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Serum leptin levels and the risk of stroke: The Framingham Study

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Abstract

Background and Purpose—Leptin is a major adipokine that regulates weight balance and energy homeostasis. There is inconsistent evidence linking circulating leptin levels to risk of stroke. We tested the hypothesis that leptin levels are associated with risk of incident stroke in an elderly community-based sample.

Methods—Serum leptin levels were assayed in 757 stroke-free individuals (mean age 79 years, 62% women) from the Framingham Original cohort at the 22nd examination cycle (1990–1994). Incidence of all-stroke and ischemic stroke were prospectively ascertained.

Results—During a mean follow-up of 10 years, 119 individuals developed stroke (99 ischemic stroke). In multivariable Cox regression models, log-leptin levels were not associated with incidence of all-stroke or ischemic stroke (hazard ratios[HR] per standard deviation(SD) increment in log-leptin 0.9 [0.73–1.09] and 0.89 [0.72–1.11], respectively). The results were suggestive for potential effect modification by waist-hip ratio(WHR) for the association between leptin and stroke ($P=0.03$). Adjusting for age, sex and established stroke risk factors, analysis stratified by WHR quartiles revealed a lower incidence of first-ever all-stroke and ischemic stroke associated with higher leptin levels among only subjects in the top WHR quartile (HR, 0.64 [0.43,

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0.95] versus 0.98 [0.77, 1.25], for incident all-stroke and 0.61 [0.39, 0.95] versus 0.96 [0.74, 1.26] for ischemic stroke).

Conclusions—Leptin levels were not directly related to risk of incident stroke overall but there was an inverse association with stroke in the top WHR quartile. Further investigations are required to confirm these findings and explore possible mechanisms for the observed association.

Keywords

Leptin; Adipokines; Risk; Stroke; Obesity

Introduction

Leptin is an adipokine hormone secreted by adipocytes, and has structural and functional similarities with proinflammatory cytokines. It plays a key role in neuroendocrine function and metabolic processes (1). Leptin demonstrates a direct association with body mass. It is higher in obese individuals and lower in persons of normal weight. Leptin inhibits appetite and increases energy expenditure (2). However, central leptin resistance occurs in obese individuals resulting in a hyperleptinemic state (3,4). There is evidence suggesting a potential role of leptin in glucose regulation, insulin sensitivity, hematopoiesis, fatty acid catabolism, angiogenesis, and vascular and endothelial function (5–8). Leptin also induces proinflammatory cytokines such as interleukin-6 (IL6), (9) promotes platelet aggregation and may have a role in arterial thrombosis associated with obesity (10).

Higher circulating leptin levels have been associated with an increased prevalence of various vascular risk factors including insulin resistance, diabetes, hypertriglyceridemia and hypertension, and with lower levels of high-density lipoprotein cholesterol (HDL-C) (11–14). However, the association of leptin with vascular diseases is controversial. Whereas some studies suggest that higher circulating leptin levels increase risk of vascular disease including myocardial infarction and stroke, (15–20) other investigations have reported no such association (21–26), or have reported a protective role for leptin in determining overall mortality and risk of coronary disease (27–29).

Moreover, although several longitudinal studies have examined the association of circulating leptin levels with incident coronary heart disease, there are limited prospective data on their association with the risk of stroke (30). Accordingly, we longitudinally evaluated the association of baseline circulating levels of leptin with the risk of first ever any and ischemic stroke in the Framingham Heart Study to determine to what extent leptin may be an independent risk marker for first-ever stroke.

Materials and Methods

Study sample

The design and details of the Framingham Heart Study cohort have been published elsewhere (31). A total of 5209 participants were included in this study in 1948 to identify cardiovascular disease (CVD) risk factors. Participants are seen in the Heart Study research clinic every 2 years. Visits include laboratory testing, anthropometric measurements, as well

as a standardized medical history and physical examination by a study physician. The 1166 participants who were alive at the time of the 22nd biennial examination cycle (between 1992 and 1994) were eligible for the present investigation. Leptin levels were measured in 796 participants. In our study sample, participants who did not have leptin levels measured were significantly older than those with leptin measured. However, after adjustment for age, participants without and with leptin levels did not differ with respect to their baseline clinical or biochemical characteristics. We excluded 39 participants for prevalent stroke. After the exclusions, 757 participants (mean age, 79 years; 470 women) were included in this analysis.

The study protocol was approved by the Institutional Review Board of the Boston University Medical Center and all participants provided written informed consent.

Outcome

Our protocols for stroke surveillance and diagnosis have been published elsewhere (32). Stroke was defined as an acute onset focal neurological deficit of vascular etiology, persisting more than 24 hours. Ischemic stroke was diagnosed if a focal neurological deficit was documented and the imaging showed no hemorrhage, the imaging showed an ischemic infarct that correlated with the clinical deficit, or an ischemic infarct was documented at autopsy.

Leptin Assay

A commercial radioimmunoassay (Linco Research, Inc., St. Louis, MO) was used to determine leptin concentrations from non-fasting plasma samples. The inter-assay coefficient of variation ranged from 3.0–6.2%. The lower sensitivity limit of the assay was 0.5 ng/ml.

Statistical Analysis

Serum leptin levels had a right skewed distribution in each sex and mean values differed between the sexes. Therefore, leptin levels were logarithmically transformed and standardized within each sex (mean=0, standard deviation (SD)=1). Cox regression models were used to relate baseline sex-standardized log-leptin levels to the incidence of stroke after confirming that the assumption of proportionality of hazards was met. Initial analyses were adjusted for age- and sex alone (Model A). Model B was additionally adjusted for Framingham Stroke Risk Profile (FSRP) that includes age, sex, systolic blood pressure, antihypertensive therapy, smoking status, diabetes mellitus and cardiovascular disease. In Model C, we further adjusted for waist-hip ratio (WHR). We chose to include WHR in our model as an index of body fat content because it is more strongly correlated with plasma leptin levels and risk of stroke than body mass index (33). We performed an exploratory analyses to evaluate whether age, sex, or WHR affected relationships of plasma leptin levels with stroke, with a Bonferroni-corrected, 2-tailed α level of 0.033 (3 effect modifiers \times 1 exposure = 3 exploratory comparisons; with an alpha level of 0.10 for tests of interaction) and then stratified analyses when significant. Covariates were selected based on biologic interest and associations with exposures. For a sharp focus, we selected sex, age and adiposity as the main determinants of plasma leptin levels. A systematic review of studies

published before September 2014 with over 1 year's follow-up was conducted using a Pubmed search and all published data relating circulating leptin levels to the specific end-point of stroke were abstracted. Odds ratios (OR) were shown with 95% confidence intervals (CI), and 2-tailed probability estimates were used. Statistical analyses were performed using SAS, version 9.3. A p-value <0.05 was considered statistically significant.

Results

A total of 757 individuals were followed for up to 10 years, in which time 119 developed stroke, including 99 individuals with ischemic stroke. Baseline characteristics of our study sample are displayed in Table 1. Log-leptin levels showed no association with incident any or ischemic stroke in any of our models (Table 2). In a meta-analysis of prior studies, the combined risk ratio across all studies was 1.09 (95% CI: 0.87 to 1.37) in the adjusted analyses (Figure 1).

Estimates from our multiplicative models were suggestive for an interaction by WHR (interaction term $P=0.031$). Therefore, we evaluated this possibility by comparing stratum-specific estimates that provides a more robust assessment of both biologic and statistical interaction. Analyses stratified by WHR revealed an inverse relationship between plasma leptin levels and risk of incident any (Hazard ratio, $HR=0.64$ (95% CI: 0.45–0.92), $P=0.01$) and ischemic stroke ($HR=0.65$ (95% CI: 0.44–0.95), $P=0.02$) among subjects in the top WHR quartile, but not in others ($HR=1.02$ (95% CI: 0.82–1.28), $P=0.84$ and $HR=1.03$ (95% CI: 0.80–1.32), $P=0.82$ for incident any and ischemic stroke, respectively) (Table 3). These associations remained unchanged after adjustment for traditional stroke risk factors.

Discussion

In this study of older individuals, higher baseline leptin levels were not prospectively associated with total or ischemic stroke incidence in multivariable adjusted models. However, our analysis stratified by WHR was suggestive for a potential protective role for leptin against risk of incident all-stroke and incident ischemic stroke among persons in the top WHR quartile.

Although high circulating leptin levels have been shown to be correlated with established vascular risk factors such as obesity, hypertension, hyperlipidemia and diabetes and an inflammatory state, few prospective studies have investigated the association of serum leptin with incidence of stroke, and prior cross-sectional and case-control studies had inconsistent findings (16,18,34). Recent studies have suggested a leptin surge as an acute response to stress or CVD events (35); therefore, retrospective and cross sectional study designs may not be optimal to study the association. For example, whereas high leptin was reported as a significant risk factor for prevalent stroke in an initial cross-sectional report from the Jackson Heart study (JHS) (18), it was not prospectively associated with incident coronary heart disease or incident stroke during 6 years of follow up in a subsequent report from the same cohort (26). Furthermore, high leptin levels may not be casually linked to vascular risk and may merely reflect a state of hypothalamic leptin resistance in obesity and the co-occurrence of multiple vascular risk factors with obesity.

Our observation of the lack of an overall association between circulating leptin and risk of stroke is in line with 3 recent prospective studies (22,25,26). In a recent prospective nested case-control study among healthy men within the PRIME study, increased plasma leptin was not shown to be associated with an increased 10-year risk of ischemic stroke (25). Similarly, results from a prospective nested case-control study within the Women's Health Initiative Observational Study cohort with 972 stroke cases suggested no association between serum leptin levels and risk of development of ischemic stroke among postmenopausal women independently of established stroke risk factors (22). On the other hand, in a longitudinal follow-up of the British Regional Heart Study participants with 192 prospectively ascertained cases of stroke, increased leptin was associated with a higher risk of the stroke in older men while obese subjects showed the lowest stroke risk (36). One previous study from the same population observed no association between leptin levels and risk of coronary heart disease, suggesting that this cohort may not be representative of other population samples (37). In our meta-analysis of prior prospective studies of leptin levels and stroke, we did not find any significant association between leptin levels and risk of stroke. None of the previous prospective studies have examined the association of leptin with risk of stroke in different strata of WHR. Our observation of an inverse association between leptin levels and risk of stroke for subjects in the top WHR quartile is intriguing. Recent studies have suggested that leptin can explain the lower rate of cardiovascular events and mortality in the obese (the obesity paradox) (38, 39). Hyperleptinemia generated in the course of diet induced obesity may protect non-adipose tissues from lipotoxicity and oxidative damage (40). Also, leptin is significantly correlated with serum nitric oxide (NO) metabolite concentrations and plays an important role in NO regulation and production in humans (41). Thus, low leptin levels in states of relative leptin resistance, such as obesity may increase risk of vascular events.

Inconsistent findings of the association between leptin and vascular events may be explained by the threshold levels of various leptin-mediated physiological effects in different conditions (42). Recent studies have supported the concept of selective leptin resistance in human and animal models, with a retained sensitivity to leptin in peripheral and vascular tissue in hyperleptinemic states through differential post-receptor signaling feedback loops or brain site-specific mechanisms (4,43,44). In a recent study, leptin was reported to mediate an increase in blood pressure in diet induced obese rodents (45). However, in another study in diet induced obese mice, leptin did not increase blood pressure despite increasing sympathetic activity suggesting that leptin is implicated in other vascular counter-regulatory pathways against hypertension. (46). These concepts suggest the wide array of pathways, beyond blood pressure regulation, by which leptin may differentially modulate the risk of stroke in obese individuals and call for further research, stratified by weight and insulin resistance, to elucidate a possible clinically significant role of leptin resistance in obese individuals.

The prospective ascertainment of first ever stroke in a community-based sample and comprehensive assessment of covariates comprising the Framingham stroke risk profile strengthen our study. However, these results should be interpreted with caution since our data were based on an observational study and cannot yield causal relationship. Also, the study is representative of an elderly population of white individuals that limits the

generalizability of our findings to other age-groups or ethnicities. Leptin concentrations were measured from non-fasting plasma samples. However, mean postprandial leptin levels have been shown not to alter from fasting leptin levels after either the low-fat or the high-fat diet (47). Leptin levels were determined in a single measurement, which may not capture a diurnal variation in leptin levels and may result in a misclassification of exposure that may have biased our results toward the null, but this does not invalidate our finding in the obese. Moreover, leptin levels have been consistently shown to be relatively stable over time in an individual (48,49).

Summary

Our findings indicate that serum leptin levels are inversely associated with risk of stroke among subjects within the top WHR quartiles. Additional population studies stratified by weight and experimental studies on the possible protective effects of leptin, particularly in obese individuals, are required to confirm our findings.

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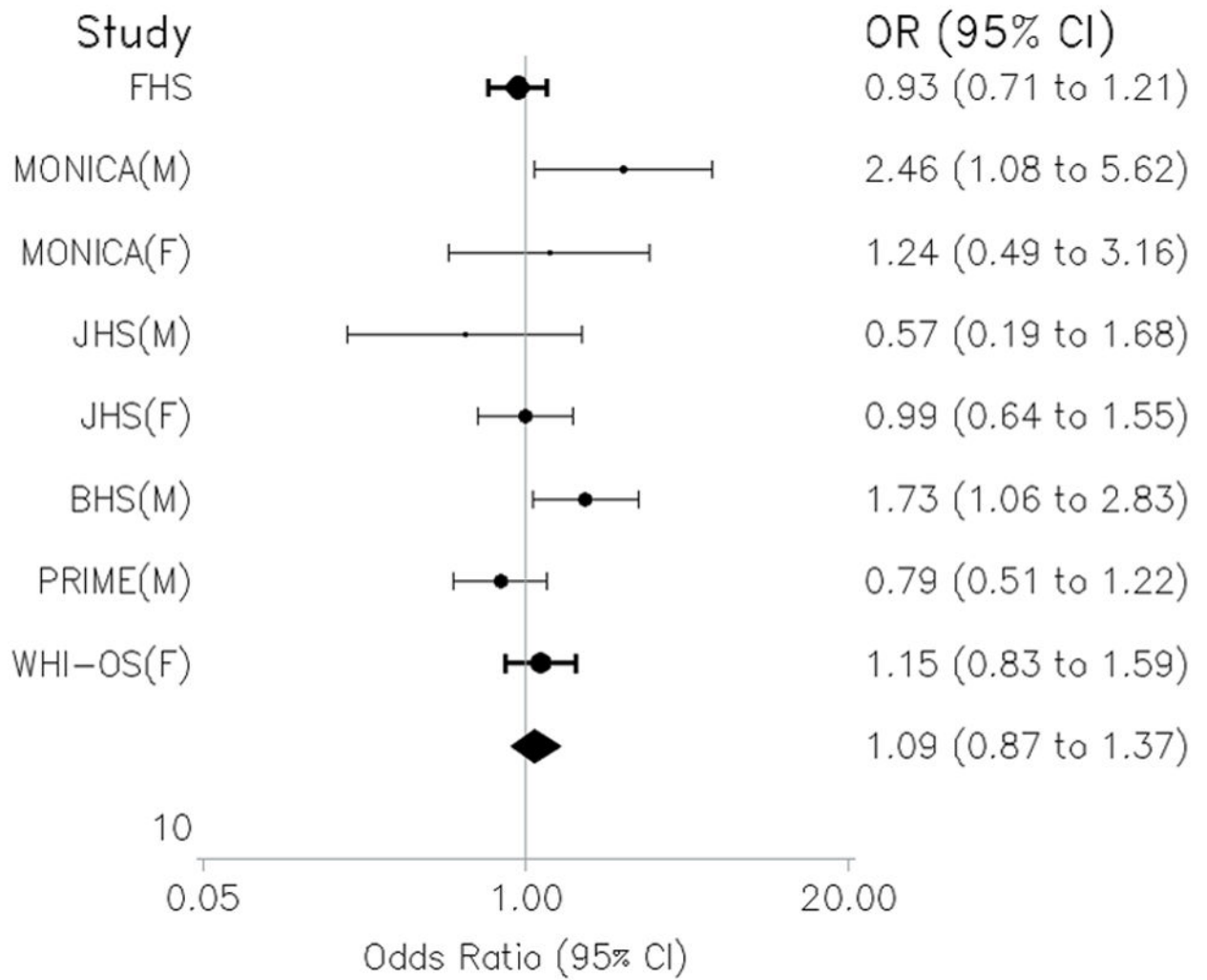


Figure 1. Random effects meta-analysis of prospective studies of circulating leptin levels and Stroke. M: Male; F: Female; FHS: Framingham Heart Study; JHS: Jackson Heart Study; BHS: British Heart Study; WHI-OS: Women Health Initiative – Observational Study.

Table 1

Baseline Characteristics of entire study sample, Framingham Heart Study.

N	757
Age, mean±SD	79±5
Female, n(%)	470 (62)
Systolic Blood Pressure, mean±SD	143±21
Body Mass Index, mean±SD	26.78±4.68
Waist-Hip Ratio, mean±SD	0.93±0.09
Q1, mean±SD	0.83±0.07
Q2, mean±SD	0.90±0.05
Q3, mean±SD	0.95±0.04
Q4, mean±SD	1.02±0.04
Current Smoking, n(%)	62 (8)
Diabetes Mellitus, n(%)	86 (12)
Anti-hypertensive, n(%)	363 (49)
Prevalent CVD, n(%)	241 (32)
Leptin, Median (Q1, Q3)	12.75(6.90, 22.35)
Incident Stroke, n(%)	119 (16)
Ischemic Stroke, n(%)	99 (13)

CVD: Cardiovascular disease; IGF1: Insulin like growth factor1; N: Number; SD: Standard deviation; Q: Quartile

Table 2

Association of leptin* with incident any and ischemic stroke (IS) **

	HR [CI] (p-value)	
	Stroke	IS
Model 1	0.94 [0.78, 1.13] (0.49)	0.96 [0.78, 1.17] (0.65)
Model 2	0.90 [0.73, 1.09] (0.27)	0.89 [0.72, 1.11] (0.28)
Model 3	0.93 [0.71, 1.21] (0.57)	0.93 [0.69, 1.23] (0.59)

* Sex-standardized natural logarithm of leptin;

** Using an indicator of top sex-specific quartile.

HR: Hazard ratio; IS: Ischemic stroke;

CI, confidence interval; HR, hazard ratio.

Model1: adjusted for age and sex; Model2: Model 1 plus systolic blood pressure, smoking, diabetes mellitus, Anti-hypertensive treatment, prevalent cardiovascular disease; Model3: Model 2 plus waist-hip ratio.

Table 3

Stratified analysis by WHR for the association between leptin* and incident any and ischemic stroke (IS)**

Model	Top Quartile of WHR				Bottom 3 quartiles of WHR			
	Stroke		IS		Stroke		IS	
	n	HR [CI] (p-value)	n	HR [CI] (p-value)	n	HR [CI] (p-value)	n	HR [CI] (p-value)
Model 1	31/189	0.64 [0.45, 0.92] (0.01)	27/189	0.65 [0.44, 0.95] (0.02)	87/564	1.02 [0.82, 1.28] (0.84)	71/564	1.03 [0.80, 1.32] (0.82)
Model 2	31/189	0.64 [0.43, 0.95] (0.02)	27/189	0.61 [0.39, 0.95] (0.02)	87/564	0.98 [0.77, 1.25] (0.89)	71/564	0.96 [0.74, 1.26] (0.78)

* Sex-standardized natural logarithm of leptin;

** Using an indicator of top sex-specific quartile.

HR: Hazard ratio; CI: Confidence interval; WHR: Weight-to-hip ratio;

Model 1: adjusted for age and sex; Model2: Model 1 plus systolic blood pressure, smoking, diabetes mellitus, Anti-hypertensive treatment, prevalent cardiovascular disease.