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NON-INSULIN-DEPENDENT DIABETES
AND
CORONARY HEART DISEASE
IN MIDDLE-AGED ~~BRITISH~~ MEN

by

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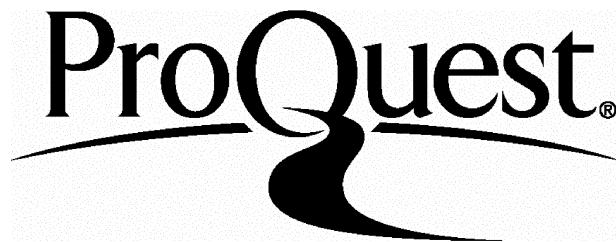
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ABSTRACT

This thesis addresses the aetiology of non-insulin dependent diabetes (NIDDM) and the association between NIDDM and coronary heart disease (CHD). Hypotheses regarding the causal role of obesity, physical inactivity, alcohol consumption and cigarette smoking in the development of NIDDM have been tested. The hypothesis that NIDDM shares common risk factors with coronary heart disease, factors which are associated with insulin resistance has also been addressed.

Associations between lifestyle and biological CHD risk factors and the risk of developing NIDDM were examined in a longitudinal study, involving a representative sample of 7735 middle-aged British men, followed for an average of 12.8 years. In a cross-sectional study, associations between baseline non-fasting serum insulin levels and CHD risk factors were examined in 5556 men. In further longitudinal studies, insulin levels at baseline were related to the subsequent development of both NIDDM and major CHD events and associations between both established NIDDM and non-fasting serum glucose and incident major CHD events in this cohort were also examined.

There were 194 new cases of NIDDM in the longitudinal study. Obesity and physical inactivity were associated with higher risk and moderate alcohol intake with lower risk of NIDDM (relative to occasional drinkers) in multivariate analysis. Men destined to develop NIDDM during follow-up had an adverse coronary risk factor profile at baseline. However some important CHD risk factors, notably serum total cholesterol concentration did not predict NIDDM and the effect of cigarette smoking was equivocal.

In cross-sectional analyses, CHD risk factors such as physical inactivity and hypertriglyceridaemia, which were predictive of NIDDM in the longitudinal study, were associated with hyperinsulinemia at the baseline examination.

A J-shaped relation between serum insulin levels and risk of NIDDM was observed which is consistent with an early and fundamental role for insulin resistance in the development of this condition.

Established NIDDM was associated with an approximately 2-fold increased risk of CHD events during follow-up, independent of other CHD risk factors. In non-diabetics, a weak non-linear association between non-fasting glucose and incident CHD events (N=704) was observed which was non-significant in multivariate analysis. A similar non-linear association between serum insulin concentration at screening and major CHD events during follow-up was observed. There was an almost 2-fold increased relative risk of major CHD events in the 10th decile of the serum insulin distribution relative to the 1st to the 9th deciles combined which was significant in multivariate analysis.

The findings support the hypothesis that non-insulin dependent diabetes and coronary heart disease share common lifestyle and biological risk factors which are associated with hyperinsulinemia and insulin resistance. There is however a major component of CHD risk which is not related to insulin resistance and diabetes.

To Mary, the Sunday in my every week

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DECLARATION OF AUTHORSHIP

The candidate has taken responsibility for all aspects of the work presented in this thesis from its inception. He initiated the work on diabetes upon which the thesis is based and has supervised the assembly of the relevant data. He has planned and conducted all of the analysis, with assistance from a professional statistician (Dr SG Wannamethee) and he has written all sections of the thesis.

The thesis includes some material which has been submitted for the membership diploma of the Faculty of Public Health Medicine. This material is based on a subset of the data presented in Chapter 4.

ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION, OBJECTIVES AND TERMINOLOGY

Introduction

Non-insulin-dependent diabetes (NIDDM) is a common condition affecting at least 3% of the middle-aged and elderly population of Britain (Neil *et al*, 1987; Williams, 1994), with considerably higher prevalence rates in specific ethnic groups, (Cruickshank, 1990). NIDDM is associated with considerable morbidity and premature mortality, both from specific diabetic complications and from cardiovascular disease, which is accelerated in the presence of diabetes (Jarrett, 1983; Pyörälä & Laakso, 1983). Though considerable work has been undertaken to identify the major risk factors for coronary heart disease (CHD) such as raised blood cholesterol, cigarette smoking and raised blood pressure, there has been relatively little epidemiological work on risk factors for NIDDM in the general population. Moreover, the nature of the relation between NIDDM and CHD has not been well elucidated.   Advancing age, obesity, body fat distribution and a positive family history of diabetes are among the well established risk factors for this condition (Everhart *et al*, 1985). There is now increasing evidence that in some populations NIDDM shares common causal factors with cardiovascular disease and in particular with coronary heart disease (Jarrett & Shipley, 1988). This observation focuses attention on a wider range of environmental factors in the development of NIDDM, such as physical activity level, alcohol intake and cigarette smoking and on the possible role of biological CHD risk factors such as dyslipidaemia and hypertension as predictors of NIDDM.

Elevated circulating insulin levels (hyperinsulinaemia) reflect resistance to insulin-mediated glucose uptake from the circulation into skeletal muscle and other tissues, i.e. insulin resistance. There is considerable evidence that hyperinsulinemia antedates NIDDM (Lillioja *et al*, 1993) and that elevated circulating insulin levels are linked with dyslipidaemia, hypertension and a number of other CHD risk factors (Abbott *et al*, 1987;

Zavaroni *et al*, 1989; Stout, 1990; Laws & Reaven, 1993; Savage & Saad, 1993). Insulin

resistance with compensatory hyperinsulinemia has therefore been proposed as the

unifying link between cardiovascular disease and NIDDM (Reaven, 1988; Stern, 1995).

However this model of the pathogenesis of NIDDM (with insulin resistance as an early

"primary" abnormality) and of the relation between NIDDM and CHD is controversial

(Taylor *et al*, 1994; O'Rahily *et al*, 1994).

Research objectives

This thesis addresses the aetiology and pathogenesis of non-insulin dependent diabetes

(NIDDM) and examines the association between NIDDM and coronary heart disease. The

research upon which the thesis is based has had two major inter-related objectives:

(i) to test, in a longitudinal study, hypotheses regarding the causal role of specific

lifestyle related factors in the development of NIDDM (obesity, physical inactivity,

alcohol consumption and cigarette smoking)

and

(ii) to test the hypothesis that NIDDM shares common risk factors[1] with coronary heart

disease, factors which cluster with insulin resistance.

Details of the specific studies which address these research objectives are set out

in the methods section, *Chapter 3*.

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[1]

The distinction between risk factors and causal factors is important. A cause of a disease
can be defined as an event, condition or characteristic that plays an essential role in
producing **an** occurence of the disease (Rothmann, 1986). Hence physical inactivity may
be a causal factor in the development of NIDDM. The term "risk factors" is used to
describe factors that are associated with risk of development of a disease and includes
factors which may be causal, together with markers of increased susceptibility, secondary
manifestations of underlying causal factors and early symptoms of disease.

Research setting

The work which forms the subject of this thesis was carried out in the University Department of Public Health, Royal Free Hospital, London. The work is based on a 12 year follow-up study of predictors of NIDDM and of coronary heart disease in a population based sample of 7735 middle-aged men, recruited for the British Regional Heart Study (Shaper *et al*, 1981; Shaper *et al*, 1985[a]).

Terminology and diagnostic criteria for diabetes and impaired glucose tolerance

Until the late 1970's epidemiological work in diabetes was hampered by the lack of both uniform diagnostic criteria for diabetes and a uniform, rational classification system. Although Himsworth had distinguished between "insulin-sensitive and insulin-insensitive types of diabetes" in 1942 (Himsworth & Kerr, 1942), there was a failure to appreciate that diabetes is not a single disease entity but rather a syndrome of sustained hyperglycaemia associated with a heterogenous group of disorders.

Diagnosis

Using current WHO criteria, diabetes may be diagnosed on the basis of fasting, post-glucose load or random blood glucose levels (WHO Study Group, 1985). A fasting venous plasma (or serum) glucose level ≥ 7.8 mmol/L and/or a 2 hour post load (75 g glucose in adults) level ≥ 11.1 mmol/L are considered diagnostic of diabetes. A random plasma glucose level ≥ 11.1 mmol/L is also regarded as diagnostic of diabetes mellitus. Impaired glucose tolerance is diagnosed where the 2 hour venous plasma glucose concentration is between 7.8 - 11.1 mmol/L and the fasting glucose level is less than 7.8 mmol/L. Slightly lower glucose levels are specified for venous whole blood as opposed

to venous plasma and higher levels for capillary samples.

In all cases confirmatory measurements are recommended to establish the diagnosis at the individual level. For epidemiological purposes however, certainty of diagnosis at the individual level is obviously neither attainable nor necessary and in the current WHO guidelines a single 2-hour post load sample $\geq$ 11.1 mmol/L is regarded as sufficient to diagnose diabetes in epidemiological studies. The distribution of plasma glucose both fasting and post glucose load is conveniently bimodal in some populations, notably the Pima Indians, Nauruans and Mexican Americans (Bennett *et al*, 1976; Zimmet *et al*, 1977). In Caucasian populations the two hour post glucose challenge level, has a unimodal positively skewed distribution, with a steep increase in the risk of subsequent "diabetic" microvascular disease at levels exceeding 11.1 mmol/L (Jarrett & Keen, 1976; Al Sayegh & Jarrett, 1979). Accordingly the current diagnostic criteria for diabetes are not entirely arbitrary, though it must be emphasised they are based on a relatively small number of population studies.

Classification of diabetes mellitus

The classification of diabetes mellitus remains largely clinical rather than aetiological. Sustained hyperglycaemia, the defining characteristic of the diabetic syndrome, may be due to either absolute or relative deficiency of insulin. While a large number of underlying causes of this syndrome are known, two major clinical classes of diabetes mellitus are described in the current (1985) WHO classification system: insulin dependent diabetes (IDDM) where insulin deficiency is generally absolute and non-insulin dependent diabetes (NIDDM), characterised (generally) by relative insulin deficiency, i.e. insulin resistance. The current WHO classification system includes malnutrition related diabetes

mellitus (MRDM) and gestational diabetes in a separate "other type" category. Among the "related diagnostic categories" in the current classification system is impaired glucose tolerance (IGT).

Insulin dependent diabetes (IDDM) is most common in youth and is associated with autoimmune phenomena such as islet cell antibodies. IDDM generally has an abrupt onset with ketoacidosis, and it is lethal without insulin. Non-insulin dependent diabetes mellitus (NIDDM) by contrast, is generally a disease of middle age and autoimmune phenomena are not prominent. The onset of NIDDM is usually insidious, is rarely associated with ketoacidosis and insulin is not required for long term survival. Obese and non-obese sub classes are described with a BMI of 27 kg/m² in men and 25 kg/m² in women as the arbitrary dividing line (WHO, 1980; WHO, 1985).

Impaired glucose tolerance, may be regarded as the transition zone between normal glucose tolerance and diabetic hyperglycaemia. IGT is an inherently unstable diagnostic label given the variability of serum glucose levels post glucose load (McDonald *et al*, 1965) and the relatively small section of the population glucose distribution (1 SD approx.) which is encompassed by the defining glucose levels. For instance, in a study of 67 subjects with IGT, it was found that only 56% remained in that category when retested 2-4 months later (Riccardi *et al*, 1985). IGT progresses to overt NIDDM at rates of 2-3% per year (Jarrett *et al*, 1982). As with overt NIDDM, there is evidence that mortality from macrovascular disease is increased (Kannel & McGee, 1979[b]; Fuller *et al*. 1983), but specific "diabetic" microvascular complications are rarely observed. The risk of progression to NIDDM is related to the degree of hyperglycaemia and is not a simple function of having IGT (Jarrett *et al*, 1982). Hence, at the population level there is a continuum of glucose intolerance ranging from IGT to

clinically overt NIDDM.

Jarrett has suggested that we should dispense with the label IGT and think rather in terms of the degree of glucose intolerance (Jarrett, 1987). Zimmet went further in suggesting that in view of the inter-relationships between NIDDM, obesity, hypertension, and dyslipidaemia and their probable common genetic and lifestyle antecedents, that NIDDM should probably not be regarded as a discrete disease entity but rather a "risk factor state" (Zimmet, 1989). This argument is difficult to sustain. It ignores the cardinal feature of diabetes, microvascular disease or the "spot in the eye in 20 years time" (Alberti, 1993). It also ignores the evidence that NIDDM is a heterogenous condition and the extent to which the clustering of vascular risk factors with NIDDM varies within and between populations (Chaturvedi et al, 1994; Cowie et al, 1994). These issues, the clustering of vascular disease risk factors with NIDDM and the relation between NIDDM and cardiovascular disease are central to this thesis.

Problems with the classification and diagnosis of diabetes

Diabetes, in common with virtually all other diseases, is a quantitative phenomenon. Attempts to place individuals into discrete diagnostic categories, while necessary for clinical decision making and for case definition in research, inevitably obscures the subtleties of the expression of disease both in individuals and in populations. Alberti emphasises the extent to which the current definition of NIDDM is one of exclusion (Alberti, 1993). Essentially all individuals with sustained hyperglycaemia who do not require insulin for survival and who do not meet the criteria for one of a small number of special categories such as "maturity onset diabetes of the young" (MODY), are regarded as having NIDDM. This obviously increases the probability that the NIDDM

category contains a mixture of aetiologically distinct conditions, a proposition for which there is increasing evidence (Alberti, 1993). For instance, it is currently estimated that 2- 4% of all cases of diabetes presenting in adult life are associated with specific, identifiable gene mutations, such as glucokinase gene mutations, mutations involving mitochondrial DNA and other mutations involving insulin, the insulin receptor, glycogen synthase and the β-3 adrenergic receptor (Zimmet, 1995).

Even the distinction between IDDM and NIDDM is less than sacrosanct. For instance, certain autoimmune phenomena, characteristic of IDDM, are seen in some patients with NIDDM and the offspring of persons with NIDDM are at increased risk of developing both types of diabetes. HlA haplotypes, previously regarded as specifically associated with IDDM have been shown to be also associated with NIDDM (Tuomilehto-Wolf *et al*, 1993). In older patients, intermediate insulin dependency is increasingly recognised. Zimmet and colleagues have presented data which suggest that in some populations up to 20% of cases of adult onset diabetes and possibly 50% of cases of "non-obese NIDDM", have a variant of insulin dependent diabetes of insidious onset. This form of diabetes, referred to as "latent autoimmune diabetes in adults" (LADA), is associated with long-term persistence of auto-antibodies against glutamic acid decarboxylase (GAD) a pancreatic β-cell constituent (Zimmet, 1995). Hence the preference, in the later 1985 WHO recommendations, for descriptive clinical terms without aetiopathogenic implications such as insulin-dependent and non-insulin dependent diabetes rather than "type 1" and "type 2" diabetes as in the earlier 1980 classification system was well founded (WHO, 1985). In essence, the inadequacies of the current classification systems for diabetes reflect our limited knowledge of aetiological factors and pathophysiological mechanisms. Further refinements in the classification of NIDDM

will undoubtedly emerge as understanding of the molecular genetics of insulin resistance and insulin hypo-secretion increases (Weir, 1995). Additional data which clarify the relative importance of specific markers of autoimmune phenomena and specific genetic defects at the population level will also be required.

The diagnosis of diabetes poses additional problems which are critical to the interpretation of epidemiological studies of NIDDM and which have been well reviewed by Alberti (Alberti. 1995). There are particular problems with the diagnosis of NIDDM in asymptomatic individuals whose blood glucose is at the lower end of the diabetic range. Despite the precise cut-off points for diagnosis as set out in the current recommendations (WHO, 1985), there is confusion regarding the difference between venous whole blood and plasma glucose levels. For instance it has been shown that a venous whole blood glucose level of 10 mmol/L is equivalent to a plasma value of 11.4 mmol/L rather than 11.1 mmol/L as implied in the current WHO recommendations. There is also controversy regarding the precise fasting glucose level which corresponds with a post load glucose of 11.1 mmol/L, a relatively consistent predictor of diabetic microvascular disease. Though these are relatively trivial differences at the individual level, it is clear that the prevalence of NIDDM at the population level will vary depending on whether the diagnosis is based on venous whole blood or plasma glucose levels or on fasting or post-load glucose levels.

Additional problems relate to the inherent variability of the oral glucose tolerance test (OGTT), regarded as the "gold standard" diagnostic test in epidemiological studies. The OGTT has a coefficient of variation of up to 40% on a day-to-day basis within individuals (McDonald et al. 1965). In a population of elderly people (64 to 87 years),

examined annually for 5 years the reliability[2] coefficient of the OGTT for the diagnosis of diabetes according to WHO criteria was 0.62 (Feskens *et al, 1991*). As with blood pressure measurement, it appears that habituation to the procedure contributes to this within subject variation. Yudkin and co-workers have presented data from an epidemiological study of diabetes prevalence in Tanzania in which over 6000 people had a standard glucose tolerance test, with an 8% stratified sub-sample having a repeat test within seven days. When the population distribution of 2-hour blood glucose concentration was reconstructed from this sub-sample there was a highly significant decrease in mean blood glucose level and the estimated population prevalence of impaired glucose tolerance fell by almost 60% (Yudkin *et al*, 1990). The authors point out that rather than merely showing glucose tolerance test variability and regression to the mean, this population demonstrated an apparent stress effect on glucose tolerance analogous to the "arousal" or defence" reaction described for blood pressure (Yudkin *et al*, 1990). This observation has clear implications both the reliability of the OGTT and for international or inter-cultural comparative data on NIDDM prevalence.

There are further problems with the OGTT which contribute to between study and between subject measurement variability or error. Although the WHO and the U.S. National Diabetes Data Group (NDDG, 1979) have agreed on the size of the glucose load (at 75g) it has become apparent that many epidemiological studies have been carried out with glucose monohydrate which contains only 68g of glucose. There is also the difficulty of ensuring that subjects are truly fasting and the fact that although the dose of oral

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Reliability refers in essence to the ratio of within subject measurement variation to between subject variation. It is study specific and is increasingly recognised as a key issue in epidemiological study design.

glucose may be standardised there will be marked variation in glucose load expressed per kilogram body weight.

Given the increased awareness of problems with the OGTT (Stolk *et al,* 1995), there is increasing interest in the use of fasting glucose or glycated haemoglobin to define glycaemic status in both the clinical and epidemiological setting. In recent work from the Pima Indians population in Arizona, McCance and colleagues (1994[a]) compared the sensitivity and specificity of 2-hour plasma glucose, fasting plasma glucose, and glycated haemoglobin against prevalent retinopathy and nephropathy and incident retinopathy and nephropathy during 5 years of follow-up. In the cross-sectional data, frequency distributions of logarithms of the three measures of glycaemic status were bimodal and for each measure antimodal cut-off points had comparable sensitivity and specificity for the prevalence and incidence of retinopathy. No measure performed well as a predictor of nephropathy (McCance *et al,* 1994 [a]).

Insulin resistance

Insulin resistance has been defined succinctly as " a state (of a cell, tissue, system or body) in which greater than normal amounts of insulin are required to elicit a quantitatively normal response (Berson & Yalow, 1970). The hyperinsulinaemic euglycemic clamp technique is currently regarded as the gold standard for the assessment of insulin resistance (DeFronzo *et al,* 1979). In this method insulin is infused systemically (usually at a rate of 40 mU/m^2 per min) and plasma glucose is maintained constant by means of a glucose infusion. Glucose is "clamped" at the ambient glucose concentration. The glucose infusion rate required to maintain euglycemia at a given insulin concentration reflects the combined effect of an insulin induced decrease in

glucose production from the liver and stimulation of glucose uptake into extra-hepatic tissues such as skeletal muscle. Since the liver takes up very little glucose in the presence of euglycemia, an increase in glucose uptake reflects enhanced glucose disposal by extra-hepatic tissues. When combined with isotope dilution methods which measure glucose turnover, this method can provide a direct assessment of the effects of insulin on hepatic and extra-hepatic tissues (Alzaid & Rizza, 1993). Clearly this method, which is labour intensive and requires considerable investigator skill, does not lend itself to use in epidemiological studies. Moreover, glucose clamp and other methods which rely on intravenous infusions of glucose and/or insulin (Bergman *et al*, 1979; Hosker *et al*, 1985) are un-physiological, bypassing the complex regulation of insulin secretion and its hepatic extraction following glucose and nutrient absorbtion from the gut to the liver (Cruickshank, 1995). Other methods of assessing insulin sensitivity in vivo, such as the organ perfusion technique (Zierler, 1961), are even more invasive than the glucose clamp method. Earlier methods, based on the oral glucose tolerance test (Yalow & Berson, 1960; Himsworth, 1939) or on an insulin tolerance test, are either poorly reproducible or induce changes in counter-regulatory hormones which limit interpretation of the findings (Alzaid & Rizza, 1993). The homeostasis model assessment (HOMA), which is based on basal fasting glucose and insulin measurement (Mathiews *et al*, 1985), has the advantage of simplicity and a reasonable approximation to physiological conditions. However it is obviously dependent on the reliability of glucose and insulin measurement and on the assumptions of the mathematical model upon which it is based.

In a study of 155 non-diabetic offspring of diabetic parents, two relatively invasive and un-physiological measures of insulin resistance, one based on the intravenous glucose tolerance test and one on Bergman's minimal model technique, were shown to predict

NIDDM during an average follow-up period of 25 years (Martin *et al*, 1992). If we accept that insulin resistance antedates NIDDM, it could be argued that these findings support the reliability and the predictive validity of measures of insulin resistance based on intravenous infusions of glucose and/or insulin. However with regard to predictive validity, there is an obvious danger of circularity in this argument.

In epidemiological studies fasting and post-load insulin measurements are used as markers of insulin resistance. Laakso has studied correlations between fasting and post-load insulin and insulin resistance as determined using the hyperinsulinaemic euglycemic clamp technique (Laakso, 1993). In persons with normal glucose tolerance, whole-body glucose uptake was inversely correlated with fasting and post-load insulin, with correlations ranging from r=-0.58 to r=-0.74. In subjects with impaired glucose tolerance and NIDDM, only the fasting insulin level was significantly associated with whole-body glucose uptake during the glucose clamp technique. The latter finding obviously reflects the insulin secretory defect in IGT and established NIDDM. This paper is cited in support of the use of fasting and post load insulin measurements as markers of insulin resistance in persons with normal glucose tolerance even though approximately two thirds of the variation in the "gold standard" measurement was not explained by variation in fasting and post-load insulin measurements. Hence, these data do not suggest that a single measurement of insulin (whether fasting or post load) provides a highly reliable measure of insulin resistance as defined on the basis of glucose clamp methods. In this thesis data based on non-fasting insulin measurements are presented. It is accepted that measurement of non-fasting insulin is likely to provide a less reliable index of insulin resistance than measurement of fasting or post-load insulin, reflecting greater random within-subject variation. However, non-fasting (post-prandial) insulin is undoubtedly the

physiologically relevant measurement and it is arguable that with adjustment for time of sampling the extent of random error will not be appreciably greater than that associated with fasting or post-load insulin measurement.

Physician diagnosed NIDDM

The prospective study of NIDDM incidence presented in this thesis is based on physician diagnosed cases of NIDDM. Clearly, this method of case ascertainment is specific but not sensitive. NIDDM is characterised by the insidious onset over several years of progressive metabolic abnormalities that lie towards the extreme end of the population spectrum of glucose tolerance. Medical attention is generally not sought until the metabolic abnormalities are well established and the probability of 'diagnosis' is heavily influenced by the intensity of opportunistic screening. In the U.S. it is estimated that there is an average delay of between four and seven years between the onset of NIDDM and clinical diagnosis (Harris, et al, 1992) and in Britain it is estimated that the ratio of diagnosed to undiagnosed diabetics lies between 1:1 and 2:1 (Williams, 1994). Despite these problems, physician diagnosed NIDDM has been the end-point in the majority of prospective, within-population studies of aetiological factors in NIDDM to date. Much of the error due to under-ascertainment of cases will be random and should therefore not invalidate within-study comparisons. There is a need, however, to consider the possibility of systematic error (or ascertainment bias) in situations where the exposure may influence the probability of screening. However it is important to remember that the gold standard, glucose tolerance test is not without problems of random and systematic error. It should be noted also that there are remarkably consistent findings on the relation between certain exposures (such as physical activity) and physician diagnosed NIDDM in studies conducted in diverse health care settings (Helmrich et al, 1991; Burchfiel et al, 1995).

CHAPTER 2

BACKGROUND

NIDDM: the burden of disease

Current estimates of the age adjusted prevalence of clinically diagnosed diabetes in England range between 1.05% and 1.36% (Nabarro, 1988). Between 85% and 90% of cases are due to NIDDM (Neil et al, 1987). There has been an apparent increase in the age adjusted prevalence of known diabetes in Britain in recent decades, though the extent to which this reflects a true increase in incidence rather than changes in detection is uncertain (Neil et al, 1987). Nation-wide glucose tolerance test data on the prevalence of impaired glucose tolerance, undiagnosed and diagnosed NIDDM by age, sex, region and ethnic group are not available in Britain. On the basis of the second National Health and Nutrition Examination Survey (NHANES II), it is estimated that one in nine Americans (between age 20 to 74 years) meet NDDG criteria for NIDDM or impaired glucose tolerance (Harris et al, 1987).

The population characteristics that most strongly influence the prevalence of diabetes are age, sex and ethnic mix. Males, the elderly and (in Britain) people of Asian and African-Caribbean origin have higher prevalences. For instance in Britain, among Asian males in the 60-79 year age group, a prevalence of 17.3% is documented for clinically diagnosed diabetes as compared with 4.6% among European men in the same age group (Williams, 1994; Simmons et al, 1989). If we assume approximately similar ratios of diagnosed diabetes to undiagnosed NIDDM and impaired glucose tolerance as in NHANES II (Harris, 1987), then more than 50% of Asian men in this age group are likely to have clinically significant glucose intolerance.

The aetiology of NIDDM

The role of genetic factors in NIDDM aetiology has long been stressed (Barnett et al,

1981) and in a number of rare variants of the NIDDM syndrome, single gene defects have been identified (Zimmet, 1995). More recently, evidence has been presented to suggest a possible role for factors acting in utero in the development of NIDDM (Hales *et al*, 1991; McCance *et al*, 1994 [b]). It is clear however, that environmental factors interact with the genome (and possibly with factors acting in utero) to determine both the ultimate probability of disease and the age of onset (Kahn, 1994). Indeed, review of the occurrence and distribution of NIDDM, both within and between populations and over time, provides compelling evidence of a major role for environmental factors acting in adult life in the development of this condition (Zimmet, 1982; Jarrett, 1989; Zimmet, 1995). In particular, data from ecological and migrant studies of NIDDM suggest an aetiological role for obesity, a sedentary lifestyle and possibly additional factors (as yet uncharacterised) associated with "modernisation" or "westernisation" such as psychosocial stress (King & Rewers 1993).

In this chapter the epidemiology of NIDDM will be reviewed with a particular focus on modifiable factors which are addressed in this thesis, i.e. obesity, physical activity, alcohol intake and cigarette smoking. The evidence suggesting a role for these factors will be considered, using data from ecological studies and from observational studies based on individuals. Data on the relation between NIDDM and cardiovascular disease will be reviewed with a focus on the evidence that NIDDM and coronary heart disease share common risk factors. A critical review of the data on additional important and/or relevant lifestyle and biological factors in the development of CHD will not be undertaken. These factors include high dietary saturated fat intake (LaRosa *et al*, 1990), obesity (Manson *et al*. 1995), cigarette smoking (Shaper *et al*, 1985[a]), physical inactivity (Shaper & Wannamethee, 1991), alcohol intake (Shaper *et al*, 1994),

dyslipidemia [especially raised total and LDL-cholesterol (Rose & Shipley, 1986) and lower HDL-cholesterol levels (Jacobs et al, 1990) and hypertension (Collins et al, 1990).

Current knowledge of the pathogenesis of NIDDM will be reviewed at the outset. This work is both critical to an understanding of the wider epidemiological picture and it is relevant to the data which will be presented on serum insulin levels and risk of NIDDM in the British Regional Heart Study cohort.

NIDDM: Pathogenesis

NIDDM is characterised by reduced tissue sensitivity to insulin mediated glucose uptake (insulin resistance, particularly in skeletal muscle, fat and liver), abnormalities in glucose mediated insulin secretion from pancreatic β-cells and elevated hepatic glucose production (DeFronzo, 1988). A central feature of the syndrome is the inability to increase the rate of insulin mediated glucose uptake (mainly into liver and muscle) to meet the substantial demand of post prandial glucose absorbtion (Alzaid & Rizza, 1993). Insulin resistance in NIDDM reflects receptor and post receptor defects and to an uncertain extent the effects of circulating factors such as glucocorticoids, catecholamines, free fatty acids and hyperglycaemia itself (Alzaid & Rizza, 1993; Laakso, 1994). There has been prolonged debate on the primary defect in NIDDM, insulin hyposecretion due to a primary pancreatic defect of obscure origin or insulin resistance with hyperinsulinaemia and pancreatic exhaustion (O'Rahily et al, 1994; Taylor et al, 1994). Data are available to support both of these hypotheses (O'Rahily et al, 1986; Pimenta et al, 1995; Warram et al, 1990; Lillioja et al, 1993). It is accepted however that both defects are required to produce clinically manifest NIDDM. It is likely that much of the controversy in this area

reflects the varying sophistication of different methods of assessing early, subtle defects in insulin sensitivity and pancreatic hyposecretion (Weir, 1995). Until recently (Pimenta *et al*, 1995), methods for measuring insulin sensitivity seem to perform better in this regard than methods for measuring insulin secretion. However the key question relates not to which defect come first but which genetic and environmental factors (particularly the latter unless we envisage mass pharmacological interventions) are most important in the development of NIDDM. Indeed regardless of the "primary" or "most important" defect in NIDDM, there is considerable evidence that insulin resistance is an early and modifiable defect in the pathogenesis of this condition (DeFronzo, 1988). Moreover the hypothesised common risk factors which link NIDDM and CHD (as addressed in this thesis) predict an early role for insulin resistance in the pathogenesis of NIDDM. However much of the current data suggesting that insulin resistance with compensatory hyperinsulinaemia is an early event in the pathogenesis of NIDDM is based on insulin assays which have been unable to distinguish insulin from proinsulin and other insulin precursors. Hence it is argued that hyperinsulinaemia in subjects who subsequently develop NIDDM in longitudinal studies, may largely reflect increased secretion of insulin precursors due to pancreatic failure (Temple, 1989). This issue is addressed directly in this thesis, in a longitudinal study of the association between true serum insulin levels (measured with a specific assay) and the development of doctor diagnosed NIDDM during follow-up which has extended for over a decade.

NIDDM is undoubtedly a heterogenous disorder (Taylor *et al*, 1994; Weir, 1995) and it would appear that the relative importance (in terms of pathogenesis) of insulin hyposecretion or insulin resistance varies within (Banerji & Lebovitz, 1991) and possibly between populations (Chaturvedi *et al*. 1994). This hetrogeneity may explain the different

associations between NIDDM and coronary heart disease in different populations such as the Afro-caribbeans (Chaturvedi *et al*, 1994) and South Asian populations (McKeigue *et al*, 1991) in Britain.

NIDDM: Genetic studies

Neel (1965) has referred to diabetes as a "geneticist nightmare". It is a heterogenous condition, somewhat arbitrarily defined, commonly sub-clinical with a late and variable age of onset, and it is influenced in its manifestations by environmental factors. Indeed from both animal work (Gauguier *et al*, 1990) and family studies (Knowler *et al*, 1993) it is becoming clear that among the environmental factors to be considered is fetal hyperglycaemia which may lead to impaired glucose tolerance in the adult, independent of genetic transmission. There is clearly however, a major genetic contribution to the development of NIDDM with concordance rates as high as 91% reported among monozygotic twins and a four fold increased risk of disease in monozygotic as compared with dizygotic twins (Barnett *et al*, 1981; Harvald & Hauge, 1963). It is clear also that environmental factors interact with the genome to determine both the ultimate probability of disease and the age of onset (Rossini *et al*, 1988; Knowler *et al*, 1993).

Twin studies

Pyke's UK twin study (Barnett *et al*, 1981) has been particularly influential in establishing the concept that genetic factors have a major role in the aetiology of NIDDM. Of 53 confirmed monozygotic twins, 48 were concordant for NIDDM and in the 5 discordant pairs they report metabolic abnormalities suggestive of "early diabetes" in the non-diabetic twin. Unfortunately data on body weight at the time of diagnosis were

available for only 21 twin pairs. However concordance for obesity in this sub-sample was not high. Subjects recruited for this study were largely self referred from all over the United Kingdom as a result of press and television publicity. Accordingly they represent a small and highly selected sample of the population of individuals with NIDDM in the UK. As subjects were included in the study not because they were twins but because they were diabetic, concordant twin pairs had at least twice the chance of ascertainment as discordant pairs. The concordance rate therefore must be regarded as an estimate. A later US study (Newman et al, 1987) which was relatively free of ascertainment bias suggest a concordance rate of approximately 60%.

The nature of the genetic defect

Considerable speculation has focused on the precise genetic defect in NIDDM. The unimodal, positively skewed distribution of serum glucose levels in Caucasian populations suggests a heterogenous polygenic/environmental disorder. Hopes that recombinant DNA technology would lead to the chromosomal localisation of the offending gene(s) in the common variants of NIDDM have not yet been realised. The late age of onset, the somewhat arbitrary diagnostic criteria for NIDDM, the heterogenous phenotype (with defects of both insulin action and secretion, at the minimum) and the relatively high prevalence of the condition all impede efforts to find the large, well defined "pedigrees" required for linkage analysis (O'Rahilly, 1988). However this may change rapidly as the traditional candidate gene linkage study is augmented by sibling pair studies, employing "positional search" methods (Elbein et al, 1994)[3]. As discussed in *Chapter 1*, a number

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Two fundamentally different approaches have been used to study the genetics of NIDDM: the study of specific cloned genes which are plausibly linked with the pathogenesis of

of specific genetic defects which are associated with the NIDDM phenotype are described, of which the most important are mutations associated with an insulin secretory defect involving the glucokinase gene. However these defects together account for less than 5% of diabetes in adults (Zimmet, 1995). Work on major candidate genes such as those coding for glycogen synthase and GLUT4 (an intracellular glucose transporter) has been negative (Laakso, 1994). Insulin receptor substrate-1 (IRS-1) a key protein in intracellular insulin action and a potential mediator of insulin resistance is currently attracting attention. In a population based study involving a group of 380 young adults of European ethnic origin, 9% of subjects were found to be hetrozygous for one specific IRS-1 gene variant. While no direct associations with insulin resistance were detected in cross-sectional analyses, a significant interaction between obesity (BMI ≥ 25 kg/m2) and this gene polymorphism and features of the insulin resistance syndrome was observed (Clausen et al, 1995). The importance of this study lies less with the specific genetic defect than with the acknowledgement of the need to look for interactions with factors such as obesity in studies of the genetics of NIDDM. In further studies, which suggest that NIDDM and obesity may share some common genetic susceptibility factors, a common mutation for the gene encoding for the ß-adrenergic receptor in visceral fat has been show to be linked to both obesity and insulin resistance (Walston et al, 1995; Widén et al, 1995; Clément et al, 1995).

The "thrifty genotype"?

NIDDM (candidate genes) and the use of regularly spaced markers that span human chromosomes in an attempt to determine the location of genes associated with NIDDM without prior knowledge of the physiological importance of the locus (positional search). Sibling pair studies have an advantage over family linkage studies in that one does not require a well defined model of inheritance.

Neel emphasised the importance of a genetic environmental interaction in diabetes when in 1962, he proposed the "thrifty genotype" hypothesis to explain the high frequency in some populations of an apparently genetic disease with an adverse effect on survival and reproduction (Neel, 1962). He speculated that the "genetic genotype" must have conferred some survival advantage such as the ability to store food as fat when food was abundant, thereby increasing the probability of survival in conditions of alternating feast or famine. He hypothesised that the "thrifty gene" might be linked to rapid insulin secretion after a meal, a "quick insulin trigger" which would facilitate fat storage. While the genetic and pathophysiological basis for this hypothesis has clearly undergone considerable modification over the years, it has provided a useful model for work in the area of genetic environmental interaction. The rapid emergence of NIDDM and other features of the insulin resistance syndrome with Westernisation in diverse populations such as the Pima Indians (King & Rewers, 1993), Pacific Islanders (Reed, 1973; Zimmet *et al,* 1990), Australian Aborigines (O'Dea, 1991) and Melanesians in Papua New Guinea (Dowse *et al,* 1994) is regarded as consistent with Neel's hypothesis. There are also animal models of NIDDM which appear to fit the "thrifty genotype" scenario, such as the Westernised Israeli sand rat (Zimmet & O'Dea, 1993). The problem with this hypothesis however (which may paradoxically explain its longevity), is that it makes few specific, testable predictions which advance understanding beyond the general model of an interaction between ill-defined genetic factors and a "Western" environment in the development of NIDDM and other features of the insulin resistance syndrome. It is not clear as to how this hypothesis can be refuted. It is also noteworthy that by substituting ill-defined intra-uterine factors for genetic factors, advocates of the "Thrifty phenotype" hypothesis have adopted an essentially similar model to explain geographic variation and

secular trends in the incidence of NIDDM (Phillips & Barker, 1993).

Thrifty phenotype hypothesis

Barker and colleagues have presented data which suggests that NIDDM and a range of additional cardiovascular risk factors including central obesity, hypertension, dyslipidaemia and elevated fibrinogen and factor VII, all have common origins in either sub-optimal development in utero as a result of poor maternal nutrition or poor nutrition in early infancy (Barker *et al*, 1990; Barker *et al*, 1993). This group have proposed that these major risk factors for cardiovascular disease in adult life are "programmed" by an adverse intrauterine and early childhood environment. They have suggested that "Syndrome X" (i.e. the insulin resistance syndrome) should be re-named "the small-baby syndrome" (Barker *et al*, 1993). In a series of studies, glucose intolerance, central obesity, hypertension and other cardiovascular risk factors have been variously related to birth weight, placental to birth weight ratio (placental ratio), measures at birth of length, head circumference and Ponderal Index[4], weight at one year and dental eruption at one year of age (Barker, 1992; Barker, 1994).

These associations are reported as independent of alcohol intake, cigarette smoking and of social class whether defined in adult life or at birth. Social class at birth and in early childhood, proxy measures for poverty and social disadvantage, are potentially important factors in the development of cardiovascular disease in adult life. Hence the adequacy of adjustment for social class at birth and in early childhood, given the limitations of available data, has emerged as a major concern in the interpretation of this

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[4]

Length/(height cubed)

work (Ben-Shlomo & Davey Smith, 1991; Paneth & Susser, 1995). Further concerns relate to the range of cardiovascular risk factors which have been examined and the diverse indices of growth retardation in utero and early infancy to which they have been related. The key refutable elements of the hypothesis have not yet been well defined. While the importance of adequate nutrition in pregnancy is not disputed, there is scepticism regarding the hypothesised role of maternal nutrition as the dominant influence on fetal growth and development in developed countries (Perry et al, 1995). The hypothesis has been well described as an "inductionist's delight" in which "example is piled on example, each somewhat consistent with the hypothesis but none seriously testing it" (Paneth & Susser, 1995).

The Barker hypothesis is based on a relatively small number of studies conducted in Britain. Corrobration of the major findings in other populations is clearly required. With regard to the specific association between birth weight and risk of NIDDM in adult life, McCance and colleagues (1994[b]) observed a U-shaped association among Pima Indians in Arizona. Presumably, heavier infants who subsequently developed NIDDM were the offspring of mothers with impaired glucose tolerance and it was argued that the association with low birth weight might have been due to the selective survival of smaller infants who were relatively insulin resistant. In this particular population low birth weight accounted for an extremely small proportion of cases of NIDDM. By contrast, in a bi-ethnic population in San Antonio, Texas, consisting of Mexican-Americans and non-Hispanic Caucasians, the prevalence of the insulin resistance syndrome (clearly defined) fell with increasing birth weight in both ethnic groups (Valdez et al, 1994).

Regarding the pathophysiological basis of the association between birth weight

(and other indices of fetal growth such as Ponderal Index) and glucose intolerance, the focus in early publications from the Barker group was on defective β-cell growth *in utero* as a cause of later pancreatic hyposecretion of insulin whereas in later publications (based on similar data) the focus has shifted to fetal growth retardation as a cause of later insulin resistance (Phillips & Barker, 1993). Indeed with typical ingenuity, abnormal blood vessel development *in utero*, leading to reduced capillary density, has been invoked as a possible mediator of both pancreatic hyposecretion and insulin resistance in peripheral tissues such as skeletal muscle (Phillips & Barker, 1993). As discussed in earlier sections, there is considerable evidence that insulin resistance is an early pathophysiological event in the development of NIDDM and it is hypothesised that insulin resistance mediates the common causal factors linking NIDDM and CHD (to which we must now add intrauterine programming as a putative common causal factor). However, given the acknowledged importance of genetic and post natal environmental factors in the development of insulin resistance and the fact that mothers with hypertension and moderate degrees of insulin resistance in pregnancy give birth to lighter infants (Breschi *et al*, 1993), it will be difficult to establish a specific independent role for intrauterine nutritional programming in the development of insulin resistance.

Ecological studies of NIDDM

Studies across populations (and within populations over time) are of particular value in the elucidation of environmental factors in the development of conditions such as NIDDM. There are considerable data on the occurrence and the distribution of NIDDM world wide and to a lesser extent over time. Interpretation of these studies however is fraught with difficulties given the problems with the classification, diagnosis and case

detection of NIDDM to which I have alluded in *Chapter 1*. The glucose load used in glucose tolerance tests and the diagnostic criteria for diabetes have varied widely in different studies. In some studies, known diabetics were included irrespective of the level of plasma glucose on screening, whereas in others they were included only if the glucose level was in the diabetic range (Haman, 1983). Additional problems in the interpretation of ecological data include those associated with inaccurate and imprecise glucose measurements, non-random population samples and variable case fatality rates (Alberti, 1993). Studies of NIDDM mortality are particularly suspect as it is estimated that only 50 percent of persons with diabetes will have the diagnosis listed on the death certificate (Hamman, 1983). In prevalence studies, age groups studied have varied widely and in many studies insufficient data have been provided to allow for age standardisation. Urine analysis for glycosuria has been used as a screening test prior to formal glucose tolerance test in some studies. Unfortunately the sensitivity of this test is variable and is particularly influenced by age.

Despite these problems, ecological studies provided early and compelling evidence of the interaction between environmental and genetic factors in the aetiology of NIDDM. This work has laid the foundation for analytical studies (including the study presented in this thesis) which have addressed the role of specific environmental factors in the development of NIDDM.

NIDDM mortality

Methodological caveats aside, it is likely that large differences in age adjusted mortality rates, as in the 1976-1978 period between Belgium 13.9 and Iceland 3.0 per 100,000 persons per year. reflect real differences in disease risk (Hamman, 1983). In Britain

diabetes mortality data (which reflects largely deaths from NIDDM, as it accounts for over 80% of all diabetes) has been used to explore the influence of obesity and socioeconomic status on disease risk (Hamman, 1983). Of interest is the data cited by Hamman (1983), which suggests that an increase in body mass index (BMI) in British males between World War II and the mid 1960's was associated with a rise in diabetes mortality whereas BMI and diabetes mortality in females were stable over the period. Between 1920 and 1972 a complete reversal of the socioeconomic gradient in diabetes mortality has been documented in England and Wales. The male standardised mortality ratio (SMR) for deaths from diabetes in 1972 was 128 in social class V with an almost uniform fall to 83 in social class I, whereas in 1920 the SMR in social class I was 129 with an equally uniform fall to 66 in social class V (Haman, 1983). Reservations regarding the validity of mortality and social class data notwithstanding, this provides fairly compelling evidence for the role of environmental and lifestyle factors in the occurrence of diabetes in Britain.

NIDDM Prevalence

There are major differences in the prevalence of NIDDM within and between populations, ethnic groups, geographic regions and time periods. West compared the prevalence of "abnormal glucose tolerance" (2-hour blood glucose level of 8.3 mmol/L or above) in 12 populations world wide ranging from Panamanians to Cherokee Indians (West, 1972). Prevalence ranged from 2% to 25% and was related in a linear fashion to the frequency of obesity expressed as mean percent of standard weight. Zimmet (1982) has presented data on NIDDM prevalence from 15 population groups where methodology and diagnostic criteria were sufficiently similar to make "cautious" comparisons.

Prevalence rates ranged from 1%-1.5% in Japanese, Indonesian and rural Indian

populations to 2.5% in Caucasian populations (Australia and New Zealand), to 29%-35%

in Micronesian, Cherokee Indian and Pima Indian populations. That genetic as well as

environmental factors contribute to the differences observed is evidenced by the different

prevalence rates seen in different ethnic groups in similar environments, with intermediate

prevalence rates observed in mixed racial groups. In South Africa for instance NIDDM

prevalence ranges from 3.6% in African, 6.6% in Malay and 10.4 % in Indian ethnic

groups (Jackson, 1978). Inter-ethnic differences of similar or greater magnitude are

described in Fiji, Singapore, Trinidad, Malaysia, and New Zealand (Zimmet, 1982). In

the United States, age adjusted NIDDM prevalence is lowest in the "Anglos" and

increases steadily with increasing "native American admixture" to the high rates seen in

Pima Indians (Everhart,et al, 1985). It is difficult to separate ethnic from

environmental/socio-cultural effects, even in the US "melting pot". Accordingly these

studies will overestimate the contribution of genetic susceptibility to diabetes prevalence

in these populations.

Environmental influences are also highlighted by the uniformly adverse effects of

modernisation or acculturation on NIDDM prevalence. From available records it appears

that NIDDM was rare among the Pima Indians at the turn of the century, but by 1967

they had the highest prevalence yet recorded in the literature. In 10 years between 1967

and 1977 age adjusted prevalence increased by a further 42% (Bennett & Knowler,

1980). As mentioned previously, similar trends are described with modernisation in

diverse populations (King & Rewers, 1993) including Guam, Nauru, and a number of

other Pacific island populations (Reed, 1973; Zimmet et al, 1990), Australian Aborigines

(O'Dea, 1991), Melanesians in Papua New Guinea (Dowse et al, 1994) and African

Americans (Harris *et al*, 1987).

Migrant studies

Studies of migrants provide unique opportunities to assess the effect of lifestyle change on NIDDM incidence and prevalence. However, as changes associated with migration are generally multiple and complex, one is not able to examine the effects of specific environmental factors. Migration is generally associated with a combination of relatively rapid improvement in economic circumstances, dietary change, the development of obesity, less physical activity and (insofar as it can be measured) psychological stress. Additional problems of interpretation of migrant studies relate to the self selection of migrants and the variable degree to which migrants form an ethnic enclave, with preservation of cultural, economic and religious structures, within their adopted country. Indeed, paradoxically where there is intermarriage among a small cohesive migrant community, diseases with a genetic component such as NIDDM may become especially prominent.

The effects of migration on NIDDM prevalence are particularly striking in Asian Indians. Age standardised prevalence is low in rural Indians, less than 2%, higher in internal migrants to urban centres, of the order of 10%, and also higher in migrants to diverse corners of the globe, including Fiji, South Africa, Singapore and the United Kingdom (Taylor & Zimmet, 1983; Ramachandran *et al*, 1988). Rates are generally higher than in the local population of the countries to which they have migrated, again emphasising the interaction between genetic susceptibility and environmental factors.

Yemenite migrants to Israel had a lower prevalence of diabetes at 0.06% than earlier settlers of the same origin. Twenty years later prevalence had increased to 11.6%.

These rates have not been age standardised but we are told that the age distribution of the population was similar at the two time points (Cohen *et al*, 1979).

Diabetes was found to be twice as prevalent in Japanese migrants to Hawaii than in residents of Hiroshima (Kawate *et al*, 1979). However as is usual in these situations, individuals left to their own devices change several potential confounding variables simultaneously! The migrants were more sedentary, more obese and ate a diet containing a higher proportion of fat and simple carbohydrate. Similar findings emerged from a comparative study of NIDDM prevalence in Mexican Americans in San Antonio and residents of Mexico City (Stern *et al*, 1992). Of interest in the latter study is the fact that even among non-diabetic subjects, fasting and 2-hour post load glucose and fasting insulin concentrations were higher among Mexicans in San Antonio than in Mexico City.

As increased obesity and reduced levels of physical activity are prominent features in migrants, studies which attempt to "adjust" for these factor would be of particular interest in the elucidation of additional factors associated with migration such as dietary change and psychosocial stress. Taylor and Zimmet (1983), who have contributed substantially to the migrant literature in a series of carefully standardised studies in the south Pacific, have examined diabetes prevalence in the 25 to 64 age group in Wallesian migrants to New Caledonia compared with native Wallesian in three BMI strata. In each stratum diabetes prevalence was higher in the migrant group, though there was clearly a BMI migration interaction effect. The age adjusted relative risk (relative to non-migrant Wallesians) of diabetes in male migrants was estimated at 8.0 (confidence intervals not quoted) and the age and BMI adjusted relative risk was 6.6. It is more difficult to adjust for physical activity than BMI as it is obviously less reliably measured, and there are no migrant studies which have adequately addressed this issue.

Compelling evidence of the importance of environmental factors associated with acculturation is provided by O'Dea's work with Australian Aborigines, another ethnic group which has developed a high prevalence of diabetes associated with recent changes of lifestyle. In this relatively small but methodologically rigorous study, improvement or complete reversion of metabolic abnormalities associated with NIDDM was observed during a temporary reversion to a traditional hunting and gathering lifestyle (Zimmet & O'Dea, 1993).   It is clear that as with most disease, genetic susceptibility to NIDDM varies considerably world wide and that the importance of environmental factors is modulated by the genetic back ground. In the Tokelau island migrant study for instance, changes in BMI were clearly related to the higher NIDDM incidence observed (Ostbye *et al*, 1989), whereas in South Africa, diabetes prevalence rates among Asian Indians were found to be approximately 5 times greater than in whites or Bantus, despite a lower prevalence of obesity in this ethnic group (Jackson, 1978). In Fiji a rural urban gradient in NIDDM prevalence, (with higher prevalence in the urban setting in each of two levels of physical activity) was seen in native Fijians, whereas the reverse pattern is seen in Asian Indians (Taylor & Zimmet, 1983).

NIDDM incidence

Determining the incidence rate of glucose intolerance is difficult due to its insidious onset. Some investigators have managed to derive a community based estimate of person time rate, notably in Minnesota, where person years at risk were determined from a community register that did not exclude those with the disease, an acceptable approximation of time at risk where the incidence of disease is low (Melton *et al*, 1983). An alternative approach is to study a fixed population at two separate points in time,

preferably five or more years apart, and then estimate the cumulative incidence rate over the period between the examinations. An approximation of the person year rate can then be derived by dividing the cumulative incidence rate (risk) by the length of the study period, provided the incidence rate is low and the period relatively short. Employing this methodology, the age/sex standardised incidence of NIDDM was 19 times higher in Pima Indians, than in Rochester, Minnesota (Knowler *et al*, 1978). While genetic factors are clearly of major importance in the high incidence of NIDDM in the Pima Indian population, the importance of environmental factors is highlighted by the rising incidence of NIDDM seen in this population in recent decades. NIDDM incidence rates increased by 40% in Pima Indians between 1965 and 1975 (Knowler *et al*, 1993), an observation which suggests that the high and rising prevalence of NIDDM in this population is not simply due to increased longevity of subjects with diabetes. Similar trends of an increasing incidence of NIDDM have been documented in the urban Hispanic population in the USA in recent decades (Haffner, 1991), trends which are also likely to reflect lifestyle change in a population which is genetically predisposed to develop high rates of NIDDM.

Risk factors for NIDDM: analytical studies

Socio-economic status and NIDDM

Data on socio-economic status and NIDDM are limited. Given the inverse association between socio-economic status and obesity in developed countries one would expect to observe a similar inverse association with NIDDM prevalence. There is some evidence from developed countries which supports this prediction (Knowler *et al*, 1993). However there are no prospective data on socio-economic status and risk of NIDDM.

Obesity and NIDDM

Obesity is the most widely recognised and the most important "environmental" risk factor

for NIDDM. As early as 1921 Joslin, using life insurance data, estimated that those

between 6-20% above average weight were between 6-12 times as liable to diabetes as

those below average weight (cited by Mann, 1983). Over the intervening years

innumerable studies have been reported which support this association, to the extent that

in 1980 a WHO Expert Committee was able to agree that obesity constituted the most

powerful risk factor for NIDDM (WHO, 1980). Several biologically plausible

mechanisms link obesity with NIDDM. Obesity has been convincingly shown to be

associated with insulin resistance (Bonadonna *et al*, 1990) and there are data which

suggest a number of specific mechanisms such as a reduced number of insulin receptors

and increased fat oxidation and glucose-fat-substrate competition in skeletal muscle

(Spelsberg & Manson, 1993). The best epidemiological evidence for the role of obesity

comes from prospective incidence studies such as those performed among the Pima

Indians, middle-aged Norwegian men and US white female nurses (Knowler *et al*, 1981;

Westlund & Nicolaysen, 1972; Colditz *et al*, 1990). In each of these populations the risk

of NIDDM increased with increasing obesity, in a log-linear fashion with a relatively

modest increase over a wide range of BMI or relative weight and a massive increase in

the very obese. In the Norwegian study the point of inflection of the incidence curve was

at a relative weight of 135 (Westlund & Nicolaysen, 1972) and in the Pima Indians at a

BMI of approximately 35 kg/m^2 (Knowler *et al*, 1981). In the Nurses Health Study, in

which 113,861 women aged 30 to 55 years were followed for 8 years, obese women in

the highest BMI categories (BMI ≥ 29 kg/m^2) had a 20 to 60 fold increased risk of

clinically diagnosed diabetes relative to lean women (BMI ≤ 22 kg/m^2) (Colditz *et al*,

1990). Weight change since the age of 18 years was also an important, though less strong predictor of diabetes in this cohort.

The distribution of obesity is important with evidence of higher risk associated with truncal obesity. In the US Tops Club study the prevalence of NIDDM increased with increasing waist hip ratio in three strata of relative weight (Hartz *et al*, 1984). Among residents of London of either European or South Asian ethnic origin, waist hip ratio was more strongly related in a cross-sectional study to diabetes and impaired glucose tolerance than was BMI (McKeigue *et al*, 1992). In the Gothenburg study of NIDDM incidence, both BMI and waist-hip ratio made independent contributions to the probability of NIDDM (Ohlson *et al*, 1985). In Japanese-American men living in Seattle, the best antropometric variable predicting the development of NIDDM was the intra-abdominal body fat area determined by computerised tomography (Bergstrom *et al*, 1990). Of interest in this context is the data suggesting that central or truncal obesity is associated mainly with hypertrophy rather than hyperplasia of adipocytes and that such cells are relatively insulin resistant (Barrett-Connor, 1989). By contrast however, in the US male health professionals study, BMI was a much stronger predictor of NIDDM than waist circumference which was a better predictor than waist hip ratio (Chan *et al*, 1994). Measurement of waist and hip circumference in this study was based on self report, using a paper tape measure supplied by the investigators. Hence the effect of waist-hip ratio on risk of NIDDM may have been diluted by measurement error, though this is unlikely to fully explain the discrepancy between this and earlier studies. In a later longitudinal study from the Pima Indian population, a number of different antropometric measures were compared, using ROC curves, as potential predictors of NIDDM. In this study, BMI performed as well as a number of measures of central fat distribution, including waist-hip

and waist-thigh ratio and percentage body fat as estimated by bioelectric resistance (Warne et al, 1995). Clearly one cannot necessarily generalise from the findings in this study to other ethnic groups as there may be ethnic variation in the relation between different measures of body fat and risk of NIDDM.

Where studies have failed to demonstrate an association between obesity and NIDDM, explanations have generally been forthcoming. Prevalence studies tend to underestimate the importance of obesity due to the effect of weight loss with onset of the disease and with therapy (Zimmet, 1982). The duration of obesity appears to be as important as the extent (Knowler et al, 1993), accordingly studies of the recently obese will yield lower relative risks.

Where the genetic predisposition is marked, a lesser degree of obesity is required to induce NIDDM. For instance among the Pima Indians, the risk of NIDDM in those with one or both parents diabetic compared with those with neither parent diabetic, is higher at every level of body mass index (Knowler et al, 1981). Kobberling (1971), in an elegant study, found that the frequency of NIDDM in the siblings of diabetics fell with increasing obesity of the diabetic subjects.

Though the association between obesity and NIDDM is well established, it is not without controversy. Jarrett has argued that only severe obesity is associated with NIDDM and that therefore a relatively small proportion of cases of NIDDM in Britain are due to this factor (Jarrett, 1986). Moreover, in the Whitehall Study, obesity did not predict deterioration to NIDDM in men with impaired glucose tolerance (Jarret et al, 1979), though clearly this is not necessarily incompatible with a role for obesity at an earlier stage in the development of NIDDM. The data from the British Regional Heart Study, as presented in this thesis, allow these particular issues to be addressed in detail.

Physical activity

A protective effect of physical activity on the risk of developing NIDDM is biologically plausible (Spelsberg & Manson, 1993). Skeletal muscle represents the predominant site of insulin resistance in NIDDM (DeFronzo *et al*, 1992), and exercise training has been shown to improve insulin sensitivity in these tissues (Spelsberg & Manson, 1993). Exercise has a favourable effect on glucose tolerance and insulin sensitivity, both in individuals with established NIDDM and in non-diabetics, an effect which has been shown to persist for up to 72 hours after cessation of exercise (Schneider *et al*, 1984; Burstein *et al*, 1985). In clinical trials, obese individuals with impaired glucose tolerance have been shown to have lower insulin levels and evidence of improved glucose metabolism after moderate exercise regimens, even without weight loss (Björntorp *et al*, 1970; Soman *et al*, 1979).

However, epidemiological data on the role of physical activity in the development of NIDDM are relatively inconsistent. Taylor and colleagues examined the relationship between NIDDM prevalence and physical activity in two ethnic groups, Indian and Melanesian, living in Fiji (Taylor *et al*, 1984). Physical activity was graded on the basis of occupation with sporting activity taken into account where it was substantial. The relative risk of NIDDM. in the sedentary/light as opposed to the moderate/heavy activity categories in the Indians was 2.1 and in the Melanesians 2.5. Both estimates were adjusted for age, triceps skinfold thickness and rural/urban residence. Though there is likely to have been residual confounding due to obesity and possibly bias due to reduced activity following diagnosis in the previously diagnosed diabetics included in the study, this study provides reasonably good evidence of a protective effect of physical activity in NIDDM. Annuzzi et al (1985), reported a similar protective effect of leisure time

physical activity on glucose intolerance (clearly defined) independent of age, sex, BMI and skinfold thickness in a carefully conducted, matched case control study where cases and controls were drawn from an industrial screening programme which included an oral glucose tolerance test. In cross-sectional data from the British Regional Heart Study, an inverse association between physical activity level and non-fasting serum glucose has been reported (Perry et al, 1993).

By contrast however, in the Whitehall study no significant relationship was observed in either univariate or multivariate analysis between leisure time physical activity and 2-hour post load glucose level in a group of 6672 middle-aged civil servants who completed an activity questionnaire (Jarret et al, 1986). It may be that there was insufficient heterogeneity of exposure to adequately test the hypothesis in this particular study. However in US data from the National Health and Nutrition Examination Survey, where NDDG criteria were applied to the result of 2-hour, 75 gm glucose tolerance test, the proportion of individuals in three physical activity strata did not differ by glucose tolerance (Everhart et al, 1985).

To date there have been few prospective studies of the relation between physical activity and physician diagnosed NIDDM (Helmrich et al, 1991; Manson et al, 1991[a]; Manson et al, 1992; Burchfiel et al, 1995). In the Nurses' Health Study cohort, in which physical activity data were available from 87,253 women who were followed for eight years, the risk of developing NIDDM was lower by a third among those who engaged in vigorous exercise at least once per week. This effect was independent of age, BMI, family history of diabetes and early myocardial infarction, personal history of hypertension, cigarette smoking and alcohol intake (Manson et al, 1991[a]). Exercise was associated with a similar reduction in the risk of NIDDM among obese and non-obese

women. However, among women who exercised at least once per week there was no clear dose-response gradient according to the frequency of exercise. This does not seem biologically plausible and must raise concerns regarding possible confounding due to unmeasured life-style factors related to both physical activity and risk of NIDDM. Similar independent associations between higher exercise level and reduced risk of NIDDM were observed in a cohort of 5990 U.S. male alumni followed for approximately 14 years (Helmrich *et al*, 1991) and in 21,271 male U.S. physicians followed for 5 years (Manson *et al*, 1992). Reassuringly, in both of the latter studies a clear dose-response gradient was observed. In both studies also the apparent benefit of exercise was most marked in the obese. Indeed in the study involving the University of Pennsylvania alumni, the reduced risk of NIDDM associated with increased physical activity was confined to those defined as at high risk of NIDDM on the basis of high BMI, a parental history of diabetes or a personal history of hypertension (Helmrich *et al*, 1991). In the Honolulu Heart Program data, an inverse association between physical activity level and the incidence of clinically diagnosed diabetes is also described. In this study of over 6000 Japanese-American men, a significant effect of physical activity on diabetes risk was not seen in those whose BMI was in the upper quintile of the distribution (Burchfiel *et al*, 1995). Interpretation of this finding is difficult however, as in this study diabetes was defined on the basis of self-reported use of diabetic medication which may be less frequently prescribed for obese patients.

Hence the findings from prospective studies broadly support the hypothesis of a protective role for physical activity in the development of NIDDM. However, given that these studies were largely conducted on US health professionals and college alumni, there is a need to examine this association in a general population sample such as that provided

by the British Regional Heart Study. In addressing this issue we need to remember that as physical inactivity is now regarded as the major cause of obesity in Britain and other developed countries (Prentice & Jebb, 1995), the relation between physical inactivity and NIDDM, unadjusted for obesity, will be more relevant from the public health perspective.

Cigarette smoking and NIDDM

A possible association between cigarette smoking and risk of NIDDM is suggested by the finding (in a population based study of middle-aged men) that smoking decreases fasting insulin and causes a transient increase in blood glucose level after an oral glucose load (Janzon et al, 1983). Smokers are generally thinner and in a number of prospective studies of the association between insulin level and cardiovascular disease it has been observed that insulin levels are lower in smokers (Ferrara et al, 1994; Yarnell et al, 1994). These observations suggest that smoking may exert direct "toxic" effects on insulin secretion. However, smoking is associated with increased waist-hip ratio (Shinokata et al, 1989) which is plausibly linked with insulin resistance and Facchini et al.(1992) have provided direct evidence to suggest that chronic cigarette smokers are insulin resistant and hyperinsulinemic. In a clinical study of 20 smokers and 20 non-smokers, plasma insulin levels post glucose load were higher in smokers as were steady-state plasma glucose concentrations in response to a continuous infusion of glucose, insulin and somatostatin. The smokers in this small study had additional features suggestive of insulin resistance, including significantly higher VLDL-triglycerides and lower HDL-cholesterol levels. It is difficult to reconcile these findings with the epidemiological data linking cigarette smoking with lower insulin levels and with the

observation that smokers are thinner. However, this was a volunteer sample and although smokers and non-smokers had a similar distribution of age, body mass index and family history of diabetes, the differences in insulin sensitivity reported were not adjusted for these factors. Hence, the generalisability and interpretation of these findings is uncertain.

Data from observational studies on cigarette smoking and risk of diabetes are also somewhat inconsistent. In the Zutphen Study of middle-aged men, those who smoked >20 cigarettes daily had a relative risk of diabetes of 3.3 (95% C. I. 1.4 to 7.9) at 25 years follow-up, adjusted for age, subscapular skinfold thickness, resting heart rate, alcohol and energy intake (Feskens & Kromhout, 1989). Given that smokers tend to be thin, it is unlikely that a more complete adjustment for obesity would have attenuated this association. However adjustment for physical activity in this study was also incomplete. In prospective data from the Nurses' Health Study, current smokers were also at increased risk of NIDDM during 12 years of follow-up and a statistically significant dose response trend was observed (Rimm *et al*, 1993). The magnitude of the association in this population was less, with a relative risk of NIDDM of 1.4 (95% C. I. 1.18 to 1.72) among women smoking ≥25 cigarettes daily compared to non-smokers after adjustment for age, BMI, family history of diabetes, physical activity and other risk factors. Rimm and colleagues (1995) have reported broadly similar findings from the US male health professionals' study (Rimm *et al*, 1995). At 6 years follow-up, men who smoked ≥25 cigarettes daily had a relative risk of NIDDM of 1.9 (95% C. I. 1.25 to 3.03) compared with non-smokers, adjusted for important potential confounders. In this study, which included men aged 40 to 75 years, former smokers had a significantly higher risk of NIDDM than never smokers and a strong dose response relation between lifetime pack years of cigarettes and risk of diabetes was observed. The relative risk of NIDDM

associated with smoking was greater among men who were less obese (Rimm *et al*, 1995).

By contrast, in the Framingham study, smoking was not associated with higher risk of NIDDM in the elderly (Wilson *et al*, 1986) and smoking did not emerge as a risk factor for diabetes in a prospective study of Israeli civil servants (Medalie *et al*, 1975). However relative to the studies of US nurses and health professionals both of these studies were small and may have lacked power to detect a significant effect. Hence, the data from the British Regional Heart Study provide a valuable opportunity to study this association with considerable power.

Alcohol intake and NIDDM

There are conflicting reports as to the acute effect of alcohol on glucose tolerance (Nikkila & Taskinen, 1975; McMonagle & Felig, 1975) and inconsistent findings from cross-sectional observational studies which have examined the relation between alcohol intake and glucose tolerance/NIDDM (Gerard *et al*, 1977; Ostrander *et al*, 1974). The findings from prospective studies of alcohol intake and NIDDM are also inconsistent (Ohlsen *et al*, 1988; Holbrook *et al*, 1990; Stampfer *et al*, 1988). In the Rancho Bernardo study alcohol consumption was an independent predictor of NIDDM in men aged 40 to 79 years but not in women over a follow-up period of up to 14 years (Holbrook *et al*, 1990). In men a daily alcohol intake of $\geq$ 24 g (2-3 U.K. units) was associated with 50% increased risk of NIDDM compared to non-drinkers. In the Swedish Study of men born in 1913, Ohlsen and colleagues (1988), reported a marginal and non-significant increased risk of NIDDM associated with "high" alcohol intake over 13.5 years of follow-up. By contrast, prospective data from the Nurses Health Study cohort are

consistent with a protective effect of alcohol intake on the risk of NIDDM (Stampfer *et al*, 1988). In this study there was a steady fall in the risk of NIDDM with increasing increasing alcohol intake. Among women consuming 15 g or more of alcohol daily (approximately 2 U.K. units), the age adjusted relative risk of NIDDM was 0.3 (95% C.I. 0.2 to 0.4) relative to non-drinkers and 0.6 (95% C.I. 0.3 to 0.9) after adjustment for age, body mass index, family history of diabetes and total calorie intake. In this study the non-drinkers carried the heaviest burden of cardiovascular disease and ill-health. This raises the possibility that the higher risk of NIDDM in this group relative to moderate drinkers may be an artefact, reflecting behaviour change associated with ill-health (Shaper, 1990). However even if the authors had used light drinkers (< 1.5g/day) as the reference category, they would have found a significantly reduced risk of NIDDM in the heaviest drinking category in the age-adjusted data. In a later study based on the US male health professionals cohort a similar linear inverse association between between alcohol intake and risk of NIDDM was observed (Rimm *et al*, 1995).

Hence, the balance of available evidence from prospective studies of alcohol consumption and risk of NIDDM suggests that drinkers may be at lower risk. As with the physical activity and cigarette smoking, data from the British Regional Heart study provide a unique opportunity to examine this relationship in a population-based sample of middle- aged men with a broad spectrum of exposure and data on a wide range of potential confounding variables.

Haematocrit and NIDDM

High haematocrit levels are associated with hypertension (Tibblin *et al*, 1966) and independently with increased risk of vascular disease including myocardial infarction and

stroke (Knotterus *et al*, 1988; Wannamethee *et al*, 1994[a]; Wannamethee *et al*, 1994[b]).

The haematocrit level is an important determinant of whole blood viscosity (Wills & Merrill, 1962). There is evidence that haematocrit levels are increased in established diabetes, as one of a range of haemorheological abnormalities which enhance the risk of vascular disease in this condition (MacRury & Lowe, 1990). In cross-sectional data from the Tecumseh Study, raised haematocrit levels were associated with major components of the insulin resistance syndrome, such as obesity, hyperglycaemia, hyperinsulinemia, hypertension, and hypertriglyceridemia in healthy non-diabetic men aged 18 to 42 years (Smith *et al*, 1994). There is also evidence from a clinical study of an association between haematocrit and whole blood viscosity and insulin resistance (Moan *et al*, 1994). These observations raise the possibility that an elevated haematocrit level might predict NIDDM, thereby forming part of "the common soil" (Stern, 1995) linking NIDDM and cardiovascular disease. Direct evidence linking haematocrit and risk of diabetes is limited. In the Framingham study, high haemoglobin levels (highly correlated with haematocrit) predicted glucose intolerance in women but not in men (Wilson *et al*, 1981). In a five year follow-up of Israeli men, mean haemoglobin was significantly higher in those who developed diabetes (Medalie *et al*, 1975). However no other prospective study of risk factors for NIDDM has examined the role of haematocrit in the development of this condition.

Height and forced expiratory volume (Fev_{1sec}) and NIDDM

In a small study involving a population based sample of 58 subjects, it was observed that 7 subjects with glucose intolerance were significantly shorter than controls, matched for age and sex and of similar BMI (Williams *et al*, 1991). It was suggested that this finding

may be consistent with the Barker hypothesis linking retarded growth in utero with glucose intolerance in adult life (Barker, 1992). In the Isle of Ely Diabetes Project, a population study of the aetiology of NIDDM in middle-aged men and women, subjects with newly diagnosed glucose intolerance (either impaired glucose tolerance or NIDDM; 21% of 1122) were significantly shorter than those with normal glucose tolerance and in both men and women a significant, independent, inverse association between height and plasma glucose level at 120 minutes post glucose load was observed (Williams et al, 1995). Short stature is probably a marker for socio-economic disadvantage in childhood (Walker et al, 1988) and the latter may confound associations between height and glucose intolerance. It is noteworthy for instance that in the prospective data from the US Health Professionals' Follow-up Study (a population which is relatively homogenous in socio-economic terms), no association between height and risk of NIDDM was observed in a group of over 27,000 men (Chan et al, 1994). Diminished vital lung capacity was associated with increased risk of NIDDM in an early report from Pafenbarger study of college alumni (Paffenbarger & Wing, 1973). Lung function is obviously influenced by cigarette smoking and reduced lung volumes are also regarded as a relatively non-specific marker of "overall vigour and general health". Of relevance to the question of possible common risk factors linking NIDDM and CHD, it is worth noting that both short stature and reduced lung volumes have been associated with increased risk of cardiovascular disease, in particular myocardial infarction in several studies (Moris et al, 1966; Marmot et al, 1978; Cook & Shaper, 1988; Walker et al, 1989; Persson et al, 1986). As with glucose intolerance, the association between adult height and coronary heart disease may reflect intrauterine growth retardation and /or childhood deprivation. The mechanisms linking pulmonary volume and function with CHD remain obscure. It seems clear that

this association does not simply reflect residual confounding due to cigarette smoking (Persson *et al*, 1986). It is suggested that reduced lung volumes may also reflect adverse intrauterine or early childhood circunstances (Barker *et al*, 1991). Height and lung volumes are closely inter-related and the latter is usually standardised for the former. In the British Regional Heart Study, associations between height and CHD were no longer significant after adjustment for height standardised FEV_{1sec} (Walker *et al*, 1989).

Baseline data on both height and Fev_{1sec} from the British Regional Heart Study will be related to the subsequent risk of NIDDM in the cohort.

Diet and risk of NIDDM

Given the importance of obesity as a risk factor for NIDDM, the role of diet in the development of this condition merits brief review, although associations with diet are not addressed directly in this work. Ecological and migrant studies suggest a positive association between the prevalence of diabetes and total fat, animal fat and protein consumption and an inverse association with total carbohydrate intake (Spelsberg & Manson, 1993), though one must be mindful of the caveats that attend such data. Marshall and colleagues examined dietary habits, based on 24-hour recall and the prevalence of IGT and NIDDM, determined by oral glucose tolerance test, in a group of 1317 undiagnosed Hispanic and non-Hispanic whites in Colorado (Marshall *et al*, 1991). A positive association between high fat and low carbohydrate intake and glucose intolerance was found. After adjustment for age, sex, ethnicity, BMI and energy intake, the risk of NIDDM increased by 45% with every 40g per day increment in total fat intake and by 31% with every 90g per day decrement in carbohydrate intake.

Data from prospective studies are limited and the studies are of varying power and

methodological rigor. Meat consumption was associated with increased mortality from diabetes in male Sevent-Day Adventists (Snowden & Phillips, 1985). In a 12-year follow-up study of Swedish women (Lundgren *et al*, 1989) and in a 25 year study of Dutch men (Feskens *et al*, 1989), dietary factors were not associated with the incidence of clinicaly diagnosed diabetes. In the Nurses' Health Study, the risk of diabetes at 6 years follow-up fell with increasing intake of vegetable fat, potassium, calcium and magnesium (Colditz *et al*, 1992). In a recent prospective study, based on 20 year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study with an oral glucose tolerance test for case ascertainment, the intake of dietary fat (especially saturated fat) was an independent risk factor for IGT and NIDDM after adjustment for age, body mass index and energy intake (Feskens *et al*, 1995).

Hence, given the difficulty of measuring dietary intake with adequate reliability and validity and the extent to which dietary constituents are inter-correlated, the data linking saturated fat intake with risk of NIDDM are reasonably consistent. Clinical and animal studies have suggested several plausible biological mechanisms by which increased dietary fat might contribute to the genesis of NIDDM, including effects on body weight, fat distribution and effects on the level of circulating free fatty acids (FFA's) (Spelsberg & Manson). Intracellular FFA oxidation is a powerful inhibitor of glycogen storage and glucose oxidation in the cell. Hence insulin antagonises FFA oxidation. In obese non-diabetics and in obese patients with NIDDM, increased postabsorbative plasma FFA levels and a weakened antagonistic effect of insulin on lipid oxidation has been demonstrated (Lillioja *et al*, 1985: Groop *et al*, 1991). It is argued therefore, that an elevated rate of FFA oxidation, associated with obesity and/or a high fat diet, is theoretically able to induce all of the major intracellular abnormalities of glucose

metabolism in NIDDM (DeFronzo *et al*, 1992). However the use of plasma FFA concentrations as a marker of dietary fat intake is controversial and studies based on the measurement of fatty acids in adipose tissue, which may provide a better marker of long-term dietary fat intake (Hunter, 1990), are awaited with interest.

It should be noted that in some populations, particularly those of African or Afro-Caribbean origin, the prevalence of NIDDM (and obesity) is high (Harris *et al*, 1987) despite relatively low saturated fat intake (Douglas, 1990) and blood cholesterol levels (Chaturvedi, 1994). It seems likely therefore that although there may be a specific association between saturated fat intake and NIDDM, obesity will increase the risk of this condition regardless of the source of excess calories. However, it is tempting to speculate that the association between NIDDM and CHD in "Westernised" populations may at least partially reflect a common aetiological role for dietary saturated fat. In the US, CHD mortality rates have fallen steadily in recent decades despite a rising prevalence of obesity (Kuczmarski *et al*, 1994) and an extremely high prevalence of NIDDM (Harris *et al*, 1987). It is accepted that cholesterol levels are falling in the US (Johnson *et al*, 1993) and it is now apparent that the rising prevalence of obesity reflects increased intake of foods low in fat combined with low levels of physical activity (Byers, 1995).

Glucose intolerance and atheromatous vascular disease

NIDDM

NIDDM has been shown to be associated with a substantially increased risk of vascular disease, including myocardial infarction, peripheral vascular and cerebrovascular disease (Kannel & McGee, 1979[a]; Fuller *et al*, 1983; Manson *et al*, 1991[b]). In the Bedford study for instance, the prevalence of arterial disease as reflected by WHO questionnaire

history of angina, myocardial infarction and intermittent claudication and by Minnesota-

coded ECG findings, increased with increasing glucose intolerance (Keen *et al*, 1965).

In a subsequent follow-up study, coronary artery disease mortality was higher in those

with "borderline diabetes" and NIDDM (Jarret *et al*, 1982). In North America, it is

estimated that about three-quarters of deaths among diabetic patients are due to

atherosclerotic vascular disease compared with about one-third of deaths in the general

North American population (Wittels & Gotto, 1992). In women, NIDDM appears to

abolish the protective effect of female sex on the risk of cardiovascular disease. In the

U.S. Nurses' Health Study the age-adjusted total cardiovascular mortality rate was

increased more than 6-fold in women with NIDDM (Manson *et al*, 1991[b]). With

adjustment for CHD risk factors linked with NIDDM such as hypertension and

dyslipidemia (a dubious exercise given the potential to confuse mediating with

confounding factors), there remained a 3-fold increased risk of death from cardiovascular

disease in this group of women, aged 30 to 55 years. Diabetes (both NIDDM and IDDM)

is associated with increased risk of CHD in all population groups, it tends to amply the

underlying population risk but does not abolish the differences between populations. This

is particularly evident in Japan, where diabetes is associated with an approximate

doubling of CHD risk as elsewhere but to a level lower than that observed in Western

non-diabetics (Keen & Ashton, 1989). In Finland, where the population is regarded as

genetically homogenous, NIDDM has been shown to be associated with a similar increase

in the relative risk of CHD in Eastern Finland, where underlying CHD rates are high as

in Western Finland, where underlying CHD rates are relatively low (Laakso *et al*, 1995).

Asymptomatic hyperglycaemia and CHD

As one might expect, the data linking lesser degrees of glucose intolerance to CHD are less consistent than that linking NIDDM with CHD. In the International Collaborative Group Report, no significant association of glucose quintile with CHD mortality was detected in eight of eleven studies of middle-aged men in eight countries and in only one study was a strong independent association observed (Stamler & Stamler, 1979). Protocols and methods for assessing glycaemia varied considerably between these studies and in all but two studies there were less than 200 CHD events.

By contrast, in the 10-year follow-up data from the Whitehall Study, there was clear evidence of a threshold effect, with an approximately two-fold increased risk of CHD observed in the upper 5% of the post-load glucose distribution. This effect was independent of major cardiovascular risk factors, including age, obesity, blood pressure, smoking, cholesterol level and electrocardiograph abnormalities (Fuller *et al*, 1983). A similar threshold effect of post load-glucose level on CHD mortality was reported from the Paris Prospective Study data (Eschwege *et al*, 1980). Barrett-Connor and colleagues (1984) reported a continuous, independent relationship between fasting plasma glucose and ischaemic heart disease mortality in men aged 40-79 years in a Southern California community. Similarly in the Honolulu Heart Program data a linear relationship between 1-hour post load glucose and CHD mortality was observed (Donahue *et al*, 1987). Other major studies reported in the last decade include the Tecumseh study in which at 18 years follow-up there was a weak linear relation between asymptomatic hyperglycaemia and CHD in men (with 142 deaths) but not in women (with 71 deaths) (Butler *et al*, 1985). However, not all studies reported since the collaborative group analysis support an independent association between asymptomatic hyperglycaemia and CHD. In the Chicago

Heart Association Detection Project data, asymptomatic hyperglycaemia (post-load) was not an independent predictor of CHD mortality (286 events) at 9 years follow-up in 11,220 middle-aged men (Pan *et al*, 1986). An independent association of borderline significance was observed in women. In the Bedford study borderline post-challenge hyperglycaemia was predictive of CHD mortality only among women (Jarret *et al*, 1982). However, in 12 year data from the Gothenburg study no association between fasting blood glucose level and CHD end points was detected in a group of 1462 women, aged 38 to 60 years with 28 events (Lapidus *et al*, 1985).

The British Regional Heart Study data allow the association between non-fasting glucose and risk of CHD to be addressed with considerable power, with adjustment for a wide range of potential confounding factors. The association between established NIDDM and CHD will also be addressed in these data.

Glucose intolerance and CHD: possible mechanisms

The association between NIDDM and atheromatous vascular disease is only partially explained by the higher prevalence of established vascular risk factors associated with diabetes, such as obesity, hypertension and common forms of dyslipidaemia (Kannel & McGee, 1979[a]; Fuller *et al*, 1983; Manson *et al*, 1991[b]). Several additional, specific lipid abnormalities are described in patients with NIDDM which probably contribute to the increased CHD risk. These include an elevation of triglycerides (particularly post prandial levels) and very low density lipoprotein (VLDL) concentrations and reduced HDL-cholesterol (Wittels & Gotto, 1992; Stewart *et al*, 1994). Increases in atherogenic intermediate density lipoproteins are also reported (Stewart *et al*, 1994). LDL-cholesterol levels, which are the best predictor of CHD in non-diabetics are not consistently higher

in patients with NIDDM (Barrett-Conner *et al*, 1982). There is evidence however, that LDL-cholesterol is more athergenic in this setting due to glycation which interferes with catabolism and to oxidation which primes LDL particles for uptake by macrophages in the vessel wall (Stewart *et al*, 1994). There is also evidence that the composition of LDL particles is shifted towards a preponderance of "small dense" LDL particles which are more readily oxidised (Stern, 1995). NIDDM subjects also have altered apolipoprotein concentrations. However, lipoprotein (a) (Lp(a)) concentrations, which are regarded as a genetic marker for CHD risk are not consistently altered in NIDDM (Stewart *et al*, 1994).

A large number of additional mechanisms by which NIDDM increases the risk of CHD have been proposed. Among these factors are increased blood viscosity, enhanced activity of the polyol pathway, direct endothelial injury from hyperglycaemia, the accumulation of advanced glycosylation end-products in the arterial wall and abnormalities of platelet function and the coagulation system, including abnormalities of fibrinolysis due to elevated plasminogen activator inhibitor-1 (PAI-1) activity in plasma (Ganda, 1980; Salonen, 1989; Manson *et al*, 1991[b]; Wittels & Gotto, 1992). Other potentially relevant abnormalities include myocardial microvascular disease and cardiac autonomic neuropathy, which may predispose to ventricular arrhythmias and sudden death (Wittels & Gotto, 1992; Manson *et al*, 1991[b]). Both gross proteinuria and microalbuminuria which are common in NIDDM are associated with increased mortality from vascular disease in patients with this condition (Mogensen, 1984) and it is postulated that increased urinary albumin protein loss reflects a generalised increase in vascular permeability which predisposes to greater penetration of atherogenic lipoprotein particles into the arterial wall (Mykkänen *et al*, 1994[b].

In the Whitehall study, no relation between duration of NIDDM and the risk of death from CHD was detected. This led Jarrett and Shipley (1988) to argue that NIDDM and CHD are not causally linked but share common, possibly genetic antecedents. In subsequent work however an effect of NIDDM duration on CHD risk was observed in women (Manson et al, 1991[b]), and given the plethora of potential mechanisms it is difficult to argue against a cumulative deleterious effect of NIDDM on CHD risk. There is evidence however, that NIDDM and CHD may in fact share common genetic antecedents which influence the development of insulin resistance (Hein et al, 1992) and there is considerable evidence that several important CHD risk factors (such as low HDL-cholesterol and possibly hypertension) are associated with insulin resistance and compensatory hyperinsulinaemia (Reaven, 1988). If insulin resistance (which is obviously influenced by environmental as well as genetic factors) antedates NIDDM, it is reasonable to hypothesise that vascular risk factors which cluster with insulin resistance will also antedate NIDDM, forming part of a common causal pathway linking NIDDM and CHD. It follows from this hypothesis that the increased risk of atheromatous vascular disease (in particular CHD) seen in individuals with NIDDM may be well established before the onset of clinically manifest disease.

The evidence that insulin resistance and hyperinsulinaemia antedate NIDDM has already been reviewed and, as discussed, the question of whether hyperinsulinaemia is an early abnormality in the development of NIDDM is addressed directly in this thesis. The question of whether major CHD risk factors (both lifestyle related and biological) predict NIDDM, forming part of a common causal pathway linking NIDDM and CHD, is also addressed in this thesis. In further analyses, inter-relations between serum insulin and major CHD risk factors at baseline (both lifestyle related and biological) are explored

and finally, the association between circulating insulin levels and incident major CHD events is examined.

As the insulin resistance syndrome has provided a conceptual framework for much of the recent work on NIDDM and CHD, the data on associations between insulin and CHD risk factors which support this concept will be reviewed initially. Then the evidence linking insulin with CHD (which forms part of the insulin resistance model of the relation between NIDDM and CHD) will be considered and finally, the evidence that CHD risk factors predict NIDDM will be reviewed. In reviewing the latter question, the evidence that CHD risk factors predict NIDDM, the focus will be on biological risk factors considered in this thesis such as dyslipidemia and hypertension. The role of key lifestyle factors in the development of CHD, obesity, physical inactivity, alcohol intake and cigarette smoking has been mentioned briefly in the introduction to this chapter and the evidence linking these lifestyle related factors to NIDDM has already been reviewed in detail. The data on other risk factors which predict both NIDDM and CHD, notably increased urinary microalbumin excretion (Mykkänen *et al,* 1994 [b]; Yudkin *et al,* 1988), will not be reviewed.

The insulin resistance syndrome

Many of the abnormalities which contribute to the increased CHD risk in patients with NIDDM, such as obesity, dyslipidaemia and hypertension are known to cluster within individuals with and without diabetes. It is almost 40 years since Vague suggested that central obesity may be a marker for cardiovascular disease risk factors "determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease" (cited by Walker & Alberti, 1993). Other workers highlighted associations between obesity,

hyperlipidaemia and NIDDM and it was suggested that these factors might have common dietary origins (Walker & Alberti, 1993). In the 1960's, Stout and others developed the hypothesis that elevated circulating insulin levels play a central, unifying role in the aetiology of atheromatous vascular disease in both diabetic and non-diabetic subjects (Stout, 1990). Reaven (1988), articulated (or perhaps revived) the concept of a cluster of CHD risk factors, centred on insulin resistance with compensatory hyperinsulinaemia, which includes glucose intolerance, dyslipidaemia (increased VLDL triglyceride, decreased HDL-cholesterol) and hypertension. This syndrome, inelegantly named "Syndrome X" or the insulin resistance syndrome has recently been updated by Reaven and now includes increased small dense LDL-cholesterol particles, hyperuricemia and abnormalities of fibrinolysis (Reaven, 1994). Others have added central obesity (Zimmet, 1989), which has been shown to be independently associated with features of Syndrome X, including dyslipidaemia and hypertension (Björntorp, 1991). In his later version of Syndrome X, Reaven (1994) continues to argue for a central role for insulin resistance and hyperinsulinaemia as the driving force in the development of an adverse CHD risk factor profile and ultimately CHD events. However, the role of "environmental and behavioural factors", such as obesity and physical inactivity in the development of insulin resistance is somewhat reluctantly acknowledged (Reaven, 1994). Unfortunately, in this further reincarnation of the insulin resistance syndrome the distinction between aetiology and pathogenesis is not similarly acknowledged.

There is undoubtedly considerable population-based data to support associations between serum insulin and raised triglyceride and low HDL-cholesterol (Abbott et al, 1987; Zavaroni et al, 1989). The reported associations with other lipid abnormalities, uric acid and PAI-1 are largely based on clinical studies (Laws & Reaven, 1993). Data

on the association between serum insulin and blood pressure are markedly inconsistent within and between populations (Saad *et al*, 1991; Collins *et al*, 1990; Dowse *et al*, 1993). There is however some evidence that the major components of the insulin resistance syndrome do in fact "cluster" in at least some populations (Ferrannini *et al*, 1991).

It should be emphasised however, that the concept of the insulin resistance syndrome is based largely (though not entirely Haffner, 1992) on cross-sectional data and that associations between insulin and other components of the syndrome are not necessarily one way. Thus for instance, it has been shown that acute elevations of triglyceride levels induce insulin resistance (Thiebaud *et al*, 1982) and both acute and chronic lowering of triglyceride levels lead to improvements in insulin sensitivity and glucose tolerance (Jones *et al*, 1990). At a more fundamental level, it is arguable that the development of the insulin resistance hypothesis in its current form reflects the dominance of the measurable (i.e. insulin) over the important (i.e. obesity and other lifestyle related factors such as physical activity, diet and possibly psycho-social stress) and the related preoccupation in contemporary clinical science with pathogenesis rather than aetiology (Cruickshank, 1995). In *Chapter 5*, in which data on association between insulin and CHD risk factors are presented, the focus is on lifestyle related variables (obesity, physical activity, alcohol intake and cigarette smoking) and evidence linking these factors with insulin resistance and hyperinsulinaemia is reviewed.

Insulin and CHD events

The role of insulin in the development of atherosclerotic cardiovascular disease is contentious (Stout, 1990; Jarrett, 1988; Savage & Saad, 1993; McKeigue & Davey,

1995). It is hypothesised that hyperinsulinemia, reflecting resistance to insulin-mediated glucose uptake (insulin resistance) contributes to the development of atherosclerosis both directly via the atherogenic effects of insulin on the vessel wall and indirectly through associations between insulin and established cardiovascular disease risk factors (Stout, 1990). There is laboratory evidence supporting direct atherogenic effects of insulin (Sato *et al*, 1989) and as discussed above, there are consistent data linking elevated insulin levels with some cardiovascular risk factors (Abbott *et al*, 1987; Zavaroni *et al*, 1989). However, epidemiological data linking insulin with specific cardiovascular disease end-points are inconsistent. In particular, there is uncertainty about the relation between circulating insulin levels and the development of coronary heart disease (CHD). This issue has been addressed in a number of prospective studies in western populations, with some findings suggesting an association with fasting insulin (Ducimetière *et al*, 1980) or post-load insulin (Fontbonne & Eschwege, 1991; Fontbonne *et al*, 1991; Pyorala, 1979; Pyorala *et al*, 1985) and some indicating no independent association between CHD and insulin (Welborn & Wearne, 1979: Cullen *et al*, 1983; Hargreaves *et al*, 1992; Welin *et al*, 1992; Yarnell *et al*, 1994; Orchard *et al*, 1994; Rewers *et al*, 1992). In a recent study Ferrara and colleagues (1994) observed a significant inverse association between 2-hour post load insulin and fatal cardiovascular disease in elderly men, with no association seen in elderly women.

In the previous studies, insulin concentrations were determined by radioimmunoassay methods which are not specific for insulin but cross-react to a variable degree with pro-insulin and pro-insulin split products. In this thesis, the association between non-fasting true insulin levels and major CHD events is addressed with considerable power in the British Regional Heart Study cohort.

NIDDM and CVD: evidence for common risk factors

There is increasing evidence that in some population the adverse coronary risk factor profile, which clusters with insulin resistance (Reaven, 1988), also antedates NIDDM, i.e. that "the clock for coronary heart disease (starts) ticking before the onset of clinical diabetes" (McPhillips et al, 1990; Haffner et al, 1990). For instance, in a prospective study of elderly Finnish men, the risk of NIDDM was related to levels of triglyceride, HDL-cholesterol and blood pressure at baseline in addition to measures of obesity, glucose and insulin (Mykkänen et al, 1993 [a]). In this study impaired glucose tolerance at baseline was associated with a 10-fold increased risk of NIDDM on follow-up. However, the clustering of impaired glucose tolerance, with elevated triglyceride levels (> 2.5 mmol/L), hypertension (BP ≥ 160/95 mmHg or on therapy), and decreased HDL-cholesterol level (< 1.0 mmol/L) was associated with a 59-fold increased risk of NIDDM relative to individuals with none of these risk factors.

It is noteworthy that in this Finnish study (Mykkänen et al, 1993[a]), and in the Zutphen and Rancho Bernardo studies (Feskens et al, 1989; McPhillips et al, 1990), plasma cholesterol levels were not elevated in men who subsequently developed NIDDM. Indeed Stern (1995), in a recent comprehensive review of prospective studies in this area, has stated that neither total cholesterol or LDL-cholesterol have ever been shown to be risk factors for NIDDM whereas both triglyceride and HDL-cholesterol concentrations has been associated with risk of NIDDM (in the direction anticipated) in all of the studies in which these lipids have been measured. As one might have expected the "never" in Stern's statement does not survive scrutiny. Total-cholesterol and LDL-cholesterol did in fact emerge as independent predictors of NIDDM in one small study of Mexican Americans, the San Antonio Heart Study (Haffner, 1990). Similarily the data on whether

elevated blood pressure predicts NIDDM are inconsistent, with positive (Mykkänen *et al*, 1993[a]; McPhillips *et al*, 1990) and negative studies (Sicree *et al*, 1987; Jarrett *et al*, 1984), reflecting perhaps the inconsistent findings in different population on the association between blood pressure and serum insulin levels (Collins *et al*, 1990).

In this thesis, the relation between CHD risk factors at baseline in the British Regional Heart Study and the development of clinically diagnosed NIDDM during a follow-up period of over a decade has been examined. The question of the extent to which CHD risk factors predict NIDDM and whether it is those risk factors which cluster (or are said to cluster) with insulin resistance which emerge in multivariate analysis is addressed with considerable power.

Summary

NIDDM is a heterogenous syndrome characterised by abnormalities of both insulin secretion and action. There is considerable evidence from ecological and migrant studies and from observational studies based on individuals that the risk of NIDDM is substantially influenced by factors acting in adult life, which are broadly associated with industrialisation or "Westernisation". There are unresolved issues regarding the form and magnitude of the association between obesity and NIDDM. Prospective data on the role of physical activity in the development of NIDDM are currently largely confined to graduates and health care professionals. The data on alcohol intake and NIDDM are inconsistent and the association with cigarette smoking has been examined in relatively few studies, also with inconsistent findings. Insulin resistance is an early event in the pathogenesis NIDDM. There is evidence that NIDDM and CHD share common risk factors, linked via insulin resistance. However there are discrepant findings on the

associations between CHD risk factors and NIDDM and in particular on the issues of whether elevated blood pressure and total cholesterol predict NIDDM. Although established NIDDM is associated with increased risk of CHD, the data on associations between both asymptomatic hyperglycaemia and hyperinsulinaemia (a marker for insulin resistance) and CHD are inconsistent.

The BRHS cohort provides a unique opportunity to examine these issues in a prospective study involving over 7000 men, followed for the development of both NIDDM and CHD for over 12 years. This large cohort is representative of British middle- aged men of European ethnic origin. There is a broad spectrum of exposure to the life-style and biological factors of interest (including serum insulin levels) and data are available on a wide range of potential confounding variables.

CHAPTER 3

METHODS

Research design

The hypotheses regarding lifestyle-related factors in the development of NIDDM, the pathogenesis of NIDDM and the association between NIDDM and coronary heart disease (as set out in Chapter 1) have been addressed in five inter-related studies involving the BRHS cohort:

(i) A longitudinal study of the aetiology of NIDDM, examining the role of lifestyle and biological CHD risk factors in the development of this condition during 12.8 years of follow-up of the BRHS cohort. A wide number of factors which have been linked with CHD have been examined as potential predictors of physician diagnosed NIDDM, including:

> a) lifestyle factors linked with CHD, such as body mass index, physical activity, alcohol intake, cigarette smoking, and

> b) established biological CHD risk factors, such as dyslipidaemia and hypertension.

(ii) A cross-sectional study of inter-relations between serum insulin concentration (measured with a specific assay) and CHD risk factors (both lifestyle and biological) at the baseline BRHS examination in a group of 5665 men (members of the BRHS cohort) from whom serum samples were saved at the baseline examination for later insulin measurement.

(iii) A longitudinal study of the association between non-fasting serum insulin level and the development of NIDDM during 12.8 years of follow-up in a group of 5550 non-diabetic men from whom serum samples were saved at the baseline examination.

(iv) A longitudinal study of the association (in non-diabetics) between serum glucose at the baseline examination and the risk of major CHD events during 11.5 years of follow-

up. Data are also presented on the effect of established NIDDM on the subsequent risk of major CHD events in the British Regional Heart Study cohort.

(v) A longitudinal study of the associations between serum insulin levels at the baseline examination (in the group of 5550 non-diabetics) and the risk of major CHD events during 11.5 years of follow-up.

Table 1 shows the personal, lifestyle and biological variables which were examined as possible predictors of NIDDM in the longitudinal study and/or as explanatory variables in the cross-sectional study of factors influencing non-fasting serum insulin levels. These variables have also been examined where appropriate as possible confounders or mediating factors in the studies of associations between established NIDDM, serum glucose and insulin levels and major CHD events.

The British Regional Heart Study

The British Regional Heart Study is a prospective study of cardiovascular disease in a representative sample of British middle-aged men. The primary aims of the British Regional Heart Study (BRHS) are (i) to explain the substantial geographic variations in cardiovascular mortality, by assessing the role of enviromental, socioeconomic and personal risk factors and (ii) to examine aetological factors in ischaemic heart disease. In the (BRHS), 7735 men aged 40 to 59, were selected at random from the age-sex register of one general practice in each of 24 towns in England, Wales and Scotland for examination between January 1978 and June 1980. The criteria for selecting the towns, general practices and subjects and details of the respondents and data collection have been described (Shaper *et al*, 1981; Shaper *et al*, 1985[a]). The towns were chosen to represent all major geographic regions in Britain and to reflect the variation in

cardiovascular disease and water hardness (a major interest at the outset of the study). Within regions an attempt was made to identify towns with a population of 50 000 to 100 000, which were representative of the region in socioeconomic terms. Towns with recent or ongoing population movement or with an unusual population structure were avoided. When a number of towns met the above criteria random selection was employed. Within each town, a group practice, representative of the socioeconomic composition of the town population, was chosen. From the age sex register of the chosen practice, 450 men were selected at random, stratified into four equal-sized five-year age groups. Men with cardiovascular or other disease or those receiving regular medication were not excluded. Between 6 and 10 men in each practice were excluded by the general practitioners on the grounds of severe mental or physical disability. The overall response rate was 78%, ranging from 70% to 85% across the 24 towns.

Data collection: base-line assessment

Research nurses administered a standard questionnaire and completed an examination of each man, including an electrocardiogram (Shaper *et al*, 1984). All measurements were made by a team of three nurses. Training for standardisation of procedures, including administration of the questionnaire. was carried out before the study and repeated at intervals throughout. Prior to commencement of the project, a feasibility study was carried out to test and a pilot study to finalise all methods and procedures. The questionnaire *(See Appendix 1)* included questions on occupational status, the usual pattern of physical activity, alcohol intake, smoking habits, medical history (including heart disease other than CHD) and use of medication, including anti-hypertensive drugs. Details of specific anti-hypertensive agents were not recorded. Age was recorded to the

day of examination. Details of the classification of social class, physical activity, alcohol intake, smoking habits, pre-existing coronary heart disease have been reported, together with details of the measurement of height, weight, blood pressure, heart rate, forced expiratory volume (FEV_{1sec}) and haematocrit (Shaper *et al*, 1981; Shaper *et al*, 1985 [a]; Shaper *et al*, 1991; Shaper & Wannamethee, 1991; Wannamethee & Shaper 1992; Wannamethee *et al*, 1993; Wannamethee & Shaper, 1994).

Social class

The longest held occupation of each man was recorded and the men were grouped into one of the six social classes of the Registrar-General: I, II,III non-manual, III manual, IV and V. Those whose longest held occupation was in the Armed Forces form a separate group. In some of the analyses social classes I, II, and III non-manual have been combined as "non-manual workers" and social classes III manual, IV and V have been combined as "manual workers". Social class data were missing for 13 men.

Physical activity

The men completed an exercise questionnaire at baseline. The exercise questions referred to (i) the type and duration of exercise while travelling to work, (ii) the participants assessment of occupational physical activity, (iii) a grading (1-5) of week-end physical activity, (iv) the frequency of participation in active physical exercise such as running, digging, and tennis and (v) the number of years the subject had been involved in such activity.

A physical activity score was derived, based on the frequency and intensity of the activities reported, using recommendations of a National Heart, Lung, and Blood Institute

workshop and the Minnesota intensity codes (Shaper & Wannamethee, 1991; Wannamethee & Shaper, 1992). This score, which has been validated against heart rate and lung function, is predictive of major cardiovascular end points, i.e. myocardial infarction and stroke (Shaper & Wannamethee, 1991; Wannamethee & Shaper, 1992). The men were grouped into six physical activity categories: inactive (N=686), occasionally active (N = 2345), light (N = 1761), moderate (N = 1205), moderately vigorous (N = 1120) and vigorous activity (N = 513). Data on physical activity were not available for 105 men. Men whose level of activity was moderate or higher were characterised as physically active.

Alcohol intake

Questions on alcohol intake formed part of a general enquiry into dietary, drinking and smoking habits. Alcohol consumption was recorded using questions on frequency, quantity and type, similar to those used in the 1978 General Household Survey. The men were classified into five groups according to their alcohol intake: never (N=466), occasional (< 1 unit/week; N=1845), light; 1-15 units/week; N=2544), moderate (16-42 units/week; N=2041) and heavy (> 42 units/week; N=832). Data on alcohol intake were not available for 7 men. A unit of alcohol was half a pint of beer, a glass of wine, or a single measure of spirits (Shaper et al, 1988; Shaper et al, 1994). One U.K. unit is equivalent to 8-10g of alcohol. The reported alcohol consumption has been correlated with 25 biochemical and haematological measurements performed on a single blood sample taken when the questionnaire was completed. Several measurements showed substantial dose-response relations with the reported alcohol consumption, including gamma glutamyl transferase (GGT), high-density lipoprotein-cholesterol (HDL-C), mean

cell volume (MCV), aspartate transaminase (AspT) and blood lead, measurements recognised to be indicators of alcohol intake (Shaper et al, 1985 [b]). These findings strongly support the validity of the reported alcohol consumption on a group basis.

Cigarette smoking

The men were classified according to their current smoking status into one of five categories: never smoked (N=1822), ex-cigarette smokers (N=2715), and current smokers (1-19/day (N=1188), 20/day (N=835), and $\geq$ 21/day (N=1162). Those who had smoked only a pipe or cigars were classified as never smokers. Former cigarette smokers who smoked a pipe or cigars were regarded as former cigarette smokers. Data on cigarette smoking status were missing for 13 men. Smoking status in this cohort has been shown to be highly correlated with blood cadmium levels (Pocock et al, 1988), which are regarded as a useful biological marker of smoking status. Smoking status has also been shown to be strongly associated with physiological variables known to be influenced by smoking, such as lung function, haematocrit and heart rate (Cook et al. 1990; Wannamethee & Shaper, 1994).

Pre-existing coronary heart disease (CHD)

Using the WHO (Rose) chest pain questionnaire and a 3-orthogonal lead electrocardiogram, pre-existing CHD at screening was defined on the basis of any of the following criteria: recall of doctor diagnosis of angina or heart attack, a WHO (Rose) chest pain questionnaire response indicating angina or possible myocardial infarction, or electrocardiographic evidence of definite or possible myocardial ischaemia or infarction (Shaper et al. 1984; Phillips et al. 1988). 25% of the men (N=1943) were characterised

as having prevalent CHD at screening. In some analyses, the men have been separated into three groups according to the degree of evidence of prevalent CHD at screening, (i) no evidence of CHD (N=5767), (ii) evidence of CHD short of a definite myocardial infarction (N=1515) and (iii) men with evidence of a definite myocardial infarction on electrocardiogram or recall of a doctor diagnosis of myocardial infarction (N=428). Men in groups (ii) and (iii) have been categorised as having pre-existing coronary heart disease. ECG recordings were unavailable from 52 men.

Body mass index

Body mass index calculated as weight/height^2 (kg/m^2) was used as an index of relative weight. Each man was weighed in trousers and socks to the nearest 0.1 kg on an MPS110 field survey scale (beam balance) and height was measured to the nearest millimetre with a Harpenden Stadiometer with digital meter. Men with BMI $\geq$ 28 kg/m^2, the top fifth of the BMI distribution in this cohort, were characterised as obese. Body mass index was not available for 3 men.

Blood pressure

Blood pressure was taken by the team of nurses. The London School of Hygiene sphygmomanometer was used to measure blood pressure twice in succession with the subject seated and the arm supported on the cushion. For each man the mean of the two readings was used. Diastolic blood pressure was recorded at the disappearance of sounds (phase V). All blood pressure readings were adjusted for observer variation within each town to allow for differences between the three observers (Bruce *et al*, 1988). Systolic and diastolic blood pressure data were missing from 8 and 10 men respectively.

Heart rate (Wannamethee *et al*, 1993)

Heart rate was determined from a three lead orthogonal electrocardiogram. All men were supine and before the recording were resting while answering the questionnaire. On the basis of the eight second recording, the average RR interval (seconds) during that period was calculated: heart rate = 60/ (average RR interval) beats per minute. If a ventricular extrasystole occured during the eight second recording, it would have been used in deriving the estimate of heart rate (N=209; 2.7% of the men). A group of 49 men (0.6%) were in atrial fibrillation. Data on heart rate were missing from 52 men.

Forced expiratory volume

Forced expiratory volume in one second (FEV_1) was measured in the seated position, using a Vitalograph spirometer. The maximum volume recorded was used. The data have been height standardised as follows: adjusted FEV_{1sec} = FEV_{1sec} * ($1.733/height^2$); where 1.733 = mean height in the cohort (Cook, 1988). Fev_{1sec} data were not available for 85 men.

Biochemical and haematological measurements

Non-fasting blood samples were obtained between 8.30 am and 6.30 pm. The time of arrival at the examination centre was noted and the estimated time of venepuncture was 35 minutes later. Details of venepuncture, serum separation and storage have been described (Pocock *et al,* 1989). Serum was separated on site within 30 minutes of venepuncture and stored at -4° C until the following day. With the exception of serum insulin measurements (see below), analyses were performed within 24 hours of sampling. Glucose, total cholesterol and uric acid were analyzed in serum using an automated

analyzer (Technicon SMA 12/60) (Pocock *et al*, 1989; Cook *et al*, 1986). HDL-cholesterol and triglyceride concentrations were measured using enzymatic methods (Pocock *et al*, 1989). Haematocrit levels were estimated using a Coulter S electronic particle counter (Coulter Electronics Ltd., Luton) which was calibrated daily. As triglyceride concentrations were not determined for men in the first six towns, data on this variable were available for only 5675 men. Data on haematocrit were missing from 389 men. Glucose data were missing from 46 men, total cholesterol from 45 men, HDL-cholesterol from 316 men and uric acid from 47 men. Diurnal variation in glucose levels was modest, with a peak-trough difference of 0.4 mmol/L (Pocock *et al*, 1989).

Insulin measurement

Serum insulin concentration was determined by a two-site enzyme-linked immuno-adsorbent assay (ELISA) using commercially available monoclonal antibodies raised against human insulin (Novo Nordisk A/S: Denmark) which do not cross react with proinsulin (Anderson *et al*, 1993). Analyses were performed in the Department of Medicine, University of Newcastle upon Tyne, UK. on non-fasting samples from 5665 men in 18 of the 24 BRHS towns (towns 7 to 24 in the order in which the towns were visited). These samples were stored at -20°C for between 13 to 15 years. In this laboratory (Newcastle upon Tyne), no change in insulin levels was detected in repeat assays of 34 samples, stored at -20°C over an 8 year period (mean difference 0.19 mU/L, paired t=0.7,p=0.5). The lower limit of detection for the ELISA was 1 mU/L and the interassay coefficients of variation were 5.5% at 8.8 mU/L, 5.9% at 21.6 mU/L and 7.5% at 44.8 mU/L. The distribution of serum insulin was markedly skewed, range 1 to 479.5 mU/L, median 11.9 mU/L, geometric mean (log sd) 12.7 (0.76) mU/L. There was obvious diurnal variation in serum insulin levels, with peaks between 8-9 am, 1-2 pm and

6-7 pm, presumably related to meals, and troughs between 11-12 am. and 4-5 pm. The highest levels (geometric mean) were observed between 1-2 pm., (18.4 mU/L) and the lowest levels between 4-5 pm.,(10.3 mU/L).

Questionnaires at year 5 of follow-up and in 1992

Five years after the initial screening a questionnaire, similar to the baseline questionnaire, was mailed to all of the surviving men in the British Regional Heart Study. This questionnaire included questions regarding morbidity during the five year-period. A total of 7275 men, 98.4% of all available survivors, completed the questionnnaire satisfactorily. The available survivors exclude those men who had died (n=297) or emigrated (n=42) prior to the fifth year questionnaire. Non-responders included men who had moved and not yet registered with a new doctor (1%) and those who declined to participate. A further questionnaire was mailed in 1992 to 6582 surviving members of the cohort who were resident in Britain. This questionnaire achieved a response rate of 90% (N= 5934), with 1% declining to participate, 1% not traced and 8% non-responders.

In both the 5th year and the 1992 questionnaire the men were asked whether they had ever been told by a doctor that they had diabetes and whether they were using tablets and/or insulin injections for diabetes. In the 1992 questionnaire there were additional questions on whether a "diet for diabetes" had been prescribed, on the year of diagnosis, and on attendance at diabetic clinics.

Follow-up for development of NIDDM

The men have been followed for the development of doctor diagnosed cases of diabetes

up to December 1991, a mean period of 12.8 years (range 11.5 to 13.0 years) (Walker & Shaper, 1984). Less than 1 % (N=73) of men were lost to follow-up, of whom 44 (0.6%) have emigrated from the United Kingdom. New cases of NIDDM were ascertained by means of (i) the postal questionnaire sent to the men at year 5 of follow-up for each individual, (ii) systematic reviews of primary care records in 1990 and 1992, (iii) the 1992 questionnaire, and (iv) review of all death certificates for any mention of diabetes.

In the primary care record review, the records of each study participant (including discharge letters from hospital) were examined for a number of specific diagnoses including diabetes. Inconsistencies between the questionnaire data and the clinical records were resolved by means of further review of the primary care records. A diagnosis of diabetes was not accepted on the basis of questionnaire data, unless confirmed in the primary care records. Information on deaths (including copies of death certificates) was obtained through the established "tagging" procedures provided by the National Health Service registers in Southport (England and Wales) and Edinburgh (Scotland).

Ascertainment of incident cases of NIDDM (N=194)

Men who reported a diagnosis of diabetes at the baseline questionnaire (N=121) were excluded as prevalent cases of NIDDM. Men with a non-fasting glucose at screening in the diabetic range ($\geq$ 11.1 mmol/L, N=23,) were also excluded as were men with a date of NIDDM diagnosis in the same calender year as the year of screening (N=14). Thus 7577 men were at risk of developing NIDDM during follow-up.

Incident cases of NIDDM were ascertained as follows:

i) cases of diabetes identified in the primary care record reviews of 1990 and 1992 which

occured in men at risk of developing NIDDM (as defined above), with a date of diagnosis up to 31st December 1991.

ii) cases of self reported diabetes on the 1992 questionnaire among men at risk of developing NIDDM, with a year of diagnosis of 1991 or earlier, provided diabetes was confirmed on subsequent review of primary care records,

iii) cases of self reported diabetes on the 5th year questionnaire among men at risk of developing NIDDM who had died before the 1992 questionnaire, provided the diagnosis of diabetes was confirmed on review of the primary care records or mentioned in the man's death certificate.

Agreement between self reported diabetes and primary care record review

In 96.7% of men who reported a diagnosis of diabetes in the 1992 questionnaire, the diagnosis was confirmed on review of the primary care records. This level of agreement between self reported cases of diabetes and medical records is similar to that reported in studies of diabetes incidence in health care professionals (Helmrich *et al*, 1991).

Follow-up for major CHD events

Over 99% of study participants have been followed for major CHD events (fatal and non-fatal myocardial infarction) for a period of 11.5 years. Full details of follow-up procedures have been published and the criteria for fatal and non-fatal major ischaemic heart disease events have been described (Shaper *et al*, 1985 [a]; Walker & Shaper, 1984). Information on death was obtained through the established "tagging" procedures as detailed in the preceeding section.

Morbidity follow-up

To facilitate reporting of non-fatal major CHD events to the study coordinator (MW), a blue report card was placed in the medical record, held by the general practice of each member of the cohort. When a man in the study consults or is visited by a doctor for a cardiovascular event (myocardial infarction, angina, transient ischaemic heart attack and stroke), the report card is completed and returned to the study coordinator in the Department of Public Health at the Royal Free Hospital School of Medicine. A similar process takes place when a death occurs. All participating doctors have been asked to review their records, every 2 years between 1980 and 1992 (Walker & Shaper, 1984). A coordinator in each of the original 24 practices is responsible for mailing the blue cards, organising the biennial inspection of primary care records (which in 1990 and in 1992 included a specific request to look for cases of diabetes) and notifying the study coordinator of removals from the practice. Any person who moves to a new area and who is registered with another General Practitioner is traced through the relevant Family Health Service Authority (formerly the Family Practitioners Committee). The new General Practitioner is contacted by letter and telephone and requested to notify the study coordinator of any cardiovascular events that have occurred (Walker and Shaper 1984).

Case definition for major CHD events

Non-fatal events- A non-fatal myocardial infarction was diagnosed according to WHO criteria, i.e. an event which satisfied at least two of the following criteria: (a) preceded by severe prolonged chest pain, (b) electrocardiograph evidence of myocardial infarction, (c) cardiac enzyme changes associated with myocardial infarction.

Fatal events- Fatal events were defined as deaths from ischaemic heart disease

(International Classification of Disease 9th Revision: Codes 410-414) as the underlying cause. Any death recorded on the death certificate as being due to ischaemic heart disease (ICD 410-414) and not contradicted by the medical history or postmortem examination was classified as a fatal CHD event. Sudden death for which no other cause was apparent and which had been certified as being due to ischaemic heart disease was included in this category. Individuals who first had a non-fatal event and then a fatal myocardial infarction during the follow-up period were classified as having had a fatal event.

Statistical analyses

Details of the statistical analyses undertaken will be provided in the relevant Chapters.

Table 1- Personal, lifestyle and biological variables which were examined as possible predictors of NIDDM in the longitudinal study and/or predictors of serum insulin in the cross-sectional study.

Age (years)

Occupational status (Social class)

Pre-existing coronary heart disease

History of "other heart disease"

Use of anti-hypertensive medication

Regular use of medication

Glucose (mmol/L)

Body mass index (kg/m^2)

Height (m)

Physical activity

Alcohol intake

Cigarette smoking

Forced expiratory volume $_{(1 \text{ second})}$(L)

Systolic and diastolic blood pressure (mmHg)

Heart rate (beats/min)

Serum total cholesterol and HDL-cholesterol (mmol/L)

Serum triglyceride (mmol/L)

Serum uric acid (μmol/L)

Haematocrit level (%)

Serum insulin (mU/L)

CHAPTER 4

RISK FACTORS FOR THE DEVELOPMENT OF NON-INSULIN-DEPENDENT DIABETES IN MIDDLE-AGED BRITISH MEN

ABSTRACT

Objectives:- To determine the risk factors for NIDDM in a cohort representative of middle-aged British men with particular reference to the hypothesis that NIDDM shares common risk factors with coronary heart disease.

Design:- A prospective study.

Subjects and setting:- 7735 men aged 40-59, drawn from one group practice in each of 24 towns in Britain. Known and probable cases of diabetes at screening were excluded (N=158).

Main outcome measure:- Incidence of physician-diagnosed non-insulin dependent diabetes over a mean follow-up period of 12.8 years.

Principal Results:- There were 194 new cases of non-insulin dependent diabetes. Body mass index was the dominant risk factor for diabetes with an age adjusted relative risk (5th to 1st quintile) of 11.6; 95% C.I. 5.4 to 16.8. Men engaged in moderate levels of physical activity had a substantially reduced risk of diabetes, relative to the physically inactive, after adjustment for age and BMI (RR 0.4; 95% C.I. 0.2 to 0.7), an association which persisted in full multivariate analysis. A non-linear relation between alcohol intake and diabetes was observed with the lowest risk among moderate drinkers (16-42 U.K. units/week), relative to the baseline group of occasional drinkers, RR 0.6 (95% C.I. 0.4 to 1.0). Additional significant predictors of diabetes in multivariate analysis included serum triglycerides, HDL-cholesterol (inverse association) and uric acid concentration, heart rate, haematocrit level, FEV_{1sec}, and prevalent coronary heart disease (CHD). However a number of important CHD risk factors, notably total cholesterol concentration, systolic blood pressure and cigarette smoking did not predict NIDDM.

Conclusion:- Non-insulin dependent diabetes and coronary heart disease share some common lifestyle and biological risk factors. There are however several important CHD risk factors which are not related to the risk of developing NIDDM.

Introduction

In this chapter the findings from the prospective study of risk factors for NIDDM in the British Regional Heart Study cohort are presented. This study addresses the role of lifestyle related factors in the development of NIDDM, i.e. body mass index, physical activity, alcohol intake, cigarette smoking. It also addresses the hypothesis that in this population CHD risk factors which cluster with insulin resistance and hyperinsulinaemia will antedate NIDDM. These factors include hypertension, dyslipidemia (specifically decreased HDL-cholesterol and elevated triglyceride levels) and hyperuricemia (Laws & Reaven, 1993).

A number of additional potential predictors of NIDDM, which have also been linked with CHD in longitudinal studies, were examined, i.e. haematocrit, height, and FEV_{1sec}. As discussed in *Chapter 2* there is evidence from cross-sectional data of an association between raised haematocrit levels and the insulin resistance syndrome (Smith *et al*, 1994; Moan *et al*, 1994) and there is evidence from the Framingham study of an association between high haemoglobin level and glucose intolerance (Wilson *et al*, 1981). Short stature and FEV_{1sec} have also been linked with increased risk of glucose intolerance (Williams *et al*, 1995; Paffenbarger *et al*, 1973). It is suggested that adult height reflects, amongst other factors, foetal growth and that the associations between height and risk of glucose intolerance and CHD may be consistent with the Barker hypothesis linking retarded growth *in utero* to these disease end-points in later life (Williams *et al*, 1991). It has also been suggested that FEV_{1sec} may be a marker of retarded foetal growth (Barker *et al*, 1991), though clearly there are additional factors which influence FEV_{1sec} (notably cigarette smoking) which are relevant to this study.

Subjects and methods of data collection

The group of 7577 men who were at risk of developing NIDDM (following the exclusion of known and probable diabetics as defined in (*Chapter 3*) form the subjects of this study. Details of the exposure variables examined and of the methods of measurement have been set out in *Chapter 3*. Details of the ascertainment of cases of NIDDM and of the measurement of age, occupational status, body mass index, height, alcohol intake, physical activity, cigarette smoking, pre-existing CHD and other heart disease, use of antihypertensive drugs and other medication, blood pressure, serum glucose, uric acid, total cholesterol, HDL-cholesterol and triglyceride concentration, heart rate, haematocrit and FEV_{1sec} have been set out in *Chapter 3*.

Statistical analysis

All analyses were carried out using the Statistical Analysis System (S.A.S.). Cox's proportional hazards models were used to assess the independent contributions of the risk factors to the subsequent risk of NIDDM and to obtain the relative risks adjusted for the other risk factors (Cox, 1972). The Cox proportional hazards model has a number of advantages over the logistic regression model which is widely used in longitudinal studies of cardiovascular disease. It accomodates unequal follow-up data and censoring of data due to death and withdrawal from the study. No assumptions are made regarding the distribution of survival times (i.e. time to develop NIDDM in the present study). In the standard Cox's model it is assumed that for a given exposure, such as cigarette smoking, the hazard (essentially the risk of the end-point event at time t) is a constant multiple of the baseline hazard (e.g. the hazard in non-smokers) over the period of follow-up.

Physical activity, cigarette smoking, alcohol intake and pre-existing coronary heart

disease were fitted as categorical variables in the proportional hazards model. Physical activity was fitted as 5 dummy variables (6 levels: none, occasional, light, moderate, moderately vigorous, vigorous), smoking as 4 variables (5 levels: never, ex-smokers, and 1-19, 20, ≥ 21 cigarettes daily), alcohol intake as 4 variables (5 levels: none, occasional, light, moderate, heavy) and pre-existing CHD as a dichotomous (yes/no) variable. The adjusted relative risks in *Figures 1 to 5 and 7* were obtained by fitting body mass index, blood glucose, systolic blood pressure, heart rate, HDL-cholesterol, triglyceride, urate and FEV_{1sec} as four dummy variables for the five quintiles of each variable. Tests for trend and adjustments were carried out by fitting the quantitative variables in their continuous form. Haematocrit was fitted as four dummy variables for five haematocrit groups, based on absolute levels of hematocrit: $<42.0\% = 1183$ men, $42.0\text{-}43.9\% = 1623$ men, $44.0\text{-}45.9\% = 1974$ men, $46.0\text{-}47.9\% = 1388$ men and $\geq 48.0\% = 1025$ men, *(Figure 6)*. Tests for trend were carried out fitting haematocrit in its original continuous form. In *Table 4* analysis of covariance was used to derive age and body mass index adjusted means. Logistic regression was used to calculate age and body mass index adjusted prevalence rates based on conversion of adjusted odds ratios to estimated proportions *(Table 4)*. For the data presented in **Tables 5** and **Table 6**, the validity of the proportional hazards assumption in the Cox's models was checked by fitting a time-dependent interaction variable $X = X(t)$, where $X(t) = \log (t)$. Subjects with missing values for covariates in the various adjustments using Cox's model, were excluded from that particular analysis.

As glucose and triglyceride concentrations were not normally distributed, log transformation and geometric means were used. Because of the marked diurnal variation in serum triglyceride levels (Pocock *et al*, 1989), the log transformed data on this

variable were adjusted for time of sampling, using the mean triglyceride level for each

hour in which samples were taken and the grand mean. From each individual value, the

mean for the hour of sampling for that individual was subtracted, and the result was

added to the grand mean (Phillips, 1986).

Results

After a mean follow-up period of 12.8 years there were 194 new cases of NIDDM in the

7577 men, an incidence of 2.15 per 1000 person years of follow-up. Men who developed

NIDDM had significantly higher mean body mass index than other men (27.9 vs 25.4

kg/m^2; p < 0.0001). Nearly half of NIDDM cases (44%) were obese ($\geq$28 kg/m^2)

compared to about 18% in the rest of the men. Men who subsequently developed

NIDDM also had significantly higher mean blood glucose levels (6.2 vs 5.4 mmol/l;

p < 0.0001) at baseline. There was little difference in mean age between the two groups

(50.4 vs 50.2).

Body mass index and blood glucose

Table 2 and *Table 3* show the incidence rate of doctor diagnosed NIDDM by quintile of

serum glucose and body mass index respectively, the dominant predictors of NIDDM.

Figure 1 shows the age-adjusted relative risk of NIDDM by quintile of serum glucose and

body mass index. There was a more than 4-fold increased risk of NIDDM in the upper

quintile of serum glucose ($\geq$ 6.1 mmol/L and < 11.1 mmol/L) relative to the first

quintile, (< 4.8 mmol/L), RR=4.7; 95% C.I. 2.9 to 7.6. The risk of NIDDM

increased exponentially with increasing body mass index, with an over 11-fold excess risk

in the upper quintile (BMI $\geq$ 27.9 kg/m^2) relative to the first quintile, (BMI $\leq$ 22.9

Kg/m²); RR=11.6; 95% C.I. 5.6 to 23.8. Among a group of 2614 men (34% of the participants), with either serum glucose or BMI in the fifth quintile, there were 137 cases of NIDDM, 71% of the cases. Among a high risk group of 346 men (5% of participants) who were in the upper quintile of both glucose and BMI, there were 42 cases of NIDDM during follow-up, 22% of the total number of cases.

Baseline characteristics of NIDDM cases compared with the rest of the cohort

Table 4 shows the baseline characteristics of the men who subsequently developed NIDDM compared with the rest of the cohort, adjusted for age and body mass index. No adjustments were made for serum glucose which is assumed to be in the causal pathway between the relevant exposures and NIDDM. Among those who developed NIDDM, there were significantly fewer moderate drinkers and physically active men than in the rest of the cohort. There was no difference in the proportion of manual and non-manual workers. Men who developed NIDDM were more likely to be smokers (p=0.06) and had a significantly higher prevalence of pre-existing CHD, but not of "other heart disease" or stroke. A higher proportion were using anti-hypertensive therapy but the difference was not significant. No significant difference in use of other medication between the groups was observed. Systolic and diastolic BP, triglyceride levels, uric acid, heart rate and haematocrit level were all significantly higer in the cases than in the rest of the cohort. FEV$_{1sec}$ and HDL-cholesterol concentrations were significantly lower in the cases. There were no significant difference in mean height or total cholesterol concentration between the cases of NIDDM and the rest of the men.

Social class and risk of NIDDM

Figure 2 shows the relative risk of NIDDM with 95% confidence intervals in each social

class group relative to Social Class III (manual), adjusted for age and separately for age

and BMI. There was no evidence of an association between social class and risk of

NIDDM in either the age or the age and body mass index adjusted data.

Physical activity, alcohol, cigarette smoking and NIDDM

Figure 3 shows the relative risk of NIDDM with 95% confidence intervals at different

levels of physical activity and alcohol intake, adjusted for age and separately for age and

BMI. An inverse association was seen with physical activity. The age and BMI adjusted

risk of NIDDM decreased with increasing levels of physical activity to moderate levels

(RR=0.4; 95% C.I. 0.2 to 0.7, relative to the physically inactive) with no further decline

thereafter. The additional adjustment for BMI produced some attenuation of the

association between physical activity and NIDDM as compared with the age adjusted

relative risks, (*Figure 4*). A shallow "U-shaped" relation was seen between alcohol intake

and the risk of NIDDM with the lowest risk seen in moderate drinkers. Relative to

occasional drinkers (the baseline group), the age and BMI adjusted relative risk of

NIDDM was 0.64 (95% C.I. 0.43 to 0.96) among moderate drinkers. Current smoking

was associated with a 50% increase in the risk of NIDDM relative to those who had

never smoked, adjusted for age and BMI, (RR=1.5; 95% C.I. 1.0 to 2.2;p=0.04). No

significant association with the number of cigarettes smoked was observed, (*Figure 4*).

Ex-smokers were at similar risk of NIDDM as never smokers, (RR=1.2; 95% C.I. 0.8

to 1.8).

Pre-existing CHD and the risk of NIDDM

Men with evidence of CHD at screening showed a 50% increase in risk of NIDDM on adjustment for age and BMI (RR=1.53 95% C.I. 1.13 to 2.07). This raises the question of whether the findings on physical activity, alcohol intake and cigarette smoking were influenced by behaviour change or by increased surveillance associated with a diagnosis of CHD. This issue was addressed in multivariate analysis and by examining the associations separately in men with and without CHD at screening. Before describing these analyses, data will be presented on the biological CHD factors which were associated with NIDDM after adjustment for age and BMI.

Biological CHD risk factors and NIDDM

Figure 5 shows the age and BMI adjusted relative risks of NIDDM, with 95% confidence intervals, by quintile of systolic BP, diastolic BP, HDL-cholesterol, triglyceride and urate concentrations and heart rate. The age adjusted risk of NIDDM increased significantly with increasing systolic BP, an association which was markedly attenuated on adjustment for BMI, although the trend remained significant, p=0.02. A similar though weaker association was seen for diastolic BP. For HDL-cholesterol, the age and BMI adjusted risk of NIDDM was raised in the lowest quintile but there was relatively little decline in risk with increasing HDL-cholesterol therefter (test for trend p=0.004). A particularly strong association between triglyceride concentration and the subsequent development of NIDDM was observed with a more than 3-fold increased risk in the top relative to the bottom quintile on adjustment for age and BMI (test for trend p<0.0001). The risk of NIDDM tended to increase with increasing urate although in the age and BMI adjusted data the lowest risk was seen in the 2nd quintile. For heart rate the risk of NIDDM was

markedly increased in the top relative to the lowest quintile (age and BMI adjusted RR=2.9; 95% C.I. 1.7 to 4.9).

Haematocrit and risk of NIDDM

Figure 6 shows the age and BMI adjusted relative risks of NIDDM, with 95% confidence intervals in the four haematocrit groups relative to the baseline group (< 42%). There was a more than four-fold increased relative risk (RR) of diabetes among men with a haematocrit of 48% or higher relative to those with a haematocrit below 42%, adjusted for age and body mass index, (RR 4.5; 95% C.I. 2.5 to 6.3). In the age adjusted data there was an almost 6-fold increased risk of NIDDM in the highest relative to the baseline group.

FEV_{1sec} and NIDDM

A linear inverse association between FEV_{1sec} and risk of NIDDM was observed, (*Figure 8*), with a substantially lower risk in the fifth relative to the first quintile after adjustment for age and BMI (RR 0.4; 95% C.I. 0.24 to 0.7).

Risk factors for NIDDM in multivariate analysis

Many of the factors shown to be significantly associated with risk of NIDDM after adjustment for age and BMI are interrelated. viz pre-existing CHD. physical activity level, alcohol intake, smoking status. systolic BP, HDL-cholesterol, heart rate, serum urate and triglyceride concentrations. haematocrit and FEV_{1sec}. To identify independent predictors of NIDDM we have examined these factors adjusting for age and for each of the other variables. As serum triglyceride data were only available in 5327 men (130

cases), we have adjusted for this variable in a separate model (below). The adjusted relative risks are presented in **Table 5**. There remained an over 6-fold increase risk of NIDDM in the fifth quintile of BMI relative to the first in multivariate analysis and the strong, linear, inverse association with physical activity was not attenuated. The associations with moderate drinking, heart rate, HDL-cholesterol level remained significant, whereas the associations with uric acid and pre-existing CHD were somewhat attenuated. The associations with haematocrit and FEV_{1sec} were also significant in the fully adjusted proportional hazard model with an over three-fold increased risk of NIDDM in the highest relative to the lowest haematocrit group and a 2-fold decreased risk in the fifth relative to the first quintile of the distribution of FEV_{1sec}. The associations between current smoking and systolic BP and the subsequent development of NIDDM were completely attenuated in this multivariate model.

Additional multivariate analysis: adjustment for triglyceride

Variables which emerged as significant (or borderline) predictors of NIDDM in the multivariate model presented in **Table 5**, were further adjusted for triglyceride in a separate model based on data from a subgroup of 5031 men with serum triglyceride data and data on the relevant variables. **Table 6**. Again, BMI was the dominant predictor of NIDDM and the association with moderate drinking was unchanged. The association with physical activity was of borderline significance in this model. However this may reflect the reduced sample size and there was no evidence that the physical activity-NIDDM association was attenuated on adjustment for triglyceride. The associations between pre-existing CHD, uric acid, haematocrit and FEV_{1sec} and risk of NIDDM were essentially unchanged in this model after adjustment for triglyceride. The association with

heart rate was attenuated. Although men in the top heart rate quintile were at somewhat higher risk of NIDDM than men in the bottom quintile after adjustment for triglyceride concentration, a significant linear trend was not observed. The inverse association between HDL-cholesterol and the risk of NIDDM was markedly attenuated after adjustment (with a change in the direction of the effect), reflecting the strong inverse association between HDL-cholesterol and triglyceride.

Effect of pre-existing CHD

The factors shown to be independently associated with NIDDM, i.e. BMI, physical activity, alcohol intake, uric acid, triglyceride, haematocrit and FEV_{1sec} were also examined separately in men without pre-existing CHD (n=3841 men;82 cases) and in men with pre-existing CHD (n=1296 men;45 cases) at baseline. This analysis was confined to the subset of men with available data on triglyceride and the other relevant covariates. Given the reduced sample size and number of cases within these subgroups the focus of this analysis was on the direction and magnitude of effects rather than the significance of linear trends. Associations of similar direction and magnitude were seen in both groups between BMI, physical activity, triglyceride and uric acid concentration and risk of NIDDM.

The positive association between haematocrit and risk of NIDDM was clearly evident in men without pre-existing CHD with a substantially increased risk in the highest relative to the baseline group, (RR 4.4; 95% C.I. 2.1 to 11.7) and a relatively smooth linear trend. This association was weaker and less consistent in men with evidence of pre-existing CHD and the linear trend did not attain statistical significance. A test for difference in trend between the two groups was of marginal significance (p=0.09 for

interaction). By contrast, the association between FEV_{1sec} and risk of NIDDM was more apparent in men with pre-existing CHD at baseline.

Similarly for alcohol, the lower risk of NIDDM seen in moderate drinkers was more apparent in men with pre-existing CHD than in men without evidence of CHD at screening, raising the possibility of confounding due to changes in alcohol consumption following the diagnosis of CHD. In men without pre-existing CHD at baseline, moderate alcohol intake showed a small, non-significant reduction in risk of subsequent NIDDM, relative risk, 0.8 (95% C.I. 0.4 to 1.5) relative to occasional drinkers whereas in men with pre-existing disease the relative risk was 0.4 (95% C.I. 0.1 to 0.9). However these difference in the associations between FEV_{1sec} and alcohol and risk of NIDDM between men with and without evidence of pre-existing CHD at baseline were not significant on formal testing for interaction, possibly because of the small number of men involved.

Predictors in the upper serum glucose quintile

It is likely that a high proportion of men with serum glucose in the upper quintile (≥ 6.1 mmol/L) had glucose intolerance. Data on all covariates (including triglyceride) were available for 1042 men with serum glucose in the 5th quintile, of whom 65 subsequently developed NIDDM. BMI, physical activity, moderate alcohol intake, triglyceride concentration, haematocrit, FEV_{1sec} and prevalent CHD all showed similar association with risk of NIDDM in this subgroup as in the rest of the cohort. However no association with uric acid was observed.

Discussion

This study confirms the value of the simple measures of body mass index and non-fasting glucose in the identification of men in middle-age at high risk for the development of NIDDM. There was a sharp increase in risk of subsequent NIDDM at relatively low non-fasting glucose levels, i.e. $\geq$ 6.1 mmol/L. A strong, graded relation between body mass index and the risk of NIDDM was observed and the findings do not support the suggestion that modest levels of obesity are not associated with NIDDM (Jarrett, 1986). The findings support the hypothesis that physical activity reduces the risk of developing NIDDM. There was a more than two-fold increased risk of disease among men who were physically inactive relative to those engaged in regular moderate activity, an association that was independent of age, obesity, and other potential confounding factors. Moderately heavy alcohol intake (between 16 and 42 units per week) was also associated with a lower risk of NIDDM. This finding however should be interpreted cautiously, given that it was significant only in men without evidence of CHD at baseline. There was at most a weak association between cigarette smoking and NIDDM. Specific lipid abnormalities which are linked with insulin resistance, (raised triglyceride and low HDL-cholesterol concentration) (Abbott et al, 1987; Zavaroni et al, 1989), were shown to predict NIDDM and there was an association with high serum urate, which is also linked with insulin resistance (Facchini et al, 1991). Haematocrit and FEV_{1sec}, which are established predictors of CHD (Knotterus et al, 1988; Wannamethee et al, 1994; Walker et al, 1989), also emerged as strong and independent predictors of non-insulin dependent diabetes in this cohort. However, no association between total serum cholesterol and the risk of subsequent NIDDM was observed. Similar factors predicted deterioration to NIDDM in men with relatively high non-fasting glucose levels (in the 5th quintile) at

screening as in the rest of the glucose distribution.

No independent association between either blood pressure or the use of anti-hypertensive therapy and the subsequent risk of NIDDM was observed. With regard to anti-hypertensive therapy, which has been incriminated in previous studies of risk factors for NIDDM (Skarfors et al, 1991), it should be noted that less than 5% of men in the Regional Heart study were exposed to this factor and we did not have data on specific anti-hypertensive agents. Hence it is likely that the study lacked power to address this issue.

The study sample.

Relative to other cohort studies which have examined risk factors for NIDDM (Helmrich et al, 1991; Manson et al, 1991[a]; Manson et al, 1992; Charles et al, 1991), the BRHS participants are uniquely representative of the population from which they are drawn, i.e. "European" middle-aged men in Britain. Hence the external validity, or generalisability of the findings to this population is probably high. However the generalisability of the findings to women or to ethnic minority groups in Britain is less certain and is obviously a matter of judgement (Rothman, 1986).

Measurements of exposure and disease

Reliability / random error

Inevitably, a number of key exposures in this study will have been measured with less than optimal reliability. In particular, fasting blood samples would have provided more reliable measures of glycemic status, and of lipid levels (particularly triglycerides) at baseline. However it is worth noting that low reliability in the measurement of exposures

such as physical inactivity or elevated triglycerides will dilute aetiological association and in particular will attenuate estimates of the independence of effects in multivariate analysis. Indeed, with regard to physical activity, given the inherent problem of measurement error, it is likely that the magnitude of the association with NIDDM has been underestimated.

Validity/ potential sources of bias

Reliance on physician diagnosed cases of NIDDM represents an important limitation of this study, given the prevalence of undiagnosed NIDDM. Clearly, glucose tolerance test data would have been preferable both at initial screening (to ensure that all prevalent cases were excluded) and at the end of the follow-up period to minimise disease misclassification. However, the key methodological issue in this study is the possibility of ascertainment bias. It may be argued that case ascertainment was biased with regard to some of the exposures of interest such as obesity and physical inactivity, i.e. the over-weight and physically inactive may be more likely to have had contact with their general practitioner and to have had disgnostic tests for diabetes. However, the smooth linear form of the NIDDM-BMI association does not suggest a case ascertainment artefact. Moreover. it is difficult to apply the same argument with regard to the associations between NIDDM and physical activity, triglyceride and HDL-cholesterol. These associations persisted after adjustment for BMI and for other factors which might increase the probability of a NIDDM diagnosis such as hypertension and prevalent CHD at screening. The weak association in these data between NIDDM and elevated blood pressure and the absence of an association with serum cholesterol or an independent association with cigarette smoking, provide further evidence against ascertainment bias.

It is worth noting that in the initial design of this study, it was planned to recall

a sub-sample of men for formal glucose tolerance testing. The data from this additional work would have provided an estimate of the extent and direction of ascertainment bias due to different factors such as prevalent CHD at screening. Unfortunately this proposal had to be abandoned for logistical reasons.

Confounding

The lack of data on body fat distribution, a potentially important predictor of NIDDM (Ohlson *et al*, 1985), is a further limitation of this study. It should be noted however, that the association with physical activity was minimally attenuated on adjustment for body mass index and the association with alcohol intake was stronger. Hence for these variables, substantial residual confounding due to body fat distribution is unlikely, although it cannot be excluded. The study also lacked data on family history of diabetes. In previous prospective studies the association between physical activity and NIDDM was independent of family history (Helmrich *et al*, 1991; Manson *et al*, 1991[a]; Manson *et al*, 1992). However it is conceivable (but unlikely) that exposure to alcohol or cigarette smoking was influenced by knowledge of family history.

On a more general issue, there is a need for cautious interpretation of findings from observational studies on relations between lifestyle factors such as physical activity and disease end-points such as diabetes where potentially important confounders are measured with low precision (Phillips & Davey-Smith, 1991). In particular, the extent to which one can adequately adjust for poor health and health related behaviour, potential sources of ascertainment bias (as above) and confounding in this study, is uncertain.

Physical activity, alcohol intake and cigarette smoking

The data on physical activity and NIDDM in this study complement and extend the

findings from previous prospective studies which have addressed this issue in selected populations (Helmrich *et al*, 1991; Manson *et al*, 1991[a]; Manson *et al*, 1992; Burchfiel *et al*, 1995). We have studied a representative British population-based sample, using a validated measure of physical activity, and we were able to adjust for a considerably wider range of potential confounding factors than in the previous studies. It is noteworthy that there was no further decline in the risk of NIDDM once moderate levels of physical activity had been achieved. A similar pattern has emerged in previous prospective studies (Manson *et al*, 1991[a]; Manson *et al*, 1992). No significant interaction with BMI was detected, which suggests that the effect of physical activity on NIDDM risk is similar in the obese and non-obese. We have also shown an association between heart rate, which may be regarded as a proxy measure of physical fitness and NIDDM. Indeed lower heart rate was an independent predictor of NIDDM in multivariate models which included physical activity. Though this may simply reflect the relatively high precision with which heart rate was measured, the possibiliity that heart rate is also acting as a marker for sympathetic nervous system activation (which is linked with insulin resistance (Saad *et al*, 1991; Björntorp, 1991) should be considered.

The data from this study provide, at most, weak and inconclusive evidence of a protective effect of alcohol on the risk of NIDDM. However it may be argued that the data provide no support for the hypothesis of a causal, positive link between alcohol intake and NIDDM.

The current study had considerable power to examine the association between cigarette smoking and NIDDM. The findings do not suggest a major role for cigarette smoking in the development of this condition. However there was a small but significant increased risk of NIDDM among current smokers after adjustment for age and BMI

(RR=1.5). This is broadly similar to the relative risks associated with current smoking status reported from the Nurses Health Study and the US Health Professionals' follow-up study, both of which were larger than the current study (Rimm *et al*, 1993; Rimm *et al*, 1995). The lack of a gradient in risk with number of cigarettes smoked may reflect the relatively low precision with which this variable is measured due to terminal digit preference. Although the association with smoking was attenuated in full multivariate analysis, this model may well have included mediating factors such as dyslipidaemia.

CHD risk factors and NIDDM

As clinically overt NIDDM is preceeded by a prolonged period of insulin resistance (Lillioja *et al*, 1993), cardiovascular disease risk factors which cluster with insulin resistance should predict NIDDM. As discussed in *Chapter 2*, Reaven and others have developed the concept of a metabolic syndrome based on insulin resistance which includes glucose intolerance, hypertriglyceridemia, decreased HDL-cholesterol concentration, hypertension and hyperuricemia (Reaven, 1988; Stout *et al*, 1990; Laws & Reaven, 1993). The positive association between triglyceride concentration and the risk of NIDDM and the inverse association with HDL-cholesterol observed in the current study are consistent with data from cross-sectional studies linking insulin resistance with elevated triglyceride and decreased HDL-cholesterol concentrations (Abbott *et al*, 1987; Zavaroni *et al*, 1989). By contrast, the results of the current study provide little support for the hypothesis of a fundamental link between elevated blood pressure and insulin resistance. We observed weak associations between blood pressure and NIDDM which were markedly attenuated on adjustment for BMI and non-significant in multivariate analysis. The link between blood pressure and insulin resistance/ hyperinsulinemia in

cross-sectional studies is more tenuous and inconsistent (Savage & Saad, 1993; Collins et al, 1990; Cigolini et al, 1991), than that between dyslipidemia and insulin resistance. In a number of studies of subjects with impaired glucose tolerance an inverse relation between diastolic BP and the risk of NIDDM has been observed (Jarrett et al, 1984; Charles et al, 1991). The association of higher uric acid levels with increased risk of NIDDM supports the proposed link between hyperuricemia and insulin resistance (Facchini et al, 1991).

Haematocrit and NIDDM

With adjustment for age and body mass index there was a more than four-fold increased risk of diabetes among the 14% of men with a haematocrit of 48% or higher relative to those with a haematocrit below 42%. In full multivariate analysis, there remained a three-fold increased risk in the highest haematocrit group. Putting this in context, the relative risk of NIDDM associated with elevated haematocrit levels at screening in this cohort was of greater magnitude than that associated with elevated triglyceride and reduced HDL-cholesterol concentration, risk factors which are regarded as major elements in the insulin resistance syndrome (Laws & Reaven, 1993). Only glucose, insulin and body mass index at baseline were superior to haematocrit as predictors of NIDDM in this cohort. There was little association between haematocrit and blood glucose (Wannamethee & Shaper, 1994) and adjustment for blood glucose made little difference to the relationship seen. It should be noted however that the magnitude of associations in multivariate analysis is heavily influenced by reliability of measurement, which is likely to be higher for haematocrit than for other biological markers of increased risk of diabetes, thereby increasing the apparent importance of haematocrit. Moreover the magnitude of relative

risk does not necessary imply a "stronger" or more fundamental aetiological role. It depends on the manner in which the exposure is categorised and on the relative prevalence of different exposures in the study population.

The mechanisms which underly this association between haematocrit and risk of non-insulin dependent diabetes are unclear. Clearly a single unifying mechanism is unlikely. We need to consider both physiological effects of insulin which are likely to influence the haematocrit level and fundamental causal factors in the development of insulin resistance and NIDDM. For instance it is reported that intravenous infusion of insulin in doses which increase plasma insulin to physiological levels is associated with increased transcapilliary escape rate of albumin and reduced plasma volume (Hilsted & Christensen, 1992). This may partially explain the association between haematocrit and hyperinsulinemia reported in the Tecumseh Study (Smith *et al*, 1994) and the association between haematocrit and insulin sensitivity as described by Moan and colleagues (1994). Physical training has been shown to decrease haematocrit levels (Suzuki *et al*, 1992) and in the British Regional Heart Study there is an inverse association between self reported physical activity and haematocrit (Wannamethee & Shaper, 1994). Though we have adjusted for physical activity in this study, there may well be residual confounding due to random measurement error, i.e. the adjustment for physical activity may have been incomplete.

In the Tecumseh Study, haematocrit levels were associated with markers of increased sympathetic tone such as higher heart rate and more specifically, elevated plasma norepinephrine levels after mental arithmetic exercises (Smith *et al*, 1994). There are plausible mechanisms linking psychosocial stress and increased sympathetic tone with obesity (particularly central obesity), insulin resistance and NIDDM (Björntorp, 1991).

Direct evidence linking psychosocial stress and increased sympathetic activity with NIDDM is lacking. However, in this study, higher heart rate, which has also been shown to be associated with haematocrit (Wannamethee & Shaper, 1994), was an independent predictor of NIDDM in multivariate analysis which included adjustment for body mass index and physical activity. Hence it may be that increased sympathetic tone is one of the mechanisms linking haematocrit with increased risk of diabetes. However, the haematocrit-NIDDM relationship appeared to be independent of heart rate in multivariate analysis. Clearly further work is required to elucidate the underlying mecganisms. However, these findings suggest that a raised haematocrit level, which is a major determinant of whole blood viscosity, should be added to the cluster of risk factors which link non-insulin dependent diabetes with atheromatous vascular disease.

FEV_{1sec}:- and NIDDM

The inverse association between reduced lung volume and risk of NIDDM is of uncertain biological significance, given that (in contrast to the association between haematocrit and NIDDM) it was mainly evident in men with pre-existing CHD at baseline.

Significant negative findings

Total cholesterol, systolic blood pressure and cigarette smoking are the major independent predictors of CHD in this study (Shaper et al, 1985[a]). The failure of these factors to emerge as independent predictors of NIDDM merits comment. The lack of an association between total serum cholesterol and NIDDM, even in univariate analysis, is of particular interest given the fundamental role of cholesterol in the development of CHD (Shaper, 1988; Monique Verschuren et al, 1995). It is consistent with the findings from all of the

previous studies (bar one), as discussed in *Chapter 2.* It suggests that although NIDDM

and CHD share a number of common causal factors, linked by insulin resistance, there

are fundamental differences in the aetiology of these conditions.

The lack of an association between height and risk of NIDDM, is at variance with

the findings from cross-sectional studies (Williams *et al,* 1995) but consistent with

prospective data from the large US Health Professionals' Study (Chan *et al,* 1994).

Clearly this finding is not consistent with the Barker hypothesis linking retarded growth

in utero to insulin resistance and NIDDM, on the basis of which one would have

predicted an association between short stature and risk of NIDDM.

The lack of an association between social class and NIDDM (even in the age

adjusted data) is also somewhat surprising. It should be acknowledged however, that the

measure of social class employed in this study, based solely on occupational status, is

imprecise and the study may have lacked power to address this issue.

Conclusions

The increased prevalence of NIDDM which generally occurs with industrialisation,

suggests a role for life-style factors in the development of this condition. In this study,

we have shown that obesity and physical inactivity are important independent risk factors

for NIDDM. There is no evidence that alcohol intake increases the risk of NIDDM,

indeed the findings are consistent with lower risk in moderate than in occasional drinkers.

Cigarette smoking was associated with a small increase in risk of NIDDM after

adjustment for the effect of smoking on body weight, though not in full multivariate

analysis. It has been shown that there are derangements in a number of established

cardiovascular disease risk factors, which are linked via insulin resistance, over a decade

on average before the onset of clinically manifest NIDDM. It has also been shown that

an elevated haematocrit level is associated with an increased risk of NIDDM. The latter

is an acknowledged CHD risk factor but is not currently regarded as forming part of the

insulin resistance syndrome. However, there was no evidence that total serum cholesterol

lavels are associated with risk of NIDDM and systolic BP did not emerge as a significant

predictor of NIDDM in multivariate analysis. These findings suggest that although

NIDDM and atherosclerotic vascular disease share common risk factors, some of which

are amenable to modification, there is a major component of CHD risk which is unrelated

to risk of NIDDM.

Table 2- Incidence of doctor diagnosed non-insulin dependent diabetes per 1000 person years of follow-up by quintile of serum glucose (non-fasting).

Glucose (mmol/L)	No of men	Events (n)	Rate/1000 person-years
< 4.8	1473	(20)	1.14
4.8-	1770	(22)	1.03
5.3-	1276	(24)	1.57
5.6-	1497	(35)	1.95
6.1-	1512	(93)	5.32

Table 3- Incidence of doctor diagnosed non-insulin dependent diabetes per 1000 person years of follow-up by quintile of body mass index.

BMI (kg/m²)	No of men	Events (n)	Rate/1000 person-years
< 22.9	1527	(8)	0.44
22.9-	1508	(18)	0.98
24.6-	1533	(30)	1.63
26.0-	1507	(50)	2.78
27.9-	1500	(88)	5.06

Table 4- Baseline values of selected variables [%, mean (se)] in 7577 middle-aged men, initially free of diabetes, by incidence of physician diagnosed non-insulin dependent diabetes during a mean follow-up of 12.8 years. All variables have been adjusted for age and body mass index.

	Developed non-insulin dependent diabetes during period of follow-up		
	No	Yes	
Number of men	7383	194	p-value
Manual occupation (%)	57	56	0.66
Current smokers (%)	41	48	0.06
#Moderate drinkers (%)	27	20	0.05
Physically Active (%)	37	28	0.01
Evidence of CHD (%)*	24	32	0.01
Other heart disease (%)	5.9	8.5	0.11
Anti-hypertensive therapy (%)	4.7	6.7	0.22
Any regular medication (%)	28	32	0.22
Heart rate (beat/min)	70.6 (0.15)	73.2 (0.90)	0.005
Systolic BP (mmHg)	145.0 (0.23)	148.2 (1.43)	0.03
Diastolic BP (mmHg)	82.2 (0.14)	84.10 (0.90)	0.04
Cholesterol (mmol/L)	6.30 (0.01)	6.34 (0.07)	0.50
HDL-cholest. (mmol/L)	1.15 (0.003)	1.11 (0.02)	0.004
##Triglyceride (mmol/L)	1.73	2.16	<0.0001
Uric acid (μmol/L)	359.1 (0.8)	370.5 (4.8)	0.02
Height (m)	1.732	1.734	0.78
FEV_{1sec}(L)	3.32	3.13	0.0001
Haematocrit (%)	44.4	45.6	<0.0001

Daily 3-6 units of alcohol and weekend > 6 units

##Geometric mean (5197 vs. 130)

Table 5- Predictors of non-insulin dependent diabetes in multivariate analysis. The analysis include 6693 men, 171 cases with data on all covariates in the table. Each variable has been adjusted for age and for each of the other variables in the model.

Variable	Adjusted relative risk	p-value ##
Body mass index ~ ~	6.5 (2.9-14.2)	0.0001
Prevalent CHD		
(yes/no)	1.3 (0.9-1.8)	0.1
Physical activity		
(Mod vs None)	0.5 (0.3-0.9)	0.02
Alcohol intake		
(mod. vs. occasional)	0.6 (0.4-0.98)	0.04
Current smoking		
(yes vs. never)	0.98 (0.6-1.5)	0.9
Systolic BP ~ ~	1.2 (0.7-2.0)	0.3
HDL-cholesterol ~ ~	0.7 (0.4-1.2)	0.04
Heart rate ~ ~	2.3 (1.3-3.9)	0.07
Uric acid ~ ~	1.4 (0.8-2.5)	0.02
Haematocrit		
(< 42% vs ≥48%)	3.3 (1.7-6.5)	0.002
FEV_{1sec} ~ ~	0.5 (0.3-0.9)	0.003

Test for linear trend for BMI, systolic BP, HDL-cholesterol, uric acid, heart rate, haematocrit and FEV_{1sec}, with each variable fitted in a continuous form. For the categorical variables the p value refers to the relative risk in the highest relative to the baseline category.

~ ~ Upper fifth versus lowest fifth.

Table 6- Predictors of non-insulin dependent diabetes in subset of men with available data on triglyceride and other covariates (5031 men; 124 cases).

Variable	Relative risk		
	Adjusted (+)	Adjusted (+ +)	## p-value
Body mass index ~ ~	5.8	5.4 (2.3-12.4)	<0.0001
Prevalent CHD	1.4	1.4 (0.9-2.0)	0.1
Physical activity			
(Moderate vs None)	0.5	0.5 (0.3-1.0)	0.06
Alcohol intake			
(moderate vs occasional)	0.6	0.6 (0.3-1.0)	0.05
HDL-cholesterol ~ ~	0.9	1.4 (0.7-2.7)	0.8
Heart rate ~ ~	2.1	1.8 (0.9-3.7)	0.6
Uric acid ~ ~	1.4	1.3 (0.7-2.4)	0.03
Haematocrit			
(< 42% vs ≥48%)	3.3	3.0 (1.4-6.5)	0.03
FEV_{1sec} ~ ~	0.6	0.7 (0.3-1.2)	0.07
Triglyceride ~ ~		2.5 (1.2-5.3)	0.004

Adjusted (+): Adjusted for age, BMI, cigarette smoking, pre-existing CHD, alcohol intake, physical activity, systolic BP, HDL-cholesterol, heart rate, uric acid haematocrit and FEV_{1sec}.

Adjusted (+ +): Adjusted for above + serum triglyceride concentration.

Test for linear trend (as in *Table 3*) in the model which includes serum triglycerides.

~ ~ Upper fifth versus lower fifth.

Figure 1- Relative risk of non-insulin dependent diabetes adjusted for age with 95% confidence intervals, by quintile of blood glucose and body mass index relative to the first quintile of each variable.
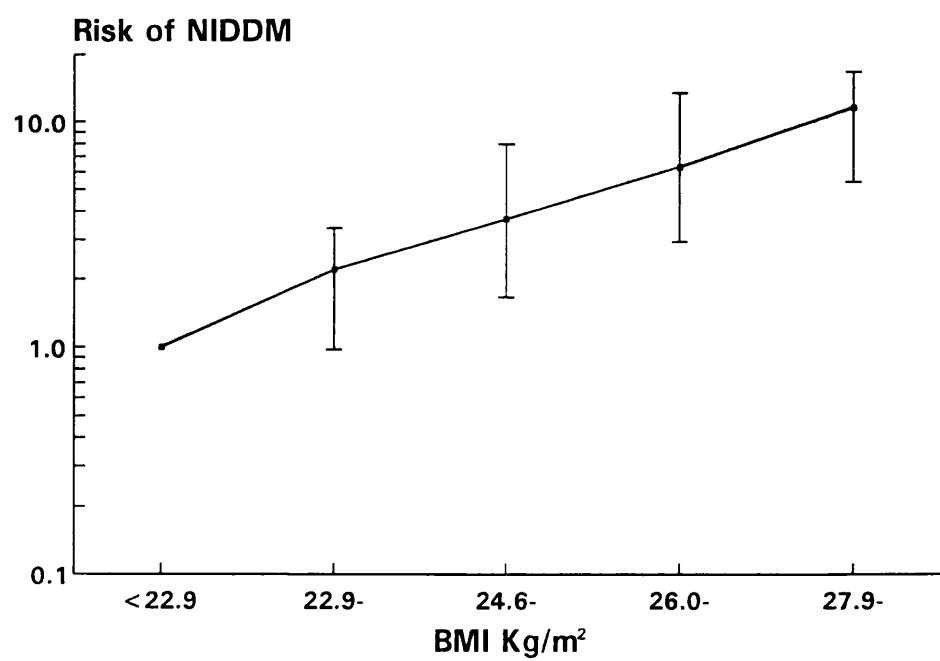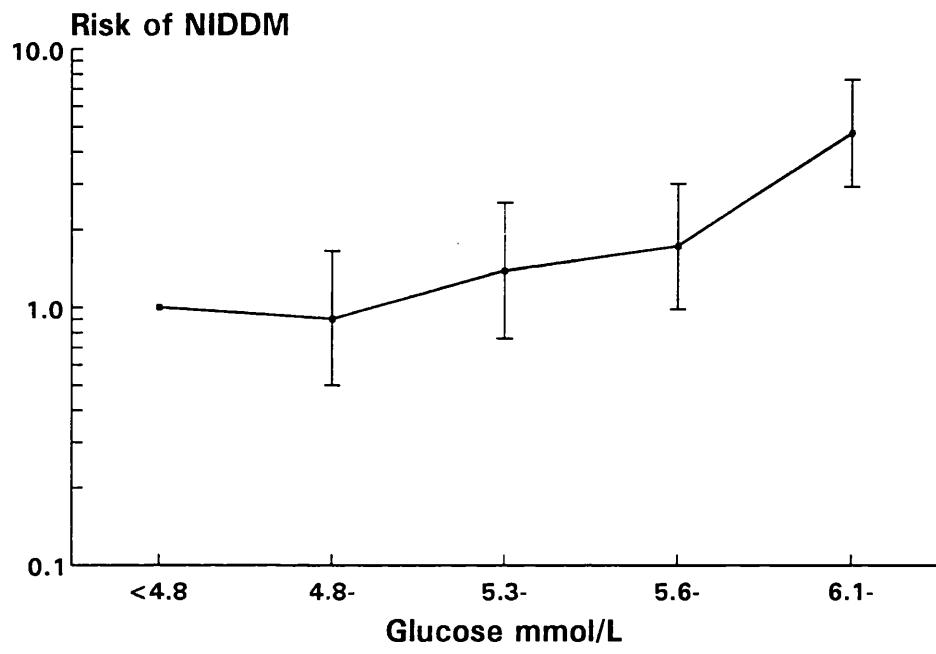
Figure 2- Relative risk of non-insulin dependent diabetes adjusted for age and BMI with 95% confidence intervals, in each social class group relative to Social Class III (manual). The x shows each relation adjusted for age alone.

Risk of NIDDM
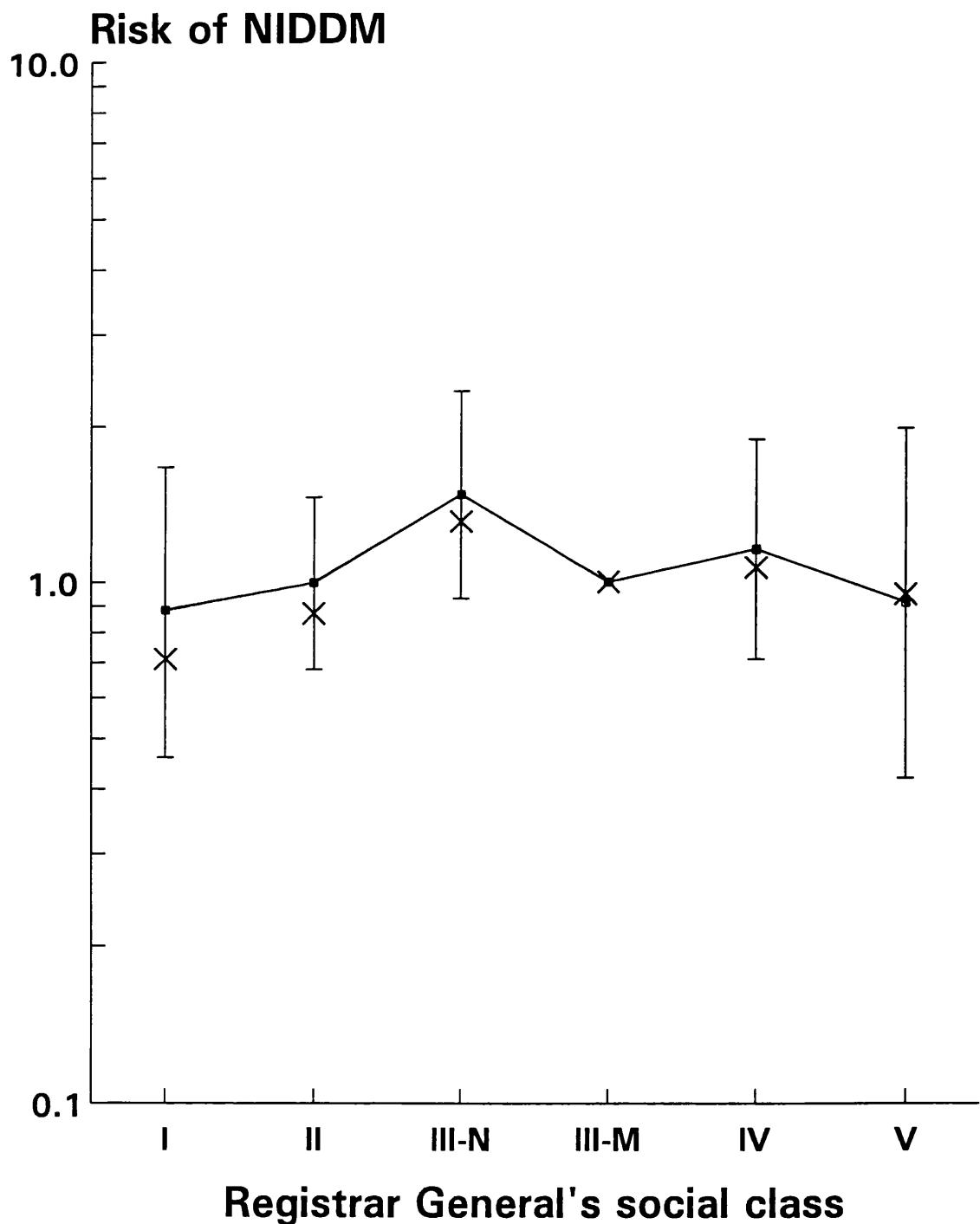
Figure 3- Relative risk of non-insulin dependent diabetes adjusted for age and BMI with 95 % confidence intervals, by physical activity level and alcohol intake. The x shows each relation adjusted for age alone. The lowest physical activity group ("none") form the baseline group for this variable and occasional drinkers form the baseline alcohol intake group.
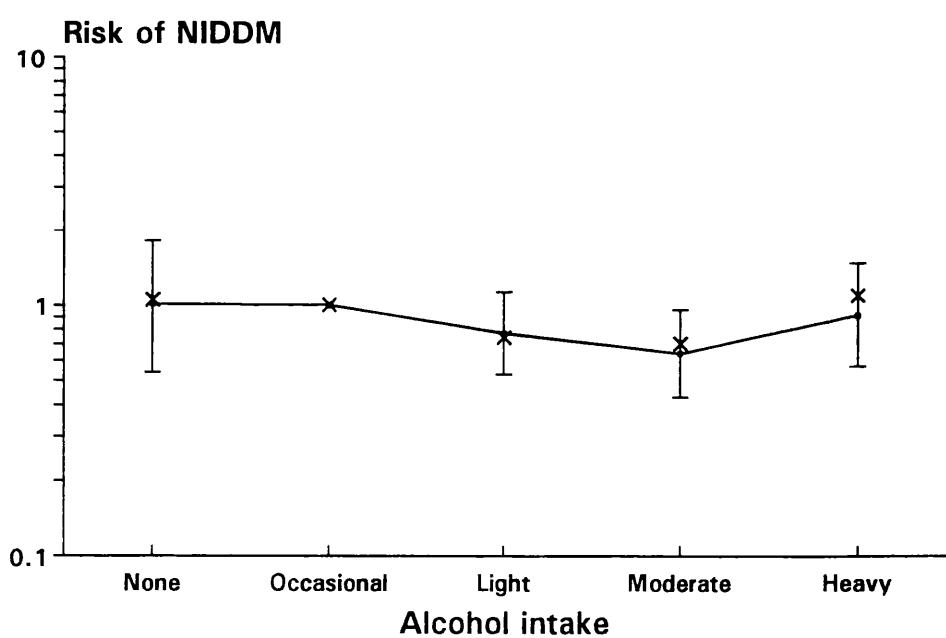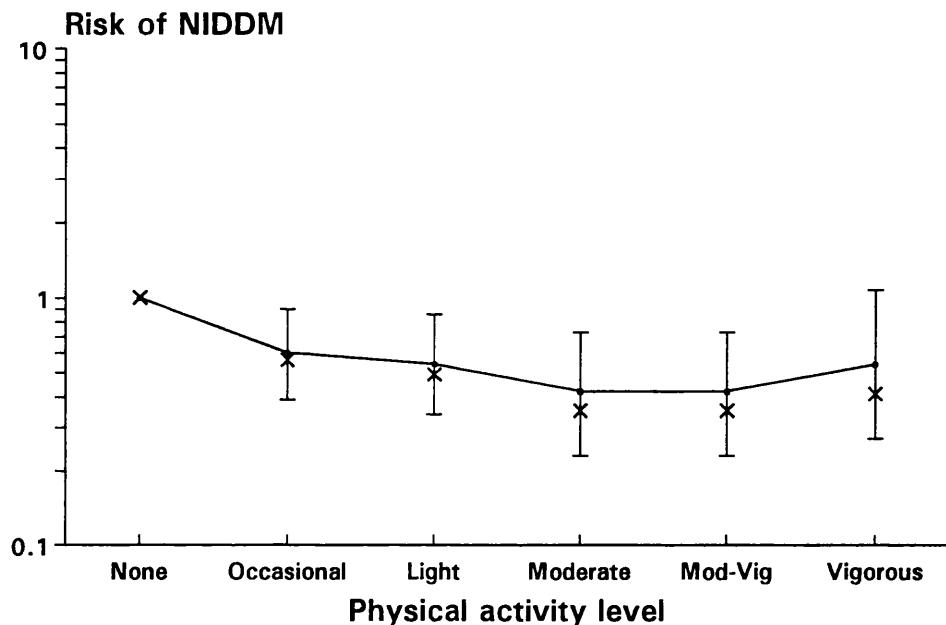
Figure 4- Relative risk of non-insulin dependent diabetes adjusted for age and BMI with 95% confidence intervals, by cigarette smoking status. The x shows the association adjusted for age alone.
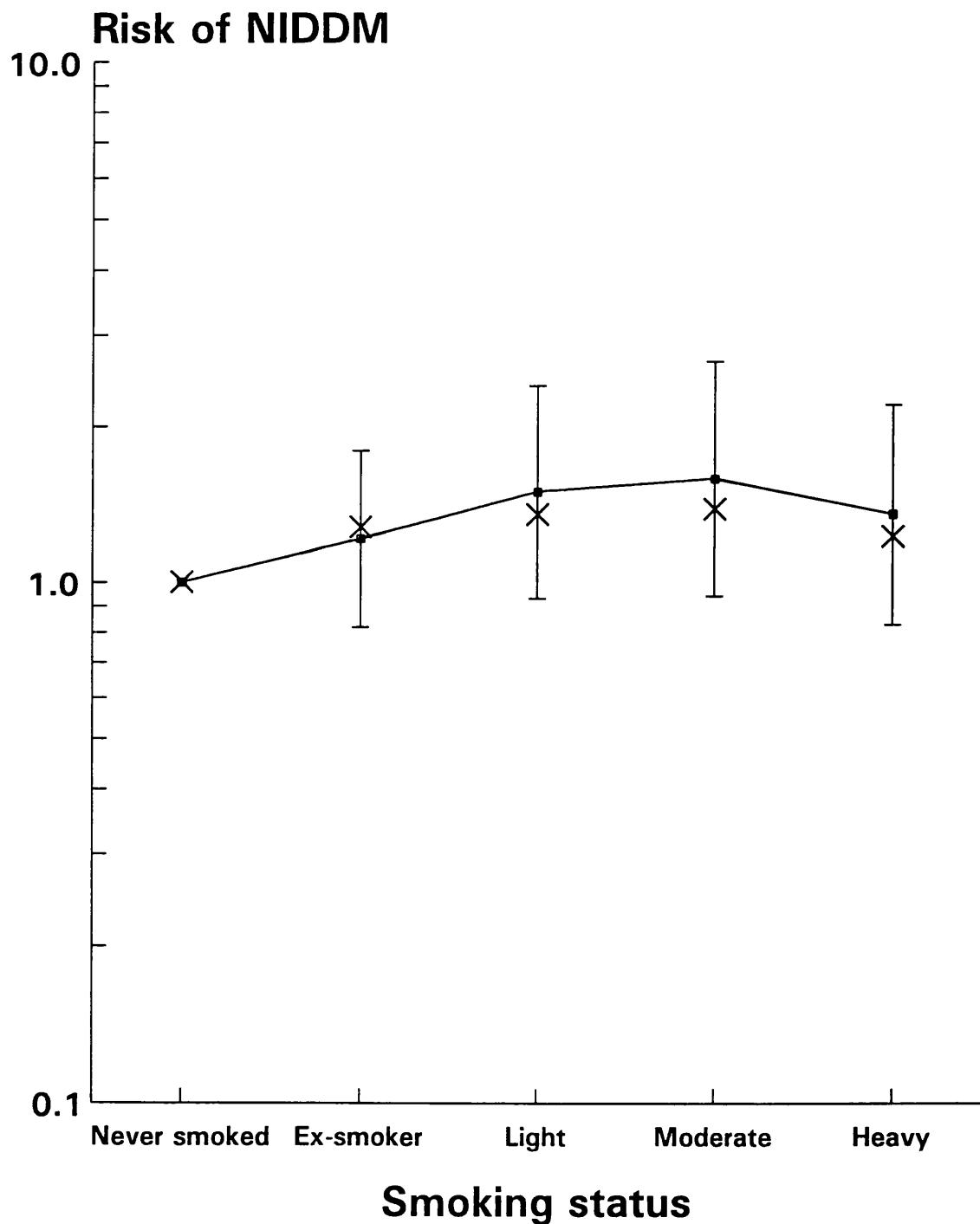
Figure 5- Relative risk of non-insulin dependent diabetes adjusted for age and BMI with 95% confidence intervals, by quintiles of systolic and diastolic blood pressure, HDL-cholesterol, triglyceride and uric acid levels and heart rate, relative to the first quintile of each variable. The x shows each relation adjusted for age alone.
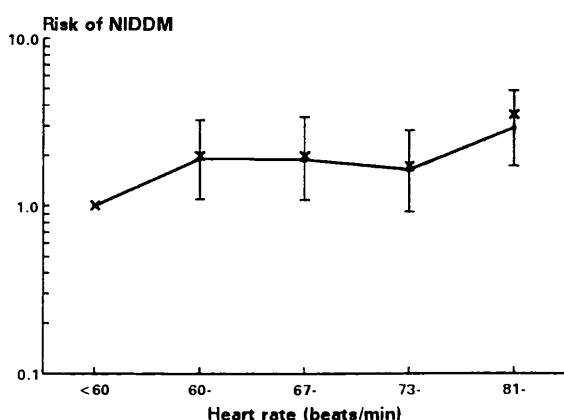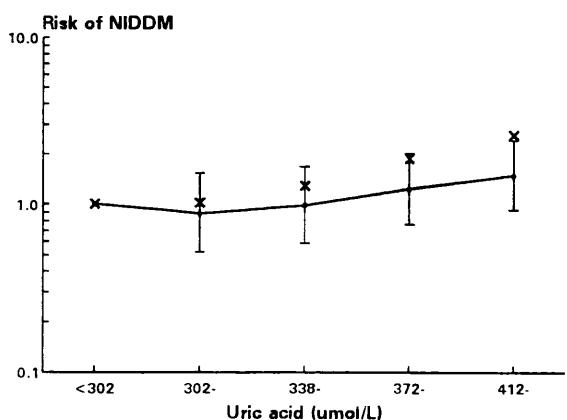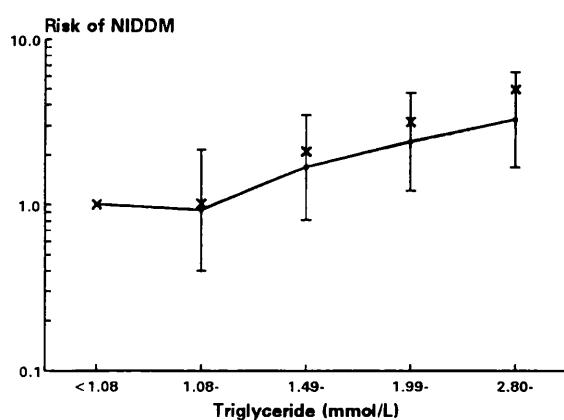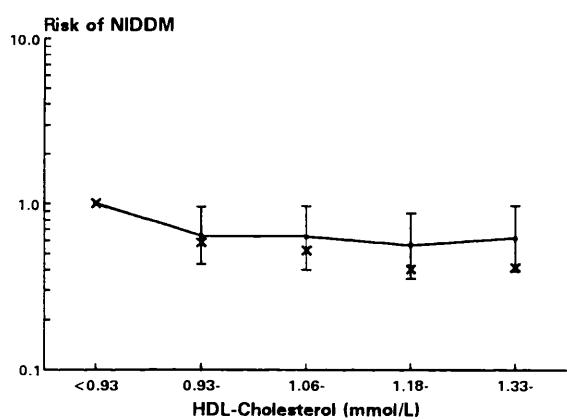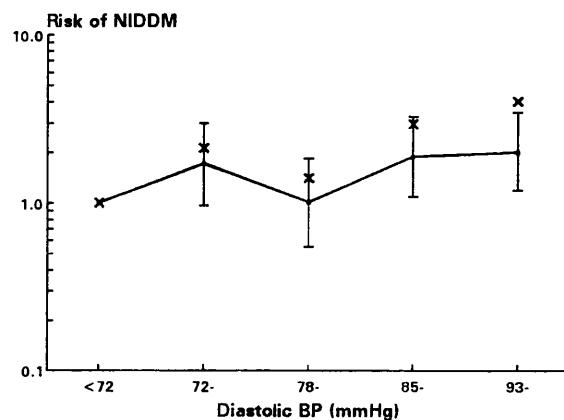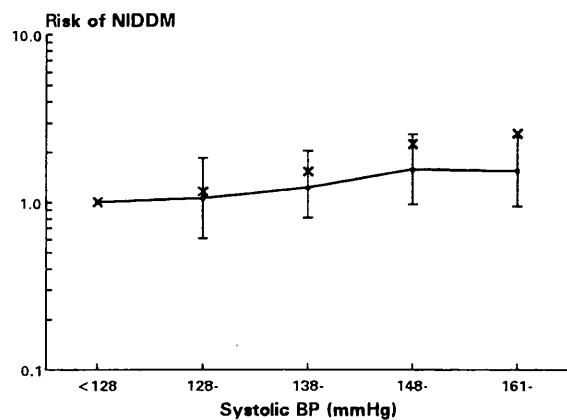
Figure 6- Relative risk of non-insulin dependent diabetes adjusted for age and BMI with 95 % confidence intervals, by haematocrit level. The x shows the association adjusted for age alone.

Risk of NIDDM
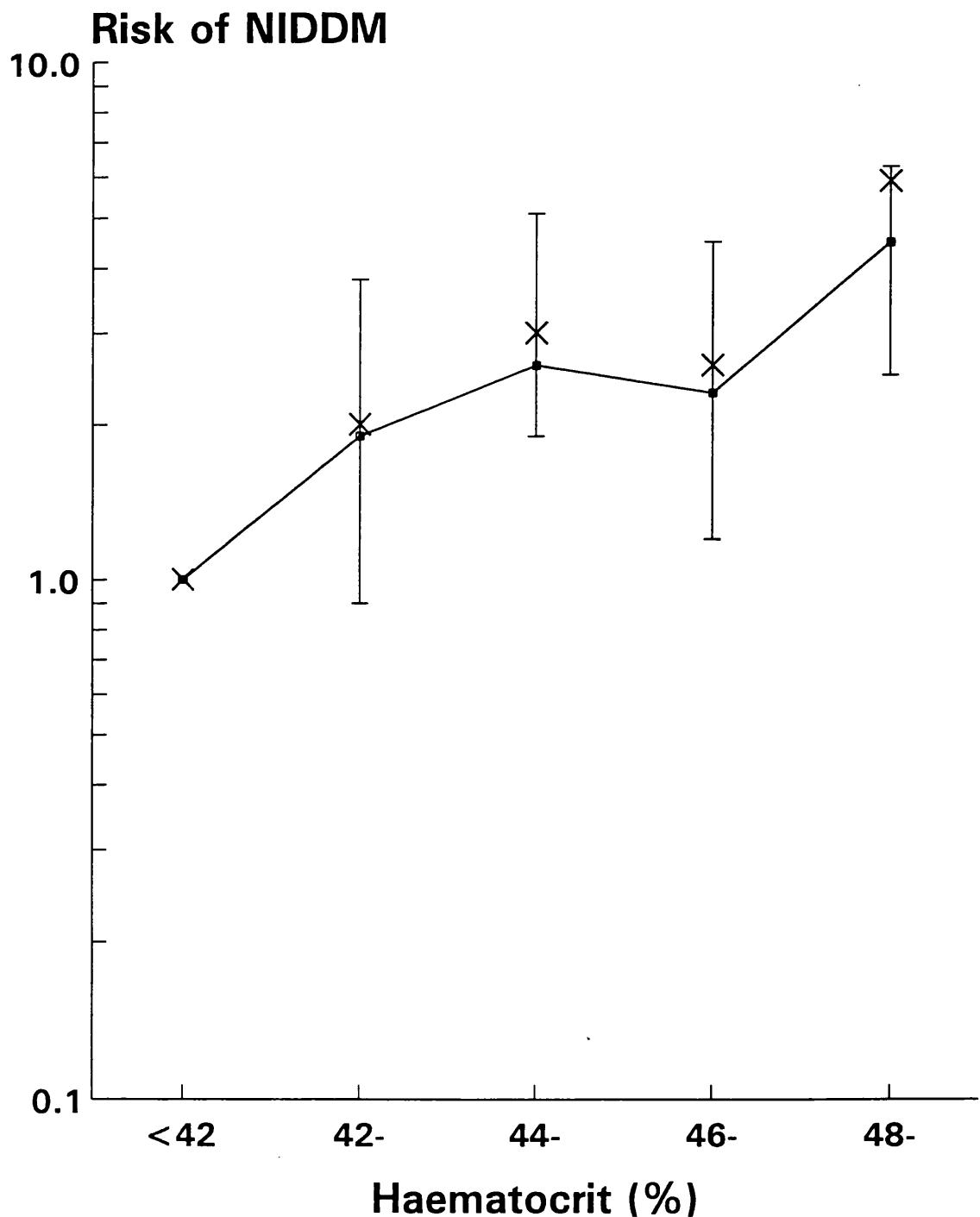
Figure 7- Relative risk of non-insulin dependent diabetes adjusted for age and BMI with 95% confidence intervals, by quintile of forced expiratory volume (FEV_{1sec}). The x shows the association adjusted for age alone.

Risk of NIDDM

Forced expiratory volume (1 sec)(L)
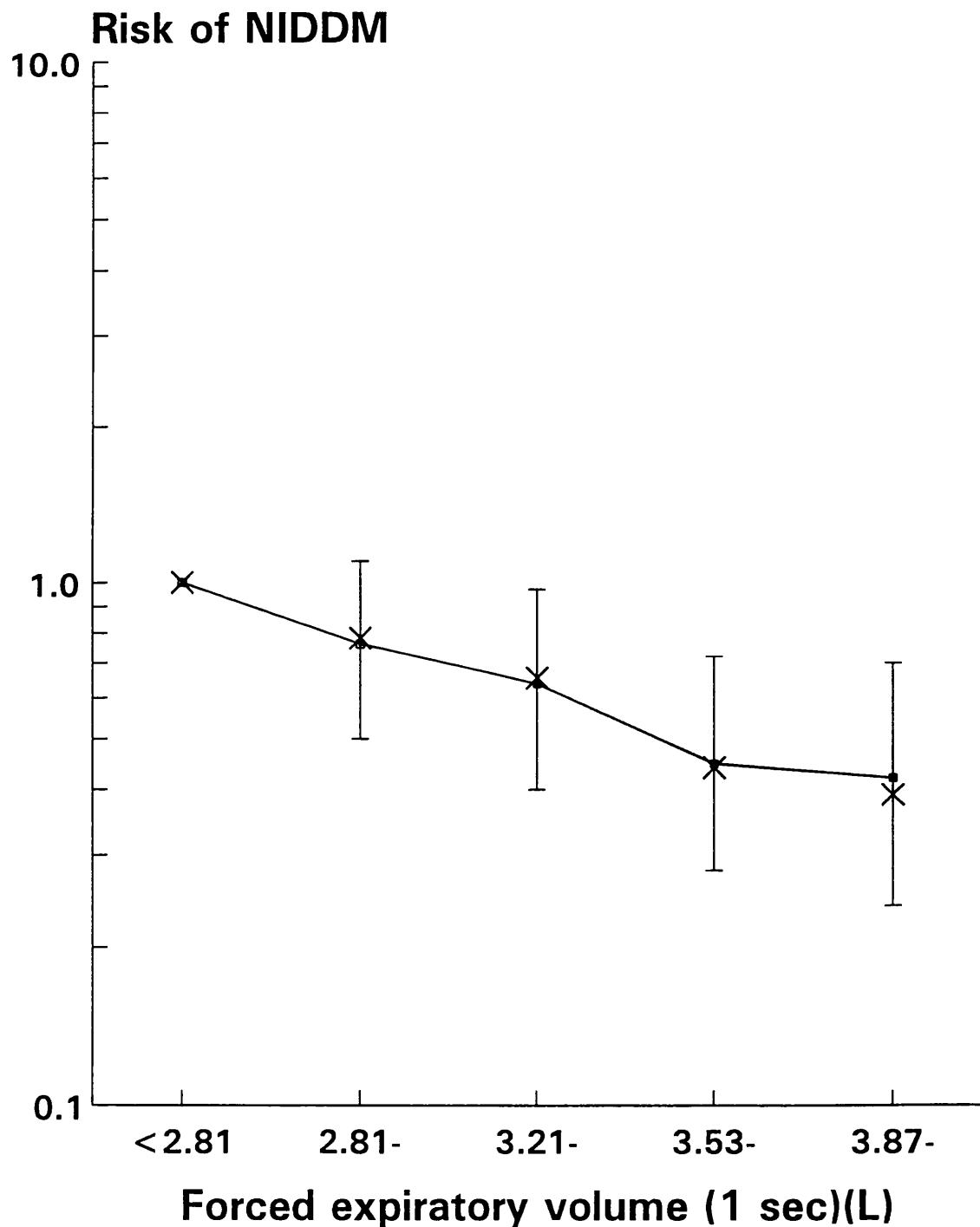
CHAPTER 5

INSULIN AND CORONARY HEART DISEASE RISK FACTORS

ABSTRACT

Objectives:- To test the hypothesis that CHD risk factors which emerged as predictors of NIDDM in the longitudinal data from the British Regional Heart Study (BRHS) would be positively associated with serum insulin (as a marker of insulin resistance) in the baseline cross-sectional data.

Design:- A cross-sectional study.

Subjects and setting:- 5550 non-diabetic men, aged 40-59 years, from 18 of the 24 BRHS towns, from whom non-fasting serum was saved for measurement of serum insulin.

Main outcome measures:- The independent associations between non-fasting serum insulin and several lifestyle and biological CHD risk factors, with a particular focus on associations with obesity, physical inactivity, alcohol intake and cigarette smoking.

Principal Results:- A strong, positive, linear association between insulin levels and BMI was observed. There were significant inverse associations between serum insulin and physical activity, alcohol intake and cigarette smoking. These associations were independent of BMI and were significant in full multivariate analysis. Insulin was also strongly associated with serum glucose. triglyceride and HDL-cholesterol (inverse association) and heart rate. Associations with blood pressure, serum cholesterol, uric acid, haematocrit and Fev_{1sec} were weak and of uncertain biological significance in multivariate analysis. A significant association between serum insulin and prevalent CHD after adjustment for age, BMI and other lifestyle characteristics was observed. This was attenuated on further adjustment for CHD risk factors such as HDL-cholesterol, with which insulin is highly correlated. There was a highly significant

positive association between insulin and height in multivariate analysis.

Conclusion:- CHD risk factors, which predict NIDDM, cluster with insulin resistance as reflected by elevated serum insulin levels. The data suggest that the associations between alcohol and risk of both NIDDM and CHD in prospective studies are mediated via effects of alcohol on insulin sensitivity. The findings do not support the hypothesis that cigarette smoking is associated with insulin resistance.

Introduction

This chapter address inter-relations between insulin and CHD risk factors which were examined as possible predictors of NIDDM in the longitudinal study (*Chapter 4*). Given the focus of this research on fundamental causal factors in NIDDM and on common causal factors linking NIDDM and CHD, there was a particular focus on associations between insulin and lifestyle related factors (obesity, physical inactivity, alcohol intake and cigarette smoking). *It was hypothesised that factors which emerged as predictors of NIDDM in the longitudinal study would be positively associated with serum insulin (as a marker of insulin resistance) in the baseline cross-sectional data.*

There are considerable data to suggest that serum insulin levels increase with obesity (Cigolini *et al,* 1991) and with decreased physical activity (Feskens *et al,* 1994) reflecting the well documented effects of obesity and exercise on insulin resistance. Few studies have examined the relation between alcohol consumption and markers of insulin resistance. While the available data suggests that fasting and post-load insulin levels are lower in drinkers (Manolio *et al,* 1990; Razay *et al,* 1992; Mayer *et al,* 1993; Facchini *et al,* 1994) there are contrary reports (Feskens *et al,* 1994). As discussed in *Chapter 2,* Facchini and colleagues have reported that cigarette smoking is associated with insulin resistance and hyperinsulinaemia (Facchini *et al.* 1992) and this finding has subsequently been replicated in middle-aged men (Eliasson *et al,* 1994). However in our prospective study we did not detect a significant independent effect of cigarette smoking on risk of NIDDM and one would not therefore anticipate a strong independent effect of smoking on serum insulin levels in this population.

Associations between insulin and high serum triglyceride levels and low HDL-cholesterol levels have been consistently found in cross-sectional studies (Abbott *et al,*

1987; Zavaroni *et al*, 1989) and this pattern of dyslipidemia is regarded as integral to the insulin resistance syndrome (Reaven, 1988). Hyperuricemia is also correlated with hyperinsulinaemia and other components of the insulin resistance syndrome (Facchini, *et al*, 1991). By contrast, data on the association between insulinaemia and blood pressure are markedly inconsistent (Ferrannini *et al*, 1987; Saad *et al*, 1991; Cigolini *et al*, 1991; Collins *et al*, 1990). Nearly all studies reporting strong associations between serum insulin levels and blood pressure were conducted among groups of northern European origin (Savage & Saad, 1993) and even among this relatively small sub-group of the world population, some data are discrepant (Manolio *et al*, 1990).

As discussed in **Chapter 2**, a positive association between serum insulin and haematocrit was reported from the Tecumseh Study (Smith *et al*, 1994), and in our prospective study of risk factors for NIDDM (**Chapter 4**) the haematocrit level at baseline was a strong and independent predictor of NIDDM. Associations between serum insulin and FEV_{1sec} and height have not been reported. A significant inverse association between FEV_{1sec} and NIDDM was found in our prospective study. On the basis of the Barker hypothesis (Barker, 1992) one would anticipate an inverse association between serum insulin and height as a marker of fetal growth retardation. However height was unrelated to risk of NIDDM in the prospective study.

Subjects and methods of data collection

Serum insulin was measured in 5665 men, of whom 111 were known or probable diabetics at screening (as defined as in **Chapter 3**). The time of examination was not available in 4 men. Hence, serum insulin data were available from 5550 non-diabetic men. Details of the measurement of insulin and other variables: age, body mass index,

height, social class, cigarette smoking, alcohol intake, physical activity, pre-existing

CHD, use of anti-hypertensive therapy, blood pressure, serum glucose, uric acid, total

cholesterol, HDL-cholesterol and triglyceride concentration, heart rate, haematocrit and

FEV_{1sec} have been set out in *Chapter 3.*

Statistical analysis

Inter-relations between serum insulin and CHD risk factors were examined in the group

of 5550 non-diabetic men. Multiple regression analyses were used to obtain partial

correlation coefficents and to assess the independent effects of CHD risk factors on serum

insulin levels. Tests for trend with age, body mass index and height were carried out,

fitting these variables continuously. Analysis of variance was used to assess the

differences in geometric mean serum insulin at different levels of the categorical

variables: social class, cigarette smoking, alcohol intake, physical activity, use of anti-

hypertensive therapy and pre-existing CHD *(Tables 7-9)*. Tests for trend for the

categorical variables were assessed by assigning quantitative values, e.g 1-6 for the 6

physical activity groups and 1-5 for the 5 alcohol intake categories. The relative

magnitude of associations between insulin and continuous biological variables was

estimated by means of standardised regression coefficients derived from the product of

the regression coefficient and the standard deviation of the relevant variable *(Table 11)*.

Analysis of covariance was used to estimate mean serum insulin in the fifth and first

quintiles of BMI and height and at different levels of physical activity and other

categorical variables, adjusted for the full range of predictors of serum insulin *(Table 12)*.

As insulin and triglyceride concentrations were not normally distributed, log

transformation and geometric means were used. Because of the marked diurnal variation

in serum insulin and triglyceride levels (Pocock *et al,* 1989), the log transformed data on these variables was adjusted for time of sampling, using the mean level of each variable for each hour in which samples were taken and the grand mean. From each individual value, the mean for the hour of sampling for that individual was subtracted, and the result was added to the grand mean (Phillips, 1986).

Results

Serum insulin levels, geometric mean (95% C.I.), were lower among the group of 5550 non-diabetic men than among the group of 111 known known diabetics, 12.4 mU/L (12.2 to 12.7) versus 27.9 mU/L (22.4 to 34.8). Among the latter diabetic group, 24 were receiving insulin therapy and insulin levels, geometric mean (95% C.I.), were higher in this small group than in the group of 87 diabetics who were not recieving insulin therapy, 154.5 mU/L (110.7 to 215.6) versus 17.5 mU/L (14.8 to 20.5).

Insulin and CHD risk factors: personal characteristics and pre-existing disease

Table 7 shows serum insulin levels, geometric mean (95% C.I.), by 5-year age groups, by quintile of body mass index and height and by social class. Serum insulin was weakly associated with age in univariate analysis. However, no evidence of a significant trend across the four age groups was detected following adjustment for body mass index. A strong linear association between insulin and BMI was observed, *(Table 7 and Figure 8).* There was no association between insulin and height in univariate analysis. Manual workers had lower insulin levels than non-manual workers but the difference (in univariate analysis) was not significant. However, since taller men and non-manual workers tend to be thinner, following adjustment for body mass index, there was a

significant positive association between serum insulin and height and a significant difference was seen between manual and non-manual workers, *(Table 7)*.

Lifestyle related variables

Insulin levels were higher in ex-smokers than in never smokers but fell with increasing number of cigarettes smoked *(Table 8 and Figure 9)*. There was a linear fall in insulin levels with increasing physical activity and increasing alcohol intake. These inverse associations with physical activity, alcohol intake and cigarette smoking were highly significant and minimally attenuated on adjustment for age and body mass index *(Table 8)*.

Anti-hypertensive therapy and pre-existing CHD

Insulin levels were higher in men taking anti-hypertensive therapy than in those who were not using anti-hypertensive therapy, but the differences were markedly attenuated on adjustment for age and BMI, *(Table 9)*. Relative to men without evidence of CHD at baseline, insulin levels were higher in men with evidence of CHD short of a definite myocardial infarction (MI) and were highest in those with a definite MI on electrocardiogram or recall of a doctor diagnosis of MI, $p < 0.001$, *(Table 9)*. These differences remained significant (though partially attenuated) on adjustment for age and body mass index, *(Table 9)*.

Insulin and CHD risk factors: biological factors

Table 10 shows correlations (unadjusted and adjusted for age and BMI) between serum insulin and a number of continuous CHD risk factors which were examined as potential predictors of NIDDM in the longitudinal study. In addition to the association with BMI, insulin was strongly associated with serum glucose, triglyceride and HDL-cholesterol

(inverse association) and heart rate. These associations were minimally attenuated on adjustment for age and body mass index. Weaker, though significant associations were also seen with cholesterol, uric acid, haematocrit and FEV_{1sec}. These latter associations were substantially attenuated and were of uncertain biological significance after adjustment for age and body mass index.

Insulin and blood pressure

Insulin was significantly associated with systolic blood pressure in univariate analysis. However the association was substantially attenuated on adjustment for age and body mass index. A weak positive association between serum insulin and diastolic blood pressure was observed in univariate analysis and on adjustment for age and body mass index the direction of this effect was reversed *(Table 10)*.

Magnitude and independence of associations with serum insulin

Many of the variables shown to be correlated with insulin in *Table 10* are influenced by the personal and lifestyle characteristics which have been examined as predictors of serum insulin concentration in *Tables 7,8 and 9*. In *Table 11* standardised regression coefficients for the associations between insulin and the continuous biological CHD risk factor are presented. In assessing the independence of associations, the effects of adjustment for personal and lifestyle characteristics have been presented separately.

Serum glucose, triglyceride, HDL-cholesterol and heart rate were strongly associated with insulin after adjustment for personal and lifestyle characteristics, *(Table 11*, Column A), whereas the associations with haematocrit, uric acid, Fev_{1sec} and cholesterol were weak. The inverse association between insulin and diastolic blood pressure which emerged on adjustment for body mass index was unchanged on adjustment

for the other personal and lifestyle characteristics. No association was seen with systolic blood pressure following adjustment for these factors.

The magnitude of the association between serum insulin and triglyceride was second only to that between insulin and glucose. Triglyceride is significantly associated with most of the biological CHD risk factors, in particular with HDL-cholesterol (r=-0.46), cholesterol (r=0.36) and uric acid (r=0.27). The effects of adjustment for triglyceride are shown in *Table 11*, Column B. The association between insulin and heart rate was unchanged on adjustment for triglyceride. The inverse association with diastolic blood pressure strengthened. Adjustment for triglyceride markedly attenuated the associations seen with HDL-cholesterol and uric acid and the weak associations with haematocrit and Fev_{1sec} were further attenuated. The weak positive association between insulin and total cholesterol became negative following adjustment for triglyceride. When all these factors (with the exception of systolic BP which was non-significant in the first model [model A] and glucose) were included in a further the multiple regression model (*Table 11*, Column C), these associations were not greatly altered. The association between insulin and uric acid became non-significant. Further adjustment for glucose also made little difference to these associations (*Table 11*, Column D).

Many of the personal and life-style related characteristics with which serum insulin was associated in univariate analysis and on adjustment for age and BMI (height, cigarette smoking, alcohol intake, physical activity and pre-existing CHD) are also associated with serum lipids and other biological CHD risk factors. The relationship between serum insulin and these personal and lifestyle related characteristics was therefore further examined in multivariate analysis which included potential biological mediating factors, i.e. biological factors shown to be independently associated with

insulin: serum triglyceride, HDL-cholesterol and total cholesterol, diastolic blood pressure, heart rate, haematocrit and FEV1. To illustrate the effects of adjustment and for simplification, the difference in log (mean) between the highest and lowest levels of these personal and lifestyle related characteristics have been presented, *(Table 12)*. In these analyses the associations between serum insulin and body mass index, height, physical activity, alcohol consumption and cigarette smoking remained significant. While there was some attenuation of the inverse association between insulin and physical activity, the positive association with height and the inverse associations with alcohol consumption and cigarette smoking were not attenuated on adjustment for other personal, lifestyle related and biological predictors of insulin *(Table 12)*. The higher insulin level observed in men with pre-existing CHD (definite myocardial infarction) on adjustment for age and BMI remained significant on further adjustment for height and for cigarette smoking, physical activity and alcohol intake, *(Table 12, column A)*. However this association was attenuated and was not significant after adjustment for the biological CHD factors *(Table 12, column B)*. The addition of glucose to this multivariate model *(Table 12, column C)* produced slight attenuation of the association between insulin and body mass index but did not alter the associations between insulin and other personal and lifestyle related variables.

Discussion

In these cross-sectional analyses, CHD risk factors such as BMI, physical inactivity and hypertriglyceridaemia, which were predictive of NIDDM in the longitudinal study, were associated with hyperinsulinemia at the baseline examination. Raised blood pressure and total serum cholesterol levels, which were not independent predictors of NIDDM in the

longitudinal study, were not linked with higher insulin in the baseline data. The inverse associations between serum insulin and both physical activity and HDL-cholesterol and the positive associations with triglyceride, heart rate and uric acid are entirely consistent with a considerable body of data from previous studies (Fontbonne et al, 1991; Hargreaves et al, 1992; Yarnell et al, 1994; Ferrara et al, 1994; Cigolini et al, 1991; Mykkanen et al, 1993[b]). The significant inverse association between serum insulin and diastolic blood pressure is intriguing and does not support the hypothesis of a fundamental role for insulin in the development of essential hypertension (Reaven & Hoffman, 1987). It is however consistent with the inverse associations between insulin levels and risk of NIDDM reported in several prospective studies (Jarrett et al, 1984; Charles et al, 1991).

The use of non-fasting insulin measurements, adjusted for time of sampling, has almost certainly increased the amount of random error or "noise" in the data relative to the use of fasting or post-load insulin measurement. It is arguable however that post prandial insulin levels, adjusted for time of day, are more relevant physiologically than measures of either fasting or post-load insulin. Moreover, the correlations of non-fasting insulin with cardiovascular disease risk factors such as BMI, lipids and blood pressure described in this study, are very similar to those reported with fasting and post-load insulin in other studies, as cited above. For example, the data on inter-relations with biological risk factors are virtually identical to those reported from a population-based study in eastern Finland which had data on fasting and 2-hour plasma insulin (Mykkanen et al, 1993[b]). This suggests that the use of non-fasting insulin has not been associated with systematic measurement error.

The strong inverse association between serum insulin and alcohol intake merits comment. This is by far the largest study to have addressed this association to date. The

data suggest that part of the "protective" effect of alcohol on both NIDDM and CHD may be mediated via effects of alcohol on insulin sensitivity. The findings are consistent with the data from previous smaller studies which have examined associations between fasting and post load insulin and alcohol intake (Manolio et al, 1990; Razay et al, 1992; Mayer et al, 1993; Facchini et al, 1994). Razay and colleagues (1992) in a cross-sectional study of young and middle-aged British women, described a U-shaped relation between fasting insulin and alcohol consumption, similar to that described between alcohol and CHD and (in the BRHS), alcohol and risk of NIDDM. It is well established that alcohol intake is associated with higher levels of HDL-cholesterol, with effects on both of the major sub-fractions, HDL_2 and HDL_3 (Gaziano et al, 1993), and that insulin and HDL-cholesterol are inversely correlated (Zavaroni et al, 1985). In the Kaiser Permanente Women Twins Study, alcohol consumption, within the range of light, (2 drinks per month) to moderate (1.5 drinks per day) drinking, was inversely related to fasting and post load insulin levels (Mayer et al, 1993). This relation however did not explain the association between alcohol and HDL-cholesterol and it was argued that effects of alcohol on insulin sensitivity might represent an additional, independent mechanism whereby alcohol reduces CHD risk. However, given the likely complexity of inter-relations between alcohol, insulin and lipids and the unreliability of measures of independence in multivariate models, the latter conclusion may be premature.

The findings in this study are at variance with data from Reaven's group and others (Facchini et al, 1992; Eliasson et al, 1994), suggesting that smoking causes insulin resistance and that chronic smoking is associated with other manifestations of the insulin resistance syndrome, including dyslipidaemia and "lipid intolerance", i.e. marked post-prandial hypertriglyceridaemia (Axelsen et al, 1995). However the data are consistent

with the lack of an independent effect of smoking on the risk of NIDDM in the British Regional Heart Study and with data from several population-based studies in which smoking has been associated with lower fasting and post load insulin levels (Yarnell *et al*, 1994; Ferrara *et al*, 1994; McKeigue & Davey, 1995). As discussed earlier, the data from Reaven's group are based on a small and unrepresentative clinic sample (Facchini *et al*, 1992). Small clinical studies of the acute effects of smoking on insulin sensitivity are of uncertain relevance (Attvall *et al*, 1993), while the data from larger studies of the chronic effects of smoking on insulin resistance and on "lipid intolerance" are more persuasive (Eliasson *et al*, 1994; Axelsen *et al*, 1995). Although smoking is associated with a central distribution of body fat (Shinokata *et al*, 1989) which is a good marker for insulin resistance (Barrett-Connor E, 1989), smoking is a strong and consistent predictor of low body mass index. With more complete adjustment for generalised obesity, including duration, the inverse association between insulin and cigarette smoking might have been attenuated, although it is unlikely that the direction of the effect would have been reversed. The association between smoking and low BMI is not well understood, although it seems clear that smoking reduces the perception of hunger. It is difficult to reconcile the observation that smokers are thinner with the notion that smoking caused insulin resistance. It may be however, that smoking causes insulin resistance via activation of the sympathetic nervous system (Cryer *et al*, 1976) and release of counter-regulatory hormones (Attvall *et al*, 1993) but that these effects are counteracted by effects of smoking (however mediated) on obesity.

In univariate analysis, taller men in this study had higher insulin levels and the association was stronger and highly significant in multivariate analysis. Again these findings are consistent with the lack of an association between short stature and risk of

NIDDM in the longitudinal study. By contrast, the association between insulin and haematocrit was somewhat weaker (although in the appropriate direction) than one would have expected from the strong independent association between haematocrit and risk of NIDDM

In summary, CHD risk factors which were associated with increased risk of NIDDM in the longitudinal study were associated with higher insulin levels in the baseline data. These findings are consistent with the hypothesis that the risk factors which NIDDM and CHD share in common, cluster with insulin resistance.

Table 7- Serum insulin levels (mU/L) by 5-year age groups, by quintile of body mass index and height and by social class. Values are geometric means (95% Confidence Interval).

	Unadjusted	Age and BMI adjusted	p (trend) for adjusted data
Age group (years)			
40-	11.9 (11.5-12.4)	12.2	
45-	12.4 (12.1-12.9)	12.6	
50-	12.6 (12.1-13.1)	12.4	
55-	12.6 (12.1-13.1)	12.6	0.18
BMI quintiles (Kg/m²)			
<22.9	8.8 (8.4-9.1)		
22.9-	11.1 (10.6-11.6)		
24.6-	12.6 (12.1-13.1)		
26.0-	13.9 (13.3-14.4)		
27.9-	17.8 (17.1-18.5)		<0.0001
Height quintile (cm)			
<167.7	12.1 (11.6-12.6)	11.7	
167.7-	12.4 (11.9-12.9)	12.4	
171.6-	12.3 (11.8-12.8)	12.3	
174.9-	12.8 (12.3-13.3)	12.8	
178.9-	12.6 (12.1-13.1)	12.8	0.003
Social class			
Non-manual	12.6 (12.1-13.1)	12.8	
Manual	12.4 (12.1-12.7)	12.2	
Armed forces	11.4 (9.9-13.1)	11.4	0.02

Table 8- Serum insulin levels (mU/L) by smoking status, alcohol intake and physical activity. Values are geometric means (95% Confidence Interval).

	Unadjusted	Age and BMI adjusted	p (trend) for adjusted data
Smoking status (Cigs/day)			
Never	13.2 (12.7-13.7)	12.9	
Ex	13.7 (13.2-14.3)	13.2	
1-19	11.5 (10.8-12.2)	11.9	
20	11.2 (10.6-11.9)	11.7	
$\geq$21	10.7 (10.1-11.4)	11.1	<0.0001
Alcohol intake			
None	13.7 (12.7-14.9)	13.7	
Occ	13.3 (12.8-13.9)	13.3	
Light	12.4 (11.9-12.9)	12.7	
Mod	11.8 (11.4-12.3)	11.7	
Heavy	11.4 (10.7-12.1)	11.0	<0.0001
Physical activity			
None	13.9 (12.7-14.9)	13.7	
Occ	12.9 (12.4-13.5)	12.7	
Light	12.1 (11.6-12.6)	12.2	
Mod	11.9 (11.5-12.4)	12.1	
Mod-Vig	12.2 (11.5-12.9)	12.3	
Vig	11.1 (10.4-12.1)	11.5	<0.0001

Figure 8- Serum insulin concentration by quintile of body mass index.

Insulin mU/L (95% C.I.)
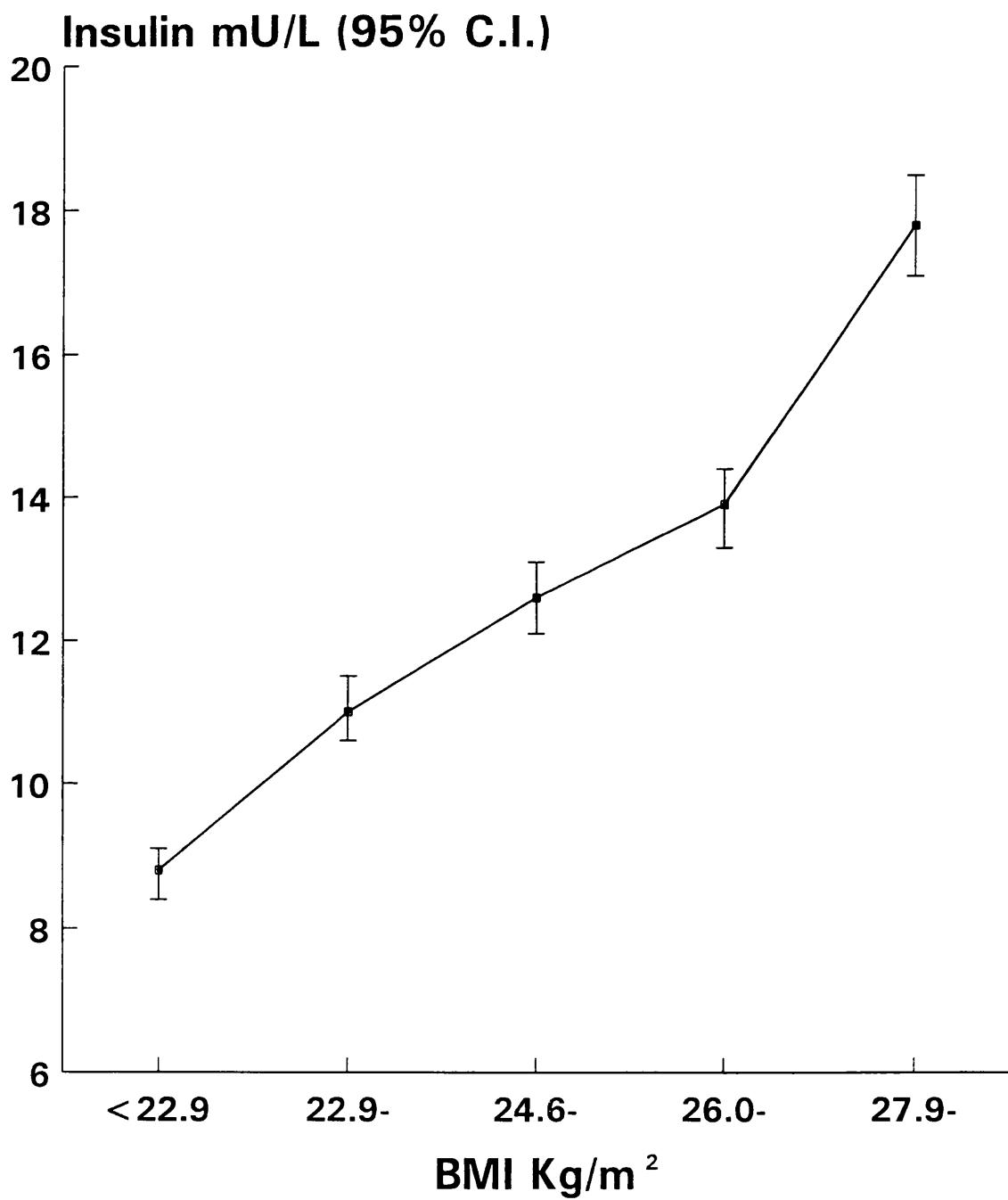
Figure 9- Serum insulin by physical activity level (6 groups), alcohol consumption (5 groups) and cigarette smoking status (5 groups).
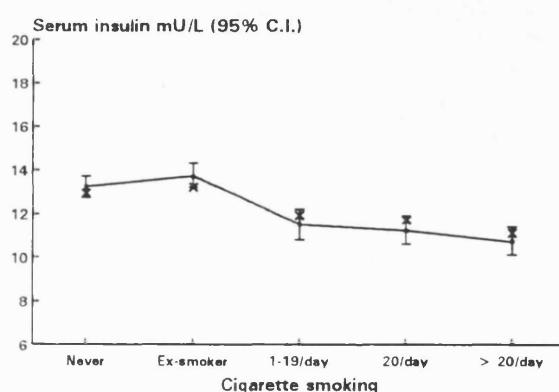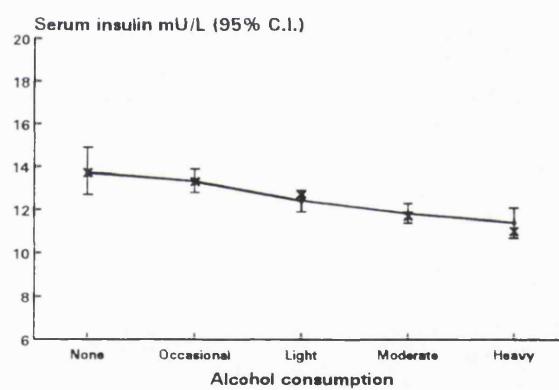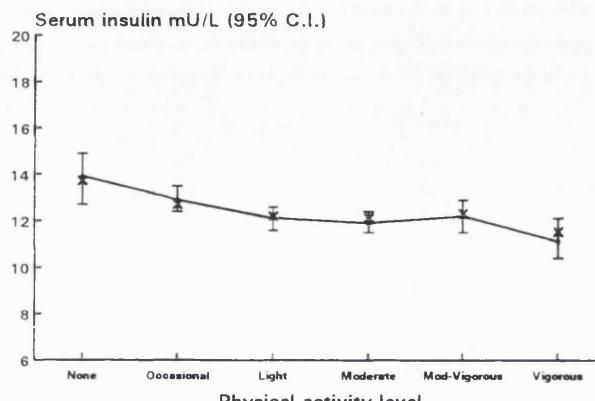
Table 9- Serum insulin levels (mU/L) by use of antihypertensive therapy and by pre-existing CHD status. Values are geometric means (95% Confidence Interval).

	Unadjusted	Age and BMI adjusted	p (trend) for adjusted data
BP therapy			
Using BP therapy (N=270)	15.0 (13.6-16.6)	13.5	
Not using BP therapy (N=5280)	12.3 (12.1-12.6)	12.4	0.05
CHD status			
No evidence of CHD (N=4139)	12.1 (11.8-12.3)	12.2	
Evidence of CHD short of definite M.I. (N=1098)	13.2 (12.7-13.7)	12.8	
Definite M.I. (N=313)	14.3 (12.9-15.8)	13.7	0.004

Table 10- Correlation between coronary heart disease risk factors and non-fasting serum insulin.

	Correlation coefficient (r)	#Adjusted r
Age	0.03*	
Body mass index	0.33**	
Glucose	0.41**	0.39**
Systolic BP	0.12**	0.03*
Diastolic BP	0.06**	-0.06**
Heart rate	0.19**	0.19**
$FEV_{1\ sec}$	-0.03*	-0.03*
Total cholesterol	0.10**	0.05**
HDL-Cholesterol	-0.27**	-0.20**
Triglyceride	0.40**	0.34**
Urate	0.16**	0.08**
Haematocrit	0.09**	0.06**
Height	0.02	0.04*

#Adjusted for age and BMI

*p <0.05

**p <0.001

Table 11- Association between insulin and continuous biological CHD risk factors. Values are adjusted standardised regression coefficients.

	A	B	C	D
Glucose	0.28 ***	0.25 ***	0.25 ***	0.25 ***
Systolic BP	0.02	0.002	0.002	-0.03 **
Diastolic BP	-0.05 ***	-0.08 ***	-0.08 ***	-0.08 ***
Cholesterol	0.04 **	-0.05 ***	-0.05 ***	-0.04 ***
Triglyceride	0.25 ***	0.23 ***	0.23 ***	0.21 ***
HDL-cholesterol	-0.14 ***	-0.03 **	-0.03 **	-0.04 ***
Heart rate	0.13 ***	0.12 ***	0.12 ***	0.07 ***
Uric acid	0.06 ***	0.02 *	0.018	0.01
Haematocrit	0.06 ***	0.03 **	0.03 **	0.04 ***
FEV_{1sec}	-0.04 ***	-0.04 ***	-0.03 **	-0.03 **

Test for trend: *** $p < 0.0001$; ** $p < 0.01$; * $p < 0.05$

A:- Adjusted for pre-existing CHD and personal characteristics viz cigarette smoking, physical activity, alcohol intake, body mass index and height.

B:- Adjusted for above and triglyceride.

C:- Adjusted for above and all biological/physiological variables excluding glucose and systolic BP.

D:- Adjusted for above and glucose.

Table 12- Association between insulin and selected personal and life-style related characteristics. Values are adjusted mean difference in log insulin.

	(A)	(B)	(C)
BMI (5th quintile vs 1st)	+0.69 ***	+0.45 ***	+0.42 ***
Smoking (heavy vs never)	-0.14 ***	-0.24 ***	-0.19 ***
Alcohol (Heavy vs None)	-0.18 ***	-0.19 ***	-0.20 ***
Physical activity (Vigorous vs none)	-0.21 ***	-0.12 ***	-0.14 ***
Height (5th quintile vs 1st)	+0.08 ***	+0.10 ***	+0.13 ***
Pre-existing CHD (Evidence short of M.I. vs none)	0.05	0.04	0.03
(Definate M.I. vs no evidence of CHD)	0.10 *	0.05	0.06

Test for difference between groups: *** $p < 0.0001$; ** $p < 0.01$; * $p < 0.05$

A:- Adjusted for age and each of the other factors in this Table.

B:- Adjusted for the above and for diastolic BP, total cholesterol, HDL-cholesterol and triglyceride concentration, heart rate, haematocrit and FEV_{1sec}.

C:- Adjusted for the above and glucose

CHAPTER 6

SERUM INSULIN AND RISK OF NIDDM

DURING LONG-TERM FOLLOW-UP

ABSTRACT

Objectives:- To test the hypothesis that an elevation of serum true insulin levels, reflecting insulin resistance, is an early event in the pathogenesis of NIDDM.

Design:- A prospective study.

Subjects and setting:- 5550 non-diabetic men, aged 40-59 years, from 18 of the 24 BRHS towns, from whom non-fasting serum was saved for measurement of insulin.

Main outcome measure:- Incidence of physician-diagnosed non-insulin dependent diabetes over a mean follow-up period of 12.8 years.

Principal Results:- There were 138 cases of NIDDM among the group of 5550 men during follow-up. Mean serum insulin at baseline (geometric mean and 95% range) was significantly higher in men who subsequently developed NIDDM than in the rest of the cohort, 19.9 mU/L (4.5 to 88.8) versus 12.2 mU/L (3.8 to 40.1), p <0.0001. A J-shaped association between serum insulin concentration and the subsequent risk of NIDDM was observed with a steady (approximately 6-fold) increase in the risk of NIDDM from the 2nd to the 5th quintile of the insulin distribution but a non-significantly higher risk in the first than in the second quintile. Among men in the lowest BMI tertile, (BMI $\leq$ 24 kg/m^2) and in the fifth quintile of the non-fasting glucose distribution ($\geq$6.1 mmol/L), the risk of NIDDM (adjusted for age and BMI) was higher in the first insulin quintile than in the other quintiles.

Conclusion: These findings are consistent with the hypothesis that the majority of cases of adult onset NIDDM in this population are characterised by the early development of insulin resistance with compensatory hyperinsulinaemia and later pancreatic failure as a preclinical event. Pancreatic hyposecretion of insulin may be a better predictor of NIDDM in men who are relatively thin.

Introduction

In this chapter the question of whether circulating insulin levels are higher in persons destined to develop NIDDM during long term follow-up is addressed. As discussed in **Chapter 2,** Temple and colleagues have shown that in established NIDDM true insulin levels are decreased and that much of the insulin as measured with immuno-assays is in fact pro-insulin and other insulin precursors (Temple *et al*, 1989). In two prospective studies, involving Japanese-Americans (Kahn *et al*, 1994) and elderly Finns (Mykkänen *et al*, 1994[a]), pro-insulin emerged as a stronger predictor of subsequent diabetes than insulin during follow-up which extended for five and four years respectively. Though these findings may be regarded as a challenge to the concept of insulin resistance with compensatory hyperinsulinaemia as an early abnormality in the pathogenesis of NIDDM, it is clear that prospective data on serum insulin and risk of NIDDM from studies with longer follow-up are required. In no previous study has it been possible to measure insulin levels with a specific assay over a decade before the onset of clinically manifest disease.

The objective of this study was to examine the relation between serum insulin levels at screening and the risk of NIDDM during 12.8 years follow-up. The primary focus was on the pathogenesis of NIDDM, on the question of whether true insulin levels (in univariate analysis) were higher or lower in men who developed this condition during follow-up. Adjustment for potential confounding and mediating factors has also been undertaken, i.e. factors which were predictors of serum insulin in the cross-sectional analyses and of NIDDM in the prospective study. Clearly the issue of the independence of insulin as a risk factor for NIDDM in multivariate analysis is of questionable significance given that insulin resistance with compensatory hyperinsulinaemia are likely

to be mediating factors in the causal pathway between factors such as obesity and physical inactivity and the risk of NIDDM. However, examination of the "independent" effects of insulin on risk of NIDDM after adjustment for the lifestyle and biological predictors of both NIDDM and serum insulin identified in *Chapter 4* and *Chapter 5* will provide an indication of the relative importance of these factors as predictors of NIDDM. The effect of adjustment for BMI and serum triglyceride level on the insulin-NIDDM association is of particular interest.

To assess possible heterogeneity in the aetiology of NIDDM in this population between obese and non-obese men, the insulin-NIDDM association has also been examined in each tertile of body mass index.

Subjects and methods of data collection

With exclusion of 111 known and probable diabetic men (as in the previous chapter), serum insulin data were available from 5550 men who were at risk of developing NIDDM during 12.8 years of follow-up. Details of the measurement of insulin, the ascertainment of cases of NIDDM and of the measurement of body mass index and other potential confounders or mediating factors (age, alcohol intake, physical activity, pre-existing CHD, blood pressure, uric acid, HDL-cholesterol and triglyceride concentration, heart rate, haematocrit and FEV_{1sec} have been set out in *Chapter 3*.

Statistical analysis

Cox's proportional hazards models were used to assess the independent contribution of serum insulin concentration at baseline to the subsequent risk of NIDDM and to estimate the relative risk of NIDDM in each quintile of insulin relative to the first quintile,

adjusted for other risk factors. Age, body mass index, systolic BP, heart rate, uric acid, FEV_{1sec}, HDL cholesterol and triglyceride concentration were fitted as continuous variables in the proportional hazards model. Haematocrit was fitted as four dummy variables for five haematocrit groups, based on absolute levels of hematocrit (as detailed in *Chapter 4*): <42.0%, 42.0-43.9%, 44.0-45.9% , 46.0-47.9% and $\geq$ 48.0%. Physical activity was fitted as 5 dummy variables (6 categories: none, occasional, light, moderate, moderately vigorous, vigorous) and alcohol as 4 variables (5 categories: none, occasional, light, moderate, heavy). Pre-existing coronary heart disease was fitted as a dichotomous (yes/no) variable. The data on serum insulin and triglyceride concentrations were log transformed and adjusted for time of sampling as described in *Chapter 5*.

To illustrate the separate effects of allowing for key life-style related and biological variables, the insulin- NIDDM relation was adjusted for potential confounding factors in three cumulative stages, i) age and BMI, ii) pre-existing coronary heart disease, physical activity, alcohol consumption, heart rate, forced expiratory volume, systolic blood pressure, uric acid, haematocrit and HDL-cholesterol level and iii) triglyceride level (*Table 14*). Possible interactions between insulin and body mass index and insulin and glucose in the development of NIDDM were explored in stratified analyses and by fitting interaction terms in Cox's proportional hazards models (*Tables 15-17*).

Results

At 12.8 years of follow-up there were 138 cases of NIDDM among the group of 5550 non-diabetic men with serum insulin data. Mean serum insulin at baseline (geometric mean and 95% range) was significantly higher in men who subsequently developed NIDDM than in the rest of the cohort, 19.9 mU/L (4.5 to 88.8) versus 12.2 mU/L (3.8

to 40.1), p <0.0001. *Table 13* shows the incidence of doctor diagnosed NIDDM per 1000 men/year (unadjusted) by quintile of serum insulin and *Figure 10* shows the relative risk of NIDDM with 95% confidence intervals in each quintile of the serum insulin distribution relative to the first adjusted for age and separately for age and body mass index. The data were consistent with a J-shaped association between serum insulin concentration and the subsequent risk of NIDDM (see also **Table 14**). There was a steady increase in the risk of NIDDM from the 2nd to the 5th quintile of the insulin distribution but a non-significantly higher risk in the first than in the second quintile. The age adjusted association between insulin and risk of diabetes was substantially attenuated on adjustment for body mass index, *(Figure 9)*, though it remained significant, *(Table 14)*. Adjustment for additional potential confounders and mediating factors (including triglyceride which is shown separately lead to further attenuation of the association between non-fasting insulin and the subsequent risk of NIDDM, *(Table 14)*. However in the full multivariate analysis there remained a significant independent association between insulin levels and risk of NIDDM with a fourfold increased risk in the fifth relative to the second quintile and a significant linear trend between the second and fifth quintiles, *(Table 14)*. There was no evidence of a sharp increase in risk of NIDDM in the upper decile of the insulin distribution. On adjustment for age and body mass index the relative risk of NIDDM in the 10th decile of the serum insulin distribution relative to the first quintile was 3.1 (95% C.I. 1.6 to 6.0), similar to the risk in the fifth relative to the first quintile, 2.9 (95% C.I. 1.6 to 5.5).

Table 15 shows the age-adjusted risk of NIDDM in each quintile of insulin relative to the first by tertile of body mass index. A similar J-shaped relation between serum insulin concentration and risk of NIDDM was seen among men in the third BMI

tertile (BMI ≥ 26.6 kg/m²). No clear pattern was discerned in the second BMI tertile. Among the group of 1862 men in the lowest BMI tertile, (BMI ≤ 24 kg/m²) there were only 17 cases of NIDDM. In this group of men the risk of NIDDM was higher in the first insulin quintile (serum insulin < 6.6 IU/L) than in each of the other quintiles including the fifth quintile (serum insulin ≥ 23.7 IU/L). However on formal testing for interaction no significant difference in the insulin-NIDDM association across the BMI tertiles was detected, (p=0.3).

The insulin-NIDDM association was further examined in two strata of non-fasting glucose, <6.1 mmol/L and 6.1 to 11.09 mmol/L. *Table 16* shows the age adjusted data and *Table 17* the age and body mass index adjusted data. A J-shaped relation was seen in the lower glucose stratum, in both the age adjusted and the age and BMI adjusted data, whereas in the upper glucose stratum the risk of NIDDM was higher in the first insulin quintile (serum insulin < 6.6 IU/L) than in the other quintiles . Tests for interaction were non-significant.

On exclusion of cases diagnosed within the first five years of follow-up from the analysis, the J-shaped association between insulin and risk of NIDDM was unchanged. The association between insulin/glucose ratio and risk of NIDDM was similar to that between insulin and NIDDM with a lower risk (RR=0.7) in the second than in the first quintile of insulin-glucose ratio but with a steady increase in relative risk from the second to the fifth quintile relative to the first (RR's 0.7, 1.6, 1.8, 2.1).

Discussion

In this study we found that men destined to develop NIDDM had higher true insulin levels than the rest of the cohort more than a decade before the onset of clinically

manifest disease. We observed a J-shaped association between serum insulin levels and risk of NIDDM with a linear increase in risk from the 2nd to the 5th quintile of the insulin distribution but with a higher (though non-significant) risk in the first than in the second quintile. The association between insulin and NIDDM was substantially attenuated after adjustment for body mass index, reflecting the importance of obesity as a predictor of current insulin levels and future risk of NIDDM. By contrast the effect of adding serum triglyceride to the multivariate model was relatively modest, although one needs to remember the relatively low precision with which the latter variable was measured. Among men in the fifth glucose quintile, who were at particularly high risk of developing NIDDM, the risk was highest (non-significantly) among those with low serum insulin levels <6.6 IU/L. These findings are consistent with the hypothesis that NIDDM is preceded by a prolonged period of true hyperinsulinaemia (reflecting insulin resistance), leading to pancreatic failure and hyposecretion of insulin at a late stage in the pathogenesis of this condition. One might have anticipated that the J-shaped association between insulin and risk of NIDDM would have been no longer evident on exclusion of men diagnosed within the first five years of follow-up. However, given the prolonged period of insulin resistance which antedates NIDDM, possibly extending from childhood (McCance et al, 1994[c]), one would require a longer period of follow-up to adequately address this issue.

This finding of true hyperinsulinaemia over a decade before the diagnosis of NIDDM is consistent with data from a number of other studies. Haffner and colleagues have shown true hyperinsulinaemia and a normal insulin: pro-insulin ratio in Mexican Americans (Haffner et al, 1994 [a]), a high risk population for NIDDM in which hyperinsulinaemia on standard radioimmunoassay is well documented (Haffner et al,

1986). This group have also reported elevated true insulin levels in subjects with impaired glucose tolerance (Haffner *et al*, 1994 [b]).

As has been pointed out, the finding that true hyperinsulinaemia rather than hyperproinsulinaemia antedates NIDDM has implications for wider issue of inter-relation between cardiovascular disease and NIDDM (Stern, 1995). In particular this finding is relevant to the hypothesis that insulin has direct atherogenic effects (Stout, 1990), i.e. that elevated circulating insulin levels should be included with the common causal factors (clustering with insulin resistance) which link NIDDM and coronary heart disease. This argument is based substantially on animal work and *in vitro* studies of the effects of insulin on the development of atheroma (Stout, 1990). However proinsulin lacks biological activity (relative to insulin) and it would be difficult to sustain the argument that it exerts direct atherogenic effects, thereby contributing to the "common soil" (Stern, 1995) linking NIDDM and coronary heart disease. This question, of the relation between circulating true insulin levels and coronary heart disease is addressed directly in *Chapter 8.*

There was a suggestion in these data that pancreatic hyposecretion of insulin is a better predictor of NIDDM in non-obese men (first BMI tertile). This is consistent with the suggestion that NIDDM in the non-obese may represent an aetiologically distinct condition (Alberti, 1993). However, interpretation of the data from this sub-group of men must be guarded, given the small number of cases and the fact that differences between the BMI strata were not significant on formal testing for interaction.

In conclusion we have found a J-shaped relation between circulating true insulin levels (non-fasting) and risk of NIDDM. This is consistent with the hypothesis that the majority of cases of adult onset NIDDM in this population are characterised by the early

development of insulin resistance with compensatory hyperinsulinaemia and later pancreatic failure as a preclinical event. As discussed in *Chapter 2,* arguments about the relative importance of insulin resistance and pancreatic failure as causal factors in NIDDM are of limited relevance. That both abnormalities contribute to the development of NIDDM, interacting at multiple levels, is not disputed. However, if insulin resistance is an early event in the pathogenesis of NIDDM extending over many years or decades before the onset of pancreatic decompensation, as the current data suggest, then lifestyle and environmental factors which reduce insulin resistance will help to prevent NIDDM. In *Chapter 4* it has been shown that absence of obesity, higher levels of physical activity and moderate alcohol intake are associated with decreased risk of NIDDM and in *Chapter 5,* these factors were shown to be associated with lower insulin levels.

Table 13- Incidence of doctor diagnosed non-insulin dependent diabetes per 1000 person years of follow-up by quintile of serum insulin (non-fasting).

Insulin	No of men	Events (n)	Rate/1000 person years
< 6.6 IU/L	1113	(13)	1.00
6.6 - IU/L	1118	(8)	0.60
9.6 - IU/L	1108	(26)	2.01
14.3 - IU/L	1111	(29)	2.25
23.7 IU/L	1100	(62)	4.95

Figure 10- Relative risk of non-insulin dependent diabetes (log scale) adjusted for age and BMI with 95% confidence intervals, by quintile of baseline non-fasting serum insulin. The 〈〉 shows each relation adjusted for age alone.
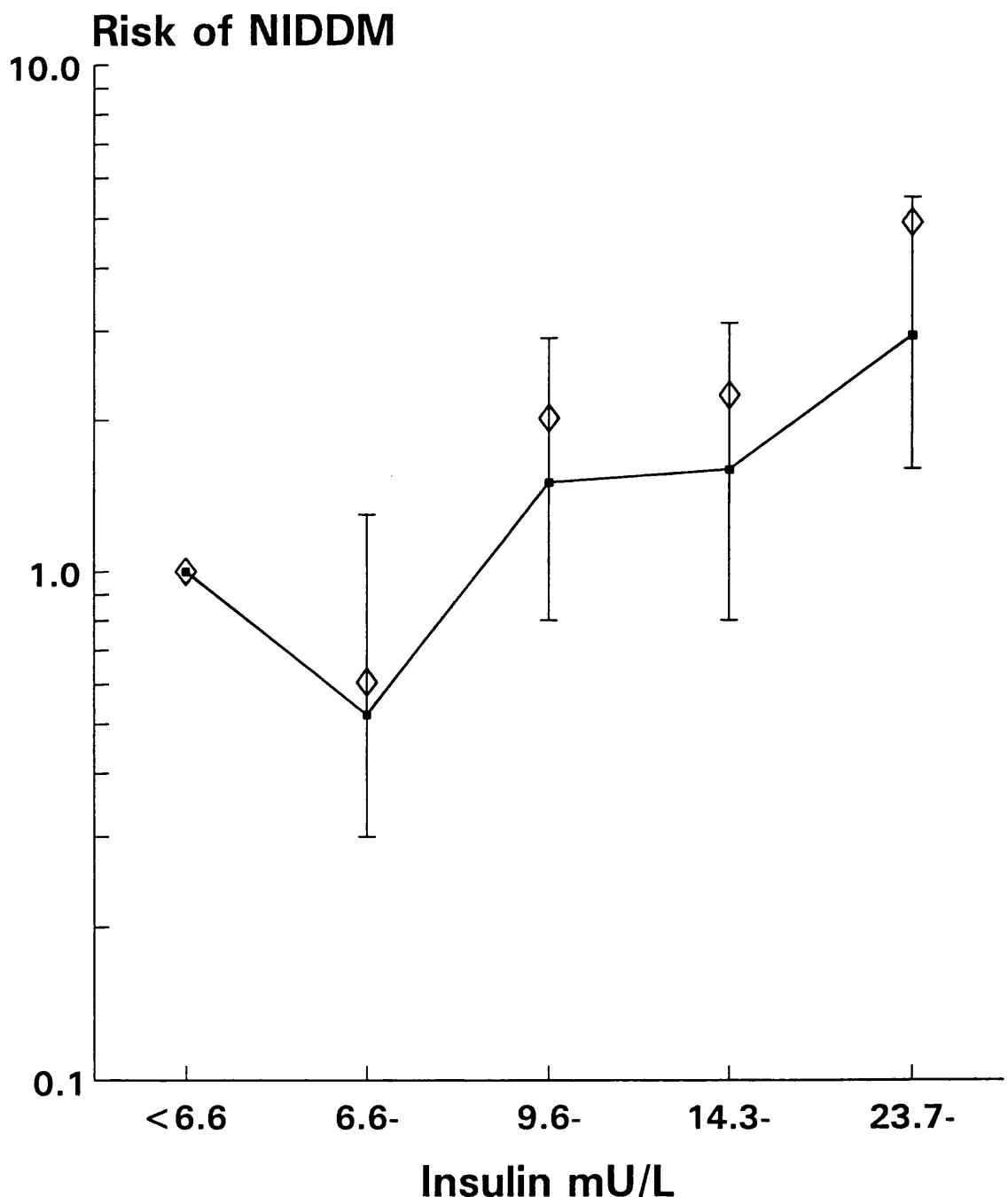
Table 14- Relative risk (95% confidence intervals) of incident cases of doctor diagnosed NIDDM by quintile of serum insulin (non-fasting).

Insulin IU/L	A	B	C
< 6.6	1.0	1.0	1.0
6.6-	0.5 (0.2-1.3)	0.6 (0.2-1.4)	0.5 (0.2-1.4)
9.6-	1.5 (0.8-2.9)	1.6 (0.8-3.4)	1.4 (0.7-2.9)
14.3-	1.6 (0.8-3.1)	1.6 (0.8-3.2)	1.3 (0.6-2.7)
23.7-	2.9 (1.6-5.5)	2.5 (1.3-5.1)	2.2 (1.1-4.0)
Trend	p=0.0001	p=0.0001	p=0.0006

Adjustments

A = Adjusted for age and BMI (N=5549; 138 cases).

B = Adjusted for the factors in A plus physical activity, alcohol consumption, systolic blood pressure, heart rate, uric acid FEV_{1sec}, haematocrit, HDL-cholesterol and pre-existing coronary heart disease (N=5036; 127 cases).

C = Adjusted for the factors above plus triglyceride level (N=4942; 121 cases).

Table 15- Age adjusted relative risk (95% confidence intervals) of incident cases of doctor diagnosed NIDDM by quintile of serum insulin in three BMI strata (tertiles).

Insulin IU/L	BMI I	BMI II	BMI III
< 6.6	1.0	1.0	1.0
6.6-	0.39 (0.1-2.0)	1.3 (0.3-5.5)	0.2 (0.02-2.1)
9.6-	0.75 (0.2-3.0)	3.5 (1.0-12.4)	1.7 (0.5-5.9)
14.3-	0.78 (0.2-3.1)	1.2 (0.3-5.1)	3.2 (0.9-10.6)
23.7-	0.81(1.2-3.9)	3.8 (1.1-13.5)	5.6 (1.8-18.1)
Trend	**p=NS**	**p=0.NS**	p=0.001

BMI I (≤ 24.05 kg/m^2; N=1862; 17 cases).

BMI II (24.06- kg/m^2; N=1836; 36 cases).

BMI III (26.6- kg/m^2; N=1851; 85 cases).

Table 16- Relative risk (95% confidence intervals) of incident cases of doctor diagnosed NIDDM by quintile of serum insulin in two glucose strata. The data are adjusted for age.

Insulin IU/L	Glucose I	Glucose II
< 6.6	1.0	1.0
6.6-	0.7 (0.3-1.9)	0.2 (0.02-1.7)
9.6-	2.3 (1.1-4.9)	0.6 (0.1-2.3)
14.3-	2.1 (0.9-4.5)	0.7 (0.2-2.4)
23.7-	3.1 (1.4-6.9)	1.3 (0.4-4.2)
Trend	p=0.0005	p=0.01

Glucose I = First to the fourth glucose quintiles combined (<6.1 mmol/L; N=4411; 70 cases).

Glucose II = Upper glucose quintile (6.1 to 11.09 mmol/L; N=1125; 68 cases).

Table 17- Relative risk (95% confidence intervals) of incident cases of doctor diagnosed NIDDM by quintile of serum insulin in two glucose strata. The data are adjusted for age and BMI.

Insulin IU/L	Glucose I	Glucose II
< 6.6	1.0	1.0
6.6-	0.6 (0.2-1.6)	0.2 (0.02-1.5)
9.6-	1.8 (0.8-3.8)	0.4 (0.1-1.8)
14.3-	1.5 (0.7-3.3)	0.5 (0.1-1.8)
23.7-	1.9 (0.8-1.4)	0.8 (0.2-2.4)
Trend	p=0.09	p=0.5

Glucose I = First to the fourth glucose quintiles combined (<6.1 mmol/L; N=4410; 70 cases).

Glucose II = Upper glucose quintile (6.1 to 11.09 mmol/L; N=1125; 68 cases).

CHAPTER 7

ASYMPTOMATIC HYPERGLYCAEMIA AND THE INCIDENCE

OF MAJOR CHD EVENTS

ABSTRACT

Objective:- To test the hypothesis that asymptomatic hyperglycaemia is an independent risk factor for major coronary heart disease events (fatal and non-fatal myocardial infarction).

Design:- A prospective study.

Subjects:- A population based sample of 7735 British middle-aged men. With exclusion of men with diabetes (n=158) and missing glucose values (n=49), there were 7528 men available for analysis.

Main outcome measures:- The association between serum glucose level at baseline and incident major CHD events during 11.5 years follow-up.

Results:- There were 704 major CHD events. A weak non-linear relation between glucose level and the risk of major CHD events was observed, with the excess risk in the upper quintile of the glucose distribution (> 6.1 mmol/L). The age adjusted relative risk in the upper glucose quintile compared with the 1st to the 4th quintiles combined was 1.2 (95% C.I. 1.0 to 1.4). Adjustment for age, body mass index, cigarette smoking, alcohol intake, occupational status, physical activity, use of anti-hypertensive therapy and pre-existing CHD did not attenuate this association, RR 1.2 (95% C.I. 1.0 to 1.4), but on further adjustment for systolic blood pressure, heart rate, FEV_{1sec} and HDL-cholesterol it was non-significant, RR 1.15 (95% C.I. 0.96 to 1.4). The association between glucose and major CHD events was not appreciably different at 5 years of follow-up and similar associations were observed in men with and without evidence of pre-existing CHD at baseline.

Conclusion:- The findings do not suggest a strong independent effect of elevated glucose levels in the development of coronary heart disease. The findings are consistent with the hypothesis that hyperglycaemia is a marker for derangements of carbohydrate and lipid metabolism centred on insulin resistance and hyperinsulinaemia which link NIDDM and coronary heart disease.

Introduction

In this chapter data are presented on the relation between both established NIDDM and asymptomatic hyperglycaemia at baseline and subsequent major CHD events (fatal and non-fatal myocardial infarction). There were relatively few cases of established NIDDM at baseline and the association between established NIDDM and risk of CHD events is well established (Kannel & McGee, 1979[a]; Fuller *et al*, 1983; Manson *et al*, 1991[b]). Hence, the primary focus of these analyses was on the association between serum glucose level (non-fasting) and CHD events at 11.5 years follow-up. In subsidiary analyses associations between non-fasting glucose and subsequent CHD events have also been examined in early (5-year) follow-up.

Asymptomatic hyperglycaemia and risk of CHD events

By contrast with the data on established NIDDM, data on the nature and form of the association between asymptomatic hyperglycaemia and CHD are inconsistent, with negative findings (Stamler & Stamler, 1979), threshold effects (Fuller *et al*, 1983; Fuller & Shipley, 1989; Eschwege *et al*, 1980) and positive linear associations (Barrett-Connor *et al*, 1984; Donahue *et al*, 1987) reported. Many previous studies have lacked power to study the blood glucose-CHD relation. The majority of studies have had less than 200 CHD events, a relatively small number given the low reliability with which glucose is measured (Ganda *et al*, 1978; Yudkin *et al*, 1990). Relatively few studies have adequately adjusted for the range of possible confounding factors. In particular, most studies have lacked data on HDL-cholesterol concentration, a potential confounding factor of fundamental importance in studies of the association between measures of glucose tolerance and coronary heart disease (Fuller & Shipley, 1989). In this study of the

relation between asymptomatic hyperglycaemia and risk of CHD events there were over 700 major CHD events and we have adjusted for all major coronary risk factors including HDL-cholesterol, triglycerides and physical activity.

Subjects and methods of data collection

There were 158 men with prevalent NIDDM (as defined in *Chapter 3*) at baseline. Of these, 121 reported a diagnosis of diabetes at the baseline questionnaire, 23 men had a non-fasting glucose in the diabetic range ($\geq$ 11.1 mmol/L) and 14 men had a date of NIDDM diagnosis in the same calender year as the year of screening. Of the 121 men who reported a diagnosis of diabetes 36 men were taking insulin. These men have been excluded from this analysis as definite or probable cases of insulin dependent diabetes (IDDM)[5]. Hence there were 122 men with prevalent NIDDM available for follow-up for major CHD events.

With exclusion of all 158 diabetic men and an additional 49 men with missing glucose data, the association between serum glucose and major CHD events over 11.5 years of follow-up was examined in 7528 of the 7735 men.

Details of the measurement of glucose and of the ascertainment of prevalent cases of NIDDM and of incident cases of major CHD events have been set out in *Chapter 3*. Details of the measurement of relevent covariates have also been set out in *Chapter 3* (age, body mass index, occupational status, cigarette smoking, alcohol intake, physical activity, pre-existing CHD, use of anti-hypertensive therapy, blood pressure, total cholesterol, HDL-cholesterol and triglyceride concentration, heart rate, haematocrit and

---

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The data did not permit reliable identification of cases of NIDDM for whom insulin had been prescribed.

FEV_{1sec}).

Statistical analysis

Cox's proportional hazards models were used to assess the independent contributions of

prevalent NIDDM and serum glucose concentration at baseline to the risk of major CHD

events at 11.5 years of follow-up (and in separate analyses at 5 years follow-up). The risk

of major CHD events was examined in men with prevalent NIDDM at baseline relative

to non-diabetic men and by quintile of serum glucose. Age, body mass index, systolic

blood pressure, heart rate, Fev_{1sec}, HDL-cholesterol were fitted as continuous variables

in the proportional hazards model. Occupational status was fitted as 6 dummy variables

(7 categories: 6 Registrar General groups and Armed Forces), physical activity as 5

variables (6 categories), alcohol as 4 variables (5 categories) and smoking as 4 dummy

variables (5 categories). Pre-existing coronary heart disease and use of anti-hypertensive

therapy were fitted as dichotomous (yes/no) variables. As glucose and triglyceride

concentrations were not normally distributed, log transformed data were used. The data

on serum triglyceride concentrations were adjusted for time of sampling as described in

Chapter 5.

To illustrate the separate effects of allowing for key biological and life-style

variables, the NIDDM-CHD and the glucose-CHD relations were adjusted for potential

confounding factors in cumulative stages, *(Tables 18, 21 and 23).* Serum triglyceride

level was negatively correlated with HDL-cholesterol (r=-0.46) and was not an

independent predictor of CHD events in this study. Therefore triglyceride was not entered

with HDL-cholesterol in Cox's proportional hazards models.

Results

Of the group of 122 men with NIDDM at baseline, 28 men experienced a major CHD event during 11.5 years of follow-up, an incidence rate of 23.4 per 1000 person-years of follow-up as compared with a rate of 8.8 per 1000 person years in men without diabetes. *Table 18* shows the association between established NIDDM at baseline and major CHD events during 11.5 years of follow-up with the effect of cumulative adjustment for established CHD risk factors. There remained an almost 2-fold increased risk of CHD events in the fully adjusted model.

A non-linear relation between glucose level and the risk of major CHD events was observed with an increased risk at or above a serum glucose level of 6.1 mmol/L, the 80th centile, *Table 19*. The age adjusted relative risk for major CHD events in the upper glucose quintile relative to the 1st to the 4th quintiles combined was 1.2 (95% C.I. 1.0 to 1.4).

The distribution of major coronary risk factors was examined in the upper quintile of the glucose distribution relative to the other four quintiles *Table 20*. Men in the upper quintile were older and significantly more obese. A significantly higher proportion of men in the upper quintile were engaged in manual occupations. There were significantly fewer smokers in the upper glucose quintile, systolic blood pressure and triglyceride level were significantly higher and HDL-cholesterol significantly lower. Total cholesterol and haematocrit levels were similar in the two groups. FEV_{1sec} was significantly lower in the upper glucose quintile. Similar proportions of men in the two groups were physically active, moderate drinkers and using anti-hypertensive therapy. The prevalence of pre-existing CHD at the initial examination was significantly higher in the upper glucose quintile. Differences in the distribution of blood pressure and serum lipids and in the

prevalence of CHD between the upper and four lower serum glucose quintiles remained significant following adjustment for age, occupational status and body mass index (BMI).

Adjustment for age, body mass index, cigarette smoking, alcohol intake, occupational status, physical activity, use of anti-hypertensive therapy and pre-existing CHD did not attenuate the increased risk of CHD events in the upper quintile of the glucose distribution, *Table 21.* However on further adjustment for systolic blood pressure, heart rate, FEV_{1sec} and HDL-cholesterol, this weak association of borderline significance was non-significant, *(Table 21).*

A similar non-linear association between glucose and major CHD events was observed at 5 years of follow-up, *(Table 22)* and *(Table 23)*. In a further analysis the data were stratified by pre-existing CHD at baseline. Similar associations between glucose and major CHD events were seen at 5 and 11.5 years in men with and without evidence of pre-existing CHD.

We detected no evidence of a further increase in risk of CHD at the 90th or 95th glucose centiles. The glucose- CHD association was unchanged on exclusion of the 194 men, known to have developed non-insulin-dependent diabetes during an average follow-up period of 12.8 years to December 1991. In this latter analysis, the age adjusted relative risk for major CHD events in the upper glucose quintile relative to the rest was 1.2 (95% C.I. 1.0 to 1.4) at 11.5 years and 1.3 (95% C.I. 1.0 to 1.7) at 5 years of follow-up.

Discussion

In this study men with established NIDDM at baseline were, as expected, at substantially increased risk of major CHD events during follow-up. This association was attenuated on adjustment for other CHD risk factors which cluster with NIDDM, although there remained an almost 2-fold increased risk in full multivariate analysis. By contrast, a weak non-linear relationship was observed between serum glucose concentration (non-fasting) and the risk of major CHD events in this population-based sample of middle-aged British men. There was a relatively small increase in risk among men in the upper decile of the distribution which was of borderline significance in the age adjusted analysis and was non-significant after adjustment for established CHD risk factors.

These data do not support the hypothesis that hyperglycaemia is an important independent predictor of CHD via direct atherogenic mechanisms such as glycosylation of lipoproteins. It may be argued however, that despite the relatively large number of events (over 700), this study lacked power to detect a significant, linear, independent relation between glucose and CHD given the low reliability of non-fasting glucose as a measure of glycemic status. Caution in declaring independence (or lack of independence) in observational studies is undoubtedly warrented where effects of highly inter-correlated variables, measured with uncertain reliability are assessed in multivariate analysis. It is noteworthy that in an earlier report based on 505 events at 9.5 years follow-up of this cohort a similar increased risk of CHD events was observed in the upper decile of the glucose distribution which. in multivariate analysis at that duration of follow-up, attained statistical significance at the conventional 5% level, RR 1.30 (95% C.I. 1.04 to 1.6) (Perry *et al,* 1994). Hence the inconsistency in the literature on asymptomatic hyperglycaemia and CHD, as reviewed in *Chapter 2,* is mirrored in the British Regional

Heart Study data.

This inconsistency in the data on glucose and CHD almost certainly reflects the limited precision and reliability of any single measure of blood glucose (whether fasting, post load or non-fasting) as an index of glucose tolerance. In essence we need a measure of glucose tolerance in which the random within subject error is small relative to the difference in glucose tolerance which we hypothesise exists between subjects, i.e. men who do and do not present with major CHD events during the particular period of follow-up. If there is an association between glucose tolerance and CHD, it is likely that differences in glucose tolerance between cases and the rest of the cohort will be greater for cases which develop in early follow-up than in later follow-up, though relative risks will probably be less than 2 given the approximately 2-fold increased risk of CHD observed in men with established NIDDM. However studies lack power in early follow-up due to the small number of events, whereas in later follow-up, as the number of events increases, the reliability of a single measure of non-fasting glucose (with regard to the specific between subject differences which we are trying to detect)[6] decreases. From this it follows that for variables such as glucose, which are measured with low reliability, there is likely to be an optimum point of follow-up in terms of study power, a cross over point between the declining reliability of the exposure measurement and the increasing number of disease end-points. Hence one would expect inconsistent findings in studies of variable power which have attempted to estimate an effect which hovers at the border of epidemiological visibility.

We need to consider the possibility that the increased risk of CHD observed in the

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[6]

As noted previously, reliability is study specific.

upper glucose quintile (in this and previous studies) simply reflects the inclusion of undiagnosed diabetics, in whom an increased risk of heart disease would be expected. This is unlikely, given that we excluded men with glucose > 11.1 mmol/L at screening from the study and that the findings were unaltered in an analysis in which we also excluded men developing non-insulin-dependent diabetes during the subsequent 12.8 years of follow-up. Undoubtedly our group of "non-diabetics" includes a number of undiagnosed diabetics. However I would argue that among a group of men with random glucose < 11.1 mmol/L, none of whom were subsequently diagnosed as diabetic over 12.8 years of follow-up, the number of undiagnosed diabetics will be small and unlikely to alter the findings from a study of this size. The stability of the relative risk estimates following exclusion of the 194 men who developed NIDDM during the period of follow-up, supports this argument. Moreover, if the small excess of CHD events (observed at the 80th centile, a serum glucose level > 6.1 mmol/L) was simply due to misclassification of undiagnosed diabetics, one would expect to find evidence of a further increase in risk at higher glucose levels, such as at the 90th or 95th centiles where the risk of subsequent NIDDM (and presumably the prevalence of undiagnosed NIDDM) rises sharply. No evidence of a further increase in risk at these higher glucose levels was found.

Although it is unlikely that undiagnosed cases of NIDDM explain the association between glucose and CHD, it may be that this association depends largely on "latent" cases of NIDDM. It is clear from the data presented in *Chapter 4* that a high proportion of men in the upper quintile of the glucose distribution will eventually develop NIDDM. Hence it may be that hyperglycaemia is simply a marker for more fundamental abnormalities in carbohydrate and lipid metabolism, centered on insulin resistance with

hyperinsulinaemia which link NIDDM and CHD (Reaven, 1988). Thus in the Paris

Prospective Study data, glucose was not an independent predictor of CHD following

adjustment for plasma insulin (Fontbonne & Eschwege, 1991). This issue will be

addressed in the next chapter, *Chapter 8.*

Table 18- Relative risk of major CHD events in men with prevalent NIDDM at baseline (N=122) relative to non-diabetic men.

Adjusted for	N (Cases)	CHD events RR (95% C.I.)	p
Age	7699 (738)	2.36 (1.6-3.4)	<0.0001
Age, smoking, alcohol intake, occupational status, physical activity, and body mass index.	7563 (725)	2.02 (1.4-3.0)	<0.0001
Use of anti- hypertensive therapy, pre-existing CHD	7563 (725)	1.75 (1.2-2.6)	0.004
Above + systolic BP, heart rate, haematocrit, FEV_{1sec} total cholesterol, and HDL-cholesterol.	6794 (647)	1.80 (1.2-2.7)	0.003

Table 19- Rate of CHD events (unadjusted) during 11.5 years of follow-up per 1000 person years of follow-up by quintile of serum glucose (non-fasting).

Glucose	No of men	Events (n)	Rate
< 4.8 mmol/L	1473	(133)	8.49
4.9 - mmol/L	1770	(163)	8.56
5.3 - mmol/L	1276	(112)	8.16
5.6 - mmol/L	1497	(129)	8.04
> 6.1 mmol/L	1512	(167)	10.50

Table 20- Distribution of cardiovascular risk factor, mean (sd) or %, in the 1st to the 4th glucose quintiles combined compared with the 5th glucose quintile.

	Glucose < 6.1 mmol/L	Glucose ≥ 6.1 mmol/L
Number of men	6016	1512
Age**	50.1 (5.8)	50.7 (5.8)
Body mass index**	25.4 (3.1)	25.9 (3.3)
Current smokers (%)**	42.1	37.4
Manual occupation (%)**	57.8	63.5
Active (%)	37.4	37.7
Moderate drinkers (%)	26.3	27.5
Systolic BP (mmHg)**	143.6 (20.4)	151.0 (21.9)
Diastolic BP (mmHg)*	82.1 (13.0)	83.0 (14.0)
Heart rate**	69.5 (12.2)	75.1 (13.9)
Cholesterol (mmol/L)	6.30 (1.04)	6.30 (1.05)
HDL-cholesterol (mmol/L)**	1.15 (0.26)	1.13 (0.27)
#Triglyceride (mmol/L)**	1.67	1.93
Haematocrit	44.48 (0.04)	44.47 (0.08)
FEV_{1sec} (L)*	3.33 (0.68)	3.29 (0.68)
BP therapy	4.6	5.5
Evidence of CHD (%)*	23.9	27.1

#Geometric mean *p ≤ 0.05, **p ≤ 0.001.

Table 21- Relative risk of major CHD events at 11.5 years of follow-up in the upper quintile of serum glucose compared with the 1st to 4th quintiles combined.

Adjusted	N (Cases)	CHD events RR (95% C.I.)	p
Age	7528 (704)	1.19 (1.0-1.4)	0.04
Age, smoking, alcohol intake, occupational status, physical activity, and body mass index.	7408 (692)	1.21 (1.0-1.4)	0.03
Use of anti-hypertensive therapy, pre-existing CHD	7408 (692)	1.21 (1.0-1.4)	0.03
Above + systolic BP, heart rate, FEV_{1sec}, total cholesterol, and HDL cholesterol.	7020 (648)	1.15 (0.96-1.4)	0.2

Table 22- Rate of CHD events (unadjusted) during 5 years of follow-up per 1000 person years of follow-up by quintile of serum glucose (non-fasting).

Glucose	No of men	Events (n)	Rate
< 4.8 mmol/L	1473	(57)	7.96
4.9 - mmol/L	1770	(58)	6.72
5.3 - mmol/L	1276	(40)	6.41
5.6 - mmol/L	1497	(57)	7.81
> 6.1 mmol/L	1512	(74)	10.12

Table 23- Relative risk of major CHD events at 5 years of follow-up in the upper quintile of serum glucose compared with the 1st to 4th quintiles combined.

Adjusted	N (Cases)	CHD events RR (95% C.I.)	p
Age	7528 (286)	1.32 (1.01-1.7)	0.03
Age, smoking, alcohol intake, occupational status, physical activity, and body mass index.	7408 (281)	1.32 (1.01-1.7)	0.04
Use of anti-hypertensive therapy, pre-existing CHD	7408 (281)	1.31 (1.02-1.7)	0.04
Above + systolic BP, heart rate, FEV_{1sec}, and HDL-cholesterol.	7020 (263)	1.24 (0.95-1.6)	0.1

CHAPTER 8

SERUM INSULIN AND INCIDENT MAJOR CHD EVENTS

ABSTRACT

Objectives:- To test the hypothesis that elevated circulating insulin levels are independently related to the development of coronary heart disease.

Design:- A prospective study.

Subjects and setting:- 5550 men aged 40-59 years, drawn from one group practice in each of 18 towns in Britain. The men were members of the British Regional Heart Study cohort, whose baseline, non-fasting serum samples were analysed for insulin, using a specific ELISA method. Known and probable cases of diabetes at baseline were excluded.

Main outcome measure:- The relation between serum insulin concentration at baseline and incident major coronary heart disease events (fatal and non-fatal myocardial infarction) during 11.5 years follow-up.

Results:- There were 522 major coronary heart disease, 261 fatal and 260 non-fatal, during follow-up. A markedly non-linear relation between serum insulin and CHD events was observed with an almost 2-fold increased relative risk (RR) in the 10th decile of the serum insulin distribution (≥ 35.3 mU/L) relative to the 1st to the 9th deciles combined, (age-adjusted RR, 1.9; 95% C.I. 1.6 to 2.4). There was some attenuation of this association on cumulative adjustment for a wide range of lifestyle and biological CHD risk factors, including physical activity level and HDL-cholesterol concentration, although it remained significant in the fully adjusted proportional hazards model, (RR, 1.6; 95% C.I. 1.1 to 2.3). Similar associations between insulin and CHD events were seen in men with and without evidence of CHD at baseline.

Conclusions:- These data are consistent with the hypothesis that a high level of serum insulin (hyperinsulinemia) is atherogenic. However, the markedly non-linear form of the association and the attenuation in multivariate analysis, strongly suggests that the elevated insulin levels observed may only be a marker for common aetiological factors in the development of both ischaemic heart disease and non-insulin dependent diabetes.

Introduction

The association between serum insulin concentration and major CHD events during 11.5 years of follow-up in the BRHS cohort is examined in this chapter. As discussed in *Chapter 2,* in previous studies which have examined this question, insulin concentrations were determined by radioimmunoassay methods which are not specific for insulin but cross-react with pro-insulin and pro-insulin split products. In this study, insulin was measured using a specific ELISA method and adjustments were made for major coronary risk factors including HDL-cholesterol, triglycerides and physical activity. In most of the previous studies, data on HDL-cholesterol were not available. As we have seen in *Chapter 5,*the latter is inversely associated with insulin in cross-sectional studies and has been consistently advanced as a confounding factor of fundamental importance in studies of the link between insulin and CHD (Jarrett, 1988). There were over 500 major CHD events (fatal and non-fatal), more than the combined total of events in the previous studies that have addressed this issue (as reviewed in *Chapter 2*). The data were examined for evidence of a non-linear association between insulin and CHD (Fontbonne & Eschwege, 1991; Fontbonne *et al,* 1991; Pyorala *et al,* 1985) and for evidence of interaction between insulin and the established CHD risk factors. In a separate analysis we have also examined the insulin-CHD association at 5 years follow-up, as there were discrepant findings in the Paris prospective study between the early (5 years) and late (11 and 15 years) follow-up studies (Ducimetiere *et al,* 1980; Eschwège *et al,* 1985; Fontbonne & Eschwège. 1991; Fontbonne *et al,* 1991).

Subjects and methods of data collection

As detailed in *Chapters 5 and 6,* serum insulin data were available from 5550 men.

Details of the measurement of glucose and insulin and of the ascertainment of cases of major CHD events have been set out in *Chapter 3*. Details of the measurement of relevent covariates have also been set out in *Chapter 3* (age, body mass index, social class, cigarette smoking, alcohol intake, physical activity, pre-existing CHD, use of anti-hypertensive therapy, blood pressure, total cholesterol, HDL-cholesterol and triglyceride concentration, heart rate, haematocrit and FEV_{1sec}).

Statistical analysis

Cox's proportional hazards models were used to assess the independent contributions of serum insulin concentration at baseline to the risk of major CHD events at 11.5 years of follow-up (and in separate analyses at 5 years follow-up). The risk of major CHD events was examined by quintile of serum insulin, with the top quintile of this variable further divided into deciles. Age, body mass index, systolic blood pressure, total cholesterol, heart rate, FEV_1, HDL cholesterol and triglyceride concentration were fitted as continuous variables in the proportional hazards model. Haematocrit was fitted as four dummy variables for the five haematocrit groups, as described in *Chapter 4*. Social class was fitted as 6 dummy variables (7 categories: 6 Registrar General groups and Armed Forces), physical activity as 5 variables (6 categories), alcohol as 4 variables (5 categories) and smoking as 4 dummy variables (5 categories). Pre-existing coronary heart disease and use of anti-hypertensive therapy were fitted as a dichotomous (yes/no) variables. As insulin, glucose and triglyceride concentrations were not normally distributed, log transformed data were used. The data on serum insulin and triglyceride concentrations were adjusted for time of sampling as described in *Chapter 5*.

To illustrate the separate effects of allowing for key biological and life-style

variables, the insulin-CHD association was adjusted for potential confounding factors in cumulative stages, *(Tables 25 and 27)*. As in the previous analysis of the association between serum glucose and CHD events *(Chapter 7)*, serum triglyceride level was not entered with HDL-cholesterol in Cox's proportional hazards models. Possible interactions between insulin and established risk factors (body mass index, physical activity, hypertension, hypercholesterolemia and cigarette smoking) in the development of CHD were explored in stratified analyses and by fitting interaction terms in Cox's proportional hazards models.

Results

After 11.5 years follow-up for all 5550 non-diabetic men with serum insulin data, 521 major CHD events had occured, 261 fatal and 260 non-fatal. Mean serum insulin at baseline (geometric mean and 95% Confidence Interval) was significantly higher in men who subsequently developed a major CHD event than in the rest of the cohort, 14.7 mU/L (13.9 to 15.6) versus 12.3 mU/L (12.1 to 12.6), p <0.0001. *Table 24* shows the CHD event rates per 1000 person-years (unadjusted) by quintile of serum insulin. Event rates were lowest in the first quintile, intermediate in the 2nd to the 4th quintiles and highest in the fifth quintile. CHD event rates in the fifth quintile were significantly raised relative to the first quintile (age adjusted relative risk, 1.9; 95% C.I. 1.4 to 2.5). To determine whether CHD risk increased further at higher levels of insulin, the 5th quintile was divided into deciles (23.7-35.2 mU/L and ≥ 35.3 mU/L), *Figure 11*. Relative to the 1st quintile baseline group, a steep increase in the age adjusted risk of CHD was observed in the 10th decile (relative risk, 2.4; 95% C.I. 1.8 to 3.2), whereas risk in the 9th decile was only slightly raised (relative risk, 1.4; 95% C.I. 1.0 to 1.9), *Figure 11*.

The age adjusted risk of major CHD events among men whose serum insulin was in the upper decile of the distribution was increased almost two-fold relative to those with insulin levels at or below the 90th centile, (relative risk, 1.9; 95% C.I. 1.6 to 2.4). In subsequent analyses, showing the effects of adjustment for potential confounding factors, attention has been focused on the risk of CHD events in the upper decile of the insulin distribution relative to the rest of the distribution.

Insulin and CHD risk factors

As presented in *Chapter 5*, strong independent association between serum insulin and established CHD risk factors were observed, in particular with glucose, BMI, triglycerides and HDL-cholesterol. Insulin levels, were lower in manual workers and in the Armed Forces group than in non-manual workers and inverse associations with physical activity, cigarette smoking and alcohol intake were observed. A strong positive association between serum insulin and heart rate was observed and there were weak associations with haematocrit (positive) and FEV_{1sec} (negative). Insulin levels were higher in men taking anti-hypertensive therapy than in those who were not using anti-hypertensive therapy (see *Chapter 5*).

Insulin and prevalent CHD

Relative to men without evidence of CHD at baseline, insulin levels were higher in men with evidence of CHD short of a definite myocardial infarction (MI) and were highest in those with a definite MI on electrocardiogram or recall of a doctor diagnosis of MI, *(Chapter 5; Table 9)*.

Insulin and CHD events in multivariate analysis

Table 25 shows the relation between insulin and incident major CHD events, with the effects of successive adjustments for potential confounding variables. The significant increase in risk in the 10th decile relative to the rest of the serum insulin distribution was minimally attenuated on adjustment for the major life-style and biological CHD risk factors, including HDL-cholesterol. In a separate analysis, in which serum triglyceride was entered into the full proportional hazards model instead of HDL-cholesterol, the insulin-CHD association was unchanged, adjusted relative risk in the upper decile of insulin relative to the 1st to the 9th deciles combined, 1.7; 95% C.I. 1.3 to 2.2).

Insulin and CHD events by CHD status at baseline

Similar non-linear relations between serum insulin levels and subsequent CHD events were observed in men without and in men with evidence of CHD at baseline. In the men without CHD at baseline, the risk of subsequent major events in the upper decile of serum insulin relative to the rest of the distribution was 1.7 (95% C.I. 0.8 to 3.2) and in the men with CHD at baseline, the risk was 1.5 (95% C.I. 1.0 to 2.2), after adjustment for all potential confounding factors.

Insulin-CHD relation and glucose tolerance

We considered whether serum insulin levels in the 10th decile were simply a marker for undiagnosed or "latent" non-insulin dependent diabetes (NIDDM), which one would expect to be associated with increased CHD risk. The insulin-CHD relation was examined in two glucose strata, at or below the 80th centile and above the 80th centile ($<$ 6.1 mmol/L, and $\geq$ 6.1 mmol/L). An excess of CHD events in the 10th decile relative to

the rest of the serum insulin distribution was observed in the lower glucose stratum, relative risk 2.1 (95% C.I. 1.4 to 3.3) but not in the upper glucose stratum, relative risk 1.1 (95% C.I. 0.4 to 3.4), adjusted for potential confounding factors, including HDL-cholesterol. In a further analysis we excluded 138 men who developed non-insulin dependent diabetes (NIDDM) during an average follow-up period of 12.8 years (see *Chapter 6)*. On exclusion of this latter group, the fully adjusted relative risk of CHD in the 10th serum insulin decile relative to the 1st to the 9th deciles was 1.6 (95% C.I. 1.1 to 2.4).

Insulin-glucose ratio, glucose and CHD

The form and magnitude of the relation between insulin-glucose ratio and CHD was similar to that of insulin and CHD. Comparing the top decile of the insulin-glucose ratio with the rest of the distribution, the fully adjusted relative risk was 1.6 (95% C.I. 1.2 to 2.0). The association between serum glucose and major CHD events was re-examined in this sub-group of 5550 men. As in the entire cohort there was an increase in risk in the upper quintile relative to the lower four quintiles combined, age adjusted RR 1.5 (95% C.I. 1.1 to 2.1). This association between serum glucose and CHD events was abolished on addition of insulin to the age adjusted model, RR 1.0 (95% C.I. 0.7 to 1.4).

Interactions with other CHD risk factors

The data were examined for evidence of interactions between insulin and other risk factors in the development of CHD. The fully adjusted relative risk of CHD in the 10th serum insulin decile relative to the rest of the insulin distribution was greater in smokers, 2.0 (95% C.I. 1.4 to 2.9) than in non-smokers, 1.4 (95% C.I. 0.9 to 2.0), a difference

which was significant on formal testing for interaction (p=0.04). The relative risk in the top decile of serum insulin versus the rest of the distribution was similar in each tertile of BMI (1.9, 1.6, 1.6), in each tertile of total serum cholesterol (1.8, 1.9, 1.9), and in two physical activity strata (1.8, 1.5). In normotensives the relative risk in the top decile was 1.4 and in hypertensives (systolic $\geq$ 160 mmHg and/or diastolic $\geq$ 90 mmHg and/or receiving anti-hypertensive therapy) it was 1.8.

Insulin and CHD events at 5 years of follow-up

At 5 years of follow-up in the British Regional Heart Study there were 201 major CHD events. A stronger and somewhat more continuous association between insulin and CHD events was observed over the first 5 years of follow-up, *(Table 26 and Figure 12)*. In the upper decile of serum insulin the age adjusted risk of CHD events was increased more than 3-fold relative to the 1st quintile, relative risk 3.7 (95% C.I. 1.8 to 5.5) and more than 2-fold, relative to the rest of the insulin distribution, *(Table 27)*. As at 11.5 years follow-up, there was some attenuation of the increased risk in the upper decile on adjustment for the full range of life-style and biological CHD risk factors, but there remained a substantial excess risk of CHD events in the upper decile of serum insulin in the fully adjusted proportional hazards model, *(Table 27)*.

Additional predictors of CHD

Table 28 shows the major independent predictors of CHD at 5 and 11.5 years follow-up. Relative to established CHD risk factors, there was marked attenuation of the association between serum insulin and risk of CHD events between 5 and 11.5 years of follow-up.

Discussion

An elevated circulating insulin level was an independent predictor of major CHD events at 5 and 11.5 years of follow-up in this representative sample of British middle-aged, non-diabetic men. The magnitude of the insulin-CHD association was greater and somewhat more curvilinear in the first 5 years than in the later period of follow-up. However, the association was markedly non-linear at 11.5 years and in both early and late folow-up, the excess risk of CHD events was concentrated largely in the upper decile of the serum insulin distribution. Although insulin levels were raised in men with pre-existing CHD at baseline, the relation between insulin and subsequent CHD events was similar in men with and without evidence of preexisting CHD at baseline. In contrast to the previous prospective studies which have addressed this question, insulin levels were determined using a specific assay which does not cross-react with pro-insulin.

The lack of fasting insulin data may be regarded as a limiting factor in this study. However, it has been suggested that the non-fasting state may be more relevant to the study of insulin and CHD than the fasting state (Yarnell *et al,* 1994). In clinical studies of the link between insulin and cardiovascular disease, consistent associations with post-load insulin have been observed but not with fasting insulin or with the insulin response to intravenous stimuli (Stout, 1990). Inevitably, within-subject variability in insulin measurement will be higher with random (non-fasting) samples than with timed, post-load samples. It is likely for instance, that the attenuation in the insulin-CHD association between 5 and 11.5 years simply reflects measurement error or (more specifically) the declining reliability of a single measurement of non-fasting insulin as a measure of the difference between cases and the rest of the cohort with increasing duration of follow-up. However as discussed in *Chapter 1,* all measurements of insulin in epidemiological

studies are beset by problems of high within-subject variability and it is arguable that differences in this regard are probably small between post- load and non-fasting samples (adjusted for time of sampling). High within-subject variability in the measurement of a putative risk factor such as insulin will dilute etiological association and in particular will attenuate estimates of the independence of effects in multivariate analysis. Indeed, given the inherent problem of variability (or random error) in insulin measurement in this as in other studies, it is likely that the magnitude of associations with cardiovascular risk factors and with CHD events has been underestimated.

As discussed in *Chapter 5*, correlations of non-fasting insulin with cardiovascular disease risk factors such as BMI, lipids and blood pressure described in this study, are very similar to those reported with fasting and post-load insulin in other studies. Similarly, the associations reported in this study between insulin levels and lifestyle factors such as physical activity, cigarette smoking and alcohol intake (as presented in *Chapter 5)* are consistent with findings from earlier studies (Fontbonne *et al,* 1991; Yarnell *et al,* 1994; Ferrara *et al,* 1994; Mykkänen *et al,* 1993[b]). It is difficult therefore to concieve of a source of systematic error related to the conditions of sampling which has produced a biased estimate of the association between insulin and coronary heart disease events but not of the association between insulin and cardiovascular risk factors.

The lack of data on body fat distribution represents a limitation in this study. Central obesity is considered to be a better marker of CHD risk than body mass index (Deprès *et al.* 1990) and is independently associated with insulinemia (Cigolini *et al,* 1991). However, there was little attenuation of the insulin-CHD association on adjustment for BMI and physical activity level, variables which are correlated with central obesity

(Marti *et al*, 1991), and there was no evidence of significant variation in the magnitude of the association in different strata of BMI or physical activity level. Hence substantial residual confounding due to central obesity is unlikely.

The findings in the present study are consistent with the 15 year follow-up data from the Paris Prospective Study, the largest of the previous studies which have examined the relation between insulin and CHD (Fontbonne *et al*, 1991). In the 15 year data from the Paris study (6093 subjects, 174 CHD deaths), 2-hour post load insulin in the upper quintile (entered as a categorical variable) emerged with systolic blood pressure, plasma cholesterol and cigarette smoking as a significant independent predictor of death from CHD. A similar non-linear relation between post-load insulin and CHD was observed in the 11 year follow-up data from the Paris study (Fontbonne & Eschwège, 1991). Post-load insulin (both 1-hour and 2-hour) was also a significant predictor of CHD events in the Helsinki Policemens' Study (1059 subjects) at 5 years (36 events) and 9.5 years of follow-up (63 events), independent of its association with other risk factors such as obesity, blood pressure and hyperlipidaemia (Pyörälä, 1979; Pyörälä *et al*, 1985). As in the Paris study, the relation in the Helsinki Study was non-linear with increased risk in the upper quintile. In the 9.5 year data from this Finnish study the upper quintile was divided into deciles and a further marked increase in CHD event rates was observed in the 10th decile (Pyörälä *et al*, 1985). Thus we now have evidence from three prospective studies of middle-aged men (Paris Prospective Study, Helsinki Policemen Study and the British Regional Heart Study) of a significant, non-linear relation between post load (or non-fasting) insulin and coronary heart disease, an association which in this study was independent of HDL-cholesterol. Negative studies in this area have either had few CHD endpoints (Cullen *et al*, 1983; Hargreaves *et al*, 1992; Welin *et al*, 1992; Rewers *et al*,

1992; Ferrara *et al*, 1994) or have been based on fasting insulin (Yarnell *et al*, 1994; Orchard *et al*, 1994). Data on women are sparse (Cullen *et al*, 1983; Rewers *et al*, 1992; Ferrara *et al*, 1994). In the Busselton study (724 women aged 40-74, 18 CHD deaths), no independent effect of 1-hour post load insulin on CHD mortality was detected at 13 years follow-up (Cullen *et al*, 1983). Subsequent negative studies in women have been based on a total of 36 coronary heart disease endpoints (Rewers *et al*, 1992; Ferrara *et al*, 1994). The reported inverse association between 2-hour insulin and cardiovascular disease mortality in elderly men, is not necessarily generalisable to younger age groups (Ferrara *et al*, 1994). Indeed, the inverse association between insulin levels and CHD risk in the Rancho Bernardo study probably reflects confounding by comorbidity given that those who died of a CHD event were leaner than those who survived (McKeigue & Davey, 1995).

In studies of subjects with established NIDDM, insulin levels have not emerged as a significant predictor of CHD (Wingard *et al*, 1995). This is hardly surprising, given that insulin levels in this setting largely reflect β-cell hyposecretion and cannot be regarded as providing a reliable measure of either insulin resistance or previous hyperinsulinaemia.

It is suggested that insulin may only accelerate the development of atheroma in the presence of other risk factors such as hypertension or hypercholesterolaemia (Modan *et al*, 1991; Durrington. 1992). This hypothesis is suggested by the low rates of CHD in populations such as the Pima Indians. despite marked hyperinsulinemia (Ingelfinger *et al*, 1976). There was no evidence of an interaction between insulin and serum cholesterol in these data and although CHD rates were higher among hypertensives than normotensives with serum insulin levels in the upper decile, the difference was not significant.

Similarily, there was no evidence of an interaction with BMI. There was however, a significant interaction between insulin and cigarette smoking, with higher CHD risk among smokers with raised insulin levels compared with non smokers. In this, as in previous data (Yarnell *et al*, 1994), insulin levels were lower in smokers. This interaction between serum insulin level and cigarette smoking clearly merits exploration in cross-sectional data on insulin and CHD risk factors and in future prospective studies

The present study provides clear evidence that serum true insulin levels in the highest part of the distribution are associated with a sharp increase in the risk of CHD events over the ensuing 5 to 10 years, which is independent of major CHD risk factors with which insulin is correlated. However, this does not necessarily suggest a direct atherogenic role for elevated circulating insulin levels in the development of CHD. Indeed, the form of the association, with a relatively marginal increase in risk over much of the insulin distribution (even in univariate analysis) suggests that insulin is not exerting direct atherogenic effects. Moreover, there is a particular need for caution in ascribing causality and declaring independence of effects in circumstances where multiple inter-correlated and labile variables such as insulin, blood pressure, HDL-cholesterol and triglycerides are examined simultaneously (Davy-Smith & Phillips, 1990).

Elevated non-fasting insulin levels in this study can be regarded as a marker for insulin resistance, particularly in men with normal glucose tolerance (Laakso, 1993). Hence, these data cannot resolve the issue of whether insulin is associated with CHD primarily via direct atherogenic effects or as a marker for insulin resistance. Given the lack of a continuous relation between serum insulin and CHD in these data and the inconsistent data on the effect of exogenous insulin on CHD risk (The University Group Diabetes Program, 1982; Wingard *et al*, 1995), the latter hypothesis, that insulin

resistance predicts CHD with insulin as an "innocent bystander" (Savage & Saad, 1993), is more plausible.

In this thesis, the evidence that CHD and non-insulin dependent diabetes share common causal factors, genetic, environmental and possibly intrauterine (Hein *et al*, 1992; Zimmet, 1989; Hales *et al*, 1991), has been reviewed. It has been shown (*Chapter 6*) that true hyperinsulinaemia antedates the development of NIDDM (with a relatively sharp increase in the upper quintile), and it is clear that insulin is acting as a marker for insulin resistance in this setting. There is increasing evidence that the adverse coronary risk factor profile, which clusters with insulin resistance (Reaven, 1988), also antedates NIDDM (Haffner *et al*, 1990; Mykkänen *et al*, 1993[a]). In the BRHS cohort also, it has been shown that "the clock for coronary heart disease (starts) ticking before the onset of clinical diabetes" (Haffner *et al*, 1990) (*Chapter 4*) and that CHD risk factors which antedate (or predict) NIDDM, are associated with hyperinsulinaemia *(Chapter 5)*. Exclusion of men who were known to have developed NIDDM during the period of follow-up did not alter the findings in this study of a sharp increase in CHD risk in the upper decile of the insulin distribution. However, there will inevitably have been undiagnosed cases of NIDDM and (on the basis of the data presented in *Chapter 6*), it is likely that a high proportion of men in the upper 10% of the insulin distribution will develop NIDDM on further follow-up. *It is likely therefore that elevated serum insulin levels in this study, reflecting insulin resistance, are a marker for the common causal factors which link CHD and non-insulin dependent diabetes.*

For over a decade the magnitude and importance of the association between circulating insulin levels and CHD has been contested. There is a need now to focus on factors (particularly environmental factors) which influence the development of insulin

resistance. The findings presented in this thesis, are consistent with the accumulating data which implicate obesity and physical inactivity in the development of insulin resistance and suggest a possible beneficial effect of moderate alcohol consumption on insulin sensitivity *(Chapter 5)*. The effects of diet (particularly saturated fat, Singh & Niaz, 1995) and psychosocial stress (Björntorp, 1991) on insulin sensitivity and risk of NIDDM, clearly merit examination in future studies. In future studies there is also a need to examine the factors within and between populations which modulate the progression from insulin resistance to non-insulin dependent diabetes, coronary heart disease or both of these conditions.

Table 24- Rate of CHD events (unadjusted) during 11.5 years of follow-up per 1000 person years of follow-up by quintile of serum insulin (non-fasting).

Insulin	No of men	Events (n)	Rate/1000 person years
< 6.6 IU/L	1113	(78)	6.49
6.6 - IU/L	1118	(100)	8.29
9.6 - IU/L	1108	(99)	8.32
14.3 - IU/L	1111	(102)	8.61
23.7 - IU/L	1100	(142)	12.4

Figure 11- Major CHD event rate per 1000 person years at 11.5 years follow-up in the 1st to the 4th quintile of serum insulin and in the 9th and 10th deciles. On the top of each bar the number of events is indicated and in brackets below each bar, the number of men in each quintile and in the 9th and 10th decile.
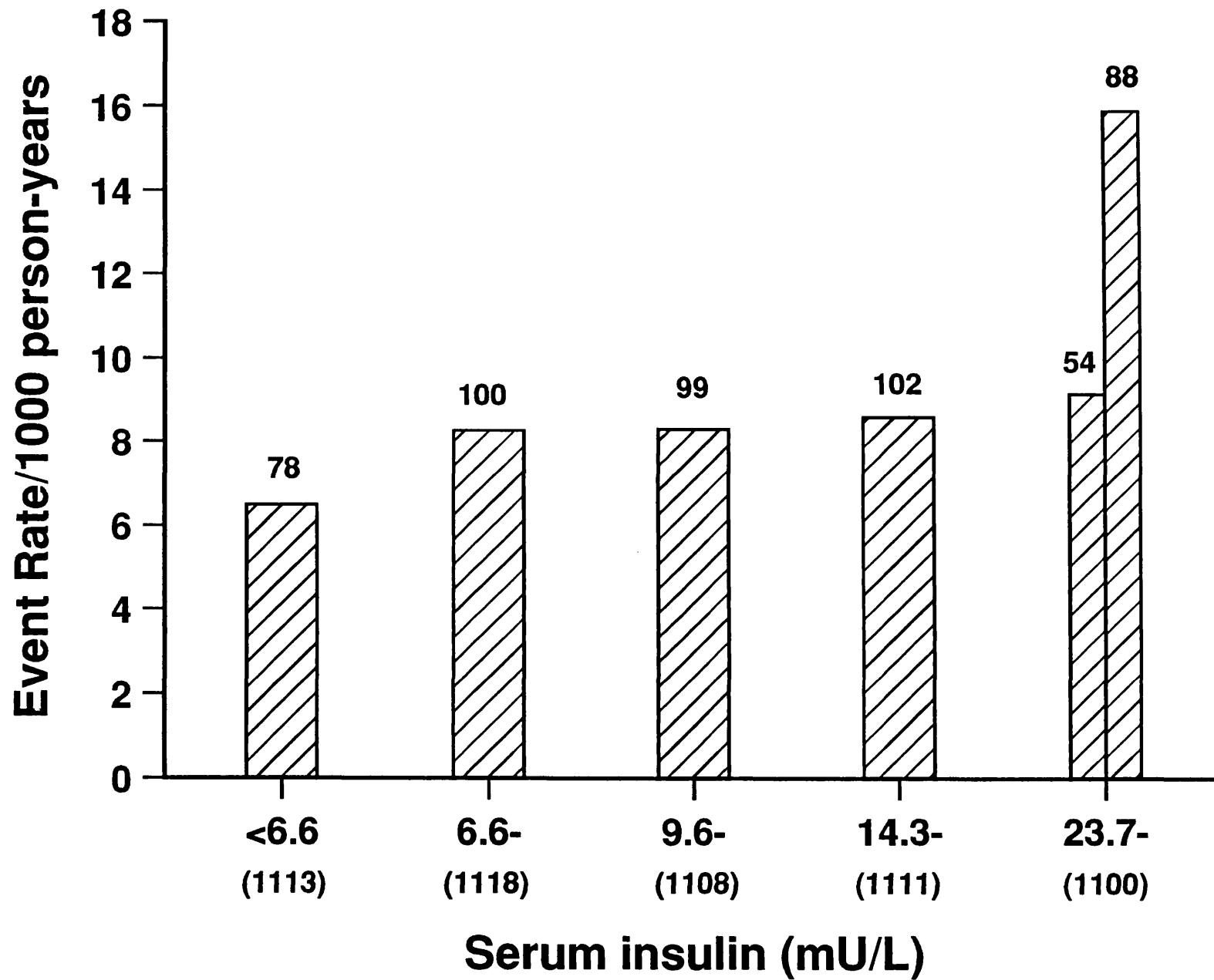
Table 25- Adjusted relative risk (95 % confidence intervals) of incident major CHD events at 11.5 years of follow-up by serum insulin level (6 groups: 1st to 4th quintiles, 9th and 10th deciles).

Insulin IU/L	A	B	C
< 6.6	1.0	1.0	1.0
6.6-	1.2 (0.9-1.6)	1.1	1.1 (0.8-1.5)
9.6-	1.2 (0.9-1.6)	1.1	1.0 (0.7-1.4)
14.3-	1.2 (0.9-1.6)	1.1	1.0 (0.7-1.4)
23.7-	1.3 (0.9-1.8)	1.1	0.9 (0.6-1.6)
35.3-	2.1 (1.5-2.1)	1.8	1.6 (1.1-2.3)

Top decile vs. rest	1.7 (1.3-2.2)	1.6 (1.3-2.1)	1.6 (1.1-2.3)

Adjustments

A = Adjusted for age, glucose* and BMI (N=5535; 520 cases).

B = Adjusted for the factors in A plus social class, smoking, physical activity, heart rate, FEV_1, alcohol consumption. systolic blood pressure, total cholesterol level, use of anti-hypertensive therapy. and prevalent coronary heart disease (N=5361; 504 cases).

C= Adjusted for the factors above plus HDL-Cholesterol level (N=5272; 493 cases).

*The findings are unchanged if glucose is fitted as a continuous variable.

Table 26- Rate of CHD events (unadjusted) during 5 years of follow-up per 1000 person years of follow-up by quintile of serum insulin (non-fasting).

Insulin	No of men	Events (n)	Rate/1000 person years
< 6.6 IU/L	1113	(25)	4.57
6.6 - IU/L	1118	(33)	6.02
9.6 - IU/L	1108	(35)	6.47
14.3 - IU/L	1111	(38)	7.02
23.7 - IU/L	1100	(70)	13.30

Figure 12- Major CHD event rate per 1000 person years at 5 years follow-up in the 1st to the 4th quintile of serum insulin and in the 9th and 10th deciles. On the top of each bar the number of events is indicated and in brackets below each bar, the number of men in each quintile and in the 9th and 10th decile.
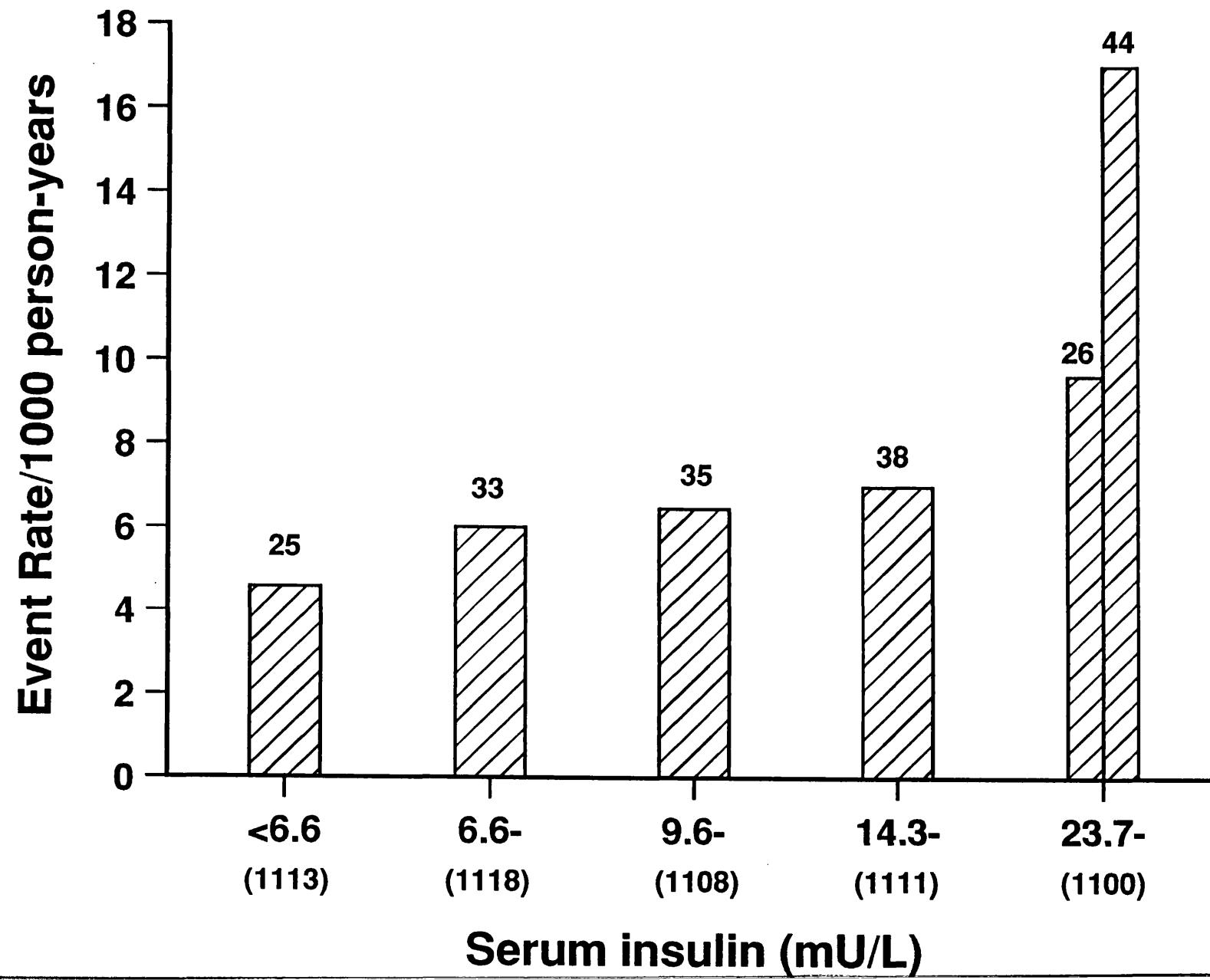
Table 27- Adjusted relative risk (95% confidence intervals) of major CHD events in the 10th serum insulin decile relative to the 1st to the 9th deciles combined at 5 years follow-up (N=5550; 201 events).

A	B	C	D
2.6 (1.9-3.6)	2.2 (1.5-3.2)	2.1 (1.4-3.1)	2.1 (1.4-3.0)

Adjustments

A = Adjusted for age (N=5550; 201 cases).

B = Adjusted for age, glucose and BMI (N=5535; 201 cases).

C = Adjusted for the factors in A and B plus social class, smoking, physical activity, heart rate, FEV_1 alcohol consumption, systolic blood pressure, total cholesterol level, use of anti-hypertensive therapy, and prevalent coronary heart disease (N=5361; 194 cases).

D= Adjusted for the factors above plus HDL-Cholesterol level (N=5272; 188 cases).

Table 28- Insulin as a predictor of CHD events compared with other predictors at 5 and 11.5 years follow-up. The results for each variable have been adjusted for age and for the other variables.

Variable	Relative risk at	
	5 years	11.5 years
Insulin (top decile versus bottom fifth)	2.7	1.6
Systolic BP (top decile versus bottom fifth)	2.1	1.8
Total cholesterol (top decile versus bottom fifth)	3.0	2.9
HDL-cholesterol (bottom decile versus top fifth)	2.1	1.8
FEV_{1sec} (bottom decile versus top fifth)	1.8	1.6
Heart rate ($\geq$ 90 beats/min versus rest)	1.3	1.4
Cigarette smoking (moderate/heavy versus never)	2.4	2.1
Use of antihypertensive medication	1.5	1.6
Prevalent CHD at screening	2.0	1.8
Physical activity (none versus moderate)	2.0	1.6

CHAPTER 9

DISCUSSION AND CONCLUSIONS

Epidemiology is concerned with advancing our understanding of the causes of disease, though as Hill (1965) reminds us, "all scientific work is incomplete, whether it be observational or experimental". Causal inferences are (by definition) tentative (Popper, 1965). The data from the British Regional Heart Study as presented in this thesis, taken with the findings from previous work, are consistent with a causal role for obesity and physical inactivity in the development of NIDDM. The data linking moderate alcohol consumption with lower risk of NIDDM require more cautious interpretation and the status of cigarette smoking as a risk factor for NIDDM is equivocal. The data also support the hypothesis that non-insulin dependent diabetes and coronary heart disease share common aetiological factors, such as obesity, physical inactivity and possibly the level of alcohol intake, which are linked by hyperinsulinemia and insulin resistance. Consistent with this hypothesis, there was evidence of a common pathway of mediating factors linking NIDDM and CHD, factors such as increased triglyceride and decreased HDL-cholesterol and raised heart rate, which are also linked by hyperinsulinemia and insulin resistance. Other mediating factors, such as hyperuricemia and raised haematocrit, which have been shown to predict CHD (Klein *et al*, 1973; Wannamethee *et al*, 1994), were shown in this work to predict NIDDM. Though strong, biologically significant associations between these latter factors and insulin were not observed in this work, there is evidence from other work that both hyperuricemia and raised haematocrit merit inclusion in the insulin resistance syndrome (Facchini *et al*, 1991; Smith *et al*, 1994). The findings suggest however, that there is a fundamental component of CHD risk which is not related to insulin resistance and diabetes. Total cholesterol levels did not predict NIDDM, and it is clear that hypertensives and smokers were at considerably greater risk of CHD (Shaper *et al*, 1985[a]) than of NIDDM in this cohort.

Lifestyle related factors and NIDDM

The association between obesity and NIDDM is strong, graded, consistently observed in diverse population, biologically plausible and supported by some evidence of reversibility (Eriksson & Lindgarde, 1991; Knowler *et al*, 1995). The association between physical activity and NIDDM is less strong, which leaves open the possibility of residual confounding due to obesity or confounding due to unmeasured behavioural, lifestyle or psychosocial factors associated with higher levels of physical activity. However the association with physical activity is graded, consistent and biologically plausible. Good evidence on the reversibility of NIDDM risk with exercise, independent of effects on obesity is lacking. Of course from the public health perspective, the question of whether physical activity is associated with reduced risk of NIDDM "independent" of effects on obesity is largely irrelevant. There is considerable evidence that in economically developed societies, such as Britain and the US, physical inactivity, rather than dietary excess, is the major contributor to the rising prevalence of obesity (Prentice & Jebb, 1995; Kuczmarski *et al*, 1994). For decades, the notion that obesity is not an important aetiological factor in the development of CHD was accepted, based on the fallacy that it had not been consistently shown to be independent of CHD risk factors such as hypertension and dyslipidaemia. Clearly in considering the role of physical inactivity in the development of NIDDM we must be careful not to confuse confounding with mediating factors.

Current data on the relation between alcohol intake and NIDDM are limited. Hence the results of this study, which (on the basis of the most cautious interpretation) suggest that alcohol consumption does not increase the risk of NIDDM, represent a significant addition to current knowledge on this issue. The lower risk of NIDDM seen

in moderate relative to occasional drinkers, is broadly consistent with findings from previous studies on alcohol and NIDDM (and CHD, Holbrook *et al*, 1990; Rimm *et al*, 1995; Shaper *et al*, 1994) and it is biologically plausible, given the associations between alcohol consumption and insulin levels *(Chapter 5)*. There was also some evidence of a biological gradient. Occasional rather than non-drinkers were taken as the reference group in these analyses, thereby reducing (but not eliminating) the potential for bias due to the inclusion in the latter category of former heavy drinkers who have stopped drinking because of illness.

The weak association between cigarette smoking and NIDDM (even in the age and age/BMI adjusted data), taken with the inverse association between the number of cigarettes smoked and serum insulin levels, do not suggest a major role for smoking in the development of insulin resistance and NIDDM. However smoking may increase risk of NIDDM via direct effects on pancreatic β-cell function leading to pancreatic hyposecretion of insulin. In this event, smoking would be associated with higher risk of NIDDM in lean rather than obese individuals, i.e. there may be an interaction with obesity, as suggested by the data from the US Health Professional's follow-up study (Rimm *et al*, 1995). This issue should be addressed in future studies based on cohorts with an adequate number of cases of NIDDM among men who can be regarded as "lean".

Insulin resistance, NIDDM and CHD

The development of insulin resistance with compensatory hyperinsulinaemia is undoubtedly central to the pathogenesis of NIDDM and as suggested by the data presented in this thesis, it acts as a marker for common causal factors shared between NIDDM and CHD. Understanding of pathogenesis is clearly of considerable importance

in the development of therapeutic agents such as, troglitazone (Nolan *et al,* 1994), which

may, via effects on insulin sensitivity reduce the risk of deterioration to NIDDM in high

risk individuals and may possibly reduce the risk of CHD in such individuals (Keen,

1994). However epidemiology and public health are primarily concerned with aetiology

and prevention at the population level rather than pathogenesis and treatment at the

individual level. It is arguable that although the insulin resistance hypothesis has provided

a useful model for exploring the links between NIDDM and CHD, there is a need now

to focus on fundamental causal factors in NIDDM and CHD rather than continue to focus

on the minutiae of pathogenesis. There is a need to keep sight of the distinction between

aetiology and pathogenesis. Keen for instance, in an editorial on troglitazone, has

suggested that "insulin resistance is now putatively responsible not only for obesity and

glucose intolerance but also for a panoply of pathologic conditions constituting what is

known as the metabolic syndrome, the insulin resistance syndrome, or Syndrome X,

whose manifestations may also include hypertension, dyslipidaemia, and coronary,

cerebral, and peripheral artery disease" (Keen, 1994). A formidable indictment of insulin

resistance! While genetically determined defects in insulin sensitivity may account for

some of the within population variation in obesity, clearly such defects cannot be

considered relevant to between population differences in obesity, to the causes of

incidence in the population. To argue that insulin resistance is responsible for obesity and

glucose intolerance not only confuses aetiology and pathogenesis but also ignores the

difference between the determinants of disease in individuals and populations. Moreover,

it is argued that in focusing on insulin resistance at the centre of a metabolic syndrome

linking NIDDM and CHD in certain populations, such as in South Asians, the role of

fundamental aetiological factors such as dietary saturated fat intake in these populations

has received insufficient attention (Singh & Niaz, 1995). For instance, there is evidence that the increasing prevalence of CHD from the low levels seen in rural North Indians to the high levels seen in South Indian immigrants to the UK is associated not only with a rising prevalence of diabetes but also with steadily increasing consumption of total fat and saturated fat (Singh & Niaz, 1995). It is clear that in considering the epidemic of CHD in South Asians and in considering the relation between NIDDM and CHD in this population, we need to focus on potentially modifiable factors associated with immigration such as changes in the amount and constituents of the diet, changes in physical activity and other factors such as psychosocial stress. Ultimately, the question of whether insulin resistance explains the link between NIDDM and CHD in this or other populations is less important than the identification of modifiable environmental factors which reduce the risk of NIDDM and CHD, whether via effects on insulin resistance or other mechanisms. In this context, it is worth noting that there are some populations, notably those of African origin (Chaturvedi et al, 1994) and the Pima Indians (Nelson et al, 1990), which have high rates of NIDDM but low rates of CHD. It may be that in some of these populations NIDDM is associated primarily with insulin hyposecretion rather than insulin resistance (Joffe et al, 1992), or there may be ethnic variation among individuals with NIDDM in resistance to the effect of insulin on lipid metabolism (Chaturvedi et al, 1994). However, these populations tend to have relatively low serum cholesterol and it may be that serum cholesterol (reflecting the amount and pattern of dietary fat intake) plays a pivotal role in modulating inter-relations between insulin resistance, NIDDM and CHD.

Public health implications

The findings from this study have implications for both policy formation and for research

with regard to the prevention of NIDDM and atherosclerotic vascular disease .

Prevention of NIDDM

Clearly, even in the affluent West, the prevention of NIDDM will depend largely on a

population based approach, which seeks to "discover and control the causes of incidence"

rather than a high risk approach with its emphasis on identifying and protecting

susceptible individuals (Rose, 1985). However, the current Health of the Nation (HoN)

targets for obesity in England focus on the tail of the BMI distribution, i.e. on reducing

the prevalence of obesity (defined as BMI $\geq$ 30 kg/m^2) rather than on achieving a

downward shift in the overall distribution (Department of Health, 1992). On the basis of

these data from the BRHS, achievement of the HoN target of an 8% reduction in the

prevalence of obesity (as defined) in middle-aged men would (assuming a causal link

between obesity and NIDDM) have lead to a 2% reduction (approximately) in the number

of men with physician diagnosed NIDDM over the period of follow-up. However 75%

of NIDDM cases in this study occurred in men with a BMI below 30 kg/m^2. If it is

accepted that the increasing risk of NIDDM across the entire range of obesity (as

reflected by BMI) represents a causal relation, these data suggest that a small downward

shift in the population mean BMI will result in a substantial reduction in the incidence

of diagnosed NIDDM. One would anticipate proportionate effects on the population

burden of undiagnosed NIDDM and impaired glucose tolerance.

The results of this study add to the accumulating evidence regarding the health

benefits of physical activity (Royal College of Physicians, 1991) and in particular the

benefits of moderate exercise. Again. if we accept that the link is causal, these data have

implications for the promotion of exercise at the population level, given the low levels of physical activity among adults (and children) in the UK (Allied Dunbar, 1992).

From a practical viewpoint however, it is unlikely that a significant downward shift in the population distribution of BMI will occur without fundamental changes in national food policy, ranging from production to marketing and labelling and changes in related areas of relevance to diet and exercise, such as education, employment, income distribution and transport policy.

The findings on alcohol and risk of NIDDM are relevant to policy formulation and the development of guidelines on "safe" drinking levels. It is important however that recommendations regarding alcohol intake are not unduly influenced by data on the association between alcohol and specific medical problems, such as NIDDM (or coronary heart disease). There is a need to focus on the effect of alcohol on total morbidity and mortality and on the wider public health issues of the psychological, social and economic cost of heavy drinking.

Clearly, population based and high risk approaches to the prevention of NIDDM are not mutually exclusive. The associations between both BMI and physical activity and NIDDM and the finding that individuals at particularly high risk of NIDDM are readily identifiable, also have implications for the development of high risk strategies for the prevention of this condition. In this context, it should be noted that detailed knowledge of family history of diabetes combined with BMI and serum glucose would probably have further enhanced the prediction of NIDDM in this study. A strong case can now be made for conducting a major intervention study to determine the effectiveness of weight loss and exercise (separately and in combination) in the prevention of NIDDM and atherosclerotic vascular disease in individuals at high risk of NIDDM. There is

preliminary evidence which supports such a strategy (Eriksson & Lindgarde, 1991).

Prevention of CHD

Given the association between CHD and NIDDM in industrialised societies, strategies to prevent NIDDM (both high risk and population-based) are likely to have a favourable impact on CHD morbidity and mortality and *vice versa*. The findings from this work support the concept of an integrated approach to the prevention of NIDDM and CHD. For example it is difficult to envisage an effective strategy for the reduction of CHD in the UK South Asian population which does not address the causes of glucose intolerance in this population (McKeigue *et al*, 1991). Furthermore from a high risk perspective, given the efficacy and relatively high cost-effectiveness of coronary risk factor reduction in diabetic subjects (Yudkin, 1993), it is likely (though not as yet proven) that early diagnosis of NIDDM combined with attention to risk factors (particularly obesity) will be of value, in postponing or preventing CHD. In this study it has been shown that the development of insulin resistance and the gradual deterioration in glucose tolerance which precedes the development of NIDDM, is related to behavioural factors associated with CHD, such as obesity and physical inactivity, and is associated with derangements in important CHD risk factors such as HDL-cholesterol. This raises the possibility of additional benefit, in terms of CHD prevention, from the primary prevention of NIDDM. Indeed it is arguable that the efficacy and cost effectiveness of coronary risk factor reduction in "pre-diabetic" subjects will approach that of risk factor intervention in diabetic subjects. Ultimately, intervention studies will need to address this hypothesis.

Further work

The findings from this work have led to additional studies examining risk factors for

NIDDM and exploring inter-relations between NIDDM and coronary heart disease in the

BRHS cohort. A proposal to recall surviving members of the BRHS cohort for a further

examination is currently under consideration. The results of this study have enabled the

author to argue for the inclusion of measures of glucose tolerance in this potentially

important national study of late middle-aged and elderly men. This further study should

contribute valuable data on the distribution of glucose intolerance in British men and it

has the potential to illuminate additional lifestyle and biological predictors of both

NIDDM and CHD.

CHAPTER 10

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APPENDIX 1

THE BRITISH REGIONAL HEART STUDY

BASELINE QUESTIONNAIRE

REGIONAL
HEART
STUDY
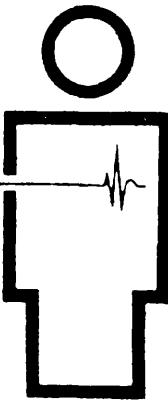
THIS IS A MEDICAL RESEARCH SURVEY

ALL THE INFORMATION IS CONFIDENTIAL

PERSONAL HEALTH RECORD

All the information recorded in this personal health record will be
treated as strictly confidential and will be available only to your own
doctor and the Regional Heart Study team. The results of the analysis
of your replies to the questionnaire and the physical measurements made
will be used by your own doctor as part of the individual health care
which he provides for you.   The results of the research involving all
the men taking part in the study will appear only in the form of general
statistics from which it will be impossible to identify you as an
individual.

If you have any questions or problems about any of the procedures included
in your examination, do not hesitate to ask the members of the Study
team.

THANK YOU FOR YOUR CO-OPERATION IN THIS STUDY. THE FINDINGS WILL HELP
TO IMPROVE THE HEALTH OF MEN THROUGHOUT THE COUNTRY.

Serial Number	☐☐☐☐☐☐☐☐	1
Card Number	0 1	9
Date of Screening	☐☐☐☐☐☐	11
Time of Screening	☐☐☐☐	17

1. GENERAL

What is your date of birth?

Day ☐☐ 21

Month ☐☐ 23

Year 19 ☐☐ 25

Where were you born? Town ....

County ....

Country ....

1.2 How many years have you lived within 10 miles of this town?
If you have moved to this area <u>within the last five years</u>,
where did you move from? ....

☐☐ years 27

1.3 What is your marital status?

Single 1

Married 2

Widowed 3

Other 4

☐ 29

1.4 How many children do you have?

	M	F	
<5 yrs.	☐	☐	30
5-10 yrs.	☐	☐	32
11-16 yrs.	☐	☐	34
>16 yrs.	☐	☐	36

---

2. YOUR FATHER

2.1 Where was your father born?

Town ....

County ....

Country ....

2.2 Is your father alive? (Y/N)

☐ 38

2.3 How old is he now? / How old was he when he died?

☐☐ 39

years

2.4 If your father has died, what were you told was the cause of his death?

Heart trouble	1
High blood pressure	2
Stroke	3
Respiratory disease	4
Cancer of lung	5
Other cancer	6
Accident or injury	7
Other	8
Don't know	9

☐ 41

3. YOUR MOTHER

3.1 Where was your mother born?

Town ....

County ....

Country ....

3.2 Is your mother alive? (Y/N)

☐ 42

3.3 How old is she now? / How old was she when she died?

☐☐ 43

years

3.4 If your mother has died, what were you told was the cause of her death?

Heart trouble	1
High blood pressure	2
Stroke	3
Respiratory disease	4
Cancer of breast	5
Other cancer	6
Accident or injury	7
Other	8
Don't know	9

☐ 45

4. OCCUPATION

4.1 What is your present job? ....

If employed go to question 4.4

4.2 If you are unemployed, for how long has this been?

<6 weeks	1
6wk.-5mo.	2
6mo.-1yr.	3
>1 year	4

☐ 46

4.3 Is this because of ill health? (Y/N)

....

4.4 What kind of work have you done for the longest period

of time? ....

4.5 What business or industry is this?

....

4.6 How many years have you done this kind of work?

years

4.7 Are/were you:

SELF-EMPLOYED	with 25 or more employees	1
	with less than 25 employees	2
	without employees	3
MANAGER	of 25 or more people	4
	of less than 25 people	5
FOREMAN	....	6
ORDINARY EMPLOYEE	....	7
ARMED SERVICES	....	8

47

48

50

5. SEVERE CHEST PAIN

5.1 Have you ever had a severe pain in your chest lasting for
half an hour or more? (Y/N)
If NO, go to question 6.

51

5.2 Where did you get this severe pain?

(Show chart.)

52

55

5.3 Did you see a doctor because of this pain? (Y/N)

6. CHEST PAIN

6.1 Do you ever have any pain or discomfort in your chest? (Y/N)
If NO, go to question 7.

56

6.2 When last did you get the pain?

Within 1 month	1
1-5 months ago	2
6-12 months ago	3
Over 1 year ago	4
Occasionally	5

57

6.3 How often do you get it?

Daily	1	
Weekly	2	
Monthly	3	□ 58
Once only	4	
Occasionally	5	

6.4 Where do you get this pain or discomfort?

(Show chart.)

□
□ 59
□

6.5 When you walk at an ordinary pace on the level, does this produce the pain? (Y/N)

□ 62

6.6 When you walk uphill or hurry, does this produce the pain? (Y/N)

□ 63

6.7 When you get any pain or discomfort in your chest on walking, what do you do?

Stop	1	
Slow down	2	□ 64
Continue at the same pace	3	

6.8 Does the pain or discomfort in your chest go away if you stand still? (Y/N)

□ 65

6.9 How long does it take to go away?

10 minutes or less	1	□ 66
More than 10 minutes	2	

7. PHLEGM, COUGH AND BREATHING

7.1 Do you usually bring up phlegm (spit) from your chest first thing in the morning in the winter? (Y/N)
If NO, go to question 7.4.

□ 67

7.2 Do you bring up phlegm like this on most days for as much as 3 months in the winter each year? (Y/N)

□ 68

7.3 In the past 3 years have you ever had a period of increased cough and phlegm lasting 3 weeks or more?

Yes, once	1	
Yes, twice or more	2	□ 69
Never	3	

7.4 Does your chest sound wheezy or whistling on most days (or nights)? (Y/N)

□ 70

7.5 Does the weather affect your breathing?
   And if so, at what season of the year is it most affected?

Not affected	1
Winter	2
Summer	3
Both	4

71

8. <u>BREATHLESSNESS</u>

8.1 Do you get short of breath walking with people your own
   age on level ground? (Y/N)

72

8.2 On walking up hills or stairs, do you get more breathless
   than people your own age? (Y/N)

73

8.3 Do you ever have to stop walking because of breathlessness?
   (Y/N)

74

S.E.G.

75

Social Class

77

Activity Score

78

Serial Number ☐☐☐☐☐☐☐☐☐

Card Number `0` `2`

9. LEG PAIN

9.1 Do you ever get pain in your calf muscles on walking at an ordinary pace, on the level?   (Y/N) ☐ 11

9.2 Do you get pain in your calf muscles when you walk uphill or hurry?   (Y/N) ☐ 12

10. MEDICAL HISTORY

10.1 Have you ever been told by a doctor that you have, or have had, any of the following?

Angina	(Y/N)	13
Heart attack	(Y/N)	14
Coronary thrombosis	(Y/N)	15
Myocardial infarction	(Y/N)	16
Other heart trouble	(Y/N)	17
High blood pressure	(Y/N)	18
Stroke	(Y/N)	19
Diabetes	(Y/N)	20
Peptic ulcer	(Y/N)	21
Gout	(Y/N)	22
Gall bladder disease	(Y/N)	23
Thyroid disease	(Y/N)	24
Arthritis	(Y/N)	25
Bronchitis	(Y/N)	26
Asthma	(Y/N)	27
Other condition(s) including surgery	(Y/N)	28

10.2 Are you on any regular medical treatment from a doctor for any condition?   (Y/N) ☐ 29

If NO, go to question 10.3.

Do you know if the pills/medicines/injections are:-

Tranquillizers	(Y/N)	30
Pain killers	(Y/N)	31
Antihypertensive drugs	(Y/N)	32
Anticoagulants	(Y/N)	33
Lipid lowering drugs	(Y/N)	34

Oral antidiabetics	(Y/N)
Injection of insulin	(Y/N)
Any others	(Y/N)
Don't know	(Y/N)

35
36
37
38

10.3 Have you taken any of these in the last 48 hours?

Tranquillizers	(Y/N)
Pain killers	(Y/N)
Antihypertensive drugs	(Y/N)
Anticoagulants	(Y/N)
Lipid lowering drugs	(Y/N)
Oral antidiabetics	(Y/N)
Injection of insulin	(Y/N)
Any others	(Y/N)
Don't know	(Y/N)

39
40
41
42
43
44
45
46
47

11. DIET & ALCOHOL

11.1 How many times during an average week would you have the following foods?

Meat (including beef, lamb, pork, bacon in any form) 48

Chicken 50

Fish 52

Eggs - how many eggs do you eat in a week? 54

Cheese - how often do you eat cheese, including cheese dishes? 56

Breakfast cereals - how often do you eat these (porridge included)? State kind 58

11.2 What kinds of bread do you eat?

White	(Y/N)
Brown	(Y/N)
Wholemeal	(Y/N)
Other	(Y/N)

60
61
62
63

11.3 Spreading fats: What kinds do you use at home?

Butter	(Y/N)
Margarine	(Y/N)

64
65

(State kind or brand name.)

11.4 Do you take sugar?

In tea	(Y/N)
In coffee	(Y/N)
In other drinks	(Y/N)

66
67
68

11.5   Do you use milk?

On cereals	(Y/N)		69
In tea	(Y/N)		70
In coffee	(Y/N)		71
As a milk drink	(Y/N)		72

11.6   (i)   Would you describe your present alcohol intake as:

None	1
On special occasions only	2
Once or twice a month	3
Weekends	4
Daily/most days	5

73

If NONE, go to question 12.

(ii)   What type of drink do you usually take?

Beer	1
Spirits	2
Wine/sherry	3
Mixed beer & spirits	4
Mixed beer, spirits, wine and sherry	5

74

(iii) How much do you usually take?

2 drinks a day or less	1
3-6 drinks a day	2
More than 6 drinks a day	3

75

(One drink is a single whisky, gin or brandy, a glass of wine, sherry or port or half a pint of beer.)

P1 ADMINISTERED BY NURSE AT EXAM

P2 MAILED FOR SELF COMPLETION.
    WITH ADDED INFO ON NON DRINKERS

Serial Number ☐☐☐☐☐☐☐☐

Card Number | 0 | 3 |

12. SMOKING

12.1 (i) Do you smoke at present?

Yes, regularly 1
No 2
Occasionally 3

☐ 11

If NO, go to question 12.6.

(ii) How old were you when you started?

☐☐ 12
years

(iii) Have you ever given up smoking? (Y/N)

☐ 14

(iv) If yes, what is the maximum time for which you have given up smoking?

☐☐ 15
years

12.2 (i) Do you smoke cigarettes now?

Yes, regularly 1
No 2
Occasionally (<1 day) 3

☐ 17

If NO, or OCCASIONALLY, go to question 12.3.

(ii) How many cigarettes do you usually smoke a day?

☐☐ 18

(iii) If hand rolled, how much tobacco do you use a week? (ozs.)

☐☐ 20
ozs.

Now proceed to 12.4.

12.3 (i) Were you previously a regular cigarette smoker?(Y/N)

☐ 22

(ii) If yes, how many cigarettes did you usually smoke a day?

☐☐ 23

(iii) At what age did you change to a pipe and/or cigars?

☐☐ 25
years

12.4 (i) Do you smoke a pipe now?

Yes, regularly 1
No 2
Occasionally 3

☐ 27

If NO, or OCCASIONALLY, go to question 12.5.

(ii) If YES, how many ozs. a week do you smoke?

☐☐ 28
ozs.

12.5 (i) Do you smoke cigars now?

Yes, regularly 1
No 2
Occasionally 3

☐ 30

(ii) If YES, how many cigars do you smoke a day? Large

☐☐ 31

Small

☐☐ 33

If you smoke ANYTHING currently, go to question 13.

12.6   (i)   Have you ever smoked for more than 1 month?   (Y/N)   ☐   35

How much did you <u>usually</u> smoke

                                         Cigarettes (per day)   ☐☐   36

                                        Pipe (ozs) (per week)   ☐☐   38

                               Cigars (per day) Large   ☐☐   40

                                                 Small   ☐☐   42

        <u>If NO, go to question 13.</u>

(ii)   At what age did you start smoking?   ☐☐   44
                                                           years

(iii) At what age did you finally stop smoking?   ☐☐   46
                                                           years

(iv) What was the maximum time between these two ages   ☐☐   48
        for which you gave up smoking?   years

---

13.   <u>EXERCISE</u>

13.1   (i)   Do you usually walk or cycle in the course of your

               journeys <u>to or from</u> work each day?   No   1

                                           Walk   2   ☐   50

                                        Cycle 3

              If YES, how many minutes do these journeys take?   ☐☐   51
                                                           mins.

(ii)   Apart from your journeys to or from work, do you

         usually walk or cycle on weekdays?   No   1

                                         Walk   2   ☐   53

                                        Cycle 3

        If YES, how many minutes do you walk/cycle each day?   ☐☐   54
                                                     mins

(iii) Would you say that in your occupation you are

        physically: Very active   1

                                   Fairly active   2

                                   Average   3   ☐   56

                                   Fairly inactive 4

                                   Very inactive   5

13.2   On average, a man of your age spends 4 hours on most

weekends on some of the following activities: walking,

gardening, household chores, DIY projects.

Compared to such a man, how physically active do you

consider yourself?   Very active   1

                                   Fairly active   2

                                   Average   3   ☐   57

                                   Fairly inactive 4

                                   Very inactive   5

13.3 Apart from these activities, do you take **active** physical exercise, e.g. running, digging, swimming, tennis, golf, sailing, etc.

No   1

Occasionally 2   ☐   58

Frequently   3

If NO or OCCASIONALLY - stop here.

13.4 Please state type of activity ....

13.5 How many years have you been involved in this activity?   ☐☐   59

years

13.6 How many times a month (on average) do you undertake these activities?

Winter   ☐☐   61

Summer   ☐☐   63

Administrator   ☐   65

Coder   ☐   66

Physical and Physiological Measurements

Serial Number | | | | | | | | |

Card Number | 1 | 1 |

		Units	Obs. Measurement
	Height	mms.							11
	Weight	1/10 kgms.						16
(1)	Blood Pressure S_1	mmHg					20
	D_5						23
(2)	Blood Pressure S_1	mmHg					26
	D_5						29
(1)	FVC	ml					32
	FEV1						35
(2)	FVC	ml					38
	FEV1						41
	Pulse Rate	per min.						44

APPENDIX 2 RELEVANT PUBLICATIONS

Perry IJ, Wannamethee SG, Whincup PH, Shaper AG. Asymptomatic hyperglycaemia and major IHD events in Britain. *J Epidemiol Commun Health* 1994;48:538-42.

Perry IJ, Wannamethee SG, Walker MK, Thomson AG, Whincup PH, Shaper AG. A prospective study of risk factors for non-insulin-dependent diabetes in middle-aged British men. *Br Med J* 1995;310:560-64.

Perry IJ, Wannamethee G, Whincup PH, Shaper AG, Walker MK, Alberti KGMM. Serum insulin and incident coronary heart disease in middle-aged British men. *Am J Epidemiol* 1995 (*In press*).

Asymptomatic hyperglycaemia and major ischaemic heart disease events in Britain

Ivan J Perry, S Goya Wannamethee, Peter H Whincup, A Gerald Shaper

Abstract

Objective – To examine the association between non-fasting serum glucose concentrations and major ischaemic heart disease (IHD) events (fatal and non-fatal myocardial infarction).

Design – A prospective study.

Subjects – A population based sample of 7735 middle aged British men. Known diabetics, men with a glucose concentration $\geq 11 \cdot 1$ mmol/l at screening, and hypertensive patients taking regular medication were excluded from the analysis. With exclusions (n = 509) and missing glucose values (n = 49), there were 7177 men available for analysis.

Main outcome measures – Major IHD events (fatal and non-fatal myocardial infarction) during 9·5 years follow up on all men.

Results – There were 505 major IHD events, 222 fatal and 283 non-fatal, in the 7177 men studied. There was a non-linear relation between the glucose concentration and the risk (per 1000 men per year) of all major IHD events and fatal IHD events, with the excess risk in the upper quintile of the glucose distribution ($\geq 6 \cdot 1$ mmol/l). The unadjusted relative risks (RR) in the upper glucose concentration quintile compared with the first to the fourth quintiles combined were 1·4 (95% CI 1·1, 1·7) for all events and 1·3 (95% CI 1·0, 1·7) for fatal events. Adjustment for age, smoking, occupational status, body mass index, physical activity, systolic blood pressure, total and high density lipoprotein cholesterol, and triglyceride concentrations had a minimal effect on these relative risk estimates. This non-linear relationship between the serum glucose concentration and the risk of a major IHD event was observed in men with no evidence of IHD at screening (n = 5518) but not in men with IHD (n = 1659). In the former group, the RR (adjusted for major coronary risk factors) for all major IHD events in the upper quintile relative to the lower quintiles combined was 1·5 (95% CI 1·2, 2·0) and for fatal IHD events was 1·8 (95% CI 1·1, 2·6).

Conclusion – These data suggest that asymptomatic hyperglycaemia is an independent risk factor for major IHD events.

Non-insulin dependent diabetes mellitus is associated with an increased risk of ischaemic heart disease (IHD).[1] The association with IHD, as with other manifestations of diabetic large vessel disease, is only partly explained by the higher prevalence of vascular risk factors associated with diabetes, such as obesity, hypertension, and hyperlipidaemia.[2][3] Diverse explanations have been proposed for the excess of IHD in non-insulin dependent diabetes mellitus. These include direct atherogenic effects of glucose, the role of insulin resistence and hyperinsulinaemia as a unifying link between non-insulin dependent diabetes mellitus and IHD, and the possibility that these conditions share common genetic or environmental antecedents, or both.[4][5]

By contrast, data on the nature and form of the association between asymptomatic hyperglycaemia and IHD are inconsistent, with negative findings,[6] threshold effects,[7][8] and positive linear associations[9][10] reported. Many previous studies have lacked power to study the relationship between blood glucose and IHD. Most have documented fewer than 200 events, a relatively small number given the low reliability with which glucose is estimated.[11][12] Relatively few studies have adequately adjusted for the range of possible confounding factors. In no previous study of this association, has it been possible to adjust simultaneously for the high density lipoprotein (HDL) cholesterol concentration, serum triglycerides, and for the level of physical activity[13][14] – potential confounding factors that are of fundamental importance. In this study we examine the relation between asymptomatic hyperglycaemia and IHD in a population based sample of middle aged British men. There were over 500 major IHD events and we have adjusted for all major coronary risk factors including high density lipoprotein (HDL) cholesterol, triglycerides, and physical activity.

Methods

In the British Regional Heart Study, 7735 men aged 40 to 59 years were selected at random from the age-sex registers of one group general practice in each of 24 towns in England, Wales, and Scotland, and examined between 1978 and 1980. The criteria for selecting the towns, general practices, and subjects and details of the respondents, data collection, and measurement of serum glucose and lipids have been described.[15-18] The overall response rate was 78%. There were 49 men with missing glucose data. Known diabetics at screening (n = 118) and hypertensives undergoing treatment (n = 362) were excluded from the analysis at the outset, the latter in view of the adverse effect of antihypertensive therapy, in particular thiazide di-

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uretics, on plasma glucose concentrations.[19] A group of 29 men with a serum glucose concentration $\geq 11\cdot1$ mmol/l at screening was also excluded, given the high probability of undiagnosed diabetes in this group. Hence, the data upon which the primary analyses were performed, refers to a total of 7177 men. In a subsidiary analysis (to minimise the possibility that men with undiagnosed diabetes were included in the study), we excluded an additional group of 100 men who developed non-insulin dependent diabetes over the subsequent 9·5 years of follow up. Cases of non-insulin dependent diabetes mellitus were ascertained by means of a questionnaire sent to the men at year 5 of follow up and by systematic periodic review of practice records.[2]

Non-fasting blood samples were obtained between 08.30 and 18.30. Serum glucose was analysed by commercially available automated analyser (Technicon SMA 12/60) and the time of sampling was noted.[17] Less than 1% of the intersubject variance in the serum glucose concentration was attributable to the time of sampling.[21]

PHYSICAL ACTIVITY

A physical activity index, which is predictive of major cardiovascular end points,[13] was derived from an exercise questionnaire administered at the initial visit. Based on this index, the men were grouped into six broad physical activity categories: inactive (n = 602), occasional (n = 2133), light (n = 1646), moderate (n = 1131), moderately vigorous (n = 1067), and vigorous (n = 503). Physical activity data were missing for 95 men. Men whose level of activity was moderate or higher were characterised as physically active.

PREVALENT IHD

The men were asked whether a doctor had ever told them that they had angina or myocardial infarction (heart attack, coronary thrombosis), stroke, and a number of other disorders. The (WHO) Rose questionnaire was administered to all men at the initial examination and a three-orthogonal lead ECG was recorded at rest. Prevalent IHD at screening was defined on the basis of any or all of the following criteria: recall of doctor diagnosis of angina or heart attack, a Rose questionnaire response indicating angina or possible myocardial infarction, and ECG evidence of definite or possible myocardial ischaemia or infarction.[22 23]

FOLLOW UP

Over 99% of study participants have been followed for morbidity and mortality for 9·5 years. Full details of follow up procedures have been published and the criteria for fatal and nonfatal major IHD events have been described.[15 20] Major IHD events refer to fatal and non-fatal myocardial infarction. Information on death was obtained through the established "tagging" procedures provided by the National Health Service registers in Southport (England and Wales) and Edinburgh (Scotland). A non-fatal myocardial infarction was diagnosed according to WHO criteria – that is, an event which satisfied at least two of the following criteria: (a) preceded by severe prolonged chest pain, (b) ECG evidence of myocardial infarction, (c) cardiac enzyme changes associated with myocardial infarction. Fatal events were defined as deaths from IHD (*International Classification of Disease* 9th revision: codes 410–414) as the underlying cause. After 9·5 years follow up on all study participants there had been 505 major IHD events, 222 fatal and 283 non-fatal. Individuals who had both a non-fatal and fatal myocardial infarction over the follow up period were classified as having had a fatal event.

STATISTICAL ANALYSIS

The risk of major IHD events was examined by quintile of serum glucose, with adjustment for confounding factors by fitting the Cox proportional hazards model. Age, body mass index, systolic blood pressure, total cholesterol, HDL cholesterol, and triglyceride concentration were fitted as continuous variables in the model. As data on triglyceride concentrations were not available for six towns, the analysis in which we adjusted for this factor was confined to a group of 5307 men. Social class was fitted as six dummy variables (seven social class groups), physical activity as five variables (six categories), alcohol as four variables (five categories, none, occasional, light, moderate, heavy), and smoking as four dummy variables (five groups, never, ex-smokers, light, moderate, and heavy).

We adjusted the IHD-glucose relation for confounding factors in three stages. Initially we adjusted for age, then body mass index, physical activity, smoking status, alcohol intake, and occupational status were added and in the third stage, systolic blood pressure, total cholesterol, HDL cholesterol, and triglycerides were added to the model. As there was an interaction with prevalent IHD at screening, men with IHD (n = 5518) and without IHD (n = 1659) at the baseline examination were considered separately in the principal analysis.

Results

A non-linear relation was observed between the glucose concentration and the risk (per 1000 men per year) of all major IHD events and fatal IHD events. There was a significant increase in risk at or above a serum glucose concentration of 6·1 mmol/l (80th centile). The unadjusted relative risk (RR) in the upper glucose quintile compared with the first to the fourth quintiles combined was 1·4 (95% CI 1·1, 1·7) for all events and 1·3 (95% CI 1·0, 1·7) for fatal events (table 1).

The distribution of major coronary risk factors was examined in the upper quintile relative to the other four quintiles. Significantly more men whose serum glucose was in the upper quintile were engaged in manual occupations and a higher proportion were overweight or obese (body mass index ≥ 28 kg/m^2) (table 2).

Table 1 *Rate of ischaemic heart disease (IHD) events (all events and fatal events, unadjusted) per 1000 men per year by quintile of serum glucose concentration (nonfasting)*

Glucose (mmol/l)	No of men	All events		IHD deaths	
		No of events	Rate (%)	No of deaths	Rate (%)
<4·8	1413	96	7·2	45	3·4
4·9–5·2	1701	116	7·2	56	3·5
5·3–5·5	1221	80	6·9	43	3·7
5·6–6·0	1407	85	6·4	24	1·8
≥6·1	1435	128	9·4	54	4·0

Table 2 *Distribution of cardiovascular risk factors (mean (SD) or %) in the first of the fourth glucose quintiles combined compared with the fifth glucose quintile*

	Glucose <6·1 mmol/l	Glucose ≥6·1 mmol/l
No of men	5742	1464
Glucose* (mmol/l)	5·1	7·0
Age (y)	49·9 (5·8)	50·6 (5·8)
Current smokers† (%)	42·6	37·5
Manual occupation† (%)	57·0	63·8
Obese† (%)	17·5	22·7
Active (%)	38·0	38·5
Moderate drinkers§ (%)	26·6	28·1
Systolic BP† (mmHg)	142·8 (19·8)	150·7 (21·7)
Diastolic BP (mmHg)	81·6 (12·8)	82·0 (13·7)
Cholesterol (mmol/l)	6·29 (1·04)	6·28 (1·04)
HDL-cholest† (mmol/l)	1·16 (0·26)	1·13 (0·27)
Triglyceride*† (mmol/l)	1·67	1·93
Evidence of IHD‡ (%)	22·3	25·3

* Geometric mean.
† p<0·001: ‡ p<0·01.
§ Daily 3–6 units of alcohol and weekend >6 units.

Table 3 *Relative risk (RR) of major ischaemic heart disease events in the upper quintile of serum glucose (n = 1435) compared with the first to fourth quintiles combined*

Adjusted for	All infarcts RR (95% CI)	Fatal infarcts RR (95% CI)
Age (y)	1·31 (1·1, 1·6)	1·21 (0·9, 1·6)
Age, smoking, alcohol intake, occupational status, physical activity, and body mass index	1·34 (1·1, 1·6)	1·27 (0·9, 1·7)
Above + systolic blood pressure, total cholesterol, and HDL cholesterol	1·29 (1·06, 1·6)	1·26 (0·9, 1·7)

**Table 4** *Relative risk (RR) of major ischaemic heart disease events in the upper quintile of serum glucose (n = 1074) compared with the first to fourth quintiles combined, in men without evidence of ischaemic heart disease at the baseline examination*

Adjusted for	All infarcts RR (95% CI)	Fatal infarcts RR (95% CI)
Age (y)	1·60 (1·3, 2·1)	1·68 (1·1, 2·5)
Age, smoking, alcohol intake, occupational status, physical activity, and body mass index	1·68 (1·3, 2·2)	1·88 (1·3, 2·8)
Above + systolic blood pressure, total cholesterol, and HDL cholesterol	1·54 (1·2, 2·0)	1·75 (1·1, 2·6)

Major ischaemic heart disease event rate in relation to serum glucose concentration in men with and without ischaemic heart disease at screening.

There were significantly fewer smokers in the upper glucose quintile, the systolic blood pressure and triglyceride level were significantly higher and the HDL cholesterol concentration was significantly lower. The proportion of physically active men and the proportion of moderate drinkers were similar in the two groups. The prevalence of IHD at the initial examination was significantly higher in the upper glucose quintile. Differences in the distribution of blood pressure and serum lipids and in the prevalence of IHD between the upper and four lower serum glucose quintiles remained significant after adjustment for age, occupational status, and body mass index (BMI).

After adjustment for the major coronary risk factors, the increased risk for all myocardial infarction and for fatal infarction in the upper glucose quintile was minimally attenuated and remained significant for all events (table 3).

The non-linear relationship between the glucose concentration and the risk of IHD observed in the entire group, was clearly evident in men free of IHD (n = 5518), but not in those with evidence of IHD at screening (figure). In those without IHD at baseline, the age adjusted RRs in the upper glucose quintile relative to the rest were 1·6 (95% CI 1·3, 2·1) for all major events and 1·7 (95% CI 1·1, 2·5) for all fatal events (table 4). These effects were not attenuated by adjustment for smoking, social class, level of physical activity, and BMI. Further adjustment for systolic blood pressure and total and HDL cholesterol had a minimal effect on the association between glucose and IHD (table 4). No relationship between glucose and the risk of subsequent myocardial infarction was observed in men with pre-existing IHD at screening.

There was a significant positive correlation between serum glucose and triglyceride concentrations (r = 0·12, p<0·0001). The triglyceride concentration, however, is not an independent predictor of IHD events in this study once adjustment for blood cholesterol has been carried out.[24] Hence, adjustment for the triglyceride concentration did not attenuate the increased IHD risk observed in the upper glucose quintile in the entire group or in men without evidence of IHD at screening.

We detected no evidence of a further increase in the risk of IHD at the 90th or 95th glucose centiles. The glucose-IHD association was unaltered when the 100 men known to have developed non-insulin-dependent diabetes during the 9·5 years of follow up were excluded. In this analysis, the age adjusted RRs for all events in the upper glucose quintile relative to the rest were 1·31, (95% CI, 1·1, 1·6) in the entire group and 1·60 (95% CI, 1·2, 2·1) in men free of IHD at screening.

Discussion

We have observed a non-linear relationship between non-fasting glucose and the risk of major IHD events in a population based sample of middle aged British men. This association was observed only in men free of IHD at the screening examination. In the latter there was a substantially increased risk of major IHD events in the upper serum glucose quintile which was independent of major cardiovascular risk factors, including physical activity and lipid

levels. In particular, the association was unaltered on adjustment for the HDL cholesterol concentration. HDL cholesterol concentrations are consistently low in subjects with abnormal glucose tolerance, both non-insulin dependent diabetes and impaired glucose tolerance,[25] and it has been suggested that confounding due to this factor might explain the association between asymptomatic hyperglycaemia and IHD.[3] Hence these findings provide further evidence of the fundamental role of abnormalities of glucose homeostasis in the development of atherosclerosis, and they highlight the need for an integrated approach to the prevention of IHD and glucose intolerance.

Non-fasting blood samples were obtained in this study to ensure a high response rate from this uniquely representative, population based sample. Though fasting samples or those taken after glucose load would have provided a more precise and reliable measure of glycaemic status, it may be argued that such imprecision will attenuate rather than exaggerate the association with IHD events which we have described.

We considered the possibility that the increased risk of IHD observed in the upper glucose quintile might have resulted from the inclusion of undiagnosed diabetics, in whom an increased risk of heart disease would be expected. This is unlikely, given that we excluded men with glucose $\geq 11 \cdot 1$ mmol l at screening from the study and that the findings were unaltered in an analysis in which we also excluded men who developed non-insulin-dependent diabetes over the subsequent 9·5 years of follow up. No single measure of glycaemic status employed in an epidemiological study, whether casual, fasting, or after glucose load, is without error. Hence a degree of misclassification of diabetic status is inevitable. We would argue, however, that in a group of men with a casual glucose concentration <11·1 mmol l, none of whom was subsequently diagnosed as diabetic over 9·5 years of follow up, the number of undiagnosed diabetics will be small and will not alter the findings from a study of this size. The stability of the RR estimates after exclusion of the 100 men who developed non-insulin dependent diabetes mellitus during the follow up, supports this argument. Moreover, if the excess of IHD events observed at the 80th centile (a serum glucose concentration $\geq 6 \cdot 1$ mmol/l) was due to misclassification of undiagnosed diabetics, one would expect to find evidence of a further increase in risk at higher glucose levels, such as at the 90th or 95th centiles where the risk of subsequent non-insulin dependent diabetes mellitus (and presumably the prevalence of undiagnosed diabetes) rises sharply. No evidence of a further increase in risk at these higher glucose concentrations was found.

In the International Collaborative Group report, no significant association between glucose quintile and IHD mortality was detected in eight of eleven studies of middle aged men in eight countries, and in only one study was a strong independent association observed.[6] Protocols and methods for assessing glycaemia varied considerably between these studies and

in all but two studies there were fewer than 200 IHD events.

In the 10 year follow up data from the Whitehall study, there was clear evidence of a threshold effect, with an approximately twofold increased risk of IHD observed in the upper 5% of the post-load glucose distribution. This effect was independent of major cardiovascular risk factors, including age, obesity, blood pressure, smoking, cholesterol level, and ECG abnormalities.[7] A similar threshold effect of the post load-glucose level on IHD mortality was reported from the Paris Prospective Study data.[8] By contrast, Barrett-Connor *et al.* reported a continuous, independent relationship between fasting plasma glucose and IHD mortality in men aged 40–79 years in a southern California community.[9] Similarly, in the Honolulu Heart Program data a linear relationship between glucose concentration one hour after glucose load and IHD mortality was observed.[10] Other major studies reported in the last decade include the Tecumseh study in which at 18 years follow up there was a weak linear relation between asymptomatic hyperglycaemia and IHD in men (142 deaths) but not in women (71 deaths).[26]

Not all studies reported since the collaborative group analysis support an independent association between asymptomatic hyperglycaemia and IHD. In the Chicago Heart Association Detection Project data, asymptomatic hyperglycaemia (after glucose load) was not an independent predictor of IHD mortality (286 events) at nine years follow up in 11 220 middle aged men.[27] An independent association of borderline significance was observed in women. In the Bedford study, borderline hyperglycaemia after glucose challenge was predictive of IHD mortality only among women.[28] In 12 year data from the Gothenburg study, however, no association between the fasting blood glucose concentration and IHD end points was detected in a group of 1462 women aged 38 to 60 years with 28 events.[29]

Factors influencing mortality and event rates are likely to differ in men with and without prevalent IHD at screening. Most previous studies have excluded men with ECG evidence of myocardial infarction[6] or have adjusted for this factor in multivariate analysis.[7] Differences in exclusion criteria for this factor may account for some of the inconsistency in current data. Additional factors of relevance in this context include the low reliability with which glucose is measured, the variable follow up periods, and, as discussed, the relatively small number of events and deaths in many of the previous studies.

In previous work we have reported a graded, inverse relation between the level of physical activity and subsequent major IHD events.[14] We have also shown in cross sectional data from the British Regional Heart Study, that physical activity is inversely associated with the serum glucose concentration.[30] One might therefore anticipate that physical activity would be an important confounder in the relation between glucose and IHD. In the British Regional Heart Study data, however, the effect of

physical activity on serum glucose is observed only among those engaged in relatively high levels of activity[ ] and is exerted predominately on those men with a non-fasting glucose level ≥ 7.8 mmol/l – that is, at or above the 96th rather than the 80th centile).

The basis for this independent association between hyperglycaemia and IHD remains obscure, though it is likely that a number of different mechanisms, both direct and indirect, are involved. Mechanisms proposed to date include increased activity of the polyol pathway, glycosylation of tissue proteins, glycosylation and altered catabolism of lipoproteins, and abnormalities of platelet function and the coagulation system.[5,31] It may be that hyperglycaemia is simply a marker for more fundamental abnormalities in carbohydrate and lipid metabolism, centered on insulin resistance with hyperinsulinaemia.[32] Thus in the Paris Prospective Study data, glucose was not an independent predictor of IHD after adjustment for plasma insulin.[33] Alternatively, it is suggested that hyperglycaemia and non-insulin dependent diabetes mellitus may be linked to IHD via common genetic antecedents[34] or perhaps via intrauterine or early environmental factors.[35]

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Prospective study of risk factors for development of non-insulin dependent diabetes in middle aged British men

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Abstract

Objective—To determine the risk factors for non-insulin dependent diabetes in a cohort representative of middle aged British men.

Design—Prospective study.

Subjects and setting—7735 men aged 40-59, drawn from one group practice in each of 24 towns in Britain. Known and probable cases of diabetes at screening (n = 158) were excluded.

Main outcome measures—Non-insulin dependent diabetes (doctor diagnosed) over a mean follow up period of 12·8 years.

Results—There were 194 new cases of non-insulin dependent diabetes. Body mass index was the dominant risk factor for diabetes, with an age adjusted relative risk (upper fifth to lower fifth) of 11·6; 95% confidence interval 5·4 to 16·8. Men engaged in moderate levels of physical activity had a substantially reduced risk of diabetes, relative to the physically inactive men, after adjustment for age and body mass index (0·4; 0·2 to 0·7), an association which persisted in full multivariate analysis. A non-linear relation between alcohol intake and diabetes was observed, with the lowest risk among moderate drinkers (16-42 units/week) relative to the baseline group of occasional drinkers (0·6; 0·4 to 1·0). Additional significant predictors of diabetes in multivariate analysis included serum triglyceride concentration, high density lipoprotein cholesterol concentration (inverse association), heart rate, uric acid concentration, and prevalent coronary heart disease.

Conclusion—These findings emphasise the inter-relations between risk factors for non-insulin dependent diabetes and coronary heart disease and the potential value of an integrated approach to the prevention of these conditions based on the prevention of obesity and the promotion of physical activity.

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Introduction

Non-insulin dependent diabetes is a common condition affecting at least 3% of the middle aged and elderly population of Britain, with a considerably higher prevalence in specific ethnic groups.[1] Advancing age, obesity, upper body fat distribution, and a family history of diabetes are among the well established risk factors for this condition.[2] Evidence is increasing that in some populations non-insulin dependent diabetes shares common causal factors with cardiovascular disease and in particular with coronary heart disease.[3]

An inverse relation between physical activity level and the risk of subsequent non-insulin dependent diabetes (reported by patients to have been diagnosed by a doctor), has been described in prospective studies from selected populations.[4-6] Data on potential confounding or mediating factors in these studies have been relatively limited. Prospective studies on the role of alcohol in the development of non-insulin dependent diabetes have produced contradictory findings.[7-9] Cigarette smoking has not been extensively investigated as a risk factor for diabetes. Smokers were at higher risk of non-insulin dependent diabetes over 25 years of follow up in the Zutphen study,[10] and evidence exists that cigarette smoking leads to insulin resistance.[11] Resistance to insulin mediated glucose uptake (insulin resistance) antedates non-insulin dependent diabetes[12] and is linked with dyslipidaemia, hypertension, and several other risk factors for coronary heart disease.[13 14]

We report on a prospective study of risk factors for non-insulin dependent diabetes among men recruited for the British regional heart study. We have focused on factors that have been linked with coronary heart disease, such as body mass index, physical activity, alcohol intake, cigarette smoking, and established biological risk factors for coronary heart disease, such as dyslipidaemia and hypertension.

Subjects and methods

In the British regional heart study 7735 men aged 40 to 59, were selected at random from the age-sex register of one general practice in each of 24 towns in England, Wales, and Scotland between January 1978 and June 1980 for a prospective study of cardiovascular disease. The criteria for selecting the towns, general practices, and subjects and methods of data collection have been described.[15 16] Men with cardiovascular or other disease or those receiving regular drug treatment were not excluded. The overall response rate was 78%, ranging from 70% to 85% across the 24 towns. Known diabetic subjects (n = 121), men diagnosed within the calendar year in which they were screened (n = 14), and those with non-fasting glucose concentrations in the diabetic range (≥11·1 mmol/l, n = 23) were excluded. Hence the analysis was based on 7577 men.

DATA COLLECTION: BASELINE ASSESSMENT

Research nurses administered a standard questionnaire and completed an examination of each man, including electrocardiography.[17] The questionnaire included questions on occupation, the usual pattern of physical activity, alcohol intake, smoking habits, medical history, and use of drugs, including antihypertensive drugs.[15-19]

Physical activity—A physical activity score was derived, based on the frequency and intensity of the activities reported.[18] The men were grouped into six physical activity categories: inactivity (n = 664), occasional activity (n = 2282), light activity (n = 1734), moderate activity (n = 1181), moderately vigorous activity (n = 1104), and vigorous activity (n = 510). Data were not available for 102 men.

Alcohol intake—The men were classified into five groups according to their current alcohol intake: none (n = 451), occasional (<1 unit/week; n = 1809), light (1-15 units/week; n = 2490), moderate (16-42 units/week; n = 2006) and heavy (>42 units/week; n = 815). Data were not available for six men.[19]

Cigarette smoking—The men were categorised as those who had never smoked (n = 1787), former

smokers (n=2649), and current smokers, (n=3125), with the latter group further subdivided by the number of cigarettes smoked daily. Data were not available for 16 men.[18]

Prevalent coronary heart disease—With the World Health Organisation's Rose chest pain questionnaire and an electrocardiogram prevalent coronary heart disease at screening was defined on the basis of any of the following crtiteria: recall of a doctor diagnosing angina or heart attack, a response to the WHO's Rose chest pain questionnaire indicating angina or possible myocardial infarction, or electrocardiographic evidence of definite or possible myocardial ischaemia or infarction.[17] In all, 24% (1834) of the men were characterised as having prevalent coronary heart disease at screening. This group did not include men who reported a history of non-specific "other heart disease."

Body mass index calculated as weight (kg)/(height (m)^2) was used as an index of relative weight. Men with a body mass index of ≥ 28 were categorised as obese. Blood pressure was recorded with a London School of Hygiene sphygmomanometer. Two successive recordings were taken, and the mean was used in the analysis with adjustment for interobserver variation. Heart rate was determined from the electrocardiogram.

Non-fasting blood samples were obtained between 8 30 am and 6 30 pm.[20] Glucose, total cholesterol, and uric acid concentrations were analysed in serum with an automated analyser (Technicon SMA 12/60).[20-21] Diurnal variation in glucose concentrations was modest, with a peak-trough difference of 0·4 mmol/l.[20] High density lipoprotein cholesterol and triglyceride concentrations were measured with enzymatic methods.[20] As triglyceride concentrations were not determined for men in the first six towns, data on this variable were available for only 5327 men.
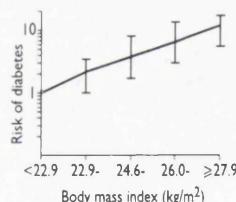
FOLLOW UP FOR DEVELOPMENT OF NON-INSULIN DEPENDENT DIABETES

The men were followed for morbidity and mortality up to December 1991, a mean period of 12·8 years.[22] Less than 1% (73) of men were lost to follow up, of whom 44 (0·6% of total) emigrated from Britain. New cases of non-insulin dependent diabetes were ascertained by means of (a) a postal questionnaire sent to the men at year 5 of follow up for each individual, (b) systematic reviews of primary care records in 1990 and 1992, (c) a further questionnaire to 6483 surviving members of the cohort resident in Britain in 1992, and (d) review of all death certificates for any mention of

diabetes. The questionnaire at year 5 achieved a response rate of 98%[22] and on the 1992 questionnaire a response rate of 91%. A diagnosis of diabetes was not accepted on the basis of questionnaire data unless confirmed in the primary care records.

STATISTICAL ANALYSIS

Cox's proportional hazards models were used to assess the independent contributions of the risk factors to the subsequent risk for non-insulin dependent diabetes and to obtain the relative risks adjusted for the other risk factors.[23]

Physical activity (six levels), smoking (three levels), alcohol intake (five levels), and pre-existing ischaemic heart disease (yes/no) were fitted as categorical variables in the proportional hazards model. The adjusted relative risks in figures 1 and 3 were obtained by fitting body mass index, systolic and diastolic blood pressure, heart rate, and concentrations of high density lipoprotein cholesterol, triglyceride, and uric acid as four dummy variables for the five equal divisions of each risk factor. Tests for trend were carried out by fitting the quantitative variables in their continuous form. For table I analysis of covariance was used to derive the means adjusted for age and body mass index, and logistic regression was used to calculate prevalences adjusted for age and body mass index on the basis of conversion of adjusted odds ratios to estimated proportions. For tables II and III the validity of the proportional hazards assumption in Cox's models was checked by fitting a time dependent interaction variable $x=x(t)$, where $x(t) = \log(t)$. Subjects with missing values for covariates in the various adjustments with Cox's model were excluded from that particular analysis.

As glucose and triglyceride concentrations were not normally distributed log transformation and geometric means were used. Because of the pronounced diurnal variation in serum triglyceride concentrations[20] the log transformed data on this variable were adjusted for time of sampling.[24]

Results

After a mean follow up period of 12·8 years there were 194 new cases of non-insulin dependent diabetes in the 7577 men, an incidence of 2·15 per 1000 person years of follow up. Men who developed diabetes had significantly higher mean blood glucose concentrations at screening than those who remained free of diabetes (6·2 v 5·4 mmol/l; P<0·0001). Little difference in mean age existed between the two groups (50·4 v 50·2). Those who developed diabetes had a significantly higher mean body mass index than those who did not (27·9 v 25·4; P<0·0001). Forty four per cent (85/194) of the men who developed diabetes were obese (body mass index of ≥ 28) compared with 18% (1328/7383) of those who did not. The risk of non-insulin dependent diabetes increased exponentially with increasing body mass index, with an over 11-fold excess risk in the upper fifth ($\geq 27·9$) relative to the lower fifth ($\leq 22·9$) (relative risk=11·6; 95% confidence interval 5·4 to 16·8) (fig 1).

Table I shows the baseline characteristics of the men who subsequently developed diabetes compared with the rest of the cohort, adjusted for age and body mass index. No adjustments were made for serum glucose concentration, which is assumed to be the causal pathway between the relevant exposures and non-insulin dependent diabetes. Significantly fewer of the men who developed diabetes compared with those who did not were moderate drinkers and physically active. Those who developed diabetes were more likely to be smokers (P=0·06), and they had a significantly higher prevalence of pre-existing coronary heart disease, but

FIG 1—*Relative risk of non-insulin dependent diabetes (log scale) adjusted for age with 95% confidence intervals, by fifth of body mass index relative to the lower fifth*

Risk of diabetes (y axis)

<22.9 22.9- 24.6- 26.0- ≥27.9
Body mass index (kg/m^2)

TABLE I—*Baseline values of selected variables (adjusted for age and body mass index) in 7577 middle aged men initially free of diabetes, by incidence of non-insulin dependent diabetes during a mean follow up of 12·8 years*

Variable	Men who did not develop diabetes during follow up (n=7383)	Men who developed diabetes during follow up (n=194)	P value
From logistic regression*:			
Manual occupation	57	56	
Current smokers	41	48	0·06
Moderate drinkers†	27	20	0·05
Physically active‡	37	28	0·01
Evidence of coronary heart disease	24	32	0·01
Other heart disease	5·9	8·5	
Treatment with antihypertensive drugs	4·7	6·7	
Any regular drug treatment	28	32	
From analysis of covariance§:			
Heart rate (beat/min)	70·6 (0·15)	73·2 (0·90)	0·005
Systolic blood pressure (mm Hg)	145·0 (0·23)	148·2 (1·43)	0·03
Diastolic blood pressure (mm Hg)	82·2 (0·14)	84·10 (0·90)	0·04
Total cholesterol (mmol/l)	6·30 (0·01)	6·34 (0·07)	
High density lipoprotein cholesterol (mmol/l)	1·15 (0·003)	1·11 (0·02)	0·004
Triglyceride (mmol/l)‖	1·73	2·16	<0·0001
Uric acid (μmol/l)	359·1 (0·8)	370·5 (4·8)	0·02

*Values are estimated percentages of men (see methods section).
†16-42 Units/week.
‡Level of activity was moderate or higher.
§Values are means (SE) (see methods section).
‖Geometric mean (5197 v 130).

Risk of diabetes

10 ⌐ (a)

1

None Occasional Light Moderate Moderately vigorous Vigorous

Physical activity

10 ⌐ (b)

Risk of diabetes

1

None Occasional Light Moderate Heavy
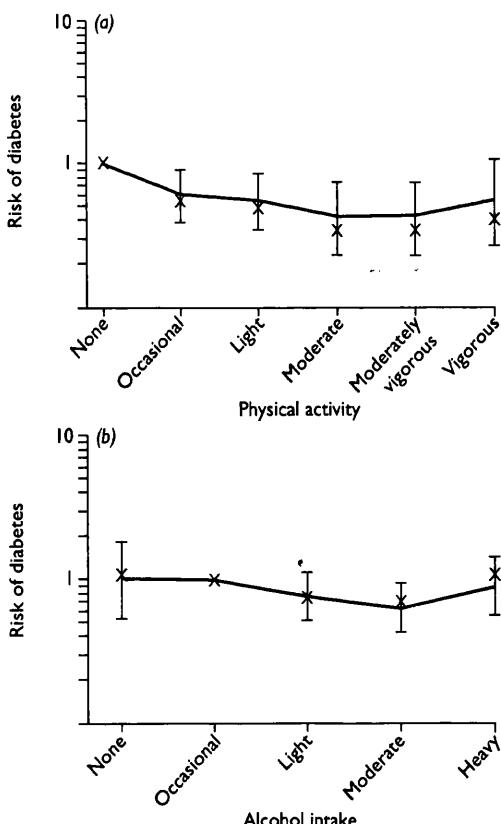
Alcohol intake

FIG 2—*Relative risk of non-insulin dependent diabetes (log scale) adjusted for age and body mass index with 95% confidence intervals, by (a) physical activity level and (b) alcohol intake. The x shows each relation adjusted for age alone. The lowest physical activity group (inactive) forms baseline group for this variable, and occasional drinkers form baseline group for alcohol intake*

10.0 ⌐

Risk of diabetes

1.0

<128   128-   138-   148-   ≥161

Systolic blood pressure (mm Hg)

10.0 ⌐

Risk of diabetes

1.0

<72   72-   78-   85-   ≥93

Diastolic blood pressure (mm Hg)

10.0 ⌐

Risk of diabetes

1.0

<0.93   0.93-   1.06-   1.18-   ≥1.33

High density lipoprotein cholesterol (mmol/l)

10.0 ⌐

Risk of diabetes

1.0

<1.08   1.08-   1.49-   1.99-   ≥2.80

Triglyceride (mmol/l)

10.0 ⌐

Risk of diabetes

1.0

<302   302-   338-   372-   ≥412

Uric acid (μmol/l)

<60   60-   67-   73-   ≥81

Heart rate (beats/min)

FIG 3—*Relative risk of non-insulin dependent diabetes (log scale) adjusted for age and body mass index with 95% confidence intervals, by fifths of systolic and diastolic blood pressure, heart rate, and concentrations of high density lipoprotein cholesterol, triglyceride, and uric acid relative to lower fifth of each variable. The x shows each relation adjusted for age alone*

not of "other heart disease." No differences existed in social class status and in the use of antihypertensive drugs or other medication. The men with diabetes had significantly higher systolic and diastolic blood pressure, heart rate, and triglyceride and uric acid concentrations and significantly lower high density lipoprotein cholesterol concentrations. No significant difference in mean total cholesterol concentration existed between the men with diabetes and those without.

PHYSICAL ACTIVITY, ALCOHOL INTAKE, AND CIGARETTE SMOKING

Figure 2 shows the relative risk of non-insulin dependent diabetes with 95% confidence intervals at different levels of physical activity and alcohol intake, adjusted for age and separately for age and body mass index. The risk of diabetes progressively decreased with increasing levels of physical activity up to moderate activity (0·4; 0·2 to 0·7) relative to the inactive group. A shallow "U shaped" relation was seen between alcohol intake and the risk of diabetes, with the lowest risk seen in moderate drinkers (0·64; 0·43 to 0·96) relative to the baseline group of occasional drinkers. Current smoking was associated with a 50% increase in the risk of diabetes relative to those who had never smoked, adjusted for age and body mass index, (1·5; 1·0 to 2·2; P=0·04). No association with the number of cigarettes smoked was detected. Former smokers were at similar risk of diabetes as men who had never smoked (1·2; 0·8 to 1·8).

ADDITIONAL RISK FACTORS FOR NON-INSULIN DEPENDENT DIABETES

Men with evidence of coronary heart disease at screening showed a 50% increase in the risk of developing diabetes on adjustment for age and body mass index (1·53, 1·13 to 2·07). Figure 3 shows the relative risks of diabetes, with 95% confidence intervals, by fifths of systolic and diastolic blood pressure, heart rate, and concentrations of high density lipoprotein cholesterol, triglyceride, and uric acid, adjusted for age and separately for age and body mass index. The positive association seen between systolic blood pressure and the age adjusted risk of diabetes was appreciably attenuated on adjustment for body mass index, although the trend remained significant (P=0·02). A similar though weaker association was seen for diastolic blood pressure. For heart rate the risk of diabetes was appreciably increased in the top fifth relative to the bottom fifth (adjusted relative ratio=2·9; 1·7 to 4·9). A significant inverse association was seen between high density lipoprotein cholesterol concentration and risk of diabetes after adjustment for age and body mass index (trend P=0·004). A particularly strong positive association was seen between triglyceride concentration and risk of diabetes after adjustment for age and body mass index (trend P<0·0001), with a more than threefold increased risk in the top fifth relative to the bottom fifth. A positive association with serum uric acid concentrations was also observed (fig 3).

MULTIVARIATE ANALYSIS

Table II shows the findings from multivariate analysis of independent predictors of diabetes. There was an over sevenfold increased risk of diabetes in the upper fifth of body mass index relative to the lowest in multivariate analysis, and the strong, linear inverse association with physical activity was not attenuated. The associations with moderate drinking, pre-existing coronary heart disease, heart rate, and concentrations of high density lipoprotein cholesterol and uric acid remained significant. The associations with current

smoking and systolic blood pressure were no longer significant in this multivariate model.

Variables which emerged as significant predictors of diabetes in the multivariate model presented in table II were further adjusted for triglyceride concentration in a separate model based on data from the subgroup of 5327 men with data on serum triglyceride concentration (table III). No association between high density lipoprotein cholesterol concentration and the risk of diabetes was observed on adjustment for triglyceride concentration, and the association with heart rate was attenuated.

EFFECT OF PRE-EXISTING CORONARY HEART DISEASE

The factors shown to be independently associated with diabetes—namely, body mass index, physical activity, alcohol intake, and uric acid and triglyceride concentrations—were also examined separately in men without pre-existing coronary heart disease (n=3981 men; 83 cases of diabetes) and in men with pre-existing coronary heart disease (n=1346 men; 47 cases) at baseline. This analysis was confined to the subset of men with available data on triglyceride concentration. Similar associations were seen in both groups between body mass index, physical activity, triglyceride and uric acid concentrations, and non-insulin dependent diabetes. The lower risk of diabetes seen in moderate drinkers was more apparent in men with pre-existing coronary heart disease than in men without evidence of coronary heart disease at screening, but a formal test for interaction was not significant.

In a separate analysis in which we excluded men with pre-existing coronary heart disease and men with a diagnosis of either hypertension or stroke at screening (n=3574, 69 cases) these findings were unchanged.

Discussion

In this study we have shown that obesity and physical inactivity are important independent risk

TABLE II—*Predictors of non-insulin dependent diabetes in multivariate analysis. Analysis includes 7097 men, 178 cases with data on all covariates in table*

Variable*	Adjusted relative risk (95% confidence interval)	P value†
Body mass index‡	7·3 (3·4 to 15·6)	0·0001
Prevalent coronary heart disease (yes/no)	1·4 (1·0 to 1·9)	0·04
Physical activity (moderate v inactive)	0·4 (0·2 to 0·8)	0·003
Alcohol intake (moderate v occasional)	0·6 (0·4 to 1·0)	0·04
Current smoker (yes v never smoked)	1·2 (0·8 to 1·8)	
Systolic blood pressure‡	1·3 (0·8 to 1·7)	
High density lipoprotein cholesterol‡	0·7 (0·5 to 1·2)	0·03
Heart rate‡	2·2 (1·1 to 4·2)	0·01
Uric acid‡	1·5 (0·9 to 2·5)	0·01

*Each variable has been adjusted for age and for each of the other variables in model.
†Test for linear trend.
‡Upper fifth v lower fifth.

TABLE III—*Predictors of non-insulin dependent diabetes in subset of men with available data on triglyceride concentration (5327 men; 130 cases)*

Variable	Relative risk (95% confidence interval)		
	Partially adjusted*	Fully adjusted†	P value‡
Body mass index§	6·6	5·8 (2·6 to 13·2)	<0·0001
Prevalent coronary heart disease (yes/no)	1·4	1·4 (1·0 to 2·0)	0·05
Physical activity (moderate v inactive)	0·5	0·5 (0·2 to 0·9)	0·02
Alcohol intake (moderate v occasional)	0·6	0·6 (0·3 to 1·0)	0·04
High density lipoprotein cholesterol§	0·8	1·4 (0·8 to 2·6)	
Heart rate§	2·3	2·0 (1·0 to 4·0)	
Uric acid§	1·4	1·3 (0·7 to 2·2)	0·06
Triglyceride§	2·8 (1·4 to 5·8)		<0·001

*Adjusted for age, body mass index, smoking, pre-existing ischaemic heart disease, alcohol intake, physical activity, systolic blood pressure, high density lipoprotein cholesterol concentration, heart rate, uric acid concentration.
†Adjusted as for partially adjusted model together with serum triglyceride concentration.
‡Test for linear trend in fully adjusted model.
§Upper fifth v lower fifth.

factors for non-insulin dependent diabetes in middle aged men, whereas evidence incriminating allcohol intake and cigarette smoking is lacking. We have shown that several cardiovascular risk factorss that are linked with insulin resistance (triglyceride,, high density lipoprotein cholesterol and uric acid comcentrations)[13] predict the development of non-imsulin dependent diabetes over a decade before the onset of clinically manifest disease. These assocications persisted after adjustment for body mass index and for other factors that might increase the probability of diagnosis, such as hypertension and prewalent coronary heart disease at screening. Additional important coronary heart disease risk factors—nottably, serum total cholesterol concentration and lblood pressure—did not predict non-insulin depemdent diabetes.

The data on physical activity and non-imsulin dependent diabetes in this study complementt and extend the findings from previous prospective sttudies that have addressed this issue in selected populaticons.[4-6] We have also shown an association between heartt rate, which may be regarded as a proxy measure of phyysical fitness, and diabetes. Indeed lower heart rate wras an independent predictor of diabetes in multivrariate models which included physical activity. Thougjh this may simply reflect the relatively high precision with which heart rate was measured, the possibility that heart rate is also acting as a marker for sympatthetic nervous system activation (which is linked with imsulin resistance[25]) should be considered.

The data from this study provide evidence of a protective effect of alcohol on the risk of non-insulin dependent diabetes. This finding, however, shouild be interpreted cautiously as it was significant only im men with evidence of coronary heart disease at baseline:. It is clear, however, that the data provide no support foor the hypothesis of a causal positive link between alccohol intake and non-insulin dependent diabetes.[8]

This study had adequate power to examinee the association between cigarette smoking and non-imsulin dependent diabetes, and the findings do not suggeest an important independent role for cigarette smokimg in the development of this condition.

CORONARY HEART DISEASE RISK FACTORS AND NON-INSULIN DEPENDENT DIABETES

Reaven and others have developed the conceptt of a metabolic syndrome based on insulin resistance· that includes glucose intolerance, hypertriglyceridaeemia, decreased high density lipoprotein cholesterol comcentration, hypertension, and hyperuricaemia.[13] As clinically overt non-insulin dependent diabetess is preceded by a prolonged period of insulin resistaince[12] risk factors for cardiovascular disease that cluster ·with insulin resistance should also predict diabetes. The data from this study support this hypothesis and adld to the evidence that the increased risk of vascular dissease in diabetes is well established before the onseet of clinically manifest disease.[26] We observed, howeever, weak associations between blood pressure and mon-insulin dependent diabetes that were appreciiably attenuated on adjustment for body mass index and were non-significant in the full multivariate anaalysis model. It is noteworthy that the link between bblood pressure and insulin resistance or hyperinsulinaeemia in cross sectional studies is more tenuous and inacon-sistent[14 25] than that between dyslipidaemia (or hyyper-uricaemia) and insulin resistance.[13 14]

CONCLUSIONS

The current *Health of the Nation* targets for oboesity focus on reducing the prevalence of obesity (defineed as body mass index of ≥30) rather than on achievirng a downward shift in the overall distribution of body mmass

index.[27] As 75% of cases of diabetes in this study, however, ocurred in men with a body mass index of <30, substantial progress towards the prevention of non-insulin dependent diabetes and its sequelae will require a population based rather than a high risk approach to the problem of obesity. The findings from this study support the concept of an integrated approach to the prevention of non-insulin dependent diabetes and atherosclerotic vascular disease based on the prevention of obesity and the promotion of physical activity. However, not all vascular risk factors predict non-insulin dependent diabetes. This suggests that there are critical factors within populations (as well as between populations[28]) that modulate the progression from insulin resistance to non-insulin dependent diabetes or atherosclerotic vascular disease, or both of these conditions.

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