Low-Grade Inflammation May Play a Role in the Etiology of the Metabolic Syndrome in Patients With Coronary Heart Disease: The HIFMECH Study

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Risk of coronary heart disease has been related to insulin resistance, but the mechanism for this is incompletely understood. Variables attributed to insulin resistance are associated with low-grade inflammation. A case-control study was performed of 469 male myocardial infarction (MI) survivors aged <60 years and 575 control subjects recruited from centers in northern and southern Europe. Principal factor analysis was used to explore correlations between insulin resistance and inflammatory variables. Three factors resulted: (a) "Metabolic Syndrome" (insulin/proinsulin/ triglyceride/body mass index [BMI]); (b) "Inflammation" (fibrinogen/C-reactive protein [CRP]/interleukin-6 [IL-6]); and (c) "Blood Pressure" (systolic and diastolic blood pressure). The "Metabolic Syndrome" factor was related to the "Inflammation" factor (largely independently of obesity), the "Blood Pressure" factor, smoking, and south location (all $P \leq .0002$). There were significant relationships between all 3 factors and case status ($P \leq .0002$). Markers of low-grade inflammation are strongly related to metabolic syndrome variables independently of obesity. This raises the possibility that links between insulin resistance and cardiovascular disease could, in part, represent common consequences of low-grade inflammation.

INSULIN RESISTANCE has been associated with a number of cardiovascular risk factors, including dyslipidemia, with elevated levels of triglyceride and low concentrations of high-density lipoprotein (HDL) cholesterol, hypertension[1] (the so-called metabolic syndrome), and disorders of coagulation and fibrinolysis.[2-4] Insulin resistance may also represent a cardiovascular risk factor in its own right. A meta-analysis has shown a relationship between hyperinsulinemia, a surrogate for insulin resistance,[5-7] and incident coronary heart disease (CHD).[8] However, as well as cause and effect, associations between hyperinsulinemia and CHD could represent consequences of a common antecedent.

Among variables that have been linked with insulin resistance are levels of fibrinogen[2,4,9] and C-reactive protein (CRP),[4,9,10] hepatic acute phase proteins produced in response to circulating proinflammatory cytokines, and von Willebrand factor, a product of activated endothelium.[10,11] We have proposed that insulin resistance and endothelial dysfunction may each represent the consequence of actions of proinflammatory cytokines.[4] In healthy subjects, adipose tissue is a major source of these cytokines,[4,12] thereby providing a possible mechanism linking obesity with CHD.[13]

We have used a study of myocardial infarction (MI) in men in north and south Europe (HIFMECH [Hypercoagulability and Impaired Fibrinolytic function MECHanisms predisposing to MI] study) to test the hypotheses that (a) the clustering of metabolic syndrome variables is related to measures of proinflammatory cytokines and acute phase activation; (b) obesity underlies the links between inflammatory and metabolic syndrome variables; and (c) low-grade inflammation may explain the increased insulin resistance in patients after MI.

To explore the clustering of metabolic syndrome and inflammatory variables and their interrelationships, we have used principal factor analysis, a widely accepted approach for exploring complex interrelationships of numerous interrelated variables.[14-18] The emphasis in these analyses is on clustering of inflammatory and metabolic syndrome variables within cases and controls, as inferences drawn from associations of risk factors with case status may be unreliable when subjects are studied after the event.

SUBJECTS, MATERIALS, AND METHODS

Study Population

Male caucasian survivors of a first MI aged under 60 (excluding patients with familial hypercholesterolemia and insulin-dependent diabetes mellitus) and population-based individuals of the same age were recruited from 4 centers: Stockholm and London (northern Europe) and Marseille and San Giovanni Rotondo (southern Europe). Consecutive patients were invited to participate along with randomly selected healthy individuals, matched for age, from national registration and general practitioner lists and employment registers from the same catchment areas. A total of 469 postinfarction patients and 575 controls were included. For purposes of comparability of the analyses, only those cases that were reported as nondiabetics were included in the analysis. Thus, among the cases, we excluded 60 patients (10.8%) with diabetes and 3 (0.5%) with no information on diabetes. There were no

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diabetics among the controls (fasting glucose ≥ 7.0 mmol/L). Study design has been described elsewhere.[19] Blood samples were obtained after an overnight fast, in the cases 3 to 6 months after the date of MI.

Assay Methods

Core laboratories performed the biochemical determinations on all samples from the entire HIFMECH cohort. Assay methods for lipids, insulin, and proinsulin have been described.[2] Plasma cholesterol and triglyceride concentrations were measured enzymatically using commercially available reagents (Cobas; Roche Diagnostics, Welwyn Garden City, Herts, UK). CRP was determined with an in-house enzyme immunoassay using rabbit antihuman antibodies (X0293) from Dako Diagnostics (Ely, Cambs, UK),[20] validated against the International Reference Preparation, with an assay range of 0.15 to 48mg $\cdot l^{-1}$ and with intra-assay and interassay coefficient of variation (CV) of <10%. Fibrinogen was determined by the Clauss thrombin clotting method using an automated coagulometer (ELECTRA 1800C Instrumentation Laboratory, Milan, Italy) with intra- and interassay CVs of 4.4% and 6.4%. Interleukin-6 (IL-6) was measured by 2-site high sensitivity enzyme-linked immunosorbent assay (ELISA) (R & D Systems, Oxon, UK) with a detection limit of 0.09 pg/mL and intra- and interassay CVs of 5.3% and 9.2%. Because correlation coefficients between insulin concentrations and homeostasis model assessment estimates of insulin resistance[21] of around 0.99 have been found in previous studies,[2,4,22,23] we used the fasting plasma insulin concentration as a surrogate measure of insulin resistance. This measure has been found to correlate with more invasive measures, such as the euglycemic clamp or frequently sampled intravenous glucose tolerance test with *r* values of 0.60 to 0.76.[5-7]

Statistical Analysis

The statistical analysis was conducted using SAS version 8 (SAS Institute, Cary, NC) and Intercooled Stata version 6.0. Analyses were performed on logarithmic or square root transformed values where necessary, and results presented using the geometric means for log transformed values, and the square of the mean of square root transformed values, with approximate standard deviations. Differences in variables by smoking status (current *v* ex/never), center, and north/south were examined either by analysis of variance, or by analysis of covariance when adjustment was required. Differences in distribution of categorical variables were examined by chi-squared tests, or Fisher's exact tests as appropriate

Factor analysis, a linear method of data reduction, was performed using principal factor analysis.[24] This technique reduces several original variables into fewer summary factors or principal factors and allows analyses of groupings and interrelationships of correlated variables. The variables cluster on the basis of the linear correlations between them with the consequent principal factors calculated to keep as much of the common variance that existed between the original variables as possible. For prior communality estimates, the squared multiple correlation was used. The principal factors are transformed (rotated) to enhance interpretation using the Promax oblique rotation method producing a series of "summary factors", which retain intercorrelation.[24,25] We used a variety of criteria to determine the number of factors.[26] A modification of the Kaiser-Guttman rule,[24] the scree plot,[26] and interpretability criteria were mutually agreeable in suggesting 3 factors, although retaining a fourth factor, comprising solely smoking status, was briefly considered. The factor loading of a variable on a summary factor is equal to the Pearson correlation coefficient between that variable and the summary factor. Only variables that share a ≥15% variance with a summary factor were used for interpretation corresponding to a loading factor of ≥0.40. When loading thresholds as low as 0.2 were considered,[26] findings were essentially identical. We have used quotations and capital initials for these factors hereafter in

Table 1. Basic Characteristics in Control and Case Subjects According to Geographic Location

	Controls			Cases		
	North	South	*P* Value for North-South Difference	North	South	*P* Value for North-South Difference
Age (yr)	52.7 (5.0) (n = 253)	50.5 (5.6) (n = 321)	<.00005	53.0 (5.1) (n = 207)	50.7 (5.7) (n = 261)	<.00005
Total cholesterol (mmol $\cdot L^{-1}$)	5.71 (0.99) (n = 233)	5.39 (0.94) (n = 321)	.0001	5.70 (1.24) (n = 190)	5.23 (1.11) (n = 251)	<.00005
Smokers n (% current)	59 (23.3)	90 (28.0)	.21	42 (20.3)	61 (23.3)	.44
Metabolic syndrome variables						
Body mass index (kg $\cdot m^{-2}$)	25.8 (3.1) (n = 253)	26.4 (3.2) (n = 322)	.02	27.0 (3.3) (n = 203)	26.8 (3.3) (n = 262)	.53
Insulin (pmol $\cdot L^{-1}$)	39.1 (25.3) (n = 234)	36.4 (22.8) (n = 213)	.24	42.4 (27.5) (n = 189)	53.9 (37.2) (n = 151)	.001
Proinsulin (pmol $\cdot L^{-1}$)	1.97 (1.31) (n = 231)	2.89 (1.96) (n = 219)	<.00005	2.90 (2.36) (n = 188)	4.26 (3.29) (n = 157)	<.00005
Systolic blood pressure (mm Hg)	130.2 (15.8) (n = 251)	126.2 (13.1) (n = 322)	.0009	128.6 (17.7) (n = 202)	125.8 (16.2) (n = 256)	.08
Diastolic blood pressure (mm Hg)	84.0 (8.6) (n = 251)	84.2 (8.6) (n = 322)	.77	81.8 (9.8) (n = 202)	81.1 (10.9) (n = 256)	.45
Triglyceride (mmol $\cdot L^{-1}$)	1.52 (0.60) (n = 233)	1.39 (0.61) (n = 321)	.01	1.93 (0.78) (n = 190)	1.79 (0.73) (n = 251)	.06
Inflammatory variables						
Fibrinogen (mg $\cdot dL^{-1}$)	343 (71) (n = 230)	339 (68) (n = 306)	.55	376 (86) (n = 188)	363 (98) (n = 246)	.14
C-reactive protein (mg $\cdot L^{-1}$)	0.91 (1.24) (n = 233)	1.53 (1.36) (n = 320)	<.0005	1.92 (2.62) (n = 195)	2.29 (2.16) (n = 251)	.11
Interleukin-6 (ng $\cdot mL^{-1}$)	1.29 (0.74) (n = 227)	1.20 (0.81) (n = 202)	.23	1.72 (1.18) (n = 17.4)	2.23 (1.50) (n = 159)	.0006

NOTE. Data are shown as mean (SD) (n = number of observations).

Table 2. Correlation Coefficients Between Variables in Controls and Cases

	BMI	Insulin	Proinsulin	Systolic BP	Diastolic BP	Triglyceride	Fibrinogen	CRP	IL-6
BMI	X	0.45§	0.28§	0.27§	0.25§	0.14‡	0.12*	0.21§	0.15†
Insulin	0.45§	X	0.46§	0.15†	0.13*	0.26§	0.09	0.23§	0.17†
Proinsulin	0.32§	0.38§	X	0.12*	0.08	0.23§	0.07	0.18‡	0.16†
Systolic BP	0.31§	0.26§	0.28§	X	0.71§	0.09	0.001	0.05	−0.005
Diastolic BP	0.29§	0.23§	0.23§	0.67§	X	0.10*	−0.04	0.04	−0.05
Triglyceride	0.33§	0.37§	0.37§	0.29§	0.27§	X	0.03	0.16‡	0.09
Fibrinogen	0.16§	0.05	0.05	0.15†	0.13†	0.15§	X	0.40§	0.29§
CRP	0.25§	0.20§	0.12*	0.16§	0.17§	0.23§	0.48§	X	0.45§
IL-6	0.16†	0.14†	0.06	0.14†	0.12*	0.19§	0.36§	0.39§	X

NOTE. Correlation coefficients are adjusted for center. Controls are shown below the diagonal and cases above the diagonal.
Abbreviations: BP, blood pressure; CRP, C-reactive protein; IL-6, interleukin-6.
*$P < .05$.
†$P < .01$.
‡$P < .001$.
§$P < .0005$.

order to clarify the fact that the term is being applied to these summary factors. Regression analysis was used to explore differences in a chosen factor by specified explanatory variables after adjusting for case/control and for north/south status. Correlations were very similar within individual centers to those in the complete data set, implying that such analyses were valid. To allow for comparability between β coefficients, variables were standardized. Waist-to-hip ratio was available in only 8 control subjects in Marseille and was omitted from the analyses. In San Giovanni Rotondo, problems with the shipment of a batch of samples led to data being unavailable for insulin, proinsulin, and IL-6 concentrations in the first 100 cases and controls. In consequence, complete data were available for 393 controls (204 north and 189 south) and 295 cases (152 north and 143 south). When the analyses were repeated using a data set using imputed values for missing data, results were virtually identical to those using the restricted data set.

Ethical Considerations

The study was approved by the local Ethics Committees of the 4 centers and all subjects gave informed consent.

RESULTS

The characteristics of the study subjects are shown in Table 1 according to case/control status and geographical area. Among the controls, there were highly significant differences ($P \leq .005$) between the 4 centers in all variables except IL-6 ($P = .1$). Among the cases, these between-center differences were significant ($P < .005$) for proinsulin and IL-6 and for insulin ($P = .01$), but not for CRP ($P = .17$). North-south differences explained a minority of the between-center variance for all measures except proinsulin and CRP (controls), and insulin and IL-6 (cases). At the time of investigation, the proportion of subjects on aspirin was 87.5% (cases) and 2.1% (controls), on angiotensin-converting enzyme (ACE) inhibitors 30.8% and 1.4%, β blockers 59.6% and 1.4%, and lipid-lowering agents 27.6% and 0.4%, respectively.

There were significant correlations between many of the metabolic syndrome and inflammation variables in both cases and controls. These correlations existed within and between the metabolic syndrome and inflammation variables, as well as between both and body mass index (BMI) (Table 2).

To explore the associations between the metabolic and inflammation variables, we performed factor analysis in the 393 controls with complete data using a method allowing exploration of relationships between the factors. Three summary fac-

Table 3. Results of Factor Analysis for BMI, Metabolic Syndrome, and Inflammation Variables in Controls

	Factor		
Variable	"Metabolic Syndrome"	"Inflammation"	"Blood Pressure"
Smoking	−0.18	0.32	0.06
BMI	0.60*	0.03	0.05
Triglyceride	0.45*	0.05	0.16
Insulin	0.71*	−0.11	−0.03
Proinsulin	0.50*	−0.03	0.03
Systolic blood pressure	0.06	0.009	0.74*
Diastolic blood pressure	0.04	0.006	0.73*
Fibrinogen	−0.07	0.63*	0.03
C-reactive protein	0.18	0.58*	−0.07
Interleukin-6	0.05	0.54*	0.002

NOTE. N = 393.
*Significant bleeding of variable on summary factor.

Table 4. Correlation Matrix Between Factors in Controls (n = 393) and Myocardial Infarction Cases (n = 295) With Body Mass Index as a Separate Variable

	Body Mass Index	"Metabolic Syndrome"	"Inflammation"	"Blood Pressure"
Body mass index	X	0.49	0.31	0.39
"Metabolic Syndrome"	0.54	X	0.45	0.61
"Inflammation"	0.33	0.48	X	0.16
"Blood Pressure"	0.40	0.74	0.39	X

NOTE. Coefficients for controls are shown below the diagonal, and for cases above the diagonal. Adjusted for center. All coefficients are significant at $P < .0001$.

tors resulted, representing (1) obesity/insulin/triglycerides ("Metabolic Syndrome"); (2) "Inflammation"; and (3) "Blood Pressure" (Table 3). Although smoking status loaded most strongly on the "inflammation" factor, this was not significant when a cut-off of 0.4 was considered, but was if a loading threshold of 0.2 was employed[26] (loading = 0.32). Altering the loading threshold did not influence the loading of any other variable.

There were significant correlations between the "metabolic syndrome" and "inflammation" factors in controls and cases ($r = 0.48$, $r = 0.45$, $P < .0001$). The "Metabolic Syndrome" factor also correlated with the "Blood Pressure" factor (controls $r = 0.74$, $P < .0001$; cases $r = 0.61$, $P < .0001$). The "Blood Pressure" and "Inflammation" factors were also related (controls $r = 0.39$, $P < .0001$; cases $r = 0.16$, $P < .0001$). The "Metabolic Syndrome" and "Inflammation" factor scores were similar between north and south, although the value for the "Blood Pressure" factor was significantly lower in the south ($P = .007$).

To explore the contribution of obesity to the clustering and associations of these factors, the analyses were repeated without the inclusion of BMI. Obesity related significantly to all 3 factors, but there remained significant and strong relationships between the "Inflammation" factor and each of the other factors independent of obesity (Table 4). In a multiple regression model in all subjects, with the "Inflammation" factor score as the dependent variable, the "Inflammation" factor was related to the "Metabolic Syndrome" factor and to case status ($F = 88.5$, $P < .0001$ and $F = 19.6$, $P < .001$, respectively), but not to BMI, north/south location or the "Blood Pressure" factor. In a separate analysis, with the "Metabolic Syndrome" factor score as the dependent variable, the "Inflammation" factor ($\beta = 0.24$, SE 0.03; $P < .0001$), "Blood Pressure" factor ($\beta = 0.51$, SE 0.03; $P < .0001$), south location ($\beta = 0.20$, SE 0.05; $P < .0001$), BMI ($\beta = 0.24$, SE 0.03; $P < .0001$), and case status ($\beta = 0.38$, SE 0.05; $P < .0001$) all contributed independently to its variance.

The factor scores in the controls and cases are shown in Fig 1. The mean values for "Metabolic Syndrome" factor (0.43, SE 0.049) and the "Inflammation" factor (0.49, SE 0.049) were significantly higher in the cases than in controls (0, SE 0.042; and 0, SE 0.040, respectively). As is seen in Fig 1, the higher values for the "Metabolic Syndrome" factor in the cases than the controls is not fully accounted for by the higher "Inflam-

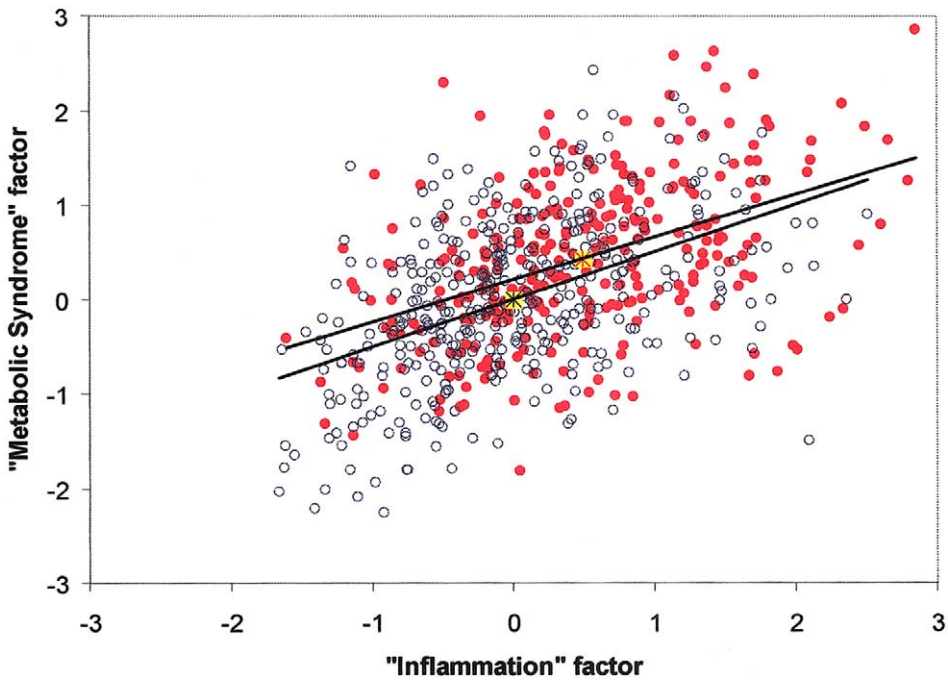

Fig 1. Scores for "Metabolic Syndrome" factor and "Inflammation" factor in cases and controls.

mation" factor score, the intercept of the regression line being significantly different between cases and controls ($P < .0001$). In a model containing case status, center, and "Inflammation" factor, with "Metabolic Syndrome" factor as the dependent variable, "Inflammation" factor explained 20.1% and case status 1.0% of the variation in "Metabolic Syndrome" factor. The "Blood Pressure" factor was similar in cases and controls. Cases treated with aspirin, β blockers, ACE inhibitors, or lipid-lowering agents showed no significant difference in any of the factor scores from untreated subjects.

DISCUSSION

We have shown powerful relationships between low-grade inflammation and measures usually ascribed to insulin resistance. Significant relationships were found between components of the metabolic syndrome and several inflammation variables. Because our data were derived from a case-control study, the interpretation is likely to be more reliable when based on relationships of variables within groups as compared with comparisons between groups, an approach we have used in this study. We have recently described strong associations between components of the metabolic syndrome and circulating markers of inflammation in a population study in healthy subjects.[4] Although it is not possible to attribute causation to associations seen in cross-sectional studies, insulin resistance and dyslipidemia might both represent consequences of actions of proinflammatory cytokines on insulin signalling and lipid metabolizing pathways.[4] Adipose tissue-generated IL-6 may play a prominent role in linking insulin resistance, endothelial dysfunction, and CHD.[10] Factor analysis, using a method permitting exploration of associations between factors,[24,25] confirmed associations between the "Metabolic Syndrome" factor and the "Inflammation" factor, this relationship being independent of obesity. In the cases, scores for both the "Metabolic Syndrome" and the "Inflammation" factors were higher than in controls, but multiple regression analysis showed that higher "Inflammation" factor score in cases is not sufficient alone to explain the higher scores for the "Metabolic Syndrome" factor. Furthermore, the strength of the relationship between the "Inflammation" and "Metabolic Syndrome" factors was similar in cases and in controls (Fig 1), suggesting that the contribution of inflammation to insulin resistance is not solely a phenomenon restricted to patients after MI.

While previous studies have used factor analysis to explore clustering of risk factors ascribed to insulin resistance,[14-18] only one has studied the link of inflammation markers with insulin resistance variables using this method.[14] Sakkinen et al[14] found univariate correlations between CRP levels and both measures of obesity and insulin resistance, but, employing a method which retains independence between factors, they found discrete factors representing insulin/glucose and inflammation. Other studies have shown that an "insulin resistance factor" is able to predict incident events independently from classic risk factors.[16-18] These studies differ from the present case-control study both in design (and consequently in analytical approach) and in the absence of measures of low-grade inflammation. It is possible that inclusion of glucose, HDL-cholesterol, and waist circumference, variables unavailable in the HIFMECH study, might have provided additional information.

Adipose tissue in vitro expresses and secretes both IL-6 and tumor necrosis factor-α,[27-29] and we have shown that up to 30% of circulating levels of IL-6 in healthy subjects might arise from adipose tissue.[12] For this reason, the associations of obesity with inflammation are to be expected. The factors, which modulate adipose tissue production of proinflammatory cytokines, remain to be explored, although our inability to find differences in levels of inflammation markers in controls between northern and southern Europe suggests that major differences in patterns of fat intake do not play an important role.

The design of the HIFMECH study was a case-control study, with MI survivors being investigated 3 months after the event. As this raises the possibility that differences between cases and controls are the consequence, rather than the cause, of the event, we have not performed such comparisons in this report. A prospective, and not a case-control, study would be needed to explore etiologic associations of insulin-like molecules and inflammation markers with MI. A number of studies have suggested that elevated concentrations of insulin predict disease.[8] Both in cross sectional[22] and prospective studies,[23,30] proinsulin was a powerful risk marker for CHD. While levels of proinflammatory cytokines and acute phase proteins increase acutely after MI,[31,32] the continuing elevation of these levels at 3 months is compatible with prospective studies implicating fibrinogen,[33] CRP,[34] and IL-6[35] as risk factors for MI. The molecular and cellular mechanisms underlying these associations and the explanation for differences between north and south Europe in their associations remain to be explored.

HIFMECH INVESTIGATORS

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REFERENCES

1. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-607, 1988

2. Mohamed-Ali V, Gould MM, Gillies S, et al: Association of proinsulin-like molecules with lipids and fibrinogen in non-diabetic subjects—Evidence against a modulating role for insulin. Diabetologia 38:1110-1116, 1995

3. Juhan-Vague I, Alessi MC, Vague P: Increased plasma plasminogen activator inhibitor 1 levels: A possible link between insulin resistance and atherothrombosis. Diabetologia 34:457-462, 1991

4. Yudkin JS, Stehouwer CDA, Emeis JJ, et al: C-reactive protein in healthy subjects: Association with obesity, insulin resistance, and endothelial dysfunction. Arterioscler Thromb Vasc Biol 19:972-978, 1999

5. Laakso M: How good a marker is insulin level for insulin resistance? Am J Epidemiol 137:959-965, 1993

6. Hermans MP, Levy JC, Morris RJ, et al: Comparison of insulin

sensitivity tests across a range of glucose tolerance from normal to diabetes. Diabetologia 42:678-687, 1999

7. Hanson RL, Pratley RE, Bogardus C, et al: Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. Am J Epidemiol 151:190-198, 2000

8. Ruige JB, Assendelft WJ, Dekker JM, et al: Insulin and risk of cardiovascular disease: A meta-analysis. Circulation 97:996-1001, 1998

9. Festa A, D'Agostino R Jr, Howard G, et al: Chronic subclinical inflammation as part of the insulin resistance syndrome: The Insulin Resistance Atherosclerosis Study (IRAS). Circulation 102:42-47, 2000

10. Juhan-Vague I, Thompson SG, Jespersen J: Involvement of the hemostatic system in the insulin resistance syndrome: A study of 1500 patients with angina pectoris. The ECAT Angina Pectoris Study Group. Arterioscler Thromb 13:1865-1873, 1993

11. Pinkney J, Coppack SW, Yudkin JS: Endothelial dysfunction: Cause of the insulin resistance syndrome? Diabetes 46:S9-S13, 1997 (suppl 2)

12. Mohamed-Ali V, Goodrick S, Rawesh A, et al: Subcutaneous adipose tissue secretes interleukin-6 but not tumour necrosis factor-α in vivo. J Clin Endocrinol Metab 82:4196-4200, 1997

13. Yudkin JS, Kumari M, Humphries S, et al: Inflammation, obesity, stress and coronary heart disease: Is interleukin-6 the link? Atherosclerosis 148:209-214, 2000

14. Sakkinen PA, Wahl P, Cushman M, et al: Clustering of procoagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. Am J Epidemiol 152:897-907, 2000

15. Meigs JB, D'Agostino RB Sr, Wilson PW, et al: Risk variable clustering in the insulin resistance syndrome. The Framingham Offspring Study. Diabetes 46:1594-1600, 1997

16. Lempiäinen P, Mykkänen L, Pyörälä K, et al: Insulin resistance syndrome predicts coronary heart disease events in elderly nondiabetic men. Circulation 100:123-128, 1999

17. Lakka HM, Laaksonen DE, Lakka TA, et al: The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 288:2709-2716, 2002

18. Pyörälä M, Miettinen H, Halonen P, et al: Insulin resistance syndrome predicts the risk of coronary heart disease and stroke in healthy middle-aged men: The 22-year follow-up results of the Helsinki Policemen Study. Arterioscler Thromb Vasc Biol 20:538-544, 2000

19. Juhan-Vahue I, Morange PE, Aubert H, et al: Plasma thrombin-activatable fibrinolysis inhibitor antigen concentration and genotype in relation to myocardial infarction in the north and south of Europe. Arterioscler Thromb Vasc Biol 22:867-873, 2002

20. de Maat MPM, Pijl H, Kluft C, et al: Consumption of black and green tea has no effect on inflammation, haemostasis and endothelial markers in smoking healthy individuals. Eur J Clin Nutr 54:757-763, 2000

21. Matthews DR, Hosker JP, Rudenski AS, et al: Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419, 1985

22. Yudkin JS, Denver AE, Mohamed-Ali V, et al: The relationship of concentrations of insulin and proinsulin-like molecules with coronary heart disease prevalence and incidence. A study of two ethnic groups. Diabetes Care 20:1093-1100, 1997

23. Yudkin JS, May M, Elwood P, et al: Concentrations of proinsulin like molecules predict coronary heart disease risk independently of insulin: Prospective data from the Caerphilly study. Diabetologia 45:327-336, 2002

24. Hatcher L: A Step-by-Step Approach to Using the SAS System for Factor Analysis and Structural Equation Modelling. Cary, NC, SAS Institute, 1994

25. Hu FB: Clustering of procoagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. Am J Epidemiol 153:717-718, 2001

26. Meigs JB: Invited commentary: Insulin resistance syndrome? Syndrome X? Multiple metabolic syndrome? A syndrome at all? Factor analysis reveals patterns in the fabric of correlated risk factors. Am J Epidemiol 152:908-911, 2000

27. Kern PA, Ranganathan S, Li C, et al: Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol 280:E745-751, 2001

28. Hotamisligil GS, Arner P, Caro JF, et al: Increased adipose tissue expression of tumor-necrosis factor-α in human obesity and insulin resistance. J Clin Invest 95:2409-2415, 1996

29. Kern PA, Saghizadeh M, Ong JM, et al: The expression of tumor necrosis factor in human adipose tissue: Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 95:2111-2119, 1995

30. Lindahl B, Dinesen B, Eliasson M, et al: High proinsulin concentration precedes acute myocardial infarction in a nondiabetic population. Metabolism 48:1197-1202, 1999

31. Miyao Y, Yasue H, Ogawa H, et al: Elevated plasma interleukin-6 levels in patients with acute myocardial infarction. Am Heart J 126:1299-1304, 1993

32. Pannitteri G, Marino B, Campa PP, et al: Interleukins 6 and 8 as mediators of acute phase response in acute myocardial infarction. Am J Cardiol 80:622-625, 1997

33. Meade TW, Ruddock V, Stirling Y, et al: Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. Lancet 342:1076-1079, 1993

34. Ridker PM, Cushman M, Stampfer MJ, et al: Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 336:973-979, 1997

35. Ridker PM, Rifai N, Stampfer MJ, et al: Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 101:1767-1772, 2000