Multiple Abnormalities in Glucose and Energy Metabolism and Coordinated Changes in Levels of Adiponectin, Cytokines, and Adhesion Molecules in Subjects With Metabolic Syndrome

Urpu Salmenniemi, MD; Eija Ruotsalainen, MD; Jussi Pihlajamäki, MD; Ilkka Vauhkonen, MD; Sakari Kainulainen, MD; Kari Punnonen, MD; Esko Vanninen, MD; Markku Laakso, MD

Background—Detailed metabolic defects in glucose and energy metabolism and abnormalities in a variety of cardiovascular risk factors are largely unknown in subjects with the metabolic syndrome.

Methods and Results—We characterized the metabolic syndrome in 119 nondiabetic offspring of diabetic probands. Cardiovascular risk factors, including cytokines and adhesion molecules, were measured. Insulin sensitivity was assessed by the euglycemic hyperinsulinemic clamp and indirect calorimetry; intra-abdominal fat and subcutaneous fat were assessed by CT; and maximal oxygen consumption was measured with a bicycle ergometer test. By applying factor analysis, we identified a single factor, the metabolic syndrome factor, from the following variables: 2-hour glucose, fasting insulin, body mass index, waist, HDL cholesterol, triglycerides, and mean blood pressure. Subjects with the highest factor score were defined as having the metabolic syndrome. During hyperinsulinemia, the highest factor score was associated with decreased rates of glucose oxidation and nonoxidative glucose disposal, high rates of lipid oxidation, low energy expenditure, and impaired suppression of free fatty acids during hyperinsulinemia. Furthermore, the metabolic syndrome was associated with a high amount of visceral fat, hypoadiponectinemia, a low maximum oxygen uptake, and high levels of C-reactive protein, proinflammatory cytokines, and adhesion molecules.

Conclusions—The metabolic syndrome is characterized by an excess of intra-abdominal fat, hypoadiponectinemia, insulin resistance in skeletal muscle and adipose tissue, multiple defects in glucose and energy metabolism, and elevated levels of cytokines and adhesion molecules. (Circulation. 2004;110:3842-3848.)

Key Words: adiponectin ■ cell adhesion molecules ■ cytokines ■ insulin resistance ■ metabolic syndrome X

The Metabolic Syndrome (MetS), a clustering of cardiovascular risk factors, is a powerful predictor of cardiovascular disease. When Reaven introduced this concept (“syndrome X”), he included in this constellation a clustering of abnormal glucose tolerance, dyslipidemia (low HDL cholesterol, high total triglycerides), and elevated blood pressure (BP). According to his interpretation, the underlying cause of the syndrome was insulin resistance. Recently, several other candidates for this syndrome—obesity, central obesity, microalbuminuria, high levels of proinflammatory cytokines, prothrombotic and fibrinolytic factors, and oxidative stress—have been proposed.

The importance of risk factor clustering with hyperinsulinemia as a predictor of type 2 diabetes and cardiovascular disease has been shown in many prospective studies. However, the pathophysiology of the MetS has remained unknown, although insulin resistance and visceral obesity have been proposed as underlying causes for this syndrome.

For clinical purposes, the MetS has been defined on the basis of different cutoff points of cardiovascular risk factors, a method that does not take into account the fact that cardiovascular risk factors are continuous variables. Furthermore, the components of the MetS are highly intercorrelated, and conventional statistical methods cannot be used to investigate this syndrome. Recently, factor analysis, allowing the analysis of interrelated continuous variables, has been applied in studies of the MetS.

In the present study, we characterized the MetS in the offspring of diabetic probands by applying factor analysis. Detailed metabolic and other measurements allowed us to quantify for the first time defects in glucose and energy metabolism and abnormalities in a variety of cardiovascular...
risk factors in subjects with the MetS. We also analyzed whether simple clinical and laboratory measurements (waist, insulin) are accurate enough to be used as surrogate markers for “gold standard” measurements (visceral fat evaluated by CT, insulin sensitivity evaluated by the euglycemic hyperinsulinemic clamp) to define the MetS for clinical practice.

Methods

Subjects
The subjects were healthy nondiabetic offspring of patients with type 2 diabetes. The diabetic patients (proband) were randomly selected from type 2 diabetic subjects living in the region of the Kuopio (Finland) University Hospital. Spouses of the probands had to have a normal glucose tolerance in an oral glucose tolerance test. A total of 119 offspring (1 to 3 from each family) were studied. The Ethics Committee of the University of Kuopio approved the study protocol. All study subjects gave informed consent.

Study Design
On the first day, BP was measured in subjects a sitting position after a 5-minute rest with a mercury sphygmomanometer. The average of 3 measurements was used to calculate systolic and diastolic BPs, as well as the mean BP [(2 × diastolic BP + systolic BP) / 3]. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist (at the midpoint between the lateral iliac crest and lowest rib) and hip circumference (at the level of the trochanter major) were measured to the nearest 0.5 cm. Fasting blood samples were drawn after 12 hours of fasting, followed by an oral glucose tolerance test (75 g glucose). Subjects with nondiabetic glucose tolerance were included in further studies. On the second day, after a 12-hour fast, an intravenous glucose tolerance test (IVGTT) and the hyperinsulinemic euglycemic clamp, including indirect calorimetry, were performed. A CT scan was performed to evaluate the amount of abdominal fat, and an exercise test was done to determine maximum oxygen uptake.

Metabolic Studies
An IVGTT was performed to determine the first-phase insulin secretion capacity after an overnight fast. After baseline blood collection, a bolus of glucose (300 mg/kg in a 50% solution) was given within 30 seconds into the antecubital vein. Samples for the measurement of blood glucose and plasma insulin (arterialized venous blood) were drawn at −5, 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, and 60 minutes.

After an IVGTT, the degree of insulin sensitivity was evaluated with the euglycemic hyperinsulinemic clamp technique (insulin infusion rate of 40 mU·min⁻¹·m⁻² body surface area) as previously described. Blood glucose was clamped at 5.0 mmol/L for the next 120 minutes by infusion of 20% glucose at various rates according to blood glucose measurements performed at 5-minute intervals. The mean amount of glucose infused during the last hour was used to calculate the rates of whole-body glucose uptake (WBGU).

Indirect calorimetry was performed with a computerized flow-through canopy gas analyzer system (DELTATRAC, TM Datex) as previously described. The mean value of the data during the last 20 minutes of the clamp was used to calculate glucose and lipid oxidation. The rates of nonoxidative glucose disposal during the clamp were estimated by subtracting the rates of glucose oxidation from the glucose infusion rate.

Body Composition, Fat Distribution, and Cardiopulmonary Exercise Test
Body composition was determined by bioelectrical impedance (RJL Systems) in subjects in the supine position after a 12-hour fast. Abdominal fat distribution was evaluated by CT (Siemens Volume Zoom) at the level of fourth lumbar vertebra. Subcutaneous and intra-abdominal fat (IAF) areas were calculated as previously described. The cardiopulmonary test was performed with a bicycle ergometer (Siemens Elema 380) until exhaustion. Respiratory gas exchange was analyzed continuously during the test with a computer-based system (Sensor Medic 2900, Metabolic Measurement Cart/System). The average values of oxygen uptake measured during the last 20 seconds of the exercise were used to calculate maximum oxygen uptake.

Laboratory Determinations
Blood and plasma glucose were measured by the glucose oxidase method (Glucose & Lactate Analyzer 2300 Stat Plus, Yellow Springs Instrument Co, Inc), and plasma insulin and C-peptide were determined by radioimmunoassay (Phadeseph Insulin RIA 100, Pharmacia Diagnostics AB; 125I RIA Kit, Incstar Co, respectively). Cholesterol and triglyceride levels from whole serum and from lipoprotein fractions were assayed by automated enzymatic methods (Roche Diagnostics). Serum free-fatty acids (FFAs) were determined by an enzymatic method from Wako Chemicals GmbH. Serum adiponectin was measured with an enzyme immunossay (Human Adiponectin ELISA Kit, B-bridge International Inc). Plasma concentrations of tumor necrosis factor-α (TNF-α) and cytokines (interleukin [IL]-1β, IL-1 receptor antagonist [IL-1RA], IL-6, IL-10) and serum levels of soluble adhesion molecules (intercellular adhesion molecule [ICAM-1], vascular cell adhesion molecule [VCAM-1], E-selectin, and P-selectin) were measured with high-sensitivity assay kits from R & D systems. IL-8 was measured with a kit from Biosource International. C-reactive protein (CRP) was measured with an Immulite analyzer and a DPC high-sensitivity CRP assay. Nonprotein urinary nitrogen was measured by automated Kjeldahl method.

Statistical Analysis
All data analyses were performed with SPSS 11.0 for Windows programs. The results for continuous variables are given as mean±SD and for cytokine levels and insulin response in an IVGTT as mean±SEM in the figures. The differences between the 3 groups were assessed by ANOVA for continuous variables and by the χ² test for noncontinuous variables. ANCOVA was used to adjust for family relationship (all comparisons) and other confounding factors. Variables with skewed distribution were logarithmically transformed for statistical analyses. Factor analysis was used to reduce a large set of intercorrelating variables into a smaller set of latent underlying factors as previously described. We used the principal component method for extraction of the initial components. Factors with eigenvalues ≥1 were retained, and varimax rotation was applied. Variable loadings ≥0.40 were considered significant in the interpretation of factors. The factor score from the analysis was categorized into the factor tertiles. The incremental insulin area under the insulin curve in an IVGTT was calculated by the trapezoidal method.

Results
Table 1 presents anthropometric and metabolic characteristics of the study population. Of the 119 participants, slightly more than half were women (102 study subjects had a normal glucose tolerance, 15 had impaired glucose tolerance, and 2 had impaired fasting glucose).

Cardiovascular risk factors (120-minute glucose, fasting insulin, BMI, waist, HDL cholesterol, total triglycerides, mean BP, rates of WBGU, IAF) correlated significantly, and the highest correlations were among the parameters measuring obesity (waist and BMI, r = 0.523, P < 0.001), whereas mean BP correlated only weakly with other components of the MetS (<0.40). Pearson correlation coefficient between fasting plasma insulin and the rates of WBGU during the clamp was −0.572 (P < 0.01) and between waist and IAF area was 0.700 (P < 0.01).
TABLE 1. Characteristics of the Study Subjects

<table>
<thead>
<tr>
<th></th>
<th>Men/women, n</th>
<th>55/64</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT/IFG/IGT, n</td>
<td>102/2/15</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>35.5±6.0 (25–50)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1±4.7 (17.6–44.2)</td>
<td></td>
</tr>
<tr>
<td>Waist, cm</td>
<td>88±12 (60–134)</td>
<td></td>
</tr>
<tr>
<td>Body fat, %</td>
<td>30±8 (14–51)</td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>126±11 (108–160)</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>84±9 (60–106)</td>
<td></td>
</tr>
<tr>
<td>Oral glucose tolerance test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.1±0.4 (4.1–6.4)</td>
<td></td>
</tr>
<tr>
<td>120-min Plasma glucose, mmol/L</td>
<td>6.2±1.4 (3.5–10.4)</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>46.2±22.5 (18.0–175.8)</td>
<td></td>
</tr>
<tr>
<td>120-min Insulin, pmol/L</td>
<td>245.6±195.2 (24.0–1345.2)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.90±0.87 (3.0–7.04)</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.19±0.78 (1.40–5.34)</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.27±0.28 (0.67–2.17)</td>
<td></td>
</tr>
<tr>
<td>Total triglycerides, mmol/L</td>
<td>1.13±0.60 (0.34–3.91)</td>
<td></td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

NGT indicates normal glucose tolerance; IFG, impaired fasting glucose; and IGT, impaired glucose tolerance. Data are mean±SD (range). n=119.

Table 2 presents the results of factor analyses. Model 1, including, among other cardiovascular risk factors, simple clinical measures of insulin resistance (fasting insulin) and abdominal fat (waist), resulted in one factor, the MetS factor, that explained 46.2% of the total variance. Waist (0.830) and fasting insulin (0.760) had the highest loadings. Substituting waist by IAF area assessed by CT and fasting insulin by the rates of WBGU during the clamp also resulted in one factor solution having the highest loading for IAF (0.802) (Model 2). Percentage of variance explained was quite similar to that in model 1 (43.3%). However, when IAF was replaced by body fat, a 2-factor solution was obtained. When both systolic and diastolic BPs having significant loadings (>0.4) on the second factor. We also performed factor analysis including fasting glucose, 120-minute insulin, systolic BP, and diastolic BP, in addition to variables in model 1, in the analysis. This model resulted in 4 separate factors (factor 1, glucose/insulin factor; factor 2, obesity/insulin factor; factor 3, lipid factor; factor 4, BP factor) (see Data Supplement Table I for details).

When adiponectin, CRP, ICAM, and maximal oxygen uptake also were included in the model, the 4-factor solution was obtained (adiponectin loaded with fasting glucose and insulin and lipids; CRP and ICAM with obesity, and maximal oxygen uptake with glucose and insulin) (see Data Supplement Table II for details).

Subjects were divided into the tertiles of factor scores based on model 1 (Table 2); the highest factor score tertile represented subjects having the MetS. Glucose oxidation (P<0.001, adjusted for gender) and nonoxidative glucose disposal during the clamp (P<0.001 adjusted for gender; Figure 1A) decreased and compensatory hyperinsulinemia in an IVGTT (P=0.003) increased with increasing factor score. The amount of IAF and subcutaneous fat also increased with increasing MetS factor score (Figure 1C and 1D). In contrast, adiponectin level decreased significantly (P=0.001, adjusted for gender and IAF; Figure 1B). Energy expenditure during the clamp decreased linearly among the MetS factor score tertiles (P=0.031 adjusted for gender; Figure 2), as well as maximum oxygen uptake (P<0.001 adjusted for gender). In contrast, the rates of lipid oxidation increased (P=0.001 adjusted for gender), which was also seen as a decrease in respiratory quotient (P<0.001 adjusted for gender). FFA levels during the clamp increased over the MetS factor score tertiles (P=0.003, adjusted for gender).

The associations between fasting cytokine levels and the MetS factor score tertiles are shown in Figure 3. A statistically significant increase in high-sensitivity CRP level (P<0.001, adjusted for gender and IAF) was found with increasing MetS factor score. In addition, cytokines increased (all probability values adjusted for gender and IAF) with increasing MetS factor score (IL-1β, P=0.015; IL-1RA, P=0.002; IL-6, P=0.042; IL-8, P=0.014). There were no significant differences in TNF-α and IL-10 levels after the adjustment for gender and IAF. P-selectin (P=0.056) and ICAM-1 (P=0.006) increased with increasing MetS score, whereas no change was observed in E-selectin and VCAM-1 (Figure 4).

We also compared all gender-adjusted parameters measured in those with and without MetS according to the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (NCEP ATPIII) criteria. Compared with subjects without the MetS, those with the MetS according to this definition had higher amount of IAF (P<0.001), lower rates of WBGU (P=0.001) and oxidative (P=0.002) and nonoxidative (P=0.001) glucose disposal, lower energy expenditure (P=0.040), and higher FFA levels (P=0.001) and lipid oxidation (P=0.006) during hyperinsulinemia, as well as lower adiponectin levels (P=0.002) and maximum oxygen uptake (P=0.001).

TABLE 2. Results of Factor Analyses Using Different Measurements of Insulin Sensitivity (Fasting Insulin or Rates of WBGU) and Visceral Obesity (Waist Circumference or IAF in CT)

<table>
<thead>
<tr>
<th></th>
<th>Model 1, Factor 1</th>
<th>0.532</th>
<th>0.670</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 2, Factor 1</td>
<td>0.574</td>
<td>0.666</td>
</tr>
<tr>
<td>120-min Plasma blood</td>
<td>0.532</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (log)</td>
<td>0.781</td>
<td>0.830</td>
<td>0.637</td>
</tr>
<tr>
<td>BMI</td>
<td>0.646</td>
<td>0.719</td>
<td>0.637</td>
</tr>
<tr>
<td>Waist</td>
<td>0.460</td>
<td>0.802</td>
<td>0.678</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.502</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total triglycerides, mmol/L</td>
<td>0.502</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean BP</td>
<td>0.502</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBGU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAF in CT (log)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance explained, %</td>
<td>46.2</td>
<td>43.3</td>
<td></td>
</tr>
</tbody>
</table>
Discussion
Our study demonstrated that subjects with the MetS have multiple defects in glucose and energy metabolism, an excess of IAF, and hypoadiponectinemia. Furthermore, high levels of cytokines and adhesion molecules were associated with the MetS, indicating that low-grade inflammation and endothelial dysfunction are essential findings in subjects with the MetS.

Factor analysis is a particularly useful statistical method in studies of highly intercorrelating variables, as is the case with the putative components of the MetS. Our study showed that one factor, the MetS factor, explained almost half of the total variance among the variables included in statistical analysis. Furthermore, we demonstrated for the first time that fasting insulin and waist circumference gave results similar to insulin sensitivity measured directly by the hyperinsulinemic euglycemic clamp and IAF assessed by CT. These results indicate that fasting insulin level and waist can reliably be used to define the MetS for clinical practice.

Results of factor analysis, yielding only one factor for the MetS, differ somewhat from previous studies. Most studies have yielded 2 to 4 factors. However, the finding of an obesity-hyperinsulinemia factor is rather consistent throughout different studies, and in most cases, this factor has also included dyslipidemia (HDL cholesterol and triglycerides). A separate BP factor having high loadings for systolic and diastolic BPs has been a rather consistent finding. However, almost all these analyses have included both systolic and diastolic BPs. When we...
repeated statistical analyses similarly, we also ended up with 2 separate factors. Furthermore, when we included 0- and 120-minute glucose and 120-minute insulin levels in the model, we obtained 4 separate factors often reported in previous studies.

Presenting the results of factor analysis as factor scores gave us an opportunity to obtain important information on metabolic abnormalities associated with the MetS defined as the highest tertile of the factor score. According to the NCEP ATPIII criteria the prevalence of the MetS in our study was 10.9% in men and 9.2% in women. All 13 men and 9 of 11 women who had the MetS according to the NCET ATPIII criteria belonged in the highest MetS factor score tertile, indicating that the NCEP ATPIII criteria are quite specific for the MetS but that their sensitivity is likely to be rather low.

Our novel findings were that during hyperinsulinemia the MetS was associated with reduced rates of glucose oxidation and nonoxidative disposal, high rates of lipid oxidation, low energy expenditure, and impaired suppression of FFAs. Furthermore, the MetS was associated with a low adiponectin level, a high amount of IAF and subcutaneous fat, low maximum oxygen uptake, and high levels of CRP, proinflammatory cytokines, and adhesion molecules.

The tight link between insulin resistance and visceral fat in the MetS seems to be the basis of this syndrome, although we cannot conclude which is the primary abnormality. In addi-

Figure 3. Fasting CRP (A) and cytokine levels (B–F) according to factor score tertiles (I=lowest, II=middle, III=highest) derived from factor analysis. Probability values are unadjusted.
tion to skeletal muscle, we observed insulin resistance in adipose tissue, because hyperinsulinemia was not able to suppress FFAs among subjects with MetS. Consequently, lipid oxidation was significantly elevated, which could, at least in part, be responsible for low rates of glucose oxidation during hyperinsulinemia. Impaired suppression of FFAs during hyperinsulinemia in subjects with the MetS contributes to elevated production of VLDL particles in the liver and thus hypertriglyceridemia. However, causes of hypertriglyceridemia in the MetS are likely to be multifactorial, and other factors, in addition to the FFA flux into the liver, probably contribute to the dyslipidemia observed in these subjects.

Lower energy expenditure during the hyperinsulinemic clamp in subjects with the MetS is a novel finding. This finding may indicate that subjects with this syndrome have a lower increase in meal-induced thermogenesis and thus a tendency to gain weight. In addition, low energy expenditure during hyperinsulinemia could indicate central insulin resistance.

Adipose tissue secretes a variety of molecules and adipocytokines, including TNF-α, IL-6, and adiponectin. Adiponectin is produced abundantly in adipocytes, and in subjects with an excess of IAF, adiponectin levels are low. High adiponectin level correlates with high insulin sensitivity. We found that the MetS was associated with a high amount of IAF, a low adiponectin level, and elevated levels of cytokines and adhesion molecules. Adiponectin inhibits the expression of ICAM-1, VCAM-1, and E-selectin and has several antiatherogenic and antiinflammatory properties. Thus, hypoadiponectinemia can be responsible for endothelial damage and a low-grade systemic chronic inflammatory state.

Previous studies have shown that CRP, IL-6, and TNF-α predict type 2 diabetes and coronary heart disease. In our study, the most marked elevations were found in IL-1RA and IL-1β, whereas TNF-α did not differ between the factor score tertiles. Therefore, conventionally determined cytokines, TNF-α and IL-6, may not be the best markers for the MetS. P-selectin and ICAM-1 were also associated with the MetS, whereas E-selectin and VCAM-1 were not. The association of adhesion molecules with the MetS is logical because they have a close interaction with proinflammatory cytokines. Adhesion molecule expression is induced by proinflammatory cytokines such as IL-1β, TNF-α, and CRP produced by the liver in response to IL-6.

In conclusion, our findings add new information for the understanding of metabolic abnormalities in the MetS. Our results show for the first time that insulin resistance in people with the MetS is seen not only in skeletal muscle but also in adipose tissue, leading to multiple defects in glucose and energy metabolism, leading to multiple defects in glucose and energy metabolism, hypoadiponectinemia, and elevated levels of proinflammatory cytokines and adhesion molecules. These results give further evidence that the MetS is an important risk factor for cardiovascular disease, but follow-up studies are needed to confirm this hypothesis.

![Figure 4](http://circ.ahajournals.org/)

Figure 4. Fasting adhesion molecule levels (A–D) according to factor score tertiles (I=lowest, II=middle, III=highest) derived from factor analysis. Probability values are unadjusted.

A  
P-Selectin

B  
E-Selectin

C  
ICAM

D  
VCAM

Probability values are unadjusted.
Acknowledgments

This study was supported in part by grants to Dr Laakso from the Academy of Finland, the Diabetes Research Foundation, and the European Union (QLG1-CT-1999-00674).

References

Multiple Abnormalities in Glucose and Energy Metabolism and Coordinated Changes in Levels of Adiponectin, Cytokines, and Adhesion Molecules in Subjects With Metabolic Syndrome
Urpu Salmenniemi, Eija Ruotsalainen, Jussi Pihlajamäki, Ilkka Vauhkonen, Sakari Kainulainen, Kari Punnonen, Esko Vanninen and Markku Laakso

_Circulation._ 2004;110:3842-3848; originally published online December 13, 2004; doi: 10.1161/01.CIR.0000150391.38660.9B
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/25/3842

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2004/12/20/01.CIR.0000150391.38660.9B.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/