Triglyceride Levels and Fracture Risk in Midlife Women: Study of Women's Health Across the Nation (SWAN)

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Context: Unfavorable lipid levels contribute to cardiovascular disease and may also harm bone health.

Objective: To investigate relationships between fasting plasma lipid levels and incident fracture in midlife women undergoing the menopausal transition.

Design and Setting: A 13-year prospective, longitudinal study of multi-ethnic women in five US communities, with near-annual assessments.

Participants: At baseline, 2062 pre- or early peri-menopausal women who had no history of fracture.

Exposures: Fasting plasma total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C), at baseline and follow-up visits 1, and 3 through 7.

Main Outcome Measure(s): Incident non-traumatic fractures 1) 2 or more years after baseline, in relation to a single baseline level of lipids and 2) 2 to 5 years later, in relation to time-varying lipid levels. Cox proportional-hazards modellings estimated hazard ratios (HR) and 95% confidence interval (CI).

Results: Among the lipids, TG levels changed the most, with median levels increased by 16% during follow-up. An increase of 50 mg/dL in baseline TG level was associated with a 1.1-fold increased hazards of fracture (adjusted HR = 1.11, 95% CI: 1.04–1.18). Women with baseline TG $\geq$ 300 mg/dL had an adjusted 2.5-fold greater hazards for fractures (95% CI: 1.13–5.44) than women with baseline TG $<$ 150 mg/dL. Time-varying analyses showed a comparable TG level-fracture risk relationship. No associations between TC, LDL-C or HDL-C levels and fractures were observed.

Conclusions: Midlife women with high fasting plasma triglycerides had an increased risk of incident non-traumatic fracture.

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Abbreviations:

Women have an estimated 40% risk of nontraumatic fracture after ages 50 years (1, 2). Diabetes and cardiovascular disease (CVD), including peripheral arterial disease and abdominal aortic calcification, are associated with a 2- to 5-fold greater risk of incident hip fracture (2–6). Nontraumatic fractures and CVD share some risk factors, such as postmenopausal status, older age, smoking, and alcohol consumption. Unfavorable lipid profiles are major contributors to CVD and could influence bone health and fracture risk via multiple mechanisms, including oxidative stress, increased inflammation, reduced blood supply to bone, and alterations in bone remodeling (7, 8). Minimally oxidized lipids have been shown to inhibit bone-forming osteoblasts and stimulate bone-resorptive osteoclasts in mice (8). Bone marrow fat, which is triglyceride-rich (9), is an emerging risk factor for lower bone mineral density (BMD) in older women (10) and vertebral fracture (11); higher circulating triglyceride (TG) appears correlate with higher fat-to-water ratio in bone marrow (12).

Results from the few prospective epidemiological studies of the relationship between lipid levels and risk of nontraumatic fracture have been equivocal (13–16). In women, fasting plasma TG level typically increases before menopause (17). In contrast, increases in low-density lipoprotein (LDL)-cholesterol (LDL-C), decreases in high-density lipoprotein cholesterol (HDL-C) (17) and accelerated loss in BMD tend to emerge after the final menstrual period (18). We hypothesized that unfavorable fasting plasma lipid levels, particularly high TG, are associated with an increased risk of incident nontraumatic fractures in midlife women. We leveraged 13 years of data collected from women undergoing the menopausal transition in the Study of Women's Health Across the Nation (SWAN).

Materials and Methods

Study Population

SWAN is a prospective, longitudinal community-based study of women's health as women undergo the menopausal transition. SWAN enrolled 3302 premenopausal or early menopausal multiethnic women, ages 42 to 52 years, at seven U.S. sites between 1995 and 1997. Women were eligible if they did not use exogenous sex hormones, had at least one menstrual period in the last three months, had an intact uterus, had at least one intact ovary, and were not currently pregnant or lactating at the time of enrollment (19). All women provided written informed consent. At each near-annual follow-up visit, participants completed standardized questionnaires (administered by interviewers) about socioeconomic status, menstrual periods, exogenous hormone use, lifestyle factors, medical and surgical conditions, and use of medications and supplements.

Women at five study sites (n = 2413) underwent BMD assessment by dual-energy X-ray absorptiometry at each visit. All five sites enrolled non-Hispanic white women and women of other races/ethnicities, including African-American (Boston, MA; Pittsburgh, PA; Detroit, MI), Chinese (Oakland, CA) and Japanese (Los Angeles, CA). The current study included 2062 women (**Figure 1**), after excluding 313 women who had a fracture history at baseline, 4 women who became pregnant during follow-up, and 34 women who self-reported any traumatic (n = 19) or nontraumatic (n = 15) fracture at visit 1 (to make sure baseline lipid measurements preceded fracture occurrence).

Assessment of Incident Non-Traumatic Fracture

We defined a woman as having an "incident fracture" when she self-reported her first nontraumatic fracture at any skeletal site, except the toe, digit or face. At each follow-up visit, participants answered the questions: "Since your last study visit, how many times did you break or fracture a bone?"; "Which bones did you break or fracture"; and "Was it for any of the following reasons: after a fall from a height above the ground greater than six inches, in a motor vehicle accident, while moving fast (like running, bicycling or skating), while playing sports, or because something heavy fell on you or struck you?" Fractures that were not related to any of these reasons were considered to be nontraumatic fractures. Fractures at visits 2 to 6 were self-reported, and factures from visits 7 through 13 were confirmed by reviewing radiology reports in medical records. The false-positive rate of self-reported any fracture by SWAN participants was 4.6% (20).

Assessment of Fasting Lipid Levels

Plasma levels of lipids (TC, TG, LDL-C and HDL-C) were measured in blood drawn after 12-hour fast, at baseline, visits 1, and 3 through 7. TC and TG were analyzed by standard enzymatic methods on Hitachi 747 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, Indiana), and HDL-C was isolated using heparin-2M manganese chloride (17, 21). LDL-C level was calculated using Friedewald equation in women with fasting TG $\leq$ 400 mg/dL (17, 21). Prespecified extreme values of TC were those outside the range 100–500 (mg/dL); TG, outside the range 20–2000 (mg/dL); HDL-C, outside the range 20–150 (mg/dL); and LDL-C, outside the range 25–400 (mg/dL). Lipid panels from each study site were measured centrally at Medical Research Laboratories International, Inc. (Highland Heights, Kentucky).

Assessment of Pertinent Characteristics

At all follow-up visits, weight (kilograms), height (meters), and blood pressure (BP) were measured. Body mass index (BMI) was calculated as weight in kilograms/(height in meters) (2). Blood pressure was obtained from the right arm with participants seated with feet flat on the floor for at least 5 minutes before measurement. Self-reported current smoking status, alcohol use, family income, diabetes status, hypertension status were assessed at each visit. Smoking status and alcohol use were assessed by questions: "Since your last study visit, have you smoked cigarettes regularly (at least one cigarette a day)?" and "Since your last study visit, did you drink any beer, wine, liquor, or mixed drinks?" Having diabetes was defined as having a fasting blood glucose $\geq$ 126 mg/dL, self-reported diabetes, or use of insulin or other antidiabetes agent (22). Having hypertension was defined as having systolic BP $\geq$ 140 mmHg, diastolic BP $\geq$ 90 mmHg, self-reported hypertension, or use of any antihyper-

tensive medication. Medication use at each visit included major bone-affecting agents (bisphosphonate, oral or inhaled corticosteroids, thiazide, calcitonin, recombinant parathyroid hormone, calcium supplements and vitamin D supplements) and lipid-lowering medications (statins, fibric acid, niacin and bile acid resins).

Menopausal stage incorporated self-reported menstrual cycle characteristics and exogenous hormone use at each visit (23). Premenopause was defined as menstruation in the past 3 months with menstrual regularity in the past year. Early peri-menopause was defined as menstruation in the last 3 month with decreased regularity in the last year. Late peri-menopause was defined as no menstruation for 3–11 months, and postmenopause was defined as no menstruation for 12 months. Menopausal stage at each visit was grouped by the SWAN Coordinating Center into the following stages: postmenopause by bilateral salpingo-oophorectomy (BSO), hormone therapy (HT) user; postmenopause by bilateral salpingo-oophorectomy (BSO), non-HT user; natural postmenopause, non-HT user; late natural postmenopause, HT user; early peri-menopause; late peri-menopause; premenopause; unknown due to HT use; and unknown due to hysterectomy.

BMD of the posterior-anterior lumbar spine and total hip were measured using Hologic QDR 2000 densitometers (Pittsburgh and Oakland) and 4500A (Boston, Detroit area, and Los Angeles) (Hologic, Waltham, MA, USA) (18, 23). Quality-control procedures included daily phantom measurements and bi-annual cross-calibration with a circulating anthropomorphic spine standard. All scans were reviewed at the study site. Scans flagged with problems and a 5% random sample of scans were centrally reviewed by Synarc (Newark, CA, USA). Short-term in vivo BMD measurement variability was 0.014 g/cm^2 (1.4%) for the lumbar spine and 0.016 g/cm^2 (2.2%) for the femoral neck (23). High-sensitivity C-reactive protein (CRP) (hsCRP) levels were quantified using an ultrasensitive rate-immunonephelometric method (hsCRP on a BN 100, Dade-Behring) (24).

Recreational physical activity was derived from questionnaire data collected at baseline, visits 3, 5 and 6, and grouped into three clinical relevant levels (25): "not active" (0–1 time/mo), "nonclinically significant" (2–3 times/mo) and "clinically significant" physical activity (≥ 4 times/mo).

Statistical analysis

Continuous variables (median, interquartile range, IQR) and categorical variables (counts and percentages) were presented for the analytic sample overall and by four racial/ethnic groups: non-Hispanic white, African-American, Japanese, and Chinese; and were assessed by Kruskal-Wallis test (for continuous variables) and χ^2 test (for categorical variables). For regression modeling, physical activity at visits 1, 4 and 7, was imputed using the last observation carried forward (LOCF). TG levels had a skewed distribution and were natural log-transformed (26). Levels of TC, LDL-C and HDL-C showed normal distributions and were not log-transformed. Missing lipid values at visit 2 were excluded for regression.

Several Cox regression modeling approaches were used to investigate longitudinally the risk of incident fracture in relation to fasting lipid levels. Firstly, we addressed the question: Is a lipid level at a single time-point (baseline) related to the risk of incident fracture between 2 to 13 years later? A woman contributed her follow-up time from baseline visit to the date of incident fracture,

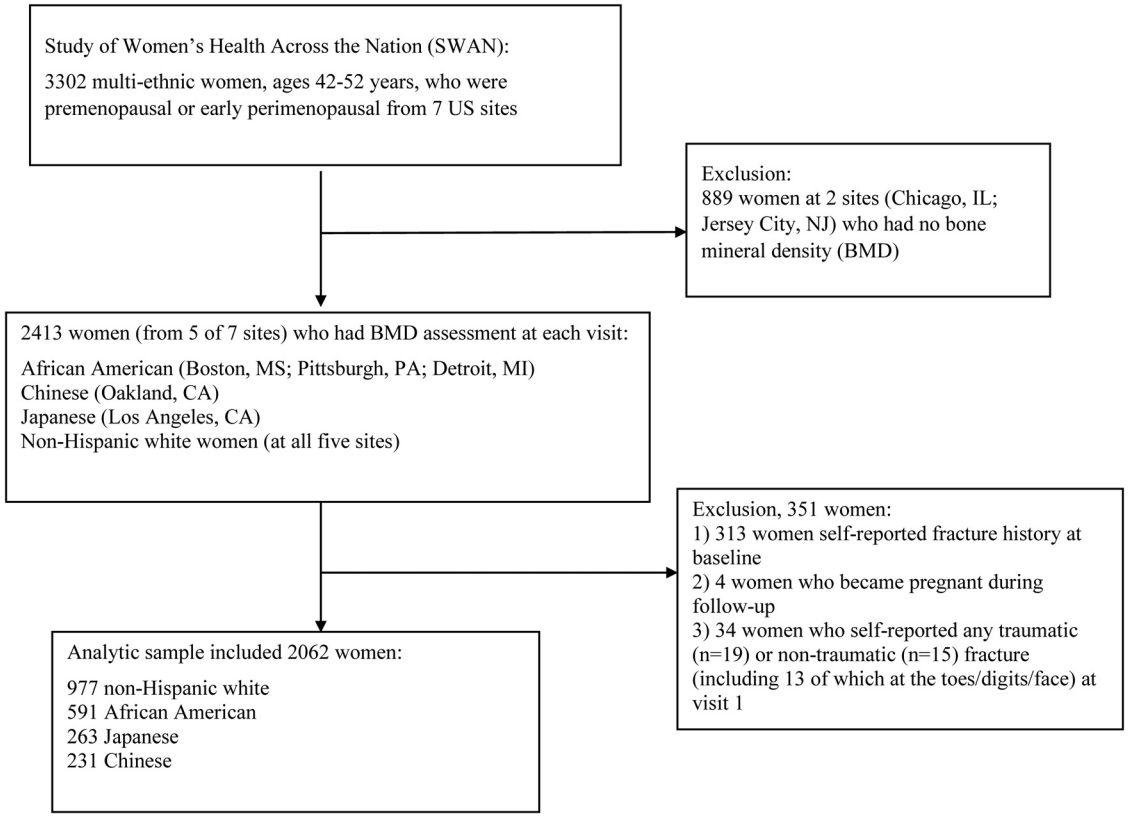

Figure 1. Flow diagram of 2062 women who were included in the current study from the Study of Women's Health Across the Nation (SWAN).

date of last follow-up, date of death, or visit 13, whichever occurred first. Women who self-reported any nontraumatic fracture within 2 years after baseline were excluded. Secondly, we utilized the available repeated measures of lipids through follow-up visit 7, to address the question: Is a lipid level of interest (time-varying) related to the risk of incident fracture that occurred 2 to 5 years later? We incorporated a 2-year lag in time-varying (repeated measures) analyses (a) to ensure that lipid levels preceded the fracture outcome (temporality) and (b) investigate whether results from time-varying (repeated measures) analyses corroborated those from Cox regression with a single baseline lipid level. Robust variance estimates were used to account for correlations between repeated measures (27). Using time-varying analyses with a 2-year lag, we also addressed the question: Is the mean lipid level from two consecutive measurements from baseline through visit 7, associated with the risk of incident fracture 2 to 5 years later?

For each approach, we examined continuous lipid levels (with or without natural log transformation) and categorical TG level (300+ mg/dL, 150–299 mg/dL, and < 150 mg/dL as reference) to provide clinically informative results. The TG level less than 150 mg/dL is the clinically desirable concentration, and TG level greater than 150 mg/dL is one of five component criteria for metabolic syndrome. The TG level of 300 mg/dL is a well-perceived, less-arguably high TG concentration. For each approach, model 1 adjusted for age, study site, and race/ethnicity; model 2 additionally included potential confounders: menopausal stage, current smoking (yes/no), alcohol use (yes/no), physical activity, diabetes (yes/no) and BMI. The "full" model added lumbar spine BMD to model 2 because BMD is a fracture risk factor in midlife women (20) and also could be a mediator for lipids-fracture associations. Covariates at baseline were used in models assessing the single baseline lipids. In time-varying analyses, these covariates were time-varying, where applicable. All models excluded lipid measurements that were known to be measured in nonfasting blood, had missing fasting information or were extreme values. Proportionality assumption was tested by including product-terms between follow-up time and covariates in multivariable models. No obvious violation was observed.

All analyses were performed in SAS v9.4 (SAS Institute Inc., Cary, NC, USA). A two-sided P-value < 0.05 was considered statistical significance.

Results

More than 75% of the 2062 women at SWAN baseline were either non-Hispanic white (47%) or African-American (29%) (Table 1). The median age was 46 years (IQR: 44–48 years). Compared to Japanese and Chinese women, higher proportions of non-Hispanic white and African American women were aged 42–45 years, were early peri-menopausal, had diabetes, and ever smoked. A higher fraction of African-American women used bone-affecting medications, particularly corticosteroids, compared to women of other race/ethnicities. The baseline median BMI, hip BMD, lumbar spine BMD, and hsCRP were highest in African-American women (all p-values < 0.001). Overall, participants were mildly overweight at baseline, with a median BMI of 26 kg/m^2 (ranging from 22 kg/m^2 in Japanese and Chinese women to 30 kg/m^2 in African-American women). Median BMD at the total hip and lumbar spine were within the nonosteopenic and nonosteoporotic range (by WHO criteria (28)) in all racial/ethnic groups. The SWAN participants had favorable lipid profiles at baseline. Median TC was 192 mg/dL and median LDL-C was 114 mg/dL. Median TG was 89 mg/dL and 75% of women having a TG level of 128 mg/dL or lower. Median HDL-C level was 55 mg/dL (IQR: 47–66 mg/dL). Japanese and Chinese women had higher median TG and HDL-C levels and lower median LDL-C level than non-Hispanic white and African-American women.

During follow-up, median TG levels increased the most, by 16% from baseline (89 mg/dL) to visit 7 (103 mg/dL); whereas median levels of TC, HDL-C and LDL-C increased 7%, 7% and 9%, respectively. The retention rate of SWAN participants ranged from 76% (visit 13) to 89% (visit 1). From visits 2 to 13, 147 had an incident (nontraumatic) fracture (**Figure 2**), which consisted of fractures of the foot (33%), ankle (16%), wrist (13%), ribs (12%) and legs (9%). At the time of incident fractures, 30% of women with incident fracture were peri-menopausal or early postmenopausal, 55% were postmenopausal, and 15% had undetermined menopausal status due to HT and/or hysterectomy. Seventy-four percent (109 of 147) of women with incident fractures reported their fractures between visits 7 and 13, a period in which self-reported fractures were also confirmed by medical records review.

An increase of single baseline TG level by 50 mg/dL was associated with increased hazards of incident fractures (adjusted HR: 1.11, 95% CI: 1.04–1.18) after adjustment for smoking status, alcohol use, physical activity, menopausal stage, diabetes, BMI and lumbar spine BMD ("full model", Table 2). No associations were observed between a single baseline level of natural log-transformed TG (lnTG), TC, LDL-C or HDL-C and incident fracture. In time-varying analyses (Table 3), every 50 mg/dL increase in TG at a given visit was associated with incident fracture 2 to 5 years later, with an adjusted HR of 1.07 (95% CI: 1.02–1.12, "full model"). For example, women with TG of 200 mg/dL at visit 3 had a 7% greater risk of nontraumatic fracture 2 to 5 years later, compared to women with TG of 150 mg/dL at visit 3. One unit change in lnTG (or 2.7-fold increase in TG level) at a given visit, the hazards of incident fracture between 2 to 5 years later was 1.31 (95% CI: 1.00–1.71, "model 2"); adjustment for lumbar spine BMD attenuated the association (HR = 1.28, 95% CI: 0.97–1.70, "full model").

Women with TG levels ≥ 300 mg/dL, whether at baseline or in time-varying analyses, consistently had increased

Table 1. Baseline characteristic and fasting lipid levels of 2062 women without a history of fracture at baseline in SWAN

Characteristics	Total $n = 2062$	Non-Hispanic white, $n = 977$	African-American $n = 591$	Japanese $n = 263$	Chinese $n = 231$	*P-value
	N (%)	N (%)	N (%)	N (%)	N (%)	
Age at baseline, year						0.015
42–45	880 (43)	435 (45)	267 (45)	92 (35)	86 (37)	
46–49	897 (43)	399 (41)	252 (43)	130 (49)	116 (50)	
50–52	285 (14)	143 (15)	72 (12)	41 (16)	29 (13)	
Menopausal stage						<0.001
Pre-menopause	1123 (55)	517 (54)	299 (51)	163 (63)	144 (63)	
Early perimenopause	914 (45)	446 (46)	287 (49)	97 (37)	84 (37)	
Hypertension[#]	540 (26)	200 (21)	245 (42)	50 (19)	45 (19)	<0.001
Diabetes[##]	92 (4)	37 (4)	53 (9)	0	2 (1)	<0.001
Tobacco smoking						<0.001
Current smoker	330 (16)	147 (15)	146 (25)	32 (12)	5 (2)	
Past smoker	519 (25)	311 (32)	140 (24)	28 (11)	3 (1)	
Never smoker	1211 (59)	519 (53)	305 (51)	201 (76)	223 (97)	
Any alcohol drinks, yes	983 (48)	572 (59)	251 (42)	113 (43)	47 (19)	<0.001
Physical activity, time/month						<0.001
0–1 (not active)	842 (41)	336 (35)	312 (53)	87 (33)	107 (48)	
2–3	864 (42)	414 (43)	226 (39)	131 (50)	93 (41)	
≥ 4	331 (16)	216 (22)	45 (8)	45 (17)	25 (11)	
Bone-affecting medications[¶]	164 (8)	65 (7)	83 (14)	7 (3)	9 (4)	<0.001
Lipid-lowering medications[¶¶]	21 (1)	11 (1)	8 (1)	2 (1)	9 (4)	0.131
Calcium or vitamin D supplement	34 (2)	17 (2)	12 (2)	4 (2)	1 (0)	0.315
Characteristics	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	
BMI, kg/m^2	26 (22–32)	26 (23–31)	30 (26–36)	22 (20–25)	22 (21–25)	<0.001
Weight, kg	69 (58–85)	71 (61–84)	82 (69–98)	55 (50–61)	56 (51–63)	<0.001
hsCRP levels, mg/liter	1.3 (0.5–4.2)	1.4 (0.6–3.7)	3.1 (1.0–7.7)	0.5 (0.2–1.1)	0.7 (0.4–1.6)	<0.001
BMD, g/cm^2						
Total hip	0.95 (0.86–1.06)	0.95 (0.87–1.04)	1.05 (0.95–1.14)	0.88 (0.80–0.95)	0.85 (0.78–0.92)	<0.001
Lumbar spine	1.08 (0.98–1.17)	1.07 (0.98–1.15)	1.14 (1.05–1.23)	1.02 (0.94–1.09)	1.04 (0.94–1.11)	<0.001
Lipids, baseline, mg/dL						
Total cholesterol	192 (171–215)	192 (171–214)	191 (168–217)	196 (174–212)	189 (171–208)	0.626
Triglyceride	89 (66–128)	89 (68–138)	85 (63–121)	94 (67–137)	97 (68–128)	0.018
HDL-C	55 (47–66)	54 (46–64)	53 (45–63)	60 (51–69)	60 (52–70)	<0.001
LDL-C	114 (94–135)	114 (95–135)	117 (96–141)	112 (91–130)	105 (90–127)	<0.001

*P-value: covariates distribution across racial/ethnic groups, using χ-square test (categorical variables) or Kruskal-Wallis test (continuous variables).

[#] Hypertension: any use of anti-hypertensive medications, self-reported hypertension or systolic/diastolic blood pressure ≥ 140/90 mmHg

[##] Diabetes definition: fasting blood glucose ≥ 126 mg/dL, self-reported diabetes, or any use of insulin or anti-diabetes agent.

[¶] Bone-affecting medications: corticosteroids, thiazide, bisphosphonates, calcitonin, or recombinant parathyroid hormone.

[¶¶] lipid-lowering medications: statins, fibric acid, niacin, or bile acid resins

hazards of incident fracture. Women with baseline TG levels 300 mg/dL or higher had 2.5-fold greater hazards (full model: HR: 2.48, 95% CI: 1.13–5.44) of fracture at visit 2 and onward, compared with women with baseline TG < 150 mg/dL (Table 4). In time-varying analyses, women with TG levels ≥ 300 mg/dL still had 1.94-fold (95% CI: 1.14–3.30) increased hazards of incident fracture, compared with women with TG < 150 mg/dL. No association with fractures was observed for TG levels of 150–299 mg/dL. Mean TG level ≥ 300 mg/dL for two consecutive follow-up visits was associated with fracture between 2 to 5 years later. Additional adjustment for TC and HDL-C in the full model did not change associations between categorical TG and incident fracture. In sensitivity analyses comparing women with TG ≥ 208 mg/dL (upper 10%) vs women with TG < 208 mg/dL, adjusted HR for incident fracture was 1.90 ("full model": 95% CI: 1.05–3.43).

Full models did not include sex hormone levels, saturated fat intake (calculated from food frequency questionnaire), hsCRP, hypertension, bone-affecting medications or supplements, lipid-lowering medications and total hip BMD. Less than 5% of participants used lipid-lowering medications. Total hip BMD was highly correlated with lumbar spine BMD (Pearson's r = 0.74, $P < .0001$) and did not alter results. Sex hormone levels, hsCRP, hypertension, and bone-affecting medications either were not associated with lipid levels or did not change the associations. TG levels were not related to saturated fat intake (r = 0.019 and 0.017, respectively) at baseline and visit 5 (where dietary intake data were available).

Discussion

Midlife women who had a fasting plasma TG levels ≥ 300 mg/dL consistently had 2- to 2.5-fold increased hazards of

Table 2. Hazard ratio estimates of incident fracture associated with a single baseline level of each fasting plasma lipid.

Models*	Hazard ratio (95% CI) for incident fractures two or more years later				
	Total Cholesterol (per 50 mg/dL)	TG (per 50 mg/dL)	ln(TG) (per unit change)**	LDL-C (per 50 mg/dL)	HDL-C (per 10 mg/dL)
Model 1: age, race/ethnicity, study site	1.03 (0.78–1.36)	1.11 (1.05–1.18)	1.42 (0.95–2.13)	0.87 (0.47–1.61)	0.95 (0.81–1.12)
Model 2: Model 1 + menopausal stage#, smoking, alcohol use, physical activity, diabetes, BMI.	1.02 (0.78–1.35)	1.12 (1.05–1.19)	1.42 (0.97–2.10)	0.90 (0.48–1.67)	0.95 (0.81–1.11)
Full model: Model 2 + lumbar spine BMD.	1.02 (0.75–1.38)	1.11 (1.04–1.18)	1.30 (0.85–1.97)	0.85 (0.43–1.68)	0.99 (0.84–1.16)

*Full model included potential confounders in model 2 plus putative fracture risk factors and/or mediators. BMI could also be a confounder. We did not log-transform levels of total cholesterol, LDL-C and HDL-C because they showed normal distribution.

**1-unit change in ln(TG) represented 2.7-fold change in TG levels. For example, compared with a woman who had TG 100 mg/dL at baseline, a woman with TG 270 mg/dL at baseline had 1.3-fold greater hazards for fracture during follow-up.

#All women were pre-menopausal or peri-menopausal and had no exogenous hormone use at baseline.

Table 3. Hazard ratio estimates of incident fracture associated with time-varying levels of each fasting plasma lipid

Models*	Hazard ratio (95% Confidence Intervals) for incident fractures occurring 2 to 5 yr later**				
	Total Cholesterol (per 50 mg/dL)	TG (per 50 mg/dL)	ln(TG) (per unit change)#	LDL-C (per 50 mg/dL)	HDL-C (per 10 mg/dL)
Model 1: age, race/ethnicity, study site.	0.94 (0.79–1.11)	1.08 (1.04–1.12)	1.37 (1.06–1.76)	0.92 (0.75–1.12)	0.93 (0.85–1.02)
Model 2: Model 1 + menopausal stage##, smoking, alcohol use, physical activity, diabetes, BMI	0.92 (0.77–1.10)	1.06 (1.04–1.12)	1.31 (1.00–1.71)	0.93 (0.76–1.13)	0.92 (0.83–1.01)
Full model: Model 2 + lumbar spine BMD.	0.89 (0.74–1.08)	1.07 (1.02–1.12)	1.28 (0.97–1.70)	0.88 (0.71–1.09)	0.93 (0.84–1.02)

*Model 2 and full model included time-varying covariates. Full model included potential confounders in model 2 and BMD (putative fracture risk factor and/or mediator).

**Lipid level at a given visit *i* in relation to incident fracture risk between *i*+2 and *i*+5 yr. Levels of total cholesterol, LDL-C and HDL-C were not log-transformed because they showed normal distribution.

#1-unit change in ln(TG) represented 2.7-fold change in TG levels.

Time-varying menopausal stage (including exogenous hormone therapy (HT) use): post-menopause by bilateral salpingo-oophorectomy (BSO), non-HT user; post-menopause by BSO, HT user; natural post-menopause, non-HT user; late natural post-menopause, HT user; early peri-menopause; late peri-menopause; pre-menopause; unknown due to HT use; and unknown due to hysterectomy.

nontraumatic fracture, after adjusting for BMD and potential confounders. No associations were observed between other plasma lipids and fractures. Using three analytical approaches, higher TG levels were consistently associated with an increased risk of incident fracture. To our knowledge, this prospective, longitudinal, multiethnic cohort study was the first to assess time-varying lipid levels, obtained at multiple time-points across 7 years, in relation to incident nontraumatic fracture among midlife women undergoing the menopausal transition. Specifically, TG levels at a given time-point were associated with increased fracture risks 2 to 5 years later, after adjusting for the earliest TG values and time-varying menopausal status. This study raises the possibility that high fasting TG levels in midlife women may be an indicator for increased fracture risk.

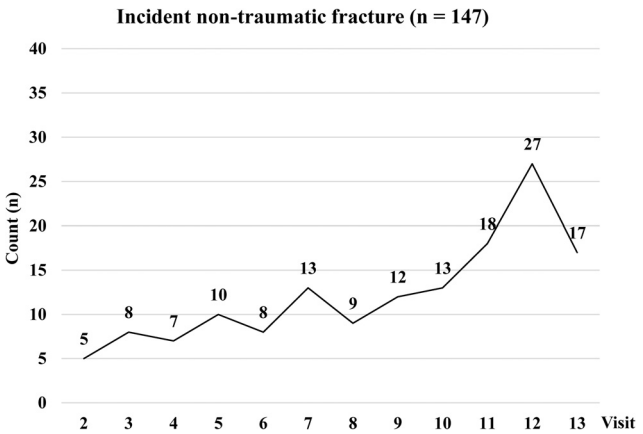

Figure 2. Counts of incident nontraumatic fracture at each near-annual visit. All nontraumatic fractures excluded those at the face, toes or digits. Incident nontraumatic fractures were self-reported fractures not related to a fall from a height above the ground greater than six inches, a motor vehicle accident, moving fast (like running, bicycling or skating), playing sports, or something heavy fell or struck. Between visits 7 and 13, self-reported fractures were further confirmed by medical records review: 74% of all (109 of 147) nontraumatic fractures were confirmed in medical records.

Few prospective studies have examined lipid levels in relation to fracture risk (13–16) and findings in women were scarce. The Sweden Gothenburg Project studied men and women combined, ages 25–64 years (53% women, less than a quarter of whom were postmenopausal) and reported positive associations between total cholesterol and fracture (16). Yet, associations in women specifically were not reported. The current study results are consistent

Table 4. Multivariable-adjusted hazard ratio (HR) estimates for incident fracture by fasting plasma TG levels at baseline and in time-varying analyses

TG level (mg/dL)	Number (%) of repeat-measures that contributed to fracture *vs.* non-fracture	*Full Model Hazard Ratios (95% Confidence Intervals)		
		For Fracture incidence two or more years later, based on baseline fasting TG level	Time-varying analyses: TG level at visit *i* and fracture risk 2 to 5 yr later	
			Single TG level	Mean TG level of two consecutive measures**
<150	793 (73)/ 7776 (77)	1 (reference)	1 (reference)	1 (reference)
150–299	242 (22)/ 2067 (20)	0.89 (0.48–1.65)	1.13 (0.80–1.59)	1.03 (0.70–1.52)
≥ 300	49 (5)/ 298 (3)	2.48 (1.13–5.44)	1.94 (1.14–3.30)	2.21 (1.29–3.79)

*Full model included age at baseline, race/ethnicity, study site, smoking, drinking, physical activity, menopausal stage (including exogenous hormone use), BMI, diabetes and lumbar spine BMD.

**Mean TG level for TG at visit *i* and the last TG level measured before visit *i* in relation to fractures between *i*+2 and *i*+5 yr.

with a post hoc report from the MORE (Multiple Outcomes of Raloxifene Evaluation) trial (29). Postmenopausal women in the placebo group with high TG or low BMD at baseline had an increased risk of subsequent vertebral fracture, and raloxifene was more effective in reducing vertebral fracture in these women, compared to women who had a normal TG or high BMD (29). Other prospective studies focused on men (14, 15) or examined nonfasting lipids (13), with inconsistent results.

Triglyceride may link CVD and nontraumatic fractures. Adults with diabetes (3) or CVD (4, 5) had a higher risk of nontraumatic fractures than those without. Women with diabetes often have elevated TG levels (30). Fracture risk was associated with high TG levels in midlife women in the current study, even after controlling for diabetes, BMD, and putative fracture risk factors. The underlying mechanisms for TG in association with fracture were unclear. We postulated that TG could influence nontraumatic fracture through its relationship with low-grade chronic inflammation that involves several pathways, including Osteoprotegerin/RANKL/RANK in bone remodeling (31–33), IL-6 (34) and/or tumor necrosis factor (TNF) soluble receptors (35). CRP, an acute phase biomarker of inflammation, did not alter the risk estimates when included in the models, but does not rule out a role for chronic inflammation.

Osteoprotegerin (OPG) is a decoy receptor that inhibits bone resorption by competing against receptor activator of nuclear factor κB ligand (RANKL) to bind to RANK (36). Serum OPG concentrations were observed to be higher in postmenopausal women with diabetes than those without diabetes (37). Higher serum OPG levels were cross-sectionally related to lower nonfasting TG levels, greater HbA1C, and higher prevalence of diabetes (33). On the other hand, studies have reported lower TG levels in postmenopausal women with elevated levels of total soluble RANKL (32) and similar TG levels across OPG concentrations (31, 32). In addition, serum TG levels were positively related to circulating levels of IL-6 (34) and TNF receptors (35), which were inflammatory markers associated with increased hip fracture risks in postmenopausal women (38).

Women with vertebral fractures, compared with those without vertebral fractures, had a higher mean marrow fat (lipid-water ratio) (10), of which the majority is composed of TG (9). Circulating TG specifically, may in part explain the variability in bone marrow lipid-water ratio (12). It is plausible that circulating TG levels are associated with fracture at least in part because it is an indicator of greater bone marrow fat content.

The current study observed consistently increased fracture risks based on a single baseline TG level and separately, repeat-measures of TG. This consistency may be due to the modest variability in fasting TG levels across the follow-up visits. The distributions of lipid levels in the SWAN participants suggests a relatively health cohort regarding lipids, and less than 10% of women had TG levels greater than 300 mg/dL. Sensitivity analyses using the 90th percentile (208 mg/dL) as cut-point for TG suggested associations similar to those in original analyses. We acknowledge the possibility of a chance finding due to the small numbers of women with a TG > 300 mg/dL and advocate for more studies. Underlying mechanisms remain to be clarified for nontraumatic fracture in midlife women. Any nontraumatic fracture occurred during the menopausal transition is a risk factor for another future (39). Our results may not be generalizable to other populations, such as older postmenopausal women or men. Although self-reported fractures from visits 2 through 6 (about 26% of all incident fractures) were not confirmed by medical records review, the rate of false-positive finding of any fracture was low: 5% (9 of 193) of all self-reported fractures were incorrect in medical records (20). The bias from inaccurate years of follow-up (for women who self-reported fracture at visits 2 through 6), measurement errors for lipids, or false-positive self-reported fractures were likely small and nondifferential.

In conclusion, midlife women with high fasting plasma TG (300 mg/dL or more) had about a 2- to 2.5-fold increased risk of nontraumatic fractures, after controlling

for BMD, diabetes, BMI, menopausal status and other potential confounders. Further investigation of the roles of TG in bone remodeling, BMD, and the risk of fracture in midlife women are warranted. If the current results are confirmed, high fasting plasma TG could be a modifiable risk factor and help to identify midlife women at-risk of nontraumatic fracture.

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