Leptin and heart sympathetic activity in normotensive obese and non-obese subjects

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Autonomic nervous system; Obesity.

Background. **In rats leptin increases sympathetic activity, and an inhibitory effect on leptin synthesis and release has been demonstrated for the catecholamines, both in adipocyte cell cultures and in healthy experimental animals. The aim of this study was to evaluate the relationship between leptin and heart sympathetic activity as well as changes in leptin levels after the administration of drugs that modify sympathetic activity.**

Methods. **We performed a randomized, blinded, before-after trial in 81 normotensive obese and non-obese subjects. They were studied before and after treatment with enalapril (5 mg every 12 hours) or clonidine (0.1 mg every 12 hours) for 7 days.**

Results. **Obese subjects had higher values for percent body fat ($p < 0.0005$), triglycerides ($p < 0.05$), leptin ($p < 0.0005$), and low frequency/high frequency ratio at night (LF/HFn, $p = 0.05$). After enalapril or clonidine treatment, leptin levels were not modified. Both drugs significantly diminished the systolic and diastolic blood pressures. In the obese group, clonidine and enalapril diminished the LF/HFn ratio ($p < 0.05$). The LF/HF index showed a univariate correlation with body mass index, leptin, systolic blood pressure, insulin, age and triglyceride levels. In the multiple regression analysis for factors associated with the LF/HF ratio, only leptin, age and insulin were included in the model. The r^2 of the model was 0.3 ($p = 0.0003$).**

Conclusions. **A higher level of heart sympathetic activity is found in normotensive obese as compared with non-obese subjects. Both clonidine and enalapril reduced heart sympathetic activity in obese subjects without a change in fasting leptin levels.**

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Introduction

Obesity is a severe health problem affecting more than one third of the adult population both in developing and in industrialized countries[1]. The increase in fat mass is closely associated with an augmented risk for hypertension, diabetes, dyslipidemia, and cardiovascular mortality[2]. Although the association between obesity and cardiovascular disease is strong, the mechanism underlying this relationship is not well understood. The sympathetic nervous system is the main regulatory mechanism of cardiovascular function and it is strongly implicated in this association[3]. The adipose tissue is the main source of leptin production. Leptin shows a variety of effects in the hypothalamus: it reduces appetite and induces weight loss and thermogenesis[4]. Leptin also has peripheral sites of action: it stimulates vascular smooth muscle proliferation and migration[5]. In rats, leptin infusion increases the sympathetic activity in the kidneys, adrenals and brown adipose tissue. As a result, vascular resistance and blood pressure are increased[6]. Previous studies on the association of sympathetic activity with human obesity offer some important information on this issue. Paolisso et al.[7] reported that serum leptin levels are associated with the sympathetic activity independently of the amount of fat; however they did not include obese subjects.

Carulli et al.[8] found a decrease in serum leptin levels and in its mRNA expression after adrenergic activation by epinephrine infusion in humans; thus a reciprocal relationship could exist between leptin and sympathetic activity.

Considering the importance of elucidating the effects of leptin on sympathetic activity in obese subjects, we decided to examine the relationship between serum leptin levels and heart sympathetic activation in obese and non-obese normotensive subjects as well as the effect of drugs that modify sympathetic activity and the renin-angiotensin system.

Enalapril is a competitive angiotensin-converting enzyme (ACE)-inhibitor. It also reduces serum aldosterone and potentiates the vasodilator kallikrein-kinin system. ACE-inhibition is accompanied by a decrease in plasma catecholamine concentrations and muscle sympathetic nerve activity in patients with heart failure and essential hypertension[9,10]. Clonidine stimulates postsynaptic α_2-adrenergic receptors in the central nervous system by activating inhibitory neurons to decrease sympathetic outflow; this reduces the peripheral vascular resistance, heart rate and blood pressure[11]. In view of the above, we decided to study the response of the heart sympathetic activity and leptin levels to these drugs in obese and non-obese normotensive subjects.

Methods

Subjects. For the study we selected normal subjects aged 30 to 45 years with a constant body weight for the last 3 months. All subjects had a blood pressure ≤ 139/85 mmHg, were non-smokers and did not have thyroid dysfunction or chronic-degenerative illnesses. None of them had received anorexigenic drugs, beta-blockers, calcium antagonists or vasodilator drugs during at least 6 months before the study. All subjects were informed about the purpose of the study and gave their informed consent to participate. The protocol of the study was approved by the local ethical committee.

The body mass index was ≥ 30 kg/m^2 in obese volunteers and ≤ 27 kg/m^2 in non-obese subjects. Each group was paired by gender, and randomly assigned to two treatment regimens, one with clonidine and the other with enalapril lasting 1 week. Patients were evaluated immediately before and after treatment. In women, the study was initiated during the early follicular phase, on days 1 to 5 of the cycle[12].

One group of volunteers received clonidine 0.1 mg every 12 hours. The other group received enalapril 5 mg every 12 hours. Both groups included obese and non-obese subjects. During treatment, patients continued with their previous feeding habits. Before, and at the end of the study, a morning fasting blood sample was obtained to determine the glucose, leptin, insulin, and lipid serum levels. A 24-hour Holter recording was carried out.

The weight and standing height were obtained to calculate the body mass index. The skin folds were measured to calculate the percent of body fat as reported by Jackson and Pollock[13], and the girth values to obtain the waist to hip ratio (WHR). Blood pressure was obtained using a mercury sphygmomanometer with a cuff covering two thirds of the right arm. Each volunteer was maintained in a recumbent position for at least 30 min. Thereafter, two readings were made within a 5 min interval, and the average was registered.

Serum insulin was measured with a solid phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra- and interassay coefficients of variation were 3.0 and 4.5% respectively. Serum leptin was measured with an immunoradiometric assay (IRMA) (Diagnostics Systems Laboratories, Inc., Webster, TX, USA). The intra- and interassay coefficients of variation were 3.4 and 6.7% respectively. The insulin resistance was estimated using the HOMA-IR index according to the following formula[14]: HOMA-IR index = fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5.

Sympathetic activity was evaluated by means of 24-hour ambulatory electrocardiographic monitoring, using a three-channel Holter recorder (model GBI-3S, Galix Biomedical Instrumentation Inc., Miami Beach, FL, USA). Although this evaluation is certainly controversial, measures of cardiovascular autonomic function have been used to evaluate the clinical status in adults at risk for myocardial morbidity and mortality[15,16]. Holter monitoring is used not only to compute the measurement of the standard deviations of the heart periods on the basis of the sinus R-R intervals over time, but also to quantify and discriminate between the sympathetic and parasympathetic autonomic functions by recording the frequency (Hz) of the R-R variation, also referred to as power spectral analysis[17]. The tapes were analyzed in a Holter Galix software, to obtain the heart rate variability, considered as the standard deviation of all the normal RR intervals in 24 hours (SDNN). Spectral analysis was carried out with a direct fast Fourier transform providing the total (TP, from 0.01-1.00 Hz), high (HF, from 0.15-0.4 Hz), and low (LF, from 0.05-0.15 Hz) frequency power to obtain the LF/HF ratio in the 24-hour register[17]. The LF and HF components are reported in normalized units, which represent the relative value of each power component in proportion to the TP minus the very LF component. Normalized units tend to minimize the effect of the changes in TP on the values of the LF and HF components[17]. Ectopic beats were identified and excluded from the analysis. We analyzed the LF/HF ratio at night (LF/HFn) from 10:00 p.m. to 6:00 a.m. to standardize a period of rest for all the volunteers.

Statistical analyses. The descriptive statistics is reported as means ± SD. A p value of < 0.05 was considered as statistically significant. The fitness to the normal distribution was determined using the χ^2 test. Differences between obese and non-obese subjects were tested using the unpaired Student's t-test or the Mann-Whitney U-test when data showed significant departure from normality. We evaluated changes before and after clonidine or enalapril treatment using the Student's t-test for paired samples with the Bonferroni correction for multiple comparisons. The interactions among leptin, insulin, HOMA-IR and the LF/HF ratio before and after clonidine or enalapril in

obese and non-obese subjects were tested using three-way ANOVA analysis.

The association of the LF/HF ratio with the body mass index, HOMA-IR, insulin, and leptin was studied using the Pearson's simple correlation. The interaction with diverse covariates was studied by means of a multiple linear stepwise regression analysis with forward inclusion of variables. The LF/HF ratio was taken as the dependent variable, and variables significantly associated with the LF/HF ratio as candidate regressors. For analysis insulin, leptin, HOMA-IR, TP and the frequency domain measures were log-transformed to improve normality for statistical testing and back-transformed for presentation in tables and figures. All data were analyzed using the Statistics software version 6.0 (Statsoft Inc., Tulsa, OK, USA).

Results

A total of 81 subjects were included in the study: 41 obese (20 in the clonidine and 21 in the enalapril group) and 40 non-obese (20 in the clonidine and enalapril groups each). The characteristics of obese and non-obese subjects are shown in table I. The group of obese subjects showed a higher body mass index, percent body fat, leptin fasting levels, insulin, HOMA-IR and triglycerides, and lower HDL cholesterol levels. The values of systolic and diastolic blood pressure, age and WHR did not differ between the two groups. The 24-hour LF/HF index was similar in both groups, but the LF/HFn index was higher for the obese group.

For women, the percent body fat and serum leptin levels were significantly higher and the WHR lower in non-obese than in obese volunteers. Non-obese women had lower TP and higher LF band values than non-obese men. However, in the obese group no significant differences in the mean values of heart rate variability between men and women were discernible (Table II). There was no difference in the basal heart sympathetic activity between groups assigned to clonidine or enalapril treatment.

Enalapril and clonidine decreased the systolic and diastolic blood pressures in both groups (Fig. 1). In the obese group, both drugs diminished the LF/HFn index (Table III). We did not find any changes in the fasting leptin levels after either treatment (Table III). Three-way ANOVA analysis did not reveal any influence of leptin, insulin, HOMA-IR or LF/HFn ratio on the dif-

Table I. Basal characteristics of obese and non-obese subjects.

Variable	Non-obese (n=40)	Obese (n=41)
Age (years)	38.1 ± 5.8	38.3 ± 5.4
BMI (kg/m^2)	24.2 ± 1.7	32.5 ± 4.3*
SBP (mmHg)	114 ± 10.4	115.4 ± 10.6
DBP (mmHg)	75.8 ± 6.0	77.6 ± 6.0
Body fat (%)	27.1 ± 5.3	35.4 ± 4.1*
WHR	0.88 ± 0.05	0.93 ± 0.07
Total cholesterol (mmol/l)	4.4 ± 0.8	4.7 ± 0.8
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.0 ± 0.2**
LDL cholesterol (mmol/l)	2.6 ± 0.7	2.8 ± 0.6
VLDL cholesterol (mmol/l)	0.6 ± 0.3	0.9 ± 0.4**
Triglycerides (g/l)	1.25 ± 0.7	1.93 ± 0.9**
Leptin (ng/ml)	14.8 ± 10.2	30.8 ± 18.7
Insulin (pmol/l)	44.8 ± 20.0	99.7 ± 72.4*
HOMA-IR	1.34 ± 0.66	3.2 ± 2.5
Total power (ms^2)	3781.8 ± 1848	3110.5 ± 1652
LF (nU)	75.4 ± 9.5	73.4 ± 13.0
HF (nU)	24.1 ± 9.0	23.1 ± 10.8
LF/HF ratio (24 hours)	3.7 ± 1.8	4.3 ± 3.2
LF/HFn ratio (ms)	2.7 ± 1.7	3.9 ± 3.1**

Values are expressed as means ± SD. BMI = body mass index; DBP = diastolic blood pressure; HF = high frequency; HOMA-IR = insulin resistance index; LF = low frequency; LF/HFn = low frequency to high frequency ratio at night; SBP = systolic blood pressure; WHR = waist to hip ratio. * $p < 0.0005$; ** $p < 0.05$.

Table II. Basal characteristics according to gender and body mass index (BMI).

Variable	Obese		Non-obese	
	Men (n=21)	Women (n=20)	Men (n=20)	Women (n=20)
Age (years)	39.0 ± 5.7	37.7 ± 5.1	39.8 ± 6.2	36.4 ± 5.0
BMI (kg/m^2)	32.7 ± 4.0	32.3 ± 2.8	24.9 ± 1.8	23.8 ± 2.2
Body fat (%)	33.1 ± 3.6	37.8 ± 2.8*	27.2 ± 5.2	32.2 ± 4.2*
WHR	0.99 ± 0.04	0.86 ± 0.05**	0.95 ± 0.05	0.86 ± 0.05**
Leptin (ng/ml)	19.1 ± 12.3	43.2 ± 16.3**	7.4 ± 4.4	22.1 ± 9.1**
Insulin (pmol/l)	121 ± 4	78 ± 29	42 ± 21	47 ± 20
HOMA-IR	3.9 ± 3.2	2.4 ± 1.0	1.3 ± 0.69	1.3 ± 0.69
Total power (ms^2)	3578 ± 1896	2719 ± 1211	4308 ± 2077	3194 ± 1312*
LF (nU)	74.9 ± 14.2	71.8 ± 11.7	19.6 ± 5.0	28.6 ± 9.9*
HF (nU)	20.9 ± 10.7	25.4 ± 10.7	8.3 ± 0.4	7.9 ± 0.4
LF/HFn ratio (ms)	4.6 ± 4.0	3.1 ± 1.4	3.2 ± 1.6	2.2 ± 1.7

Values are expressed as means ± SD. HF = high frequency; HOMA-IR = insulin resistance index; LF = low frequency; LF/HFn = low frequency to high frequency ratio at night; WHR = waist to hip ratio. * $p < 0.05$; ** $p < 0.005$.

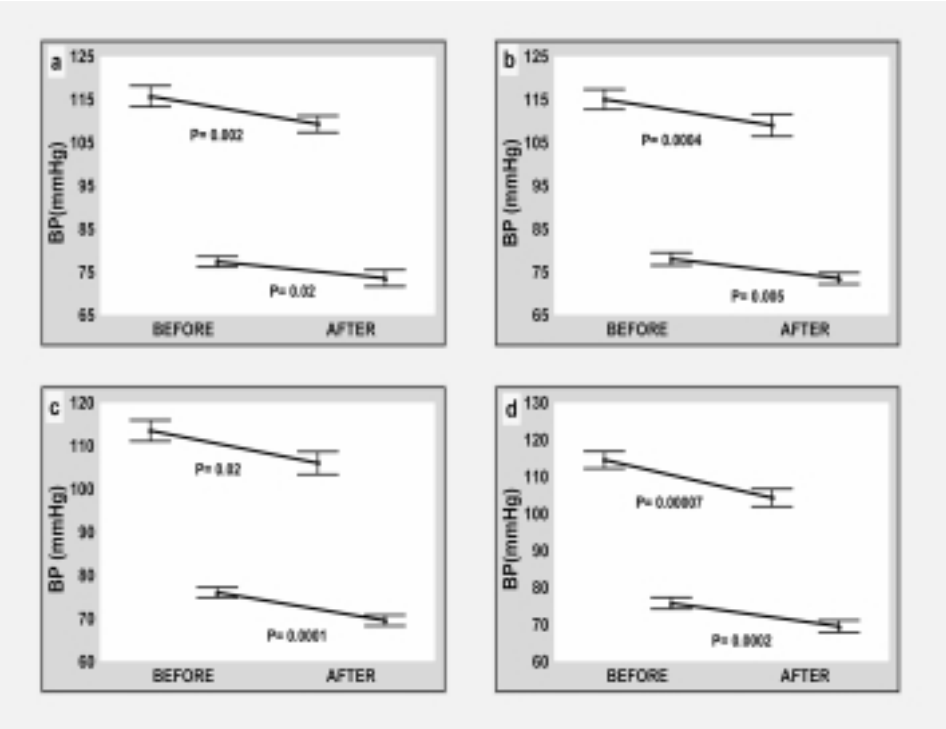

Figure 1. *Comparison of systolic and diastolic blood pressures (BP) before and after treatment.* a: *obese subjects with enalapril treatment;* b: *obese subjects with clonidine treatment;* c: *non-obese subjects with enalapril treatment;* d: *non-obese subjects with clonidine treatment.* Vertical bars *indicate SEM. Data were analyzed using the paired Student's t-test.*

Table III. Effects of clonidine and enalapril among the study groups.

Variable	Obese		Non-obese	
	Before	After	Before	After
Clonidine treatment				
Leptin (ng/ml)	30.2 ± 17.3	32.8 ± 23.6	15.4 ± 9.4	15.5 ± 9.8
Insulin (pmol/l)	83.2 ± 57.4	86.8 ± 63.1	43.0 ± 17.9	56.6 ± 36.5
LF (nU)	71.4 ± 12.6	66.9 ± 13.1	79.1 ± 7.6	76.2 ± 9.5
HF (nU)	23.8 ± 9.6	28.9 ± 11.4*	21.0 ± 7.3	25.8 ± 10.8
LF/HFn ratio (ms)	3.4 ± 2.4	2.6 ± 1.0*	3.1 ± 1.9	2.5 ± 1.3
Enalapril treatment				
Leptin (ng/ml)	31.4 ± 20.4	32.0 ± 20.9	14.1 ± 11.2	13.2 ± 8.9
Insulin (pmol/l)	114.8 ± 82.5	85.3 ± 45.9	45.9 ± 22.2	49.5 ± 23.6
LF (nU)	74.8 ± 13.0	71.6 ± 10.3	71.7 ± 9.9	71.0 ± 12.3
HF (nU)	22.7 ± 12.2	26.1 ± 10.3	27.2 ± 9.5	27.8 ± 10.8
LF/HFn ratio (ms)	4.3 ± 2.9	3.0 ± 1.4*	2.2 ± 1.3	2.2 ± 1.4

Values are expressed as means ± SD. HF = high frequency; HOMA-IR = insulin resistance index; LF = low frequency; LF/HFn = low frequency to high frequency ratio at night. * $p < 0.05$.

ferences observed between obese and non-obese subjects within each treatment subgroup.

At simple linear regression analysis we found the LF/HF ratio to be significantly associated with body mass index, log (leptin), log (insulin), triglyceride levels, age, and systolic blood pressure (Table IV). At multiple regression analysis for the factors associated with the LF/HF ratio, only leptin, age and insulin were included in the model. The r^2 of the model was 0.3 ($p = 0.0003$).

Discussion

In this study, we compared obese and non-obese subjects of both genders with a similar age. As expected, obese subjects had higher insulin resistance, insulinemia, triglyceridemia and serum leptin levels[18]. At any level of body mass index, women had a higher percent body fat and serum leptin levels. In contrast, the WHR was smaller than that observed in men. All these findings are explained by the women's increased sub-

Table IV. The relationships of low frequency to high frequency ratio and some parameters studied.

	r	p
Age	0.38	0.002
BMI	0.32	0.01
SBP	0.31	0.01
Triglycerides	0.30	0.04
Leptin (log)	0.3	0.004
Insulin (log)	0.30	0.01

BMI = body mass index; SBP = systolic blood pressure.

cutaneous fat deposition that is due to the effects of estrogen and genetic factors[12]. In the non-obese group, women had a lower heart sympathetic activity than men, but this difference disappeared in the obese group. Thus, obesity seems to increase the heart sympathetic activity independently of gender. This could be explained by the sexual dimorphism in the baroreflex function related to the ability of the male hormone testosterone to enhance the baroreflex responsiveness[19]. However, reduced testosterone levels have been reported in obese men[20].

There was no significant difference in the 24-hour LF/HF ratio between obese and non-obese subjects, but this index was higher in the obese group. A possible explanation for the lack of difference in the 24-hour LF/HFn ratio is the wide variability of the subjects' physical activities during the day. The heart rate variability represents an end-organ response to the sympathetic nervous system determined by nerve firing and electrochemical coupling but also by cardiac adrenergic receptor sensitivity, and postsynaptic signal transduction[21]. The study of the sympathetic overactivity has important applications in clinical practice. For instance, after a myocardial infarction the excessive sympathetic activation may induce electric instability and a higher mortality[15].

We examined the sympathetic influence in obese and non-obese subjects by means of pharmacological modifications of the adrenergic and the renin-angiotensin systems. In our study, clonidine and enalapril reduced the systolic and diastolic blood pressures both in obese and in non-obese subjects. However, in spite of this change, the heart sympathetic activity was diminished only in obese subjects. These results are in accordance with a higher sympathetic tone in the obese subjects that renders them more sensitive to the inhibitory effect of the drugs. Similar results have been reported for normotensive patients with heart failure, after clonidine treatment[22], and for patients with chronic renal failure after enalapril treatment[23].

The main point of interest in this study is the possible relationship between serum leptin levels and sympathetic activity. In the analysis of the factors associated with the LF/HF ratio, simple correlation analysis showed significance for age, serum leptin levels, body mass index, insulin levels and systolic blood pressure. The independent role of the fasting plasma leptin concentrations on the baseline LF/HF ratio investigated by means of multivariate linear regression analysis demonstrated that age, insulin and leptin concentrations were all significantly and independently associated with the baseline LF/HF ratio. This point is of interest because several reports suggest a possible association of leptin with sympathetic activity and probably with hypertension. In agreement with our findings, Paolisso et al.[7] reported serum leptin levels to be associated with heart sympathetic activity independently of the mass of fat. Recently, Eikelis et al.[24] reported that renal norepinephrine spillover was correlated with plasma leptin levels in obese subjects. However, they did not find any correlation with other parameters of the sympathoadrenal function. Thus, a racial effect may be implied. Hirose et al.[25] found that serum leptin levels were highly correlated with the mean arterial pressure and body mass index in male adolescents. Suter et al.[26] also found that systolic blood pressure correlated with the plasma leptin levels after adjustment for body mass index in women and in non-hypertensive men. Lawrence et al. (American Diabetes Association Annual Meeting, San Francisco, CA, USA, 2002, unpublished data) reported that the systemic nervous system activity was greater with obesity, and correlated with blood pressure, while adipose tissue sympathetic activity was almost 50% lower with obesity. Moreover, the experimental infusion of high doses of leptin in rats induced overactivity of the sympathetic nervous system at a central level[6]. This could be a pharmacological or species-specific effect, considering that in non-obese human subjects, leptin administration for a short period did not modify the autonomous activity[27]. However, we are not aware of reports on the chronic administration of leptin or on its effect in obese subjects.

We did not find any changes in the serum leptin levels after enalapril or clonidine treatments. In previous studies, fasting morning plasma leptin concentrations were affected only by prolonged fasting and changes in body adiposity[28,29]. Carulli et al.[8] found a decrease in serum leptin levels and in its mRNA expression following adrenergic activation by epinephrine infusion in humans. We attempted to achieve the opposite effect with enalapril and clonidine, and as expected the catecholamine serum levels decreased. In agreement with our results, Eikelis et al.[24] did not find any changes in leptin levels after clonidine treatment in different subjects. Thus, our and Eikelis' results provide some support for the view that leptin stimulates the sympathetic nervous system, but do not confirm the concept of a regulatory feedback inhibition of leptin by the sympathetic nervous system. It is possible that if a reciprocal relationship between leptin and sympathetic activity exists it could be affected in obese subjects. Recently, it has been demonstrated that alterations in the regula-

tory elements of the ob gene may lead to an abnormal response to cold in mice, and the researchers asserted that in some cases abnormal regulation of the leptin gene may be an etiological factor in the pathogenesis of obesity and diseases linked to obesity in humans[30]. The relationship between these factors is a complex one and requires that additional factors be taken into account. For example, the Pima Indians have high plasma insulin and leptin levels and a high prevalence of diabetes mellitus, but they do not have sympathetic overactivity or a higher prevalence of hypertension[31,32]. A factor that could explain the different results in different populations is the leptin receptor polymorphism. Rosmond et al.[33] found that obese men with high serum leptin levels have hypertension only when they had the more prevalent genotype of the leptin receptor.

Although leptin has been associated with impaired sympathetic activity, most studies have focused on rodents and men, and they do not include therapeutic drugs. One of the advantages of this study over previous ones is that we did not include smokers and hypertensive subjects or menopausal women since such statuses could affect sympathetic activity and hormonal measurements. However, a potential limitation of our study was that only the association among different variables was determined, and thus no results on the cause-effect relationship could be drawn.

In conclusion, a higher level of sympathetic activity is found in normotensive obese as compared with non-obese subjects. Age, levels of serum leptin and insulin correlate with the sympathetic activation. Clonidine and enalapril showed their hypotensor effects in obese and non-obese subjects; however, they only decreased the heart sympathetic activity in the obese group, without modifying serum leptin levels.

Further studies are necessary in order to determine the risk for cardiovascular events in this population of normotensive obese subjects with increased heart sympathetic activity.

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