# Adipocytokines and the risk of ischemic stroke  The PRIME Study

# Adipocytokines and the Risk of Ischemic Stroke: The PRIME Study

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**Objective:** Adipocytokines are hormones secreted from adipose tissue that possibly link adiposity and the risk of cardiovascular disease, but limited prospective data exist on plasma adipocytokines and ischemic stroke risk. We investigated associations and predictive properties of 4 plasma adipocytokines, namely resistin, adipsin, leptin, and total adiponectin, with regard to incident ischemic stroke in the PRIME Study.
**Methods:** A cohort of 9,771 healthy men 50 to 59 years of age at baseline was followed up over a period of 10 years. In a nested case–control study, 95 ischemic stroke cases were matched with 190 controls on age, study center, and date of examination. Hazard ratios (HRs) per standard deviation increase in plasma adipocytokine levels were estimated using conditional logistic regression analysis. The additive value of adipocytokines in stroke risk prediction was evaluated by discrimination and reclassification metrics.
**Results:** Resistin (HR, 1.88; 95% confidence interval [CI], 1.16–3.03), adipsin (HR, 2.01; 95% CI, 1.33–3.04), and total adiponectin (HR, 1.53; 95% CI, 1.01–2.34), but not leptin, were independent predictors of ischemic stroke. The performance of a traditional risk factor model predicting ischemic stroke was significantly improved by the simultaneous inclusion of resistin, adipsin, and total adiponectin (c-statistic: 0.673 [95% CI, 0.631–0.766] vs 0.826 [95% CI, 0.792–0.892], $p < 0.001$; net reclassification improvement: 38.1%, $p < 0.001$).
**Interpretation:** Higher plasma levels of resistin, adipsin, and total adiponectin were associated with an increased 10-year risk of ischemic stroke among healthy middle-aged men. Resistin, adipsin, and total adiponectin provided incremental value over traditional risk factors for the prediction of ischemic stroke risk.

ANN NEUROL 2012;71:478–486

The accumulation of abdominal fat mass is associated with an increased risk of coronary heart disease (CHD) and stroke,[1,2] yet the underlying pathogenic mechanisms are not well understood. Plasma adipocytokines are possible biological links between adiposity and cardiovascular disease. Among these adipose-derived hormones, leptin, adipsin, and total adiponectin are primarily secreted by adipocytes, and resistin predominantly originates from macrophages.[3] A number of studies have examined the association of these adipocytokines with incident CHD[4–7] and combined cardiovascular outcomes,[8,9] but no firm conclusions have been reached at present. With regard to future ischemic stroke, associations of plasma adipocytokines have not been investigated extensively, and most studies have examined the relationship between individual adipocytokines and stroke risk. Two prior studies explored the association between total adiponectin and future ischemic

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.22669

Received Jun 28, 2011, and in revised form Oct 3, 2011. Accepted for publication Nov 4, 2011.

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stroke,[10,11] whereas resistin and leptin were each subject to 1 prior study on incident ischemic stroke.[12,13] Because adipocytokines operate on distinct pathways of atherothrombosis, assessing their simultaneous contribution to future ischemic stroke may provide valuable epidemiological and clinical insights. Only 1 prior study that was conducted among postmenopausal women has jointly investigated the association between resistin, leptin, and total adiponectin with regard to future ischemic stroke.[14] Furthermore, we are not aware of any previous study that has investigated the additive value of plasma adipocytokines over traditional risk factors in predicting ischemic stroke risk.

Thus, the aim of the present study was, first, to quantify the association of resistin, adipsin, leptin, and total adiponectin with incident ischemic stroke among healthy middle-aged men and, second, to evaluate the value of these adipocytokines for the prediction of ischemic stroke risk using data from the PRIME study (Prospective Epidemiological Study on Myocardial Infarction).

## Subjects and Methods

### Sampling Frame

PRIME is a prospective cohort study of male European Caucasians.[15] Briefly, the study was conducted in 4 collaborating centers of the World Health Organization (WHO) MONICA (Multinational MONItoring trends and determinants in CArdiovascular disease) project in Northern Ireland (Belfast) and France (Lille, Strasbourg, and Toulouse). Individuals were recruited from various employment groups and health care settings.

### Baseline Assessment

Between 1991 and 1994, a total of 10,602 men 50 to 59 years of age were interviewed and examined using standardized methods. Information on medical history, medication use, and tobacco as well as alcohol consumption was obtained using interviewer-administered questionnaires. Height and weight were measured in individuals standing tall, wearing light cloths, and without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was taken midway between the lower rib bow and the superior border of the iliac crest. Blood pressure was measured after 5 minutes of rest in an upright sitting position using an automated device (Spengler, Asnières-sur-Seine, France). Diabetes mellitus was defined as self-reported diabetes and/or use of antidiabetic medication. Cigarette smoking and alcohol drinking were defined as the average number of cigarettes smoked over the past 5 years and the average alcohol intake in grams per day during the past week, respectively. Venous blood samples were drawn after a minimum fasting time of 12 hours. Total and high-density lipoprotein (HDL) cholesterol were determined at the central study laboratory (Pasteur Institute of Lille, Lille, France) using an automated analyzer (Boehringer, Mannheim, Germany). Plasma aliquots were stored locally in liquid nitrogen at −80°C for subsequent laboratory analysis of biomarkers.

### Follow-up and Case Ascertainment

Study participants were contacted annually over a period of 10 years either by mail or by telephone and completed self-report questionnaires on clinical events. Among individuals with possible events, clinical information was obtained from medical records in hospital and general practice. All available data were gathered concerning hospital admission, electrocardiogram, laboratory results, medical treatment, and interventions. Deceased patients' families and practitioners were contacted to clarify death circumstances, and death certificates were checked to complement clinical and postmortem information. Stroke events were adjudicated by an independent committee, and stroke was defined as previously reported[16,17] according to WHO MONICA criteria. Transient or permanent cerebral focal deficit caused by a blood disease, a cerebral tumor, or metastasis, or secondary to a trauma was not considered by the medical committee. Clinical information, computerized tomodensitometry scans (compatible signs), and angiographic and autopsy data were used to distinguish between ischemic and hemorrhagic stroke events. After 10 years of follow-up, the stroke event status was available for 95.1% of the cohort. The study was approved by local ethics committees, and participants provided written informed consent.

### Nested Case–Control Study

Among 9,771 men free of CHD and stroke at baseline, 98 first ischemic stroke cases were validated during 10 years of follow-up, but baseline plasma samples were available for only 95 cases. A total of 190 controls, 2 individuals for each stroke case, were randomly selected from the initial cohort. Controls were free of CHD and stroke at the time of event and matched on age (±3 years), study center, and date of recruitment (±3 days). Plasma levels of adipocytokines and high-sensitivity C-reactive protein (hs-CRP) were determined in baseline samples blind to the case-control status with a multiplex bioassay using commercially available kits.[7] Multiplex beads were purchased from the following 2 manufacturers: R&D Systems (Billerica, MA) for measurements of hs-CRP (LOB1707), resistin (LOB1359), adipsin (LOB1824), and leptin (LUB398); and Linco Research Inc. (Billerica, MA) for total adiponectin (human cardiovascular disease panel 1 multiplex immunoassay). Each multiplex assay was performed according to the manufacturers' specifications. The plates were read on a Luminex 200 instrument system. The coefficients of variation were 9.9%, 6.6%, 7.2%, 12.4%, and 9.7% for resistin, adipsin, leptin, total adiponectin, and hs-CRP, respectively. The study protocol was approved by the institutional review board of Broussais Hospital, Paris, France.

### *Statistical Analysis*

Only complete case–control pairs without missing information on plasma adipocytokines were considered in the analysis. Univariate conditional logistic regression analysis was applied to compare baseline characteristics of ischemic stroke cases and controls. In the control data set, correlations between plasma adipocytokines and selected cardiovascular risk factors were examined by means of partial Spearman rho adjusted for matching variables. Hazard ratios (HRs) of ischemic stroke per standard deviation increase in adipocytokine levels were estimated using conditional logistic regression analysis. First, each adipocytokine was examined separately in univariate analysis accounting for matching variables only (model 1) and with sequential adjustment for systolic blood pressure, antihypertensive treatment, cigarette smoking, alcohol drinking (model 2), total cholesterol, HDL cholesterol, waist circumference, diabetes mellitus (model 3), and hs-CRP (model 4). Interaction between each adipocytokine and systolic blood pressure, adiposity (BMI or waist circumference), and hs-CRP was explored in the fully adjusted model (model 4) by including interaction terms, and their statistical significance was evaluated using the Wald test. Second, adipocytokines independently associated with ischemic stroke in separate models were mutually analyzed using backward elimination from a multivariate model additionally containing traditional risk factors and hs-CRP. To assess discrimination and reclassification metrics, predicted probabilities were obtained from unconditional logistic regression analysis adjusted for matching variables. C-statistic indices from a traditional risk factor model and models additionally including adipocytokines both separately and in combination were compared with the method described by DeLong et al[18] and internally evaluated by bootstrapping with 240 replications.[19] Net reclassification improvement (NRI) by the novel model with adipocytokines was quantified as opposed to the traditional risk factor model after adjustment for the case–control design.[20,21] Risk categories for NRI estimation were based on tertiles of risk as predicted by the traditional risk factor model. All tests were 2-tailed, and an alpha level of 0.05 was chosen to indicate statistical significance. The statistical analysis was carried out using Statistical Analysis Software version 9.2 (SAS Institute, Cary, NC).

## Results

### *Study Population*

Overall, 15 case–control pairs were excluded from the original data set due to missing information on plasma adipocytokines, leaving 80 complete pairs for the present analysis. No significant differences in baseline characteristics were observed between included and excluded individuals both among cases and controls.

### *Baseline Characteristics*

Ischemic stroke cases showed higher levels of systolic and diastolic blood pressure, cigarette smoking, and plasma levels of resistin, adipsin, and total adiponectin when compared to control individuals (Table 1).

### *Correlates of Adipocytokines*

Correlation coefficients for the association of cardiovascular risk factors with resistin, adipsin, leptin, and total adiponectin ranged from −0.093 to 0.093, from −0.102 to 0.241, from −0.235 to 0717, and from −0.092 to 0.206, respectively (Table 2). Plasma adipsin and leptin positively correlated with BMI, waist circumference, and hs-CRP. A positive correlation was found between plasma total adiponectin and HDL cholesterol. Correlation coefficients between the 4 plasma adipocytokines ranged from 0.001 to 0.321. No significant correlations were observed between resistin and the selected cardiovascular risk factors.

### *Associations of Adipocytokines with Future Ischemic Stroke*

Table 3 presents HRs of ischemic stroke separately for each adipocytokine and per standard deviation increase in plasma levels. In the analysis accounting for matching variables only, higher plasma concentrations of resistin (HR, 2.76; 95% CI, 1.78–4.28), adipsin (HR, 2.22; 95% CI, 1.57–3.15), and total adiponectin (HR, 2.05; 95% CI, 1.38–3.05) were associated with an increased risk of future ischemic stroke. These associations were not attenuated by subsequent adjustment for systolic blood pressure, antihypertensive treatment, cigarette smoking, alcohol drinking, total and HDL cholesterol, waist circumference, diabetes mellitus, and hs-CRP (see also Supplementary Table 1). There was no significant interaction of plasma adipocytokines with systolic blood pressure, adiposity (waist circumference or BMI), and hs-CRP. Estimated HRs for each plasma adipocytokine were similar when adjusting for BMI instead of waist circumference (data not shown). In analyses stratified by country, we observed similar results as in the pooled analysis of adipocytokines' association with ischemic stroke (Supplementary Table 2).

When we assessed resistin, adipsin, and total adiponectin together in a single multivariate model including putative confounding factors, the HRs per standard deviation increase of resistin (HR, 1.88; 95% CI, 1.16-3.03), adipsin (HR, 2.01; 95% CI, 1.33-3.04), and total adiponectin (HR, 1.53; 95% CI 1.01-2.34) were attenuated but remained statistically significant (Table 4). In this model, systolic blood pressure and cigarette smoking were the remaining risk factors independently associated with ischemic stroke.

**TABLE 1: Baseline Characteristics of Study Participants with Incident Ischemic Stroke During 10 Years of Follow-up and Matched Controls**

| Characteristic | Cases, n = 80 | Controls, n = 160 | $p$[a] |
|---|---|---|---|
| Age, mean yr (SD) | 55.5 (2.9) | 55.4 (2.7) | Matched |
| Systolic blood pressure, mean mmHg (SD) | 141.5 (20.8) | 133.9 (18.7) | 0.002 |
| Diastolic blood pressure, mean mmHg (SD) | 87.3 (13.9) | 83.7 (12.0) | 0.026 |
| Antihypertensive treatment, No. [%] | 16 [20.0] | 27 [16.9] | 0.556 |
| Cigarette smoking, mean per day (SD) | 6.4 (9.3) | 3.1 (8.5) | 0.008 |
| Alcohol drinking, mean g/day (SD) | 40.6 (33.7) | 35.5 (42.9) | 0.358 |
| Total cholesterol, mean mg/dl (SD) | 226.1 (41.9) | 222.9 (40.2) | 0.570 |
| HDL cholesterol, mean mg/dl (SD) | 48.9 (13.5) | 49.3 (11.7) | 0.775 |
| Body mass index, mean kg/m$^2$ (SD) | 27.0 (4.0) | 26.6 (3.3) | 0.424 |
| Waist circumference, mean cm (SD) | 96.6 (10.7) | 94.6 (8.9) | 0.128 |
| Diabetes mellitus, No. [%] | 6 [7.5] | 6 [3.8] | 0.191 |
| Resistin, mean ng/ml (SD) | 7.3 (3.5) | 5.0 (3.7) | <0.001 |
| Adipsin, mean ng/ml (SD) | 4.2 (1.6) | 3.1 (1.3) | <0.001 |
| Leptin, mean ng/ml (SD) | 8.9 (7.9) | 7.8 (6.9) | 0.477 |
| Total adiponectin, mean mg/dl (SD) | 19.1 (12.4) | 13.5 (10.8) | <0.001 |
| hs C-reactive protein, mean mg/l (SD) | 3.5 (2.7) | 3.1 (2.8) | 0.207 |

[a]Probability values from univariate conditional logistic regression analysis.
HDL = high-density lipoprotein; hs = high sensitivity; SD = standard deviation.

**TABLE 2: Spearman Partial Correlation Coefficients between Plasma Adipocytokines and Selected Cardiovascular Risk Factors in the Control Data Set**

| Factor | Adipocytokines | | | |
|---|---|---|---|---|
| | Resistin | Adipsin | Leptin | Total Adiponectin |
| Systolic blood pressure | −0.015 | 0.107 | 0.397[a] | 0.019 |
| Cigarette smoking | 0.093 | −0.013 | −0.235[b] | −0.007 |
| Total cholesterol | 0.074 | 0.005 | 0.131 | −0.068 |
| HDL cholesterol | −0.016 | −0.102 | −0.080 | 0.206[b] |
| Body mass index | −0.093 | 0.241[b] | 0.662[a] | −0.034 |
| Waist circumference | −0.058 | 0.232[b] | 0.717[a] | −0.092 |
| hs C-reactive protein | 0.066 | 0.209[b] | 0.331[a] | 0.064 |
| Resistin | | 0.301[a] | 0.060 | 0.135 |
| Adipsin | | | 0.321[a] | 0.152 |
| Leptin | | | | 0.001 |

Spearman partial correlation coefficients adjusted for matching variables.
[a]$p < 0.001$.
[b]$p < 0.01$.
HDL = high-density lipoprotein; hs = high sensitivity.

**TABLE 3: Univariate and Multivariate HRs of Ischemic Stroke Presented Separately for Each Plasma Adipocytokine**

| Adipocytokine | Model 1, HR (95% CI) | Model 2, HR (95% CI) | Model 3, HR (95% CI) | Model 4, HR (95% CI) |
|---|---|---|---|---|
| Resistin | 2.76 (1.78–4.28) | 2.78 (1.72–4.49) | 2.79 (1.70–4.59) | 2.82 (1.72–4.65) |
| Adipsin | 2.22 (1.57–3.15) | 2.57 (1.73–3.82) | 2.68 (1.77–4.10) | 2.67 (1.76–4.04) |
| Leptin | 1.10 (0.85–1.42) | 1.01 (0.75–1.36) | 0.81 (0.53–1.25) | 0.79 (0.51–1.22) |
| Total adiponectin | 2.05 (1.38–3.05) | 1.99 (1.29–3.07) | 2.17 (1.35–3.47) | 2.18 (1.37–3.48) |

HRs from conditional logistic regression analysis per standard deviation increase in adipocytokine levels accounting for matching variables (model 1) and after sequential adjustment for systolic blood pressure, antihypertensive treatment, cigarette smoking, alcohol drinking (model 2), total cholesterol, high-density lipoprotein cholesterol, waist circumference, diabetes mellitus (model 3), and high-sensitivity C-reactive protein (model 4).
CI = confidence interval; HR = hazard ratio.

### *Adipocytokines and Prediction of Ischemic Stroke Risk*

As shown in Table 5, the separate inclusion of resistin, adipsin, and total adiponectin in the traditional risk factor model containing systolic blood pressure and cigarette smoking in addition to matching variables significantly increased the c-statistic from 0.673 up to 0.789 ($p = 0.001$). A more pronounced increase in the c-statistic from 0.673 to 0.826 ($p < 0.001$) was observed with the simultaneous inclusion of resistin, adipsin, and total adiponectin in the traditional risk factor model. Likewise, when simultaneously including resistin, adipsin, and total adiponectin in the traditional risk factor model, risk classification among cases and controls improved by 13.8% and 24.4%, respectively, yielding an NRI of 38.1% ($p < 0.001$). When additionally including waist circumference in the traditional risk factor model, only marginal changes were observed in NRI (40.0%, $p < 0.001$) and c-statistic indices (Supplementary Table 3).

## Discussion

In a cohort of Caucasian middle-aged men free of CHD and stroke, elevated plasma levels of resistin, adipsin, and total adiponectin, but not leptin, were independent predictors of incident ischemic stroke and significantly improved the prediction of 10-year ischemic stroke risk over traditional risk factors and hs-CRP.

Only a limited number of studies have so far investigated the association of adipocytokines with future ischemic stroke, and these studies mostly examined a single adipocytokine. The Women's Health Initiative Observational Study (WHI-OS) recently published associations of several adipocytokines including resistin, leptin, and adiponectin with incident ischemic stroke among postmenopausal women.[14] Our study complements previous studies in the field in 2 novel ways. First, we extend the concurrent investigation of several adipocytokines and their association with future ischemic stroke to a sample of healthy middle-aged men. Second, to the best of our knowledge, our study is the first to evaluate the additive value of several plasma adipocytokines for the prediction of ischemic stroke risk.

Our finding of increased ischemic stroke risk with higher resistin concentration is consistent with the results

**TABLE 4: Mutually Adjusted HRs of Ischemic Stroke for Traditional Risk Factors and Plasma Adipocytokines**

| Factor | HRs | 95% CI | *p* |
|---|---|---|---|
| Traditional risk factors | | | |
| Systolic blood pressure, mmHg | 1.75 | 1.16–2.66 | 0.008 |
| Cigarette smoking, No./day | 1.43 | 1.03–1.99 | 0.035 |
| Plasma adipocytokines | | | |
| Resistin, ng/ml | 1.88 | 1.16–3.03 | 0.010 |
| Adipsin, ng/ml | 2.01 | 1.33–3.04 | 0.001 |
| Total adiponectin, mg/dl | 1.53 | 1.01–2.34 | 0.047 |

HRs from conditional logistic regression analysis per standard deviation increase in risk factor and adipocytokine levels. Model was fitted using backward selection from a model including systolic blood pressure, antihypertensive treatment, cigarette smoking, alcohol drinking, total cholesterol, high-density lipoprotein cholesterol, waist circumference, diabetes mellitus, high-sensitivity C-reactive protein, resistin, leptin, and total adiponectin.
CI = confidence interval; HR = hazard ratio.

**TABLE 5: Comparison of c-Statistic Indices from Nested Multivariate Models Predicting Ischemic Stroke Risk**

| Model | c-Statistic | 95% CI | *p* |
|---|---|---|---|
| Traditional risk factor model | 0.673 | 0.631–0.766 | — |
| + resistin | 0.763 | 0.729–0.833 | 0.007 |
| + adipsin | 0.789 | 0.744–0.859 | 0.001 |
| + total adiponectin | 0.725 | 0.677–0.816 | 0.029 |
| + resistin + adipsin + total adiponectin | 0.826 | 0.792–0.892 | <0.001 |

c-Statistic indices from unconditional logistic regression analysis adjusted for matching variables; 95% CI from bootstrapping with 240 replications. Probability values are for the comparison with the traditional risk factor model using the DeLong test. The traditional risk factor model includes systolic blood pressure and cigarette smoking.
CI = confidence interval.

of the WHI-OS cited above. In that nested case–control study including 972 cases, women in the third and fourth quartile of resistin concentration respectively had a 55% and 39% increased risk of ischemic stroke when compared to women in the first quartile.[14] In contrast, in a case–control study nested within the EPIC-Potsdam Study including 97 ischemic stroke cases, there was a nonsignificant association between resistin and incident ischemic stoke.[12] The adverse relationship of high total adiponectin with future ischemic stroke as observed in the present study was unexpected, given its putative antiatherogenic properties, but might also be due to chance. This finding is inconsistent with results of a nested case–control study among Japanese community-dwelling adults of all ages,[10] a post hoc analysis among older individuals at increased cardiovascular risk from the PROSPER study,[11] and the abovementioned WHI-OS.[14] In these studies, the inverse association between total adiponectin and ischemic stroke disappeared after adjusting for cardiovascular risk factors. Nevertheless, significant adverse associations of high total adiponectin with future CHD[22] and cardiovascular mortality[23,24] have already been reported. Various hypotheses have been proposed for such counterintuitive findings, including possible confounding by kidney dysfunction and wasting syndrome. However, prior studies have observed an increased cardiovascular risk with elevated total adiponectin despite adjustments for renal function and weight change.[22,24] Furthermore, compensatory increase in total adiponectin among individuals with subclinical disease has been suggested as a possible explanation.[25] Unfortunately, information on renal function, weight change, and subclinical atherosclerosis was not available for the present analysis. We are not aware of any previous study that prospectively evaluated the relationship between plasma adipsin and incident ischemic stroke. However, our group recently reported a nonsignificant association between adipsin and the risk of CHD over a period of 10 years.[7] Finally, the lack of an association between leptin and ischemic stroke in our study is in line with results of the WHI-OS, the findings of a case–control analysis nested within a population-based study in Sweden,[13] and more generally with a recent meta-analysis of studies on leptin and incident CHD.[4]

Although the exact mechanisms by which adipocytokines may increase vascular disease risk require further investigation, preclinical evidence has recently been accumulating for adipocytokines' involvement in several pathways of atherothrombosis. Resistin increases the uptake of oxidized low-density lipoprotein cholesterol by macrophages, thereby promoting foam cell formation,[26] and it promotes endothelial cell activation through release of endiothelin-1 and upregulation of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1.[27] In addition, resistin upregulates expression and secretion of cytokines including interleukin-6, tumor necrosis factor-alpha (TNF-$\alpha$), and interleukin-1$\beta$ through nuclear factor-$\kappa$B and mitogen-activated protein kinase pathways likely initiated by resistin binding to the toll-like receptor 4.[28] Finally, resistin may enhance thrombus formation during plaque formation.[29] In contrast, total adiponectin is thought to exert antiatherogenic effects by reducing early atherogenic processes and endothelial cell adhesion in particular.[30] Further, adipsin is related to the alternative pathway of complement activation.[31]

Adipocytokines' involvement in different pathways of atherothrombosis may explain the larger improvement in discrimination and reclassification accuracy with the simultaneous inclusion of adipocytokines when compared to their separate addition to the traditional risk factor model.[32,33] The results of our study with regard to the predictive properties of resistin, adipsin, and total adiponectin may have clinical implications in the near future, because they suggest that these adipocytokines may be useful to better identify individuals at increased risk of ischemic stroke. Plasma concentrations of resistin and total adiponectin have been shown to remain stable over time,[34,35] which is an important characteristic of biomarkers suitable for risk prediction purposes. Of course, a number of issues need to be addressed before advocating the use of these

adipocytokines for stroke risk prediction. First, our findings on the usefulness of resistin, adipsin, and total adiponectin need to be validated in independent data. Second, whether these adipocytokines are causally involved in the disease process is a challenging but important question. Epidemiological studies utilizing the Mendelian randomization approach may be helpful in examining this issue. So far, a polymorphism in the resistin promoter region at −420 has been suggested to be associated with both serum resistin levels and history of stroke in type 2 diabetic patients.[36] Finally, clinical trials on the efficacy of modifying plasma adipocytokine levels to reduce ischemic stroke risk may provide further evidence for adipocytokines' clinical importance. Although drugs specifically targeting adipocytokines are currently not available, atorvastatin has been suggested to reduce resistin levels in type 2 diabetic patients and in vitro to lower resistin mRNA levels in adipocytes and human monocytes/macrophages.[37] Furthermore, anti-TNF-α monoclonal antibodies have been shown to reduce resistin concentration in patients with rheumatoid arthritis.[38] Also, experimental data have suggested that high-dose folic acid consumption is associated with a significant reduction of resistin levels in obese diabetic mice.[39]

Our study has some limitations. The number of ischemic stroke events precluded us from conducting subgroup analyses by phenotype. Adiponectin and resistin exist in various isoforms, which were not considered in the present investigation. It should be noted that high–molecular-weight adiponectin was not associated with incident ischemic stroke in postmenopausal women from the WHI-OS.[40] We acknowledge the possibility of residual confounding by diabetes and insulin resistance owing to imprecision in its definition and unavailable information, respectively. The choice of cutpoints is crucial in reclassification analysis, and results regarding NRI should therefore be interpreted with caution. The narrow age range of our study population may have contributed to the predictive properties of plasma adipocytokines relative to traditional risk factors of ischemic stroke.[41] Our study results should be confirmed for older age groups, women, and other ethnic groups.

In conclusion, increased plasma levels of resistin, adipsin, and total adiponectin, but not leptin, were independently associated with an increased 10-year risk of ischemic stroke among healthy middle-aged men. The 3 adipocytokines provided incremental value over traditional risk factors and hs-CRP in the prediction of ischemic stroke risk. Further studies are needed to elucidate the pathogenic mechanisms behind these associations, if they are confirmed by independent studies.

## Acknowledgment

The PRIME Study was funded by the INSERM and Merck, Sharp, and Dohme-Chibret Laboratory. The PRIME Study was also supported by a 3-year grant from the Fondation Coeur et Artères (No. FCA 06 T2). Support was also provided by the Alliance Partnership Program. The postdoctoral fellowship of C.P. was supported by the Ile de France region under the program CODDIM (Coeur Obesité Diabète- Domaine d'Intérêt Majeur). Further support was provided by the Health and Social Care Research and Development Office (F.K.) and Agence Nationale de la Recherche (G.L.).

We thank the following organizations, which allowed the recruitment of the PRIME subjects: the health screening centers organized by the Social Security of Lille (Institut Pasteur), Strasbourg, Toulouse, and Tourcoing; Occupational Medicine Services of Haute-Garonne of the Urban Community of Strasbourg; the Association Inter-entreprises des Services Médicaux du Travail de Lille et environs; the Comité pour le Développement de la Médecine du Travail; the Mutuelle Générale des PTT du Bas-Rhin; the Laboratoire d'Analyses de l'Institut de Chimie Biologique de la Faculté de Médecine de Strasbourg; and the Department of Health and Social Services and Personal Safety (Northern Ireland), its Research and Development Office, and the Northern Ireland Chest Heart and Stroke Association.

We also thank Drs L. Guize (PRIME Validation Event Committee, deceased), C. Morrison (PRIME Validation Event Committee), M.-T. Guillanneuf (PRIME Validation Event Committee), and M. Giroud (PRIME Validation Event Committee) and A. Belot (statistical support).

## Potential Conflicts of Interest

P.A.: board membership, Total; consultancy, Ipsen; grants/grants pending, Ipsen, Pfizer, Sanofi-Aventis, AstraZeneca, Fondation Plan Alzheimer; speaking fees, Sanofi-Aventis, Pfizer, Servier; stock/stock options, Genoscreen. J.Y.: travel expenses, British Council/Alliance Francaise.

## Authorship

C.P. conducted the statistical analysis and drafted the paper. G.L., E.M., J.Y., J.-.B.R., M.M., and B.H. were responsible for substantial contributions to acquisition of data and critical revision of the manuscript for important intellectual content. J.F., F.K., D.A., P.A., and P.D. were responsible for substantial contributions to conception and

design and critical revision of the manuscript for important intellectual content. P.D. and A.B. were responsible for general coordination and critical revision of the manuscript for important intellectual content. J.-P.E. was responsible for supervision of statistical analysis, data interpretation, and writing of the manuscript. C.P., D.A., J.F., P.A., F.K., P.D., and J.-P.E. take responsibility for the integrity of the work as a whole, from inception to publication.

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