Published in final edited form as:

J Stroke Cerebrovasc Dis. 2018 January ; 27(1): 68–75. doi:10.1016/j.jstrokecerebrovasdis.2017.08.003.

Plasma retinol-binding protein 4 levels and the risk of ischemic stroke among women

Pamela M. Rist^{1,2}, Monik C. Jiménez¹, Shelley S. Tworoger^{2,3}, Frank B. Hu^{2,3,4}, JoAnn E. Manson^{1,2}, Qi Sun^{3,4}, and Kathryn M. Rexrode¹

¹Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

²Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA

³Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School

⁴Department of Nutrition, Harvard T. H. Chan School of Public Health, Boston, MA

Abstract

Background—Plasma retinol-binding protein 4 (RBP4) levels have been associated with cardiovascular risk factors and risk of coronary heart disease, but little is known about the association between RBP4 and risk of ischemic stroke. We hypothesized that elevated RBP4 levels would be associated with an increased risk of ischemic stroke among women.

Methods—We performed a nested case-control study among women enrolled in the Nurses' Health Study who provided blood samples between 1989–1990 and were free of prior stroke and cancer. We measured pre-diagnostic RBP4 levels in 471 ischemic stroke cases who were confirmed by medical record review and in 471 controls who were matched 1:1 to the cases on age, race, blood collection date, menopausal status, postmenopausal hormone use, and smoking status. We analyzed the association between RBP4 levels and ischemic stroke using multivariable conditional logistic regression conditional on the matching factors and adjusted for physical activity, body mass index, aspirin use, alcohol consumption, diet, history of diabetes, high cholesterol, high blood pressure, or heart disease, and cholesterol and hemoglobin A1C levels.

Results—Median levels of RBP4 were similar in cases (31.1 µg/mL) and controls (31.0 µg/mL; p-value from Wilcoxon rank sum test=0.82). Quartiles of RBP4 were not associated with an increased risk of ischemic stroke (highest quartile compared to lowest quartile: multivariate-adjusted odds ratio=0.75; 95%CI: 0.48, 1.17). We also did not observe associations between RBP4 and ischemic stroke of thrombotic or embolic origin.

Conclusions—Elevated levels of RBP4 were not associated with an increased risk of ischemic stroke.

Corresponding Author: Pamela M. Rist, 900 Commonwealth Ave, 3rd floor, Boston, MA 02215, Telephone: 617-278-0835, Fax: 617-731-3843, prist@mail.harvard.edu.

Disclosures: The authors have no relevant disclosures.

Keywords

epidemiology; ischemic stroke; retinol-binding protein 4; women

INTRODUCTION

Stroke is a leading cause of morbidity and mortality worldwide. Given the high morbidity burden of stroke, there is interest in finding biomarkers that may identify individuals at high risk. One potential biomarker may be retinol-binding protein 4 (RBP4). RBP4 is a cytokine secreted by adipocytes and hepatocytes and carries retinol (vitamin A alcohol) in blood. A study among mice suggested that elevated RBP4 levels may play an essential role in the development of insulin resistance.[1] Although cross-sectional studies on RBP4 and risk of insulin resistance or diabetes have been mixed[2, 3], recent prospective cohort studies observed an association between an RBP4 promoter polymorphism and risk of type 2 diabetes[4] and between RBP4 and risk of prediabetes[5] and type 2 diabetes.[6]

In addition to associations with insulin resistance and diabetes, elevated RBP4 levels have been associated with several cardiovascular disease risk factors including metabolic syndrome, inflammatory markers, and hypertension [2, 7–15], atherosclerosis severity [16], prevalent cardiovascular disease (CVD)[17], and with incident coronary heart disease among women.[18] However, few studies have explored the association between RBP4 and stroke, which shares similar risk factors to these cardiometabolic diseases. One study observed that elevated RBP4 levels were associated with an increased prevalence of cerebrovascular disease among elderly men.[8] Another suggested that RBP4 may be higher among individuals with a history of cerebral infarction, although this association was attenuated after adjustment for CVD risk factors.[19] A proteomics analysis using data from the Women's Health Initiative reported evidence that levels of RBP4 may be elevated among women who develop total stroke compared to those who do not develop a stroke during their discovery phase, but this association was not explored further because the false discovery rates for this association were high.[20]

Thus, we conducted a prospective study examining the association between RBP4 and incident ischemic stroke risk among women. We specifically studied women because prior research has suggested that diabetes is a stronger risk factor for stroke among women than men [21] and RBP4 may be associated with the risk of developing diabetes[4, 6]. Using a nested case-control study design, we explored the association between RBP4 levels and risk of total, thrombotic, and embolic ischemic stroke after adjustment for CVD risk factors. Additionally, we explored whether this association may be modified by age, body mass index, smoking, postmenopausal hormone therapy, history of diabetes, and hypertension.

METHODS

The Nurses' Health Study (NHS) was started in 1976 when 121,700 female registered nurses aged 30 to 55 years living in 11 U.S. states completed questionnaires about lifestyle factors and medical history. [22] During follow-up, women were sent biennial questionnaires asking about lifestyle factors and medical history, including incident stroke events. In addition,

between 1989 and 1990, 32,826 participants provided blood samples that were processed, archived, and stored in liquid nitrogen freezers as previously described. [23, 24]

We performed a nested case-control study among women with available blood samples without a history of cancer or stroke at the time of blood collection. Cases included in this study were defined as incident ischemic stroke cases occurring between the time of blood collection through 2008. Each case was matched with a control without a history of stroke prior to the index date (i.e., the date the case experienced a stroke event) on age (± 2 years), race/ethnicity (white/ black/ Asian/ Hispanic/ other/ unknown), smoking (never, past, and current), date of blood draw, fasting status (< 8 vs. > 8 hours), menopausal status (premenopausal, postmenopausal, unknown), and hormone use (yes/no). This study was approved by the Institutional Review Board of Brigham and Women's Hospital and all procedures followed were in accordance with institutional guidelines. Informed consent was implied by receipt of completed self-administered questionnaires and blood samples.

Blood Sample Assay

Case-control pairs were handled identically and shipped together; pairs were randomly ordered and assayed in the same batch. All batches included replicate blinded plasma samples to assess laboratory precision. The analysis laboratory (Clinical and Epidemiological Research Laboratory at the Children's Hospital (Boston, MA)) is certified by the Centers for Disease Control and Prevention/National Heart, Lung, and Blood Institute Lipid Standardization Program with commercially available analytic systems.

RBP4 was measured by an ELISA assay from R & D Systems, Minneapolis, MN. The assay employs the two-step quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for RBP4 was pre-coated onto a microtitre plate. Samples, standards and controls are added to the wells of the plate, binding RBP4 to the plate. After a wash to remove unbound substances, a second monoclonal RBP4 antibody linked to an enzyme was added. RBP4 is 'sandwiched' between the immobilized antibody and the enzyme-linked antibody specific to RBP4. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added. Color is generated that is proportional to the amount of RBP4 present in the sample. The mean intra-assay CV of our blinded quality control samples was 8.7%

In addition to the measurement of RBP4, several other biomarkers were considered as covariates and were measured as follows. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and glycohemoglobin (HbA_{1c}) were measured in the Clinical and Epidemiological Research Laboratory at Children's Hospital (Boston, MA) for all cases and controls. Total cholesterol was measured enzymatically with a mean coefficient of variation (CV) of 4% and HDL-C concentration was determined using a direct enzymatic colorimetric assay with a mean CV of 3%. HbA_{1c} levels were determined by turbidimetric immunoinhibition using packed red blood cells with a Hitachi 911 analyzer (Roche Diagnostics) with a mean intra-assay CV of 1.2%.

Cerebrovascular Disease Ascertainment

If a woman reported a non-fatal stroke on her biennial questionnaire, we asked for permission to review her medical records. Deaths were reported by next of kin or postal authorities or determined by systematic searches of the National Death Index. Fatal strokes were confirmed by review of death certificates, hospital records, or autopsy records. Medical records were reviewed by physicians blinded to exposure status. Strokes were confirmed according to the National Survey of Stroke criteria which requires evidence of a neurologic deficit with sudden or rapid onset that persisted for >24 hours or until death. Strokes were classified as ischemic stroke when there was evidence of thrombotic or embolic occlusion of a cerebral artery. Imaging data from computed tomography or MRI or data on autopsy was available for 95% of events. Reproducibility of our classification system is high.[25] Thrombotic strokes were defined as infarction involving the cortical artery regions in the cerebrum and the cerebellum (cortex and subcortical areas) or the focal, small, and deep areas such as the internal capsule, corona radiata, basal ganglia, and brain stem, without involvement of the cortex. Strokes were defined as embolic if evidence of an embolic source was present in the medical record and if imaging studies or neurology consult supported the diagnosis. In the event of incomplete evidence or competing causes of stroke, strokes were considered to be unclassified ischemic stroke.

Statistical Analysis

RBP4 was measured for 471 cases and 471 matched controls. We created quartiles of RBP4 levels based on the distribution in the controls. We compared baseline characteristics of individuals across RBP4 quartiles by using chi-squares tests for proportions (except for race where a Fisher's exact test was used) and Kruskal Wallis tests for continuous variables.

Lifestyle covariates were assessed in the 1990 questionnaire or the closest year prior. Model 1 was conditional on matching factors (age, race-ethnicity, smoking, menopausal status, hormone therapy use, and date of sample collection). Model 2 additionally adjusted for physical activity (in tertiles), body mass index (normal, overweight, obese), aspirin use (none, 1–5 tabs/wk, ≥6 tabs/wk), alcohol consumption (0, 0.1–4.9, 5.0–14.9, ≥15.0 g/day), and Alternative Health Eating Index (aHEI 2010; in quartiles). The aHEI 2010 score was used as an indicator of adherence to healthy eating behavior. Higher scores correspond to a diet lower in trans fat, red and processed meats, sodium, and higher in fruits and vegetables, nuts and legumes, polyunsaturated fats and whole grains.[26] Model 3 adjusted for all covariates in Model 2 and history of diabetes, history of high blood pressure, history of coronary heart disease or revascularization and history of high cholesterol. Model 4 adjusted for all covariates in Model 3 as well as log transformed cholesterol ratio (total cholesterol to HDL-C; continuous) and HbA_{1c} (continuous). Those missing information on physical activity (N=8), body mass index (N=6), alcohol consumption (N=19), and aHEI 2010 (N=19) were assigned to the median value by case-control status. Fourteen matched pairs were missing information on cholesterol ratio and twenty-one pairs were missing information on HbA_{1c} and median values were imputed by case-control status. Those with missing information on smoking (N=3) or aspirin use (N=3) were assigned to never smoker and no aspirin use, respectively, the most frequent category for each variable.

A prior study on RBP4 levels and coronary heart disease (CHD) suggested that time to event may modify the association between RBP4 and CHD because an association was only observed during the first 8 years of follow-up. [18] Therefore, we explored whether the time to event may modify the association between RBP4 levels and risk of ischemic stroke by including an interaction term between follow-up time (less than nine years versus nine or more years) and our exposure. Significance of the interaction was assessed using the likelihood ratio test.

A priori, we proposed to examine effect modification of RBP4 (highest quartiles compared to all others) by age (<65/ ≥ 65 years), postmenopausal hormone therapy (use versus no use), body mass index (< 25 kg/m² versus 18.5 to 24.9 kg/m²), smoking (current versus past/never), history of diabetes at baseline (yes versus no), and history of hypertension at baseline (yes versus no). Significance of the interaction was assessed using the likelihood ratio test.

All p-values were 2-sided. Analyses were conducted with SAS for UNIX statistical software (version 9.4; SAS Institute).

RESULTS

The mean age at baseline was 60.8 years with a median of 9.0 years to stroke event. Median levels of RBP4 were similar in cases (31.1 ng/mL) and controls (31.0 ng/mL; p-value from Wilcoxon rank sum test=0.82). Baseline characteristics of the participants by case-control status are shown in Table 1. Women who developed ischemic stroke were more likely to have a history of diabetes or high blood pressure and had higher total cholesterol/HDL ratios at baseline compared to controls. Table 2 presents the baseline characteristics of participants by RBP4 quartiles. Women in the highest RBP4 quartile were more likely to have a history of high blood pressure, higher total cholesterol/HDL ratios, more likely to use postmenopausal hormones, and more likely to consume alcohol than women in the lowest quartile.

Time until stroke did not significantly modify the association between RBP4 quartiles and risk of ischemic stroke (p-value from likelihood ratio test=0.63) and we therefore present results for the full time period of follow-up. Conditional on the matching factors, women in the highest quartile of RBP4 did not have a statistically significant increase in the risk of developing an ischemic stroke (odds ratio (OR)=1.00; 95% CI: 0.68, 1.49) compared to those in the lowest quartile. The other quartiles were not associated with an increased risk of ischemic stroke compared to the lowest quartile (Table 3). Additional adjustment for lifestyle factors such as physical activity, BMI, aspirin use, alcohol consumption, and diet did not impact our results (Model 2). Further adjustment for medical history variables such as history of diabetes, high blood pressure, coronary heart disease, or high cholesterol suggested a slight and statistically nonsignificant inverse relationship between the highest quartile of RBP4 and risk of ischemic stroke (Model 3: OR=0.77; 95% CI: 0.49, 1.19). Finally, we also adjusted for total cholesterol/HDL ratio and hemoglobin A_{1c} and observed similar results (Model 4: OR=0.75; 0.48, 1.17)

We did not find evidence that the association between RBP4 and risk of ischemic stroke was modified by age, body mass index, smoking, postmenopausal hormone therapy, history of diabetes, or hypertension (all p-values for interaction > 0.05).

We also explored the association between RBP4 and risk of ischemic stroke subtypes. We observed similar results to those seen for all ischemic strokes. Women with RBP4 levels in the highest quartile were not at increased risk of embolic (OR=0.56; 95% CI: 0.20, 1.53) or thrombotic (OR=0.77; 95% CI: 0.43, 1.36) subtypes of ischemic stroke (Table 4).

DISCUSSION

In this large nested case-control study of middle-aged women, we observed no association between RBP4 levels and the risk of total ischemic stroke or ischemic stroke of thrombotic or embolic origin.

Similar to results from other studies showing that elevated RBP4 levels may be associated with cardiovascular disease risk factors [1, 2, 4, 5, 7–15], we observed that elevated RBP4 levels were associated with an increased prevalence of hypertension and elevated cholesterol. However, studies of the association between elevated RBP4 levels and cardiovascular disease risk have been mixed [8, 18, 27]. One reason for the discrepant results across studies may be differences in the assays used to measure RBP4. For example, Mallet et al. used a particle-enhanced immunonephelometry assay and found no association between total serum RBP4 levels and CHD after controlling for CVD risk factors [27]. In contrast, a prior study using data from NHS used a novel mass spectrometry immunoassay and found a significantly increased risk of CHD with higher total RBP4 levels [18]. Unfortunately we were not able to measure RBP4 using the novel mass spectrometry immunoassay because the reagents needed for this assay were no longer available. However, RBP4 as measured by the novel mass spectrometry immunoassay has been shown to be comparable to RBP4 as measured by ELISA [28].

A few studies examined the association between RBP4 levels and prevalent cerebrovascular disease. A small case-control study (58 cases and 53 controls) among Japanese participants observed a higher mean RBP4 levels among cases (16.4 ± 2.8 ug/mL) than among controls (10.1 ± 1.2 ug/mL) but this association did not persist after multivariate adjustment [19]. A study by Ingelsson et al among 507 elderly men (mean age=81.7) used commercially available ELISA kits to measure RBP4 levels and observed a borderline significant association between RBP4 levels and the prevalence of cerebrovascular disease (OR per 1 standard deviation increase=1.37; 95% CI: 1.00, 1.88) [8]. In addition to demographic differences between our study and the study by Ingelsson et al, the mean level of RBP4 was much higher in the study by Ingelsson et al (56.2 ± 19.8 ug/mL) [8]. It is important to note that these studies included prevalent stroke events which capture not only stroke incidence but also survival after stroke. It is possible that RBP4, which transports retinol, may play a role in stroke survival or recovery. In the brain, retinol is metabolized to retinoic acid which plays an essential role in neurogenesis and may also impact neuroplasticity [29]. Additionally, studies among rats have observed that retinoic acid may reduce ischemic-induced cerebral injury [30, 31].

Limited evidence exists on the association between RBP4 levels and the risk of incident ischemic stroke. A proteomic discovery analysis using data from the Women's Health Initiative suggested that RBP4 levels may be higher at baseline among women who later develop a stroke (ischemic or hemorrhagic) than those who do not develop a stroke (nominal p -value<0.05).[20] However, after adjustment for multiple comparisons, this association was no longer statistically significant and they did not explore this association further or report measures of effect adjusted for other cardiovascular disease risk factors. Our study expands upon the results of this study by exploring the association between RBP4 and incident ischemic stroke adjusted for cardiovascular disease risk factors.

Strengths of our study include the prospective nested case-control design which allowed us to assess RBP4 levels years prior to the stroke event, unlike prior studies in which RBP4 levels were measured after the stroke event. Additionally, medical records were used to verify all stroke events. We were able to explore the association between RBP4 and specific ischemic stroke subtypes although power for these analyses was limited. Limitations include the inability to examine change in RBP4 levels over time. Additionally, this study was performed primarily among middle-aged white women, which may limit the generalizability of our results.

We did not find any evidence of an association between RBP4 levels and risk of ischemic stroke among women. Further research is needed to explore whether RBP4 levels may influence functional recovery and survival after ischemic stroke events.

Acknowledgments

FUNDING

The Nurses' Health Study is funded by UM1 CA186107, R01 CA49449, and R01 HL088521. Dr. Rist is funded by K01 HL128791. Dr. Jiménez is funded by K01 HL124391. Dr. Sun is supported by HL035464. Plasma retinol-binding protein 4 analysis planning and interpretation was supported by P30DK7521 – Metabolic Physiology Core (Director BB Kahn).

We would like to thank Dr. Barbara Kahn for her feedback on this manuscript.

References

1. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*. 2005; 436:356–62. [PubMed: 16034410]
2. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *The New England journal of medicine*. 2006; 354:2552–63. [PubMed: 16775236]
3. Kotnik P, Fischer-Posovszky P, Wabitsch M. RBP4: a controversial adipokine. *European journal of endocrinology / European Federation of Endocrine Societies*. 2011; 165:703–11.
4. van Hoek M, Dehghan A, Zillikens MC, Hofman A, Witteman JC, Sijbrands EJ. An RBP4 promoter polymorphism increases risk of type 2 diabetes. *Diabetologia*. 2008; 51:1423–8. [PubMed: 18496666]
5. Meisinger C, Ruckert IM, Rathmann W, Doring A, Thorand B, Huth C, et al. Retinol-binding protein 4 is associated with prediabetes in adults from the general population: the Cooperative Health Research in the Region of Augsburg (KORA) F4 Study. *Diabetes care*. 2011; 34:1648–50. [PubMed: 21617096]

6. Sun L, Qi Q, Zong G, Ye X, Li H, Liu X, et al. Elevated plasma retinol-binding protein 4 is associated with increased risk of type 2 diabetes in middle-aged and elderly Chinese adults. *The Journal of nutrition*. 2014; 144:722–8. [PubMed: 24647386]
7. Alkharfy KM, Al-Daghri NM, Vanhoutte PM, Krishnaswamy S, Xu A. Serum retinol-binding protein 4 as a marker for cardiovascular disease in women. *PloS one*. 2012; 7:e48612. [PubMed: 23119072]
8. Ingelsson E, Sundstrom J, Melhus H, Michaelsson K, Berne C, Vasan RS, et al. Circulating retinol-binding protein 4, cardiovascular risk factors and prevalent cardiovascular disease in elderly. *Atherosclerosis*. 2009; 206:239–44. [PubMed: 19339013]
9. Solini A, Santini E, Madec S, Rossi C, Muscelli E. Retinol-binding protein-4 in women with untreated essential hypertension. *American journal of hypertension*. 2009; 22:1001–6. [PubMed: 19556974]
10. Cabre A, Lazaro I, Girona J, Manzanares J, Marimon F, Plana N, et al. Retinol-binding protein 4 as a plasma biomarker of renal dysfunction and cardiovascular disease in type 2 diabetes. *Journal of internal medicine*. 2007; 262:496–503. [PubMed: 17875187]
11. Barazzoni R, Zanetti M, Semolic A, Pirulli A, Cattin MR, Biolo G, et al. High plasma retinol binding protein 4 (RBP4) is associated with systemic inflammation independently of low RBP4 adipose expression and is normalized by transplantation in nonobese, nondiabetic patients with chronic kidney disease. *Clinical endocrinology*. 2011; 75:56–63. [PubMed: 21521262]
12. Yao-Borengasser A, Varma V, Bodles AM, Rasouli N, Phanavanh B, Lee MJ, et al. Retinol binding protein 4 expression in humans: relationship to insulin resistance, inflammation, and response to pioglitazone. *The Journal of clinical endocrinology and metabolism*. 2007; 92:2590–7. [PubMed: 17595259]
13. Tschoner A, Sturm W, Engl J, Kaser S, Laimer M, Laimer E, et al. Retinol-binding protein 4, visceral fat, and the metabolic syndrome: effects of weight loss. *Obesity*. 2008; 16:2439–44. [PubMed: 18719670]
14. Qi Q, Yu Z, Ye X, Zhao F, Huang P, Hu FB, et al. Elevated retinol-binding protein 4 levels are associated with metabolic syndrome in Chinese people. *The Journal of clinical endocrinology and metabolism*. 2007; 92:4827–34. [PubMed: 17878249]
15. Liu Y, Wang D, Li D, Sun R, Xia M. Associations of retinol-binding protein 4 with oxidative stress, inflammatory markers, and metabolic syndrome in a middle-aged and elderly Chinese population. *Diabetology & metabolic syndrome*. 2014; 6:25. [PubMed: 24559154]
16. Kadoglou NP, Lambadiari V, Gastounioti A, Gkekas C, Giannakopoulos TG, Koulia K, et al. The relationship of novel adipokines, RBP4 and omentin-1, with carotid atherosclerosis severity and vulnerability. *Atherosclerosis*. 2014; 235:606–12. [PubMed: 24956535]
17. Lambadiari V, Kadoglou NP, Stasinou V, Maratou E, Antoniadis A, Kolokathis F, et al. Serum levels of retinol-binding protein-4 are associated with the presence and severity of coronary artery disease. *Cardiovascular diabetology*. 2014; 13:121. [PubMed: 25142320]
18. Sun Q, Kiernan UA, Shi L, Phillips DA, Kahn BB, Hu FB, et al. Plasma retinol-binding protein 4 (RBP4) levels and risk of coronary heart disease: a prospective analysis among women in the nurses' health study. *Circulation*. 2013; 127:1938–47. [PubMed: 23584360]
19. Sasaki M, Otani T, Kawakami M, Ishikawa SE. Elevation of plasma retinol-binding protein 4 and reduction of plasma adiponectin in subjects with cerebral infarction. *Metabolism: clinical and experimental*. 2010; 59:527–32. [PubMed: 19846170]
20. Prentice RL, Paczesny S, Aragaki A, Amon LM, Chen L, Pitteri SJ, et al. Novel proteins associated with risk for coronary heart disease or stroke among postmenopausal women identified by in-depth plasma proteome profiling. *Genome medicine*. 2010; 2:48. [PubMed: 20667078]
21. Regensteiner JG, Golden S, Huebschmann AG, Barrett-Connor E, Chang AY, Chyun D, et al. Sex Differences in the Cardiovascular Consequences of Diabetes Mellitus: A Scientific Statement From the American Heart Association. *Circulation*. 2015; 132:2424–47. [PubMed: 26644329]
22. Stampfer MJ, Colditz GA, Willett WC, Speizer FE, Hennekens CH. A prospective study of moderate alcohol consumption and the risk of coronary disease and stroke in women. *The New England journal of medicine*. 1988; 319:267–73. [PubMed: 3393181]

23. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2–3-year period. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 1995; 4:649–54.
24. Larsson SC, Virtamo J, Wolk A. Potassium, calcium, and magnesium intakes and risk of stroke in women. *American journal of epidemiology*. 2011; 174:35–43. [PubMed: 21540318]
25. Iso H, Rexrode K, Hennekens CH, Manson JE. Application of computer tomography-oriented criteria for stroke subtype classification in a prospective study. *Annals of epidemiology*. 2000; 10:81–7. [PubMed: 10691061]
26. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, et al. Alternative dietary indices both strongly predict risk of chronic disease. *The Journal of nutrition*. 2012; 142:1009–18. [PubMed: 22513989]
27. Mallat Z, Simon T, Benessiano J, Clement K, Taleb S, Wareham NJ, et al. Retinol-binding protein 4 and prediction of incident coronary events in healthy men and women. *The Journal of clinical endocrinology and metabolism*. 2009; 94:255–60. [PubMed: 18854400]
28. Kiernan UA, Phillips DA, Trenchevska O, Nedelkov D. Quantitative mass spectrometry evaluation of human retinol binding protein 4 and related variants. *PloS one*. 2011; 6:e17282. [PubMed: 21479165]
29. Shearer KD, Stoney PN, Morgan PJ, McCaffery PJ. A vitamin for the brain. *Trends in neurosciences*. 2012; 35:733–41. [PubMed: 22959670]
30. Choi BK, Kim JH, Jung JS, Lee YS, Han ME, Baek SY, et al. Reduction of ischemia-induced cerebral injury by all-trans-retinoic acid. *Experimental brain research*. 2009; 193:581–9. [PubMed: 19050858]
31. Harvey BK, Shen H, Chen GJ, Yoshida Y, Wang Y. Midkine and retinoic acid reduce cerebral infarction induced by middle cerebral artery ligation in rats. *Neuroscience letters*. 2004; 369:138–41. [PubMed: 15450683]

Table 1**Baseline Characteristics By Case Control Status in 1990**

	Case Control Status			p-value
	cases (n=471)	controls (n=471)	Matching factor (MF)	
Age ^a , <i>b</i>	60.8 ± 6.0	60.8 ± 6.0		
White, % <i>b</i>	97	98	MF	
Smoking <i>b</i>				
- Never, %	42	42	MF	
- Past, %	40	42		
- Current, %	18	16		
Postmenopausal HT status <i>b</i>			MF	
- Premenopausal, %	6	6		
- Postmenopausal, No PMH use, %	47	47		
- Postmenopausal, Use PMH, %	42	42		
- Dubious, %	5	5		
History of diabetes, %	13	6		<0.01
History of high blood pressure, %	48	35		<0.01
History of heart disease, %	5	7		0.34
History of elevated cholesterol, %	48	46		0.51
Physical activity				0.50
- Tertile 1, %	37	33		
- Tertile 2, %	33	34		
- Tertile 3, %	30	33		
BMI				0.08
- Healthy weight, %	49	56		
- Overweight, %	35	29		
- Obese, %	16	15		
Aspirin				0.65
- None, %	53	50		
- 1–5 tabs/wk, %	31	33		
- ≥6 tabs/wk, %	16	17		

Case Control Status			
	cases (n=471)	controls (n=471)	p-value
Alcohol			0.74
- 0 g/d, %	39	42	
- 0.1–4.9 g/d, %	32	31	
- 5.0–14.9 g/d, %	18	16	
- 15.0+ g/d, %	10	11	
AHEI Quartiles			0.32
- First quartile, %	28	25	
- Second quartile, %	27	25	
- Third quartile, %	26	25	
- Fourth quartile, %	20	25	
Total cholesterol to HDL cholesterol ratio	4.3 ± 1.5	4.0 ± 1.4	<0.01
Hemoglobin A1C (%)	5.9 ± 1.0	5.7 ± 0.7	0.03
Retinol-binding protein 4 (µg/mL)	32.4 ± 9.0	32.0 ± 8.0	0.82

Values are means ± SD or percentages and are standardized to the age distribution of the study population.

Values of polytomous variables may not sum to 100% due to rounding

^aValue is not age adjusted

^bMatching factor

Table 2

Characteristics at or near blood collection By RBP4 Quartiles^a

	RBP4 Quartiles				p-value
	First quartile (n=226)	Second quartile (n=244)	Third quartile (n=244)	Fourth quartile (n=228)	
Age ^b	60.3 ± 6.4	60.7 ± 6.2	60.9 ± 5.8	61.3 ± 5.4	0.68
White, %	99	96	99	98	0.13
Smoking					0.44
- Never, %	45	42	40	43	
- Past, %	37	41	41	45	
- Current, %	19	17	19	12	
Postmenopausal HT status					<0.01
- Premenopausal, %	7	8	6	2	
- Postmenopausal, No PMH use, %	51	53	42	43	
- Postmenopausal, Use PMH, %	38	34	49	48	
- Dubious, %	4	5	4	6	
History of diabetes, %	11	11	7	10	0.46
History of high blood pressure, %	31	36	41	58	<0.01
History of heart disease, %	4	5	7	8	0.12
History of elevated cholesterol, %	36	39	50	63	<0.01
Physical activity					0.32
- Tertile 1, %	36	38	32	33	
- Tertile 2, %	37	32	31	35	
- Tertile 3, %	27	30	37	31	
BMI					0.16
- Healthy weight, %	57	55	48	49	
- Overweight, %	29	27	38	34	
- Obese, %	15	18	14	17	
Aspirin					0.37
- None, %	54	48	55	49	
- 1–5 tabs/wk, %	32	37	29	31	
- ≥6 tabs/wk, %	14	16	16	20	

	RBP4 Quartiles				p-value
	First quartile (n=226)	Second quartile (n=244)	Third quartile (n=244)	Fourth quartile (n=228)	
Alcohol					<0.01
- 0 g/d, %	45	46	36	37	
- 0.1–4.9 g/d, %	35	31	28	33	
- 5.0–14.9 g/d, %	11	15	24	18	
- 15.0+ g/d, %	10	8	13	13	
AHEI Quartiles					0.32
- First quartile, %	27	29	23	26	
- Second quartile, %	21	24	29	27	
- Third quartile, %	27	29	24	24	
- Fourth quartile, %	26	18	24	23	
Total cholesterol to HDL cholesterol ratio	3.9 ± 1.3	4.3 ± 1.5	4.1 ± 1.5	4.4 ± 1.6	<0.01
Hemoglobin A1C (%)	5.8 ± 1.0	5.8 ± 1.0	5.7 ± 0.7	5.7 ± 0.8	0.31

Values are means ± SD or percentages and are standardized to the age distribution of the study population.

Values of polytomous variables may not sum to 100% due to rounding

^a Quartile cutpoints are based on distribution among controls.

^b Value is not age adjusted

PMH = Post-menopausal hormone use

Table 3

Multivariate adjusted odds ratios (95% CI) of ischemic stroke by quartiles RBP4 in NHS I

	Quartiles of RBP4				P for linear trend
	Q1	Q2	Q3	Q4	
Range, mg/mL	26.12	26.12–31.02	31.02–37.20	37.20	
Cases/Controls	109/117	126/118	126/118	110/118	
Model 1	1.00	1.15 (0.79, 1.67)	1.15 (0.79, 1.66)	1.00 (0.68, 1.49)	>0.99
Model 2	1.00	1.12 (0.77, 1.64)	1.05 (0.71, 1.55)	0.97 (0.65, 1.46)	0.82
Model 3	1.00	1.06 (0.72, 1.58)	0.96 (0.64, 1.45)	0.77 (0.49, 1.19)	0.22
Model 4	1.00	1.00 (0.67, 1.49)	0.95 (0.63, 1.43)	0.75 (0.48, 1.17)	0.21

Model 1: conditional on matching factors (age, race/ethnicity, menopausal status, hormone use, smoking, and date of sample collection)

Model 2: Model 1 + physical activity (in tertiles), body mass index (normal, overweight, obese), aspirin use (none, 1–5 tabs/wk, ≥6 tabs/wk), alcohol consumption (0, 0.1–4.9, 5.0–14.9, ≥15.0 g/day), and aHEI 2010 (quartiles)

Model 3: Model 2 + history of diabetes, history of high blood pressure, history of coronary heart disease or revascularization and history of high cholesterol

Model 4: Model 3 + log transformed cholesterol ratio (continuous) and HbA1C (continuous)

Table 4
Multivariable adjusted* OR (95% CI) of ischemic stroke subtypes by RBP4 quartiles.

	Quartiles of RBP4				P for linear trend
	Q1	Q2	Q3	Q4	
Range, mg/mL	26.12	26.12–31.02	31.02–37.20	37.20	
Thrombotic stroke					
Cases/Controls	78/81	82/76	78/82	72/71	
Odds ratio	1.00	0.88 (0.53, 1.46)	0.82 (0.49, 1.39)	0.77 (0.43, 1.36)	0.35
Embolic stroke					
Cases/Controls	26/29	39/34	36/32	28/34	
Odds ratio	1.00	1.23 (0.50, 3.03)	0.86 (0.34, 2.18)	0.56 (0.20, 1.53)	0.19

* Conditional on matching factors (age, race/ethnicity, menopausal status, hormone use, smoking, and date of sample collection) and adjusted for physical activity, body mass index, aspirin use, alcohol consumption, aHEI 2010, history of diabetes, history of high blood pressure, history of coronary heart disease or revascularization and log transformed cholesterol ratio.