Original Contribution

Follicle-Stimulating Hormone Levels and Subclinical Atherosclerosis in Older Postmenopausal Women

Elizabeth R. Bertone-Johnson*, Jyrki K. Virtanen, Tarja Nurmi, Leo Niskanen, Jaakko Mursu, Sari Voutilainen, Kimmo Ronkainen, Jussi Kauhanen, and Tomi-Pekka Tuomainen

* Correspondence to Dr. Elizabeth R. Bertone-Johnson, Department of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts, 715 North Pleasant Street, Arnold House, Amherst, MA 01003-9304 (e-mail: ebertone@schoolph.umass.edu).

Recent studies of perimenopausal women suggest that follicle-stimulating hormone (FSH) levels may be associated with atherosclerosis, independent of estradiol. Whether FSH is related to atherosclerosis in older postmenopausal women, who have completed the menopausal transition, remains unknown. We assessed the relationship of serum FSH and estradiol levels with carotid artery intima-media thickness (IMT) among 587 postmenopausal participants in the Kuopio Ischemic Heart Disease Risk Factor Study (Kuopio, Finland). Participants were aged 53–73 years and not using hormone therapy at baseline (1998–2001). Mean IMT was measured via high-resolution ultrasonography. We observed a significant inverse association between FSH levels and IMT. Mean IMTs among women in quartiles 1–4 of FSH were 0.94 mm, 0.91 mm, 0.87 mm, and 0.85 mm, respectively (P-trend < 0.001). After adjustment for age, estradiol, testosterone, body mass index (weight (kg)/height (m)^2), lipids, and other factors, FSH levels remained significantly associated with IMT (regression coefficients for quartiles 2–4 vs. quartile 1 were −0.038, −0.045, and −0.062, respectively; P-trend = 0.01). Findings were strongest in women aged 64–73 years (P-trend = 0.006) and did not vary by body mass index. In contrast, estradiol levels were not related to IMT. In summary, high postmenopausal FSH levels were associated with a lower atherosclerotic burden, independent of estradiol, adiposity, and other factors. Our findings warrant replication and the further exploration of potential underlying mechanisms.

atherosclerosis; carotid intima-media thickness; estradiol; follicle-stimulating hormone; postmenopausal women

Abbreviations: BMI, body mass index; CCA, common carotid artery; CVD, cardiovascular disease; FSH, follicle-stimulating hormone; IMT, intima-media thickness; KIHD, Kuopio Ischemic Heart Disease Risk Factor.

The role of sex steroids in cardiovascular health in women after menopause remains controversial. While higher levels of estradiol across the menopausal transition have been associated with a lower atherosclerotic burden (1–3), recent studies suggest that follicle-stimulating hormone (FSH) levels may be positively associated with atherosclerosis, independent of estradiol (1). However, these studies have almost exclusively considered FSH levels in women still undergoing the menopausal transition, when sex steroid levels are highly dynamic (1–6). The relationship of serum FSH and residual circulating estradiol levels to cardiovascular health in late postmenopausal women, after the most pronounced fluctuations in ovarian estrogen production have stabilized, remains virtually unknown.

We assessed the relationship of serum FSH and estradiol levels with common carotid artery (CCA) intima-media thickness (IMT), a measure of atherosclerosis, among women aged 53–73 years participating in the Kuopio Ischemic Heart Disease Risk Factor (KIHD) Study who were not using hormone therapy.

METHODS

Study population

The KIHD Study is an ongoing prospective population-based cohort study of risk factors for cardiovascular disease (CVD) and metabolic conditions in men and women living in eastern

Finland. While male participants were enrolled in 1984–1989, female participants were first enrolled between March 1998 and February 2001, corresponding to the 11-year follow-up visit for male participants. Women eligible for enrollment were a random sample of 1,173 postmenopausal women living in the city of Kuopio and surrounding rural communities. Women from 4 specific age groups were selected: 53–56 years, 59–62 years, 64–68 years, and 71–73 years. Of eligible women, 920 completed baseline clinical assessments and joined the cohort (78.4%). Among nonparticipants, 168 refused participation, 51 could not be contacted, and 34 were excluded for other reasons. The study protocol was approved by the Research Ethics Committee of the University of Kuopio. Informed consent was obtained from all participants.

Clinical measurements

At a clinical interview, participants completed a comprehensive clinical examination, during which CCA IMT was measured by high-resolution B-mode ultrasonography (Biosound Phase 2; Biosound Inc., Indianapolis, Indiana), as described previously (7). Briefly, measurements were made at the distal end of the right and left CCAs, proximal to the carotid bulb, and videotaped for computerized analysis with PROSOUND software (University of Southern California, Los Angeles, California) (8). Measurements of the intima-lumen and media-adventitia interfaces were made at approximately 100 points along a 1.0- to 1.5-cm section of the right and left CCAs by edge-detection algorithm. For each participant, approximately 100 measurements from the right CCA and 100 from the left CCA were averaged to calculate mean IMT. Interrater reliability of IMT measurements among male KIHD participants was assessed previously, with correlation coefficients across raters ranging from 0.90 to 0.99 (9).

Resting systolic and diastolic blood pressures were calculated as the average of 6 measurements: 3 taken while the participant lay supine after 5 minutes' rest; 1 taken while the participant was standing; and 2 taken while the participant was sitting, with 5 minutes between measurements. Height and weight were directly measured and used to calculate body mass index (BMI; weight (kg)/height (m)^2). Waist and hip circumferences were measured with a standard measuring tape and used to calculate waist:hip ratio.

Blood collection and biochemical measurements

At the clinical interview, fasting blood samples were collected between 8:00 AM and 10:00 AM, after participants had abstained from eating or smoking cigarettes for 12 hours and consuming alcohol for 3 days. Serum was separated from other blood components within 60 minutes and stored at −20°C or −80°C until assay.

Samples were assayed for FSH between June 2001 and February 2002. Serum FSH concentration was determined with a sandwich technique applying an immunoradiometric assay manufactured by Diagnostic Products Corporation (Coat-A-Count FSH IRMA; Diagnostic Products Corporation, Los Angeles, California). For quality control, triplicates of low, medium, and high Lyphochek controls (Bio-Rad Laboratories, Anaheim, California) were included in each run. Coefficients of variation were 5% for each set of controls. Serum 17β-estradiol was assayed between 1999 and 2001 with a radioimmunoassay manufactured by DiaSorin (DiaSorin S.p.A., Stillwater, Minnesota). Four different types of controls were included in each run: Lyphochek low and medium controls, an in-house serum pool, and a control provided by the kit manufacturer. Concentrations of 17β-estradiol were in the physiological range for the in-house pool and higher for other types of controls. Coefficients of variation ranged from 7.6% (in-house pool) to 12.0% (manufacturer's control). Serum testosterone (17β-hydroxy-4-androsten-3-one) concentration was determined with the Spectria Testosterone ^{125}I radioimmunoassay kit (Orion Diagnostica, Espoo, Finland). Three different controls were included in each assay: Lyphochek low, medium, and high. Coefficients of variation ranged from 7.9% (high control) to 12.2% (low control). ^{125}I-labeled measurements for FSH, estradiol, and testosterone were carried out by means of the Wallac 1261 MultiGamma gamma counter (PerkinElmer Wallac Oy, Turku, Finland) using RiaCalc LM software (PerkinElmer Wallac Oy). Sex hormone-binding globulin was evaluated using the 1235 AutoDELFIA automatic immunoassay system (PerkinElmer Wallac Oy) based on a time-resolved fluoroimmunoassay (10).

Samples from all participants were assayed for levels of total, high-density lipoprotein, and low-density lipoprotein cholesterol, triglycerides, glucose, insulin, and C-reactive protein using laboratory methods described in detail previously. Briefly, the cholesterol contents of lipoprotein fractions and serum triglycerides were measured enzymatically; high-density lipoprotein fractions were separated from serum by combined ultracentrifugation and precipitation (11, 12). Glucose was measured using a glucose dehydrogenase method after precipitation of proteins by trichloroacetic acid (13). Serum insulin was determine with a Novo BioLabs radioimmunoassay kit (Novo Nordisk, Bagsvaerd, Denmark). C-reactive protein was measured via high-sensitivity immunometric assay (Immulite High Sensitivity CRP Assay; Diagnostic Products Corporation).

Questionnaire assessments

Participants reported data on demographic, behavioral, reproductive, and health-related factors on questionnaires, which were then reviewed by a trained interviewer for completeness and clarity. Reproductive factors included age at menarche, history and duration of oral contraceptive use, number of full-term pregnancies, age at last menses, and history of hysterectomy and oophorectomy; use of hormone therapy (ever use, current use, and total duration) was also assessed. Postmenopausal status and age at menopause were defined by the absence of menses for at least 12 months, or at the time of oophorectomy for women who reported having undergone surgery prior to menopause. Physical activity was assessed with the KIHD 12-Month Leisure Time Physical Activity History questionnaire (14) and was used to estimate amount of physical activity (metabolic equivalent of task hours) per day.

Study physicians conducted interviews to record each participant's history of CVD and metabolic disorders and her use of medications for hypertension, diabetes, heart disease, angina pectoralis, and high cholesterol. Each participant also completed 4 days of diet records and a detailed alcohol use questionnaire, which were used to estimate total energy and alcohol intake (15).

Statistical analysis

The present analysis was limited to women not using hormone therapy ($n = 588$). One woman was missing an IMT measurement and was therefore excluded, which gave us a final analytical sample size of 587. We divided participants into quartiles of FSH and estradiol and compared baseline characteristics across quartiles, using analysis of variance for continuous variables and χ^2 tests for categorical factors.

We then used multivariable linear regression to assess the relationships of FSH and estradiol levels with mean IMT. IMT values were transformed (negative reciprocal transformation) to improve normality and meet model assumptions. To evaluate the potential for confounding, we then built a series of models. In model 1, we adjusted for age and year of examination; models for estradiol also adjusted for FSH, and models for FSH adjusted for estradiol. Model 2 included model 1 variables plus measures of adiposity (BMI and waist:hip ratio). Model 3 included model 2 variables plus behavioral and biochemical factors associated with IMT, FSH, and/or estradiol levels in this population. These included physical activity, past use of hormone therapy, duration of use of hormone therapy, smoking status (current, past, or never smoker), pack-years of cigarette smoking, alcohol intake (g/week), number of full-term pregnancies, age at menopause, systolic and diastolic blood pressure, and levels of testosterone, sex hormone-binding globulin, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, glucose, insulin, and C-reactive protein. Model 4 included model 3 variables and further adjusted for CVD history and medication use, including history of hypertension, type 2 diabetes, symptomatic heart disease, or stroke and current use of medication for hypertension, coronary heart disease, high cholesterol, or diabetes. Linear trends across quartiles of FSH and estradiol were assessed by modeling the median values of all quartiles as a continuous variable.

We conducted stratified analyses to evaluate whether associations between hormones and IMT in model 4 varied by age and BMI. Effect modification was assessed with multiplicative interaction terms, and a Wald test result of $P < 0.05$ was considered significant. In sensitivity analyses, we excluded women reporting any history of CVD and those reporting use of CVD medication. We also repeated our main analysis evaluating associations between hormones and maximum IMT values. Statistical analyses were completed with SPSS (SPSS Inc., Chicago, Illinois), version 21.0 for Windows (IBM Corporation, Armonk, New York).

RESULTS

Characteristics of study participants by quartile of serum FSH concentration are presented in Table 1. In univariate analyses, women with the highest FSH levels were younger and had a lower BMI, waist:hip ratio, and estradiol level than women with lower FSH levels. Mean levels of systolic blood pressure, triglycerides, fasting insulin, and glucose were lower in women with higher FSH levels, while levels of total and high-density lipoprotein cholesterol were significantly higher. Women with higher FSH levels were less likely to report medication use for hypertension, symptomatic heart disease, type 2 diabetes, or angina pectoralis and had lower prevalences of hypertension and type 2 diabetes than women with lower FSH levels. The distributions of other factors did not differ significantly across FSH quartiles.

Relationships between baseline characteristics and estradiol levels are shown in Table 2. Women in the highest quartile of estradiol had significantly higher BMI, waist:hip ratio, and weight at age 20 years than women with lower levels. Mean testosterone, triglyceride, glucose, and insulin levels and mean systolic and diastolic blood pressure were higher in women with higher estradiol levels, while FSH levels were lower. Women with higher estradiol levels were more likely to report hypertension and use of antihypertensive medication but not other cardiometabolic conditions.

In unadjusted analyses, mean IMTs decreased across quartiles of FSH (Table 3). Mean IMTs of women in quartiles 1–4 of FSH were 0.94 mm, 0.91 mm, 0.87 mm, and 0.85 mm, respectively ($P < 0.001$). Mean IMT decreased with increasing FSH levels in models adjusting for age, examination year, and estradiol (model 1; P for trend < 0.001). Adjustment for BMI and waist:hip ratio attenuated results only modestly (model 2), and associations remained significant (P for trend $= 0.001$). Additional adjustment for behavioral and biochemical factors (model 3) and comorbid conditions and medication use (model 4) had a minimal impact on results, and a higher FSH level remained significantly associated with lower IMT. Regression coefficients for quartiles 2–4 of FSH versus quartile 1 (model 4) were −0.038, −0.045, and −0.062, respectively (P for trend $= 0.01$).

Estradiol levels were not linearly related to mean IMT (Table 3). Unadjusted mean IMTs across quartiles 1–4 of estradiol were 0.88 mm, 0.88 mm, 0.87 mm, and 0.93 mm, respectively ($P = 0.03$). In models that adjusted only for age, year, and FSH, high estradiol levels were associated with higher IMT (model 1; P for trend $= 0.04$). After further adjustment, results were attenuated, and high estradiol levels were not associated with higher IMT (model 4; P for trend $= 0.33$).

To facilitate comparison of our findings with those from previous studies of younger postmenopausal women, we stratified the results by age group (ages 53–62 years vs. 64–73 years) (Table 4). Mean FSH levels were marginally lower in older women than in younger women (50.1 (standard deviation, 16.6) IU/L vs. 53.2 (standard deviation, 22.0) IU/L; $P = 0.05$). Among older postmenopausal women, higher FSH levels were related to lower mean IMT, with regression coefficients being larger than those in the main analysis (P for trend $= 0.006$). In contrast, among younger postmenopausal women (ages 53–62 years), FSH levels were not linearly associated with IMT (P for trend $= 0.73$). The association between estradiol levels and mean IMT also varied by age. Among younger postmenopausal women, women with the highest estradiol levels had a greater mean IMT than those with the lowest levels (P for trend $= 0.03$). In older postmenopausal women, associations with estradiol were not significant. Results stratified by median time since menopause (15.3 years) were similar, with associations with FSH being strongest in women who had been postmenopausal longer (results not shown).

Because associations of FSH levels with IMT could potentially be driven by adiposity, we then stratified our population according to World Health Organization BMI category (<25.0 (normal-weight), 25.0–29.9 (overweight), or ≥30.0 (obese); Table 5). We continued to adjust for BMI and waist:hip ratio

Table 1.   Characteristics of Women Aged 53–73 Years Who Were Not Using Hormone Therapy, by Quartile of Follicle-Stimulating Hormone Concentration, Kuopio Ischemic Heart Disease Risk Factor Study, 1998–2001

Characteristic	Quartile of Follicle-Stimulating Hormone Level												P Value
	Quartile 1 (1.0–39.2 IU/L) (n = 147)			Quartile 2 (39.3–50.0 IU/L) (n = 149)			Quartile 3 (50.1–61.8 IU/L) (n = 144)			Quartile 4 (61.9–150.0 IU/L) (n = 147)			
	Mean (SD)	No. of Women	%	Mean (SD)	No. of Women	%	Mean (SD)	No. of Women	%	Mean (SD)	No. of Women	%	
Age, years	64.5 (6.8)			65.1 (5.9)			64.4 (6.1)			62.9 (6.9)			0.02
Body mass index[a]	31.1 (5.7)			29.0 (5.4)			28.6 (4.7)			26.8 (4.4)			<0.001
Waist:hip ratio	0.87 (0.07)			0.85 (0.06)			0.8 (0.1)			0.83 (0.06)			<0.001
Estradiol, pmol/L	55.8 (77.6)			35.1 (19.8)			32.3 (12.3)			34.1 (19.5)			<0.001
Testosterone, nmol/L	1.5 (2.9)			1.1 (0.5)			1.1 (0.5)			1.1 (0.5)			0.06
Sex hormone-binding globulin, nmol/L	55.7 (25.7)			56.0 (27.9)			54.5 (26.6)			57.8 (27.9)			0.77
Systolic blood pressure, mm Hg	141 (20.4)			141 (17.3)			136 (16.3)			136 (16.4)			0.008
Diastolic blood pressure, mm Hg	80.9 (9.7)			80.5 (8.7)			79.5 (8.2)			80.3 (8.5)			0.60
Total cholesterol, mmol/L	5.6 (0.7)			5.8 (1.0)			5.8 (0.9)			5.9 (1.0)			0.009
Triglycerides, mmol/L	1.4 (0.7)			1.4 (0.8)			1.2 (0.5)			1.2 (0.6)			0.007
HDL cholesterol, mmol/L	1.3 (0.3)			1.3 (0.3)			1.4 (0.3)			1.4 (0.3)			<0.001
LDL cholesterol, mmol/L	3.6 (0.7)			3.8 (1.0)			3.8 (0.9)			3.9 (1.0)			0.15
Glucose, mmol/L	5.3 (1.6)			5.2 (1.3)			5.0 (1.0)			4.9 (1.3)			0.03
Insulin, mU/L	12.0 (12.4)			9.5 (6.3)			8.3 (4.6)			7.3 (3.9)			<0.001
C-reactive protein, mg/L	3.7 (7.7)			2.9 (4.7)			3.1 (4.9)			2.4 (2.8)			0.19
Physical activity (MET-hours/day)	45.2 (5.9)			46.1 (7.1)			46.4 (6.1)			47.4 (7.5)			0.09
Alcohol intake, g/week	15.4 (32.2)			23.5 (53.2)			15.3 (29.5)			13.6 (26.1)			0.09
Age at menarche, years	13.7 (1.6)			14.0 (1.4)			14.1 (1.5)			13.9 (1.6)			0.18
Parity (no. of full-term pregnancies)	2.5 (1.6)			2.4 (1.8)			2.5 (1.8)			2.0 (1.5)			0.02
Age at menopause, years	49.5 (4.5)			49.5 (4.4)			48.9 (5.3)			49.2 (4.3)			0.78
Years postmenopausal	15.0 (8.2)			15.6 (7.0)			15.4 (7.9)			13.6 (8.5)			0.14
Pack-years of smoking[b]	6.2 (11.6)			6.4 (13.9)			6.8 (11.1)			4.9 (11.4)			0.89
Smoking status													
Never smoker		111	76		110	74		110	76		113	77	
Current smoker		13	9		18	12		16	11		7	5	0.13
Past smoker		23	16		21	14		18	12		27	18	0.71
Ever use of oral contraceptives		41	28		37	25		51	35		50	34	0.16
Past use of hormone therapy		41	28		47	32		50	34		53	36	0.45
Medical history[c]													
Heart disease		51	35		51	34		42	29		34	23	0.11
Diabetes		27	18		24	16		11	8		9	6	0.002
Hypertension		100	68		89	60		73	51		65	44	<0.001
Current medication use[c]													
Heart disease		27	18		20	13		12	8		11	7	0.01
Diabetes		14	10		6	4		6	4		2	1	0.01
Hypertension		83	56		77	52		62	43		51	35	0.001
High cholesterol		5	3		11	7		7	5		4	3	0.23
Angina		25	17		18	12		11	8		7	5	0.004

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent of task; SD, standard deviation.

[a] Weight (kg)/height (m)^2.

[b] Pack-years of cigarette smoking among ever smokers.

[c] Categories for medical history and current medication use were not mutually exclusive.

Table 2.   Characteristics of Women Aged 53–73 Years Who Were Not Using Hormone Therapy, by Quartile of Estradiol Concentration, Kuopio Ischemic Heart Disease Risk Factor Study, 1998–2001

| Characteristic | Quartile of Estradiol Level | | | | | | | | | | | | P Value |
| | Quartile 1 (1.0–26.0 pmol/L) (n = 158) | | | Quartile 2 (26.1–32.0 pmol/L) (n = 160) | | | Quartile 3 (32.1–40.0 pmol/L) (n = 127) | | | Quartile 4 (40.1–788 pmol/L) (n = 142) | | | |
	Mean (SD)	No. of Women	%	Mean (SD)	No. of Women	%	Mean (SD)	No. of Women	%	Mean (SD)	No. of Women	%	
Age, years	64.5 (6.4)			64.1 (6.7)			64.6 (6.6)			63.7 (6.3)			0.70
Body mass index[a]	26.2 (3.8)			27.6 (4.2)			29.1 (4.2)			33.1 (6.1)			<0.001
Waist:hip ratio	0.82 (0.05)			0.84 (0.06)			0.86 (0.06)			0.87 (0.07)			<0.001
Follicle-stimulating hormone, IU/L	56.5 (20.1)			54.8 (17.7)			48.7 (15.8)			44.1 (19.6)			<0.001
Testosterone, nmol/L	0.8 (0.4)			1.0 (0.5)			1.2 (0.5)			1.7 (3.0)			<0.001
Sex hormone-binding globulin, nmol/L	54.5 (25.6)			60.1 (30.4)			56.0 (26.8)			53.1 (24.1)			0.12
Systolic blood pressure, mm Hg	136 (18.0)			137 (16.1)			139 (19.6)			142 (17.2)			0.01
Diastolic blood pressure, mm Hg	78.8 (8.2)			80.1 (8.5)			80.0 (10.0)			82.7 (8.1)			0.001
Total cholesterol, mmol/L	5.6 (0.9)			5.8 (0.9)			5.9 (0.9)			5.7 (0.9)			0.09
Triglycerides, mmol/L	1.1 (0.5)			1.2 (0.6)			1.4 (0.7)			1.5 (0.8)			<0.001
HDL cholesterol, mmol/L	1.4 (0.3)			1.4 (0.3)			1.3 (0.3)			1.3 (0.3)			0.04
LDL cholesterol, mmol/L	3.6 (0.9)			3.8 (0.9)			3.9 (0.9)			3.8 (0.9)			0.04
Glucose, mmol/L	4.8 (0.8)			4.9 (1.1)			5.2 (1.2)			5.5 (1.9)			<0.001
Insulin, mU/L	6.9 (3.4)			8.7 (10.9)			9.7 (6.4)			12.1 (7.4)			<0.001
C-reactive protein, mg/L	2.7 (3.9)			2.8 (4.7)			3.7 (7.9)			3.2 (4.3)			0.45
Physical activity, MET-hours/day	47.6 (7.5)			45.7 (6.2)			45.4 (6.5)			46.0 (6.4)			0.02
Alcohol intake, g/week	14.7 (38.8)			13.7 (27.1)			23.1 (43.0)			17.7 (38.5)			0.15
Age at menarche, years	14.0 (1.5)			14.0 (1.5)			13.9 (1.3)			13.8 (1.7)			0.76
Parity (no. of full-term pregnancies)	2.4 (1.7)			2.2 (1.4)			2.6 (1.8)			2.4 (1.6)			0.23
Age at menopause, years	49.0 (4.5)			49.5 (4.2)			49.5 (4.3)			49.4 (4.7)			0.76
Years postmenopausal	15.6 (7.9)			14.6 (7.7)			15.0 (8.0)			14.5 (8.2)			0.63
Pack-years of smoking[b]	7.1 (11.5)			4.6 (9.0)			8.6 (16.6)			3.4 (8.1)			0.25
Smoking status													
Never smoker		120	76		123	77		89	70		112	79	
Current smoker		18	11		13	8		15	12		8	6	0.38
Past smoker		20	13		24	15		23	18		22	15	0.23
Ever use of oral contraceptives		47	30		52	33		40	31		40	28	0.86
Past use of hormone therapy		50	32		65	41		37	29		39	27	0.07
Medical history[c]													
Heart disease		42	27		49	31		35	28		52	37	0.24
Diabetes		12	8		16	10		18	14		25	17	0.04
Hypertension		71	45		83	52		76	60		97	68	<0.001
Current medication use[c]													
Heart disease		13	8		19	12		17	13		21	15	0.33
Diabetes		5	3		6	4		7	6		10	7	0.39
Hypertension		54	34		65	41		69	43		85	60	<0.001
High cholesterol		7	4		8	5		5	4		7	5	0.97
Angina		12	8		14	9		15	12		20	14	0.25

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent of task; SD, standard deviation.
[a] Weight (kg)/height (m)^2.
[b] Pack-years of cigarette smoking among ever smokers.
[c] Categories for medical history and current medication use were not mutually exclusive.

Table 3. Associations of Follicle-Stimulating Hormone and Estradiol Levels With Mean Carotid Intima-Media Thickness Among Women Aged 53–73 Years Who Were Not Using Hormone Therapy, Kuopio Ischemic Heart Disease Risk Factor Study, 1998–2001

Hormone and Quartile	Quartile Median Value	Mean IMT (SD), mm[a]	Model 1[b] β (SE)[f]	Model 1[b] P Value	Model 2[c] β (SE)[f]	Model 2[c] P Value	Model 3[d] β (SE)[f]	Model 3[d] P Value	Model 4[e] β (SE)[f]	Model 4[e] P Value
Follicle-stimulating hormone, IU/L										
Q1	32.1	0.94 (0.20)	0 (Referent)		0 (Referent)		0 (Referent)		0 (Referent)	
Q2	45.1	0.91 (0.21)	−0.044 (0.023)	0.06	−0.041 (0.023)	0.08	−0.038 (0.023)	0.09	−0.038 (0.023)	0.10
Q3	55.4	0.87 (0.16)	−0.068 (0.023)	0.004	−0.065 (0.024)	0.006	−0.052 (0.023)	0.03	−0.045 (0.023)	0.05
Q4	72.8	0.85 (0.19)	−0.085 (0.023)	<0.001	−0.079 (0.024)	0.001	−0.066 (0.024)	0.007	−0.062 (0.024)	0.01
P for trend		<0.001	<0.001		0.001		0.006		0.01	
Estradiol, pmol/L										
Q1	22.0	0.88 (0.18)	0 (Referent)		0 (Referent)		0 (Referent)		0 (Referent)	
Q2	30.0	0.88 (0.20)	0.004 (0.022)	0.84	0.000 (0.022)	0.99	0.004 (0.022)	0.86	0.000 (0.022)	0.99
Q3	36.0	0.87 (0.17)	−0.021 (0.024)	0.39	−0.027 (0.024)	0.26	−0.035 (0.025)	0.17	−0.036 (0.024)	0.16
Q4	49.0	0.93 (0.22)	0.049 (0.023)	0.04	0.037 (0.026)	0.16	0.031 (0.028)	0.27	0.028 (0.027)	0.31
P for trend		0.03	0.04		0.21		0.32		0.33	

Abbreviations: IMT, intima-media thickness; MET, metabolic equivalent of task; Q, quartile; SD, standard deviation; SE, standard error.

[a] Unadjusted mean IMT (not transformed).

[b] Model 1 adjusted for age (years), date of examination (year), and estradiol (pmol/L) or follicle-stimulating hormone (IU/L) level (quartiles).

[c] Model 2 further adjusted for body mass index (weight (kg)/height (m)^2) and waist:hip ratio (continuous).

[d] Model 3 further adjusted for physical activity (MET-hours/day), past use of hormone therapy (yes, no), duration of use of hormone therapy (5 categories), smoking status (current, past, or never smoker), pack-years of smoking (continuous), alcohol use (g/week), parity (number of full-term pregnancies), age at menopause (years; continuous), oophorectomy (yes, no), hysterectomy (yes, no), systolic and diastolic blood pressure (mm Hg; continuous), and levels of testosterone (nmol/L), sex hormone-binding globulin (nmol/L), total cholesterol (mmol/L), low-density lipoprotein cholesterol (mmol/L), high-density lipoprotein cholesterol (mmol/L), triglycerides (mmol/L), glucose (mmol/L), insulin (mU/L), and C-reactive protein (mg/L) (all natural log-transformed; continuous).

[e] Model 4 further adjusted for history of hypertension, diabetes, symptomatic heart disease, and stroke (each dichotomous) and current use of medication for hypertension, heart disease, high cholesterol, and diabetes (each dichotomous).

[f] Mean IMT values were transformed (negative reciprocal transformation) to improve normality.

Table 4. Associations of Follicle-Stimulating Hormone and Estradiol Levels With Mean Carotid Intima-Media Thickness Among Women Aged 53–73 Years Who Were Not Using Hormone Therapy, by Age Group[a], Kuopio Ischemic Heart Disease Risk Factor Study, 1998–2001

Hormone and Quartile	Quartile Median Value	Age Group							
		53–62 Years				64–73 Years			
		No. of Women	Mean IMT (SD), mm[b]	β (SE)[c]	P Value	No. of Women	Mean IMT (SD), mm[b]	β (SE)[c]	P Value
Follicle-stimulating hormone, IU/L[d]									
Q1	32.1	61	0.87 (0.17)	0 (Referent)		86	0.99 (0.20)	0 (Referent)	
Q2	45.1	49	0.84 (0.25)	−0.036 (0.044)	0.42	100	0.94 (0.19)	−0.028 (0.029)	0.33
Q3	55.4	59	0.81 (0.12)	−0.033 (0.042)	0.44	85	0.91 (0.17)	−0.047 (0.030)	0.12
Q4	72.8	74	0.81 (0.19)	−0.024 (0.041)	0.56	73	0.89 (0.17)	−0.089 (0.032)	0.007
P for trend			0.28	0.73			0.003	0.006	
Estradiol, pmol/L[e]									
Q1	22.0	63	0.78 (0.11)	0 (Referent)		95	0.94 (0.18)	0 (Referent)	
Q2	30.0	67	0.83 (0.20)	0.043 (0.039)	0.27	93	0.92 (0.18)	−0.019 (0.029)	0.50
Q3	36.0	49	0.80 (0.12)	0.023 (0.044)	0.60	78	0.91 (0.18)	−0.061 (0.032)	0.06
Q4	49.0	64	0.90 (0.24)	0.109 (0.048)	0.02	78	0.96 (0.20)	−0.004 (0.036)	0.90
P for trend			0.002	0.03			0.37	0.83	

Abbreviations: IMT, intima-media thickness; MET, metabolic equivalent of task; Q, quartile; SD, standard deviation; SE, standard error.

[a] Women from 4 specific age groups were selected for the study: 53–56 years, 59–62 years, 64–68 years, and 71–73 years.

[b] Unadjusted mean IMT (not transformed).

[c] Mean IMT values were transformed (negative reciprocal transformation) to improve normality. Results were adjusted for age (years; continuous), date of examination (year), body mass index (weight (kg)/height (m)^2; continuous), waist:hip ratio (continuous), estradiol (pmol/L) or follicle-stimulating hormone (IU/L) level (quartiles), physical activity (MET-hours/day), past use of hormone therapy (yes, no), duration of use of hormone therapy (5 categories), smoking status (current, past, or never smoker), pack-years of smoking (continuous), alcohol use (g/week), parity (number of full-term pregnancies), age at menopause (years; continuous), oophorectomy (yes, no), hysterectomy (yes, no), systolic and diastolic blood pressure (mm Hg; continuous), levels of testosterone (nmol/L), sex hormone-binding globulin (nmol/L), total cholesterol (mmol/L), low-density lipoprotein cholesterol (mmol/L), high-density lipoprotein cholesterol (mmol/L), triglycerides (mmol/L), glucose (mmol/L), insulin (mU/L), and C-reactive protein (mg/L) (all natural log-transformed; continuous), history of hypertension, diabetes, symptomatic heart disease, and stroke (each dichotomous), and current use of medication for hypertension, heart disease, high cholesterol, and diabetes (each dichotomous).

[d] Interaction with age: $P = 0.13$.

[e] Interaction with age: $P = 0.02$.

(both continuous) to minimize residual confounding within strata. We did not observe evidence that the association of FSH or estradiol with mean IMT varied significantly by BMI (all P's for interaction > 0.05). Although sample sizes for some comparisons were relatively small, we observed lower IMTs among women with higher FSH levels in all strata.

Results from analyses limited to women without prevalent CVD ($n = 395$) were similar to those from the main analysis (complete results not shown); regression coefficients for quartiles 2–4 of FSH versus quartile 1 (model 4) were −0.057, −0.055, and −0.098, respectively (P for trend = 0.002). Results from analyses excluding women who were currently using medication for CVD, hypertension, high cholesterol, or diabetes ($n = 301$) were slightly attenuated (complete results not shown); regression coefficients for quartiles 2–4 of FSH versus quartile 1 (model 3) were −0.034, −0.007, and −0.056, respectively (P for trend = 0.06). Furthermore, we did not observe evidence of effect modification by prevalent hypertension (results not shown).

FSH levels were significantly associated with maximum IMT (results not shown); for example, regression coefficients for quartiles 2–4 versus quartile 1 (model 4) were −0.030, −0.038, and −0.050, respectively (P for trend = 0.009). Estradiol was not associated with maximum IMT (results not shown).

DISCUSSION

We observed a significant inverse association between FSH levels and measures of subclinical atherosclerosis among postmenopausal women. Findings were most pronounced among women aged 64–73 years. To our knowledge, the association between FSH and IMT in older postmenopausal women who have completed the menopausal transition has not been previously evaluated. Importantly, our findings were not explained by confounding by adiposity, estradiol, or sex hormone-binding globulin levels or prevalent cardiometabolic disorders.

The magnitude of observed associations between FSH and IMT was difficult to interpret given our transformation of IMT data to improve normality. In unadjusted analyses of untransformed IMT data, mean IMT differed by 0.09 mm between the highest and lowest quartiles of FSH, a difference of 9.6%. For comparison, unadjusted mean IMT was 3.1% higher in obese

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Table 5. Associations of Follicle-Stimulating Hormone and Estradiol Levels With Mean Carotid Intima-Media Thickness Among Women Aged 53–73 Years Who Were Not Using Hormone Therapy, by Body Mass Index, Kuopio Ischemic Heart Disease Risk Factor Study, 1998–2001

Hormone and Quartile	Quartile Median Value	Body Mass Index[a]											
		<25.0 (Normal-Weight)				25.0–29.9 (Overweight)				≥30.0 (Obese)			
		No. of Women	Mean IMT (SD), mm[b]	β (SE)[c]	P Value	No. of Women	Mean IMT (SD), mm[b]	β (SE)[c]	P Value	No. of Women	Mean IMT (SD), mm[b]	β (SE)[c]	P Value
Follicle-stimulating hormone, IU/L[d]													
Q1	32.1	22	0.94 (0.20)	0 (Referent)		43	0.93 (0.23)	0 (Referent)		81	0.94 (0.18)	0 (Referent)	
Q2	45.1	34	0.89 (0.19)	−0.144 (0.064)	0.03	60	0.93 (0.24)	−0.019 (0.044)	0.67	55	0.90 (0.19)	−0.038 (0.032)	0.24
Q3	55.4	33	0.86 (0.16)	−0.108 (0.065)	0.10	61	0.88 (0.15)	0.003 (0.044)	0.94	51	0.86 (0.16)	−0.081 (0.034)	0.02
Q4	72.8	57	0.84 (0.18)	−0.107 (0.060)	0.08	61	0.84 (0.16)	−0.085 (0.045)	0.06	29	0.90 (0.24)	−0.026 (0.039)	0.51
P for trend				0.41				0.10				0.24	
Estradiol, pmol/L[e]													
Q1	22.0	62	0.88 (0.18)	0 (Referent)		75	0.87 (0.17)	0 (Referent)		21	0.90 (0.20)	0 (Referent)	
Q2	30.0	43	0.85 (0.18)	−0.074 (0.051)	0.16	74	0.91 (0.23)	0.037 (0.036)	0.30	43	0.87 (0.15)	0.011 (0.050)	0.83
Q3	36.0	26	0.85 (0.21)	−0.111 (0.061)	0.07	49	0.88 (0.17)	0.038 (0.043)	0.39	52	0.87 (0.14)	−0.045 (0.050)	0.37
Q4	49.0	15	0.90 (0.16)	−0.040 (0.073)	0.58	28	0.92 (0.25)	0.024 (0.052)	0.65	99	0.94 (0.22)	0.051 (0.049)	0.30
P for trend				0.29				0.57				0.09	

Abbreviations: IMT, intima-media thickness; FSH, follicle-stimulating hormone; MET, metabolic equivalent of task; Q, quartile; SD, standard deviation; SE, standard error.

[a] Weight (kg)/height (m)^2.

[b] Unadjusted mean IMT (not transformed).

[c] Mean IMT values were transformed (negative reciprocal transformation) to improve normality. Results were adjusted for age (years; continuous), date of examination (year), body mass index (weight (kg)/height (m)^2; continuous), waist:hip ratio (continuous), estradiol (pmol/L) or follicle-stimulating hormone (IU/L) level (quartiles), physical activity (MET-hours/day), past use of hormone therapy (yes, no), duration of use of hormone therapy (5 categories), smoking status (current, past, or never smoker), pack-years of smoking (continuous), alcohol use (g/week), parity (number of full-term pregnancies), age at menopause (years; continuous), oophorectomy (yes, no), hysterectomy (yes, no), systolic and diastolic blood pressure (mm Hg; continuous), levels of testosterone (nmol/L), sex hormone-binding globulin (nmol/L), total cholesterol (mmol/L), low-density lipoprotein cholesterol (mmol/L), high-density lipoprotein cholesterol (mmol/L), triglycerides (mmol/L), glucose (mmol/L), insulin (mU/L), and C-reactive protein (mg/L) (all natural log-transformed; continuous), history of hypertension, diabetes, symptomatic heart disease, and stroke (each dichotomous), and current use of medication for hypertension, heart disease, high cholesterol, and diabetes (each dichotomous).

[d] Interaction with body mass index: $P = 0.67$.

[e] Interaction with body mass index: $P = 0.58$.

women than in normal-weight women ($P = 0.20$) and 9.8% higher in women with hypertension versus women without it ($P < 0.001$), suggesting a level of difference comparable in magnitude to that of established risk factors for atherosclerosis.

To our knowledge, an inverse association between FSH and IMT has not been observed before. The few previous studies of FSH and atherosclerosis have assessed this relationship during the menopausal transition, when participants were probably still experiencing significant fluctuations in FSH and estradiol levels [1–5]. In 2 of these studies, investigators reported only unadjusted correlation coefficients and thus did not account for potential confounding by age, adiposity, estradiol, or other factors [4, 5].

The other studies of FSH and IMT were conducted within the Study of Women's Health Across the Nation [1–3]. In a cross-sectional analysis carried out when participants were aged 45–58 years (mean age = approximately 50 years), FSH level was not associated with IMT [3]. In subsequent work, participants were characterized by trajectory of change in FSH and estradiol levels across the menopausal transition [16]. El Khoudary et al. [1] then evaluated how the trajectory predicted mean IMT approximately 8 years after the final menstrual period (mean age = 59 years). After adjustment, IMT was significantly greater in women with the most common "medium" trajectory of FSH increase ($n = 431$) than in those with the lowest trajectory ($n = 53$). Interestingly, IMT was not significantly higher in women with the highest trajectory ($n = 372$), suggesting a non-linear relationship. Estradiol pattern was not significantly associated with mean IMT. It is notable that FSH levels continued to change for 10 years after the final menstrual period, underscoring the extended duration of the hormonal changes of menopause and the inherent difficulty of capturing associations of hormones with long-term cardiovascular health during this period. FSH levels during this dynamic period may be differently associated with atherosclerosis than the more stable levels attained after the menopausal transition is complete.

Few studies have characterized FSH in older postmenopausal women. Shaw et al. [17] reported that FSH levels were significantly lower in women aged 70–77 years (average of 27 years after menopause) than in women aged 48–57 years (average of 4 years after menopause), though estradiol levels did not vary. FSH response to gonadotropin-releasing hormone was also lower in the older postmenopausal women versus the younger ones. This is consistent with our study.

The structure of FSH may be physiologically different in postmenopausal women and premenopausal women [18]. FSH exists in multiple isoforms, with sialic acid and sulfonated *N*-acetylgalactosamine residues varying by isoform and influencing the half-life of the molecule. Wide et al. [18] report that FSH is more negatively charged after menopause due to a higher number of sialic acid molecules on postmenopausal isoforms, and this greater acidity is consistent with a longer half-life in vivo. Additionally, the distribution of isoforms changes with age and additionally varies across individuals, contributing further to individual differences.

The inverse association we observed between FSH level and IMT could plausibly be explained by relationships of FSH with estradiol and adiposity. It is well established that obese women have worse CVD risk profiles and higher IMT measurements than lean women [19, 20]. After menopause,

obesity is associated with higher estradiol levels, due to the aromatization of androgens to estrogens in adipose tissue. Estradiol produced extragonadally could then suppress FSH secretion through negative feedback, leading to lower FSH levels in women with greater adiposity. Strong inverse correlations of FSH with adiposity measures have in fact been observed in this study and in other observational studies [1, 4, 6, 21]. In a cohort study conducted within the Diabetes Prevention Project, Kim et al. [22] observed increases in FSH accompanying weight loss among overweight glucose-intolerant postmenopausal women. In our study, the inverse association between FSH and IMT that we observed was not explained by adiposity and estradiol, as associations persisted after adjustment for and stratification by these factors.

Activins and follistatin may also play a role in these relationships. Activins A, B, and AB, members of the transforming growth factor β superfamily, stimulate FSH secretion by the pituitary gland. FSH secretion via activins is inhibited by binding of the protein hormone follistatin. Ample evidence indicates that the activins and follistatin together play a role in regulating adipogenesis, inflammation, insulin resistance, and atherosclerosis [23, 24]. Interaction between the activins and follistatin has also been evaluated in the context of polycystic ovary syndrome, a reproductive condition associated with reduced FSH levels, increased adiposity, insulin resistance, and inflammation, in addition to hyperandrogenism and anovulation [25]. Teede et al. [26] observed higher follistatin levels in polycystic ovary syndrome patients than in controls, as well as significant correlations of follistatin and/or activin A with lipids, inflammatory markers, and insulin. The authors proposed that these hormones may be an important physiological link between the reproductive and metabolic features of polycystic ovary syndrome. Follistatin levels decline and activin bioavailability increases across the menopausal transition, likely contributing to the increase in FSH levels after menopause [27]. Our findings for FSH may potentially reflect downstream hormonal effects of activins or follistatin or their interaction with insulin resistance on atherosclerotic processes in late menopause, manifesting as reduced sensitivity of the hypothalamic-pituitary-gonadal axis. This is an important area for further study.

Our study had several important limitations. Because our study was cross-sectional, we were unable to assess the temporality of the relationship between FSH and IMT. Our assessment of subclinical atherosclerosis was limited to measurement of CCA IMT, which is well established as a predictor of future cardiovascular events [28, 29] but may not characterize atherosclerosis as comprehensively as measures of carotid plaques, carotid artery diameter, and coronary artery calcification [30, 31]. Prospective studies assessing how FSH levels are associated with these measures, atherosclerosis progression, and incident CVD will be essential for determining both the reproducibility of our findings and their clinical importance. Because the KIHD study population is very homogenous with respect to race and ethnicity, additional evaluation of these relationships in diverse populations is needed. Additionally, studies assessing the role of additional sex steroid and pituitary hormones such as estrone, androstenedione, and follistatin will be important for better understanding the physiology of FSH and atherosclerosis in older women. Strengths of our study include the availability of extensive data on biochemical and clinical CVD risk factors,

allowing us to comprehensively evaluate potential confounding and effect modification.

To our knowledge, this was the first study of FSH and markers of subclinical atherosclerosis in older postmenopausal women. We observed evidence of significantly lower IMT in women with the highest FSH levels, which was not explained by estradiol, adiposity, or other factors. The potential clinical implications of these findings are uncertain at this time. Additional studies of FSH and cardiovascular health outcomes in older postmenopausal women are needed to determine whether these findings are robust and, if so, to further explore potential underlying physiological explanations.

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