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Serum Ferritin Is a Risk Factor for Stroke in Postmenopausal Women

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Background and Purpose—Iron is an essential element for the human body. It has, however, been suggested that excessive iron stores may increase the risk of vascular disease. So far, epidemiologic studies on stroke are sparse.

Methods—We studied the association between iron status and stroke risk in a population-based cohort of 11 471 Dutch postmenopausal women between 49 and 70 years of age. Women were included between 1993 and 1997 and followed up until January 1, 2000, for cerebrovascular events. We conducted a case-cohort study by using all stroke cases (n=63) and a random sample of the baseline cohort (n=1134). Serum ferritin, serum iron, and transferrin saturation were measured as markers of iron status. A weighted Cox proportional-hazards model was used to estimate crude and multivariate-adjusted hazard ratios for tertiles of different iron parameters in relation to stroke.

Results—In a multivariate model, the highest tertile of serum ferritin concentration was associated with an increased risk of stroke (hazard ratio [HR], 1.45; 95% confidence interval [CI], 0.87 to 2.42) compared with the lowest tertile. For ischemic stroke, the increase was more pronounced (HR, 2.23; 95% CI, 1.05 to 4.73) and reached statistical significance.

Conclusions—Neither serum iron nor transferrin saturation was associated with an increased stroke risk. However, higher serum ferritin concentrations in postmenopausal women are associated with an increased risk of ischemic stroke. (*Stroke*. 2005;36:1637-1641.)

Key Words: iron ■ stroke, ischemic ■ menopause ■ prospective studies

As early as 1981, Sullivan proposed the “iron hypothesis,”¹ suggesting that the lower incidence rates of ischemic heart disease in premenopausal women compared with men and the increase of ischemic heart disease rates in postmenopausal women were results of the rise in iron stores after cessation of menses, with oxidative imbalance as the central biologic mechanism. In the Fenton reaction, Fe(II) catalyzes the formation of extremely reactive hydroxyl radicals. Interaction with lipids may initiate the formation of oxidized LDL that ultimately leads to the development of foam cells and progression of atherosclerosis.² Additionally, iron could also play a role in vascular disease by activating platelets via a protein kinase C mechanism.³ Although its initial focus was on ischemic heart disease, the hypothesis may also apply to cerebrovascular disease.

Another proposed mechanism by which iron may play a role in ischemic vascular disease, which might be more relevant to stroke risk, is through ischemia/reperfusion injury. During reperfusion after cerebral infarction, there is a marked increase in oxygen-radical production as well as a release of iron ions, leading to progressive tissue damage and cellular death.⁴ Because of its specific areas rich in iron, high amounts

of polyunsaturated fatty acid side chains in membrane lipids, and low concentrations of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, the brain may be especially vulnerable to oxidative stress.⁵ So far, only a few articles have reported on the association between iron and the risk of stroke in population-based studies.^{6–12}

In the present study, we investigated the relation between high body iron stores, indicated by serum ferritin levels, and the risk of stroke in a cohort of healthy, postmenopausal women.

Methods

Population

The study population consisted of participants of the Prospect-EPIC cohort,¹³ which is 1 of 2 Dutch contributions to the European Prospective Investigation Into Cancer and Nutrition (EPIC). Participants were recruited between 1993 and 1997 among women living in Utrecht and vicinity who attended the regional, population-based, breast cancer–screening program. A total of 17 357 women aged 49 to 70 years were included. At enrolment, all women underwent a physical examination, donated a nonfasting blood sample, and filled

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out a general questionnaire relating to lifestyle and medical factors and a food-frequency questionnaire.

Data on morbidity were obtained from the Dutch Centre for Health Care Information, which maintains a standardized, computerized register of hospital discharge diagnoses. Admission files have been filed continuously from all general and university hospitals in the Netherlands since 1990. Whenever a patient is discharged from a hospital, data on sex, date of birth, dates of admission and discharge, 1 mandatory principal diagnosis, and up to 9 optional additional diagnoses are recorded. All diagnoses were coded according to the *International Classification of Diseases*, 9th revision. We categorized cardiovascular disease (codes 390 to 459) as stroke (codes 430 to 438; ischemic, 433 to 435) and other cardiovascular disease. Whenever multiple events occurred, the first diagnosis was taken as the end point. Follow-up was complete until January 1, 2000. The database was linked to the cohort on the basis of birth date, sex, postal code, and general practitioner with a validated probabilistic method.¹⁴

Information on vital status was gained through linkage to the national municipal administration database. Causes of death were obtained from the women's general practitioners. All women signed an informed consent form before study inclusion. The study was approved by the Institutional Review Board of the University Medical Center Utrecht.

Design

To increase efficiency and preserve valuable biologic material, we used a case-cohort approach as introduced by Prentice.¹⁵ We selected all 108 first fatal and nonfatal strokes that arose during follow-up. From the initial 17 357 women, we took a random sample of 10% ($n=1736$). Women who did not consent to linkage with vital status registries or who were not traceable (cases, $n=3$; subcohort, $n=38$) were not included. We excluded prevalent cases of cardiovascular disease at baseline (cases, $n=24$; subcohort, $n=107$), those who had missing questionnaires or blood samples (cases, $n=7$; subcohort, $n=71$), those with a daily energy intake <500 kcal/d (cases, $n=0$; subcohort, $n=2$), and women who had missing information on menopausal status or who reported menstrual bleeding periods, either natural or hormone induced, in the 12 months before enrollment into the study (cases, $n=18$; subcohort, $n=442$). For some women, multiple reasons for exclusion applied, resulting in a total number of 63 cases (including 35 ischemic strokes) and 1134 women in the subcohort who remained in the analyses. After all exclusions were applied, the total cohort encompassed 11 471 women. For all subjects who had a cardiovascular event, follow-up ended at the date of diagnosis or death. Moving from the Netherlands ($n=1$) and death due to causes other than cardiovascular disease ($n=15$) were considered censoring events. All others ($n=1046$) were censored on January 1, 2000. Overall, only 1 first fatal stroke event occurred during follow-up.

Baseline Measurements

Biochemical measurements were performed for all subcohort members and stroke cases according to standard laboratory procedures. Sera of cases were randomly distributed among those of the subcohort, and all biochemical analyses were performed without knowledge of disease status. Total cholesterol and glucose levels were determined with an automated enzymatic procedure on a Vitros 250 (Johnson & Johnson). Serum iron, LDL cholesterol, and HDL cholesterol were measured with a colorimetric assay on a Hitachi 904 (Johnson & Johnson). As a marker of inflammation, high-sensitivity C-reactive protein (hsCRP) was measured in citrated plasma by the Behring BNII nephelometric method (Dade Behring). hsCRP values below the detection limit of 0.2 mg/L ($n=50$) were set to 0.1 mg/L. Serum ferritin was assessed with an automated immunometric assay on an Immulite apparatus (Diagnostic Products Corp). Serum transferrin values were obtained by immunochemical turbidimetry on a Hitachi 904. Total iron binding capacity (TIBC; in $\mu\text{mol/L}$) was calculated as serum transferrin (g/L) $\times 25.14$, and transferrin saturation as the ratio of iron to TIBC.

Smokers were classified as current, past, or never smokers. Alcohol intake was divided into 5 categories: <1 g/d, 1 to 5 g/d, 5 to 15 g/d, 15 to 30 g/d, and >30 g/d. Systolic and diastolic blood pressures were measured in duplicate, and the mean value was calculated. Furthermore, height and weight were measured without shoes and wearing light indoor clothing to compute body mass index, defined as weight divided by height squared (kg/m^2). Hypercholesterolemia or diabetes was defined as a self-reported physician diagnosis. Presence of hypertension was defined as a measured systolic blood pressure >160 mm Hg and/or a diastolic blood pressure >95 mm Hg and/or a self-reported physician diagnosis.

Data Analysis

Baseline characteristics for the subcohort were summarized according to the tertile distribution of serum ferritin. Means and SDs were computed for normally distributed variables, and medians and interquartile ranges were calculated for variables that showed skewed distributions. Categorical variables were expressed as frequencies. For further analyses, hsCRP concentrations were logarithmically transformed to produce approximately normal distributions.

To assess the relation between iron status and stroke, we used a Cox proportional-hazards model with an estimation procedure adapted for case-cohort designs.¹⁵ We investigated whether a dose-response relation existed between serum ferritin and stroke by dividing serum ferritin concentration values into tertile groups based on the distribution in the random sample or a high threshold relation by dichotomizing serum ferritin concentration in above and below 200 $\mu\text{g/L}$. In addition, we evaluated the association between stroke and 2 other iron parameters, serum iron and transferrin saturation. Both parameters were categorized into tertiles based on their distribution in the random sample. The fit of the proportional-hazards models was evaluated by examining the log-log plots in SPSS 11.5. Trend tests were conducted by introducing the tertiles as a continuous variable in the model.

Associations were adjusted for age (continuous), body mass index (continuous), alcohol intake (categorical), hsCRP (continuous), smoking (current/past/never), hypertension (yes/no), hypercholesterolemia (yes/no), diabetes (yes/no), glucose (continuous), LDL cholesterol (continuous), and HDL cholesterol (continuous). We also considered time since last menstrual period as a potential confounder. Effect modification of serum ferritin by hypertension was examined by calculating hazard ratios (HRs) for combined categories of serum ferritin (<200 $\mu\text{g/L}$, ≥ 200 $\mu\text{g/L}$) and hypertension (yes/no). Normotensives with a serum ferritin concentration <200 $\mu\text{g/L}$ served as the reference group.

Statistical analyses were performed with the statistical software packages SPSS (SPSS for Windows, release 11.5.0, 2002, SPSS Inc) and SAS (version 8.2, SAS Institute Inc).

Results

The cohort was followed up for a mean of 4.3 years. Table 1 shows the baseline characteristics of the subcohort according to tertiles of serum ferritin. For 2 women in the subcohort, measurement of serum ferritin failed. Serum ferritin concentrations ranged from 4.6 to 1158.0 $\mu\text{g/L}$, and the overall median concentration was 103.0 $\mu\text{g/L}$. There were 22 (1.9%) women whose serum ferritin concentration was <15 $\mu\text{g/L}$ and 49 (4.3%) whose concentration was >300 $\mu\text{g/L}$. Women in the highest tertile were, on average, older and consumed slightly more alcohol. In addition, the prevalence of the classic cardiovascular risk factors hypertension, hypercholesterolemia, and diabetes mellitus was highest in these women. Mean serum iron concentration was highest among women in the third tertile, and transferrin saturations increased in parallel with serum ferritin concentrations, whereas TIBC declined. Higher levels of serum ferritin were correlated with higher median hsCRP concentrations as well as higher mean LDL cholesterol, total cholesterol,

TABLE 1. Baseline Characteristics of the Subcohort (n=1132) According to Tertiles of Serum Ferritin Concentration

	Tertiles			P‡
	1 (n=377)	2 (n=377)	3 (n=378)	
Serum ferritin range, µg/L	4.6–75.7	75.7–136	137–1158	...
Serum ferritin concentration, µg/L*	51.8 (36.0–64.3)	103.0 (91.9–118.0)	195.0 (162.0–251.0)	<0.001
Serum iron, µmol/L†	16.2 (4.8)	16.2 (4.6)	17.0 (4.9)	0.02
TIBC, µmol/L†	74.7 (9.3)	70.4 (8.0)	70.5 (8.7)	<0.001
Transferrin saturation, %†	22.0 (6.8)	23.2 (6.6)	24.3 (7.1)	<0.001
Total cholesterol, mmol/L†	5.8 (0.9)	6.0 (1.0)	6.1 (1.0)	<0.001
HDL cholesterol, mmol/L†	1.6 (0.4)	1.6 (0.4)	1.5 (0.4)	0.05
LDL cholesterol, mmol/L†	3.9 (0.9)	4.1 (1.0)	4.1 (0.9)	0.01
Serum glucose, mmol/L†	4.4 (1.2)	4.5 (1.1)	4.9 (1.9)	<0.001
hsCRP, mg/L*	1.0 (0.5–2.4)	1.2 (0.6–2.2)	1.4 (0.7–3.0)	<0.001
Age at intake, y*	58 (53–63)	59 (55–64)	60 (55–64)	<0.001
Body mass index, kg/m †	25.2±3.8	25.9±3.9	27.0±4.0	<0.001
Alcohol intake, g/d*	2.1 (0.2–10.0)	3.6 (0.5–11.4)	3.8 (0.3–14.1)	0.67
Hypertension, %	108 (29)	99 (26)	120 (32)	0.25
Hypercholesterolemia, %	14 (4)	16 (4)	24 (6)	0.20
Diabetes, %	3 (1)	7 (2)	20 (5)	<0.001
Smoking, %				0.92
Current	90 (24)	84 (22)	80 (21)	
Past	120 (32)	121 (32)	172 (34)	
Never	167 (44)	172 (46)	171(45)	

*Median and (interquartile range).

†Mean±SD.

‡P for linear trend for continuous variables or χ^2 statistics for categorical variables.

and serum glucose concentrations. Differences in absolute values of all of these parameters were, however, small.

In the adjusted model, an increased risk of stroke was found for the highest tertile of serum ferritin compared with the lowest (Table 2; HR, 1.45; 95% confidence interval [CI], 0.87 to 2.42; $P_{\text{trend}}=0.158$). For ischemic stroke, the risk more than doubled and was statistically significant (HR, 2.23; 95% CI, 1.05 to 4.73; $P_{\text{trend}}=0.058$). Similarly, serum ferritin levels ≥ 200 µg/L increased the risk of ischemic stroke significantly (HR, 2.50; 95% CI, 1.16 to 5.37). Risk estimates hardly altered when hsCRP was omitted from the multivariate models, except that the CIs narrowed slightly. No association between serum iron or transferrin saturation and the risk of either stroke or ischemic stroke was observed (Table 3). In Table 4, an effect modification of serum ferritin by hypertension is presented. Hypertensive women who had serum ferritin concentrations ≥ 200 µg/L showed the highest risk of stroke (HR, 3.77; 95% CI, 1.56 to 9.08; $P_{\text{interaction}}=0.18$).

Discussion

In this prospective study among postmenopausal women, we examined the relation between iron status and risk of stroke. High serum ferritin concentrations are associated with an increased risk of stroke, in particular, ischemic stroke (Table 2). However, neither serum iron nor transferrin saturation was associated with the disease outcome (Table 3).

To appreciate these findings, some issues need to be addressed. The prospective design of the study provides the most valid approach to detect a causal relation between body iron status and stroke. To prevent risk factors and serum ferritin concentrations from being biased by the presence of disease, we excluded prevalent cases of cardiovascular disease from our analyses. The PROSPECT study is a population-based cohort, which makes it less susceptible to selection bias. Additional strengths are the comprehensive data and sample collection and the hospital admission and mortality follow-up that we have at our disposal for the entire cohort. Furthermore, we accounted for the acute-phase properties of serum ferritin by adjustment for hsCRP. By doing this, we attempted to reduce the possibility that the observed relations were, entirely or in part, explained by underlying low-grade inflammation instead of high body iron stores. Supporting evidence that additional inflammation was an unlikely mechanism comes from a recently published article that demonstrated the absence of a strong relation between elevated serum ferritin levels and elevated CRP levels in a non-institutionalized population.¹⁶ In our analyses, indeed, inclusion or exclusion of hsCRP in the regression models did not materially affect the findings. Another strength of our study is that it is one of a few prospective studies relating to iron stores and stroke disease involving women. Our study is, however, limited by the relatively short follow-up and small number of disease cases, which limits the potential for meaningful subgroup analyses.

Serum ferritin is generally considered the best clinical measure of body iron stores.¹⁷ Although it provides a good estimate

TABLE 2. HRs and 95% CIs for Risk of Stroke (Especially Ischemic Stroke) According to Tertiles and the 200- $\mu\text{g/L}$ Threshold of Serum Ferritin Concentration

	Cases, No.	HR (95% CI)	
		Unadjusted	Adjusted†
Stroke			
Serum ferritin, $\mu\text{g/L}$			
Tertiles*			
1	19	1.0	1.0
2	16	0.87 (0.51–1.49)	0.94 (0.53–1.68)
3	28	1.49 (0.92–2.40)	1.45 (0.87–2.42)
P_{trend}		0.113	0.158
<200 $\mu\text{g/L}$	47	1.0	1.0
$\geq 200 \mu\text{g/L}$	16	1.86 (1.06–3.24)	1.77 (1.03–3.05)
Ischemic stroke			
Serum ferritin, $\mu\text{g/L}$			
Tertiles*			
1	8	1.0	1.0
2	8	1.03 (0.58–1.84)	1.16 (0.60–2.23)
3	19	2.39 (1.40–4.09)	2.23 (1.05–4.73)
P_{trend}		0.006	0.058
<200 $\mu\text{g/L}$	23	1.0	1.0
$\geq 200 \mu\text{g/L}$	12	2.84 (1.63–4.96)	2.50 (1.16–5.37)

*Ranges: for 1, 3.2–75.7 $\mu\text{g/L}$; for 2, 75.7–136 $\mu\text{g/L}$; for 3, 137–1158 $\mu\text{g/L}$.
 †Adjusted for age, body mass index, alcohol intake, hsCRP, smoking, hypertension, hypercholesterolemia, diabetes, glucose, LDL cholesterol, and HDL-cholesterol.

of the size of the storage compartment, the part that reflects safely bound iron, it provides little information on the amount of catalytically active iron at sites relevant to stroke development. Nevertheless, we found that relatively high serum ferritin concentrations ($\geq 200 \mu\text{g/L}$) more than doubled the risk of ischemic stroke (HR, 2.50; 95% CI, 1.16 to 5.37). The ability of superoxide radicals, generated during ischemia/reperfusion injury, to release ferrous iron from ferritin¹⁸ might explain part of the observed findings. By this mechanism, a small, subclinical event, such as transient ischemic attack, could progress into a clinical event in the presence of superoxide radicals. If such a mechanism plays a role, one would also expect a larger case fatality of stroke, because clinical events will then be more massive and fatal. In our population, follow-up is at the present time too limited to study mortality separately.

Both serum iron and transferrin concentrations, which are markers of iron release into the plasma and utilization of iron incorporation in mainly hemoglobin, are less informative when it comes to body iron status. Therefore, it is not so surprising that both parameters were not associated with the occurrence of cerebrovascular events. Our negative finding for transferrin saturation contradicts the study by Gillum et al,⁷ who found a statistically significant U-shaped relation for the risk of stroke incidence and death in white women.

A plausible biologic mechanism that may explain an association between iron and stroke involves the catalytic properties of iron in the production of hydroxyl radicals. Oxygen radicals may play an important role in atherosclerosis by accelerating lipid

TABLE 3. HRs and 95% CIs for Risk of Stroke (Especially Ischemic Stroke) According to Tertiles of Serum Iron and Transferrin Saturation

	Cases, No.	HR (95% CI)	
		Unadjusted	Adjusted‡
Stroke			
Serum iron, $\mu\text{mol/L}$			
Tertiles*			
1	21	1.0	1.0
2	28	1.23 (0.76–1.99)	1.28 (0.77–2.12)
3	14	0.66 (0.41–1.06)	0.67 (0.42–1.07)
P_{trend}		0.076	0.098
Ischemic stroke			
Serum iron, $\mu\text{mol/L}$			
Tertiles*			
1	10	1.0	1.0
2	16	1.47 (0.76–2.83)	1.42 (0.53–3.77)
3	9	0.89 (0.48–1.64)	0.84 (0.38–1.87)
P_{trend}		0.697	0.669
Stroke			
Transferrin saturation, %			
Tertiles†			
1	21	1.0	1.0
2	27	1.30 (0.92–1.84)	1.44 (0.94–2.22)
3	15	0.71 (0.49–1.03)	0.80 (0.50–1.30)
P_{trend}		0.071	0.456
Ischemic stroke			
Transferrin saturation, %			
Tertiles†			
1	12	1.0	1.0
2	12	1.01 (0.50–2.03)	1.14 (0.41–3.15)
3	11	0.91 (0.44–1.88)	1.09 (0.36–3.32)
P_{trend}		0.804	0.877

*Ranges: I: 4.6–14.2 $\mu\text{mol/L}$, II: 14.2–18.3 $\mu\text{mol/L}$, III: 18.3–39.4 $\mu\text{mol/L}$.
 †Ranges for 2, 4.7–19.8%; for 3, 19.8–25.5%; for 3, 25.5–57.1%.
 ‡Adjusted for age, body mass index, alcohol intake, hsCRP, smoking, hypertension, hypercholesterolemia, diabetes, glucose, LDL cholesterol, and HDL cholesterol.

peroxidation.¹⁹ Previously, serum ferritin was shown to be a significant predictor of carotid atherosclerosis progression, especially in subjects with high levels of LDL.²⁰ In our population, there were no signs of an effect modification by LDL cholesterol

TABLE 4. Association Between Hypertension, Serum Ferritin Concentration, and Stroke Risk

	Subcohort (n=1132)	Stroke (n=63)	HR (95% CI)*
Normotensive/<200 $\mu\text{g/L}$	697	28	1.0
Hypertensive/<200 $\mu\text{g/L}$	261	19	1.42 (0.66–3.06)
Normotensive/ $\geq 200 \mu\text{g/L}$	108	6	1.16 (0.56–2.43)
Hypertensive/ $\geq 200 \mu\text{g/L}$	66	10	3.77 (1.56–9.08)

*Adjusted for age, body mass index, alcohol intake, hsCRP, smoking, hypercholesterolemia, diabetes, glucose, LDL cholesterol, and HDL cholesterol.

(data not shown). Additionally, *in vitro* experiments have shown that iron can directly interact with primed platelets via a Fenton-like reaction, resulting in the activation and subsequent aggregation of platelets.³ The importance of this mechanism *in vivo*, however, needs to be established.

The involvement of free radicals in brain ischemia and reperfusion may have effects on vasodilation and membrane permeability,²¹ resulting in a poor prognosis for stroke. Experimental studies in rats showed larger infarct volumes after middle cerebral arterial occlusion in iron-fed rats compared with controls,²² and ischemic brain injury was reduced after administration of scavengers of hydroxyl radicals in the early stage of reperfusion.²³

In this study, we found strong evidence for modification of the risk of high ferritin levels by hypertension. Women who had serum ferritin levels ≥ 200 $\mu\text{g/L}$ and were hypertensive had a 3.77-fold higher risk of stroke (95% CI, 1.56 to 9.08) compared with normotensive women who had levels < 200 $\mu\text{g/L}$. The generation of superoxide radicals in hypertension has been shown to affect the production and activity of endogenous vascular nitric oxide, which may result in impaired endothelium-dependent vasorelaxation.²⁴

In recent years, several epidemiological studies have been performed to investigate the role of iron metabolism in stroke. These studies can be categorized into those that studied the association between iron status and stroke incidence or mortality⁶ and those that studied the role of iron in the severity and prognosis of stroke.^{8–10} The only prospective study that used serum ferritin as a marker for iron status and related this to stroke events was the Busselton Health Study.⁶ The results of that study were null for stroke. It is well possible, however, that the effect on ischemic stroke has been diluted by the fact that the investigators were unable to distinguish between hemorrhagic and ischemic events. Studies related to severity and prognosis of stroke demonstrated that serum ferritin was a significant predictor of mortality and neurologic deterioration.^{8–10} These studies measured serum ferritin within 24 to 48 hours after the event. Because of this, it is difficult to disentangle whether the increased serum ferritin concentrations reflect high body iron stores or were merely due to the acute-phase response.²⁵ This limitation highlights the need for prospective studies that measure serum ferritin before the onset of disease, like the present one, and that study its effect on stroke fatality.

Additional support for a relation between iron and stroke comes from 2 studies that used the hemochromatosis genotype as a marker for iron status.^{11,12} Although only the study by Roest et al demonstrated an independent association of C282Y heterozygosity with cerebrovascular events, both provided strong evidence for an effect modification by smoking, implying a common mechanism likely mediated by oxidative stress.

In conclusion, the findings of this prospective study in a cohort of postmenopausal women support the view that raised serum ferritin concentrations increase the risk of ischemic stroke. The exact underlying mechanism of this association remains unclear and requires further research.

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