Incidence of Obesity-Associated Cardiovascular Disease Is Related to Inflammation-Sensitive Plasma Proteins
A Population-Based Cohort Study

Gunnar Engström, Bo Hedblad, Lars Stavenow, Susanna Jonsson, Peter Lind, Lars Janzon, Folke Lindgärde

Background—Although obesity is associated with increased inflammation, it is unclear whether this accounts for the increased cardiovascular risk in obesity. This population-based study explored whether inflammation-sensitive plasma proteins (ISPs) modify the cardiovascular risk in overweight or obese men.

Methods and Results—The ISPs (fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin, ceruloplasmin) were measured in 6075 healthy men, aged 28 to 61 years. The incidences of cardiovascular events (myocardial infarction, stroke, cardiovascular deaths), cardiac events (fatal or nonfatal myocardial infarction), and stroke were followed-up over 18±4 years. High ISPs were associated with an increased cardiovascular risk in all categories of body mass index (BMI). The age-adjusted relative risks for cardiovascular events in obese men (BMI ≥30) were 2.1 (95% CI, 1.4 to 3.4), 2.4 (CI, 1.5 to 3.7), 3.7 (CI, 2.3 to 6.0), and 4.5 (CI, 3.0 to 6.6), respectively, for those with 0, 1, 2, and ≥3 ISPs in the top quartile (trend P=0.002) (reference: BMI <25 and no elevated ISP). This trend persisted after adjustments for several potential confounders (P=0.02). Incidence of cardiac events showed similar relations with the number of elevated ISPs in obese men.

Conclusion—The cardiovascular risk varies widely between obese or overweight men with high and low ISPs. Relationships with ISPs contribute to, but cannot fully explain, the increased cardiovascular risk in obese men. (Arterioscler Thromb Vasc Biol. 2004;24:1498-1502.)

Key Words: obesity ■ inflammation ■ myocardial infarction ■ stroke ■ epidemiology

Several cohort studies have shown associations between obesity and an increased incidence of cardiovascular diseases.1–4 However, the risk could be very different for individuals with the same body mass. Two recent studies2,3 showed that the cardiovascular risk among obese subjects varies substantially depending on the levels of other risk factors associated with obesity. During the past years, elevated levels of inflammation-sensitive plasma proteins (ISPs) and other inflammatory markers have emerged as important cardiovascular risk factors,5–10 and it has been shown that inflammation adds important clinical information to traditional risk factors.6,7 To what extent a low-grade inflammation accounts for the increased risk of myocardial infarction and stroke in overweight and obese men is, however, largely unknown.

Cross-sectional studies have reported associations between obesity and different inflammatory markers.11–13 It has been shown that the adipose tissue is an organ with endocrine functions,14,15 and it has been proposed that proinflammatory cytokines formed in the adipose tissue increase the hepatic synthesis of ISPs.16 However, the relation between inflammation and body mass has not been fully explored. Obesity is associated with other risk factors, eg, hypertension, dyslipidemia, and diabetes, which could increase the obesity-associated inflammation. Whether body mass is similarly associated with inflammation in men without other risk factors has not been extensively studied.

Haptoglobin, fibrinogen, orosomucoid (α1-acid glucoprotein), α1-antitrypsin, and ceruloplasmin are 5 ISPs that are used in clinical practice to measure the degree of inflammation and to differentiate between inflammatory diseases. It has been shown that human endothelial cells produce orosomucoid.17 The hepatic synthesis of these proteins is, however, the main source and the production is chiefly regulated by various proinflammatory cytokines.18,19 Several studies from the population-based cohort Malmö Preventive Study have shown that these proteins are associated with an increased incidence of cardiac events and stroke.5,6,20–22 In 2 longitudinal studies, it was shown that elevated levels of these proteins predict a large weight gain23 and the development of a high blood pressure.24
This study explored the relationship between body mass index (BMI) and the ISPs, and whether these proteins modify the cardiovascular risk in obese and overweight men.

Methods

Between 1974 and 1984, 22,444 men participated in a screening program for detection of individuals at high risk for cardiovascular diseases. Complete birth cohorts from the city of Malmö were invited. Participation rate was 71%. Determination of 5 plasma proteins was part of the program for 6,193 men. These men were randomly selected from birth cohorts examined between 1974 and 1982. After exclusion of men with a history of myocardial infarction, stroke, or cancer (according to questionnaire), 6,075 men remained. Mean age was 46.8 ± 3.7 years (range, 28 to 61). The health service authority of Malmö approved and funded the screening program. All participants gave informed consent.

Baseline Examinations

Subjects were categorized as smokers and nonsmokers. The smokers were categorized as consumers of ≤9 cigarettes per day, 10 to 19 cigarettes per day, and daily consumption of ≥20 cigarettes. BMI was calculated as weight/height$^2$ (kg/m$^2$).

Blood pressure (mm Hg) was measured twice in the right arm after a 10-minute rest. The average of 2 measurements was used. A sphygmomanometer and a rubber cuff of appropriate size were used. Use of antihypertensive medication was assessed in a questionnaire.

Physical inactivity in spare time was assessed using the question, “Are you mostly engaged in sedentary activities in spare time, for example, watching TV, reading, going to the movies?” Subjects who confirmed a doctor’s diagnosis of angina pectoris or who used nitrates were considered to have angina pectoris.

Blood samples were taken after an overnight fast. Serum cholesterol was analyzed with standard methods at the laboratory of the university hospital. Men with fasting whole blood glucose ≥6.1 mmol/L, men with 2-hour glucose values ≥10.0 mmol/L (glucose load, 30g/m² body surface area)$^{19, 26}$ and men who reported treatment for diabetes were considered diabetic patients. Serum levels of γ-glutamyltransferase (γ-GT) were analyzed with standard methods at the hospital laboratory.$^27$ The γ-GT values were log transformed because of the skewed distribution.

High alcohol consumption was assessed by means of the modified shortened version of the Michigan Alcoholism Screening Test.$^{27}$ Men with ≥2 (of 9) affirmative answers were considered to have high alcohol consumption.

Low Levels of Traditional Risk Factors

The relation between BMI and ISPs was specifically studied in men with low levels of traditional risk factors. This group was defined as nondiabetic nonsmokers without hypertension ($\geq 140/90$ or treatment), dyslipidemia (cholesterol ≥6.5 mmol/L or triglycerides ≥2.3 mmol/L), and angina.$^{22}$

ISPs

Electroimmunoassay was used to assess the plasma levels of 5 ISPs.$^{28}$ The analysis was performed consecutively at the time of study entry. The detection limits were 20 mg/L for ceruloplasmin, 50 mg/L for α1-antitrypsin, and 350 mg/L for orosomucoid, haptoglobin, and fibrinogen. The precision of the analysis had a variation of 5%.$^{28}$ We have previously shown that the correlation coefficients between the individual proteins range from 0.31 to 0.56.$^6$ We have also reported that all 5 ISPs are associated with cardiovascular disease and that the cardiovascular risk increases with the number of ISPs in the top quartile.$^6, 21$ The sample was categorized according to the number of ISPs in the top quartile. Two to 5 ISPs in the top quartile were denoted “high ISPs” and 0 to 1 ISP in the top quartile were “low ISPs.”$^{6, 21}$ The top quartiles were as follows: fibrinogen ≥4.0 g/L, orosomucoid ≥0.93 g/L, α1-antitrypsin ≥1.42 g/L, haptoglobin ≥1.76 g/L, and ceruloplasmin ≥0.36 g/L.

| Table 1. Baseline Characteristics of the Study Cohort (n=6075) |
|-------------------------|-------------------------|
| Mean age, y             | 46.8 ± 3.7              |
| Smokers, %              | 48                      |
| BMI, kg/m²              | 25.0 ± 3.3              |
| Height, m              | 1.765 ± 6.6             |
| Weight, kg             | 77.9 ± 11.6             |
| SBP, mm Hg             | 129 ± 16                |
| DBP, mm Hg             | 87 ± 10                 |
| Cholesterol, mmol/L    | 5.7 ± 1.0 (220 ± 40 mg/dL) |
| Triglycerides, mmol/L  | 1.6 ± 1.1               |
| γ-Glutamyl transferase, μkat/L | 0.74 ± 1.12             |
| Diabetes, %            | 5.4                     |
| Physical inactivity, % | 56                      |
| Angina, %              | 1.3                     |

Mean ± SD or %.

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

Follow-Up

All men were followed-up from the baseline examination until death or December 31, 1997 (mean follow-up, 18.7 ± 4.2 years). A cardiac event was defined as nonfatal myocardial infarction (code 410 according to the International Classification of Diseases, ICD, 9th revision) or death caused by ischemic heart disease (ie, ICD codes 410 to 414). Stroke (fatal or nonfatal) was defined as cases coded 430 (subarachnoid hemorrhage), 431 (intracerebral hemorrhage), 434 (ischemic stroke), or 436 (unspecified) according to the ICD. A cardiovascular event was defined as nonfatal stroke, nonfatal myocardial infarction, or death from cardiovascular disease (ICD-9 codes 390 to 448).

The Swedish Hospital Discharge Register, the Stroke register of Malmö, and the Swedish Cause of Death register were used for case retrieval.$^{6, 20, 21}$ Of the 613 cardiac events, 209 were fatal within 28 days. Of the 209 fatal events, cause of death was based on autopsy in 146 (70%), on examination in hospital before death in 50 cases, examination outside hospital before death in 6 cases, and on other sources in 7 cases. A validation study from the Swedish Hospital Discharge Register showed that the diagnosis myocardial infarction was false in only 5% of the cases.$^{31}$

Statistics

Analysis of variance was used to study the relationships between BMI and ISP levels. A general linear model was used to adjust the relations for confounders and to test the linear effects across the quartiles of BMI. Cox proportional hazards model was used for the analysis of the event rates in categories of BMI and ISP with adjustment for potential confounders.

Results

ISPs in Relation to BMI

The baseline characteristics of the cohort are presented in Table 1. The ISP levels in relation to quartiles of BMI in all men are presented in the first part of Table 2. After adjustments for smoking and other confounders, fibrinogen, haptoglobin, and orosomucoid were positively and linearly associated with BMI. α1-Antitrypsin was highest in men with low BMI. Ceruloplasmin was unrelated to BMI.

With the purpose of further exploring whether the relations between ISPs and BMI were independent of other risk factors, men with low levels of the traditional risk factors were analyzed (Table 2). As expected, the mean ISP concentrations were lower in this group. Fibrinogen, haptoglobin, and orosomucoid were still positively associated with BMI. α1-Antitrypsin and ceruloplasmin were unrelated to BMI in this subgroup.
ISPs and BMI in Relation to Incidence of Cardiovascular Diseases

The men were categorized by quartile of BMI and number of ISPs in the top quartile (0 to 1 to 2 to 5). Among men in the fourth quartile of BMI, all 3 cardiovascular end points were significantly related to the ISPs. In all quartiles of BMI, the relative risks of cardiac events were significantly higher in those with elevated ISPs as compared with those with low ISPs. High BMI (quartile 4) was significantly associated with increased cardiovascular and cardiac events rates, even in men with low ISPs (Table 3).

Obese Men

A total of 437 men were obese (BMI >30). The proportion with 2 to 5 elevated ISPs was 41.6% in obese men, as compared with 33.6% in men with BMI <25.

TABLE 3. Incidence of Cardiovascular Diseases in Relation to ISPs and Quartiles of BMI

<table>
<thead>
<tr>
<th>BMI</th>
<th>Q1 (&lt;22.7)</th>
<th>Q2 (22.7–24.6)</th>
<th>Q3 (24.6–26.0)</th>
<th>Q4 (&gt;26.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ISPs</td>
<td>High ISPs</td>
<td>Low ISPs</td>
<td>High ISPs</td>
<td>Low ISPs</td>
</tr>
<tr>
<td>N</td>
<td>998</td>
<td>522</td>
<td>1019</td>
<td>501</td>
</tr>
<tr>
<td>Age</td>
<td>46.3±4.1</td>
<td>46.6±3.7</td>
<td>46.5±3.7</td>
<td>47.3±3.8</td>
</tr>
<tr>
<td>Cardiac events, %</td>
<td>5.8</td>
<td>11.9</td>
<td>6.5</td>
<td>12.6</td>
</tr>
<tr>
<td>RR*</td>
<td>Reference</td>
<td>2.2 (1.6–3.2)</td>
<td>1.1 (0.8–1.5)</td>
<td>2.2 (1.6–3.2)</td>
</tr>
<tr>
<td>RR‡</td>
<td>Reference</td>
<td>1.7 (1.2–2.5)</td>
<td>1.0 (0.7–1.5)</td>
<td>1.5 (1.1–2.2)</td>
</tr>
<tr>
<td>Stroke, %</td>
<td>3.0</td>
<td>4.6</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>RR*</td>
<td>Reference</td>
<td>1.7 (1.0–2.9)</td>
<td>0.6 (0.4–1.1)</td>
<td>1.7 (1.02–2.9)</td>
</tr>
<tr>
<td>RR‡</td>
<td>Reference</td>
<td>1.5 (0.9–2.5)</td>
<td>0.6 (0.3–1.03)</td>
<td>1.4 (0.8–2.4)</td>
</tr>
<tr>
<td>Cardiovascular events, %</td>
<td>9.0</td>
<td>16.1</td>
<td>8.7</td>
<td>17.8</td>
</tr>
<tr>
<td>RR*</td>
<td>Reference</td>
<td>2.0 (1.5–2.7)</td>
<td>0.94 (0.7–1.3)</td>
<td>2.1 (1.5–2.7)</td>
</tr>
<tr>
<td>RR‡</td>
<td>Reference</td>
<td>1.6 (1.2–2.1)</td>
<td>0.89 (0.7–1.2)</td>
<td>1.5 (1.1–2.0)</td>
</tr>
</tbody>
</table>

*Age-adjusted relative risk (95% CI).
†P<0.05 vs men with low ISPs within the same quartile of BMI.
‡Relative risk (95% CI) adjusted for age, smoking, tobacco consumption, systolic and diastolic blood pressure, blood pressure medication, high alcohol consumption, cholesterol, triglycerides, physical inactivity, diabetes, angina, and γ-GT.
The obese group was categorized into men with 0, 1, 2, and ≥3 ISPs in the top quartile. The number of cardiovascular events were 23 (16.8%), 22 (18.6%), 20 (27.4%), and 34 (31.2%), respectively, for obese men with 0, 1, 2, and ≥3 ISPs in the top quartile (P for trend = 0.003). The numbers of cardiac events were 15 (10.9%), 15 (12.7%), 14 (19.2%), and 26 (23.9%), respectively, for normal weight and obese men. This relation remained significant after full adjustments for potential founders (P = 0.02) (Figure). The multivariate-adjusted incidence of cardiac events in obese men showed similar relations with the number of elevated ISPs (P for trend = 0.04).

**Discussion**

Many studies have reported an increased incidence of cardiovascular diseases in obese subjects. To what extent this could be related to a low-grade inflammation has been unclear. The present results show that obesity or overweight is associated with elevated levels of several ISPs. Moreover, the increased incidence of cardiovascular diseases among overweight or obese men was further elevated in those who also had elevated ISPs. It is concluded that relationships with elevated ISPs contribute to, but cannot fully explain, the increased cardiovascular risk in obese or overweight men. The results suggest that measurements of ISPs may be useful in a global risk assessment of obese men to identify those who urgently need risk factor intervention and weight reduction.

Cross-sectional studies have shown correlations between various inflammatory markers and measures of body fat. Weight loss has been associated with reduced inflammation. There could be several causes for this association. Proinflammatory cytokines formed in the adipose tissue, eg, IL-6 and tumor necrosis factor-α, could increase the hepatic synthesis of ISPs. Obesity has been associated with oxidative stress, which could increase inflammation. Other risk factors associated with obesity, eg, hyperglycemia, insulin resistance, dyslipidemia, diet, and hypertension, could also increase inflammation. In accordance with a recent case-control study of obese women, this study showed a relation between BMI and inflammation, even in men with low levels of other major risk factors. However, the reasons for the increased ISPs are unclear, and obesity per se is probably not the only explanation for an increased inflammation in obese men. It has even been shown that elevated ISPs predict a large weight gain and, thus, that a low-grade inflammation often occurs before the increased weight.

The reasons for the increased cardiovascular risk in obese men with high ISP levels are also incompletely understood. Inflammation has been implicated in the pathogenesis of atherosclerosis and unstable atherosclerotic plaque in the coronary arteries. Obesity and high ISPs have been associated with persistent platelet activation, which hypothetically could increase the incidence of cardiovascular diseases. It is also possible that the relation between inflammation and incidence of cardiovascular diseases partially is mediated through an increased incidence of other risk factors during follow-up, eg, hypertension, diabetes, and dyslipidemia. It has been shown that elevated ISPs are associated with an increased incidence of hypertension and diabetes.

Three of the ISPs showed linear associations with BMI, both in all men and in men with low levels of the traditional risk factors. This is in accordance with a previous study on BMI and C-reactive protein. However, the relationships were different for ceruloplasmin and α1-antitrypsin, and these proteins were unrelated to BMI in men with low risk factor levels. Although most inflammatory conditions are associated with elevated levels of all the ISPs in this study, it can be concluded that the relations with BMI are different for the inflammatory markers. It is noteworthy, however, that the relations with mortality and cardiovascular disease are similar for all 5 ISPs.

Whether the relationships between BMI and cardiovascular diseases should be adjusted for hypertension, diabetes, dyslipidemia, physical activity, or alcohol consumption, ie, determinants or physiological effects of obesity, is debatable. It is likely that the risk factor-adjusted relative risks in Table 3 and the Figure are overadjusted, and that the Figure therefore underestimates the true health hazards of obesity.

Men with diabetes, hypertension, and hypercholesterolemia were referred for further evaluation and treatment. Smokers were recommended to stop but were not offered any help to do so. Because these factors were more common among men with high ISPs, it is likely that this group gained most from the interventions. Another limitation is that we do not know whether the subfractions of cholesterol differed between the groups with high and low ISPs. However, the ratio of low-density lipoprotein to high-density lipoprotein is correlated to levels of triglycerides, and the results were adjusted for triglyceride levels as a proxy for dyslipidemia.

The BMI was used to assess degree of overweight. We had no information on the distribution of body fat.
studies have shown that the relationships with measures of inflammation are largely similar for different measures of overweight\cite{11,13,39} and that BMI or waist-to-hip ratio accounts for \approx 10\% to 15\% of the variance in ISP levels. It is likely that the heterogeneity in ISP levels affects the prognosis similarly for men with obesity assessed by means of the waist circumference or waist-to-hip ratio.

Obesity is associated with elevated ISPs. The cardiovascular health hazard of overweight and obesity varies widely by the level of these proteins. Relationships with elevated ISPs contribute to, but cannot fully explain, the increased cardiovascular risk in obese men.

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