Leptin Is a Risk Marker for First-Ever Hemorrhagic Stroke in a Population-Based Cohort

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Background and Purpose—Leptin, important for body weight regulation, may be involved in the pathogenesis of the insulin resistance syndrome, associated with cardiovascular disease. We tested to determine whether leptin is a risk marker for first-ever stroke in a nested case-referent study.

Methods—We identified 113 patients with first-ever stroke (94 with ischemic and 19 with hemorrhagic stroke) who, before the stroke, had participated in population-based health surveys in northern Sweden. Referents were matched for sex, age, date and type of health survey, and geographic region. Blood pressure (BP), body mass index (BMI), and presence of smoking, diabetes, and hypertension were recorded. Total cholesterol, insulin, and leptin were analyzed in stored samples. Risk markers for first-ever stroke were analyzed by conditional logistic regression analysis.

Results—Patients with hemorrhagic stroke had higher levels of BMI and systolic and diastolic BPs. Leptin levels were 72% and 59% higher in males and females, respectively, with hemorrhagic stroke versus referents. Patients with ischemic stroke more often had hypertension, diabetes mellitus, and higher fasting glucose and insulin levels. A diagnosis of hypertension and elevated systolic and diastolic BPs were significant risk markers for first-ever hemorrhagic stroke in univariate analysis. High leptin (OR = 20.55; 95% CI, 1.12 to 376.7) levels together with hypertension (OR = 16.28; 95% CI, 1.49 to 177.3) remained as significant risk markers in a multivariate model. The combination of high leptin and high systolic or diastolic BP were associated with a profoundly increased risk for hemorrhagic stroke (OR = 22.11; 95% CI, 1.57 to 310.9). Diabetes, hypertension, and obesity (BMI ≥ 27), together with high levels of insulin, glucose, systolic and diastolic BP, were significant risk markers for first-ever ischemic stroke in univariate analysis. Hypertension (OR = 2.10; 95% CI, 1.14 to 3.86) remained as an independent risk marker in a multivariate model.

Conclusions—Plasma leptin is strongly associated with an increased risk for first-ever hemorrhagic stroke, independent of other risk markers for cardiovascular disease. Leptin may be an important link in the development of cardiovascular disease in obesity. (Stroke. 1999;30:328-337.)

Key Words: leptin ■ risk factors ■ stroke, hemorrhagic ■ stroke, ischemic

Well-established risk factors for stroke include hypertension, cigarette smoking, atrial fibrillation, diabetes mellitus, and alcohol abuse. The risk factors for ischemic and hemorrhagic stroke differ to some extent. A consistent finding, however, has been the association between hypertension and the risk of ischemic and hemorrhagic stroke and the protective effect of antihypertensive treatment. The cluster of risk factors named the insulin resistance syndrome, or syndrome X, has attracted considerable interest as a major contributing cause for coronary heart disease. Key features of this syndrome include hyperinsulinemia, hypertension, dyslipidemia, and dysfibrinolysis. Much less attention has been paid to the contribution of some of these factors to cerebrovascular disease. Notably, it has recently been suggested that insulin resistance with compensatory hyperinsulinemia may be an important pathogenetic factor of atherothrombotic brain infarction.

The newly discovered protein product of the ob gene leptin may be an important link between obesity, the insulin resistance syndrome, and an increased risk for vascular disease. Leptin reduces food intake and increases energy expenditure when administered to mice but has recently been suggested to have adverse effects in an “overflow” situation, including effects on blood pressure (BP) regulation.

The aim of this nested case-referent study was to examine to what extent leptin may be an independent risk marker for first-ever stroke.
Subjects and Methods

Study Populations

The two northernmost counties in Sweden (Västerbotten and Norrbotten), with a total population of 510,000, have constituted since 1985 one of 39 collaborating centers in the WHO MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) study. Population-based surveys were performed in 1986, 1990, and 1994. For each survey, a total of 2000 individuals (250 men and 250 women, randomly selected from each of the following age groups: 25 to 34, 35 to 44, 45 to 54, and 55 to 64 years) were invited. In total, 4725 men and women in the age group 25 to 64 years participated in the MONICA surveys.

A community intervention program concerning cardiovascular disease and diabetes prevention, the Västerbotten Intervention Program (VIP), was performed in 1 of the 2 counties. This program, started in 1985, combines a population- and an individual-oriented strategy. Men and women in Västerbotten county were asked to participate in a health survey (the same design as the MONICA population surveys) at their primary health care center the year they became 30, 40, 50, and 60 years of age. The total population in Västerbotten is 260,000 people. Between January 1, 1985, and August 31, 1996, approximately 40,000 individuals took part in the VIP health surveys.

Participants in both the MONICA and the VIP surveys were requested to donate a fasting blood sample to be stored at the Northern Sweden Medical Research Bank for future research.

Case Findings

All cases with acute stroke (age group 25 to 74 years) and acute myocardial infarction (AMI; age group 25 to 64 years) from the MONICA area have since 1985 been included in the Northern Sweden MONICA registries. Each case has been strictly validated according to MONICA criteria before registration. An acute stroke case was defined as “rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting more than 24 hours (unless interrupted by surgery or death) with no apparent cause other than a vascular origin.” Global clinical signs were accepted only in patients with deep coma or subarachnoid hemorrhage. This definition excluded transient ischemic attacks, subdural hemorrhage, traumatic intracerebral hemorrhage, and lesions caused by brain tumor.

Case-finding was based on clinical presentation, and cases detected by brain imaging but not presenting with any acute symptoms of stroke were excluded.

The subtypes of acute stroke, according to the MONICA manual, were based on the following findings (International Classification of Diseases, Revision 9 [ICD-9] codes are in parentheses). Subarachnoid hemorrhage (430): bloodstained cerebrospinal fluid and an aneurysm or an arteriovenous malformation found on angiography, or positive finding on CT scan or necropsy. Subarachnoid hemorrhages were excluded in this study. Hemorrhagic stroke (431): positive finding on CT scan or at autopsy. In cases of a hemorrhagic stroke, autopsy (alone or in combination with CT scan) was performed in 14.6% of the cases and CT scan alone in the remaining 85.4%. The proportion of hemorrhagic stroke events in the MONICA registry was 11.0% and 10.8% for men and for women, respectively. Ischemic stroke (434): no signs of hemorrhage on CT scan or at autopsy. Unspecified stroke (436): none of the investigations (CT scan or autopsy) were performed. In this study, 3 of 113 had a diagnosis of unspecified stroke, and these were categorized as “ischemic stroke” in the analysis.

This study used a nested case-referent design in which the incident cases were definite first-ever stroke events, classified according to MONICA criteria and identified during the period January 1, 1985, through August 31, 1996. We identified 166 individuals who, after participation in either the MONICA or VIP health survey, suffered from a first-ever ischemic or hemorrhagic stroke before the age of 74. For this study, 113 cases (94 ischemic stroke [10 from MONICA and 84 from VIP] and 19 hemorrhagic stroke [2 from MONICA and 17 from VIP]; 71 men and 42 women) remained, after exclusion of individuals with a previous AMI (n=15), stroke (n=9), or cancer diagnosis according to the Regional Cancer registry (n=13), or those for whom the amount of blood in the sample taken was inadequate for analysis (n=16).

Potential referents for each case were randomly selected among participants in the MONICA or the VIP survey. They were matched for sex, age (±2 years), type (MONICA or VIP) and date (±1 year) of health survey, and geographical region.

Individuals were excluded if they had died or had moved away from the Northern Sweden MONICA registry or before August 31, 1996. Referents were also excluded if they were known from the Northern Sweden MONICA incidence registry to have had an AMI or stroke before the health survey. An additional questionnaire was sent to all referents to further ensure absence of stroke and/or AMI in their history. Finally, 2 referents for each case were selected.

The study was approved by the Research Ethics Committee of Umeå University and the data handling procedures by the National Computer Data Inspection Board.

Biomedical Factors and Biochemical Analyses

Smokers were defined as those who reported smoking cigarettes (n=57), cigars (n=1), or a pipe (n=10) daily. Ex-smokers or “occasional smokers” were classified as nonsmokers. The presence or absence of diabetes was based on self-reported data and/or fasting glucose >7.7 mmol/L and/or postload glucose levels >11.0 mmol/L. BP was recorded after 5 minutes’ rest with a mercury sphygmomanometer. With the subject in the sitting position, Korotkoff’s 5th phase was used as the diastolic pressure. For subjects whose BP was measured only in a recumbent position, an adjustment was made for sitting posture based on comparisons between sitting and recumbent positions in 1850 subjects from the VIP health survey. Styloric BP was divided into 3 groups: <130, 130 to 160, and >160 mm Hg. Diastolic BP was grouped into <85, 85 to 95, and >95 mm Hg.

Hypertension was defined as systolic BP ≥160 mm Hg and/or diastolic BP ≥95 mm Hg and/or antihypertensive medication therapy. Weight was measured without shoes in light indoor clothing and recorded to the nearest 0.2 kilogram; height was measured to the nearest centimeter, without shoes. Body mass index (BMI) was calculated as total body weight in kilograms divided by height in meters squared. BMI was divided into 3 groups: <27, 27 to 30, and >30 kg/m², according to the classification by Bray.

Most subjects were screened in the morning after an overnight fast, even if the requested minimum fasting period was only 4 hours, for practical reasons. This period was extended to 8 hours from 1992 on. In the majority of subjects, an abbreviated oral glucose tolerance test was performed according to the standard of WHO, with a 75-g glucose load. Fasting and postload glucose (2 hours) were measured. Total serum cholesterol was measured with a benchtop analyzer (Reflotron, Boehringer Mannheim GmbH Diagnostica) at each health survey center at the time of the health survey or by an enzymatic method (Boehringer Mannheim GmbH Diagnostica) at a central laboratory. The results from the benchtop analysis have been evaluated earlier and show a high correlation to those obtained by the enzymatic method at a central laboratory. For total cholesterol, 3 groups were considered clinically relevant: <6.5, 6.5 to 7.8, and >7.8 mmol/L.

Plasma samples for the following analyses were obtained after patients fasted for a minimum of 4 hours; the samples were stored in a deep-freeze blood bank at −80°C. Insulin was measured with a double-antibody radioimmunoassay technique. Guinea pig anti-human insulin antibodies, human insulin standard, and mono-I-Tyr-human insulin (Linco Research, Inc) were used. This assay had a cross-reactivity under 0.2% with intact and des(31,32) proinsulin, according to the manufacturer. The detection limit was 10 pmol/L and the CV (both interassay and intraassay) was <3%. The leptin analysis was performed by use of a double antibody radioimmunoassay with rabbit anti-human leptin antibodies. 125I-labeled human leptin as tracer and human leptin as standard. Interassay CV was 1.9% at low levels (<5 ng/mL) and 3.2% at high levels (10 to 15 ng/mL) (Linco Research, Inc).
Leptin and Stroke

TABLE 1. Subject Characteristics

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<th>Variable</th>
<th>Ischemic Stroke</th>
<th>Hemorrhagic Stroke</th>
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<td>Cases</td>
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<td>Postload glucose, mmol/L</td>
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<td>Leptin, ng/mL</td>
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<td>Regular smokers, %</td>
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Values shown are means and SDs.

*Ischemic cases versus matched controls (t test).
†Hemorrhagic cases versus matched controls (t test, except for *Mann-Whitney nonparametric test).
§Diastolic BP ≥95 and/or systolic BP ≥160 and/or on antihypertensive medication.
¶Fasting glucose ≥7.7 and/or postload glucose >11.0 and/or reported diabetes.

Statistical Analysis

Missing values for categorical variables were treated as a separate category (omitted from tables) in the analysis, while missing continuous values in the logistic regression analysis were replaced by the mean value representing the referents, thus ensuring a conservative test when appropriate. Possible interactions between study variables were explored with bivariate and multivariate logistic regression analysis by use of the conditional maximum likelihood routine designed for matched analysis to estimate ORs and 95% CIs. (The effect of gender was tested with ordinary logistic regression because of the matched design). To test the relationship between increasing levels of risk markers and the risk of stroke, we categorized the continuous variables into tertiles by the distribution of the referent values, men and women separately. Categorical and tertiles were combined to get 1 “exposed” (usually the highest category/tertile) and 1 “unexposed” (the remaining categories/tertiles) group. This strategy was adopted because of the limited number of cases in some cells and guided by the results in the univariate analysis. For assessment of the influence of separate biomedical risk factors, we controlled for BMI, hypertension, smoking, diabetes (or fasting glucose), cholesterol, insulin, and leptin in various models. Possible interactions between leptin on one hand and BP, cholesterol, and insulin on the other were studied by visual inspection of scatterplots and construction of combination terms, including high and low levels (below and over the median value for referents, stratified for type of stroke and sex) of the studied variables. The confounding effect of gender and type of stroke was evaluated by stratification. Furthermore, the presence of other possible confounders and the stability of multivariate models were tested in multiple steps of adding/deleting all possible combinations of variables (both as continuous and as categorical variables when appropriate). All calculations were made with the statistical programs SPSS (version 6.1; SPSS, Inc.) and stata (version 5.0; Stata Corp) on a Macintosh computer (Apple Computer, Inc.).

Results

One hundred thirteen patients with a first-ever stroke were included in the study. Ninety-four patients (55 men and 39 women) had had an ischemic stroke and 19 (16 men and 3 women) a hemorrhagic stroke. The stroke event occurred on average 30 months after the health survey.

Basal characteristics are presented in Table 1. Patients with ischemic stroke were more often hypertensive and had higher systolic and diastolic BPs at the time of investigation than their matched referents. Furthermore, they more often had diabetes, and they had higher fasting glucose and insulin levels. The same pattern of differences was seen after stratification for sex, even if some parameters did not reach statistical significance (data not shown).

Patients with hemorrhagic stroke had higher diastolic and systolic BPs and BMI than their referents, a pattern also seen among men (data not shown; a separate analysis of women with hemorrhagic stroke was not meaningful because of few cases). Compared with the referents, the leptin levels were 72% higher in male and 59% higher in female patients with hemorrhagic stroke, a difference not seen between ischemic cases and their referents. Cholesterol levels and the number of smokers did not differ between cases and referents, irrespective of type of stroke and sex.
Significant correlations between study variables were found (Table 2). In men, high levels of leptin were associated with high BMI, high diastolic and systolic BPs, and high levels of cholesterol, fasting glucose, and insulin. Significant associations remained between leptin on one hand and glucose, insulin, and cholesterol on the other, after adjustment for BMI. Furthermore, high leptin levels correlated to high BMI and elevated insulin levels (after adjustment for BMI as well) in women.

Univariate logistic regression revealed that a diagnosis of diabetes mellitus as well as high fasting glucose and insulin levels, together with known hypertension and high diastolic/systolic BP levels, were significant risk markers for first-ever ischemic stroke (Table 3). A high BMI was associated with ischemic stroke (≥27) and hemorrhagic stroke (continuous). In addition, first-ever hemorrhagic stroke was associated with known hypertension and high systolic and diastolic BPs at the time of investigation (Table 4). High levels of leptin (the upper two tertiles) were associated with elevated risk for first-ever hemorrhagic stroke, although not significant (OR = 8.58; 95% CI, 0.98 to 74.9). Neither daily smoking nor high levels of cholesterol were associated with stroke of any kind. Variables associated with increased risk for future stroke in the univariate analysis retained their predictive power after stratification for sex, although not always significantly, because of the limited number of cases in some cells.

Hypertension was significantly associated with ischemic stroke in a multivariate model (Table 3). Furthermore, diabetes mellitus and high BP (diastolic BP ≥85 mm Hg or systolic BP ≥130 mm Hg) became significant risk markers for first-ever ischemic stroke if BP was analyzed categorized as diastolic or systolic BP instead of hypertension (data not shown). Leptin was not associated with ischemic stroke in any of these models.

Leptin and hypertension were the only remaining risk markers for hemorrhagic stroke in a multivariate model, including leptin, BMI, smoking, hypertension, glucose, and insulin (Table 4). The outcome was similar after controlling for cholesterol as well but at the expense of less precise estimates (wider confidence intervals). A second multivariate model including only leptin, hypertension, and cholesterol is thus presented (Table 4).

Sex did not predict stroke of any type in either univariate analysis or as an interaction term with leptin (data not shown).

The combination of high BP (systolic or diastolic) and high leptin levels was associated with a pronounced increased risk of hemorrhagic stroke (Figure 1 and 2), a pattern not seen in patients with ischemic stroke. The combination of high leptin levels and high BP (systolic or diastolic) was a significant risk marker for hemorrhagic stroke both in univariate (OR = 12.71; 95% CI, 1.25 to 129.8) and multivariate (OR = 20.11; 95% CI, 1.57 to 310.9) models compared with the combination of low leptin levels and low BP (systolic and diastolic). In contrast, high BP (systolic or diastolic) alone predicted ischemic stroke (OR = 4.79; 95% CI, 1.80 to 12.76) in a multivariate model, not in combination with high leptin levels.

**Discussion**

This study examined leptin as a possible independent risk marker for first-ever stroke in a strict population-based, nested case-referent study. The nested case-referent design unites the prospective dimension of the cohort study with the efficiency of the case-referent study. Recall and selection bias are minimized, because blood sampling and data collection are carried out before the event. All events in the population, including those not treated in the hospital, are included in the study. The identification and definition of cases is crucial, and it should be stressed that all stroke events in this study were strictly classified according to the MONICA criteria by the Northern Sweden MONICA stroke registry. Extensive quality assessments of the registry have been performed. This is, therefore, a genuine population-based study, and there are no major differences in risk-factor profiles between participants and nonparticipants in the VIP and MONICA surveys. The excess of men with hemorrhagic stroke (16 of 19 cases) was unexpected, and there are no obvious reason for this, other than chance, because of the small number of cases; this issue can be solved only by larger studies with more men and women.

The etiology of stroke is multifactorial. In the present study, circulating levels of leptin were found to be significantly associated with other risk markers, including elevated BP, BMI, glucose, insulin, and cholesterol levels. In multivariate analyses, high leptin retained its position as a powerful marker for the future risk of hemorrhagic stroke. This was independent of variables related to high leptin levels, such as high BMI and hyperinsulinemia. In fact, leptin (together...
with high BP) emerged as the strongest independent risk marker for hemorrhagic stroke. In contrast, hypertension was the only remaining risk factor for ischemic stroke in a multivariate model after adjustment for covariates, including leptin and other known risk markers for ischemic stroke. The absence of association between diabetes mellitus and smoking on one hand and ischemic stroke on the other, in the multivariate analysis, may be related to the limited study sample.

We have recently shown that a high leptin level is a strong risk marker for first-ever AMI in men independent of other known AMI risk factors in a prospective, nested case-referent study.26 The absence of association between ischemic stroke and leptin levels was thus unexpected and may seem puz-
zling. However, different pathophysiological mechanisms may be present among subgroups of ischemic stroke, including atherothrombotic, lacunar, and cardioembolic infarctions. Further studies of the predictive power of leptin in larger study populations may take this diversity into account.

In evolutionary terms, the role of leptin has been suggested to be that of a protector against the effects of starvation. However, hyperleptinemia may contribute to the development of cardiovascular disease via its effects on BP regulation, insulin sensitivity, and a number of other hormonal interactions. In experimental studies, leptin increased renal, brown adipose tissue, and adrenal gland sympathetic nerve activity, and intravenous as well as intracerebroventricular ad-

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<th>Multivariate‡</th>
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Glucose indicates fasting glucose.
*Cut-off values for categories; left = men, right = women.
†Multivariate model, including leptin, BMI, insulin, glucose, hypertension, and smoking, all categorical. Glucose used as a surrogate for diabetes.
‡Multivariate model, including leptin, hypertension, and cholesterol, all categorical.
Administration of leptin causes sustained increase of arterial BP in normal rats. Our data support a close relationship between leptin and BP, as the combination of high levels of leptin and high diastolic or systolic BP was a strong predictor for hemorrhagic stroke. In contrast, the combination of high levels of leptin and BP did not predict ischemic stroke. The prompt reduction of stroke risk after normalization of high BP related to the relatively small benefit on AMI risk suggests interruption of a precipitating factor rather than interference with atherogenesis. A leptin-mediated increase in sympathetic activity via central and peripheral effects may thus induce short-term as well as long-term BP changes.

We found a close association between circulating leptin and insulin levels, verifying data from other study groups. Impaired insulin sensitivity has been associated with cardiovascular disease. Insulin resistance has been shown to be associated with atherothrombotic stroke (but not with lacunar or cardioembolic strokes) in nonobese Japanese men and women. In another study, decreased insulin sensitivity was independently associated with increased atherosclerosis of the carotid arteries.

Leptin secretion is probably regulated by insulin in humans, but it was notable that in a multiple regression model, insulin lost its apparent predictive value for ischemic stroke when adjusted for leptin levels, whereas leptin was a predictor for hemorrhagic stroke that was independent of insulin. This raises the possibility that the reported association between hyperinsulinemia/insulin resistance and an increased risk of cardiovascular disease is mediated by leptin. Interestingly, the insulin resistance syndrome is associated with increased sympathetic activity, which has been suggested to be an important link in the development of hyper-

Figure 1. Scatterplot showing the relationship between plasma leptin (ng/mL) and diastolic BP levels for men with hemorrhagic stroke (●) and their referents (□). The upper scatterplot shows cases versus one set of referents and the lower shows cases versus the other set of randomly chosen referents. The median values for leptin and diastolic BP in the reference group are indicated as reference lines. (A separate scatterplot of women with hemorrhagic stroke was not meaningful because of the small number of cases.) Note the aggregation of cases in the quadrant corresponding to high leptin and high diastolic BP.
tension and associated metabolic abnormalities. Further-
more, weight loss reduces sympathetic activity, possibly
related to a decrease in leptin levels.

The association between cholesterol levels and stroke risk
is not clear-cut. Interestingly, low HDL cholesterol levels,
which correlate with extracranial carotid atherosclerosis
and are a key feature of the dyslipidemia associated with
insulin resistance and central obesity, are associated with high
leptin levels.

Central obesity may be of importance in determining leptin
levels in men, and indirect measures of central obesity, such as
visceral fat area or waist circumference, are associated with
increased leptin levels in men and postmenopausal women.
This is of interest, because central obesity, rather than the obesity
involving hip and thighs, relates to an increased stroke risk.

A final mechanism behind increased leptin, the insulin
resistance syndrome, and cardiovascular disease (including
stroke) may be dysfibrinolysis. Indeed, we have recently
shown that elevated leptin levels are associated with low
levels of tissue plasminogen activator activity and high levels
of plasminogen activator inhibitor-1.

There are some potential drawbacks with this study, including
the lack of standardization of glucose measurements and blood
sampling time. However, misclassification of exposure status
due to these factors would have been random in cases and
referents. The effects of high glucose, insulin, and leptin levels
may thus be underestimated in this study because of a regression
dilution bias. Furthermore, leptin levels show a diurnal variation,
but daytime levels are quite stable, with a variation of approxi-
mately 20% compared with levels at 8 AM. Our results should
be interpreted with caution owing to the relatively limited
number of hemorrhagic cases. We believe, however, that a major
strength of the study is the nested case-referent design with
strictly validated case-finding.

Figure 2. Scatterplot showing the relationship
between plasma leptin (ng/mL) and systolic BP
levels for men with hemorrhagic stroke (●) and
their referents (□). The upper scatterplot shows
cases versus one set of referents and the lower
shows cases versus the other set of randomly cho-
sen referents. The median values for leptin and
systolic BP in the reference group are indicated as
reference lines. (A separate scatterplot of women
with hemorrhagic stroke was not meaningful
because of the small number of cases.) Note the
aggregation of cases in the quadrant corre-
spending to high leptin and high systolic BP.

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In conclusion, elevated plasma leptin level is an independent predictor of first-ever hemorrhagic stroke but not ischemic stroke. The mechanisms behind this association should be further explored but may include effects of leptin directly or indirectly on sympathetic nervous system activation and BP, lipid levels, and the fibrinolytic system.

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