0.04	0.304	70.4
Waist girth (cm)	0.31	0.07	<0.001	22.4	0.05	0.05	0.266	70.2
WHR	0.47	0.08	<0.001	31.0	0.11	0.06	0.073	70.7
BMI	0.20	0.06	0.003	15.5	0.04	0.04	0.355	70.1
Percent fat (%)	0.20	0.09	0.029	11.2	0.07	0.05	0.189	68.8

* = log (natural) transformed values of these variables were used; R² : Adjusted R² values; β = standardised beta coefficient. (see methods section 5.9)

6.2. Summary of results

The results in this chapter can be summarised as follows:

1. South Asians compared with Europeans tended to have
 - Higher levels of overall obesity (greater percent fat), and central obesity (greater VFA), especially so in women.
 - Greater prevalence of insulin resistance (higher glucose and insulin levels and lower ISI)
 - Higher levels of postprandial triglyceride in men (significant at 6h, but not at 8h)
2. All measures of insulin sensitivity (ISI, fasting insulin and 2h insulin) were strongly related with obesity in all groups, especially central obesity. In particular there was a strong association between VFA and ISI that was independent of SFA and percent fat. There was also a strong association between SFA and ISI, but this association was eliminated when adjustment was made for VFA.
3. The strong association between ISI and VFA persisted when adjusted for overall obesity (percent fat), but was eliminated when adjustment was made for triglyceride level (fasting or 8h).
4. The slopes of the regression lines for the association between ISI and BMI, and between ISI and VFA were not significantly different between Europeans and South Asians. However, the slopes of the regression lines for the association between fasting insulin and VFA (as well as waist girth and WHR), and between 2h insulin and BMI, and 2h insulin and VFA (as well as waist girth and WHR) were significantly different in Europeans and South Asians, with the latter group having steeper slopes.
5. Less than half the ethnic difference in insulin sensitivity (ISI, or insulin level) was accounted for by obesity, body fat distribution and triglyceride level.

6. Postprandial triglyceride level was a correlate of the metabolic syndrome, and this association was independent of the fasting triglyceride level in both Europeans and South Asians.

6.3. Discussion of results

While it has been suggested that in South Asians central obesity is related to the high prevalence of insulin resistance, diabetes and CHD²⁹, no one has used direct measures to study the relationship between central obesity and insulin resistance in South Asians and Europeans. The results from this study are the first to address this issue using direct measures.

We found overall higher levels of total obesity (percent fat), central obesity (VFA), postprandial triglyceride level and insulin resistance (lower ISI and higher insulin level) in South Asians than Europeans who were frequency matched for BMI. The level of obesity was significantly different in women but not men; there was a trend towards higher postprandial triglyceride concentration in men (significant at 6h in a small number, but not at 8h in the whole group) but not women; the greater insulin resistance in South Asians was significant among men for ISI, but both for ISI and insulin level among women. We have shown that postprandial triglyceride level has a strong relation with both central obesity and insulin sensitivity, and statistically accounts for the association between them. We also report that the ethnic difference in insulin sensitivity is not accounted for by central obesity.

6.3.1. Ethnic differences in obesity

We have found that South Asians had higher overall obesity (total percent body fat) than Europeans at any given level of BMI. Among the sexes separately, this was significantly so in women but not men, though the trend in men was also in the same direction. This is a novel finding as there are no previous studies of South Asian - European ethnic comparison that have quantified percent body fat measured accurately by DEXA scan. However some previous studies have attempted to quantify percent body fat by measuring

sum of total skinfolds. Thus studies so far that have quantified obesity only by BMI will have not fully accounted for ethnic differences in body composition.

The greater level of percent fat in South Asians compared with Europeans could be due to genetic differences, environmental and life-style factors and lower levels of exercise, or a combination of these. In this study reported levels of physical activity were low in both groups, and even lower in South Asians than Europeans. The only exception to this was that South Asians reported significantly lower number of hours spent watching television than Europeans. This could be because of cultural differences in the way that periods of physical inactivity are spent. It is possible that South Asians spend more time socialising for example than watching television. There might also be differences in the way exercise is taken; for example it is noteworthy that none of the South Asian women rode a bicycle. There are many limitations due to cultural/ethnic factors in assessing levels of physical activity from self-report. The most objective way to quantify physical fitness would have been to measure maximal aerobic power (VO_2 -max). However, this was not possible in the current study because the study protocol already involved multiple tests on multiple visits, and it was not feasible to fit in any more formal tests.

It is also noteworthy that although there were no ethnic differences in mean waist girth or WHR, mean VFA measured by CT scan was higher in South Asians than in Europeans, although this difference was statistically significant only in women. We have previously reported that in comparison with European men and women, South Asian men and women have higher levels of central obesity, (as measured by WHR, sagittal abdominal diameter or trunk skinfolds), than Europeans²⁹. The failure to detect a significant ethnic difference in VFA in men in this and another smaller study comparing South Asians and Europeans²²⁶, may be a chance result, as differences in abdominal obesity have consistently been detected in larger surveys using anthropometry.

6.3.2. Ethnic difference in insulin sensitivity

Higher average levels of insulin resistance in South Asians compared with Europeans have been demonstrated in epidemiological studies measuring fasting and post-load insulin levels. In a steady-state study using the insulin suppression test²²⁷, the ethnic difference in insulin resistance (measured by the steady-state plasma glucose level) between weight-matched South Asians and Europeans was of comparable size to that typically observed

when patients with NIDDM are compared with weight-matched controls. As a measure of insulin-stimulated glucose uptake, the short insulin tolerance test has been validated against the euglycaemic clamp with correlation coefficients of 0.81 to 0.86¹⁰⁰⁻¹⁰². The higher insulin sensitivity in women compared with men of similar BMI in the present study is in keeping with a previous study²²⁸ which measured ISI by the frequently sampled intravenous glucose tolerance test. The ethnic difference in ISI in the current study was statistically significant among men but not women. However insulin levels (fasting and 2h) were markedly higher in South Asians than in Europeans in both men and women, as previously observed in a larger epidemiological study in this population²⁹.

This suggests that the defect in insulin-mediated glucose disposal in South Asians may be more evident after a glucose load than in the fasting state. Furthermore, although the short insulin tolerance test has been validated against the euglycaemic clamp, it has never specifically been validated in South Asians. It is likely to be valid in South Asians as one group has successfully employed it in a study of an ethnic comparison of prevalence of insulin resistance in non-diabetic relatives of patients with diabetes⁵³. Nonetheless, specific validation in South Asians should be undertaken before it is used in further studies of ethnic comparison.

6.3.3. Ethnic differences in relation between insulin sensitivity and obesity

ISI and insulin levels were correlated with VFA in each ethnic group and in the whole group, and this relationship was independent of percent body fat. This contrasts with a smaller study in India²²⁹ in which there was no significant relation of insulin level to CT-scan-measured abdominal fat areas in South Asians.

Also, the significant association between ISI and VFA was independent of SFA, but the converse was not the case. This finding is directly in contrast with a study by Goodpaster *et al*²³⁰, where SFA was the strongest correlate of insulin sensitivity (measured by the glucose clamp technique), independently of VFA. The primary importance of visceral adipose tissue versus subcutaneous abdominal obesity has also been challenged by others^{231,232}. One reason for the difference in our results could be that Goodpaster *et al*'s study was based on a smaller number of subjects (n=54), who were recruited by public advertisement.

We report that the ethnic difference in insulin sensitivity as measured by the short insulin tolerance test cannot be accounted for by central obesity based on 3 lines of evidence in this study - (A) the ethnic difference in ISI did not disappear with the addition of VFA in regression analysis; (B) the slopes of the regression lines for the association between ISI and VFA or ISI and BMI were not significantly different - i.e. the reduction in ISI with increasing BMI or VFA did not differ significantly between the ethnic groups; and (C) the results did not fit a sex pattern: in men there was a large ethnic difference in ISI but not in VFA, whereas in women there was no significant difference in ISI but a large ethnic difference in VFA.

If accumulation of visceral fat accounts for the greater susceptibility of South Asians to insulin resistance, in comparison with Europeans, we would predict that (i) adjusting for direct measures of visceral fat would account for the ethnic difference in insulin resistance, and (ii) the slope of the relationship between insulin resistance and percent body fat would be steeper in South Asians than in Europeans because in the leanest individuals visceral fat stores are low in both ethnic groups. Neither of these two predictions was confirmed. South Asians had lower insulin sensitivity (ISI) than Europeans at all levels of overall adiposity, even when lean. Central obesity did not account statistically for the ethnic difference in insulin sensitivity. Our finding that the ethnic difference in insulin sensitivity was statistically significant only in men, while the ethnic difference in VFA was statistically significant only in women is not consistent with the hypothesis that central obesity has a primary role in the ethnic difference in insulin sensitivity.

However, when insulin level was used as the measure of insulin sensitivity there was an interaction between ethnicity and VFA. This suggests that lean individuals of either ethnic group had low insulin levels, but as visceral obesity increased, South Asians had significantly higher insulin levels than Europeans. This was shown clearly by the crossing over or diverging of the regression lines (in Europeans and South Asians) in the graphs of the relationship between insulin concentration and measures of central obesity (figures 7 – 9 and 11 – 13) as well as by the differences in the correlation coefficients of the relation between insulin concentration and measures of central obesity (especially in men; Table 9). This difference in finding with insulin concentration versus ISI as the outcome measure could be due to reasons discussed before in section 6.3.2, and has also been further addressed in chapter 10 (section 10.3.6.1). The finding of greater increases among South Asians in insulin concentration with increasing levels of central adiposity is of potential

public health importance. This finding suggests that that in South Asians, even more so than in Europeans, efforts to control central adiposity should form the focus of efforts to reduce insulin resistance (as indexed by insulin concentration), and hence CHD. This subject is further discussed in section 10.5.

Even though central (visceral) obesity did not account for the *ethnic difference* in insulin sensitivity in this study, nonetheless it was strongly and independently associated with insulin sensitivity in each ethnic group. The mechanism by which accumulation of fat in visceral depots is associated with insulin resistance is not fully understood. It is postulated that greater lipolytic activity of centrally located adipocytes contributes to portal venous elevations of NEFA, because omental fat cells *in vitro* are resistant to insulin-mediated suppression of lipolysis²³³. Elevated NEFA decrease hepatic insulin extraction²³⁴ and promote synthesis of apolipoprotein B lipoproteins^{46,95,95,235,235}. Thus NEFA derived from visceral fat might selectively potentiate peripheral hyperinsulinaemia and dyslipidaemia²³⁶. Recent evidence suggests that resistance to insulin-mediated glucose uptake is strongly related to the level of triglyceride stores in muscle cells^{208,209}. Triglyceride levels in muscle are likely to depend upon plasma triglyceride levels, which in turn are highly correlated with central adiposity.

6.3.4. The role of postprandial triglyceride in the relation between insulin sensitivity and obesity

Triglyceride levels at 8h after a fat load in this study were strongly correlated with measures of central adiposity including waist/hip ratio and VFA. This relationship is what we would expect to observe: the accumulation of fat in intra-abdominal depots could lead to a sustained production of VLDL and an impaired clearance of triglyceride-rich lipoproteins in the postprandial period^{147,237}. 8h triglyceride was also strongly associated with insulin sensitivity, and accounted statistically for the association between central adiposity and insulin sensitivity.

Both at 8h and in a smaller number at 6h, there was a higher postprandial triglyceride level in South Asian men versus European men, but there was no significant ethnic difference between women. This is probably because most of the women in this study were premenopausal, and as such are probably relatively “protected” from an adverse lipaemic response. In both ethnic groups the sex difference in 8h triglyceride level persisted when

adjusted for age, percent fat or SFA, but was abolished when adjusted for the sex difference in VFA. This suggests that the higher level of visceral fat in men compared with women is related to their more adverse postprandial lipaemic response. This argument is supported by the finding that when this sex difference in body fat distribution is taken into account, no sex difference in lipaemic response remains. This has been shown before for sex differences in fasting triglyceride level²³⁸, but never before to our knowledge, for sex differences in postprandial triglyceride.

We have shown that postprandial triglyceride level was, independently of fasting triglyceride level, associated with the metabolic syndrome or the insulin resistance syndrome. Thus we propose that postprandial triglyceride forms part of the cluster of CHD risk factors associated with the metabolic syndrome in both Europeans and South Asians. This is a new finding in South Asians, not previously reported. Although in people of European descent it has been suggested previously that elevated postprandial triglyceride level is part of the cluster of features that form the metabolic syndrome^{144,146,147}, it is still not widely accepted, and it remains controversial whether this association is independent of fasting triglyceride level. Some groups have failed altogether to find an association between insulin resistance (or insulin level) and postprandial triglyceride^{133,145}. In a recent study in men, Couillard *et al*¹⁴⁷ showed that visceral obesity is associated with an impaired postprandial triglyceride clearance, and that visceral obesity may contribute to fasting and postprandial hypertriglyceridaemia by altering NEFA metabolism in the postprandial state. They reported that features of the insulin resistance syndrome were all significant correlates of an impaired postprandial TGRL clearance.

However, in the current study even after adjusting for central obesity and triglyceride level (fasting and 8h), there remained a significant ethnic difference in insulin sensitivity. For this epidemiological study, rather than undertake a full fat tolerance test with hourly blood samples, we took a single post-load sample at 8h, plus a 6h measurement in a sub-sample. This was based on previous studies showing that a single measurement at 8 h correlates with the extent of coronary disease at angiography¹³¹. As the ethnic differences in postprandial triglyceride level were greater at 6h than at 8h, it is possible that we have underestimated the ethnic difference in postprandial triglyceride level by relying on a single measurement at 8h in most participants. If so, then it is possible that we have also underestimated the extent to which differences in postprandial triglyceride levels could account for the ethnic difference in insulin sensitivity. From the limited information that

we have, it looks as if there may be ethnic differences in postprandial triglyceride earlier in the fat tolerance test that are not detectable at 8 h, at least not with total triglyceride.

There may have been subtle differences in fractions of the postprandial response that were possibly not evident in the measurement of total triglyceride level alone. We wanted to measure apoB-48 and apoB-100, as well as plasma retinyl palmitate in chylomicron and non-chylomicron plasma fractions to see whether there were ethnic differences in the exogenous (dietary; apoB-48 and retinyl palmitate) and/or endogenous (hepatic derived; apoB-100) levels of triglyceride. However, although samples were collected, due to technical problems and lack of a laboratory that was able to measure these for us on a collaborative basis, we were unable to do so. This remains a limitation of this part of the study. As a compromise we did measure triglyceride level in the chylomicron and non-chylomicron fractions of the plasma at 8 hours, but found there to be no ethnic difference in these. However the validity of the data obtained for triglyceride concentration in the two fractions is open to question, as there were technical problems associated with the preparation in the laboratory.

In conclusion, our results suggest that the greater resistance to insulin-mediated glucose uptake in South Asians compared with Europeans cannot be fully accounted for by differences in body fat pattern, or in plasma triglyceride levels. In both ethnic groups the relationships of insulin sensitivity to visceral fat were eliminated by adjusting for postprandial triglyceride level, suggesting that an underlying alteration of lipid metabolism may be responsible for the relationship between these variables. Ethnic differences in plasma lipids appear to be larger in the postprandial state than in the fasting state, and this may be relevant to the excess risk of CHD in South Asians compared with other groups.

CHAPTER 7: The relation between insulin sensitivity, body fat pattern and intramyocellular lipid content

7.1. Presentation of results

7.1.1. Basic characteristics and general associations of IMCL

Mean (\pm SEM) IMCL content (measured with creatine as the internal standard) was higher in South Asians than in Europeans [72.1 ± 7.5 vs. 53.6 ± 4.9 mmol/kg dry weight respectively, $p=0.046$]. The clinical and metabolic measurements of the participants, and their correlation with IMCL are summarised in Table 15.

South Asian men had significantly higher percent body fat and insulin resistance than European men. In Europeans IMCL was positively correlated with indices of generalised obesity and central obesity (Table 15), and inversely correlated with ISI (Figure 14). In South Asians IMCL was not significantly correlated with any measured variable. European men smoked more and had a higher mean walking score than South Asians, though both groups took little exercise. The distribution of ISI by BMI in the two groups is shown in Figure 15.

One possible reason for the lack of association of IMCL with the measured obesity and metabolic variables among South Asian men could be because of one South Asian “outlier”, who had very low IMCL and low ISI (figure 14). Thus the analysis was repeated with the exclusion of this outlier. This did not make any difference to the observed associations. In particular the slope (beta coefficient) of the ISI/IMCL association in South Asian men was $+0.05$ ($p = 0.891$) with all men, and -0.08 ($p = 0.875$) with the outlier excluded. The correlation coefficient (r) for this association changed from $+0.03$ ($p = 0.891$) to -0.04 ($p = 0.875$) with the exclusion of the outlier.

Table 15: Mean values of clinical and metabolic variables and Pearson's correlation coefficients (r) with intramyocellular lipid in men by ethnic group

	European (n =20)			South Asian (n =20)			p ^b
	Mean*	r	p ^a	Mean*	r	p ^a	
Age (years)	47.8 ± 1.0	0.45	0.048	46.3 ± 1.1	0.01	NS	NS
BMI (kg/m ²)	26.4 ± 0.8	0.72	<0.001	26.3 ± 0.6	0.25	NS	NS
% body fat	22.5 ± 1.7	0.49	0.028	26.8 ± 1.3	0.31	NS	0.052
WHR	0.91 ± 0.01	0.74	<0.001	0.92 ± 0.01	0.25	NS	NS
Visceral fat area* (cm ²)	113.5 [87- 148]	0.62	0.004	137.2 [115 - 164]	0.14	NS	NS
Fasting glucose (mmol/l)	5.3 ± 0.1	0.10	NS	5.4 ± 0.1	-0.20	NS	NS
2h glucose (mmol/l)	4.9 ± 0.2	-0.12	NS	5.9 ± 0.4	-0.08	NS	0.022
Fasting insulin* (μU /ml)	4.9 [3.7 - 6.5]	0.37	NS	9.7 [7.2 - 13.1]	-0.17	NS	0.003
2h insulin* (μU/ml)	10.3 [6.9 - 15.6]	0.15	NS	51.4 [31.6- 83.7]	-0.09	NS	<0.001
ISI (%/min)	3.4 ± 0.3	-0.53	0.016	2.4 ± 0.2	0.03	NS	0.013
Fasting triglyceride* (mmol/l)	1.46 [1.2 - 1.8]	0.13	NS	1.60 [1.3 - 2.0]	-0.08	NS	NS
Fasting NEFA* (mmol/l)	0.29 [0.24 - 0.37]	0.04	NS	0.34 [0.28 - 0.41]	-0.19	NS	NS
2h NEFA* (mmol/l)	0.06 [0.04 - 0.10]	0.01	NS	0.05 [0.04 - 0.08]	0.03	NS	NS
Physical activity (1-5)	2.2 ± 0.2	-0.33	NS	2.0 ± 0.2	-0.15	NS	NS
Walking score (1-4)	3.0 ± 0.1	-0.08	NS	2.2 ± 0.2	0.04	NS	0.009
Active in sport (%)	25	0.02	NS	25	-0.03	NS	NS
Ever smoker (%)	55	0.37	NS	10	-0.12	NS	0.002

Arithmetic means and SEM are given except where the variable has been log transformed (*), where geometric means and 95% CIs are shown. ^a: significance level associated with r; ^b: p value for ethnic difference in mean values; Exact p values are quoted for p<0.10. Ever smoker = past or current smoker

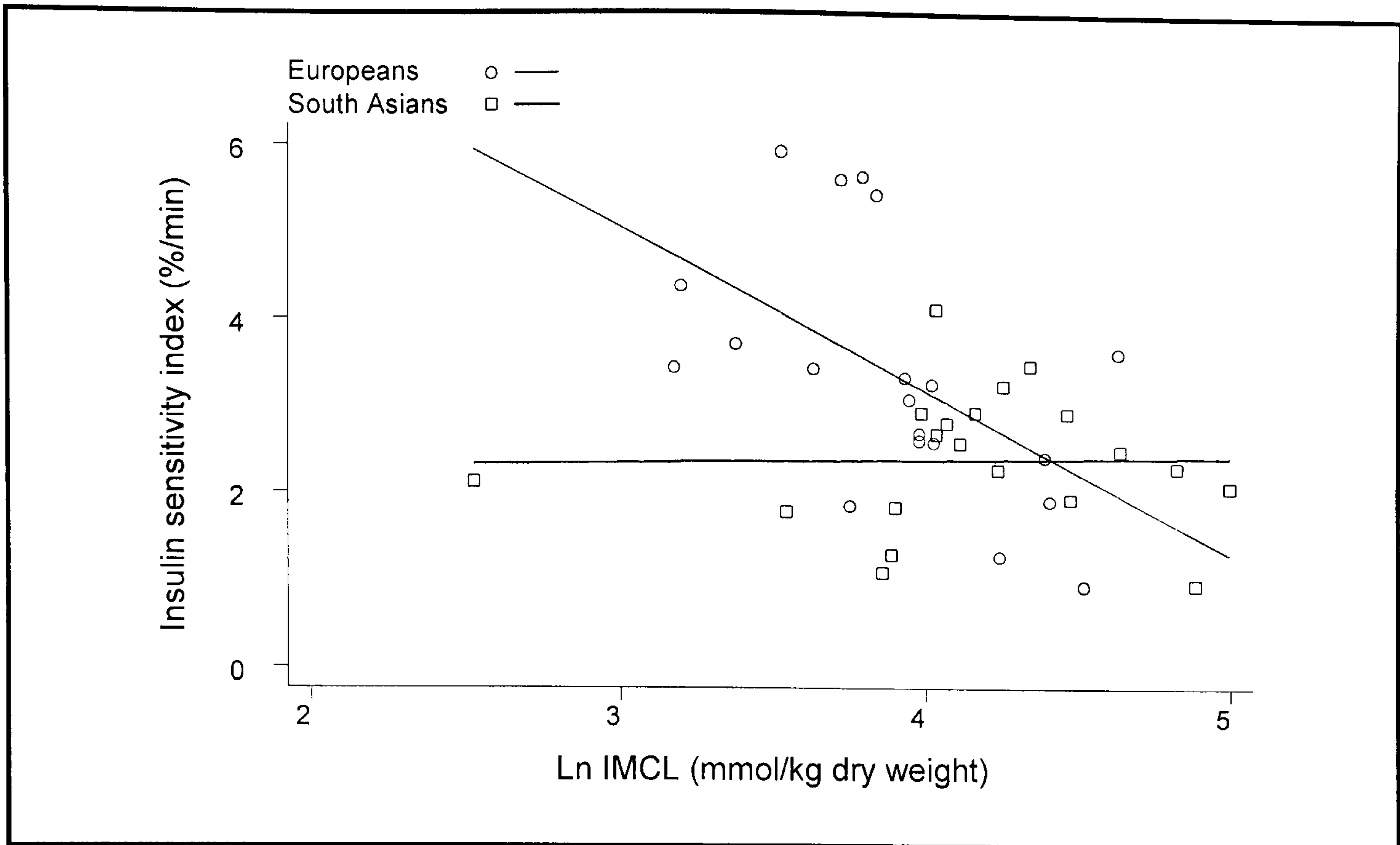


Figure 14: Relationship between insulin sensitivity index and intramyocellular lipid content in 40 European and South Asian men

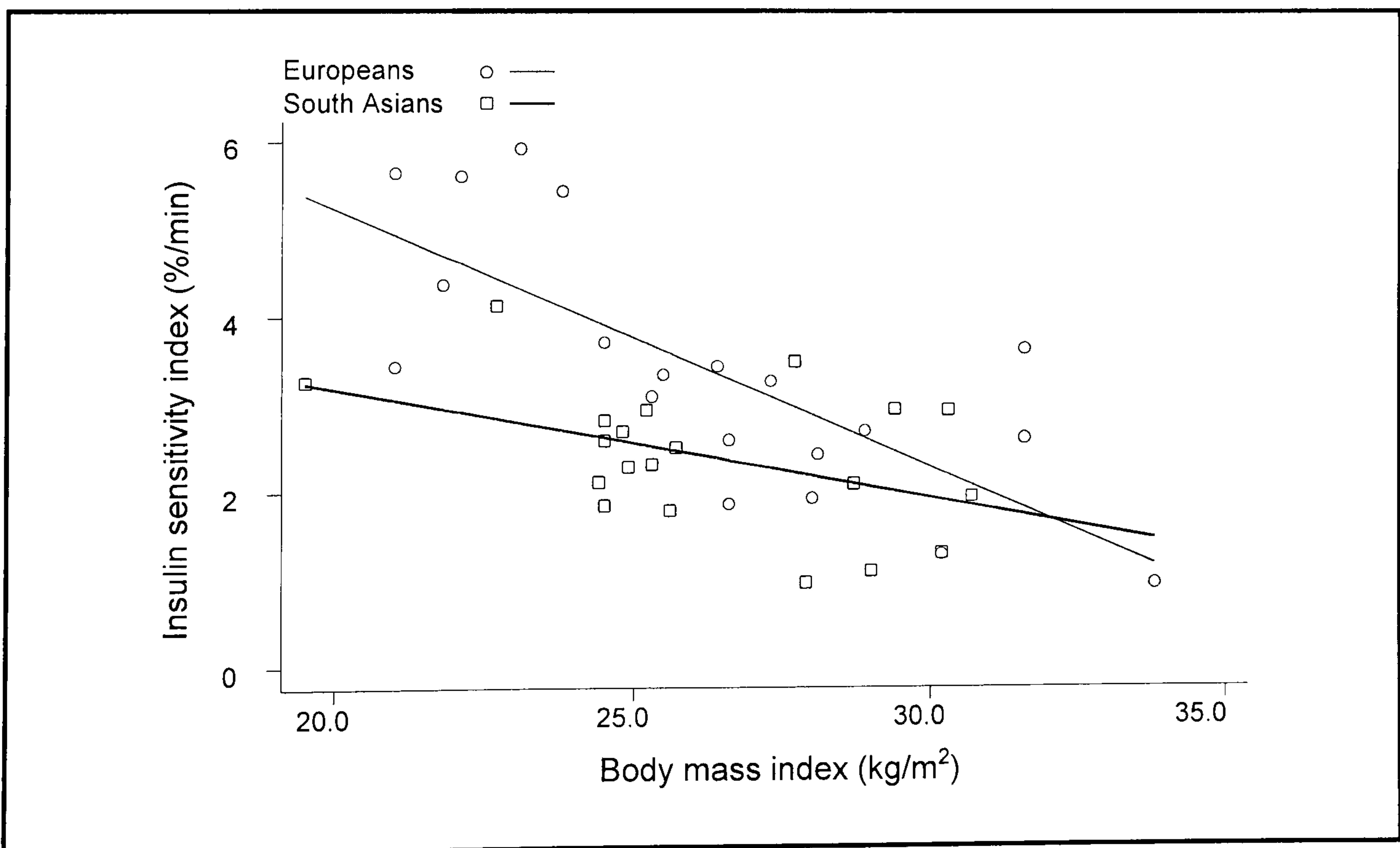


Figure 15: Relationship between insulin sensitivity index and BMI in 40 European and South Asian men

When water was used as the internal standard instead of creatine, the results were similar but associations of IMCL content with ethnic origin, obesity, insulin sensitivity and lipids were generally weaker.

7.1.2. Relation between IMCL, insulin sensitivity, obesity, and lipids

The relation of ISI to obesity, plasma lipids and IMCL was examined in each ethnic group separately. All analyses were adjusted for age, and standardised regression coefficients are presented (Table 16). The strongest (age adjusted) associations of ISI in Europeans were with: generalised obesity [percent fat and BMI], central obesity [VFA, WHR and waist girth], and IMCL. There was no significant association of ISI with plasma lipids among Europeans. Among South Asians ISI was significantly associated with plasma fasting and 8h triglyceride, and central obesity measured anthropometrically (WHR and waist girth). However ISI in South Asians was not significantly associated with VFA, IMCL, BMI or percent fat. These results were unchanged when the analyses were repeated with the exclusion of one South Asian “outlier” (figure 14).

Table 16: Age adjusted univariate regressions of insulin sensitivity index on measures of obesity, plasma lipids and IMCL content in men by ethnic group

	European men			South Asian men		
	β	se	p value	β	se	p value
VFA* (cm ²)	-0.79	0.25	0.005	-0.41	0.25	0.128
WHR	-1.01	0.44	0.034	-0.74	0.30	0.025
Waist girth (cm)	-1.09	0.28	0.001	-0.41	0.20	0.063
BMI (kg/m ²)	-1.03	0.25	0.001	-0.43	0.22	0.066
percent fat	-1.52	0.33	<0.001	-0.04	0.32	NS
Fasting TG* (mmol/l)	0.22	0.29	NS	-0.72	0.17	<0.001
8h TG* (mmol/l)	-0.12	0.32	NS	-0.67	0.17	0.001
Fasting NEFA* (mmol/l)	-0.21	0.32	NS	-0.16	0.24	NS
2h NEFA* (mmol/l)	-0.13	0.26	NS	-0.13	0.25	NS
IMCL* (mmol/kg dry wt)	-0.80	0.39	0.058	0.02	0.17	NS

*: log (natural) transformed values of these variables were used; TG = triglyceride; Standardised regression coefficient: β

Analyses with fasting and 2h insulin as the dependent variables showed a similar pattern with strong associations with central obesity (WHR, waist girth and VFA), triglyceride level (0h and 8h), and additionally BMI in South Asians. In Europeans the most significant association was with percent fat, but not with central obesity.

In multiple regression analyses we examined whether the association between insulin sensitivity and central obesity, seen in both European and South Asian men, persisted when adjusted for IMCL. In age-adjusted analyses in Europeans the significant association between ISI and VFA, and ISI and percent fat persisted even after adjustment for IMCL, in contrast to the association between ISI and WHR which was made non-significant when adjusted for IMCL (Table 17, model A). Among South Asian men the association between ISI and WHR remained significant when adjusted for IMCL. Among European men the significant association between IMCL and WHR, and IMCL and VFA persisted even after adjusting for fasting plasma triglyceride (Table 17, model B). The results with 8h triglyceride in the models were identical.

The significant relationship between ISI and IMCL in Europeans was independent of fasting and 2h glucose, insulin and NEFA, and plasma triglyceride. However, this association was no longer statistically significant when adjustment was made for generalised obesity [percent fat: $\beta = -0.40$, $p = 0.199$; BMI: $\beta = 0.03$, $p = 0.941$] or for central obesity [VFA: $\beta = -0.29$, $p = 0.493$; WHR: $\beta = -0.37$, $p = 0.494$].

There were fewer smokers among South Asians (Table 15). Adjusting for smoking did not make a difference to the relations between insulin sensitivity, central obesity and IMCL. The level of physical activity reported were low in both groups, and adjusting for it did not alter the reported results.

Table 17: Effect of (A) adjusting for IMCL on association between insulin sensitivity (ISI) and obesity, and of (B) adjusting for plasma triglyceride on association between IMCL and obesity, in men by ethnic group

	European			South Asian		
	β^a	se	p	β^a	se	p
(A) insulin sensitivity index as dependent variable						
	Slope of relationship with WHR					
age, whr	-1.01	0.44	0.034	-0.74	0.30	0.025
age, whr, IMCL*	-0.72	0.61	0.256	-0.80	0.32	0.022
	Slope of relationship with VFA					
age, VFA*	-0.79	0.25	0.005	-0.41	0.25	0.128
age, VFA*, IMCL*	-0.68	0.29	0.035	-0.42	0.26	0.129
	Slope of relationship with percent body fat					
age, %fat	-1.52	0.33	<0.001	-0.04	0.19	0.909
age, %fat, IMCL*	-1.38	0.34	0.001	-0.06	0.34	0.873
(B) IMCL as dependent variable						
	Slope of relationship with WHR					
age, whr	0.38	0.10	0.001	0.25	0.23	0.299
age, whr, plasma fasting TG*	0.36	0.11	0.006	0.32	0.26	0.242
	Slope of relationship with VFA					
age, VFA*	0.19	0.07	0.019	0.11	0.18	0.543
age, VFA*, plasma fasting TG*	0.23	0.10	0.043	0.09	0.19	0.636
	Slope of relationship with percent body fat					
age, %fat	0.18	0.13	0.174	0.28	0.20	0.188
age, %fat, plasma fasting TG*	0.11	0.14	0.441	0.17	0.24	0.484

^a β = standardised regression coefficient; *:log (natural transformed) values used; TG = triglyceride

7.2. Summary of results

1. South Asian men compared with European men had higher mean percent fat and lower insulin sensitivity.
2. Mean intramyocellular lipid content was higher in South Asian men than in European men.
3. In Europeans IMCL was strongly correlated with percent fat, WHR, visceral fat and insulin sensitivity. In South Asians IMCL was not significantly related to insulin sensitivity or obesity, and the strongest associations of insulin sensitivity were with plasma triglyceride (fasting and 8h) and WHR.
4. In Europeans the relation between IMCL and ISI was independent of plasma glucose, insulin or lipid levels, but not independent of generalised or central obesity.

7.3. Discussion of results

Our findings in Europeans are consistent with the hypothesis that IMCL content modulates insulin sensitivity. We found, like others²⁰⁸, that the relation in Europeans between ISI and IMCL was independent of fasting and 2h glucose, insulin and NEFA, and triglyceride level. However, in contrast to other studies where insulin sensitivity was correlated with IMCL independently of BMI in Europeans²⁰⁸ and independently of BMI, percent fat or waist/thigh ratio in Pima Indians²⁰², we found that the relation between ISI and IMCL was not independent of obesity and was not stronger than the relation between ISI and obesity²⁰⁹. This was the case whether we used creatine or water as the internal standard in ¹H-NMR. The reason for these differences is unclear, but it is unlikely to be related to data acquisition given that others used similar localisation techniques and have reported similar reproducibility^{208,209}. Likely possibilities may relate to differences in the population sample, and the larger sample size of our study.

Although in South Asian men IMCL content was higher than in Europeans, IMCL was not significantly related to ISI or indices of obesity. The strongest associations of ISI were with WHR and plasma triglyceride (fasting and 8h) in South Asians. These results are not

easily reconciled with the simple hypothesis that elevated IMCL content mediates the associations of central obesity and raised triglyceride with insulin resistance in South Asians. Phillips *et al*²⁰⁰ have shown that IMCL measured in biopsy specimens of gastrocnemius muscle was inversely associated with glycogen synthase (an insulin regulated enzyme that is rate limiting for insulin action in muscle), but not with insulin sensitivity measured by the short insulin tolerance test. However in our study we found an association between IMCL and ISI in Europeans but not in South Asians. Other physiological mechanisms may mediate impairment of insulin sensitivity in South Asians. For instance increased lipolysis of IMCL stores (modulated by hormone-sensitive lipase) or increased supply of triglyceride from plasma could cause insulin resistance. The strong correlation between plasma triglyceride and ISI observed in South Asians in this study is consistent with this. This may also account for our failure to detect the usual association of insulin resistance with obesity in South Asians in this small sample.

We would expect to see a close relationship between central obesity and IMCL content because VLDL triglyceride is likely to be one of the main sources of triglyceride in muscle, and the main substrate for hepatic VLDL triglyceride production is NEFA derived from lipolysis of fat stores drained by the portal vein^{46,95,235}. Because lipolysis of lipid stores in skeletal muscle is not easily suppressed by insulin, the NEFA derived from triglyceride stores would compete with glucose for oxidative metabolism and reduce insulin-mediated glucose uptake via the glucose fatty-acid cycle.

In summary, we found a negative correlation between IMCL and insulin sensitivity in Europeans but not in South Asians. To establish definitively whether insulin resistance depends upon muscle cell triglyceride stores will require experimental studies of the effects of dietary and pharmacological interventions that alter muscle triglyceride stores on insulin-mediated glucose uptake. This could open up possibilities for developing new measures to prevent and control insulin resistance and NIDDM.

CHAPTER 8: Validation and reproducibility of bio-electrical impedance analysis to measure percent body fat

8.1. Presentation of results

8.1.1. Validation of the use of bio-electrical impedance (BIA)

The correlation coefficients of the relation between percent fat measured by DEXA scan and percent fat measured by BIA in each of the 4 sex and ethnic groups were as follows (Table 18):

Table 18: Product moment correlation coefficients (r) between %fat by DEXA and BIA

	n	r	p value
South Asian men	26	0.77	<0.0001
South Asian women	30	0.85	<0.0001
European men	31	0.86	<0.0001
European women	31	0.81	<0.0001

The relationship between BIA and DEXA measurements of percent fat in European and South Asian men and women is shown in Figure 16 and Figure 17 respectively.

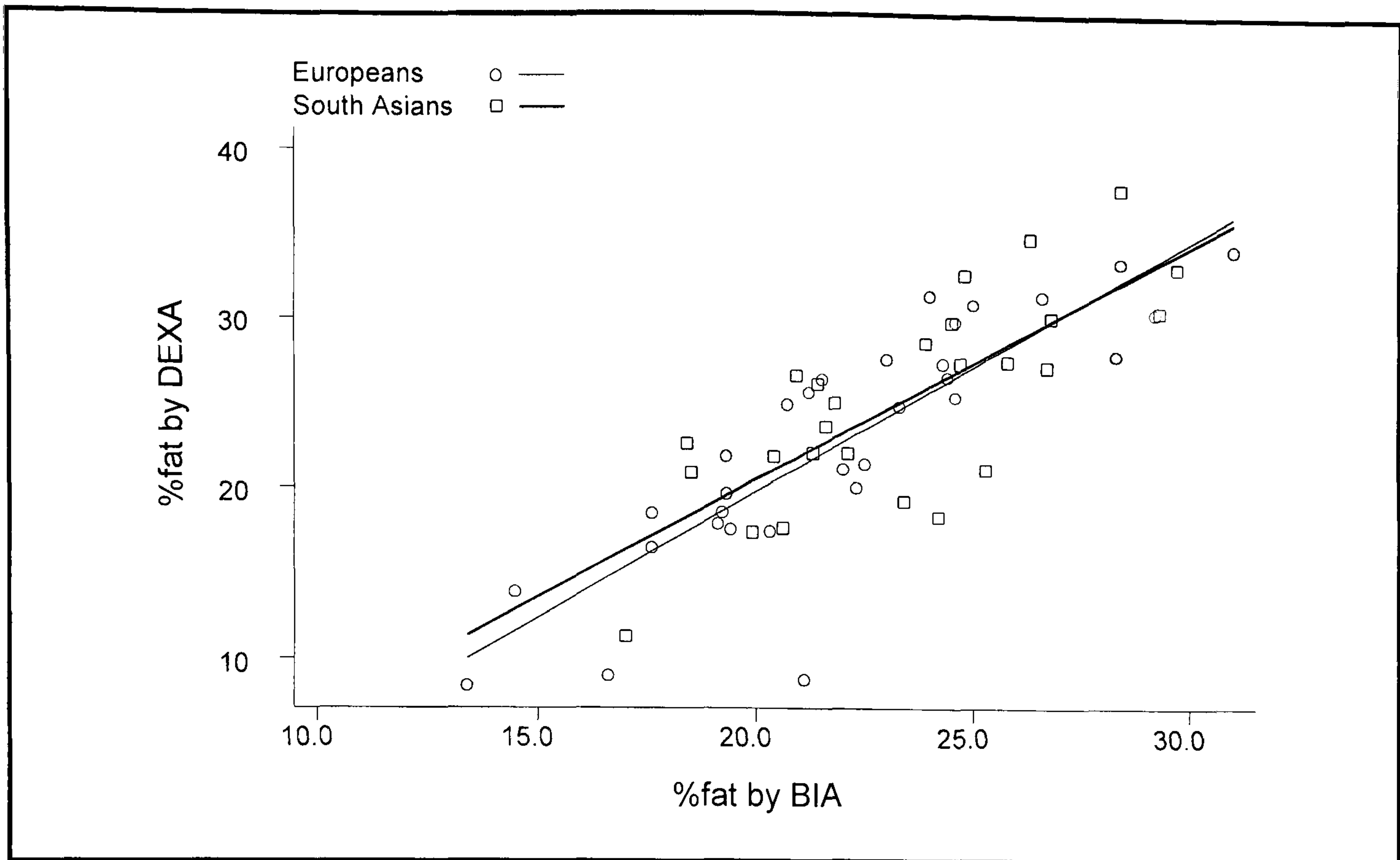


Figure 16: Relationship between measurement of %fat by DEXA and bio-electrical impedance analysis in European and South Asian men

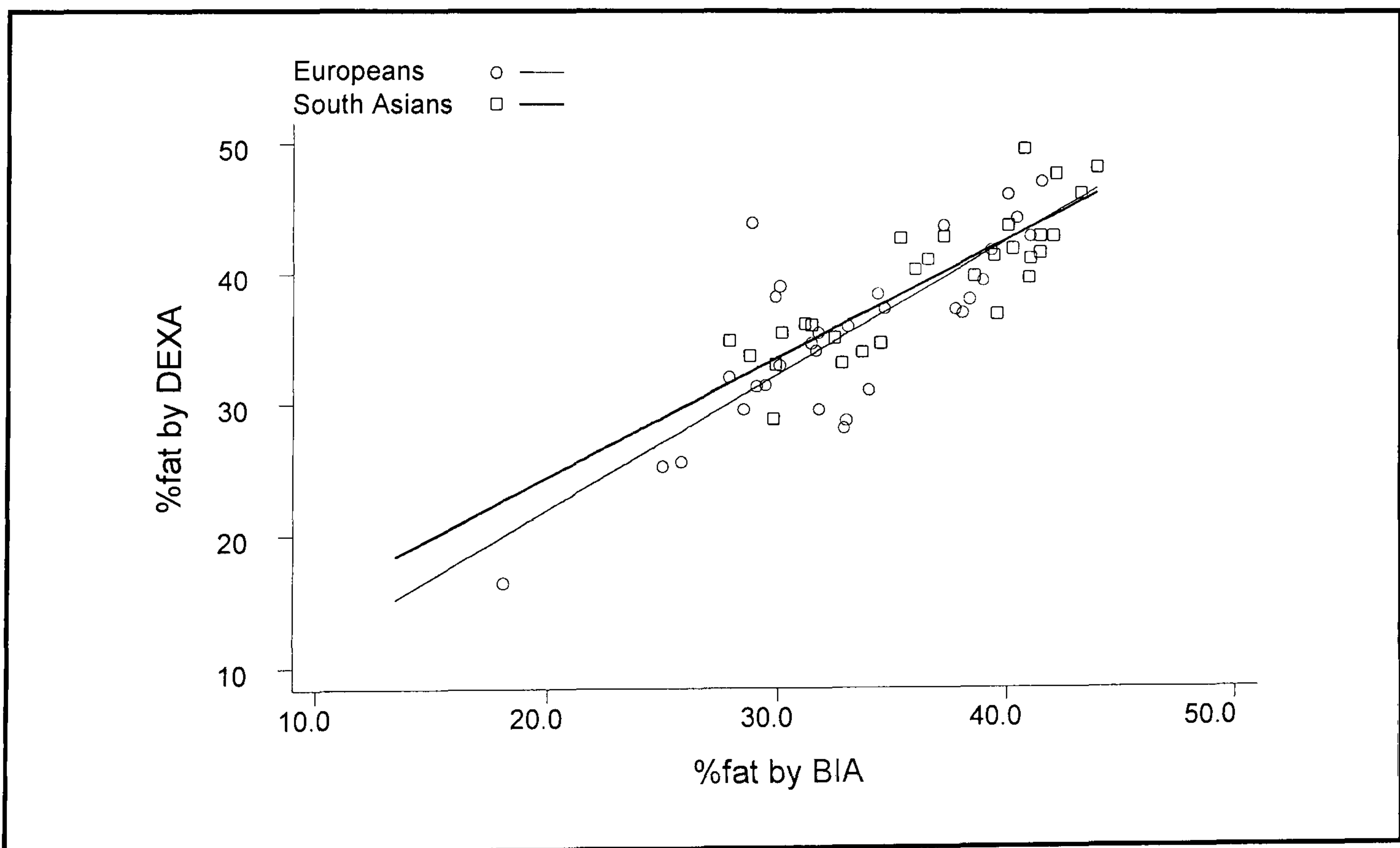


Figure 17: Relationship between measurement of %fat by DEXA and bio-electrical impedance analysis in European and South Asian women

The agreement between the two methods (DEXA and BIA) of measuring percent fat was calculated using the method of Bland and Altman²³⁹. The results of this calculation which yielded the lower and upper limits of agreement between the two methods are shown in Table 19.

Table 19: Agreement between the DEXA and BIA measures of %fat

Group	N	Mean of differences	sd of differences	Agreement: lower limit	Agreement: upper limit
All South Asians	56	2.43	2.99	-3.56	8.42
All Europeans	62	1.57	3.12	-4.67	7.81
S. Asian men	26	1.90	3.18	-4.47	8.26
S. Asian women	30	2.89	2.82	-2.75	8.53
European men	31	0.86	2.98	-5.11	6.82
European women	31	2.28	3.25	-4.23	8.79

8.1.2. Reproducibility of BIA

Additionally in 87 people we were also able to repeat the BIA measurement of percent fat during the visit for DEXA scan. There was very strong correlation between percent fat measured at the two separate visits (Table 20).

Table 20: Product moment correlation coefficients (r) between %fat at two separate visits (measured by BIA)

	n	r	p value
South Asian men	22	0.91	<0.0001
South Asian women	21	0.98	<0.0001
European men	23	0.96	<0.0001
European women	21	0.73	0.0002

The strong correlation between the two visits for BIA-percent fat measurement in European and South Asian men and women is shown in Figure 18 and Figure 19 respectively.

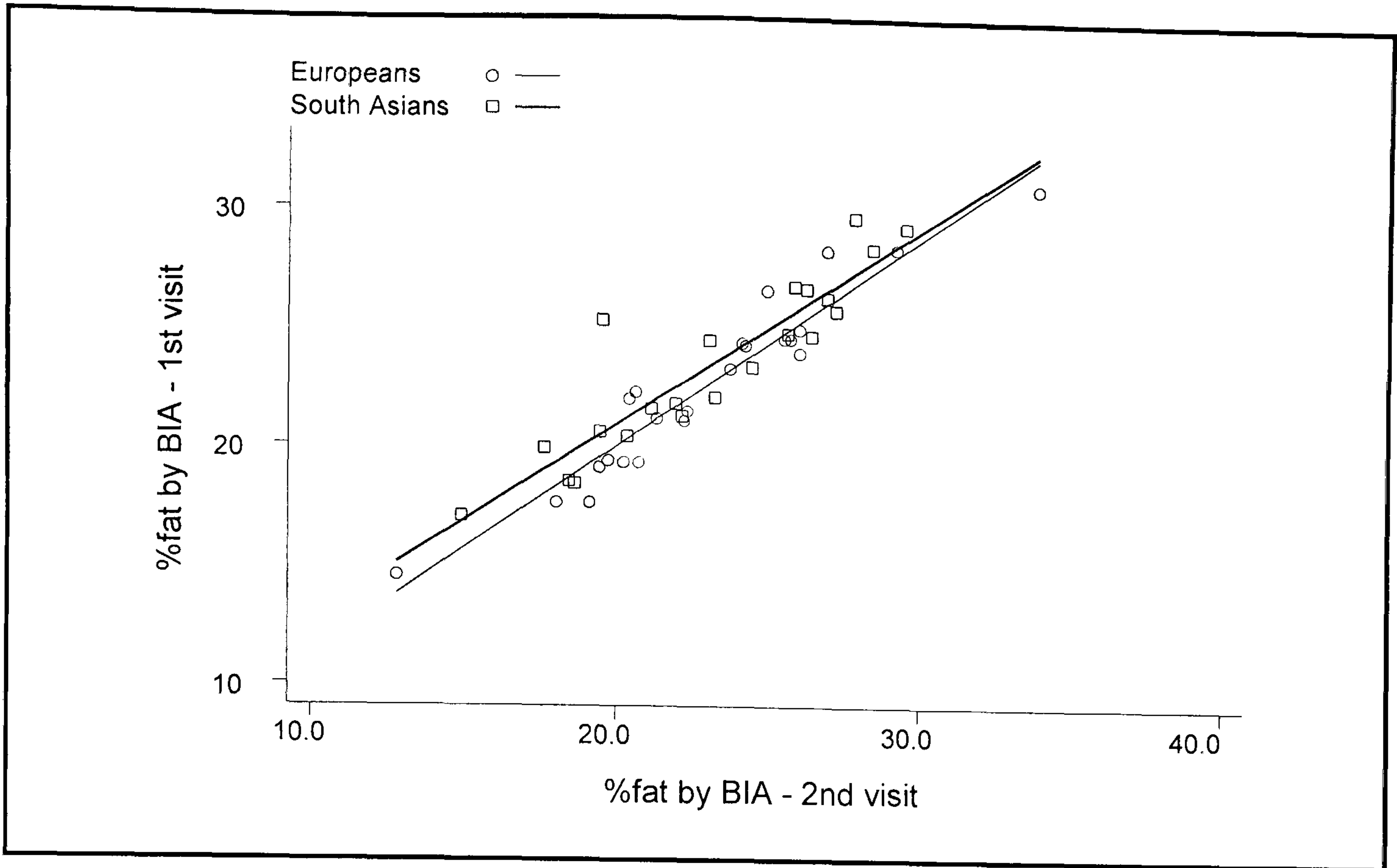


Figure 18: Relationship between two repeat measurements of %fat by bio-electrical impedance analysis in European and South Asian men

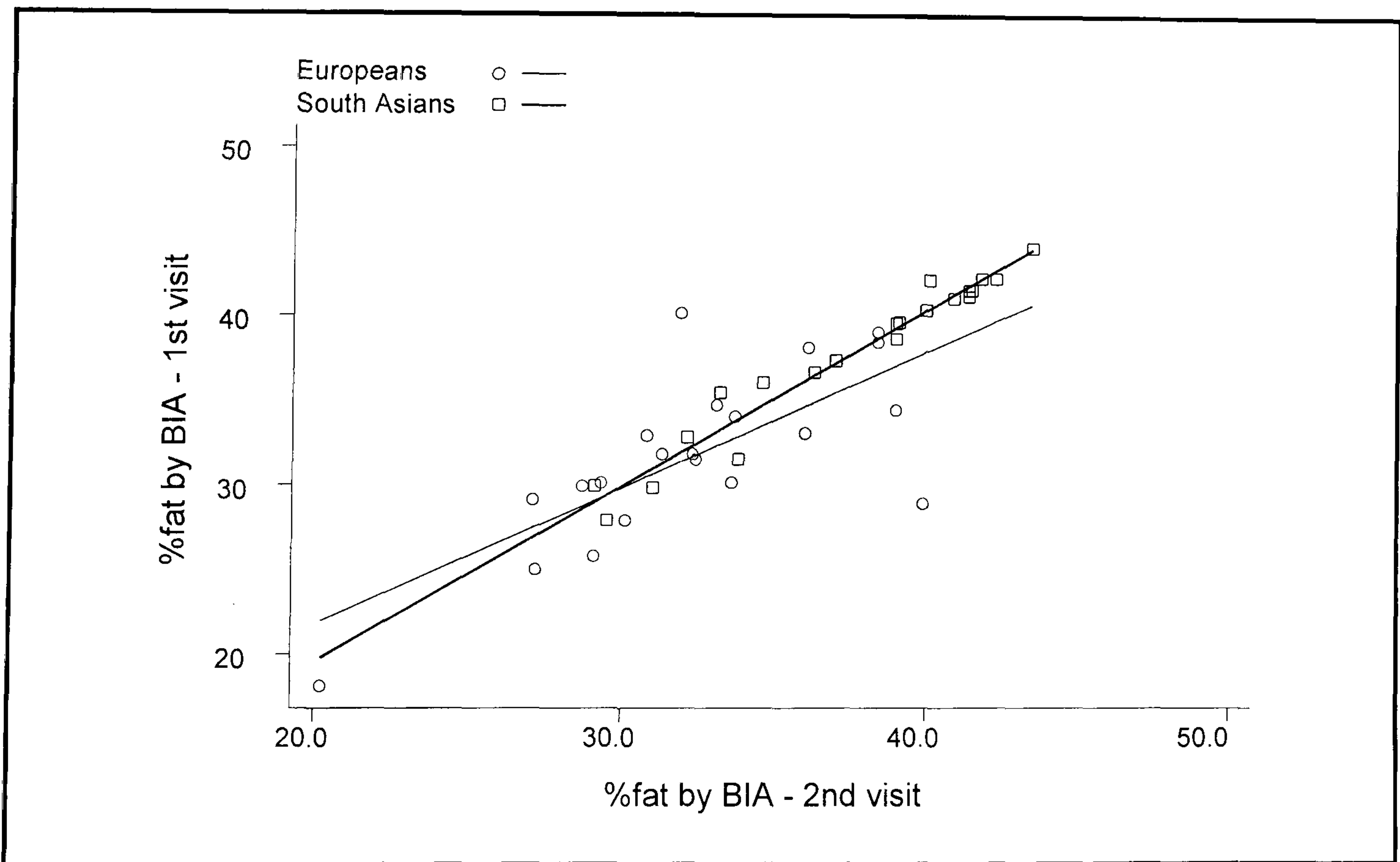


Figure 19: Relationship between two repeat measurements of %fat by bio-electrical impedance analysis in European and South Asian women

To assess the reproducibility of percent fat measurement by BIA, the coefficient of variation for between-visits was calculated for each group, using the method of Bland²⁴⁰. The coefficients of variation for two repeat measurements of percent fat by BIA in 87 subjects were as follows: 5.0% in South Asian men, 2.0% in South Asian women, 3.9% in European men and 8.0% in European women. The repeatability coefficients as defined by the British Standards Institution^{240,241} were 3.3 and 2.0 in South Asian men and women respectively, and 2.5 and 7.2 in European men and women respectively. The reliability coefficient represents the 2 x sd (of differences), the value below which the difference between two measurements will lie with a probability of 0.95²⁴¹.

8.2. Summary of results

We have shown that BIA is a valid and reliable (reproducible) method for measuring percent body fat in both South Asians and Europeans. We have validated the use of BIA against DEXA scan.

8.3. Discussion of results

BIA is now an accepted method for measuring percent body fat. Although single frequency BIA has been validated against DEXA for use in people of European descent^{223,225}, its validity against DEXA scans in South Asians has never previously been established.

Since the design and conduct of the current study, one group from India has published results for the validation of BIA against hydrodensitometry²⁴². They found that BIA estimates of percent fat were higher than those from hydrodensitometry, which they argued could be attributable to the use of inappropriate regression equations derived from studies in people of European descent. They developed their own predictive equation for the derivation of fat-free mass, using the variables of height²/impedance and fat free mass by under-water weighing. The use of this equation in BIA gave good agreement between BIA and hydrodensitometry method. We were unable to do this as the company that

manufactures the Bodystat impedance meters was unwilling to release the components of the equations it uses in computing the final formula for percent body fat or for impedance.

Single frequency bio-electrical impedance analysis can be used as an epidemiological “tool” to measure percent fat in studies of South Asians. It offers a cheap, quick and acceptable method to measure percent fat, which can be performed at the bedside.

CHAPTER 9: Relation of inflammation with risk for CHD

During the course of this study evidence started to emerge for a possible link between markers of inflammation (C-reactive protein level) and CHD. Opportunistically a collaboration also became possible (Appendix) which enabled measurement of C-reactive protein (CRP) on frozen plasma from this study. Spare aliquots of plasma that were kept frozen at -70°C from the visit for fat tolerance test (n=113) were used for this measurement. The background and rationale to this measurement is explained below.

9.1. The evidence for a link between inflammation and CHD

C-reactive protein (CRP) is an acute phase reactant which is a measurable marker of inflammation in the body. There is a growing body of evidence that low grade chronic inflammation may be associated with the development of cardiovascular disease²⁴³⁻²⁴⁶. A summary of published studies examining the association between CRP and cardiovascular disease has recently been given by Lagrand *et al*¹²². Mild elevations of CRP concentrations even when within the clinically 'normal' range are predictive of future cardiovascular events. This association holds true for individuals with multiple risk factors²⁴⁷, those with stable or unstable angina²⁴⁵, and in men with favourable coronary risk factor profiles²⁴³. More recent data also link plasma CRP concentrations to the risk of developing peripheral arterial disease²⁴⁸. New evidence published from the ARIC study (Atherosclerosis Risk in Communities) has also linked inflammation to the development of diabetes mellitus, in a population free of diabetes at baseline²⁴⁹.

Whether inflammation also contributes to this increased CHD risk in South Asians is not known as no studies comparing CRP concentrations in South Asians and other groups have been published. The only inflammatory marker to have been studied in an ethnic comparison so far is plasma fibrinogen, which has been found to be no higher in South Asians than in Europeans^{28,33}. It is certainly plausible that inflammatory markers such as CRP might be elevated in South Asians compared with Europeans, given the greater degree of total fat mass in South Asians, and the suggested link between obesity and CRP level. The latter is thought to occur through increased production of CRP by hepatocytes

in response to elevated interleukin-6 cytokine, which in turn is elevated through increased production of TNF-alpha²⁵⁰, which is overproduced by adipocytes²⁵¹.

Thus we measured CRP levels with the following aims: to test (i) whether CRP concentrations were elevated in South Asians compared with Europeans, (ii) whether CRP was associated with insulin sensitivity measured directly by the short insulin test, and indirectly by insulin level, and (iii) whether there was an association between CRP level and body fat distribution and lipid levels (fasting and postprandial) in the two ethnic groups.

9.2. Measurement of plasma CRP

C-reactive protein was measured using a sensitive double antibody sandwich ELISA with rabbit antihuman C-reactive protein and peroxidase conjugated rabbit anti-human C-reactive protein. The assay was linear up to 5mg/l and logarithmic thereafter. The interassay and intra-assay coefficients of variation were less than 10% across the range of measured results²⁵².

9.3. Presentation of results

Among women median CRP level was higher among South Asians [1.35 mg/l, (interquartile range 0.72, 3.04)] vs. Europeans [0.70 mg/l, (0.41, 1.70)], $p=0.050$. This was despite the absence of any smokers among South Asian women. Although the median CRP level was about 15% higher in South Asian men [1.07 mg/l, (0.76, 1.50)] compared with European men [0.92 mg/l, (0.34, 1.61)], this was not statistically significantly different. This is reflective of the lack of power to examine sub-group differences in this study with relatively small numbers. Only 3 people had CRP level greater than 10 mg/l, the cut point used in clinical practice to identify inflammation. There was a trend towards a significant ethnic difference in the CRP level in analysis adjusted for age, sex and smoking [$\beta=0.45$, $se=0.25$, $p=0.076$].

The age, sex and smoking adjusted standardised regression coefficients for the relation between CRP level and its determinants are shown in Table 21. CRP concentrations were generally more strongly associated with central obesity in South Asians but with both overall obesity and central obesity in Europeans. Social class was not significantly associated with CRP in either ethnic group in this study.

Table 21: Determinants of C reactive protein concentration among Europeans and South Asians.

	Europeans			South Asians		
	β	se	p	β	se	p
Age (years) ^a	0.35	0.18	0.053	0.21	0.12	0.087
BMI (kg/m ²)	0.49	0.17	0.005	0.26	0.13	0.058
Percent fat	0.55	0.24	0.026	0.34	0.20	0.096
Waist/hip ratio	0.37	0.26	NS	0.60	0.15	<0.001
Waist girth (cm)	0.49	0.21	0.027	0.44	0.13	0.002
Visceral fat area* (cm ²)	0.51	0.20	0.013	0.41	0.14	0.004

Exact p values are given for $p \leq 0.1$; *: log (ln) transformed. ^a: adjusted only for sex & smoking
Age, sex and smoking adjusted standardised regression coefficients are given for log (natural) C reactive protein as the dependent variable (per one standard deviation increase in explanatory variable).

The association of CHD risk factors with CRP concentration as the independent variable is shown in Table 22. In multiple regression analyses adjusted for age, sex and smoking, CRP level was significantly associated with insulin (0h and 2h) and triglyceride (0h and 8h) in both ethnic groups, and also with HDL-cholesterol (negative) and 0h glucose in Europeans, but not with ISI in either group. After additionally adjusting for percent fat, there remained an independent association of CRP level with fasting glucose and insulin and HDL-cholesterol in Europeans, and with triglyceride level in South Asians.

Table 22: Relationship between cardiovascular risk factors and CRP among Europeans and South Asians

Dependent variable		Europeans			South Asians		
		β	se	p	β	se	p
ISI (%/min)	<i>a</i>	-0.11	0.14	NS	-0.07	0.18	NS
	<i>b</i>	-0.04	0.15	NS	0.00	0.18	NS
Fasting insulin* ($\mu\text{u/ml}$)	<i>a</i>	0.14	0.05	0.005	0.12	0.10	NS
	<i>b</i>	0.11	0.05	0.023	0.07	0.10	NS
2h insulin* ($\mu\text{u/ml}$)	<i>a</i>	0.13	0.09	NS	0.33	0.15	0.029
	<i>b</i>	0.09	0.09	NS	0.26	0.14	0.085
Fasting glucose (mmol/l)	<i>a</i>	0.10	0.04	0.018	0.03	0.08	NS
	<i>b</i>	0.09	0.04	0.017	0.02	0.09	NS
2h glucose (mmol/l)	<i>a</i>	0.11	0.11	NS	0.38	0.25	NS
	<i>b</i>	0.10	0.11	NS	0.31	0.25	NS
Fasting triglyceride* (mmol/l)	<i>a</i>	0.07	0.05	NS	0.19	0.08	0.025
	<i>b</i>	0.05	0.05	NS	0.16	0.08	0.054
8h triglyceride* (mmol/l)	<i>a</i>	0.12	0.06	0.056	0.23	0.11	0.035
	<i>b</i>	0.11	0.07	NS	0.19	0.11	0.098
Total cholesterol (mmol/l)	<i>a</i>	0.10	0.11	NS	0.15	0.14	NS
	<i>b</i>	0.13	0.12	NS	0.17	0.14	NS
HDL-cholesterol* (mmol/l)	<i>a</i>	-0.07	0.03	0.013	0.01	0.03	NS
	<i>b</i>	-0.06	0.03	0.044	0.02	0.04	NS

log (natural) CRP as the independent variable with each measured cardiovascular risk factor as the dependent variable in turn. *a* = adjusted for age, sex and smoking; *b* = adjusted for age, sex, smoking and percent fat; * = log (natural) transformed values of these variables were used.

To account for potential residual confounding when adjusting for the effect of smoking we repeated the analyses among ever-smokers and never-smokers separately. In analyses adjusted for age and sex, among ever-smokers (n=41) there was no significant ethnic difference in CRP level [$\beta=0.06$, se=0.55, p=0.913]. In similar analyses among never-smokers (n=72) South Asians had higher CRP levels than Europeans [β for ethnic difference 0.60, se=0.28, p=0.039]. With additional adjustment for triglyceride level (0 and 8h) and BMI the ethnic difference persisted [$\beta=0.62$, se=0.26, p=0.021]. The ethnic difference was no longer statistically significant when further adjustment was made for percent fat [$\beta=0.44$, se=0.27, p=0.115] or for VFA [$\beta=0.42$, se=0.27, p=0.121].

9.4. Discussion of results

In this study healthy South Asian women and all never-smokers (men and women combined) had higher CRP levels than Europeans. Among never-smokers the higher CRP levels in South Asians vs. Europeans persisted after adjusting for age, sex, BMI and triglyceride level.

It is interesting that we found a significant ethnic difference in CRP level among never-smokers but not in ever-smokers. Our finding is at variance with that of one group who reported that the association between CRP level and increased risk for CHD death in men was limited to smokers²⁴⁷, and that lifetime smoking exposure affects the association of CRP level to cardiovascular disease risk factors²⁵³. However, our finding is in agreement with recent results from (i) IRAS (Insulin Resistance Atherosclerosis Study) where in 1560 participants there was no significant relationship between smoking status and CRP level²⁵⁴, and (ii) the Honolulu Heart Program cohort, where baseline CRP level was significantly related to the incidence of thromboembolic stroke in Japanese American non-diabetic never smokers, but not in current or past smokers²⁵⁵. We speculate that our finding of a significant ethnic difference in CRP level in never smokers is probably because most (89%) of the South Asians in this study were never smokers, but also raises the possibility that it is among the healthy non-smokers that the ethnic difference in inflammatory burden is most extreme. A high proportion of South Asians at high risk for CHD are non-smokers.

The raised CRP concentrations in both South Asians and Europeans were strongly associated with obesity (including central obesity), raised insulin levels and raised triglyceride concentration, and additionally with raised glucose levels and low HDL-cholesterol in Europeans, suggesting an association of CRP level with features of the metabolic syndrome. However, in both groups there was no direct association between CRP and insulin sensitivity index measured directly. As CRP levels are related to insulin levels and to obesity, it is surprising that they are not also related to insulin sensitivity index measured by the short insulin tolerance test in the current study. The reasons for differences between ISI and insulin level have been discussed in section 10.3.6.1.

The association of metabolic disturbances with raised C-reactive protein could reflect a higher inflammatory burden associated with increased atherosclerosis, or direct metabolic

effects on synthesis of C-reactive protein²⁵⁶. There is biological plausibility for direct metabolic effects on inflammation and acute phase response. It has been shown that adipocytes from obese humans overproduce tumour necrosis factor-alpha (TNF-alpha) mRNA and protein²⁵¹, and TNF-alpha is a potent inducer of interleukin-6 (IL-6) production by various cells²⁵⁰, which in turn causes inflammation.

A previous study has shown a correlation between plasma fasting triglyceride concentration and CRP level²⁵⁷. We have found an association between both fasting as well as 8h triglyceride and CRP level in this study, suggesting a link between inflammation and postprandial fat clearance. This may be a direct effect of cytokines (IL-6 or TNF-alpha) on the activity of lipoprotein lipase^{258,259} (the endothelial enzyme responsible for catabolism of triglyceride-rich lipoproteins), or alternatively may reflect the association of both factors with insulin resistance. CRP levels are correlated with PAI-1 levels²⁶⁰, which are also strongly related to central obesity, insulin resistance and lipid disturbances. It has been shown that PAI-1 synthesis *in vitro* is stimulated by raised NEFA levels²⁶¹. If this applies to CRP also, raised (intracellular or extracellular) NEFA levels could underlie the associations of CRP with triglyceride, insulin and central obesity. The association of CRP level with plasma lipids persisted in each of the two ethnic groups after adjustment for percent body fat, but the ethnic difference in CRP level was no longer significant when adjustment was made for percent body fat or for VFA. This may suggest that a higher fat mass level, in association with greater visceral fat, is directly responsible for an increased inflammatory burden in South Asians.

CRP level is correlated with the extent of coronary disease in patients undergoing angiography and predicts the progression of coronary atherosclerosis²⁶². It is not known whether inflammation (indexed by raised CRP level) promotes atherogenesis, or whether atheroma itself causes raised CRP levels. But, whatever the mechanism, if our observations are confirmed then there may be implications for preventive strategies. Ridker *et al*²⁴³ demonstrated that the efficacy of aspirin in reducing the incidence of myocardial infarction appears to be directly related to the level of CRP. If elevated levels of CRP account for some of the excess risk of CHD in South Asians compared with other groups in prospective studies, a possible next step might be to evaluate the efficacy of aspirin therapy in reducing the high CHD risk in this group.

PART 4

**DISCUSSION
&
CONCLUSIONS**

CHAPTER 10: Overall discussion

10.1. Main findings of the study

This study has shown ethnic differences between South Asians and Europeans in body fat distribution, insulin sensitivity, intramyocellular lipid content, lipid metabolism and inflammation, and the inter-relationships between them. Many of the findings have been shown for the first time in South Asians. The main findings from different parts of the study, in relation to the hypotheses we had on an *a priori* basis, can be summarised as following:

- South Asians had more total body fat than Europeans at a given level of BMI (significantly so in women in this study); and at a given level of body fat, the distribution of this fat was located more centrally in South Asians than in Europeans. In addition the use of bio-electrical impedance analysis as a field method for quantifying total percent body fat in South Asians was validated against DEXA scan measurement, and its reproducibility established. This provides a basis for using this quick, safe and cheap method in future studies. There are also implications for public health – criteria for “ideal weight” that are based on weight-for-height in European populations may be inappropriate for South Asian populations where for any given BMI average percent body fat is higher than in Europeans.
- As the level of central obesity increased, levels of insulin resistance (as measured by insulin concentration) increased more steeply in South Asians than in Europeans. Thus we found that the slopes of the regression lines for the association between fasting or 2h insulin and central obesity were significantly steeper in South Asians than in Europeans. This is in keeping with our original hypothesis that the tendency to insulin resistance in South Asians increases at higher levels of central adiposity. Thus lean individuals of either ethnic group are insulin sensitive, but as central obesity increases South Asians become more insulin resistant than Europeans. However the slopes of the regression lines for the association between insulin sensitivity (as measured by the short insulin tolerance test) and obesity (both generalised and central obesity) were not

significantly different between Europeans and South Asians. This points to differences in the two methods of measuring insulin resistance, and is discussed further below (section 10.3.6.1).

- The hypothesis was not confirmed that visceral obesity would account for the lower insulin sensitivity in South Asians compared with Europeans. Visceral obesity had a strong and independent association with insulin sensitivity in both Europeans and South Asians, but it failed to explain the ethnic difference in insulin sensitivity. We found that the proportion of the ethnic difference in insulin sensitivity index that was accounted for by adjusting for visceral fat area alone was 27%, adjusting for visceral fat area as well as percent body fat was 34%, and adjusting for obesity (visceral fat area and percent fat) as well as fasting and postprandial triglyceride concentration was 42%. No more than 40% of the ethnic difference in each of fasting and 2h insulin was accounted for by central obesity or obesity plus triglyceride concentration. We suggested that lipid disturbances (in the fasting or postprandial state) may be the primary defect underlying the higher insulin resistance in this group.
- The hypothesis that South Asians would have higher postprandial triglyceride concentration than Europeans was not substantiated at the single postprandial time point of 8h at which triglyceride level was measured in this study. We did however establish that an elevated postprandial triglyceride level is associated with component features of the insulin resistance syndrome in all groups.
- South Asian men had greater intramyocellular lipid content than European men. In Europeans there was a strong association between insulin sensitivity, central obesity and intramyocellular lipid content, but not in South Asians. Thus the findings in Europeans were consistent with the hypothesis that the relation between insulin-mediated glucose uptake and central obesity is mediated through triglyceride stores in skeletal muscle. However this was not demonstrated in South Asians. As this technique is new and has never been validated in South Asians, there remain uncertainties about its use in South Asians.

10.2. New hypotheses

C-reactive protein concentration was found to be higher in South Asians than Europeans, particularly in women and all non-smokers, and it was associated with features of the insulin resistance syndrome and with central obesity and percent fat, as well as triglyceride concentration in both the fasting and postprandial state. From this we hypothesise that inflammation may mediate the greater insulin resistance seen in South Asians, and also may mediate the relation between insulin resistance and CHD in South Asians. This hypothesis would need to be tested in further studies.

Other hypotheses relate to measurement issues.

Firstly we hypothesise that measurement of insulin concentration is more effective in studying ethnic differences in insulin resistance than the use of the short insulin tolerance test as used in this study. We have found that measurement of insulin resistance by the fasting and 2h insulin concentration showed much greater ethnic differences than measurement of insulin sensitivity by the short insulin tolerance test. Additionally with insulin concentration as the dependent variable the slopes of the relationship with central obesity were significantly steeper in South Asians than in Europeans, but this was not the case when the insulin sensitivity index was the dependent variable. This subject is discussed in greater detail below (section 10.3.6.1).

Secondly we hypothesise that in epidemiological studies, including those of a metabolic nature, use of anthropometric measures to quantify central obesity is adequate, and no added advantage is gained by measuring visceral fat area directly by CT scan. This is based on the following. In this small study of subjects frequency matched on BMI we found no ethnic difference in the waist/hip ratio or waist circumference in either men or in women, but visceral fat area was higher among South Asians compared with Europeans, and significantly so in women. However we found that there was high correlation between both waist/hip ratio and waist girth with visceral fat area (see section 6.1.2 for results). We also found that whatever associations were found for CT-derived visceral fat area with variables of interest such as insulin resistance (insulin sensitivity index or insulin concentration), intramyocellular lipid content, triglyceride concentration or C-reactive protein concentration, were also of similar magnitude and direction for anthropometrically derived measures of central obesity (waist girth or waist/hip ratio). Thus the use of a

relatively expensive method such as a CT scan, which also includes a small but measurable degree of radiation exposure is not justified on the basis of this study.

Although hypotheses have been generated and some *a priori* hypotheses have been substantiated or rejected by this study, they need to be seen in the light of the many limitations of the study. These limitations relate particularly to methodological issues including the study design itself, the methods employed in the study and the lack of pilot studies, and these are discussed below.

10.3. Methodological issues

10.3.1. Study design

A cross-sectional design was chosen as we wanted to generate hypotheses about the relation between obesity, body fat pattern, insulin sensitivity, postprandial lipids and intramyocellular lipid in relation to ethnicity in a preliminary study. This is the first study to examine these relationships in detail. We chose to study an age group of people (40 to 55 years) who were young enough to have not developed chronic illnesses such as diabetes and coronary heart disease. To avoid the influence of exogenous oestrogens on insulin resistance and lipid metabolism in women, we only included women who were not taking any hormonal preparations. The majority of the women included were premenopausal (section 6.1.1).

This study suffers with the usual limitations of cross-sectional studies. One of these is that the status of an individual with respect to the presence or absence of both exposure and disease is assessed at the same point in time. Thus it is difficult to distinguish the causal pathways, and to know the temporal sequence of events (such as which is the “chicken” and which is the “egg” in a sequence of events). However, associations can still be defined, and particularly if the associations are strong and independent of other effects, interpretation can be made based on biological plausibility. Thus given the current results (chapter 6) it is possible to say in this study that the findings are consistent with the idea that primary defect in South Asians is something to do with lipid metabolism.

A cross sectional study provides information about the frequency and characteristics of a disease by furnishing a “snapshot” of the health experience of the study population at a specified time. This is a limitation in that what may be the situation at one time point might be different at another time point. However, as our aim was to compare the *ethnic differences* in characteristics such as response to a fat meal and to a bolus of insulin injection, this is a valid study design. Case control studies also suffer from the same drawbacks. Prospective studies in the metabolic field are difficult to design and perform because of the dynamic nature of the metabolic variables, and are often not feasible.

We have found ethnic differences in obesity, body fat pattern, insulin sensitivity, lipid levels and intramyocellular lipid content in this cross-sectional study. Although it is difficult to tease out the temporal sequence of events, hypotheses can be generated, which can be tested in future, better designed studies. Some suggestions for these are given in chapter 11.

10.3.2. Subject recruitment

The selection of participants in this study was through inviting randomly eligible healthy people registered with general practitioners. A total of 1638 invitations were sent out and 351 people responded (22%). Of these, 210 were eligible for participating, thus representing 13% of those invited. We actually only invited 143 of those eligible, because mainly of frequency matching on BMI, and because a larger number were not needed according to our sample size calculations. Thus 9% of the original invitees attended for the first screening visit. A response rate of 22% and a participation rate of 9% is very small, but is understandable given the very involved and invasive nature of a study with multiple visits, which was clearly explained in detail as part of the original invitation. Most studies of a metabolic nature often recruit volunteers through advertisements in the media because of the expected low response rate from healthy subjects registered with family practitioners. We chose not to do that to make an attempt to draw our study sample from randomly eligible people who represented a cross-section of the population residing in the geographical area of the study.

We might speculate that the motivation for participating in the study might have been because of family history of diabetes or CHD. We did find a high prevalence of family history for CHD that was not different in the two groups. We also found a high

prevalence of family history for diabetes mellitus, nearly twice as high in South Asians than in Europeans (though this did not reach statistical significance). Although not formally recorded, it was indicated to the registrant by some participants that their motivation to participate was that they might get a “full check up” and might get advice for healthy living, and find out if “anything was wrong with them”. Some also said they just wanted to help with medical research because it is “very worthwhile”.

We do not have detailed information available about the non-responders, except that they were healthy according to their doctor’s records. To assess seriously if there were differences between the participants and non participants, one could send out questionnaires asking about family history, for example, or for reasons for participating or not. However it was not felt necessary to do that in the current metabolic study. Response rate could have been increased by following up the first invitation letter with a second one, or with a phone call or a visit. However, given the invasive and involved nature of the study, it was not considered ethical to “pressurise” people to take part. Selecting participants in this manner, particularly for a metabolic study, does not invalidate the findings, but may limit their generalisability. Since our aim was to compare the results for the two ethnic groups, this is particularly the case.

Three of the four general practices whose lists were used for the initial mailshots had previously been involved in research studies in collaboration with the London School of Hygiene and Tropical Medicine. These practices may be above average in Southall and Greenford with respect to the services they offer patients and their organisational efficiency. This may in turn be reflected in the readiness of the responders to participate in such a study. Again, these factors do not undermine the validity of the results but may affect their generalisability.

10.3.3. Adverse events

The main adverse event was that of a chemical phlebitis following the injection of 50% dextrose solution to terminate the SITT as mentioned in section 5.5.4. The precaution of giving the injection slowly and of using plenty of saline flush, combined with raising the arm after the flush minimised such incidents in anyone who complained of an “aching” or “burning” sensation in the receiving arm.

10.3.4. Drop-outs

Of the 143 people who were screened there were 11 not eligible to proceed and 12 people withdrew, leaving a total of 120 people who proceeded with the rest of the study (see section 5.5.6). Among those who withdrew the reasons given were mainly practical ones (section 5.5.6) except for 2 people who felt the tests were too invasive. This actually left us with our target number of 120 people for the study. Because of the multiple visits involved and the implication for taking time off work, not all 120 people could complete all the stages of the study. A summary of the number of people completing each of the stages of the study is given in section 5.5.6.1. For a study of this nature the compliance with attendance was actually quite good and acceptable.

10.3.5. Time lag between visits

We tried to get participants through all stages of the study in batches, so there was a minimal time delay between visits. So rather than complete the screening stage for all participants, before starting on other visits and so on, we took a batch of people through the first stage, on to the next and so on, while a new batch of people was starting on the first stage. For many participants this meant attending for the second visit within 3 to 4 weeks of the screening visit, on to the 3rd visit within another 4 weeks and so on. The clinical data collection was completed within a total of 8 months for all participants, for all stages of the study, except for the sub study involving NMR-spectroscopy in 40 men. This was completed within 15 months from starting.

The time lag between different stages of the study may introduce behavioural changes for instance which might affect the results of the study. For example some participants might increase the amount of exercise taken regularly, or improve their diet. Or changes may occur with the passing of time involuntarily. We did not measure any of these factors. In a study of this nature it was impossible to complete the many stages of the study any sooner than already accomplished.

10.3.6. Experimental measures

A description of the reasons for choice of the particular tests that were used in this study has already been given in section 5.10. However based on the results of the study, certain points arise regarding the choice of methods.

10.3.6.1. Measurement of insulin resistance

The method employed for obtaining a direct measure of insulin sensitivity in this study was the short insulin tolerance test. However it was apparent that measurement of fasting and 2h insulin concentration showed much larger ethnic differences, with elevated levels among South Asians relative to Europeans, than did measurement of the insulin sensitivity index. We also found that the ethnic differences in insulin level were present among both men and women, while those for ISI were only significant in men.

Furthermore the data failed to show a significant ethnic difference in the slope of the relationship between ISI (as measured by the short insulin tolerance test) and measures of central obesity. There was, however, a significant ethnic difference in the slope of the relationship between both fasting and 2h insulin as the dependent variable, with measures of central obesity as the independent variables.

This raises two issues. The first relates to the fact that ISI and insulin level are measuring two different things. In the short insulin test we measure the glucose disposal rate in response to a bolus of insulin, while in the measurement of 2h insulin level we measure the body's response to an oral glucose load. The SITT gives an overall assessment of whole body sensitivity to insulin, but does not allow direct quantitative measurement of insulin mediated glucose metabolism. The SITT also does not distinguish between hepatic and peripheral insulin sensitivity. It is possible that the SITT measures mainly the effect of suppression of hepatic glucose production following a bolus of exogenous insulin.

The second issue relates to the fact that there is a possibility that the defect in insulin-mediated glucose disposal in South Asians may be more evident after a glucose load than in the fasting state. It has been observed that ethnic differences in the 2h insulin level are much greater than the differences in fasting insulin. This could be extrapolated to imply that the insulin resistance in South Asians is a specific defect in the delayed response to insulin. It would be of interest to perform studies of an ethnic comparison of the time-

course relation of insulin concentration to glucose uptake. It is possible that such a relation might be shifted to the right in South Asians.

Furthermore the use of the short insulin tolerance test has never previously been validated specifically in South Asians. A pilot study validating the SITT against the “gold standard” of an euglycaemic clamp would have added to this study, but was not undertaken due mainly to the following reasons: time constraints, the fact that the SITT had been used in a study of an ethnic comparison of first degree relatives of Europeans and South Asians with diabetes⁵³, the fact that this was an already invasive study and further tests were not feasible and also because the ISI calculated from the SITT allows relative ranking of individuals with respect to insulin sensitivity, which was our main aim in this study.

This study did not find any advantage of the short insulin tolerance test over the measurement of insulin resistance by a fasting and post-challenge insulin concentration in studying the relationship of insulin resistance with obesity, metabolic measures and ethnic origin.

10.3.6.2. Fat tolerance test and postprandial triglyceride

As discussed in section 6.3.4, for this epidemiological study, rather than undertake a full fat tolerance test with hourly blood samples, we took a single post-load sample at 8h, plus a 6h measurement in 35 people. It is possible that we have underestimated the ethnic difference in postprandial triglyceride level by relying on a single measurement at 8h in most participants. If so, then it is possible that we have also underestimated the extent to which differences in postprandial triglyceride levels could account for the ethnic difference in insulin sensitivity.

With hindsight, a more detailed fat tolerance test, with measurements at perhaps 4, 6 and 8 hours would have allowed a much more comprehensive assessment of the role of postprandial triglyceride, as the area under the triglyceride curve could possibly give more information than just a single measurement. If the finding of Patsch²⁶³ in an European population suggesting that a single time point at 6 or 8 hours was as discriminatory between cases of CHD and controls is to be extrapolated to a South Asian population, and a study of an ethnic comparison, as in this study, a pilot study should have been undertaken first. This would have helped to determine the optimal time point(s) for measurement of total triglyceride concentration in the fat tolerance test which would have

discriminated between Europeans and South Asians. In this study we found that there was a significant ethnic difference in the 6h triglyceride concentration in those men who had it measured, but by 8 hours postprandially the triglyceride concentration was returning towards fasting values and was no longer significantly different between ethnic groups. A pilot study of triglyceride concentrations at different time points of a full (unmodified) fat tolerance test in each of the four ethnic and sex groups would have enabled us to avoid the problem we experienced of having missed the optimal time point for maximal ethnic differences. This should be considered before future fat tolerance test studies are undertaken.

We only measured total postprandial triglyceride level. It would have been more informative to have the measurement of apoB-48 and apoB-100, as well as retinyl palmitate in chylomicron and non-chylomicron plasma fractions, as discussed in section 6.3.4. Future studies should attempt to undertake these more detailed measurements.

10.4. Discussion of results

The results have been specifically discussed in sections 6.3, 7.3, 8.3 and 9.4.

10.5. Relevance of the findings of this study to overall strategies to reduce CHD risk in South Asians

We have found an adverse risk factor profile of factors associated with CHD risk in South Asians compared with Europeans in this study. These risk factors included: (i) greater levels of obesity, generalised and central, in South Asians as shown by the greater amount of total percent fat and visceral fat in South Asians; (ii) higher degree of insulin resistance in South Asians as shown by the lower insulin sensitivity index in South Asians (significant in men only), and higher levels of fasting and 2h insulin in South Asian men and women; (iii) a steeper rise in insulin concentration in South Asians (than in Europeans) with increasing levels of central adiposity, and (iv) a trend towards higher postprandial triglyceride level in South Asian men (significant at 6h, but not at 8h postprandially).

In this study there was no collection of detailed information on diet, and assessment of physical activity was limited, and based on self-report in response to a questionnaire. This showed that levels of physical activity were low in both groups, but even lower in South Asians, and especially so among South Asian women. Attempts to reduce the absolute risk for CHD include lifestyle interventions such as modifications to diet and physical activity, as well as therapeutic interventions such as to lower lipids. The relevance of these strategies to South Asians is discussed in the following sections.

10.5.1. Physical activity

The increased obesity in South Asians may be due to genetic reasons, or to lifestyle and environmental factors including lack of exercise. But whatever the reason, prevention of diabetes and CHD in South Asians may require control of obesity and regular exercise to be maintained throughout life.

10.5.1.1. Effect of physical activity on risk for diabetes and CHD

The effect of physical activity on the development of diabetes in people with impaired glucose tolerance (with 50% from minorities including people of Asian origin) is being tested in the United States as part of a multicentre (27 centres) randomised controlled trial, the Diabetes Prevention Program (DPP)²⁶⁴. 3000 participants are being randomised to *either* a standard advice package and placebo (as control group), *or* an intensive lifestyle intervention comprising weight-loss diet and exercise *or* to metformin. There was to be an additional treatment group, with troglitazone, a thiazolidinedione recently licensed for use in insulin resistant states in the U.S. However this has now been discontinued because of the hepato-toxicity of the drug. The main outcome measure during follow-up is the development of NIDDM. This study will yield important information on the relative effectiveness of the three interventions.

The Southall Intervention Study [Gail Davey, manuscript submitted to *Medicine and Science in Sports and Exercise*] is the first to demonstrate a significant increase in insulin sensitivity in the twenty-four hours following exercise in South Asians (and Europeans). The results of this study suggested that exercise may increase insulin sensitivity, independent of its effect on body weight or lipid profile. Over the past few years, health

promotion material has moved from encouraging vigorous exercise for twenty minutes three times per week²⁶⁵ to advocating daily or more than daily activity of lower intensity²⁶⁶. These changes reflect the trend towards increasing energy expenditure rather than fitness in interventions against coronary heart disease. The Southall Intervention Study adds support to this pattern of increased frequency of activity for improving insulin resistance. Lehman *et al*²⁶⁷ conducted a trial of exercise training over 3 months in 16 patients with NIDDM, with 13 NIDDM patients as controls. They found that in those who took regular physical exercise there was a significant amelioration of cardiovascular risk profile; in particular total percent fat, WHR and triglycerides decreased, while HDL-cholesterol levels increased independently of changes in body weight and glycaemic control.

10.5.1.2. Effect of physical activity on abdominal obesity

That exercise training can be beneficial in South Asians is highlighted by the fact that the effect of training is to reduce both percent body fat as well as visceral fat. Després *et al*²⁶⁸ have shown that when aerobic exercise is used to induce weight loss, men generally lose more fat than women. In men the loss of adipose tissue is more central. Although resistance to fat loss is noted in women, those with a “male” distribution of adipose tissue greatly benefit from aerobic exercise training. They showed further that obese premenopausal women who participated in an exercise training programme lost a greater amount of fat (measured by CT) from the abdominal area than from the mid-thigh area²⁶⁹. Another group showed that of 60-70 year old men and women who participated in a 9-12 month exercise programme, men lost more weight than women, but in both groups the largest absolute and relative changes occurred in the truncal area, indicating a preferential loss of fat from the central regions of the body²⁷⁰. This preferential loss of visceral adipose tissue with exercise in women has also been shown by others²⁷¹.

However one group showed that decrease in subcutaneous fat mass, but not visceral fat mass was proportional to the amount of aerobic exercise training in women²⁷². They argued that the change in visceral fat mass appears to be related to a deficit in caloric balance either by dietary restriction (decrease in caloric intake) or by increased caloric expenditure. Ross²⁷³ reviewed the studies of effects of diet and exercise induced weight loss on visceral adipose tissue in men and women. He concluded that for every kg of diet-induced weight loss, the corresponding reduction in visceral fat was about 3-4 cm², and that there appears to be a resistance to visceral fat area reduction in obese women but not

in men. He suggested that data on the separate effects of diet and exercise induced weight loss on visceral fat from well controlled studies are required to advance current knowledge with respect to the effects of diet and exercise on the adipose tissue depot that conveys the greatest health risk. Such studies are not only needed for the general population, but also for people from the Indian sub-continent, whose risk for visceral obesity, insulin resistance, diabetes and CHD remains high.

10.5.1.3. Effect of physical activity on postprandial lipids

It is now known that exercise can also have a beneficial effect on postprandial lipaemia. Zhang *et al*²⁷⁴ studied 21 recreationally trained men and found that exercising before a fat meal, at 60% of maximal O₂ consumption for 1 hour, had a beneficial effect on postprandial triglyceride response and HDL metabolism. Another group²⁷⁵ studied postprandial triglyceride and apoB-100 level in 54 men and women who were sedentary, recreational exercisers or endurance trained. They found that both recreational and competitive aerobic training were associated with a lower triglyceride response and apoB-100 level after a fatty meal. Further, Hardman *et al*²⁷⁶ have studied changes in postprandial lipaemia in endurance trained people during a short interruption to training. They found that postprandial lipaemia increased with detraining, and concluded that frequent exercise is needed to maintain a low level of postprandial lipaemia and insulinaemia in trained individuals.

Thus there is evidence that physical activity is beneficial both for increasing insulin sensitivity, and for reducing a postprandial lipaemic response. Although there are numerous such studies in Europeans, further such studies need to be performed in South Asians. However, it seems plausible that physical activity will be equally, if not more, beneficial in South Asians.

10.5.1.4. Public health implications of physical activity

But how can this public health message about the necessity and beneficial effect of exercise be highlighted to the public, especially the South Asian population in the U.K.? In the United Kingdom, the Health Education Authority are currently encouraging exercise among people in a national campaign called "Active for Life". The second phase of the campaign over 1997-98, had as its target people over the age of 50 years and people from

black or minority backgrounds. The Health Education Authority has made use of national and local media to promote regular moderate physical activity. It has also undertaken training for general practitioners and community nurses, and offered support including small grants to health professionals. The message being conveyed encourages modest but frequent physical activity such as cycling, dancing, walking and gardening. These have been put across as activities that do not require special preparation or facilities, but can contribute to health if pursued frequently. Another part of the campaign involves general practitioners in “exercise prescription” (free or at reduced rates) schemes in conjunction with local leisure centres. However, the aims of the campaign are broad: improving health by increasing physical activity, which makes evaluation difficult. While one could evaluate whether activity has increased by using, for instance, actometers, no such evaluation efforts have been made. Thus what proportion of South Asians, particularly women have taken the “message on-board” is currently unknown.

10.5.2. Diet

In the current study there were more vegetarians among South Asians than among Europeans. Detailed information on diet was not collected in this study. However we have information about diet in this group of people from a previous, larger study, the Southall Study³⁶. Recording of 7-day weighed dietary intakes in 173 South Asian and European men showed that overall the South Asian diet was more favourable: in South Asian men (compared with European men) mean energy intake was lower, carbohydrate intake was higher, polyunsaturated fat intake was higher, and dietary fibre intake was higher. Elevated serum 2h-insulin concentrations were positively associated with carbohydrate intake, but not with saturated fatty acid intake. The authors concluded that the high coronary risk in South Asians is not explained by any unfavourable characteristic of South Asian diets.

Leaf^{277,278} has estimated that the average diet of our ancestors consisted of fat as about 20% of total food energy, with 7 to 8% saturated fat, and a n-6 to n-3 fatty acid ratio of about 4 to 3. However, since the Industrial Revolution the saturated fat content has risen dramatically, and the n-6 to n-3 fatty acid ratio has changed to about 15 to 1. He argues that n-3 class of polyunsaturated fatty acids (PUFA) are beneficial for coronary protection^{277,279}. The Diet and Reinfarction trial (DART) was the first large trial to report

that advice (among 2033 men) to eat oily fish rich in n-3 PUFA was associated with a 29% reduction in total mortality in the first two years after myocardial infarction²⁸⁰.

It has been suggested that low intake of polyunsaturated fatty acids of the n-3 series (alpha linolenic acid) may contribute to the high coronary risk in South Asians²⁸¹⁻²⁸³. This may be one factor in causing high rates of CHD in Gujarati Hindus, but cannot account for the high risk in Bengalis¹² whose diet is high in n-3 fatty acids from fish²⁸⁴. Also, some groups from South Asia already have low average plasma cholesterol and low saturated fat intake, but still have very high CHD mortality, such as among Gujarati Hindu women living in North-West London^{35,285}.

10.5.2.1. Effects of dietary change on plasma lipids

Previous dietary advice consisted of lowering total fat intake by low fat diets. However such low fat diets were achieved by having a higher proportion of carbohydrates. Carbohydrates, however, not only decreased LDL-cholesterol, but also lowered HDL-cholesterol^{286,287}. First suggested in the 1970's³⁸, it is now well accepted that HDL-cholesterol is cardioprotective. Thus recommendations to decrease fat and increase carbohydrate intake have come under scrutiny²⁸⁸. In a recent commentary Katan²⁸⁷ discusses the evidence for whether there are "good and bad carbohydrates for HDL-cholesterol", based mainly on a recent report by Frost and colleagues²⁸⁹ suggesting that the glycaemic index of the diet is a stronger predictor than dietary fat intake, of serum HDL-cholesterol concentration. Katan concludes that as yet the effects of low-glycaemic index foods on blood lipids remain unproven²⁸⁷.

10.5.2.2. Effects of diet on CHD risk

It is known that lowering saturated fat intake by substituting it with polyunsaturated fatty acids of the n-6 series (linoleic acid; such as in vegetable oils) has adverse effects or fails to improve clinical prognosis. It is only when n-6 fatty acids are decreased and n-3 fatty acids are increased that there is clinical benefit and risk reduction for CHD.

Ravnskov²⁹⁰ has reviewed the existing literature on the influence of diet on cardiovascular disease. He examined the results of all available ecological, cross-sectional, case-control, cohort and intervention studies (including randomised trials) of the effect of fat reduction alone on cardiovascular disease. He reported that the findings from these numerous

studies including studies in different ethnic groups yielded inconsistent and contradictory results. He thus concluded that the hypothesis that dietary saturated fatty acids are harmful while polyunsaturated fatty acids are protective for CHD is questionable. However he presents one extreme view. Most epidemiological evidence now points in favour of advocating a "Mediterranean" diet, high in n-3 polyunsaturated fatty acids, mono-unsaturates, fibre, fish and fresh vegetables.

The results of the recent Lyon Diet Heart Study provide support for a cardio-protective role of the Mediterranean type diet^{277,291}. An initial report of this randomised secondary prevention trial²⁹² at 27 months of follow up had shown a striking 70% reduction in all-cause mortality due to a reduction in CHD mortality and non-fatal CHD sequelae. Thus the trial was terminated by the Ethics committee at 27 months rather than the planned 5 years. Recently published results of the final 4 year follow up of this cohort further show that the initial remarkably beneficial effects of the experimental dietary programme persisted compared with the control group consuming the usual Western type diet. The heartening thing about this diet, versus low fat diets, seems to be that there was continued good adherence to the experimental diet for up to 4 years, despite the fact that the study was officially terminated at a mean follow up of 27 months. The Mediterranean type diet consisted of about 30% total fat, with only 8% saturated fat. Participants were instructed to consume more bread, root and green vegetables, more fish and less meat, fruit at least once daily, canola-based margarine (made from rapeseed oil, and rich in n-3 PUFA) and olive oil as a fat source. The authors concluded that a cardio-protective diet should be part of a comprehensive programme to reduce CHD morbidity and mortality.

If these results can be confirmed by others, there are implications for preventive strategies. It has been argued whether 18:3[n-3] PUFA (alpha linolenic acid) derived from vegetable oils such as soya oil and rapeseed oil could have similar cardio-protective effects as 20:5[n-3] PUFA (eicosapentaenoic acid) derived from oily fish²⁹³. It would be useful to test specifically in secondary prevention trials the effect of substituting oils containing 18:3[n-3] PUFA for other sources of dietary fat. The rationale for this is that it is easier to substitute vegetable oils rich in 18:3[n-3] PUFA for other sources of dietary fat than to increase consumption of oily fish on a mass scale.

Based on the currently available evidence no specific recommendations regarding the South Asian diet can be made. However, it would do well for all people, South Asian or European to reduce their total daily fat intake. It also stands to reason that a

Mediterranean type diet would be beneficial to both Europeans and South Asians. The Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy²⁹⁴ recommended that the average intake of saturated fatty acids should be reduced to 10% of total energy. Based on the Keys equation²⁹⁵ it has been estimated by McKeigue and Sevak²⁷ that among South Asians achieving the above goal could reduce the average cholesterol to about 5.0 mmol/l. Some would extrapolate this to predict that in so doing effective control of CHD as a leading cause of death could be achieved.

10.5.3. Lipid abnormalities

10.5.3.1. Underlying defect might be related to lipid metabolism

It is intriguing that while there was a trend towards higher postprandial triglyceride level coupled with a higher intramyocellular lipid content in South Asian men, yet postprandial triglyceride failed to account for the lower insulin sensitivity, and the intramyocellular lipid content was not related to insulin sensitivity in South Asians in this study. However postprandial triglyceride was strongly related to the insulin resistance syndrome, and together with body fat distribution, accounted for almost 50% of the ethnic difference in insulin sensitivity.

This raises the possibility that there is some basic derangement in lipid metabolism or storage in South Asians, which with better measurement technique(s) could yield more detailed information. For instance, we have only been able to measure the level of triglyceride in this study in plasma and in skeletal muscle. It would be informative to be able to measure the flux of lipids between different body compartments such as plasma and muscle. It would also be informative and of interest to measure the fate of triglyceride after a fat load; thus studies of intramyocellular lipid by NMR spectroscopy before and after a fat load would be helpful. Techniques for study of uptake of NEFA in cells are becoming available²⁹⁶. Such techniques as positron emission tomography (PET) can be used to measure the uptake of NEFA into muscle cells. Using this technique a recent study²⁹⁶ showed that subjects with impaired glucose tolerance (compared with normoglycaemic controls) had similar myocardial but lowered femoral muscle NEFA uptake. Such studies performed in South Asians would be valuable to try and tease out the mechanisms involved in their high rates of insulin resistance.

The suggestion that disturbances of lipid metabolism might be primary in the development of insulin resistance and diabetes mellitus is not new. Randle *et al*¹⁸² proposed a glucose-fatty acid cycle in 1963. The pros and cons of this cycle have been discussed in section 3.1.1. McGarry raised this possibility in his classical paper in 1992²⁹⁷ entitled “What if Minkowski had been ageusic? An alternative angle on diabetes”. Boden¹⁵⁵ has reviewed the evidence for a central role of NEFA in the pathogenesis of insulin resistance and Type II diabetes.

Genetic studies in rat models are also beginning to support this hypothesis. Aitman *et al*²⁹⁸ reported earlier this year that Cd36 (Fat) is an insulin resistance gene causing defective fatty acid and glucose metabolism in the spontaneously hypertensive rat. Cd36 has been identified as a fatty acid receptor/transporter. Aitman *et al* found that the protein product of Cd36 is absent in the rat adipocyte plasma membrane, while overexpression of Cd36 in transgenic mice reduces blood triglycerides and fatty acids. This suggests that a deficiency of Cd36 (i.e. a defect in a fatty acid transport gene) underlies insulin resistance, defective fatty acid metabolism and hypertriglyceridaemia in the rat model. By extrapolation, Cd36 may play an important part in the pathogenesis of human insulin resistance syndromes. However, since the publication of Aitman’s report, there has been a further report (in July 1999) by a Japanese group²⁹⁹ showing that the Cd36 mutation is absent in the original spontaneously hypertensive rat strains, maintained since their development in Japan. This throws into question the aetiological relevance of the Cd36 mutation to insulin resistance in this rat model. However, further study of this rat line, as well as others and studies of the human genome project should shed further light into the genetic origins of insulin resistance.

10.5.3.2. Management of lipid abnormalities

Evidence from several large randomised clinical trials has shown coronary benefit of reducing LDL cholesterol, and evidence is also emerging for a beneficial effect of lowering elevated triglyceride levels. Among South Asians the predominant lipid disturbances are related to the insulin resistance syndrome and include high triglyceride level and low HDL-cholesterol, as well as increased concentrations of small dense LDL particles. When these lipid abnormalities occur together the atherogenic lipoprotein phenotype (ALP) is said to exist. The role of lipid lowering drugs specifically in South

Asians has not been evaluated. It is not known whether we should target lowering of triglycerides and the ALP, or LDL cholesterol.

10.5.3.3. Lowering plasma triglyceride levels

The data available so far suggest that treatment to lower plasma triglyceride levels may reduce cardiovascular events. In the Helsinki Heart Study (HHS) treatment with the fibrate gemfibrozil produced a 34% reduction in incident coronary events with a 35% reduction in plasma triglyceride levels, a 9% increase in HDL-cholesterol levels and an 11% reduction in plasma LDL concentration³⁰⁰. However the beneficial effect of gemfibrozil was mostly confined to overweight subjects (with BMI greater than 26 kg/m²). This may not be directly relevant to those South Asians who have the lipid abnormalities despite average/below average BMI. The formal published results of the Bezafibrate Infarction Prevention (BIP) secondary prevention trial are still awaited, in which subjects with CHD were randomised to either bezafibrate or placebo. However a preliminary report was presented at the European Society of Cardiology meeting in Vienna in 1998. This showed a non-significant 9% reduction in CHD (combined endpoint of nonfatal myocardial infarction, fatal myocardial infarction, or sudden death). Total mortality was also unaffected by bezafibrate, and only a small sub-group with high baseline plasma triglyceride levels profited from treatment.

Results of a large scale trial to address the issue of these lipid abnormalities in the secondary prevention of CHD have just been published (August 1999). The Veterans Affairs HDL-cholesterol Intervention Trial (VA-HIT) Study³⁰¹ of 2531 men with previous CHD, with HDL-cholesterol levels of 1.0 mmol/l or less and LDL cholesterol levels of 3.6 mmol/l or less showed, at one year, a relative risk reduction of 22% (95% CI 7 to 35%) for CHD (non-fatal myocardial infarction or coronary death) in those treated with a fibrate (gemfibrozil) compared with those on placebo. The effect of treatment with gemfibrozil was to increase HDL-cholesterol (6%), lower the mean triglyceride level (31%) and lower the total cholesterol level (4%). LDL cholesterol levels did not differ between the treatment groups. It was concluded that the rate of coronary events is reduced by raising HDL-cholesterol levels and lowering triglyceride levels, without lowering LDL cholesterol levels. This may be of relevance to South Asians. If these findings apply to South Asians, they may also benefit from reversing of these lipid abnormalities. Trials of fibrates in South Asians would merit consideration.

It is too early to say as yet whether those South Asians (or Europeans) who have normal fasting triglyceride levels but elevated postprandial triglyceride levels will benefit from drug therapy targeted at lowering of postprandial triglyceride levels. Further more detailed studies are needed to first establish whether this is the case in South Asians, and then randomised trials will need to be conducted to gather the evidence for any beneficial effect on CHD risk.

10.5.3.4. Lipid lowering with statins

10.5.3.4.1. Evidence for coronary benefit of statin therapy

Statins are hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that form an important group of lipid-lowering drugs for prevention of CHD. Compelling evidence for their role in cardioprotection has been shown in large trials of secondary prevention such as the Scandinavian Simvastatin Survival Study (4S)³⁰², the Cholesterol and Recurrent Events Trial (CARE)³⁰³ and the Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) study³⁰⁴. These studies showed reduction in relative risk for CHD of 34%, 24% and 24% respectively. Evidence for a cardioprotective role has also come from trials of primary prevention such as the West of Scotland Coronary Prevention Study (WOSCOPS)³⁰⁵ and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS)³⁰⁶ study in the United States. These studies showed a relative risk reduction of CHD of 31% and 37% respectively. The initial reports of these trials have largely been in people with average to high lipid levels and with no history of diabetes. However, little information has been available on the role of statins in people with diabetes or glucose intolerance until recently.

The role of pravastatin has been investigated in a subgroup analysis of patients with diabetes and glucose intolerance with average cholesterol levels in the CARE trial (of secondary prevention in myocardial infarction survivors)³⁰⁷. The trial included 586 patients with clinical diagnosis of diabetes, and 342 patients from 3553 non-diabetic patients had impaired fasting glucose at entry. The analysis showed that patients with diabetes and impaired fasting glucose were at high risk of recurrent coronary events that could be substantially reduced by pravastatin therapy (in the diabetic group: absolute risk reduction of 8.1%, and relative risk reduction of 25%; in the impaired fasting glucose group: 50% reduced recurrence rates for non-fatal MI in treatment group). Similar results

were reported from a subgroup analysis of 202 diabetic patients and 4242 non-diabetic patients in the 4S study³⁰⁸. The authors concluded that cholesterol lowering with simvastatin improves the prognosis of diabetic patients with CHD. They argued that the absolute clinical benefit achieved may be greater in diabetic than in non-diabetic patients with CHD because diabetic patients have a higher absolute risk of recurrent CHD events.

It is also been shown by Haffner *et al*³⁰⁹ in a 7 year follow-up study of 2432 subjects that diabetic patients without prior myocardial infarction had as high a risk of myocardial infarction (20.2%) as non-diabetic patients with previous myocardial infarction (18.8%). They argued that CHD risk factors in diabetic subjects should be treated as aggressively as in non-diabetic patients with a prior history of CHD. Since much of the risk for macrovascular disease (such as CHD) is associated with impaired glucose tolerance, before the onset of clinically detectable diabetes, it can be extrapolated that people with impaired glucose tolerance will have risk for CHD of similar proportions. This is important in the context of the high prevalence of insulin resistance and glucose intolerance, as well as diabetes, in people of South Asian descent.

10.5.3.4.2. *Relation between baseline cholesterol level and benefit from statin therapy*

Evidence is now accumulating that statins are beneficial regardless of the baseline cholesterol level. Results of the CARE study³⁰³ showed benefit of statins in those with average cholesterol levels (total cholesterol below 6.2 mmol/l and LDL cholesterol between 3 to 4.5 mmol/l). A further analysis of results from CARE trial showed that the LDL concentration achieved during follow-up was a significant predictor of the coronary event rate down to an LDL concentration of 3.2 mmol/l³¹⁰. However, the extent of LDL reduction (absolute or percentage reduction) had no significant relationship to coronary events. Results of the LIPID study³⁰⁴ also showed benefit of statins in subjects with a broad range of initial cholesterol levels (total cholesterol of 4 to 7 mmol/l). Benefit of statin therapy was also shown by MacMahon *et al*³¹¹ in the LIPID Carotid Atherosclerosis Sub-study of the LIPID trial among 522 patients with a history of ischaemic heart disease. Thickening of the carotid arteries increased in those given placebo, but decreased in those given pravastatin. The statin prevented substantial carotid wall thickening in patients with cholesterol levels that were high or average as well as below average (4 to 7 mmol/l). These studies suggest that there might be benefit in lowering cholesterol even in normocholesterolaemic individuals at high risk for CHD. The case for this has also been

argued before by Byrne and Wild³¹². It is not clear what the cellular mechanisms are for the beneficial effects of cholesterol lowering, especially in people with average or below average cholesterol levels. It seems likely that plaque stabilisation or improvement in endothelial function will play a part. Further studies will help to clarify this.

10.5.3.4.3. *Non lipid lowering effects of statins*

In fact evidence is now emerging that statins may also exert their effect independently of their lipid-lowering effects. Ridker *et al*³¹³ have shown this recently in a trial of secondary prevention of 472 survivors of myocardial infarction. They found that C-reactive protein concentrations (CRP - a marker for inflammation) fell by a mean of 17% in patients receiving pravastatin but rose by 4% in those treated with standard therapy plus placebo. The changes in CRP did not correlate with changes in lipid values, so for example, CRP values rose in patients who lowered their LDL-cholesterol by diet and exercise.

10.5.3.4.4. *Should statins be used in South Asians?*

South Asians have higher prevalence of insulin resistance and diabetes associated with high risk for CHD in the face of cholesterol levels in the normal or below normal range. Thus a case can be made for consideration of statin therapy in the prevention (primary and secondary) of CHD in South Asians. This is based on the evidence available for coronary benefit of statin therapy in those with glucose intolerance and diabetes, coupled with the evidence that statins are beneficial regardless of initial cholesterol levels.

At present risk for CHD is commonly calculated as a probability (%) of developing CHD (non-fatal myocardial infarction or coronary death) over 10 years; that is, the number of people per 100 expected to have a major CHD event in the next 10 years. This calculation is based on the risk equation from the Framingham study. However, this equation has not been validated for use in ethnic minorities. For example, raised serum triglycerides or evidence of impaired glucose tolerance or insulin resistance are not currently part of the risk calculation.

To test if there is specific coronary benefit in South Asians one possibility is to consider trials of statins in South Asians with evidence of insulin resistance. The selection of a group of people suitable for randomisation would have to be carefully performed as there will be some in whom it will be unethical not to treat with a statin, while there will also be

those in whom it will be unreasonable to treat with statins. For example it may not be ethical to perform a trial of secondary prevention, where perhaps everyone with a previous myocardial infarction should be on a statin, but it may be ethical to perform a trial of primary prevention.

Of course there will be cost implications for use of statins in primary prevention in South Asians. In general as the CHD risk profile becomes less favourable the cost-effectiveness increases. Thus the number of subjects who would need to be treated in order to prevent an adverse event decreases dramatically. However, specific studies of cost-effectiveness will need to be performed.

CHAPTER 11: Conclusions and suggestions for further research

From the results of this study we can conclude as follows: South Asians have more total body fat than Europeans at a given level of BMI, and it is more centrally distributed, especially in the women in this study. South Asians have lower insulin sensitivity than Europeans, especially so in men. Insulin concentration rises significantly more steeply in South Asians compared with Europeans as the level of central obesity increases. There is a trend towards a higher postprandial triglyceride response in South Asian men, and elevated postprandial triglyceride level is associated with component features of the insulin resistance syndrome in all groups. South Asian men also have greater intramyocellular lipid content than European men. In Europeans there is a strong association between insulin sensitivity, central obesity and intramyocellular lipid content, but not in South Asians. Although visceral obesity has a strong and independent association with insulin sensitivity in both Europeans and South Asians, it fails to account for the lower insulin sensitivity in South Asians compared with Europeans. Lastly, there is a trend towards higher CRP concentrations in South Asians, and CRP concentration is associated with features of the insulin resistance syndrome.

We have suggested that postprandial lipid metabolism may be important in South Asians, and that lipid disturbances (in the fasting or postprandial state) may be the primary defect underlying the higher insulin resistance in this group. However it has not been possible in this study to determine what specific lipid disturbance may be more important in South Asians. The possible defects in lipid metabolism in South Asians could include one or more of the following: (i) increased VLDL-triglyceride production or failure to shut down postprandial triglyceride production, (ii) reduced triglyceride clearance, or (iii) increased triglyceride storage in skeletal muscle.

Although we have learnt in more detail about the relationships between body fat distribution, insulin sensitivity and lipid disturbances (in plasma as well as in skeletal muscle) from this cross-sectional study, there remain large gaps in our understanding of the reasons for the excess risk for CHD and diabetes in South Asians. Some specific suggestions for further research arising from the current study are as follows.

11.1. Further research relating to measurements

Several novel techniques, not previously used in South Asians, were employed in this study. Their strengths and weaknesses have been discussed before, but specific recommendations are summarised below.

Specific validation of the short insulin tolerance test (SITT) against the euglycaemic insulin clamp should be performed in South Asians. While the validity and reproducibility of the SITT have been established in European subjects, South Asians have not been included in such studies. It is possible that in South Asians measurement of insulin concentration following an oral glucose load is a more valid measurement to use than the SITT. Until this specific validation has been performed the use of the SITT is not recommended in studies of South Asians as it is not clear that ethnic differences in insulin resistance are “captured” by the SITT.

Pilot studies should be performed with the full (unmodified) fat tolerance test to determine the optimal time point(s) for measurement of total triglyceride concentration that would discriminate best between Europeans and South Asians. To determine which component of lipid metabolism is specifically defective more detailed studies than measuring total triglyceride concentration should also be performed. This includes measuring exogenous (dietary derived; apoB-48) and endogenous (hepatic derived, apoB-100) triglyceride concentration in different plasma fractions (plasma chylomicron and non-chylomicron fractions). Measurement of a single 8h postprandial total triglyceride concentration proved to be inadequate in this study, and is not recommended for future studies.

Reproducibility and validity of nuclear magnetic resonance spectroscopy to measure IMCL content should also be performed specifically in South Asians. To date all such studies have been limited to European descent populations. The results of the current study showing higher IMCL content in South Asian men, but failure to find any association between IMCL and insulin resistance or obesity measures raise questions about the use of the technique in this ethnic group.

Measurement of central obesity by anthropometric measures of waist/hip ratio or waist girth was adequate. No additional benefit was found in measuring visceral fat area directly by CT scan for studying the associations between central obesity and various metabolic measurements. This study has validated bio-electrical impedance analysis against DEXA

scan as a method for measuring total percent body fat in South Asians. We recommend that future studies should include measurement of percent body fat, measured by this non-invasive technique.

11.2. Further experimental studies

Suggestions for specific experimental studies that build on the research findings from the current study are as follows.

Measuring plasma triglyceride concentration alone yields limited information. Studies of triglyceride flux (in the fasting state and postprandially) between plasma and the muscle compartment will add to our understanding of ethnic differences in synthesis and clearance of lipids. The availability of a non-invasive method, the nuclear magnetic resonance spectroscopy, has now opened up the possibility for performing such studies. Examples of such studies include examining what happens when one manipulates intramyocellular lipid stores by short-term experimental interventions such as running a marathon or consuming a fatty-meal. Studies can also be designed to measure changes in muscle triglyceride content before and after administration of drugs that modulate (increase or decrease) plasma triglyceride concentration. Such changes should also be measured before and after administration of drugs that modulate insulin sensitivity. It would be of interest to study whether insulin sensitising drugs affect insulin resistance at the level of skeletal muscle, and whether there are any ethnic differences in such effects.

11.3. Suggestions for other studies to take forward the current area of research

11.3.1. The use of electron beam computerised tomography (EBCT)

In the past there has been almost exclusive focus on clinical cardiovascular events as an outcome in epidemiological studies. As such we have little information on the distribution

and determinants of early atheromatous change in the coronary arteries. The advent of EBCT, a rapid, non-invasive method of detecting and quantifying calcification in the coronary arteries, provides us for the first time with a research tool that can define early (pre-symptomatic) atheromatous change in the coronary arteries. This opens up exciting possibilities in the current area of research. Specific suggestions for studies using EBCT are as follows.

To study postprandial lipid metabolism in both ethnic groups in subjects with and without evidence of coronary calcification on EBCT. This will help to determine whether postprandial triglyceride concentration is associated with the presence of coronary calcification, and whether ethnic origin is associated with calcified coronary atheroma. To date there is scant information on the relative importance of early atheroma development and later stages such as arterial occlusion in the pathogenesis of cardiovascular events. It is of scientific interest to find out whether elevated postprandial triglyceride concentration relates only to late stages of atheroma formation manifested by arterial occlusion, or whether it is also associated with early calcification (and atheroma) development.

A further separate suggestion is for a case-control study of the association, in South Asians and Europeans, between CRP concentration and coronary calcification on EBCT. CRP concentration would be measured in those with and without evidence of coronary calcification. This would help to clarify whether inflammatory markers such as CRP concentration are elevated in association with development of coronary calcification, or predate its development. This would be an important contribution as it is not known whether inflammation is a phenomenon arising as a result of development of atherosclerosis, or is causal in the development of atherosclerosis.

11.3.2. Prospective studies

Our finding of a trend towards elevated C-reactive protein concentration in South Asians should be confirmed in larger studies. The ideal setting for this would be a prospective study of stored sera from the Southall Study²⁹, where records were flagged at the ONS (Office of National Statistics) and mortality data are available from death certificates for each person enrolled in that study in 1988-1990. Association between baseline CRP concentration and CHD mortality at 10 years of follow-up can be studied prospectively. If

such an association is found in South Asians it will provide, in a strong research design, the first such data in this group.

Another separate suggestion relates to the use of the stored sera in a prospective study design. While the stored sera were thawed for analysis of CRP concentration, it would be opportune to measure antibodies to *Chlamydia pneumoniae* and *Helicobacter pylori* in the stored sera, to test if the excess CHD incidence in South Asians can be accounted for by an ethnic difference in the prevalence of chronic infections (section 1.6). Additionally the extent to which Lp(a) could account for the ethnic differences in CHD mortality has not been examined. Lp(a) should also be measured on the frozen sera. At the same time there would be the opportunity to measure serum homocysteine, raised levels of which have been shown to be associated with increased cardiovascular disease risk.

11.3.3. Randomised clinical trials

If in larger, prospective studies, elevated levels of CRP are confirmed in South Asians, and an association is found with CHD mortality as has been reported in European populations, and if elevated levels of C-reactive protein are shown to account for some of the excess risk of CHD in South Asians compared with other groups, a recommended next step would be to evaluate the efficacy of aspirin therapy in reducing the high CHD risk in South Asians. The hypothesis would be that aspirin therapy would reduce CHD mortality through reductions in CRP concentrations.

A case for possible randomised trials of fibrate therapy or statin therapy, or both, in South Asians has already been discussed in sections 10.5.3.3 and 10.5.3.4.4.

11.3.4. Studies of the vascular endothelium

The present study has raised the possibility that there is a trend towards higher postprandial lipid concentration in South Asians (which would have been better measured with a more detailed fat tolerance test as discussed before). New evidence is emerging in studies of people of European descent that postprandial lipaemia can affect endothelial function (as discussed in section 2.3.1.1). One recent study³¹⁴ of 44 men showed that endothelial function was impaired in healthy South Asians compared with Europeans, and the defect

was not accounted for by measured coronary risk factors. However, they only measured fasting triglyceride level (which was not significantly different in the two groups). Non-invasive techniques such as brachial artery ultrasound can be easily undertaken in South Asians to assess the effects on the vascular endothelium of postprandial lipaemia.

The reasons for the excess risk of diabetes and CHD in South Asians are still not understood. From the results of this study, in general we have demonstrated that insulin resistance is closely related to plasma lipid levels, but not simply to excess of central obesity or excess of intramyocellular lipid stores. To understand this further we need focused studies of lipid metabolism and its effects on insulin resistance and atheroma.

There are very few research groups addressing the issue in South Asians of metabolic and physiological disturbances associated with insulin resistance, diabetes and CHD. The results from the current study, albeit with its limitations, as well as the suggestions made for further research, should help us to understand this more clearly.

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APPENDIX: FUNDING, SITES, COLLABORATORS, PERSONNEL

Many departments and people enabled this study to be performed and completed. Those who collaborated or helped were as follows:

Funding for this study came from the Wellcome Trust, as part of a clinical training fellowship in Clinical Epidemiology for the registrant, Nita Forouhi.

Supervision was provided by Dr Paul McKeigue. The idea for the study was originally conceived by him. He was available for discussion regarding any problems. He also read the first draft of the dissertation and gave his comments for improvement.

This study was performed in the department of cardiology at Ealing Hospital, Uxbridge Road, Southall, Middx, UB1 3HWI, and was facilitated by Dr Jaspal Kooner. He also kindly arranged for Miss Mary Rowley, research nurse, to assist up to two mornings a week with clinical data collection. CT scans were kindly performed with the agreement of Dr Bill Svensson at Ealing Hospital. Mr Paul Eathorne and Mr Tim Rabbitte performed all the scans.

DEXA scans were performed by Mr Uday Bhonsle at the Medical Physics Department of the Northwick Park Hospital, Watford Road, Harrow, Middlesex. He provided the final percent fat data to the registrant.

Magnetic resonance spectroscopy was undertaken at the Robert Steiner MRI Unit, Hammersmith Hospital, Du Cane Road, London, with the collaboration of Dr Jimmy Bell. Louise Thomas, Gabby Jenkinson and S Mierisova performed the scans and analysed the computer output for the scans.

The laboratory analyses were undertaken at the Wynn Division of Medicine, Wellington Road, London, NW1. The lab work was mainly performed by Mandeep Sidhu, Melik Worthington and Tony Proudler under the supervision of Dr Ian Godsland and Dr David Crook. The chylomicron separation protocol was developed by Dr David Crook.

The analysis of C-reactive protein was very kindly performed by Dr. Naveed Sattar of the department of Pathological Biochemistry at the Glasgow Royal Infirmary.

The following general practitioners assisted with subject recruitment. Dr Chatrath and Dr Choudhury, Drs Thomas, Gill and Patel, Drs Jenkins and Heavey, and Dr Irene Ross.

Dr Saiqa Mullick attended during clinical data sessions. She helped with clinical data collection.

Mrs Jan Bibi Mazar, research assistant, helped with data entry and in booking appointments for the study participants. Mrs Sheelagh Kerr set up the FoxPro database for data entry. Mrs Christine Brandon-Jones gave advice regarding preparing small labels for subject identification, and helped with teaching the registrant how to use the File Maker Pro database.

Mr Stephen Sharp, medical statistics unit at the London School of Hygiene & Tropical Medicine, helped with writing a programme to calculate the insulin sensitivity index, and with generating the graphs for the mean glucose values against time. Mr Ian White, also of the same unit, helped with the original sample size calculations for the study.

Dr Janendrappa, a visiting clinical fellow from Bangalore, India, attended some clinical sessions during his short stay in London. During these he helped with preparing sample collection tubes.

Lucozade for the study was donated by Smith Kline Beecham.

The responsibility for the study rested with the registrant. She attended every single clinical session and did all the data collection personally. She also prepared the fat meal for the fat tolerance test. All the insulin tolerance tests were personally performed by her. She managed all the day to day running of the project, from grant proposal stage, through to completion of the study. The ordering of equipment and managing of the budget, secretarial work and all management and administrative issues were dealt with by her. The registrant performed all the statistical analyses and wrote the papers for publication. This dissertation has been written by the registrant.

