

Serum Insulin and Incident Coronary Heart Disease in Middle-aged British Men

Ivan J. Perry,¹ S. Goya Wannamethee,¹ Peter H. Whincup,¹ A. Gerald Shaper,¹ Mary K. Walker,¹ and K. George M. M. Alberti²

Earlier studies have not resolved the question of whether elevated circulating insulin levels are independently related to the development of coronary heart disease. Previous studies have not used a specific insulin assay and in all but a minority of studies that have addressed this issue it has not been possible to adjust for possible confounding due to high density lipoprotein (HDL) cholesterol. The authors examined the relation between serum insulin concentration and major coronary disease events (fatal and non-fatal myocardial infarction) in the British Regional Heart Study. The data are based on 5,550 men (aged 40–59 years) in 18 towns whose baseline, non-fasting serum samples were analyzed for insulin using a specific enzyme-linked immunoadsorbent assay (ELISA) method. Known diabetics were excluded. At 11.5 years of follow-up, 521 major coronary disease events had occurred, 261 fatal and 260 non-fatal. A nonlinear relation between serum insulin and coronary disease events was observed with an almost twofold increased relative risk in the 10th decile of the serum insulin distribution (≥ 33.8 mU/liter) relative to the 1st to the 9th deciles combined (age-adjusted relative risk (RR) = 1.9, 95% confidence interval (CI) 1.6–2.4). There was some attenuation of this association on cumulative adjustment for a wide range of biologic and life-style coronary disease risk factors, including HDL cholesterol, though it remained significant in the fully adjusted proportional hazards model (RR = 1.6, 95% CI 1.1–2.3). Similar associations between insulin and coronary disease events were seen in men with and without evidence of coronary disease at screening and in men with baseline serum glucose below the 80th percentile. These data are consistent with the hypothesis that a high level of serum insulin (hyperinsulinemia) is atherogenic, with a threshold effect. However, the markedly nonlinear form of the association and the attenuation in multivariate analysis strongly suggest that elevated insulin levels may only be a marker for common etiologic factors in the development of both coronary disease and non-insulin-dependent diabetes mellitus. *Am J Epidemiol* 1996;144:224–34.

blood glucose; coronary disease; prospective studies

The role of insulin in the development of atherosclerotic cardiovascular disease has been the subject of debate (1–3). It has been hypothesized 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 a

cluster of cardiovascular disease risk factors (1). Laboratory evidence supports direct atherogenic effects of insulin (4), and consistent data link elevated insulin levels with some cardiovascular risk factors (5, 6). However, the epidemiologic data linking insulin with specific cardiovascular disease endpoints are inconsistent. In particular, there is uncertainty about the relation between circulating insulin levels and the development of coronary heart disease. This issue has been addressed in a number of prospective studies in western populations. Some findings suggest an association with fasting insulin (7) or post-load insulin (8–11), while others indicate no independent association between coronary disease and insulin (12–19). In the Rancho Bernardo Study, Ferrara et al. (19) 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 a prospective study of elderly men and women in Kuopio, Finland, fasting insulin predicted coronary disease

Received for publication June 14, 1995, and accepted for publication January 5, 1996.

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunoadsorbent assay; FEV₁, forced expiratory volume in one second; HDL, high density lipoprotein; NIDDM, non-insulin-dependent diabetes mellitus; RR, relative risk; WHO, World Health Organization.

¹ Department of Public Health, The Royal Free Hospital School of Medicine, London, England.

² Department of Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne, England.

Correspondence to: Dr. Ivan J. Perry, Department of Public Health, The Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, England.

events only in subjects with a relatively high urinary microalbumin excretion rate (20).

In the previous studies, insulin concentrations were determined by radioimmunoassay methods that are not specific for insulin but cross-react to a variable degree with proinsulin and proinsulin split products. In most of these earlier studies, data on high density lipoprotein (HDL) cholesterol were not available. HDL cholesterol is inversely associated with insulin in cross-sectional studies (21), and it has been hypothesized to be an important confounding factor in studies on the link between insulin and coronary disease (2).

In this study, we examined the relation between serum insulin concentration and major coronary disease events over 11.5 years of follow-up in the British Regional Heart Study, a population-based sample of middle-aged men. There were over 500 major coronary disease events (fatal and non-fatal), more than the combined total of events in the previous studies that have addressed this issue (7–20). Insulin was measured using a specific enzyme-linked immunosorbent assay (ELISA) method and adjustments were made for major coronary risk factors including HDL cholesterol, triglycerides, and physical activity. The data have been examined for evidence of a nonlinear association between insulin and coronary disease (8, 9, 11) and for evidence of interaction between insulin and the established coronary disease risk factors. In a separate analysis, we have also examined the insulin-coronary disease association at 5 years follow-up, because there were discrepant findings in the Paris Prospective Study between the early (5 years) and late (11 and 15 years) follow-up studies (8, 9).

MATERIALS AND METHODS

Study subjects

In the British Regional Heart Study, 7,735 men aged 40–59 years were selected at random (using an age-sex register) from 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 details of the respondents and data collection have been described previously (22, 23). Men with cardiovascular or other disease or those receiving regular medication were not excluded. The overall response rate was 78 percent. Aliquots of serum from the men in the 7th to the 24th towns visited, a total of 5,661 men, were stored at -20°C . With exclusion of 111 men with established or probable diabetes (self-reported, physician-diagnosed, or non-fasting serum glucose ≥ 11.1 mmol/liter (200 mg/dl)), data were available for 5,550 men, who were the subjects for the present study.

Baseline assessment

Research nurses administered a standard questionnaire and completed an examination of each man, which included an electrocardiogram (24). The questionnaire included questions on smoking habits, alcohol intake, the usual pattern of physical activity, medical history, and regular medication, including use of anti-hypertensive drugs. Details of the classification of smoking habits, alcohol intake, social class, the measurement of blood pressure, and other physical measurements have been reported (22, 23, 25). Body mass index, calculated as $\text{weight}/\text{height}^2$, was used as an index of relative weight. Heart rate was determined from the electrocardiogram. A physical activity score was derived from the exercise questionnaire administered at the screening examination, based on the frequency and intensity of the activities reported (26). This score, which has been validated against heart rate and lung function, is predictive of major cardiovascular endpoints (myocardial infarction and stroke) (26, 27). Physical activity data were available for 5,481 men. Based on the score, the men were grouped into six broad physical activity categories: inactive ($n = 525$), occasional ($n = 1,618$), light ($n = 1,346$), moderate ($n = 879$), moderately vigorous ($n = 774$), and vigorous ($n = 339$). Men whose level of activity was moderate or higher were characterized as physically active. Forced expiratory volume in one second (FEV_1) was measured in the seated position using a Vitalograph spirometer (model J49-B2, Vitalograph Ltd., Buckingham, England), and values were height standardized.

Non-fasting blood samples were obtained between 8:30 AM and 6:30 PM. The time of arrival at the examination center was noted and the estimated time of venipuncture (at the end of the examination) was 35 minutes later (28). Details of venipuncture, serum separation and storage, and the methods of analysis for serum lipids have been described (28, 29).

Insulin and glucose measurement

Serum insulin concentration was determined by a two-site ELISA using commercially available monoclonal antibodies raised against human insulin (Novo Nordisk A/S, Denmark) which do not cross-react with proinsulin (30). Analyses were performed in the Department of Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne, England, on non-fasting samples which were stored at -20°C for 13–15 years. In this laboratory, no change in insulin levels was detected in repeat assays (using a standard radioimmunoassay method) of 34 samples, stored at -20°C over an 8-year period (mean difference 0.19 mU/liter,

paired $t = 0.7$, $p = 0.5$). The lower limit of detection for the ELISA was 1 mU/liter and the interassay coefficients of variation were 5.5 percent at 8.8 mU/liter, 5.9 percent at 21.6 mU/liter, and 7.5 percent at 44.8 mU/liter. The distribution of serum insulin was markedly skewed (range 1–479.5 mU/liter, median 11.6 mU/liter, geometric mean 12.4 mU/liter (log standard deviation 0.76)). There was obvious diurnal variation in serum insulin levels, related presumably to meals, with peaks at 08.00, 09.00, 13.00, 14.00, 15.00, and 18.00 hours, troughs at 11.00 and 16.00 hours, and intermediate levels at 10.00, 12.00, 17.00, and 19.00 hours. Peak levels (hourly geometric mean) ranged from 13.6 mU/liter to 18.4 mU/liter, the trough levels were 10.3 mU/liter and 10.4 mU/liter, and the intermediate levels ranged from 11.6 mU/liter to 11.7 mU/liter.

Glucose was analyzed in serum at screening using a commercially available automated analyzer (Technicon SMA 12/60, Technicon Instruments Corp., Tarrytown, New York). Diurnal variation in glucose levels was modest, with a peak-trough difference of 0.4 mmol/liter (28, 31).

Prevalent coronary disease

The men were asked whether a doctor had ever told them that they had angina or myocardial infarction (heart attack or coronary thrombosis), stroke, and a number of other disorders. The World Health Organization (WHO) (Rose) chest pain questionnaire was administered to all men at the initial examination and a 3-orthogonal lead electrocardiogram was recorded at rest. Prevalent coronary disease 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 WHO (Rose) questionnaire response indicating angina or possible myocardial infarction and electrocardiographic evidence of definite or possible myocardial ischemia or infarction (24, 32). The group of 5,550 nondiabetic men with serum insulin data were separated into three groups according to the degree of evidence of prevalent coronary disease at screening: 1) men with no evidence of coronary disease ($n = 4,139$), 2) men with evidence of coronary disease short of a definite myocardial infarction ($n = 1,098$), and 3) men with evidence of a definite myocardial infarction on electrocardiogram or recall of a doctor diagnosis of myocardial infarction ($n = 313$). In the subsequent analyses, men in groups 2 and 3 are regarded as having preexisting coronary disease.

Follow-up for major events

Over 99 percent of study participants have been followed for morbidity and mortality for 11.5 years.

Full details of follow-up procedures have been published and the criteria for fatal and non-fatal major ischemic heart disease events have been described (23, 33). Major coronary disease 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, i.e., an event that satisfied at least two of the following criteria: 1) preceded by severe prolonged chest pain, 2) electrocardiographic evidence of myocardial infarction, or 3) cardiac enzyme changes associated with myocardial infarction. Fatal events were defined as deaths from ischemic heart disease (*International Classification of Diseases* 9th Revision codes 410–414) as the underlying cause. Individuals who had first a non-fatal and then a fatal myocardial infarction during the follow-up period were classified as having had a fatal event.

Identification of incident cases of diabetes

In a subsidiary analysis, a group of 138 men who developed non-insulin-dependent diabetes mellitus (NIDDM) during the subsequent 11.5–14 years of follow-up were excluded. These new cases of NIDDM were ascertained by means of a postal questionnaire sent to the men at year 5 of follow-up (98 percent response rate), by systematic reviews of primary care records in 1990 and 1992, and a further questionnaire in 1992 (90 percent response rate) (34).

Statistical analysis

The risk of major coronary disease events at 11.5 years follow-up (and in a separate analysis at 5 years follow-up) was examined by quintile of serum insulin, with the top quintile further divided into deciles. Direct standardization was used to obtain the age-adjusted coronary disease event rate per 1,000 person-years of follow-up, using the whole population as standard (figures 1 and 2). The Cox proportional hazards model was used to obtain relative risks adjusted for confounding factors (35). Age, body mass index, systolic blood pressure, total cholesterol, heart rate, FEV₁, HDL cholesterol, and triglyceride concentration were fitted as continuous variables in the proportional hazards model. Glucose was entered as a dichotomous variable, <6.1 mmol/liter and ≥6.1 mmol/liter, reflecting the nonlinear form of the association between glucose and coronary disease in these data (36). Social class was fitted as 6 dummy variables (7 groups: 6 Registrar General groups and Armed Forces), physical activity as 5 variables (6 categories), alcohol as 4

variables (5 categories: none, occasional, light, moderate, and heavy) and smoking as 4 dummy variables (5 groups: never, ex-smokers, light, moderate, and heavy). As insulin, glucose, 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 (28), the log-transformed data on these variables were adjusted for time of sampling, using the mean level of each variable for each hour in which samples were taken and the grand mean (see Appendix) (37).

To illustrate the separate effects of allowing for key biologic and life-style variables, the insulin-coronary disease relation was adjusted for potential confounding factors in four cumulative stages, 1) age, 2) glucose and body mass index, 3) social class, smoking, physical activity, heart rate, FEV₁, use of anti-hypertensive therapy, alcohol consumption, systolic blood pressure, total cholesterol, and prevalent coronary disease at screening, and 4) HDL cholesterol level (tables 3 and 4). Serum triglyceride level was negatively correlated with HDL cholesterol ($r = -0.46$) and was not an independent predictor of coronary disease events in this study. Therefore, triglyceride was not entered with HDL cholesterol in the main Cox proportional hazards model. Possible interactions between insulin and established risk factors

(age, body mass index, physical activity, hypertension, hypercholesterolemia, and cigarette smoking) in the development of coronary disease were explored in stratified analyses and by fitting interaction terms in Cox proportional hazards models.

In further analyses, to investigate possible bias due to time of sampling, the association between serum insulin concentration, unadjusted for time of sampling, and major coronary disease events was examined in three time strata, at times of peak, trough, and intermediate insulin levels (as detailed above).

RESULTS

After 11.5 years follow-up for all 5,550 nondiabetic men with serum insulin data, 521 major coronary disease events had occurred—261 fatal and 260 non-fatal. Mean serum insulin at baseline (geometric mean and 95 percent confidence interval) was significantly higher in men who subsequently developed a major coronary disease event than in the rest of the cohort, 14.7 mU/liter (13.9–15.6) versus 12.3 mU/liter (12.1–12.6), $p < 0.0001$. Figure 1 shows the age-adjusted coronary disease event rates per 1,000 person-years of follow-up by quintile of serum insulin and in the 9th and 10th deciles. Event rates were lowest in the 1st quintile, intermediate in the 2nd to the 4th quintiles, and highest in the 5th quintile. Coronary disease event

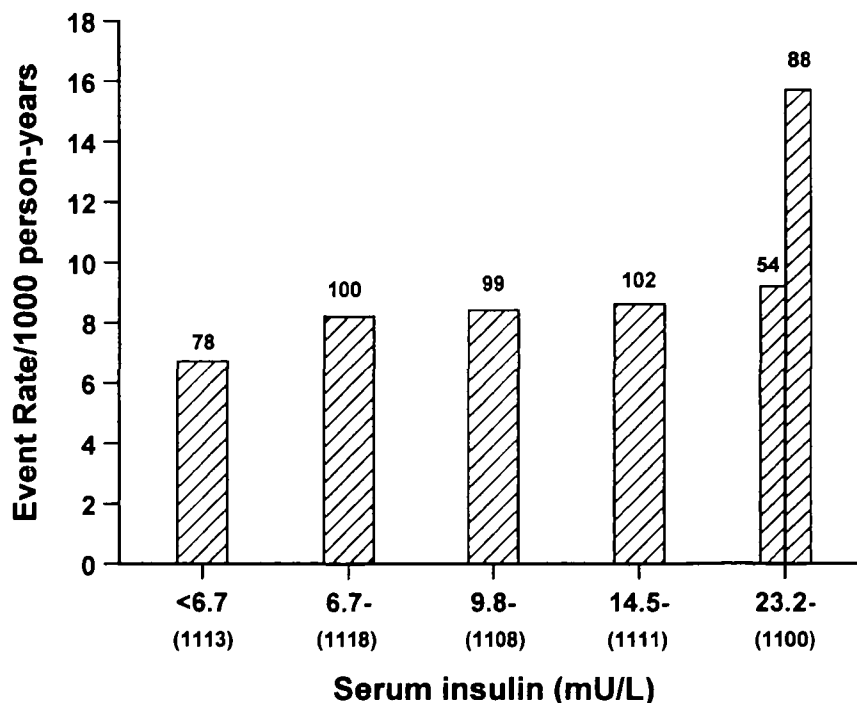


FIGURE 1. Major coronary heart disease event rates per 1,000 person-years of follow-up at 11.5 years follow-up in the 1st to the 4th quintile of serum insulin and in the 9th and 10th deciles. Above each bar is shown the number of events, and in parentheses at bottom, the number of men in each quintile and in the 9th and 10th decile.

rates in the 5th quintile were significantly raised relative to the 1st quintile (age-adjusted relative risk (RR) = 1.9, 95 percent confidence interval (CI) 1.4–2.5). To determine whether coronary disease risk increased further at higher levels of insulin, the 5th quintile was divided into deciles (23.2–33.7 mU/liter and ≥ 33.8 mU/liter). Relative to the 1st quintile baseline group, a steep increase in the age-adjusted risk of coronary disease was observed in the 10th decile (RR = 2.4, 95 percent CI 1.8–3.2), whereas risk in the 9th decile was only slightly raised (RR = 1.4, 95 percent CI 1.0–1.9) (figure 1). The age-adjusted risk of major coronary disease events among men whose serum insulin was in the upper decile of the distribution was increased almost twofold relative to those with insulin levels at or below the 90th percentile (RR = 1.9, 95 percent CI 1.6–2.4). In subsequent analyses showing the effects of adjustment for potential confounding factors, we have focused on the risk of coronary disease events in the upper decile of the insulin distribution relative to the rest of the distribution.

Insulin and coronary disease risk factors

Strong independent associations between serum insulin and established coronary disease risk factors were observed, in particular with glucose, body mass index, triglycerides, and HDL cholesterol (table 1). Insulin levels were lower in manual workers and in the Armed Forces group than in non-manual workers. Insulin levels (n ; geometric mean (95 percent confidence interval)), adjusted for age and body mass index, were higher in men who were inactive ($n = 3,489$; 12.6 mU/liter (12.4–12.9)) compared with those who were physically active ($n = 1,992$; 12.0 mU/liter (11.7–12.4), $p < 0.0001$). Insulin levels, adjusted for age and body mass index, were lower in

TABLE 1. Correlation between coronary heart disease risk factors and non-fasting serum insulin in 5,550 men aged 40–59 years in the British Regional Heart Study

Risk factor	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 ‡	-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**

* $p = 0.01$; ** $p < 0.001$.

† Adjusted for age and body mass index.

‡ BP, blood pressure; FEV₁, forced expiratory volume in one second; HDL cholesterol, high density lipoprotein cholesterol.

smokers ($n = 2,339$; 11.6 mU/liter (11.3–11.9)) than in nonsmokers ($n = 3,202$; 13.1 mU/liter (12.8–13.4), $p < 0.0001$), and in heavy drinkers ($n = 616$; 11.0 mU/liter (10.4–11.6)) compared with occasional drinkers ($n = 1,296$; 13.4 mU/liter (12.9–13.9), $p < 0.0001$). Insulin levels were higher in men taking anti-hypertensive therapy ($n = 279$; 13.5 mU/liter (12.4–14.6)) than in those who were not using anti-hypertensive therapy ($n = 5,271$; 12.4 mU/liter (12.1–12.6), $p < 0.05$).

Insulin and prevalent coronary disease

Relative to men without evidence of coronary disease at baseline, insulin levels were higher in men with evidence of coronary disease short of a definite myocardial infarction and were highest in those with a definite myocardial infarction on electrocardiogram or recall of a physician's diagnosis of myocardial infarction, $p < 0.001$ (table 2). These differences remained significant (though partially attenuated) on adjustment for the full range of coronary disease risk factors with which insulin is associated (table 2).

Insulin and coronary disease events in multivariate analysis

Table 3 shows the relation between insulin and incident major coronary disease 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 biologic coronary disease risk factors, including prevalent coronary disease at baseline and HDL cholesterol. A similar increased risk in the upper decile of the insulin distribution was observed for fatal and non-fatal events with adjusted relative risks (95 percent CIs) of 1.4 (1.0–2.0) and 1.8 (1.2–2.5). In a separate analysis, in which serum triglyceride was entered into the full proportional hazards model instead of HDL cholesterol, the insulin-coronary disease association was unchanged (adjusted relative risk for all coronary disease events in the upper decile of insulin relative to the 1st to the 9th deciles combined, 1.7 (95 percent CI 1.3–2.2)).

Insulin and coronary disease events by coronary disease status at baseline

Similar nonlinear relations between serum insulin levels and subsequent coronary disease events were observed in men without evidence of coronary disease at baseline and in men with evidence of coronary disease at baseline. In the men without coronary disease at baseline, the risk of subsequent major events in

TABLE 2. Unadjusted and adjusted mean insulin levels (95% confidence intervals (CI)) by prevalent coronary heart disease (CHD) status in men aged 40–59 years in the British Regional Heart Study

CHD status	Non-fasting serum insulin (mU/liter)			
	Unadjusted*	95% CI	Adjusted**,†	95% CI
No evidence of CHD (n = 4,139)	12.1	11.8–12.3	12.3	11.9–12.7
Evidence of CHD short of definite myocardial infarction (n = 1,098)	13.2	12.7–13.7	12.8	12.3–13.4
Definite myocardial infarction (n = 313)	14.3	12.9–15.8	13.1	12.2–14.2

* $p < 0.001$; ** $p = 0.04$ (test for trend).

† Adjusted for age, glucose, body mass index, social class, smoking, physical activity, heart rate, forced expiratory volume in one second (FEV₁), alcohol consumption, systolic blood pressure, total cholesterol, high density lipoprotein (HDL) cholesterol level, and use of anti-hypertensive therapy.

TABLE 3. Adjusted relative risks (RR) (95% confidence intervals (CI)) of incident major coronary heart disease (CHD) events by serum insulin level (6 groups; 1st to 4th quintiles, 9th and 10th deciles) in all 5,550 men aged 40–59 years in the British Regional Heart Study 11.5-year follow-up (521 CHD events)

Insulin (mU/liter)	Model A*		Model B†		Model C‡	
	RR	95% CI	RR	95% CI	RR	95% CI
<6.6	1.0		1.0		1.0	
6.6–9.5	1.2	0.9–1.6	1.1	0.8–1.5	1.1	0.8–1.5
9.6–14.2	1.2	0.9–1.6	1.1	0.8–1.5	1.0	0.7–1.4
14.3–23.6	1.2	0.9–1.6	1.1	0.8–1.5	1.0	0.7–1.4
23.7–35.2	1.3	0.9–1.8	1.1	0.7–1.6	0.9	0.6–1.6
≥35.3	2.1	1.5–2.1	1.8	1.3–2.5	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

* Model A: Relative risks adjusted for age, glucose, and body mass index (n = 5,535; 520 cases). (The findings are unchanged if glucose is fitted as a continuous variable.)

† Model B: Relative risks adjusted for the factors above plus social class, smoking, physical activity, heart rate, forced expiratory volume in one second (FEV₁), alcohol consumption, systolic blood pressure, total cholesterol level, use of anti-hypertensive therapy, and prevalent CHD (n = 5,361; 504 cases).

‡ Model C: Relative risks adjusted for the factors above plus high density lipoprotein (HDL) cholesterol level (n = 5,272; 493 cases).

the upper decile of serum insulin relative to the rest of the distribution was 1.7 (95 percent CI 0.8–3.2) and in the men with coronary disease at baseline, the risk was 1.5 (95 percent CI 1.0–2.2), adjusted for all potential confounding factors.

Insulin-coronary disease relation and glucose tolerance

We considered whether serum insulin levels in the 10th decile were simply a marker for undiagnosed or “latent” NIDDM, which one would expect to be associated with increased coronary disease risk. In an analysis confined to men with baseline serum glucose below the 80th percentile who were also without evidence of coronary disease at baseline (n = 3,189; 203 cases), a significant excess risk of incident coronary disease events was seen in the upper decile of the insulin distribution (RR = 1.9, 95 percent CI 1.5–3.0) adjusted for potential confounding factors, including HDL cholesterol. In a further analysis, we excluded 138 men who developed NIDDM over 11.5–14 years of follow-up. On exclusion of this latter group, the fully adjusted relative risk of coronary disease in the

10th serum insulin decile compared with the 1st to the 9th deciles was 1.6 (95 percent CI 1.1–2.4).

Insulin-glucose ratio, glucose, and coronary disease

The form and magnitude of the relation between insulin-glucose ratio and coronary disease was similar to that of insulin and coronary disease. Comparing the top decile of the insulin-glucose ratio with the rest of the distribution, the fully adjusted relative risk was 1.6 (95 percent CI 1.2–2.0). In an earlier analysis (at 9.5 years follow-up, before serum insulin data were available), a non-fasting serum glucose level in the top quintile was associated with an approximately 40 percent increase in coronary disease events relative to the rest of the glucose distribution, independent of major coronary disease risk factors, including HDL cholesterol (36). In this earlier analysis, no further increase in the risk of coronary disease was observed in the upper decile of serum glucose. The association between serum glucose and major coronary disease events was reexamined in this subgroup of 5,550 men. As in the entire cohort, there was an increase in risk in the upper

glucose quintile relative to the lower four quintiles combined (age-adjusted RR = 1.5, 95 percent CI 1.1–2.1). This association between serum glucose and coronary disease events was abolished on addition of insulin to the age-adjusted model (RR = 1.0, 95 percent CI 0.7–1.4).

Interactions with other coronary disease risk factors

The data were examined for evidence of interactions between insulin and other risk factors in the development of coronary disease. The fully adjusted relative risk of coronary disease in the 10th serum insulin decile compared with the rest of the insulin distribution was greater in smokers (RR = 2.0, 95 percent CI 1.4–2.9) than in nonsmokers (RR = 1.4, 95 percent CI 0.9–2.0), a difference which was significant on formal testing for interaction ($p = 0.04$). The adjusted relative risk was nonsignificantly greater in relatively young men aged <50 years at screening (RR = 2.2, 95 percent CI 1.4–3.4) than in older men aged ≥ 50 years at screening (RR = 1.4, 95 percent CI 1.0–1.9; test for interaction $p = 0.2$). The relative risk in the top decile of serum insulin versus the rest of the distribution was similar in each tertile of body mass index (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 ≥ 160 mmHg and/or diastolic ≥ 90 mmHg and/or receiving anti-hypertensive therapy) it was 1.8.

Effect of time of sampling on insulin-coronary disease association

In analyses based on serum insulin data which were unadjusted for time of sampling, the increased risk of coronary disease events observed in the upper decile of the insulin distribution was similar at times of peak, intermediate, and trough insulin levels. Among men whose blood samples were obtained at times of peak insulin levels ($n = 2,228$; 207 events), the fully adjusted relative risk of coronary disease in the 10th serum insulin decile relative to the rest of the insulin distribution was 1.7 (95 percent CI 1.2–2.5). At times of intermediate insulin levels ($n = 2,004$; 191 events) and trough insulin levels ($n = 1,318$; 123 events), the adjusted relative risks in the 10th serum insulin decile were 1.7 (95 percent CI 1.2–2.6) and 1.6 (95 percent CI 0.9–3.1), respectively.

Insulin and coronary disease events at 5 years of follow-up

At 5 years of follow-up in the British Regional Heart Study, there were 201 coronary disease events.

A stronger, more linear association between insulin and coronary disease events was observed over the first 5 years of follow-up (figure 2). In the upper decile of serum insulin, the age-adjusted risk of coronary disease events was increased more than threefold relative to the 1st quintile (RR = 3.7, 95 percent CI 1.8–5.5) and more than twofold relative to the rest of the insulin distribution (table 4). 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 biologic coronary disease risk factors, but there remained a substantial excess risk of coronary disease events in the upper decile of serum insulin in the fully adjusted proportional hazards model (table 4).

DISCUSSION

An elevated circulating insulin level was an independent predictor of major coronary disease events at 5 and 11.5 years of follow-up in this representative sample of British middle-aged, nondiabetic men. The magnitude of the insulin-coronary disease 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 nonlinear at 11.5 years, and in both early and late follow-up, the excess risk of coronary disease events was concentrated largely in the upper decile of the serum insulin distribution. Although insulin levels were raised in men with pre-existing coronary disease at baseline, the relation between insulin and subsequent coronary disease events was similar in men with and without evidence of preexisting coronary disease at baseline. A serum insulin level in the upper decile was associated with a similar increase in the risk of both fatal and non-fatal coronary disease events in multivariate analysis. Exclusion of men with a serum glucose level at or above the 80th percentile and those with evidence of coronary disease at baseline did not attenuate this association. In contrast to the previous prospective studies that have addressed this question, insulin levels were determined using a specific assay which does not cross-react with proinsulin.

The lack of fasting insulin data is a limitation of this study. It has been suggested (16), however, that the non-fasting state may be more relevant to the study of insulin and coronary disease than the fasting state. 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 (1). Inevitably, within-subject variability in insulin measurement will be higher with random (non-fasting) samples than with timed, post-load samples.

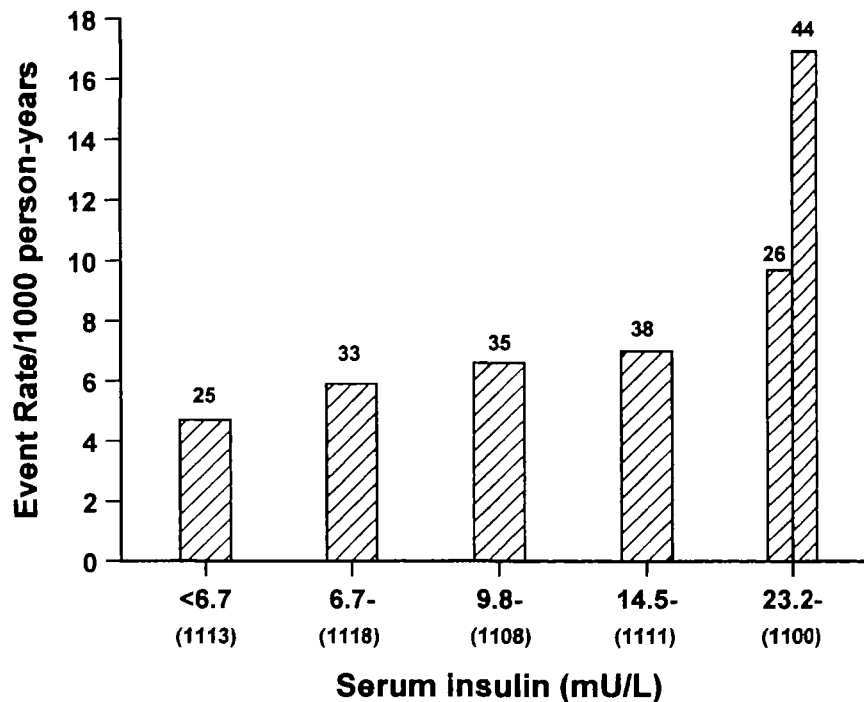


FIGURE 2. Major coronary heart disease event rates per 1,000 person-years of follow-up at 5 years follow-up in the 1st to the 4th quintile of serum insulin and in the 9th and 10th deciles. Above each bar is shown the number of events, and in parentheses at bottom, the number of men in each quintile and in the 9th and 10th decile.

TABLE 4. Adjusted relative risks (RR) (95% confidence intervals (CI)) of major coronary heart disease (CHD) events in the 10th serum insulin decile relative to the 1st to the 9th deciles combined at 5 years follow-up in all 5,550 men aged 40–59 years in the British Regional Heart Study (201 CHD events)

Model A*		Model B†		Model C‡		Model D§	
RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
2.6	1.9–3.6	2.2	1.5–3.2	2.1	1.4–3.1	2.1	1.4–3.0

* Model A: Relative risk adjusted for age ($n = 5,550$; 201 cases).
 † Model B: Relative risk adjusted for age, glucose, and body mass index ($n = 5,535$; 201 cases).
 ‡ Model C: Relative risk adjusted for the factors above plus social class, smoking, physical activity, heart rate, forced expiratory volume in one second (FEV_1), alcohol consumption, systolic blood pressure, total cholesterol level, use of anti-hypertensive therapy, and prevalent CHD ($n = 5,361$; 194 cases).
 § Model D: Relative risk adjusted for the factors above plus high density lipoprotein (HDL) cholesterol level ($n = 5,272$; 188 cases).

However, all measurements of insulin in epidemiologic studies are beset by problems of high within-subject variability. High within-subject variability in the measurement of a putative risk factor such as insulin will dilute etiologic associations 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 cardio-

vascular risk factors and with coronary disease events has been underestimated.

There was no evidence that the association between serum insulin and coronary disease events was influenced by the time of day at which samples were obtained. Moreover, correlations of non-fasting insulin with such cardiovascular disease risk factors as body mass index, lipids, and blood pressure that we observed in this study are similar to the correlations with fasting and post-load insulin reported in other studies (9, 14, 16, 19, 38, 39). For example, the data on interrelations with biologic risk factors are virtually identical to the interrelations reported from a population-based study in eastern Finland which had data on fasting and 2-hour plasma insulin (39). Similarly, the associations reported in this study between insulin levels and life-style factors such as physical activity, cigarette smoking, and alcohol intake are consistent with findings from previous work (9, 16, 19). It is difficult therefore to conceive 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 disease events but not of the association between insulin and cardiovascular disease risk factors.

The lack of data on body fat distribution represents an additional limitation of this study. Central obesity is considered to be a better marker of coronary disease

risk than body mass index (40) and is independently associated with insulinemia (38). However, there was little attenuation of the insulin-coronary disease association on adjustment for body mass index and physical activity level, variables which are correlated with central obesity (41), and there was no evidence of significant variation in the magnitude of the association in different strata of body mass index 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 coronary disease. In the 15-year data from the Paris study (6,093 subjects, 174 coronary disease 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 coronary disease (9). A similar nonlinear relation between post-load insulin and coronary disease was observed in the 11-year follow-up data from the Paris study (8). Post-load insulin (both 1-hour and 2-hour) was also a significant predictor of coronary disease events in the Helsinki Policemen Study (1,059 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 hyperlipidemia (10, 11). As in the Paris study, the relation in the Helsinki study was nonlinear 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 coronary disease event rates was observed in the 10th decile (11). Thus, we now have evidence from three prospective studies of middle-aged men (the Paris Prospective Study, Helsinki Policemen Study, and the British Regional Heart Study) of a significant, nonlinear 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 coronary disease endpoints (13–15, 18, 19) or have been based on fasting insulin (16, 17). Data on women are sparse (13, 18, 19). In the Busselton study (724 women aged 40–74 years, 18 coronary disease deaths), no independent effect of 1-hour post-load insulin on coronary disease mortality was detected at 13 years follow-up (13). Subsequent negative studies in women have been based on a total of 36 coronary disease endpoints (18, 19). The reported inverse association between 2-hour insulin and cardiovascular disease mortality in elderly men is not necessarily generalizable to younger age groups (19).

It has been hypothesized (42, 43) that insulin may accelerate the development of atheroma in the presence of other risk factors such as hypertension or hypercholesterolemia. This hypothesis is suggested by the low rates of coronary disease in populations such as the Pima Indians, despite marked hyperinsulinemia (44). There was no evidence of an interaction between insulin and serum cholesterol in our data, and although coronary disease rates were higher among hypertensives than normotensives with serum insulin levels in the upper decile, the difference was not significant. There was, however, a significant interaction between insulin and cigarette smoking, with higher coronary disease risk among smokers with raised insulin levels compared with nonsmokers. In this, as in previous data (16), 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 coronary disease risk factors and in future prospective studies. A serum insulin level in the upper decile was also associated with a non-significantly higher coronary disease risk in men aged 40–49 years than in men aged 50–59 years at screening. As McKeigue and Davey (45) have argued in relation to the finding of an inverse association between insulin and coronary disease in elderly men (19), the weaker association between insulin and coronary disease events seen in older men in this study may reflect confounding due to comorbidity.

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 coronary disease events over the ensuing 5–10 years, which is independent of major coronary disease risk factors with which insulin is correlated. This finding is consistent with a direct atherogenic role for elevated circulating insulin levels (with a threshold effect) in the development of coronary disease. However, given the form of the association, with a relatively marginal increase in risk over much of the insulin distribution (even in the age-adjusted analysis), it is arguable that direct atherogenic effects of insulin are unlikely. Moreover, there is a particular need for caution in ascribing causality and declaring independence of effects in circumstances where multiple intercorrelated and labile variables such as insulin, blood pressure, HDL cholesterol, and triglycerides are examined simultaneously (46).

Elevated non-fasting insulin levels in this study can be regarded as a marker for insulin resistance. Hence, these data cannot resolve the issue of whether insulin is associated with coronary disease primarily via direct atherogenic effects or as a marker for insulin resistance. Given the lack of a continuous relation between serum insulin

and coronary disease in these data and the inconsistent data on the effect of exogenous insulin on coronary disease risk (47, 48), the latter hypothesis, that insulin resistance predicts coronary disease with insulin as an "innocent bystander" (3), is more plausible.

There is now considerable evidence that coronary disease and NIDDM share common causal factors, genetic, environmental, and possibly intrauterine (49, 50). It is known that insulin resistance antedates the development of NIDDM (51), and there is increasing evidence that the adverse coronary risk factor profile, which clusters with insulin resistance (52), also antedates NIDDM, i.e., that "the clock for coronary heart disease (starts) ticking before the onset of clinical diabetes" (34, 53). Although exclusion of men who were known to have developed NIDDM during the period of follow-up did not alter the findings in this study, there will inevitably have been undiagnosed cases and a proportion of men in the upper 10 percent of the insulin distribution are likely to develop NIDDM on further follow-up. We suggest therefore that elevated serum insulin levels in this study, reflecting insulin resistance, are a marker for the common causal factors that link coronary disease and NIDDM. The observation from the Kuopio study (20), that the association between hyperinsulinemia and coronary disease risk was confined to subjects with microalbuminuria is consistent with this interpretation of our data, given that microalbuminuria is associated with increased risk of both coronary disease and NIDDM in longitudinal studies (54, 55).

For over a decade, the magnitude and importance of the association between circulating insulin levels and coronary disease has been contested. There is a need now to focus on factors that influence the development of insulin resistance and on the factors within and between populations that modulate the progression from insulin resistance to non-insulin-dependent diabetes mellitus and coronary heart disease.

ACKNOWLEDGMENTS

The British Regional Heart Study is a British Heart Foundation Research Group. Support is also provided by The Stroke Association and the UK Department of Health. Biochemical estimations (with the exception of insulin and triglyceride) were carried out at the Wolfson Research Laboratories, Queen Elizabeth Hospital, Birmingham. Dr. Perry was supported by the Wellcome Trust.

REFERENCES

1. Stout RW. Insulin and atheroma. 20-yr perspective. *Diabetes Care* 1990;13:631-54.
2. Jarrett RJ. Is insulin atherogenic? *Diabetologia* 1988;31:71-5.
3. Savage PJ, Saad MF. Insulin and atherosclerosis: villain, accomplice, or innocent bystander? *Br Heart J* 1993;69:473-5.
4. Sato Y, Shiraishi S, Oshida Y, et al. Experimental atherosclerosis-like lesions induced by hyperinsulinism in Wistar rats. *Diabetes* 1989;38:91-6.
5. Abbott WG, Lillioja S, Young AA, et al. Relationships between plasma lipoprotein concentrations and insulin action in an obese hyperinsulinemic population. *Diabetes* 1987;36:897-904.
6. Zaveroni I, Bonora E, Pagliara M, et al. Risk factors for coronary heart disease in healthy persons with hyperinsulinemia and normal glucose tolerance. *N Engl J Med* 1989;320:702-6.
7. Ducimetière P, Eschwege E, Papoz L, et al. Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease in a middle-aged population. *Diabetologia* 1980;19:205-10.
8. Fontbonne AM, Eschwege EM. Insulin and cardiovascular disease: Paris Prospective Study. *Diabetes Care* 1991;14:461-9.
9. Fontbonne A, Charles MA, Thibault N, et al. Hyperinsulinemia as a predictor of coronary heart disease mortality in a healthy population: the Paris Prospective Study, 15-year follow-up. *Diabetologia* 1991;34:356-61.
10. Pyörälä K. Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. *Diabetes Care* 1979;2:131-41.
11. Pyörälä K, Savolainen E, Kaukola S, et al. Plasma insulin as coronary heart disease risk factor: relationship to other risk factors and predictive value during 9 1/2 year follow-up of the Helsinki Policemen Study population. *Acta Med Scand* 1985;701(suppl):38-52.
12. Welborn TA, Wearne K. Coronary heart disease incidence and cardiovascular mortality in Busselton with reference to glucose and insulin concentrations. *Diabetes Care* 1979;2:154-60.
13. Cullen K, Stenhouse NS, Wearne KL, et al. Multiple regression analysis of risk factor for cardiovascular disease and cancer mortality in Busselton, Western Australia—13-year study. *J Chronic Dis* 1983;36:371-7.
14. Hargreaves AD, Logan RL, Elton RA, et al. Glucose tolerance, plasma insulin, HDL-cholesterol and obesity: 12-year follow-up and development of coronary heart disease in Edinburgh men. *Atherosclerosis* 1992;94:61-9.
15. Welin L, Eriksson H, Larsson B, et al. Hyperinsulinemia is not a major coronary risk factor in elderly men: the study of men born in 1913. *Diabetologia* 1992;35:766-70.
16. Yarnell JWG, Sweetnam PM, Marks V, et al. Insulin in ischaemic heart disease: are associations explained by triglyceride concentrations? The Caerphilly prospective study. *Br Heart J* 1994;71:293-6.
17. Orchard TJ, Eichner J, Kuller LH, et al. Insulin as a predictor of coronary heart disease: interaction with Apo E phenotype. A report from MRFIT. *Ann Epidemiol* 1994;4:40-9.
18. Rewers M, Shetterly SM, Baxter J, et al. Insulin and cardiovascular disease in Hispanics and non-Hispanic whites (NHW): the San Luis Valley Diabetes Study. (Abstract). *Circulation* 1992;85:865.
19. Ferrara A, Barrett-Connor EL, Edelstein SL. Hyperinsulinemia does not increase the risk of fatal cardiovascular disease in elderly men or women without diabetes: The Rancho Bernardo Study, 1984-1991. *Am J Epidemiol* 1994;140:857-69.
20. Kuusisto J, Mykkänen L, Pyörälä K, et al. Hyperinsulinemic microalbuminuria: a new risk indicator for coronary heart disease. *Circulation* 1995;91:831-7.
21. Lichtenstein MJ, Yarnell JWG, Elwood PC, et al. Sex hormones, insulin, lipids and prevalent ischemic heart disease. *Am J Epidemiol* 1987;126:647-57.
22. Shaper AG, Pocock SJ, Walker M, et al. The British Regional

- Heart Study: cardiovascular risk factors in middle-aged men in 24 towns. *Br Med J* 1981;283:179–86.
23. Shaper AG, Pocock SJ, Walker M, et al. Risk factors for ischaemic heart disease: the prospective phase of the British Regional Heart Study. *J Epidemiol Community Health* 1985; 39:197–209.
 24. Shaper AG, Cook DG, Walker M, et al. Prevalence of ischaemic heart disease in middle-aged British men. *Br Heart J* 1984;51:595–605.
 25. Shaper AG, Phillips AN, Pocock SJ, et al. Risk factors for stroke in middle-aged British men. *Br Med J* 1991;302: 1111–15.
 26. Shaper AG, Wannamethee G. Physical activity and ischaemic heart disease in middle-aged British men. *Br Heart J* 1991;66: 384–94.
 27. Wannamethee G, Shaper AG. Physical activity and stroke in middle-aged British men. *Br Med J* 1992;304:597–601.
 28. Pocock SJ, Ashby D, Shaper AG, et al. Diurnal variations in serum biochemical and haematological measurements. *J Clin Path* 1989;42:172–9.
 29. Thelle DS, Shaper AG, Whitehead TP, et al. Blood lipids in middle-aged British men. *Br Heart J* 1984;49:205–13.
 30. Andersen L, Dinesen B, Jorgensen PN, et al. Enzyme immunoassay for intact human insulin in serum or plasma. *Clin Chem* 1993;39:578–82.
 31. Cook DG, Shaper AG, Thelle DS, et al. Serum uric acid, serum glucose and diabetes: relationships in a population study. *Postgrad Med J* 1986;62:1001–6.
 32. Phillips AN, Shaper AG, Pocock SJ, et al. The role of risk factors in heart attack occurring in men with pre-existing IHD. *Br Heart J* 1988;60:404–10.
 33. Walker M, Shaper AG. Follow-up of subjects in prospective studies in general practice. *J R Coll Gen Pract* 1984;34: 197–209.
 34. Perry IJ, Wannamethee SG, Walker MK, et al. Prospective study of risk factors for development of non-insulin-dependent diabetes in middle-aged British men. *Br Med J* 1995;310: 560–4.
 35. Cox DR. Regression models and life-tables. *J R Stat Soc [B]* 1972;34:187–220.
 36. Perry IJ, Wannamethee SG, Whincup PH, et al. Asymptomatic hyperglycaemia and major IHD events in Britain. *J Epidemiol Community Health* 1994;48:538–42.
 37. Phillips AN. Statistical issues in prospective studies of risk factors for ischaemic heart disease. PhD dissertation. London: University of London, 1986.
 38. Cigolini M, Seidell JC, Charzewska J, et al. Fasting serum insulin in relation to fat distribution, serum lipid profile, and blood pressure in European women: the European Fat Distribution Study. *Metabolism* 1991;40:781–7.
 39. Mykkänen L, Laakso M, Pyörälä K. High plasma insulin level associated with coronary heart disease in the elderly. *Am J Epidemiol* 1993;137:1190–1202.
 40. Deprès J-P, Moorjani S, Lupien PJ, et al. Regional distribution of body fat, plasma lipoprotein and cardiovascular disease. *Arteriosclerosis* 1990;10:497–511.
 41. Marti B, Tuomilehto J, Salomaa V, et al. Body fat distribution in the Finnish population: environmental determinants and predictive power for cardiovascular risk factor levels. *J Epidemiol Community Health* 1991;45:131–7.
 42. Modan M, Or J, Karaski A, et al. Hyperinsulinemia, sex and risk of atherosclerotic cardiovascular disease. *Circulation* 1991;84:1165–75.
 43. Durrington PN. Is insulin atherogenic? *Diabetic Med* 1992;9: 597–600.
 44. Ingelfinger JA, Bennett PH, Liebow IM, et al. Coronary heart disease in the Pima Indians: electrocardiographic findings and post-mortem evidence of myocardial infarction in a population with a high prevalence of diabetes mellitus. *Diabetes* 1976; 25:561–5.
 45. McKeigue P, Davey G. Association between insulin levels and cardiovascular disease are confounded by comorbidity. *Diabetes Care* 1995;18:1294–8.
 46. Smith GD, Phillips A. Declaring independence: why we should be cautious. *J Epidemiol Community Health* 1990;44: 257–8.
 47. The University Group Diabetes Program. Effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. *Diabetes* 1982;31(suppl 5):1–77.
 48. Wingard DL, Barrett-Connor EL, Ferrara A. Is insulin really a heart disease risk factor? *Diabetes Care* 1995;18:1299–1304.
 49. Jarrett RJ, Shipley MJ. Type 2 (non-insulin-dependent diabetes) and cardiovascular disease—putative association via common antecedents; further evidence from the Whitehall study. *Diabetologia* 1988;31:737–40.
 50. Hales CN, Barker DJP, Clark PMS, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *Br Med J* 1991;303:1019–22.
 51. Warram JH, Martin BC, Krolewski AS, et al. Slow glucose removal rate and hyperinsulinemia precede the development of type 2 diabetes in the offspring of diabetic parents. *Ann Intern Med* 1990;113:909–15.
 52. Reaven GM. Role of insulin resistance in human disease. *Diabetes* 1988;37:1595–1606.
 53. Haffner SM, Stern MP, Hazuda HP, et al. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA* 1990;263:2893–8.
 54. Yudkin JS, Forrester RD, Jackson CA. Microalbuminuria as a predictor of vascular disease in non-diabetic subjects. *Lancet* 1988;2:530–3.
 55. Mykkänen L, Haffner SM, Kuusisto J, et al. Microalbuminuria precedes the development of NIDDM. *Diabetes* 1994;43: 552–7.

APPENDIX

Method adjustment of serum insulin and triglyceride data for time of sampling

Serum insulin and triglyceride levels were adjusted for time of sampling using a simple mathematical approach which makes no assumptions about the form of the association between these variables and time of sampling. The log-transformed data on these variables were 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. Hence, each value was adjusted to one an equal distance away from the grand mean as it was from the mean for the hour in which it was measured. The calculation for serum insulin was as follows:

$$\begin{aligned}
 &\text{adjusted log insulin level} \\
 &= (\text{unadjusted log insulin level} \\
 &\quad - \text{the mean log insulin level} \\
 &\quad \text{for the hour of sampling}) \\
 &\quad + \text{the grand mean log insulin level.}
 \end{aligned}$$