Adiponectin, Cardiac Hypertrophy, and Hypertension

Adiponectin Level and Left Ventricular Hypertrophy in Japanese Men

Hirotsugu Mitsuhashi, Hiroshi Yatsuya, Koji Tamakoshi, Kunihiro Matsushita, Rei Otsuka, Keiko Wada, Kaichiro Sugiura, Seiko Takefuji, Yo Hotta, Takahisa Kondo, Toyoaki Murohara, Hideaki Toyoshima

Abstract—A recent study has demonstrated that adiponectin inhibited hypertrophic signaling in the myocardium of mice, implying that a decrease in the blood adiponectin level could cause cardiac muscle hypertrophy. We hypothesized that a relationship might exist between the serum adiponectin level and electrocardiographically diagnosed left ventricular hypertrophy (ECG-LVH), and we examined this hypothesis by epidemiological study of 2839 Japanese male workers who were not taking medications for hypertension. ECG-LVH was defined as meeting Sokolow–Lyon voltage criteria and/or Cornell voltage-duration product. The subjects were categorized by tertiles of serum adiponectin level, and a multivariate logistic regression analysis was conducted relating left ventricular hypertrophy to adiponectin tertiles adjusting for potential confounding factors. Prevalence of ECG-LVH in the studied sample was 16.7%. Adiponectin ranged from 1.0 to 5.0 μg/mL in the lowest category and from 7.4 to 30.6 μg/mL in the highest. Compared with subjects in the highest adiponectin category, those in the lowest one had a significantly higher prevalence of ECG-LVH independent of age, body mass index, and systolic blood pressure with an odds ratio of 1.50 and a 95% CI of 1.16 to 1.94. Further adjustment for high-density lipoprotein cholesterol, triglyceride, and insulin resistance did not change the association (odds ratio: 1.68; 95% CI: 1.28 to 2.21; P<0.001). Similar results were obtained when different criteria for ECG-LVH were used or when subjects were stratified by blood pressure or body mass index. Adiponectin concentration was inversely and independently associated with ECG-LVH in Japanese men.

(Hypertension. 2007;49:1448-1454.)

Key Words: adiponectin ■ left ventricular hypertrophy ■ electrocardiography ■ epidemiologic study ■ Japanese

Adiponectin is a serum protein of 30 kDa, which is a member of the complement factor C1q family and is produced and secreted by adipose tissue.1 Its associations with cardiac risk factors, such as metabolic syndrome, type 2 diabetes, and hypertension, have been described.2–4 One recent experimental study demonstrated that adiponectin inhibited hypertrophic signaling in the myocardium by inhibiting extracellular signal regulated kinase and activating adenosine monophosphate-activated protein kinase in mice.5

Left ventricular hypertrophy (LVH) is characterized by an increase in chamber mass produced largely by an increase in the size of cardiomyocytes.6 The determinants of LVH include age, elevated blood pressure (BP), obesity, and insulin resistance.7 LVH diagnosed by ECG, as well as echocardiographically diagnosed LVH, has been known to be associated with increased risk of cardiovascular morbidity and mortality.8–13 The prevalence of ECG-LVH in Japan increased from 15.3% in 1990 to 19.7% in 2000,14,15 which warrants an investigation of LVH in relation to its determinants aiming at reducing cardiovascular morbidity and mortality.

Recently, a case–reference study enrolling a small sample mainly including hypertensive patients reported the inverse association between echocardiographically determined left ventricular mass index and blood adiponectin level.16 However, hypertensive states may have confounded the association, because hypertension could cause both LVH and reduction of adiponectin concentration via arteriosclerosis. In addition, some antihypertensive agents are known to reduce left ventricular wall thickness and also to increase adiponectin levels, further complicating the association.17–19 To gain epidemiological insight into the pathogenesis of LVH in relation to adiponectin, it is necessary to examine the association in healthy individuals not taking medication for hypertension that could influence LVH and adi-
Adiponectin concentration. Therefore, we conducted a large-scale epidemiological study in Japanese male workers, examining the association between blood adiponectin level and LVH. We also investigated the association while stratifying subjects by BP level that might confound the association.

Methods

Study Population
This study uses baseline data collected in 2002 for a workers’ cohort study on cardiovascular diseases in Aichi, Japan. The present analysis included 3168 men aged 35 to 66 years old with available information on weight, height, BP, and ECG, as well as adiponectin and other biomarker concentrations. Written consent was obtained from each person, and the study protocol was approved by the ethics review committee of the Nagoya University Graduate School of Medicine. We excluded 259 subjects who had been on medical treatment for hypertension from the initial 3168 subjects. Because the diagnosis of LVH by ECG is not reliable in subjects with myocardial infarction or complete bundle branch block, 5 subjects with ECG findings relevant for a history of myocardial infarction and 65 subjects who had ECG findings of complete bundle branch block were excluded. Finally, 2839 men were included in the present analysis.

ECG
Standard 12-lead ECG was recorded at a paper speed of 25 mm/s and 1 mV/cm of standardization. Every ECG was read independently by 2 experienced investigators blinded to the information of the health checkups according to a slightly modified 1982 revision of the Minnesota Code. QRS duration and voltage of R waves in leads aVL (RaVL), V5 (RV5), and V6 (RV6), and S waves in leads V1 (SV1) and V3 (SV3) were measured manually by a third cardiologist. LVH in this study was defined using the following 2 criteria, which the Losartan Intervention for Endpoint Reduction in Hypertension study group used20,21: the Cornell product obtained as Cornell voltage (RaVL + SV5)×QRS duration >2440 mm×ms and Sokolow–Lyon voltage (SV1 + RV5 or RV6) >38 mm. Subjects meeting either or both of these criteria were defined as having ECG-LVH.20,21

To examine the effect of change in the definition of ECG-LVH, 4 other ECG-LVH criteria were set as follows: (1) Minnesota Code, 2.6 mV; R in leads I, II, III, or RaVF; (2) Cornell voltage (RaVL + SV5) >38 mm; (3) Cornell product used20,21: the Cornell product obtained as Cornell voltage (RaVL + SV5)×QRS duration >2440 mm×ms and Sokolow–Lyon voltage (SV1 + RV5 or RV6) >38 mm. Subjects meeting either or both of these criteria were defined as having ECG-LVH.20,21

Adiponectin Concentration
Venous blood samples were withdrawn from each subject after ≥8 hours fasting. The samples were stored at −80°C until assay. Serum concentration of adiponectin was measured by an ELISA system (Otsuka Pharmaceutical Co, Ltd). Interassay coefficients of variation of adiponectin were <8.6%.

Clinical Features and Other Laboratory Measurements
Height and weight were measured in standing position. Body mass index (BMI) was calculated as weight in kilograms divided by the length in meters divided by 1.2 mV; SV5; 1.5 mV <2.0 mV; SV1 >2.0 mV, or RaVF >1.2 mV) or 3-3 (1.5 mV <2.0 mV; SV1 + RV5; or RV6 >3.5 mV)22; (2) Sokolow–Lyon voltage >38 mm; (3) Cornell voltage (RaVL + SV5) ≥28 mm20,21; and (4) Cornell product >2440 mm×ms.23

Statistical Analysis
Because distribution of adiponectin, TG, HDL-chol, and HOME-R were skewed, log-transformed value was used for the subsequent analyses to approximately normalize the distributions. These log-transformed continuous variables were expressed as geometric means with the 95% CIs. Other continuous variables were expressed as means with the 95% CI. Subjects were divided into 3 categories by tertiles of serum adiponectin level. Comparisons of continuous variables among 3 categories were performed by 1-way ANOVA and categorical variables by χ² test. The odds ratios (ORs) and their 95% CIs of LVH according to adiponectin levels were calculated by multivariate logistic regression analysis adjusting for potential confounding factors, that is, age, BMI, TG, HDL-chol, HOME-R, and either SBP or pulse pressure.24 The same analyses were conducted

| TABLE 1. Baseline Characteristics of Study Sample According to Adiponectin Tertiles |
|------------------|------------------|------------------|
| Adiponectin Ranges, µg/mL | Lowest (n=953) | Middle (n=940) | Highest (n=946) |
| Age, y | 48.1 (47.6 to 48.5) | 48.1 (47.6 to 48.5) | 48.5 (48.0 to 49.0) |
| BMI, kg/m² | 24.2 (24.0 to 24.3) | 23.3 (23.1 to 23.4) | 22.1 (21.9 to 22.3) |
| SBP, mm Hg | 127.9 (127.3 to 129.1) | 127.9 (126.9 to 128.8) | 126.1 (125.2 to 127.0) |
| Adiponectin, µg/mL | 3.72 (3.66 to 3.78) | 6.1 (6.06 to 6.14) | 9.86 (9.71 to 10.02) |
| T-chol, mmol/L | 5.54 (5.49 to 5.60) | 5.47 (5.41 to 5.53) | 5.33 (5.28 to 5.39) |
| TG, mmol/L | 3.78 (3.65 to 3.92) | 3.16 (3.05 to 3.27) | 2.38 (2.30 to 2.45) |
| HDL-chol, mmol/L | 1.27 (1.25 to 1.29) | 1.4 (1.38 to 1.43) | 1.58 (1.55 to 1.66) |
| FBG, g/mL | 5.42 (5.35 to 5.48) | 5.31 (5.26 to 5.36) | 5.27 (5.21 to 5.33) |
| HOME-R | 1.92 (1.83 to 2.02) | 1.57 (1.49 to 1.65) | 1.24 (1.18 to 1.31) |
| LVH, n (%) | 189 (19.8) | 141 (15.0) | 144 (15.2) |

* Differences in mean values were tested by 1-way ANOVA. Differences in the proportions were tested by χ² test.
TABLE 2. Association Between Adiponectin Tertiles and Prevalence of LVH by Multivariate Logistic Regression Analysis (n=2839)

<table>
<thead>
<tr>
<th>Adiponectin Tertiles</th>
<th>Crude Age, BMI, and SBP Adjusted</th>
<th>Multivariate Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest</td>
<td>OR 1.38 (1.09 to 1.75) P=0.008</td>
<td>OR 1.50 (1.16 to 1.94) P&lt;0.001</td>
</tr>
<tr>
<td>Middle</td>
<td>OR 0.98 (0.76 to 1.27) P=0.89</td>
<td>OR 1.00 (0.77 to 1.30) P=0.98</td>
</tr>
<tr>
<td>Highest</td>
<td>OR 1.00 (0.76 to 1.27) P=0.89</td>
<td>OR 1.00 (0.77 to 1.30) P=0.98</td>
</tr>
</tbody>
</table>

In samples stratified by BP and BMI. High BP was defined as SBP of ≥140 mm Hg and/or DBP of ≥90 mm Hg. Overweight was defined as BMI of ≥25 kg/m². In addition, we presented and compared the prevalence of ECG-LVH among the following 4 groups: normal weight with normal BP, normal weight with high BP, overweight with normal BP, and overweight with high BP. A P<0.05 was considered statistically significant. All of the analyses and calculations were performed using the statistical package SPSS 12.0 J for Windows.

Results

The mean age of the present sample was 48.2 years old. BMI ranged from 15.0 to 35.8 kg/m², and the mean was 23.7 kg/m². SBP ranged from 84 to 191 mm Hg, and the mean was 127.4 mm Hg. Serum adiponectin level ranged from 1.0 to 35.8 kg/m², and the mean was 23.7 kg/m². In addition, we presented and compared the prevalence of ECG-LVH among the following 4 groups: normal weight with normal BP, normal weight with high BP, overweight with normal BP, and overweight with high BP. A P<0.05 was considered statistically significant. All of the analyses and calculations were performed using the statistical package SPSS 12.0 J for Windows.

Prevalence of ECG-LVH was significantly higher in the high-BP group than in normal-BP group in both BMI categories (Figure). However, there was no statistically significant difference in the prevalence of ECG-LVH between the BMI groups.

In the category with the lowest adiponectin, multivariate-adjusted OR by which subjects had LVH was 1.65 (95% CI: 1.18 to 2.30; P=0.003) and 1.75 (95% CI: 1.07 to 2.86; P=0.027) in the normal-BP and high-BP groups, respectively (Table 3). The same analyses were conducted when the sample was divided into 2 groups depending on BMI (Table 4). In the normal-BMI group, the OR in the lowest adiponectin category was 1.45 (95% CI: 1.07 to 1.98; P=0.018). In the overweight group, the OR was 1.65 (95% CI: 0.95 to 2.86; P=0.074). Changing the definition of ECG-LVH did not substantially influence the present findings (Table 5).

Discussion

Our study revealed the significant inverse and independent association of LVH determined electrocardiographically with the serum adiponectin level in Japanese men. Although
We have here demonstrated the association observed in a small sample mainly composed of hypertensive patients.\textsuperscript{16} We excluded subjects on medical treatment for hypertension,\textsuperscript{17,18} Hypertension is acknowledged as a major determinant of LVH,\textsuperscript{30} and in the present study extended the current understanding of the association was independent of age, the degree of obesity evaluated by BMI, BP or pulse pressure, lipid parameters, and insulin resistance and lipids.\textsuperscript{24,26–29} This finding would be consistent with the experimental studies, which showed that adiponectin-knockout mice developed enhanced myocardial fibrosis and injection of adiponectin to LVH mice significantly reduced LVH.\textsuperscript{5} The present study extended the current understanding of the association observed in a small sample mainly composed of hypertensive patients.\textsuperscript{16} We have here demonstrated the association in more detail in a large sample of apparently healthy individuals and also revealed that the association was independent of age and the degree of obesity evaluated by BMI, BP or pulse pressure, lipid parameters, and index of insulin resistance.

We excluded subjects on medical treatment for hypertension and examined this association in a normal-BP subgroup in which subjects with elevated BP were also excluded to minimize the confounding effects of hypertension or medications for hypertension.\textsuperscript{17,18} Hypertension is acknowledged to be a major determinant of LVH,\textsuperscript{30} and in the present study it increased the prevalence of LVH approximately twice compared with normotensive subjects (Figure). Hypertension could cause or have an association with decreased adiponectin concentration as well.\textsuperscript{4} Thus, the present findings suggest that adiponectin may be causally associated with LVH, although this study is cross-sectional.

On the other hand, compared with the category with the highest adiponectin, in the category with an intermediate amount of adiponectin, the OR of LVH was not statistically significantly different from unity. In an additional analysis in which the higher 2 adiponectin categories were combined, the lowest tertile was significantly associated with a higher prevalence of LVH with similar magnitude (OR: 1.63; 95% CI: 1.30 to 2.04; \( P<0.001 \)). In a study of Japanese adults, a clinical entity of hypoadiponectinemia (<4.0 \( \mu \)g/mL) was suggested in relation to metabolic syndrome.\textsuperscript{31} The lowest adiponectin category in the present study included subjects whose adiponectin levels were <4.0 \( \mu \)g/mL. Taken together, the findings imply that a threshold adiponectin level to induce LVH may exist; thus, only very low levels of adiponectin may have adverse effects related to LVH. Further study is warranted to examine whether such a threshold exists.

Although elucidating the mechanism by which adiponectin might prevent LVH is beyond the scope of the present study, one possibility might be that adiponectin has the ability to suppress hypertrophic remodeling of the myocardium. Adiponectin directly inhibited hypertrophic signaling in the myocardium by activating adenosine monophosphate-activated protein kinase, which activates eukaryotic elongation factor-2 kinase and inhibits protein synthesis of cardiac myocytes.\textsuperscript{5,27,32}

In the present study, we used ECG in the diagnosis of LVH, and ECG-LVH was defined as a finding that complied with the Sokolow–Lyon voltage and/or the Cornell product, which were reported to have significantly higher sensitivity (40% to 50%) and specificity (\( \approx 95\% \)) among various criteria for diagnosis of LVH, ECG has been widely used in clinical practice and periodic health checkups because of its availability and low cost and is still considered the standard method for screening of LVH.\textsuperscript{25} ECG is also used in epidemiological studies enrolling large populations, and ECG-LVH has been confirmed as one of the risk factors of cardiovascular morbidity and mortality.\textsuperscript{11,25} Assuming the cardiovascular morbidity and mortality could cause or have an association with decreased adiponectin concentration as well.\textsuperscript{4} Thus, the present findings suggest that adiponectin may be causally associated with LVH, although this study is cross-sectional.

The major finding of the present study was the significant relationship between serum adiponectin and ECG-LVH in apparently healthy individuals with normal and high BP. The association was independent of age, the degree of obesity, variability in BP, and other potential confounding factors, including insulin resistance and lipids.\textsuperscript{24,26–29} This finding would be consistent with the experimental studies, which reported that adiponectin-knockout mice developed enhanced concentric cardiac hypertrophy, and injection of adiponectin to LVH mice significantly reduced LVH.\textsuperscript{5} The present study extended the current understanding of the association observed in a small sample mainly composed of hypertensive patients.\textsuperscript{16} We have here demonstrated the association in more detail in a large sample of apparently healthy individuals and also revealed that the association was independent of age and the degree of obesity evaluated by BMI, BP or pulse pressure, lipid parameters, and index of insulin resistance.

We excluded subjects on medical treatment for hypertension and examined this association in a normal-BP subgroup in which subjects with elevated BP were also excluded to minimize the confounding effects of hypertension or medications for hypertension.\textsuperscript{17,18} Hypertension is acknowledged to be a major determinant of LVH,\textsuperscript{30} and in the present study it increased the prevalence of LVH approximately twice compared with normotensive subjects (Figure). Hypertension could cause or have an association with decreased adiponectin concentration as well.\textsuperscript{4} Thus, the present findings suggest that adiponectin may be causally associated with LVH, although this study is cross-sectional.

The major finding of the present study was the significant relationship between serum adiponectin and ECG-LVH in apparently healthy individuals with normal and high BP. The association was independent of age, the degree of obesity, variability in BP, and other potential confounding factors, including insulin resistance and lipids.\textsuperscript{24,26–29} This finding would be consistent with the experimental studies, which reported that adiponectin-knockout mice developed enhanced concentric cardiac hypertrophy, and injection of adiponectin to LVH mice significantly reduced LVH.\textsuperscript{5} The present study extended the current understanding of the association observed in a small sample mainly composed of hypertensive patients.\textsuperscript{16} We have here demonstrated the association in more detail in a large sample of apparently healthy individuals and also revealed that the association was independent of age and the degree of obesity evaluated by BMI, BP or pulse pressure, lipid parameters, and index of insulin resistance.

We excluded subjects on medical treatment for hypertension and examined this association in a normal-BP subgroup in which subjects with elevated BP were also excluded to minimize the confounding effects of hypertension or medications for hypertension.\textsuperscript{17,18} Hypertension is acknowledged to be a major determinant of LVH,\textsuperscript{30} and in the present study it increased the prevalence of LVH approximately twice compared with normotensive subjects (Figure). Hypertension could cause or have an association with decreased adiponectin concentration as well.\textsuperscript{4} Thus, the present findings suggest that adiponectin may be causally associated with LVH, although this study is cross-sectional.

On the other hand, compared with the category with the highest adiponectin, in the category with an intermediate amount of adiponectin, the OR of LVH was not statistically significantly different from unity. In an additional analysis in which the higher 2 adiponectin categories were combined, the lowest tertile was significantly associated with a higher prevalence of LVH with similar magnitude (OR: 1.63; 95% CI: 1.30 to 2.04; \( P<0.001 \)). In a study of Japanese adults, a clinical entity of hypoadiponectinemia (<4.0 \( \mu \)g/mL) was suggested in relation to metabolic syndrome.\textsuperscript{31} The lowest adiponectin category in the present study included subjects whose adiponectin levels were <4.0 \( \mu \)g/mL. Taken together, the findings imply that a threshold adiponectin level to induce LVH may exist; thus, only very low levels of adiponectin may have adverse effects related to LVH. Further study is warranted to examine whether such a threshold exists.

Although elucidating the mechanism by which adiponectin might prevent LVH is beyond the scope of the present study, one possibility might be that adiponectin has the ability to suppress hypertrophic remodeling of the myocardium. Adiponectin directly inhibited hypertrophic signaling in the myocardium by activating adenosine monophosphate-activated protein kinase, which activates eukaryotic elongation factor-2 kinase and inhibits protein synthesis of cardiac myocytes.\textsuperscript{5,27,32}

In the present study, we used ECG in the diagnosis of LVH, and ECG-LVH was defined as a finding that complied with the Sokolow–Lyon voltage and/or the Cornell product, which were reported to have significantly higher sensitivity (40% to 50%) and specificity (\( \approx 95\% \)) among various criteria for diagnosis of LVH.
when echocardiography-diagnosed LVH was used as the gold standard.\textsuperscript{21,23,33} This criterion is known to be influenced by BMI, that is, the sensitivity has been reported to be lower in subjects with higher BMI because of the waning QRS voltage in such individuals. Waning of QRS voltage has been reported to occur in those subjects because the distance between the electrode and the left ventricle tends to become greater.\textsuperscript{20,33–37} This may be because of the increment of adipose mass and clockwise cardiac rotation, which were frequently observed characteristics of overweight and obese subjects.\textsuperscript{20,38,39} We, thus, conducted an analysis to investigate whether clockwise rotation might influence the result. OR was similar even when clockwise rotation was added to the adjustment factors, which were used in the analysis model on Table 4 in the overweight group (OR: 1.66; 95% CI: 0.95 to 2.90; P=0.075). However, because the mean BMI in the present sample was relatively low (23.7 kg/m\textsuperscript{2}), the low sensitivity would not have caused significant distortion in the association between adiponectin and LVH. Furthermore, we enhanced the reliability of the results by obtaining a similar finding when different diagnostic criteria were used.

Nevertheless, it may still be argued that only a borderline significant association was obtained in the overweight group, although the association was significant in the normal-BMI group. As stated earlier, underestimation of the prevalence of ECG-LVH might have attenuated the significance, although its magnitude was unknown. However, the point estimate of OR, which would represent the strength of the association, was greater in the overweight group than in the normal-BMI group. Thus, we speculated that a lack of statistical power because of a limited number of overweight subjects would be a major reason behind the borderline significance. In the future, studies with echocardiography to diagnose LVH are warranted, especially in overweight and obese subjects, to confirm the present findings.

Although we have adjusted for the degree of obesity by using BMI, there may be a possibility that adjustment with other measures of obesity, such as waist circumference or waist/hip ratio, alters the present association. However, BMI is known to be a useful predictor of morbidity and mortality and is highly correlated with waist circumference in Japanese subjects.\textsuperscript{40–42} BMI, as well as waist circumference, has been reported to be significantly associated with LVH to the same degree.\textsuperscript{43} They also had similar correlation coefficients with the adiponectin level.\textsuperscript{44} Because renal function has been reported to be associated with LVH and adiponectin,\textsuperscript{45,46} mild renal impairment may also be a potential confounding factor. We conducted another logistic regression analysis, further adjusting for serum creatinine level in a subsample whose creatinine level was available (n=2589) and found almost identical results (OR by which subjects had LVH in the lowest category against the highest one: 1.67; P<0.001).

We have excluded medically treated hypertensive patients and further statistically controlled the confounding effects of hypertension by adjusting for BP measured at the annual health checkup. We also conducted a stratified analysis by BP and found similar results in both strata. Because ambulatory or home BP measurements may detect other types of hypertension, such as out-of-clinic hypertension and home hypertension, which have also been associated with LVH,\textsuperscript{30,47} future studies should obtain these measurements to confirm the present findings. In conclusion, the adiponectin level was inversely associated with ECG-LVH in Japanese men independent of age, BP, obesity, lipids, and insulin resistance.

**Perspectives**

Adiponectin might be causally associated with LVH independent of age, BP, the degree of obesity, lipids, and insulin resistance. The findings should first be confirmed by studies with echocardiography. Further investigations in women, other ethnic groups, or other populations with different body habitus should also be conducted. Ideally, a prospective cohort study or interventional study should be conducted to examine whether increasing adiponectin levels in LVH patients may regress LVH in the future.

**Sources of Funding**

This work was supported by grants to H.T. (17390185), H.Y. (17790384), and K.T. (18590594) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and a research grant from the Japan Atherosclerosis Prevention Fund.

**Disclosures**

None.

**References**

Mitsuhashi et al. Adiponectin and LVH in Japanese Men

1453


Adiponectin Level and Left Ventricular Hypertrophy in Japanese Men
Hirotsugu Mitsuhashi, Hiroshi Yatsuya, Koji Tamakoshi, Kunihiro Matsushita, Rei Otsuka, Keiko Wada, Kaichiro Sugiura, Seiko Takefuji, Yo Hotta, Takahisa Kondo, Toyoaki Murohara and Hideaki Toyoshima

Hypertension. 2007;49:1448-1454; originally published online April 9, 2007;
doi: 10.1161/HYPERTENSIONAHA.106.079509

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/49/6/1448