BMJ Open

Effects of Age, Gender, and Menopausal status on Small Dense Low-Density Lipoprotein Cholesterol and Low-Density Lipoprotein Cholesterol Fractions; A Population-Based Study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-041613
Article Type:	Original research
Date Submitted by the Author:	12-Jun-2020
Complete List of Authors:	Izumida, Toshihide; Kanazawa Medical University Himi Municipal Hospital, ; University of Toyama, Yoshikazu, Nakamura; Jichi Medical University, Public Health Sato, Yukihiro Ishikawa, Shizukiyo; Jichi Ika Daigaku, Department of General Medicine
Keywords:	CARDIOLOGY, Heart failure < CARDIOLOGY, Ischaemic heart disease < CARDIOLOGY, INTERNAL MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

[TITLE] Effects of Age, Gender, and Menopausal status on Small Dense Low-Density Lipoprotein Cholesterol and Low-Density Lipoprotein Cholesterol Fractions; A Population-Based Study

Authors: Toshihide Izumida[1], Yosikazu Nakamura[2], Yukihiro Sato[3], Shizukiyo Ishikawa[2]

[1] Division of Community Medicine, Kanazawa Medical University Himi Municipal Hospital, Himi, Toyama, Japan
1130 Kurakawa, Himi, Toyama 935-0025, Japan

[2] Division of Public Health, Center for Community Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan
3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan

[3] Internal Medicine, Kamiichi General Hospital, Nakaniikawa-gun, Toyama, Japan
51 Hoonji, Kamiichi, Nakaniikawa-gun, Toyama 930-0391, Japan

Corresponding Author: Shizukiyo Ishikawa
Division of Public Health, Center for Community Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan
E-mail: i-shizu@jichi.ac.jp

E-mail address: Toshihide Izumida, m07011ti@jichi.ac.jp; Yosikazu Nakamura, nakamuyk@jichi.ac.jp; Yukihiro Sato, yukisato@fancy.ocn.ne.jp; Shizukiyo Ishikawa, i-shizu@jichi.ac.jp

Keywords: small dense low-density lipoprotein cholesterol, small dense low-density lipoprotein cholesterol / low-density lipoprotein cholesterol ratio, age, gender, menopause

Total word count: 2745 (abstract: 248, without keywords; main text: 2497, from the introduction until the conclusion)

Number of Tables: 3
Figures: 3
Supplementary materials: 5

AUTHOR CONTRIBUTIONS

All authors have participated in the research and designed the study; TI and SI performed the statistics analysis; TI contributed to the drafting of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Objectives: Small dense low-density lipoprotein cholesterol (sdLDL-C) might be a better cardiovascular disease (CVD) indicator than low-density lipoprotein cholesterol (LDL-C); however, details regarding its epidemiology remain elusive. The present study aimed at evaluating the effect of age, gender, and menopausal status on sdLDL-C levels and sdLDL-C/LDL-C ratio in the Japanese population.

Design: This was a cross-sectional study.

Setting: 13 rural districts in Japan, 2010-2017

Participants: This study included 5,208 participants (2,397 men and 2,811 women), who underwent the health mass screening that was conducted in accordance with the medical care system for the elderly and obtained informed consent for this study.

Results: In men, the sdLDL-C levels and sdLDL-C/LDL-C ratio increased during younger adulthood, peaked at 50–54 years, and then decreased. In women, relatively regular increasing trends of sdLDL-C level and sdLDL-C/LDL-C ratio until approximately 65 years, followed by a downward or pleated trend. The crossover of sdLDL-C levels for the genders occurred at 70–74 years, but the crossover of sdLDL-C/LDL-C ratio could not be observed. Standardized sdLDL-C levels and sdLDL-C/LDL-C ratio in 50-year old men, premenopausal women, and postmenopausal women were 26.6, 22.7, and 27.4 mg/dL and 0.24, 0.15, and 0.23, respectively. The differences between premenopausal and postmenopausal women were significant (*P<0.001*).

Conclusions: SdLDL-C and sdLDL-C/LDL-C ratios showed different distributions by age, gender, and menopausal status with trends different from other lipids. A subgroup-specific approach would be necessary to implement sdLDL-C for CVD prevention strategies, fully considering age-related trends, gender differences, and menopausal status.

(248 words / within 300 words)

Strengths and limitations of this study

1. To the best of our knowledge, the present study is the first to demonstrate the effects of age, gender, and menopausal status on the sdLDL-C and sdLDL-C/LDL-C ratio.
2. This study is based on a large representative sample from Japanese general population.
3. Serum lipid markers were measured by the standardized program proposed by the Clinical and Laboratory Standards Institute.
4. It is unclear whether our results of sdLDL-C would be valid for other populations.
5. This study did not control for several confounding factors, such as diet, life activity, socioeconomic status, and genetic factors.

INTRODUCTION

Although hypercholesterolemia is one of the leading causes of cardiovascular disease (CVD), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and non-high-density lipoprotein cholesterol (nonHDL-C) have not been good enough to predict risk stratification and the novel target is needed.[1-3] Small dense low-density lipoprotein cholesterol (sdLDL-C) easily penetrates into the arterial wall, has a high susceptibility to oxidation, and may exacerbate and perpetuate atherosclerosis.[4] In fact, patients with metabolic syndrome, which have been found as highly atherogenic conditions without hypercholesterolemia, have elevated sdLDL-C.[5] Current studies suggest that the sdLDL-C or sdLDL-C/LDL-C ratio might be the better factors for the prediction of CVD than total cholesterol (TC) or LDL-C in the general population or patients with CVD.[6-9]

However, almost all of the current analytical strategies might be not able to adjust accurately the interaction between age and sdLDL-C due to the association between the lipid factors and age, which might follow a curvilinear model. Few studies have evaluated how age is associated with sdLDL-C and sdLDL-C/LDL-C ratio over a wide age range and distinguished the effects of menopause and gender on sdLDL-C and sdLDL-C fraction from those of aging.[10,11]

Diet composition, which is affected by aging, is associated with blood cholesterol and the absorption, synthesis, and metabolism per se of fat and lipoproteins change with age.[12,13] Another study showed Asian age-related trends of traditional lipid profiles displayed roughly an increasing trend, followed by a decreasing one at the middle-aged stage.[14,15] Meanwhile, sdLDL-C has been regulated by more complex mechanisms than regulating traditional lipids and might be plateaued or increased even at the middle-aged by changed metabolic functions with aging influencing sdLDL-C synthesis.[5,7,12,16,17] Furthermore, the detailed multiple mechanisms of metabolizing sdLDLs are unknown in the real-world, population-based setting and the age-related trend of sdLDL-C might be different from the sdLDL-C/LDL-C ratio. In other words, the ability to generate sdLDL-C from LDL-C might be different among each generation, gender, and menopausal status. Therefore, we evaluated the effect of age, gender, and

menopausal status on sdLDL-C and sdLDL-C/LDL-C ratio in Japanese general population.

METHODS

Population

The Jichi Medical School (JMS)-II Cohort Study is a prospective, population-based cohort study of the risk factors of atherosclerosis and CVD in the Japanese general population. A total of 6,436 individuals participated in this study. Details of the methods of enrollment have been reported previously.[18,19] In brief, from April 2010 through December 2017, this study evaluated Japanese individuals who were residents of 13 rural districts in Japan, Shimotsuke, Kakara, Sue, Omori, Kamiichi, Wara, Takasu, Onabi, Nakatsu, Yame, Miwa, Ueno, and Saji areas. Local government offices in each community issued invitations to eligible residents for the mass CVD screening, and personal invitations were also sent to all potential participants by mail. All the participants in the present study provided written informed consent prior to inclusion. The study protocol and data analysis plan were approved by the institutional review board of Jichi Medical School (Tochigi, Japan, IRB No. G09-39 [G17-64 revised]).

We excluded individuals as follows: 1) taking lipid-lowering agents or anti-hyperglycemia agents (n = 1,073); 2) the use of hormone replacement therapy (n = 96); and 3) the data such as age, gender status, menopausal status, and sdLDL-C were not available (n = 73).

Measurements

A central committee, composed of the chief medical officers of all 13 participating districts, developed a detailed manual for data collection. Body weight was recorded with the subjects clothed. Height was measured with stockinged feet. Body mass index (BMI) was calculated as weight (kg) / height (m^2). Blood samples were taken after overnight fasting. TC was measured via a cholesterol dehydrogenase-ultraviolet method. Triglycerides (TG) was measured using an enzymatic method. LDL-C and high-density lipoprotein cholesterol (HDL-C) were measured by direct methods.

SdLDL-C level was directly and selectively measured using a commercial kit (sdLDL-EX from Denka Seiken, Tokyo, Japan). An external laboratory (SRL, Tokyo, Japan) measured the serum lipid markers. The markers were measured by the standardized program proposed by the Clinical and Laboratory Standards Institute. The nonHDL-C was calculated by subtracting HDL-C from TC. Information about medical history, lifestyle, and menopausal status were obtained with a self-reported questionnaire. Smoking status was classified as smoking, former smoking, or never-smoking.

Statistical analysis

Baseline characteristics were summarized as mean ± standard deviation (SD) for normally distributed continuous variables and numbers and percentages for categorical variables. SdLDL-C and TG were highly skewed; these data were expressed as the median and interquartile range and transformed into natural logarithms before statistical analysis.

The one-way analysis of variance (ANOVA) was used for comparison among three groups, and differences were tested via post hoc pairwise comparison (Bonferroni). To explore the age-related trend in sdLDL-C and sdLDL-C/LDL-C ratio with age, geometric means or means and 95 percent confidence intervals for each variable in 5-year age ranges were derived and plotted against age range in each of the three groups.

Among the three groups, correlations between age and each parameter were assessed using multiple linear regression analysis. The agreement between the estimated sdLDL-C and sdLDL-C/LDL-C ratio and measured ones was assessed by Pearson's correlation coefficient. To evaluate the effect of menopausal status on sdLDL-C and sdLDL-C/LDL-C ratio, using the beta value of each variable from the analysis in the premenopausal and postmenopausal group, data were standardized to a nominal 50 years of menopausal age, never smoking and zero alcohol for participants with normal weight (BMI 18.5-22.0). All statistical analyses were performed using SPSS version 22 (IBM, Chicago, IL, USA), and statistical significance was defined as $P < 0.05$.

Patient and public involvement

Participants of this study or members of the public were not directly and personally involved with study design, data provision, analysis and publication of the study.

RESULTS

Baseline characteristics

After exclusions, 517 premenopausal women (mean age ± SD, 45.1 ± 4.2 years), 2,294 postmenopausal women (66.5 ± 8.8 years) and 2,397 men (64.1 ± 11.2 years) were analyzed. Demographic data for the three groups are shown in Table 1. Compared with men, premenopausal women had higher HDL-C and postmenopausal women had higher TC, LDL-C, HDL-C, and nonHDL-C. Compared with premenopausal women, postmenopausal women had higher fasting glucose, TC, LDL-C, TG, nonHDL-C, TC/LDL-C, sdLDL-C, and sdLDL-C/LDL-C. TC and LDL-C didn't differ between men and premenopausal women.

Table 1 Baseline characteristics

	Group 1 (G1) Men (n=2,397)	*Group2 (G2)* Premenopausal Women (n=517)	*Group3 (G3)* Postmenopausal Women (n=2,294)	*P* G1 vs G2	*P* G1 vs G3	*P* G2 vs G3
Age, years	64.1±11.2	45.1±4.2	66.5±8.8	<0.001	<0.001	<0.001
BMI, kg/m2	23.3±3.0	22.3±3.6	22.5±3.3	<0.001	<0.001	0.631
Smoking						
Current	600 (25.1%)	40 (7.7%)	67 (2.9%)	<0.001	<0.001	0.007
EX	1204 (50.3%)	73 (14.1%)	97 (4.2%)	<0.001	<0.001	<0.001
Drinker	1869 (78.2%)	316 (61.1%)	866 (37.8%)	<0.001	<0.001	<0.001
Glucose, mg/dL	100.7±17.8	90.9±9.4	96.3±12.3	<0.001	<0.001	<0.001
TC, mg/dL	198.7±32.9	199.2±31.2	215.4±31.6	1.000	<0.001	<0.001
LDL-C, mg/dL	115.2±29.6	114.2±28.5	126.7±28.7	1.000	<0.001	<0.001
TGs, mg/dL	100 (71, 146)	68 (50, 94)	89 (67, 123)	<0.001	<0.001	<0.001
HDL-C, mg/dL	56.3±13.8	67.8±14.7	62.8±14.9	<0.001	<0.001	<0.001
Non HDL-C, mg/dL	142.4±32.6	131.4±31.2	152.5±31.3	<0.001	<0.001	<0.001
TC/HDL-C	3.7±1.0	3.1±0.8	3.6±0.9	<0.001	<0.001	<0.001
SdLDL-C. mg/dL	34.1 (24.8, 46.5)	23.0 (16.8, 30.5)	31.2 (23.5, 41.8)	<0.001	<0.001	<0.001
SdLDL-C/LDL-C	0.32±0.14	0.22±0.08	0.29±0.12	<0.001	<0.001	<0.001

Data are expressed as mean±standard deviation (SD), %, and median (25th percentile, 75th percentile). P-values were assessed in one-way analysis of variance (ANOVA) and post hoc pairwise comparison (Bonferroni). BMI=body mass index; TC= total cholesterol; LDL-C= low-density lipoprotein cholesterol; TGs= triglycerides; HDL-C=high-density lipoprotein cholesterol; non HDL-C= non high-density lipoprotein cholesterol; sdLDL-C=small dense low-density lipoprotein cholesterol.

sdLDL-C trends in 5-year age groups

To assess the age-related trend in sdLDL-C levels, a 5-year age stratification was applied, and geometric mean sdLDL-C levels for each age groups were calculated and plotted against gender.

For men, the level of sdLDL-C increased from 34.1 mg / dL in those ＜ 39 years to a maximum of 37.7 mg / dL in those of 50-54 years, followed by decreasing from 36.4 mg / dL in those of 55-59 years to 27.4 mg / dL in those of 80 ≤ years (Figure 1). For women, a relatively regular increasing trend of the sdLDL-C level was found up to 60-64 year-olds. After 65 years, a downward trend was fitted. The maximum of the sdLDL-C level of women was 32.7 mg / dL. Moreover, sdLDL-C levels in men were higher than those in women for all age groups younger than 70-74-year-olds but exceeded those in women after the age of 75-79 years.

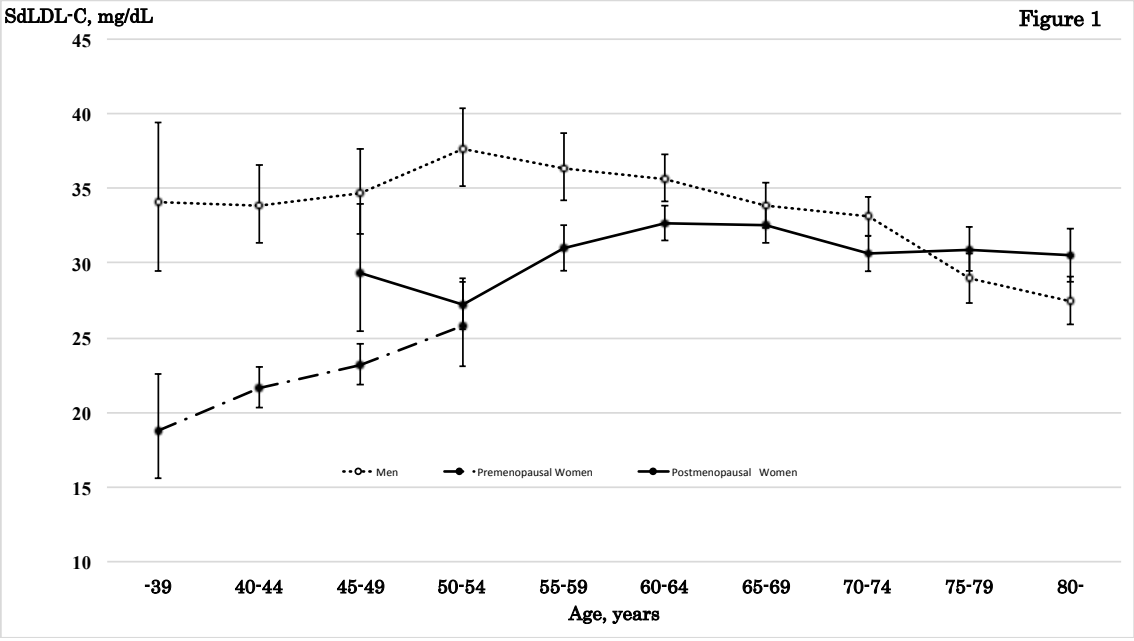
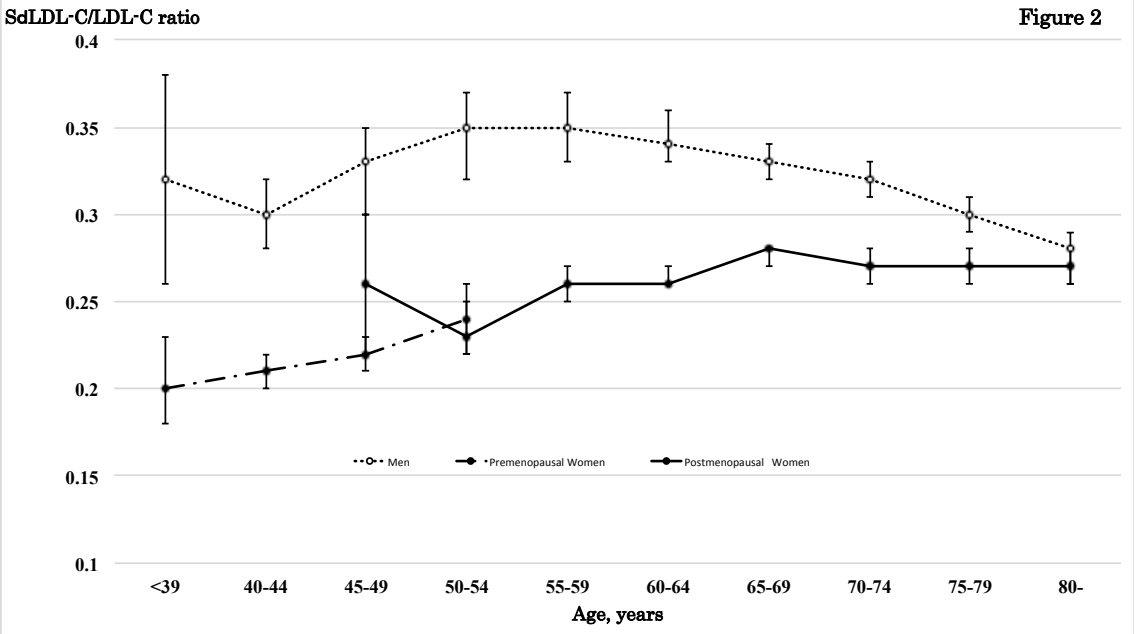


Figure 1

sdLDL-C/LDL-C ratio trends in 5-year age groups

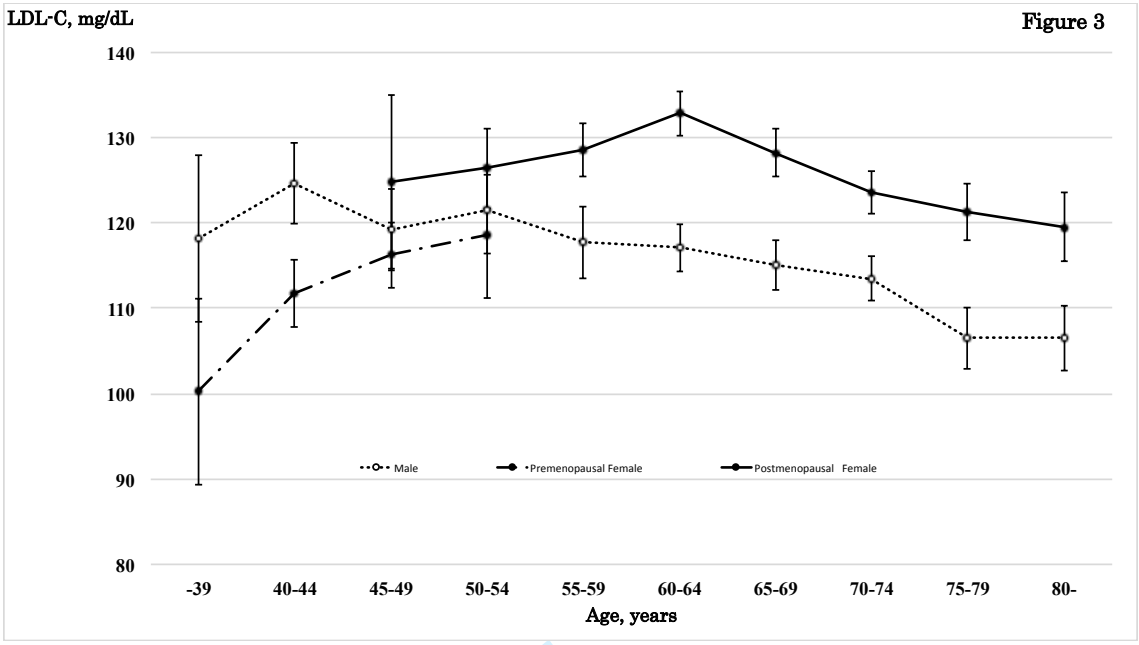
SdLDL-C/LDL-C ratio in men increased from 0.30 in 40-44-year-olds to a maximum of 0.35 in 50-54-year-olds, plateaued in those of 55-59 years, and then decreased from 0.34 in those of 60-64 years to 0.28 in those of 80 ≤ years (Figure 2). For women, these values increased from 0.20 in those < 39 years to a maximum of 0.28 in those of 65-69 years and plateaued after 70 ≤ years (with mean levels of 0.27). SdLDL-C/LDL-C ratio in men was higher than those in women for all age groups and the crossover of sdLDL-C/LDL-C ratio for the genders did not occur.



Trends in other lipoproteins (LDL-C, total cholesterol, TG, HDL-C, and total cholesterol/HDL-C ratio) in 5-year age groups

LDL-C level in men decreased almost linearly, while LDL-C level in women rapidly increased from 100.3 mg / dL in those aged < 39 years to a maximum of 132.8 mg / dL in 60-64-year-olds and decreased from 128.2 mg / dL in those aged 65-69 to 119.5 mg / dL in those 80≤ years (Figure 3). The level of TC, nonHDL-C, and TC/HDL-C ratio revealed a pattern similar to the trend of LDL-C levels (Supplementary Figure 1-3). The TG levels in men decreased almost linearly, while the level in women increased linearly (Supplementary Figure 4). HDL-C in both men and women decreased almost linearly

(Supplementary Figure 5).



SdLDL-C and sdLDL-C/LDL-C ratio in the standardized analysis among the three groups

To standardize sdLDL-C and sdLDL-C/LDL-C ratio among the three groups and validate the above-mentioned turning points, the participants were re-stratified by age ranges corresponding to increasing, plateau and decreasing phases for each marker by gender and multiple linear regression analysis was then applied.

As shown in Table 2, among men, age was positively and negatively associated with log-transformed small dense low-density lipoprotein cholesterol (LNsdLDL-C) levels in those ≤ 54 years and ≥ 55 years, with beta values of 0.006 and -0.010, respectively. Among premenopausal women, postmenopausal women ≤ 64 years, and postmenopausal women 65 ≥ years, beta values of age were 0.014, 0.014 and, -0.004, respectively. But the association between LNsdLDL-C and age was not significantly associated with men ≤ 54 years.

Table 2 Factors Associated with LN sdLDL-C Level in Age Groups by Gender

Variable	β	SE	P
Men ≤54, n=475; mean±SD, 46.7±4.9 years, Pearson's r= 0.320 (P<0.001)			
Age	0.006	0.004	0.169
BMI	0.033	0.006	<0.001
Fasting glucose	0.004	0.002	0.003
Smoker			
Current	0.018	0.054	0.747
EX	0.050	0.053	0.342
Drinker	0.144	0.059	0.015
Men ≥55, n=1,922; 68.4±7.6 years, Pearson's r= 0.316 (P<0.001)			
Age	-0.010	0.001	<0.001
BMI	0.032	0.003	<0.001
Fasting glucose	0.002	0.001	<0.001
Smoker			
Current	0.025	0.030	0.402
EX	0.032	0.024	0.192
Drinker	0.076	0.024	0.001
Women (Premenopausal), n=517; 45.1±4.2 years, Pearson's r=0.330 (P<0.001)			
Age	0.014	0.005	0.002
BMI	0.024	0.006	<0.001
Fasting glucose	0.008	0.002	<0.001
Smoker			
Current	0.021	0.072	0.775
EX	-0.005	0.056	0.934
Drinker	0.033	0.039	0.398
Women≤64 years (Postmenopausal), n=978; 58.3±4.5 years, Pearson's r=0.261 (P<0.001)			
Age	0.014	0.003	<0.001
BMI	0.019	0.004	<0.001
Fasting glucose	0.004	0.001	<0.001
Smoker			
Current	0.052	0.067	0.437

EX	0.036	0.051	0.479
Drinker	0.007	0.026	0.792
Women 65≥ years (Postmenopausal), n=1,316; 72.6±5.7 year olds, Pearson's r=0.228 (P<0.001)			
Age	-0.004	0.002	0.045
BMI	0.022	0.004	<0.001
Fasting glucose	0.003	0.001	0.001
Smoker			
Current	-0.086	0.078	0.267
EX	0.204	0.076	0.007
Drinker	-0.007	0.024	0.760

β is a coefficient indicating a one-unit increase in an independent variable in multivariable linear logic regression analyses. SE=standard error; LNsdLDL-C=log transformed small dense low-density lipoprotein cholesterol; BMI=body mass index.

As shown in Table 3, the beta values of age for sdLDL-C/LDL-C ratio in men ≤ 54 years, 55-59 years, and 60 ≥ years, were 0.003, 0.004, and -0.002, respectively. In women, the beta values of age for sdLDL-C/LDL-C ratio in premenopausal women, postmenopausal women ≤ 69 years, and 70 ≥ years were 0.001, 0.002, and 0.000, respectively. The association between sdLDL-C/LDL-C and age was not significantly associated with men 55-59 years, premenopausal women, and postmenopausal women 70 ≥ years.

Table 3 Factors Associated with SdLDL-C/LDL-C Ratio in Age Groups by Gender

Variable	***β***	**SE**	***P***
Men ≤54 years, n=475; mean±SD, 46.7±4.9 year olds, Pearson's r= 0.320 (P<0.001)			
Age	0.003	0.001	0.020
BMI	0.005	0.002	0.012
Fasting glucose	0.001	0.000	0.010
Smoker			
Current	0.029	0.016	0.081

EX	0.011	0.016	0.501
Drinker	0.049	0.018	0.007
Men 55-59 years, n=245; 57.2±1.4 years, Pearson's r= 0.222 (P<0.001)			
Age	0.004	0.007	0.589
BMI	0.003	0.003	0.385
Fasting glucose	0.001	0.001	0.285
Smoker			
Current	0.049	0.032	0.125
EX	0.062	0.030	0.042
Drinker	0.055	0.027	0.041
Men 60≥ years, n=1,677; 70.0±6.8 years, Pearson's r= 0.272 (P<0.001)			
Age	-0.002	0.000	<0.001
BMI	0.005	0.001	<0.001
Fasting glucose	0.001	0.000	<0.001
Smoker			
Current	0.029	0.009	0.001
EX	0.009	0.007	0.235
Drinker	0.055	0.007	<0.001
Women (Premenopausal), n=517; 45.1±4.2 years, Pearson's r=0.313 (P<0.001)			
Age	0.001	0.001	0.147
BMI	0.003	0.001	0.002
Fasting glucose	0.001	0.000	<0.001
Smoker			
Current	0.010	0.012	0.413
EX	0.000	0.010	0.988
Drinker	0.015	0.007	0.027
Women≤69 years (Postmenopausal), n=1,434; 61.0±5.5 years, Pearson's r=0.264 (P<0.001)			
Age	0.002	0.000	<0.001
BMI	0.004	0.001	<0.001
Fasting glucose	0.001	0.000	<0.001
Smoker			

Current	0.001	0.012	0.914
EX	0.013	0.010	0.201
Drinker	0.003	0.005	0.555
Women 70≥ years (Postmenopausal), n=860; 75.6±4.6 year olds, Pearson's r=0.167 (P<0.001)			
Age	0.000	0.001	0.704
BMI	0.004	0.001	<0.001
Fasting glucose	0.001	0.000	<0.001
Smoker			
Current	-0.049	0.025	0.052
EX	0.034	0.021	0102
Drinker	-0.004	0.006	0.501

β is a coefficient indicating a one-unit increase in an independent variable in multivariable linear logic regression analyses. SE=standard error; sdLDL-C=small dense low-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; BMI=body mass index.

Considering the beta value of each variable, 50-year old standardized sdLDL-C levels in men, premenopausal women, and postmenopausal women were 26.6 mg / dL (95 % CI; 26.4-26.9 mg / dL), 22.7 mg / dL (95 % CI; 22.5-22.9 mg / dL), and 27.4 mg / dL (95 % CI; 27.3-27.5 mg/dL), respectively. Standardized sdLDL-C/LDL-C ratio in men, premenopausal women, and postmenopausal women were 0.242 (95 % CI; 0.240-0.244), 0.154 (95 % CI; 0.153-0.156), and 0.227 (95 % CI; 0.224-0.230), respectively. These differences between premenopausal women and postmenopausal women were significant (Bonferroni analysis, $P < 0.001$).

DISCUSSION

To the best of our knowledge, the present study is the first to demonstrate the effects of age, gender, and menopausal status on the sdLDL-C and sdLDL-C/LDL-C ratio. The age-related sdLDL-C trends showed roughly an increasing phase, followed by a decreasing phase in men and a plateaued phase in middle-aged women. The age-related sdLDL-C trend in men, but not in women, was similar to traditional lipid

cholesterol profiles. The reason for this gender difference might be related to the mechanism of hypertriglyceridemia in postmenopausal women, which induced small LDL particles.[20-22] There were age or gender-related differences in the ability to generate sdLDL-C from LDL-C. This ability in men was higher than that in women for all age groups or standardized groups, which is identical to the fact that atherosclerosis is more common in men than in women, considering sdLDL-C is a highly atherogenic factor.

Our study showed three important results. First, age showed partial correlation trends with sdLDL-C levels and sdLDL-C/LDL-C ratio and non-linear trends between age and sdLDL-C and sdLDL-C/LDL-C ratio were found in both men and women. Therefore, using the sdLDL-C and sdLDL-C/LDL-C ratio, the definition of CVD risk assessment and the adaption of the lipid-lowering therapy should fully consider age-related trends and gender differences.

Second, menopausal status was an additional determinant of increasing sdLDL-C and sdLDL-C/LDL-C ratio. Many factors such as excess adiposity, free fatty acids, apo-lipoproteins, and action of lipoprotein lipase activity and cholesterol ester transfer protein affected multiple and complex mechanisms regulating sdLDL.[12,16,17] In postmenopausal women, the decrease of plasma estrogen levels plays a significant role in reducing the clearance of LDL particles via LDL receptor and increasing TG and the number of smaller LDL particles.[23] This hormone change was related to the process of regulating sdLDL-C but there was little evidence available on the association between menopausal status and sdLDL-C or sdLDL-C/LDL-C ratio in a real-world, population setting.[24] Our results showed that sdLDL-C in postmenopausal women was 0.8 or 3.9 mg / dL higher than men or premenopausal women in the standardized analysis.

Finally, the relationships between age-related trends in sdLDL-C and sdLDL-C/LDL-C ratio and gender were different from traditional lipid factors, such as LDL-C. The crossover of LDL-C for the genders occurred in middle-aged patients. On the contrary, the crossover of sdLDL-C occurred between 70-74 years and the sdLDL-C/LDL-C ratio did not occur. Rather than LDL-C, the results of the sdLDL-C and sdLDL-C/LDL-C ratio might reflect the fact that, for all age groups, men have more susceptible to CVD than

women, even with the narrowing gap of risk for CVD in postmenopausal women.[25]

Our findings suggest that a subgroup-specific approach is required to develop efficient CVD prevention strategies using the sdLDL-C and sdLDL-C/LDL-C ratio.

Limitations

Our study has several limitations. First, age-related trends and levels of traditional lipid factors were almost similar to National Health and Nutrition Survey in Japan and our age-related trends of these factors were also similar to the trends of the Korean and Chinese Singaporeans population.[14,15] But the trends of the US population or healthy Caucasian[26,27] were not similar. Especially in healthy Caucasian patients aged ≥ 70 years, the trends for TC, LDL-C, and nonHDL-C differed from our observed trends and continuously increased. Although our results could not identify the mechanism, there might be racial differences. Therefore, it is unclear whether our results of sdLDL-C would be valid for these populations. Second, compared with mean lipid levels of the Korean population from KNHANES, Japanese men showed higher mean TC, LDL-C, and HDL levels (TC 199 mg / dL; LDL-C 115 mg / dL; HDL-C 56 mg / dL) compared to Korean men (TC 183 mg / dL; LDL-C 106 mg / dL; HDL 50 mg / dL), and Japanese women also showed higher mean levels (TC 212 mg / dL; LDL-C 124 mg / dL; HDL-C 64 mg / dL) than Korean women (TC 188 mg / dL; LDL-C 111 mg / dL; HDL-C 55 mg / dL). The reason for the difference in the lipoprotein profile between Japanese and Korean populations might be due to genetics and environmental factors. It is also unknown whether these factors might affect sdLDL-C levels and sdLDL-C/LDL-C ratio because sdLDLs are regulated through complex mechanisms. Third, we did not control for the effects of diet, life activity, socioeconomic status, and genetic factors, which might be associated with changes in lipid metabolism.[28,29,30]

CONCLUSION

SdLDL-C and sdLDL-C/LDL-C ratio are differently distributed by age, gender, and menopausal status. Our findings suggest that a subgroup-specific approach is required

to develop efficient CVD prevention strategies using the sdLDL-C and sdLDL-C/LDL-C ratio.

List of abbreviations

sdLDL-C: small dense low-density lipoprotein cholesterol; CVD: cardiovascular disease; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; TGs :triglycerides; HDL-C :high-density lipoprotein cholesterol: nonHDL-C: non-high-density lipoprotein cholesterol; LNsdLDL-C: log-transformed small dense low-density lipoprotein cholesterol; JMS: Jichi Medical School; ANOVA; analysis of variance

DECLARATIONS

Acknowledgements

The authors thank the public health doctors, nurses, and officers in Shimotsuke, Kakara, Sue, Omori, Kamiichi, Wara, Takasu, Onabi, Nakatsu, Yame, Miwa, Ueno, and Saji areas, Japan, for their help, support, and contributions.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All the participants included in the present study provided written informed consent prior to inclusion, and this study was approved by the Institutional Review Board of Jichi Medical School (Tochigi, Japan, IRB No. G09-39 [G17-64 revised]).

AUTHOR CONTRIBUTIONS

All authors have participated in the research and designed the study; TI and SI performed the statistics analysis; TI contributed to the drafting of the manuscript. All authors read and approved the final manuscript.

Consent for publication

All the participants included in the present study provided written informed consent for publication.

Competing interests

The authors declare they have no conflict of interest with respect to this research study and paper.

Funding

This research was supported by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities (S0901032); a Japanese Society for the Promotion of Science KAKENHI grant (No. 16K09141); a Grant-In-Aid from the Ministry of Health, Labour, and Welfare; Health and Labor Sciences and Japan Comprehensive

Research on Cardiovascular and Lifestyle-Related Diseases grants (H26-Junkankitou-[Seisaku]-Ippan-001 and H29-Junkankitou-Ippan-003; IRB No. G09-39 [G17-64 revised]); and Jichi Medical University Almuni Association Project Grant 2020.

REFERENCES

1. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tomma- sino J, Forman DE, Goldberg R, Heidenreich PA, Hlatky MA, Jones DW, Lloyd-Jones D, Lopez-Pajares N, Ndumele CE, Orringer CE, Peralta CA, Saseen JJ, Smith SC Jr, Sperling L, Virani SS, Yeboah J. 2018AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/ NLA/PCNA Guideline on the Management of Blood Cholesterol. Circulation, 2019; 139: e1082-e1143

2. Lawler PR, Akinkuolie AO, Harada P, Glynn RJ, Chasman DI, Ridker PM, Mora S. Residual Risk of Athero- sclerotic Cardiovascular Events in Relation to Reductions in Very-Low-Density Lipoproteins. J Am Heart Assoc, 2017; 6: 1-11

3. Varbo A, Nordestgaard BG. Remnant Cholesterol and Triglyceride-Rich Lipoproteins in Atherosclerosis Progres- sion and Cardiovascular Disease. Arterioscler Thromb Vasc Biol, 2016; 36: 2133-2135

4. Ivanova EA, Myasoedova VA, Melnichenko AA, Grechko AV, Orekhov AN. Small Dense Low-Density Lipoprotein as Biomarker for Atherosclerotic Diseases. Oxidative Medicine and Cellular Longevity, 2017; 2017: 1273042

5. Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PWF, D'Agostino RB, Vasan RS, Robins SJ. Increased Small Low-Density Lipoprotein Particle Number. Circulation, 2006; 113: 20-29

6. Arai H, Kokubo Y, Watanabe M, Sawamura T, Ito Y, Minagawa A, Okamura T, Miyamato Y. Small dense low-density lipoproteins cholesterol can predict incident car- diovascular disease in an urban Japanese cohort: the Suita study. J Atheroscler Thromb, 2013; 20: 195-203

7. Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, Couper D, Virani SS, Kathiresan S, Boerwin- kle E, Ballantyne CM. Small Dense Low-Density Lipo- protein-Cholesterol Concentrations Predict Risk for Coronary Heart Disease. Arterioscler Thromb Vasc Biol, 2014; 34: 1069-1077

8. St-Pierre AC, Cantin B, Dagenais GR, Mauriège P, Ber- nard P-M, Després J-P, Lamarche B. Low-Density Lipoprotein Subfractions and the Long-Term Risk of Ischemic Heart Disease in Men. Arterioscler Thromb Vasc Biol, 2005; 25: 553-559

9. Nishikura T, Koba S, Yokota Y, Hirano T, Tsunoda F, Shoji M, Hamazaki Y,

Suzuki H, Itoh Y, Katagiri T, Kobayashi Y. Elevated small dense low-density lipoprotein cholesterol as a predictor for future cardiovascular events in patients with stable coronary artery disease. J Atheroscler Thromb. 2014;21(8):755-67.

10. Mogarekar MR, Kulkarni SK. Small Dense Low Density Lipoprotein Cholesterol, Paraoxonase 1 and Lipid Profile in Postmenopausal Women: Quality or Quantity? Arch Med Res. 2015 Oct;46(7):534-8.

11. Gentile M, Panico S, Mattiello A, Ubaldi S, Iannuzzo G, De Michele M, Iannuzzi A, Rubba P. Association between small dense LDL and early atherosclerosis in a sample of menopausal women. Clin Chim Acta. 2013;426:1-5.

12. Johnson AA, Stolzing A. The role of lipid metabolism in aging, lifespan regulation, and age-related disease. Aging Cell. 2019;18(6):e13048.

13. Toth MJ, Tchernof A. Lipid metabolism in the elderly. Eur J Clin Nutr. 2000 ;54 Suppl 3:S121-5.

14. Park JH, Lee MH, Shim JS, Choi DP, Song BM, Lee SW, Choi H, Kim HC. Effects of age, sex, and menopausal status on blood cholesterol profile in the korean population. Korean Circ J. 2015 Mar;45(2):141-8.

15. Goh VH, Tong TY, Mok HP, Said B. Differential impact of aging and gender on lipid and lipoprotein profiles in a cohort of healthy Chinese Singaporeans. Asian J Androl. 2007;9(6):787-94.

16. Qamar A, Khetarpal SA, Khera AV, Qasim A, Rader DJ, Reilly MP. Plasma apolipoprotein C-III levels, triglycer- ides, and coronary artery calcification in type 2 diabetics. Arterioscler Thromb Vasc Biol, 2015; 35: 1880-1888

17. Pechlaner R, Tsimikas S, Yin X, Willeit P, Baig F, Santer P, Oberhollenzer F, Egger G, Witztum JL, Alexander VJ, Willeit J, Kiechl S, Mayr M. Very-Low-Density Lipopro- tein-Associated Apolipoproteins Predict Cardiovascular Events and Are Lowered by Inhibition of APOC-III. Journal of the American College of Cardiology, 2017; 69: 789-800

18. Ishikawa S, Gotoh T, Nago N, Kayaba K; Jichi Medical School (JMS) Cohort Study Group.The Jichi Medical School (JMS) Cohort Study: design, baseline data and standardized mortality ratios. J Epidemiol. 2002;12:408-17.

19. Izumida T, Nakamura Y, Hino Y, Ishikawa S. Combined Effect of Small Dense Low-Density Lipoprotein Cholesterol (sdLDL-C) and Remnant-Like

Particle Cholesterol (RLP-C) on Low-Grade Inflammation. J Atheroscler Thromb. 2020;27:319-330.

20. Cho Y, Lee SG, Jee SH, Kim JH. Hypertriglyceridemia is a major factor associated with elevated levels of small dense LDL cholesterol in patients with metabolic syndrome. Ann Lab Med. 2015;35(6):586-94.

21. Hayashi T, Koba S, Ito Y, Hirano T. Method for estimating high sdLDL-C by measuring triglyceride and apolipoprotein B levels. Lipids Health Dis. 2017 Jan 26;16(1):21.

22. Dayspring TD. Understanding hypertriglyceridemia in women: clinical impact and management with prescription omega-3-acid ethyl esters. Int J Womens Health. 2011 Mar 9;3:87-97.

23. Campos H, Walsh BW, Judge H, Sacks FM. Effect of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. J Clin Endocrinol Metab. 1997 Dec;82(12):3955-63.

24. Carr MC, Kim KH, Zambon A, Mitchell ES, Woods NF, Casazza CP, Purnell JQ, Hokanson JE, Brunzell JD, Schwartz RS. Changes in LDL density across the menopausal transition. J Investig Med. 2000 Jul;48(4):245-50.

25. Bhatnagar P, Wickramasinghe K, Williams J, Rayner M, Townsend N.The epidemiology of cardiovascular disease in the UK 2014. Heart.;101(15):1182-9.

26. Anagnostis P, Stevenson JC, Crook D, Johnston DG, Godsland IF. Effects of menopause, gender and age on lipids and high-density lipoprotein cholesterol subfractions. Maturitas. 2015;81(1):62-8.

27. Schaefer EJ, Lamon-Fava S, Cohn SD, Schaefer MM, Ordovas JM, Castelli WP, Wilson PW.Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. J Lipid Res.;35(5):779-92.

28. Tenk J, Mátrai P, Hegyi P, et al. Perceived stress correlates with visceral obesity and lipid parameters of the metabolic syndrome: a systematic review and meta-analysis. Psychoneuroendocrinology 2018;95:63–73.

29. Slopen N, Goodman E, Koenen KC, Kubzansky LD. Socioeconomic and other social stressors and biomarkers of cardiometabolic risk in youth: a systematic review of less studied risk factors. PLoS One. 2013;8(5):e64418.

30. Garcia CK, Mues G, Liao Y, Hyatt T, Patil N, Cohen JC, Hobbs HH.

505 Sequence diversity in genes of lipid metabolism. Genome Res. 2001
506 Jun;11(6):1043-52.
507

FIGURE LEGENDS

Figure 1. Geometric mean and 95% confidence interval of sdLDL-C for age, gender, and menopausal status

Figure 2. Mean and 95% confidence interval of sdLDL-C/LDL-C ratio for age, gender, and menopausal status

Figure 3. Mean and 95% confidence interval of LDL-C for age, gender, and menopausal status

Supplementary Material

Supplementary Figure 1. Mean of total cholesterol for age, gender, and menopausal status

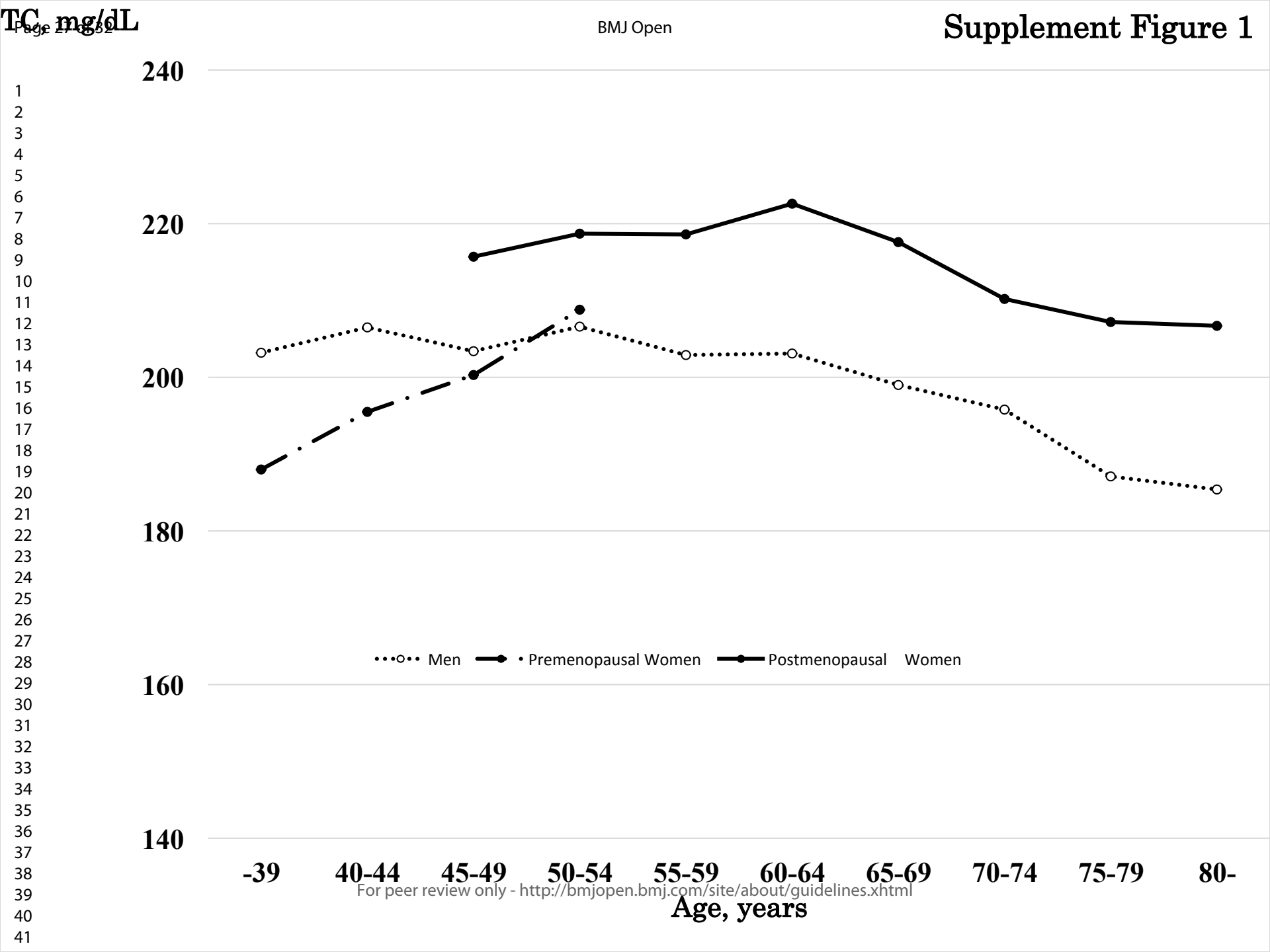
Supplementary Figure 2. Mean of non-high-density lipoprotein cholesterol for age, gender, and menopausal status

Supplementary Figure 3. Mean of total cholesterol / high-density lipoprotein cholesterol ratio for age, gender, and menopausal status

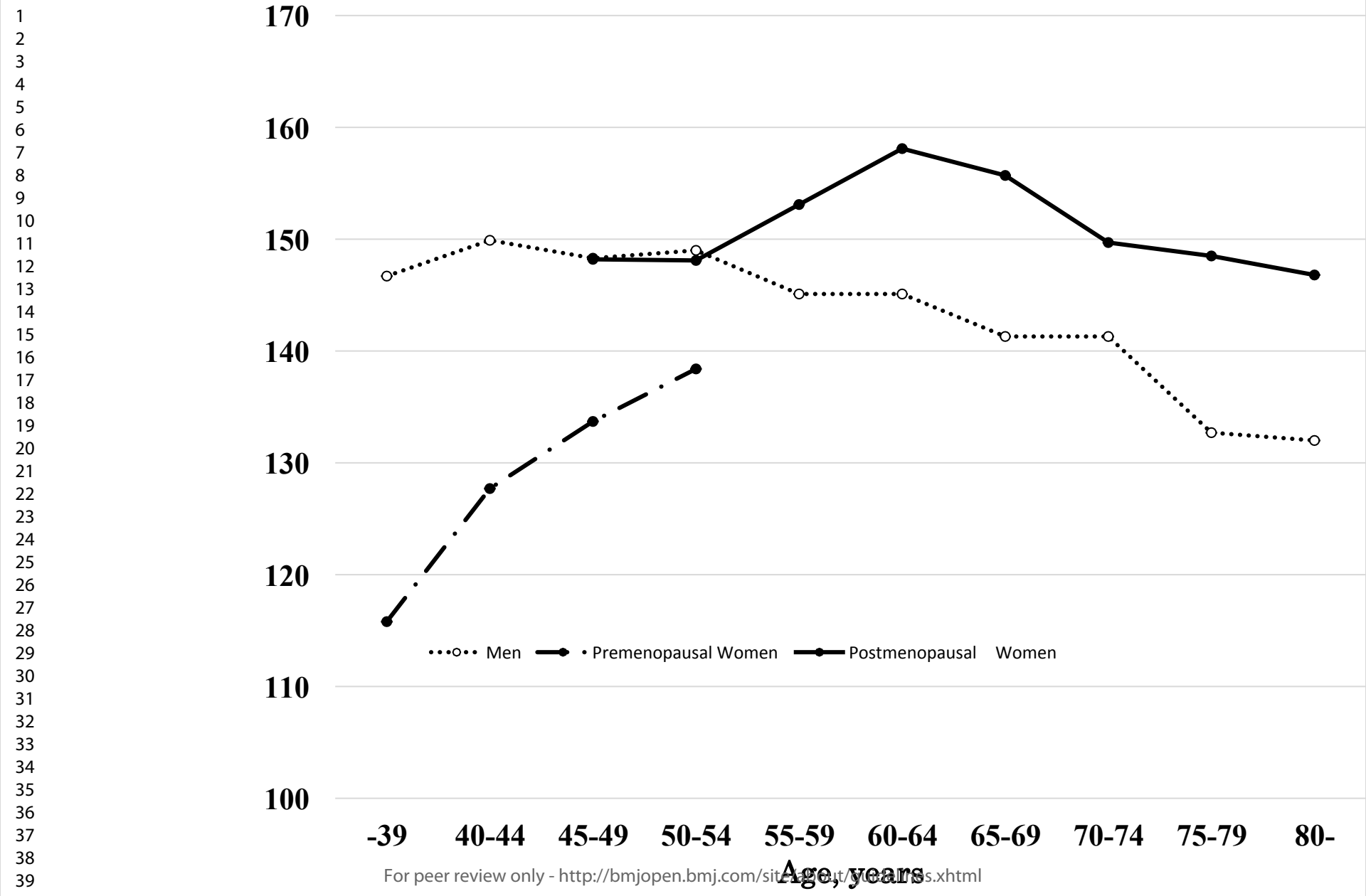
Supplementary Figure 4. Geometric mean of triglycerides for age, gender, and menopausal status

Supplementary Figure 5. Mean of high-density lipoprotein cholesterol for age, gender, and menopausal status

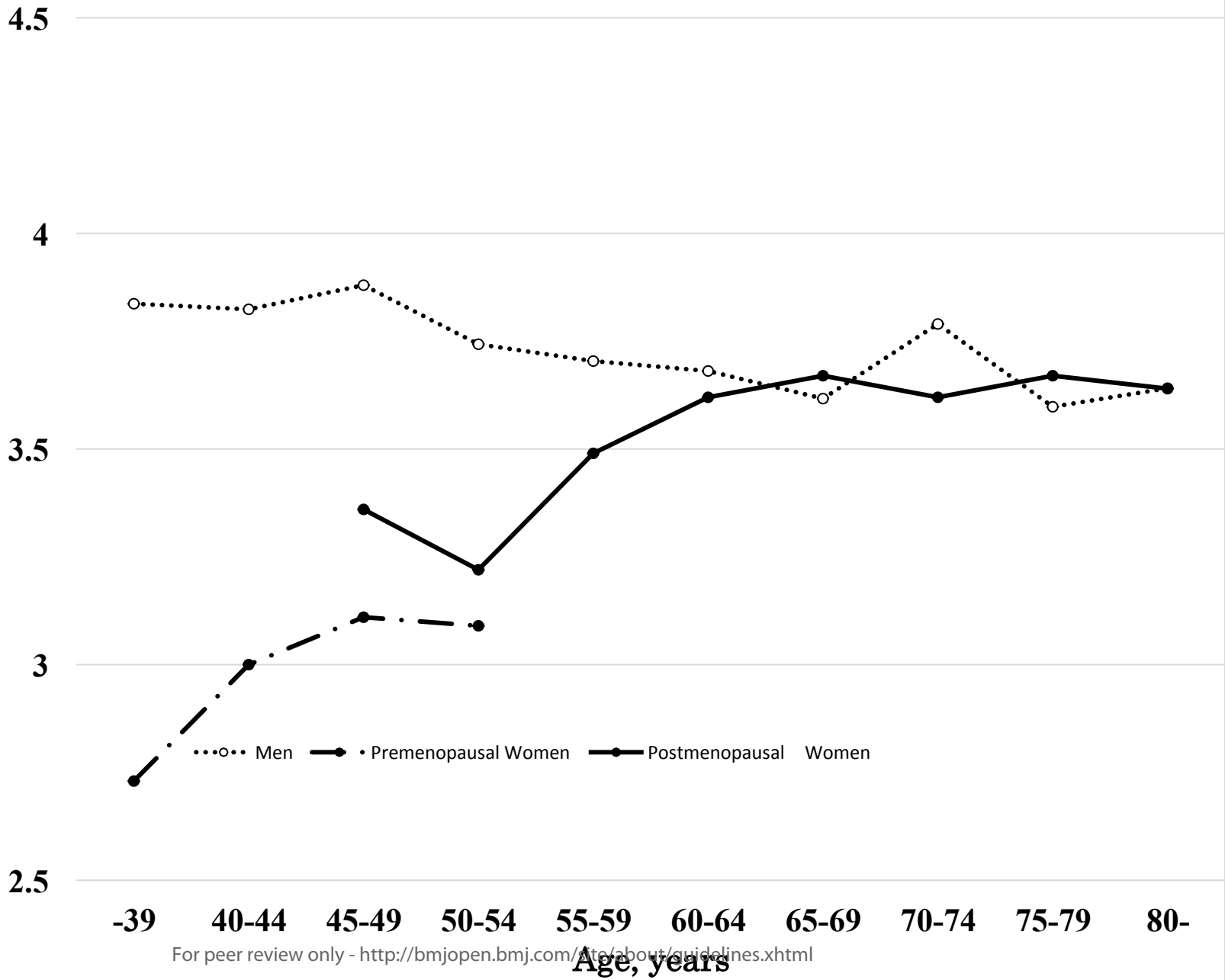
Supplement Figure 1



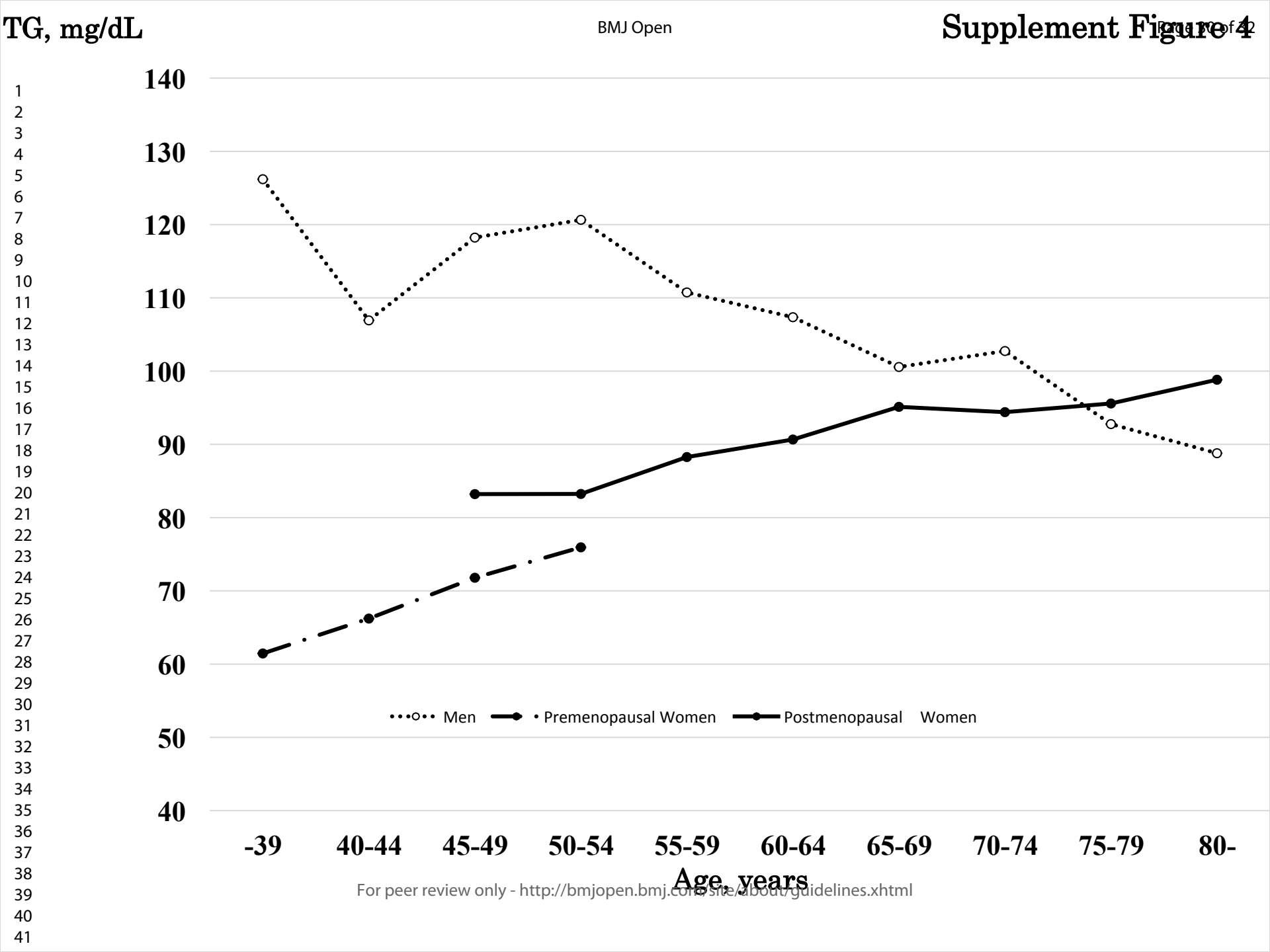
Supplement Figure 2



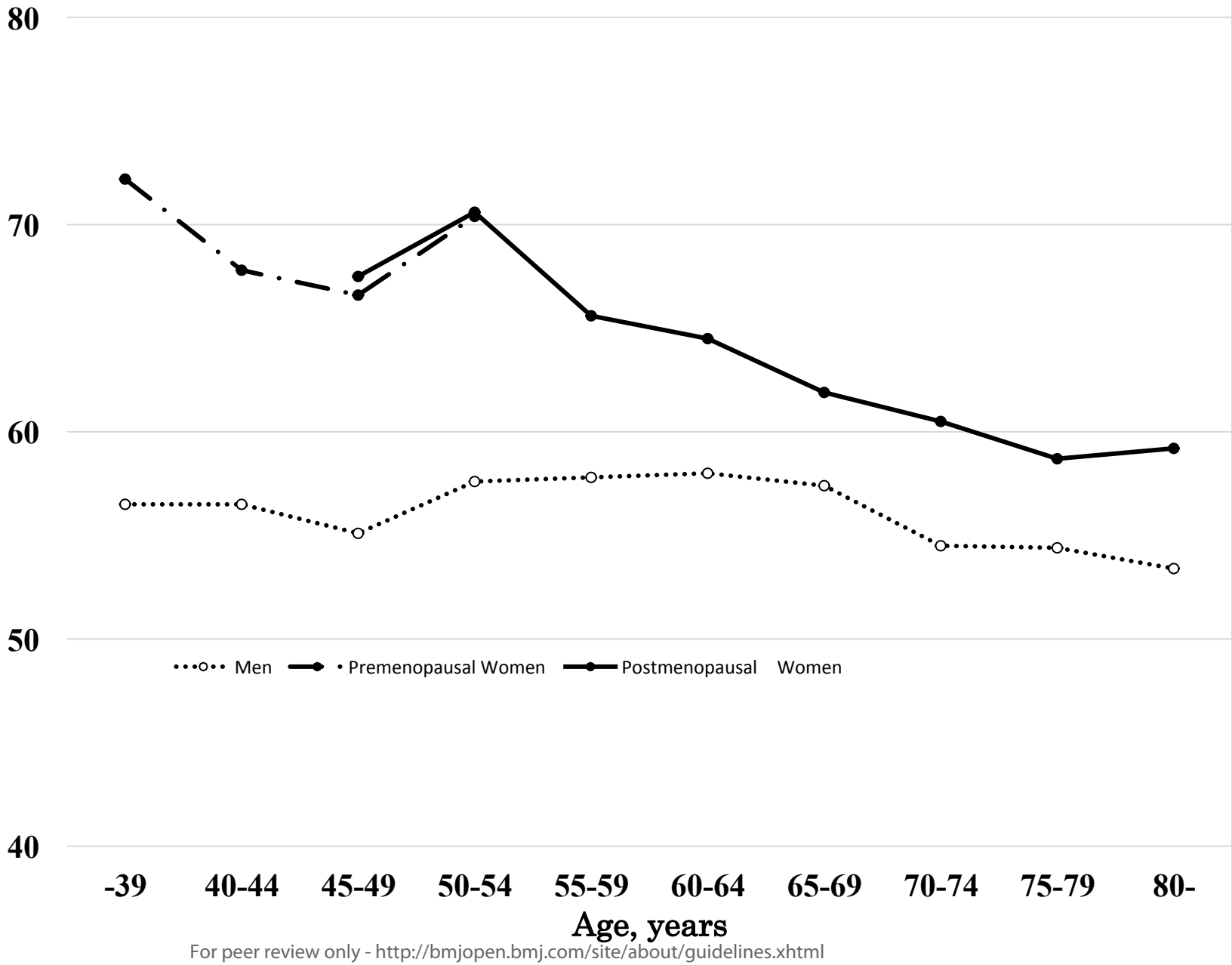
Supplement Figure 3



Supplement Figure 4



Supplement Figure 5



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	1, 3, 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6, 7*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how loss to follow-up was addressed	-
		(*e*) Describe any sensitivity analyses	-
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6, 8
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-10
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	Report numbers of outcome events or summary measures over time	-
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-16
		(*b*) Report category boundaries when continuous variables were categorized	8-16
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-16
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-16
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20-21

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The Association between Age, Gender, Menopausal Status, and Small Dense Low-Density Lipoprotein Cholesterol; A Cross-Sectional Study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-041613.R1
Article Type:	Original research
Date Submitted by the Author:	02-Sep-2020
Complete List of Authors:	Izumida, Toshihide; Kanazawa Medical University Himi Municipal Hospital; Nakamura, Yosikazu; Jichi Medical University, Public Health Sato, Yukihiro; Kamiichi General Hospital, Internal Medicine Ishikawa, Shizukiyo; Jichi Medical University, Division of Public Health, Center for Community Medicine
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Public health, Global health, Epidemiology, Cardiovascular medicine
Keywords:	CARDIOLOGY, Heart failure < CARDIOLOGY, Ischaemic heart disease < CARDIOLOGY, INTERNAL MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

[TITLE] The Association between Age, Gender, Menopausal Status, and Small Dense Low-Density Lipoprotein Cholesterol; A Cross-Sectional Study

Authors: Toshihide Izumida[1], Yosikazu Nakamura[2], Yukihiro Sato[3], Shizukiyo Ishikawa[2]

[1] Division of Community Medicine, Kanazawa Medical University Himi Municipal Hospital, Himi, Toyama, Japan
1130 Kurakawa, Himi, Toyama 935-0025, Japan

[2] Division of Public Health, Center for Community Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan
3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan

[3] Internal Medicine, Kamiichi General Hospital, Nakaniikawa-gun, Toyama, Japan
51 Hoonji, Kamiichi, Nakaniikawa-gun, Toyama 930-0391, Japan

Corresponding Author: Shizukiyo Ishikawa
Division of Public Health, Center for Community Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan
E-mail: i-shizu@jichi.ac.jp

E-mail address: Toshihide Izumida, m07011ti@jichi.ac.jp; Yosikazu Nakamura, nakamuyk@jichi.ac.jp; Yukihiro Sato, yukisato@fancy.ocn.ne.jp; Shizukiyo Ishikawa, i-shizu@jichi.ac.jp

Keywords: small dense low-density lipoprotein cholesterol, small dense low-density lipoprotein cholesterol / low-density lipoprotein cholesterol ratio, age, gender, menopause

Total word count: 3024 (abstract: 279, without keywords; main text: 2745, from the introduction until the conclusion)

Number of Tables: 3
Figures: 3
Supplementary materials: 7

AUTHOR CONTRIBUTIONS

TI, YN, YS, and SI have participated in the research and designed the study; TI and SI performed the statistics analysis; TI contributed to the drafting of the manuscript. YN, YS, and SI provided feedback on the manuscript, and all authors read and approved the final manuscript.

ABSTRACT

Objectives: Small dense low-density lipoprotein cholesterol (sdLDL-C) might be a better cardiovascular disease (CVD) indicator than low-density lipoprotein cholesterol (LDL-C); however, details regarding its epidemiology remain elusive. The present study aimed at evaluating the association between the demographic factors, such as age, gender, and menopausal status, and sdLDL-C levels and sdLDL-C/LDL-C ratio in the Japanese population.

Design: This was a cross-sectional study.

Setting: 13 rural districts in Japan, 2010-2017

Participants: This study included 5,208 participants (2,397 men and 2,811 women), who underwent the health mass screening that was conducted in accordance with the medical care system for the elderly and obtained informed consent for this study.

Results: In total, 517 premenopausal women (mean age ± SD, 45.1 ± 4.2 years), 2,294 postmenopausal women (66.5 ± 8.8 years) and 2,397 men (64.1 ± 11.2 years) were analyzed. In men, the sdLDL-C levels and sdLDL-C/LDL-C ratio increased during younger adulthood, peaked (36.4 mg/dL, 0.35) at 50–54 years, and then decreased. In women, relatively regular increasing trends of sdLDL-C level and sdLDL-C/LDL-C ratio until approximately 65 years (32.7 mg/dL, 0.28), followed by a downward or pleated trend. Given the beta value of age, body mass index, fasting glucose, and smoking and drinking status by multiple linear regression analysis, standardized sdLDL-C levels and sdLDL-C/LDL-C ratio in 50-year old men, premenopausal women, and postmenopausal women were 26.6, 22.7, and 27.4 mg/dL and 0.24, 0.15, and 0.23, respectively. The differences between premenopausal and postmenopausal women were significant (*P<0.001*).

Conclusions: SdLDL-C and sdLDL-C/LDL-C ratios showed different distributions by age, gender, and menopausal status. A subgroup-specific approach would be necessary to implement sdLDL-C for CVD prevention strategies, fully considering age-related trends, gender differences, and menopausal status.

76 (279 words / within 300 words)

Strengths and limitations of this study

1. To the best of our knowledge, the present study is the first to demonstrate the association between age, gender, and menopausal status on the sdLDL-C and sdLDL-C/LDL-C ratio.
2. This study is based on a large representative sample from Japanese general population.
3. Serum lipid markers were measured by the standardized program proposed by the Clinical and Laboratory Standards Institute.
4. It is unclear whether our results of sdLDL-C would be valid for other populations.
5. This study did not control for several confounding factors, such as diet, life activity, socioeconomic status, and genetic factors.

INTRODUCTION

Although hypercholesterolemia is one of the leading causes of cardiovascular disease (CVD), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and non-high-density lipoprotein cholesterol (nonHDL-C) have not been good enough to predict risk stratification and the novel target is needed.[1-3] Small dense low-density lipoprotein cholesterol (sdLDL-C) easily penetrates into the arterial wall, has a high susceptibility to oxidation, and may exacerbate and perpetuate atherosclerosis.[4] In fact, patients with metabolic syndrome, which have been found as highly atherogenic conditions without hypercholesterolemia, have elevated sdLDL-C.[5] The sdLDL-C/LDL-C ratio, reflecting the ability to generate sdLDL-C from LDL-C, might increase by the high activity of hepatic lipase, which was associated with higher risk of CVD. Current studies suggest that the sdLDL-C or sdLDL-C/LDL-C ratio might be the better factors for the prediction of CVD than total cholesterol (TC) or LDL-C in the general population or patients with CVD.[6-9]

However, almost all of the current analytical strategies might be not able to adjust accurately the interaction between age and sdLDL-C. Few studies have evaluated how age is associated with sdLDL-C and sdLDL-C/LDL-C ratio over a wide age range and distinguished the effects of menopause and gender on sdLDL-C and sdLDL-C fraction from those of aging.[10,11]

Diet composition, which is affected by aging, is associated with blood cholesterol and the absorption, synthesis, and metabolism per se of fat and lipoproteins change with age.[12,13] Another study showed Asian age-related trends of traditional lipid profiles displayed roughly an increasing trend, followed by a decreasing one at the middle-aged stage.[14,15] Meanwhile, sdLDL-C has been regulated by more complex mechanisms than regulating traditional lipids and might be plateaued or increased even at the middle-aged by changed metabolic functions with aging influencing sdLDL-C synthesis.[5,7,12,16,17] Furthermore, the detailed multiple mechanisms of metabolizing sdLDLs are unknown in the real-world, population-based setting and the age-related trend of sdLDL-C might be different from the sdLDL-C/LDL-C ratio. In other words, the ability to generate sdLDL-C from LDL-C might be different among each generation,

gender, and menopausal status. Therefore, we evaluated the association between the demographic factors, such as age, gender, and menopausal status, and sdLDL-C and sdLDL-C/LDL-C ratio in Japanese general population.

METHODS

Population

The present cross-sectional study was conducted as part of the Jichi Medical School (JMS)-II Cohort Study, a population-based cohort study of the risk factors of atherosclerosis and CVD in the Japanese general population. A total of 6,436 individuals participated in this study. Details of the methods of enrollment have been reported previously.[18,19] In brief, from April 2010 through December 2017, this study evaluated Japanese individuals who were residents of 13 rural districts in Japan, Shimotsuke, Kakara, Sue, Omori, Kamiichi, Wara, Takasu, Onabi, Nakatsu, Yame, Miwa, Ueno, and Saji areas. Local government offices in each community issued invitations to eligible residents for the mass CVD screening, and personal invitations were also sent to all potential participants by mail. All the participants in the present study provided written informed consent prior to inclusion. The study protocol and data analysis plan were approved by the institutional review board of Jichi Medical School (Tochigi, Japan, IRB No. G09-39 [G17-64 revised]).

We excluded individuals as follows: 1) taking lipid-lowering agents or anti-hyperglycemia agents (n = 1,073); 2) the use of hormone replacement therapy (n = 96); and 3) the data such as age, gender status, menopausal status, and sdLDL-C were not available (n = 73).

Measurements

A central committee, composed of the chief medical officers of all 13 participating districts, developed a detailed manual for data collection. Body weight was recorded with the subjects clothed. Height was measured with stockinged feet. Body mass index (BMI) was calculated as weight (kg) / height (m^2). Blood samples were taken after overnight fasting. TC was measured via a cholesterol dehydrogenase-ultraviolet

method. Triglycerides (TG) was measured using an enzymatic method. LDL-C and high-density lipoprotein cholesterol (HDL-C) were measured by direct methods using a commercial kit (Cholestest from Sekisui Medical, Tokyo, Japan). SdLDL-C level was directly and selectively measured using a commercial kit (sdLDL-EX from Denka Seiken, Tokyo, Japan). An external laboratory (SRL, Tokyo, Japan) measured the serum lipid markers. The markers were measured by the standardized program proposed by the Clinical and Laboratory Standards Institute. The nonHDL-C was calculated by subtracting HDL-C from TC. Information about medical history, lifestyle, and menopausal status were obtained with a self-reported questionnaire. Smoking status was classified as smoking, former smoking, or never-smoking.

Statistical analysis

Baseline characteristics were summarized as mean ± standard deviation (SD) for normally distributed continuous variables and numbers and percentages for categorical variables. SdLDL-C and TG were highly skewed; these data were expressed as the median and interquartile range and transformed into natural logarithms before statistical analysis. The participants were divided into three groups (men, premenopausal women, and postmenopausal women) according to gender and menopausal status.

The one-way analysis of variance (ANOVA) was used for comparison among three groups, and differences were tested via post hoc pairwise comparison (Bonferroni). To explore the age-related trend in sdLDL-C and sdLDL-C/LDL-C ratio with age, geometric means or means and 95 percent confidence intervals for each variable in 5-year age ranges were derived and plotted against age range in each of the three groups.

Among the three groups, correlations between age and each parameter were assessed using multiple linear regression analysis. Considering the beta value of age, body mass index, fasting glucose, and smoking and drinking status, we calculated the estimated sdLDL-C and sdLDL-C/LDL-C ratio. The agreement between the estimated sdLDL-C and sdLDL-C/LDL-C ratio and measured ones was assessed by Pearson's correlation coefficient. To evaluate the effect of menopausal status on sdLDL-C and sdLDL-C/LDL-C ratio, using the beta value of each variable from the analysis in the premenopausal and postmenopausal group, data were standardized to a nominal 50 years of menopausal age, never smoking and zero alcohol for participants with normal

weight (BMI 18.5-22.0). All statistical analyses were performed using SPSS version 22 (IBM, Chicago, IL, USA), and statistical significance was defined as $P < 0.05$.

Patient and public involvement

Participants of this study or members of the public were not directly and personally involved with study design, data provision, analysis and publication of the study.

RESULTS

Baseline characteristics

After exclusions, 517 premenopausal women (mean age ± SD, 45.1 ± 4.2 years), 2,294 postmenopausal women (66.5 ± 8.8 years) and 2,397 men (64.1 ± 11.2 years) were analyzed. Demographic data for the three groups are shown in Table 1. Compared with men, premenopausal women had higher HDL-C and postmenopausal women had higher TC, LDL-C, HDL-C, and nonHDL-C. Compared with premenopausal women, postmenopausal women had higher fasting glucose, TC, LDL-C, TG, nonHDL-C, TC/LDL-C, sdLDL-C, and sdLDL-C/LDL-C. TC and LDL-C didn't differ between men and premenopausal women.

Table 1 Baseline characteristics

	Group 1 (G1) Men (n=2,397)	*Group2 (G2)* Premenopausal Women (n=517)	*Group3 (G3)* Postmenopausal Women (n=2,294)	*P G1 vs G2*	*P G1 vs G3*	*P G2 vs G3*
Age, years	64.1±11.2	45.1±4.2	66.5±8.8	<0.001	<0.001	<0.001
BMI, kg/m2	23.3±3.0	22.3±3.6	22.5±3.3	<0.001	<0.001	0.631
Smoking						

Current	600 (25.1%)	40 (7.7%)	67 (2.9%)	<0.001	<0.001	0.007
EX	1204 (50.3%)	73 (14.1%)	97 (4.2%)	<0.001	<0.001	<0.001
Drinker	1869 (78.2%)	316 (61.1%)	866 (37.8%)	<0.001	<0.001	<0.001
Glucose, mg/dL	100.7±17.8	90.9±9.4	96.3±12.3	<0.001	<0.001	<0.001
TC, mg/dL	198.7±32.9	199.2±31.2	215.4±31.6	1.000	<0.001	<0.001
LDL-C, mg/dL	115.2±29.6	114.2±28.5	126.7±28.7	1.000	<0.001	<0.001
TGs, mg/dL	100 (71, 146)	68 (50, 94)	89 (67, 123)	<0.001	<0.001	<0.001
HDL-C, mg/dL	56.3±13.8	67.8±14.7	62.8±14.9	<0.001	<0.001	<0.001
Non HDL-C, mg/dL	142.4±32.6	131.4±31.2	152.5±31.3	<0.001	<0.001	<0.001
TC/HDL-C	3.7±1.0	3.1±0.8	3.6±0.9	<0.001	<0.001	<0.001
SdLDL-C. mg/dL	34.1 (24.8, 46.5)	23.0 (16.8, 30.5)	31.2 (23.5, 41.8)	<0.001	<0.001	<0.001
SdLDL-C/LDL-C	0.32±0.14	0.22±0.08	0.29±0.12	<0.001	<0.001	<0.001

Data are expressed as mean±standard deviation (SD), %, and median (25th percentile, 75th percentile). P-values were assessed in one-way analysis of variance (ANOVA) and post hoc pairwise comparison (Bonferroni). BMI=body mass index; TC= total cholesterol; LDL-C= low-density lipoprotein cholesterol; TGs= triglycerides; HDL-C=high-density lipoprotein cholesterol; non HDL-C= non high-density lipoprotein cholesterol; sdLDL-C=small dense low-density lipoprotein cholesterol.

sdLDL-C trends in 5-year age groups

To assess the age-related trend in sdLDL-C levels, a 5-year age stratification was applied, and geometric mean sdLDL-C levels for each age groups were calculated and

plotted against gender.

For men, the level of sdLDL-C increased from 34.1 mg / dL in those < 39 years to a maximum of 37.7 mg / dL in those of 50-54 years, followed by decreasing from 36.4 mg / dL in those of 55-59 years to 27.4 mg / dL in those of 80 ≤ years (Figure 1). For women, a relatively regular increasing trend of the sdLDL-C level was found up to 60-64 year-olds. After 65 years, a downward trend was fitted. The maximum of the sdLDL-C level of women was 32.7 mg / dL. Moreover, sdLDL-C levels in men were higher than those in women for all age groups younger than 70-74-year-olds but exceeded those in women after the age of 75-79 years.

sdLDL-C/LDL-C ratio trends in 5-year age groups

SdLDL-C/LDL-C ratio in men increased from 0.30 in 40-44-year-olds to a maximum of 0.35 in 50-54-year-olds, plateaued in those of 55-59 years, and then decreased from 0.34 in those of 60-64 years to 0.28 in those of 80 ≤ years (Figure 2). For women, these values increased from 0.20 in those < 39 years to a maximum of 0.28 in those of 65-69 years and plateaued after 70 ≤ years (with mean levels of 0.27). SdLDL-C/LDL-C ratio in men was higher than those in women for all age groups and the crossover of sdLDL-C/LDL-C ratio for the genders did not occur.

Trends in other lipoproteins (LDL-C, total cholesterol, TG, HDL-C, and total cholesterol/HDL-C ratio) in 5-year age groups

LDL-C level in men decreased almost linearly, while LDL-C level in women rapidly increased from 100.3 mg / dL in those aged < 39 years to a maximum of 132.8 mg / dL in 60-64-year-olds and decreased from 128.2 mg / dL in those aged 65-69 to 119.5 mg / dL in those 80≤ years (Figure 3). The level of TC, nonHDL-C, and TC/HDL-C ratio revealed a pattern similar to the trend of LDL-C levels (Supplementary Figure 1-3). The TG levels in men decreased almost linearly, while the level in women increased linearly (Supplementary Figure 4). HDL-C in both men and women decreased almost linearly (Supplementary Figure 5).

SdLDL-C and sdLDL-C/LDL-C ratio in the standardized analysis among the three groups

To standardize sdLDL-C and sdLDL-C/LDL-C ratio among the three groups and validate the above-mentioned turning points, the participants were re-stratified by age ranges corresponding to increasing, plateau and decreasing phases for each marker by gender and multiple linear regression analysis was then applied.

As shown in Table 2, among men, age was positively and negatively associated with log-transformed small dense low-density lipoprotein cholesterol (LNsdLDL-C) levels in those ≤ 54 years and ≥ 55 years. Among premenopausal women, postmenopausal women ≤ 64 years, and postmenopausal women 65 ≥ years, age was positively, positively, and negatively associated with LNsdLDL-C levels. But the association between LNsdLDL-C and age was not significantly associated with men ≤ 54 years.

Table 2 Factors Associated with LN sdLDL-C Level in Age Groups by Gender

Variable	*β*	SE	*P*
Men ≤54, n=475; mean±SD, 46.7±4.9 years, Pearson's r= 0.320 (P<0.001)			
Age	0.006	0.004	0.169
BMI	0.033	0.006	<0.001
Fasting glucose	0.004	0.002	0.003
Smoker			
Current	0.018	0.054	0.747
EX	0.050	0.053	0.342
Drinker	0.144	0.059	0.015
Men ≥55, n=1,922; 68.4±7.6 years, Pearson's r= 0.316 (P<0.001)			
Age	-0.010	0.001	<0.001
BMI	0.032	0.003	<0.001
Fasting glucose	0.002	0.001	<0.001
Smoker			

Current	0.025	0.030	0.402
EX	0.032	0.024	0.192
Drinker	0.076	0.024	0.001
Women (Premenopausal), n=517; 45.1±4.2 years, Pearson's r=0.330 (P<0.001)			
Age	0.014	0.005	0.002
BMI	0.024	0.006	<0.001
Fasting glucose	0.008	0.002	<0.001
Smoker			
Current	0.021	0.072	0.775
EX	-0.005	0.056	0.934
Drinker	0.033	0.039	0.398
Women≤64 years (Postmenopausal), n=978; 58.3±4.5 years, Pearson's r=0.261 (P<0.001)			
Age	0.014	0.003	<0.001
BMI	0.019	0.004	<0.001
Fasting glucose	0.004	0.001	<0.001
Smoker			
Current	0.052	0.067	0.437
EX	0.036	0.051	0.479
Drinker	0.007	0.026	0.792
Women 65≥ years (Postmenopausal), n=1,316; 72.6±5.7 year olds, Pearson's r=0.228 (P<0.001)			
Age	-0.004	0.002	0.045
BMI	0.022	0.004	<0.001
Fasting glucose	0.003	0.001	0.001
Smoker			
Current	-0.086	0.078	0.267
EX	0.204	0.076	0.007
Drinker	-0.007	0.024	0.760

β is a coefficient indicating a one-unit increase in an independent variable in multivariable linear logic regression analyses. SE=standard error; LNsdLDL-C=log transformed small dense low-density lipoprotein cholesterol; BMI=body mass index.

As shown in Table 3, age in men ≤ 54 years, 55-59 years, and 60 ≥ years, was positively, positively, and negatively associated with sdLDL-C/LDL-C ratio. In women, age in premenopausal women, postmenopausal women ≤ 69 years was positively associated with sdLDL-C/LDL-C ratio, whereas age in postmenopausal women 70 ≥ years was not significantly associated sdLDL-C/LDL-C ratio. The association between sdLDL-C/LDL-C and age was not significantly associated with men 55-59 years, premenopausal women, and postmenopausal women 70 ≥ years.

Table 3 Factors Associated with SdLDL-C/LDL-C Ratio in Age Groups by Gender

Variable	β	SE	P
Men ≤54 years, n=475; mean±SD, 46.7±4.9 year olds, Pearson's r= 0.320 (P<0.001)			
Age	0.003	0.001	0.020
BMI	0.005	0.002	0.012
Fasting glucose	0.001	0.000	0.010
Smoker			
Current	0.029	0.016	0.081
EX	0.011	0.016	0.501
Drinker	0.049	0.018	0.007
Men 55-59 years, n=245; 57.2±1.4 years, Pearson's r= 0.222 (P<0.001)			
Age	0.004	0.007	0.589
BMI	0.003	0.003	0.385
Fasting glucose	0.001	0.001	0.285
Smoker			
Current	0.049	0.032	0.125
EX	0.062	0.030	0.042
Drinker	0.055	0.027	0.041
Men 60≥ years, n=1,677; 70.0±6.8 years, Pearson's r= 0.272 (P<0.001)			
Age	-0.002	0.000	<0.001
BMI	0.005	0.001	<0.001
Fasting glucose	0.001	0.000	<0.001
Smoker			

Current	0.029	0.009	0.001
EX	0.009	0.007	0.235
Drinker	0.055	0.007	<0.001
Women (Premenopausal), n=517; 45.1±4.2 years, Pearson's r=0.313 (P<0.001)			
Age	0.001	0.001	0.147
BMI	0.003	0.001	0.002
Fasting glucose	0.001	0.000	<0.001
Smoker			
Current	0.010	0.012	0.413
EX	0.000	0.010	0.988
Drinker	0.015	0.007	0.027
Women≤69 years (Postmenopausal), n=1,434; 61.0±5.5 years, Pearson's r=0.264 (P<0.001)			
Age	0.002	0.000	<0.001
BMI	0.004	0.001	<0.001
Fasting glucose	0.001	0.000	<0.001
Smoker			
Current	0.001	0.012	0.914
EX	0.013	0.010	0.201
Drinker	0.003	0.005	0.555
Women 70≥ years (Postmenopausal), n=860; 75.6±4.6 year olds, Pearson's r=0.167 (P<0.001)			
Age	0.000	0.001	0.704
BMI	0.004	0.001	<0.001
Fasting glucose	0.001	0.000	<0.001
Smoker			
Current	-0.049	0.025	0.052
EX	0.034	0.021	0102
Drinker	-0.004	0.006	0.501

β is a coefficient indicating a one-unit increase in an independent variable in multivariable linear logic regression analyses. SE=standard error; sdLDL-C=small dense low-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; BMI=body mass index.

Considering the beta value of each variable, 50-year old standardized sdLDL-C levels in men, premenopausal women, and postmenopausal women were 26.6 mg / dL (95 % CI; 26.4-26.9 mg / dL), 22.7 mg / dL (95 % CI; 22.5-22.9 mg / dL), and 27.4 mg / dL (95 % CI; 27.3-27.5 mg/dL), respectively. Standardized sdLDL-C/LDL-C ratio in men, premenopausal women, and postmenopausal women were 0.24 (95 % CI; 0.24-0.24), 0.15 (95 % CI; 0.15-0.16), and 0.23 (95 % CI; 0.22-0.23), respectively. These differences between premenopausal women and postmenopausal women were significant (Bonferroni analysis, $P < 0.001$).

DISCUSSION

To the best of our knowledge, the present study is the first to demonstrate the association between age, gender, menopausal status, and sdLDL-C and sdLDL-C/LDL-C ratio. The age-related sdLDL-C trends showed roughly an increasing phase, followed by a decreasing phase in men and a plateaued phase in middle-aged women. The age-related sdLDL-C trend in men, but not in women, was similar to traditional lipid cholesterol profiles. The reason for this gender difference might be related to the mechanism of hypertriglyceridemia in postmenopausal women, which induced small LDL particles.[20-22] There were age or gender-related differences in sdLDL-C / LDL-C ratio, reflecting the ability to generate sdLDL-C from LDL-C. This ability in men was higher than that in women for all age groups or standardized groups, which is identical to the fact that atherosclerosis is more common in men than in women, considering sdLDL-C is a highly atherogenic factor.

Our study showed three important results. First, age showed partial correlation trends with sdLDL-C levels and sdLDL-C/LDL-C ratio and non-linear trends between age and sdLDL-C and sdLDL-C/LDL-C ratio were found in both men and women. Therefore, using the sdLDL-C and sdLDL-C/LDL-C ratio, the definition of CVD risk assessment and the adaption of the lipid-lowering therapy should fully consider age-related trends and gender differences.

Second, menopausal status was an additional determinant of increasing sdLDL-C and sdLDL-C/LDL-C ratio. Many factors such as excess adiposity, free fatty acids,

apo-lipoproteins, and action of lipoprotein lipase activity and cholesterol ester transfer protein affected multiple and complex mechanisms regulating sdLDL.[12,16,17] In postmenopausal women, the decrease of plasma estrogen levels plays a significant role in reducing the clearance of LDL particles via LDL receptor and increasing TG and the number of smaller LDL particles.[23] This hormone change was related to the process of regulating sdLDL-C but there was little evidence available on the association between menopausal status and sdLDL-C or sdLDL-C/LDL-C ratio in a real-world, population setting.[24] Our results showed that sdLDL-C in postmenopausal women was 0.8 or 3.9 mg / dL higher than men or premenopausal women in the standardized analysis.

Finally, the relationships between age-related trends in sdLDL-C and sdLDL-C/LDL-C ratio and gender were different from traditional lipid factors, such as LDL-C. The crossover of LDL-C for the genders occurred in middle-aged patients. On the contrary, the crossover of sdLDL-C occurred between 70-74 years and the sdLDL-C/LDL-C ratio did not occur. Rather than LDL-C, the results of the sdLDL-C and sdLDL-C/LDL-C ratio might reflect the fact that, for all age groups, men have more susceptible to CVD than women, even with the narrowing gap of risk for CVD in postmenopausal women.[25]

Our findings suggest that a subgroup-specific approach is required to develop efficient CVD prevention strategies using the sdLDL-C and sdLDL-C/LDL-C ratio.

Limitations

Our study has several limitations. First, age-related trends and levels of traditional lipid factors were almost similar to National Health and Nutrition Survey in Japan and our age-related trends of these factors were also similar to the trends of the Korean and Chinese Singaporeans population.[14,15] But the trends of the US population or healthy Caucasian[26,27] were not similar. Especially in healthy Caucasian patients aged ≥ 70 years, the trends for TC, LDL-C, and nonHDL-C differed from our observed trends and continuously increased. Although our results could not identify the mechanism, there might be racial differences. Therefore, it is unclear whether our results of sdLDL-C would be valid for these populations. Second, compared with mean lipid levels of the

Korean population from KNHANES, Japanese men showed higher mean TC, LDL-C, and HDL levels (TC 199 mg / dL; LDL-C 115 mg / dL; HDL-C 56 mg / dL) compared to Korean men (TC 183 mg / dL; LDL-C 106 mg / dL; HDL 50 mg / dL), and Japanese women also showed higher mean levels (TC 212 mg / dL; LDL-C 124 mg / dL; HDL-C 64 mg / dL) than Korean women (TC 188 mg / dL; LDL-C 111 mg / dL; HDL-C 55 mg / dL). The reason for the difference in the lipoprotein profile between Japanese and Korean populations might be due to genetics and environmental factors. It is also unknown whether these factors might affect sdLDL-C levels and sdLDL-C/LDL-C ratio because sdLDLs are regulated through complex mechanisms. Third, we did not control for the effects of diet, life activity, socioeconomic status, and genetic factors, which might be associated with changes in lipid metabolism.[28,29,30] Fourth, there might be several biases. Selection bias might come from potential non-representativeness of the study population, which was rural dwelling. There might be information bias and data misclassification due to error in measurement of the lipid parameters. Fifth, as shown in the supplementary figure 6 and 7, the results regarding the association between demographic factors and sdLDL-C and sdLDL-C/LDL-C ratio remained the same in 6,282 participants including patients taking lipid-lowering therapy. SdLDL-C/LDL-C ratio in men including patients taking lipid-lowering therapy was higher than in men excluding these patients (0.45 vs 0.35). Our assessment was limited in terms of this difference, because data regarding type and dose of medications for dyslipidemia were not available. We need to validate the association in patients taking lipid-lowering therapy in another cohort. Finally, our study could not evaluate the association between the demographic factors and other lipid markers, such as Lp(a) and oxidized LDL-C. Lp(a) was a significant risk factor for cardiovascular disorders and to be in the spotlight due to a novel therapy using antisense oligonucleotides. These lipid markers should be discussed in further study.[31]

CONCLUSION

SdLDL-C and sdLDL-C/LDL-C ratio are differently distributed by age, gender, and menopausal status. Our findings suggest that a subgroup-specific approach is required

to develop efficient CVD prevention strategies using the sdLDL-C and sdLDL-C/LDL-C ratio.

List of abbreviations

sdLDL-C: small dense low-density lipoprotein cholesterol; CVD: cardiovascular disease; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; TGs :triglycerides; HDL-C :high-density lipoprotein cholesterol: nonHDL-C: non-high-density lipoprotein cholesterol; LNsdLDL-C: log-transformed small dense low-density lipoprotein cholesterol; JMS: Jichi Medical School; ANOVA; analysis of variance

DECLARATIONS

Acknowledgements

The authors thank the public health doctors, nurses, and officers in Shimotsuke, Kakara, Sue, Omori, Kamiichi, Wara, Takasu, Onabi, Nakatsu, Yame, Miwa, Ueno, and Saji areas, Japan, for their help, support, and contributions.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All the participants included in the present study provided written informed consent prior to inclusion, and this study was approved by the Institutional Review Board of Jichi Medical School (Tochigi, Japan, IRB No. G09-39 [G17-64 revised]).

Data sharing statement

Data are available upon reasonable request.

Author contributions

TI, YN, YS, and SI have participated in the research and designed the study; TI and SI performed the statistics analysis; TI contributed to the drafting of the manuscript. YN, YS, and SI provided feedback on the manuscript, and all authors read and approved the final manuscript.

Consent for publication

All the participants included in the present study provided written informed consent for publication.

Competing interests

The authors declare they have no conflict of interest with respect to this research study and paper.

Funding

This research was supported by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities (S0901032); a Japanese Society for the Promotion of Science KAKENHI grant (No. 16K09141); a Grant-In-Aid from the Ministry of Health, Labour, and Welfare; Health and Labor Sciences and Japan Comprehensive Research on Cardiovascular and Lifestyle-Related Diseases grants (H26-Junkankitou-[Seisaku]-Ippan-001 and H29-Junkankitou-Ippan-003; IRB No. G09-39 [G17-64 revised]); and Jichi Medical University Almuni Association Project Grant 2020 (5-3).

REFERENCES

1. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tomma- sino J, Forman DE, Goldberg R, Heidenreich PA, Hlatky MA, Jones DW, Lloyd-Jones D, Lopez-Pajares N, Ndumele CE, Orringer CE, Peralta CA, Saseen JJ, Smith SC Jr, Sperling L, Virani SS, Yeboah J. 2018AHA/ACC/ AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/ NLA/PCNA Guideline on the Management of Blood Cholesterol. Circulation, 2019; 139: e1082-e1143

2. Lawler PR, Akinkuolie AO, Harada P, Glynn RJ, Chasman DI, Ridker PM, Mora S. Residual Risk of Athero- sclerotic Cardiovascular Events in Relation to Reductions in Very-Low-Density Lipoproteins. J Am Heart Assoc, 2017; 6: 1-11

3. Varbo A, Nordestgaard BG. Remnant Cholesterol and Triglyceride-Rich Lipoproteins in Atherosclerosis Progres- sion and Cardiovascular Disease. Arterioscler Thromb Vasc Biol, 2016; 36: 2133-2135

4. Ivanova EA, Myasoedova VA, Melnichenko AA, Grechko AV, Orekhov AN. Small Dense Low-Density Lipoprotein as Biomarker for Atherosclerotic Diseases. Oxidative Medicine and Cellular Longevity, 2017; 2017: 1273042

5. Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PWF, D'Agostino RB, Vasan RS, Robins SJ. Increased Small Low-Density Lipoprotein Particle Number. Circulation, 2006; 113: 20-29

6. Arai H, Kokubo Y, Watanabe M, Sawamura T, Ito Y, Minagawa A, Okamura T, Miyamato Y. Small dense low-density lipoproteins cholesterol can predict incident car- diovascular disease in an urban Japanese cohort: the Suita study. J Atheroscler Thromb, 2013; 20: 195-203

7. Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, Couper D, Virani SS, Kathiresan S, Boerwin- kle E, Ballantyne CM. Small Dense Low-Density Lipo- protein-Cholesterol Concentrations Predict Risk for Coronary Heart Disease. Arterioscler Thromb Vasc Biol, 2014; 34: 1069-1077

8. St-Pierre AC, Cantin B, Dagenais GR, Mauriège P, Ber- nard P-M, Després J-P, Lamarche B. Low-Density Lipoprotein Subfractions and the Long-Term Risk of Ischemic Heart Disease in Men. Arterioscler Thromb Vasc Biol, 2005; 25: 553-559

9. Nishikura T, Koba S, Yokota Y, Hirano T, Tsunoda F, Shoji M, Hamazaki Y,

Suzuki H, Itoh Y, Katagiri T, Kobayashi Y. Elevated small dense low-density lipoprotein cholesterol as a predictor for future cardiovascular events in patients with stable coronary artery disease. J Atheroscler Thromb. 2014;21(8):755-67.

10. Mogarekar MR, Kulkarni SK. Small Dense Low Density Lipoprotein Cholesterol, Paraoxonase 1 and Lipid Profile in Postmenopausal Women: Quality or Quantity? Arch Med Res. 2015 Oct;46(7):534-8.

11. Gentile M, Panico S, Mattiello A, Ubaldi S, Iannuzzo G, De Michele M, Iannuzzi A, Rubba P. Association between small dense LDL and early atherosclerosis in a sample of menopausal women. Clin Chim Acta. 2013;426:1-5.

12. Johnson AA, Stolzing A. The role of lipid metabolism in aging, lifespan regulation, and age-related disease. Aging Cell. 2019;18(6):e13048.

13. Toth MJ, Tchernof A. Lipid metabolism in the elderly. Eur J Clin Nutr. 2000 ;54 Suppl 3:S121-5.

14. Park JH, Lee MH, Shim JS, Choi DP, Song BM, Lee SW, Choi H, Kim HC. Effects of age, sex, and menopausal status on blood cholesterol profile in the korean population. Korean Circ J. 2015 Mar;45(2):141-8.

15. Goh VH, Tong TY, Mok HP, Said B. Differential impact of aging and gender on lipid and lipoprotein profiles in a cohort of healthy Chinese Singaporeans. Asian J Androl. 2007;9(6):787-94.

16. Qamar A, Khetarpal SA, Khera AV, Qasim A, Rader DJ, Reilly MP. Plasma apolipoprotein C-III levels, triglycer- ides, and coronary artery calcification in type 2 diabetics. Arterioscler Thromb Vasc Biol, 2015; 35: 1880-1888

17. Pechlaner R, Tsimikas S, Yin X, Willeit P, Baig F, Santer P, Oberhollenzer F, Egger G, Witztum JL, Alexander VJ, Willeit J, Kiechl S, Mayr M. Very-Low-Density Lipopro- tein-Associated Apolipoproteins Predict Cardiovascular Events and Are Lowered by Inhibition of APOC-III. Journal of the American College of Cardiology, 2017; 69: 789-800

18. Ishikawa S, Gotoh T, Nago N, Kayaba K; Jichi Medical School (JMS) Cohort Study Group.The Jichi Medical School (JMS) Cohort Study: design, baseline data and standardized mortality ratios. J Epidemiol. 2002;12:408-17.

19. Izumida T, Nakamura Y, Hino Y, Ishikawa S. Combined Effect of Small Dense Low-Density Lipoprotein Cholesterol (sdLDL-C) and Remnant-Like

Particle Cholesterol (RLP-C) on Low-Grade Inflammation. J Atheroscler Thromb. 2020;27:319-330.

20. Cho Y, Lee SG, Jee SH, Kim JH. Hypertriglyceridemia is a major factor associated with elevated levels of small dense LDL cholesterol in patients with metabolic syndrome. Ann Lab Med. 2015;35(6):586-94.

21. Hayashi T, Koba S, Ito Y, Hirano T. Method for estimating high sdLDL-C by measuring triglyceride and apolipoprotein B levels. Lipids Health Dis. 2017 Jan 26;16(1):21.

22. Dayspring TD. Understanding hypertriglyceridemia in women: clinical impact and management with prescription omega-3-acid ethyl esters. Int J Womens Health. 2011 Mar 9;3:87-97.

23. Campos H, Walsh BW, Judge H, Sacks FM. Effect of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. J Clin Endocrinol Metab. 1997 Dec;82(12):3955-63.

24. Carr MC, Kim KH, Zambon A, Mitchell ES, Woods NF, Casazza CP, Purnell JQ, Hokanson JE, Brunzell JD, Schwartz RS. Changes in LDL density across the menopausal transition. J Investig Med. 2000 Jul;48(4):245-50.

25. Bhatnagar P, Wickramasinghe K, Williams J, Rayner M, Townsend N.The epidemiology of cardiovascular disease in the UK 2014. Heart.;101(15):1182-9.

26. Anagnostis P, Stevenson JC, Crook D, Johnston DG, Godsland IF. Effects of menopause, gender and age on lipids and high-density lipoprotein cholesterol subfractions. Maturitas. 2015;81(1):62-8.

27. Schaefer EJ, Lamon-Fava S, Cohn SD, Schaefer MM, Ordovas JM, Castelli WP, Wilson PW.Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. J Lipid Res.;35(5):779-92.

28. Tenk J, Mátrai P, Hegyi P, et al. Perceived stress correlates with visceral obesity and lipid parameters of the metabolic syndrome: a systematic review and meta-analysis. Psychoneuroendocrinology 2018;95:63–73.

29. Slopen N, Goodman E, Koenen KC, Kubzansky LD. Socioeconomic and other social stressors and biomarkers of cardiometabolic risk in youth: a systematic review of less studied risk factors. PLoS One. 2013;8(5):e64418.

30. Garcia CK, Mues G, Liao Y, Hyatt T, Patil N, Cohen JC, Hobbs HH.

534 Sequence diversity in genes of lipid metabolism. Genome Res.
535 2001;11(6):1043-52.
536 31. Viney NJ, van Capelleveen JC, Geary RS, et al. Antisense oligonucleotides
537 targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised,
538 double-blind, placebo-controlled, dose-ranging trials. Lancet.
539 2016;388(10057):2239-2253
540

FIGURE LEGENDS

Figure 1. Geometric mean and 95% confidence interval of sdLDL-C for age, gender, and menopausal status

Figure 2. Mean and 95% confidence interval of sdLDL-C/LDL-C ratio for age, gender, and menopausal status

Figure 3. Mean and 95% confidence interval of LDL-C for age, gender, and menopausal status

Supplementary Material

Supplementary Figure 1. Mean of total cholesterol for age, gender, and menopausal status

Supplementary Figure 2. Mean of non-high-density lipoprotein cholesterol for age, gender, and menopausal status

Supplementary Figure 3. Mean of total cholesterol / high-density lipoprotein cholesterol ratio for age, gender, and menopausal status

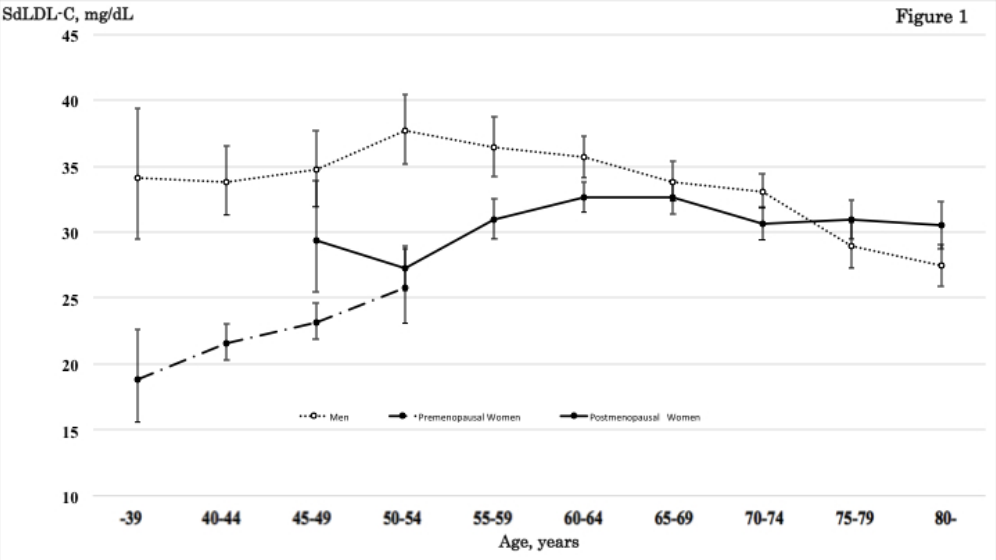
Supplementary Figure 4. Geometric mean of triglycerides for age, gender, and menopausal status

Supplementary Figure 5. Mean of high-density lipoprotein cholesterol for age, gender, and menopausal status

Supplementary Figure 6. Geometric mean and 95% confidence interval of sdLDL-C for age, gender, and menopausal status

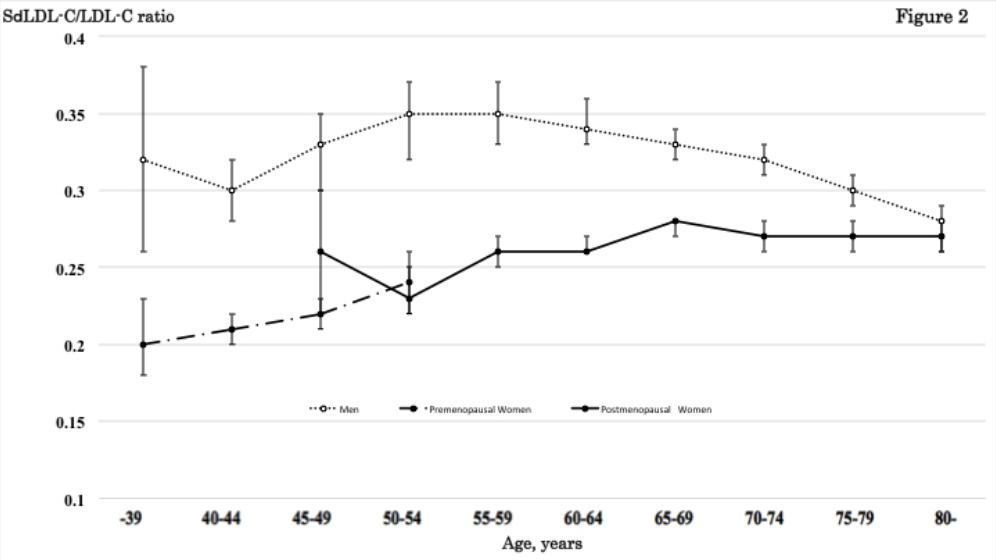
570

571 **Supplementary Figure 7. Mean and 95% confidence interval of sdLDL-C/LDL-C**
572 **ratio for age, gender, and menopausal status**



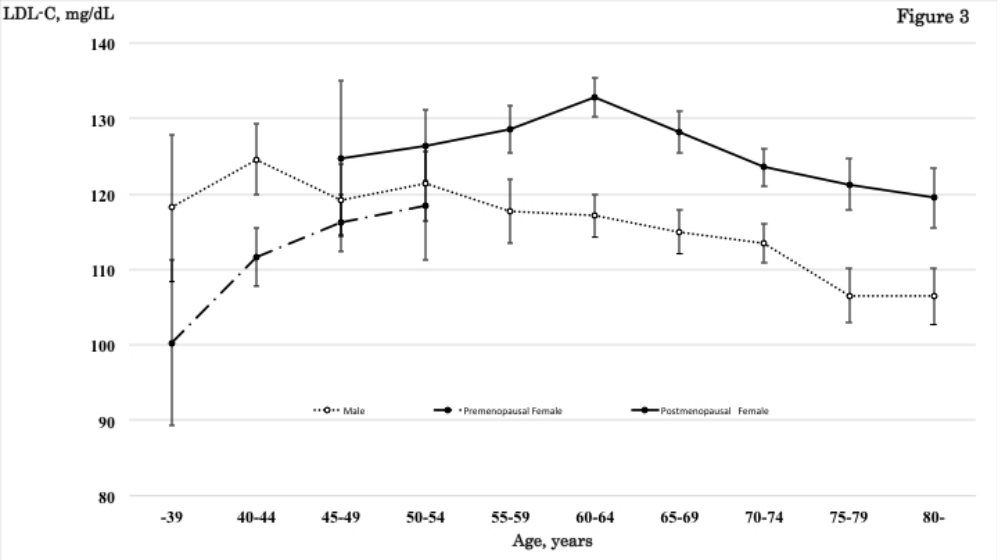
Geometric mean and 95% confidence interval of sdLDL-C for age, gender, and menopausal status

254x142mm (72 x 72 DPI)



Mean and 95% confidence interval of sdLDL-C/LDL-C ratio for age, gender, and menopausal status

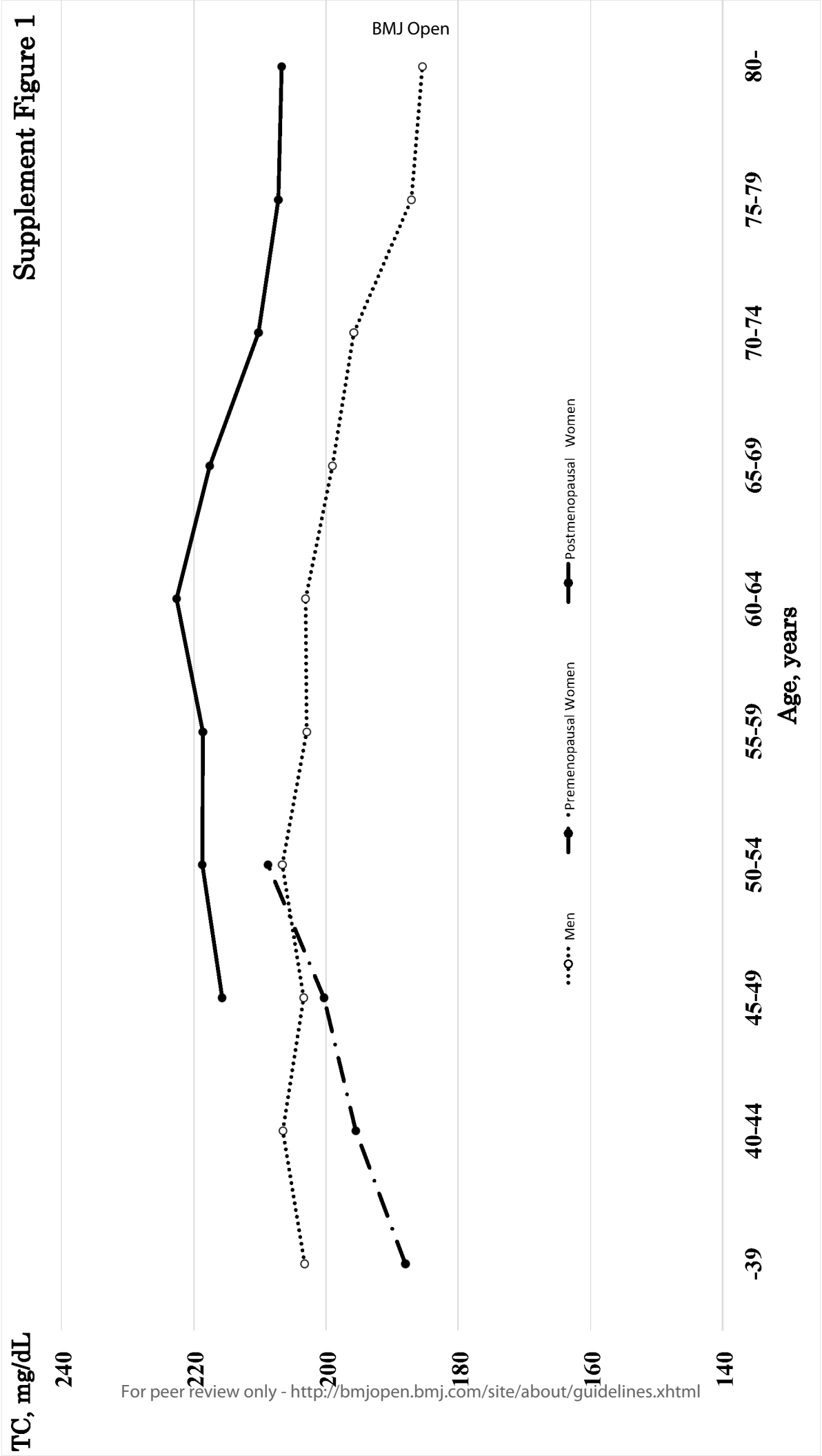
254x142mm (72 x 72 DPI)



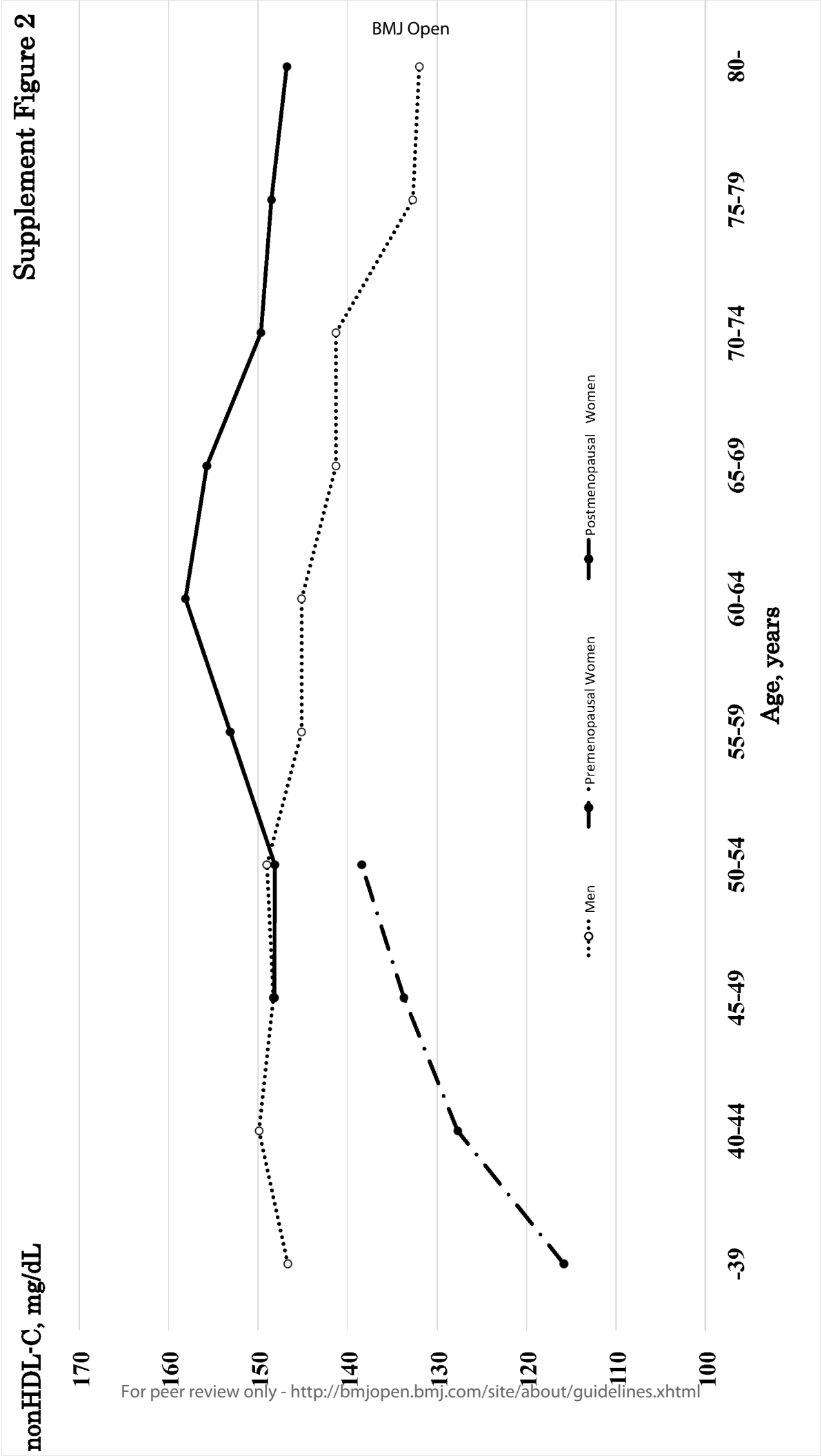
Mean and 95% confidence interval of LDL-C for age, gender, and menopausal status

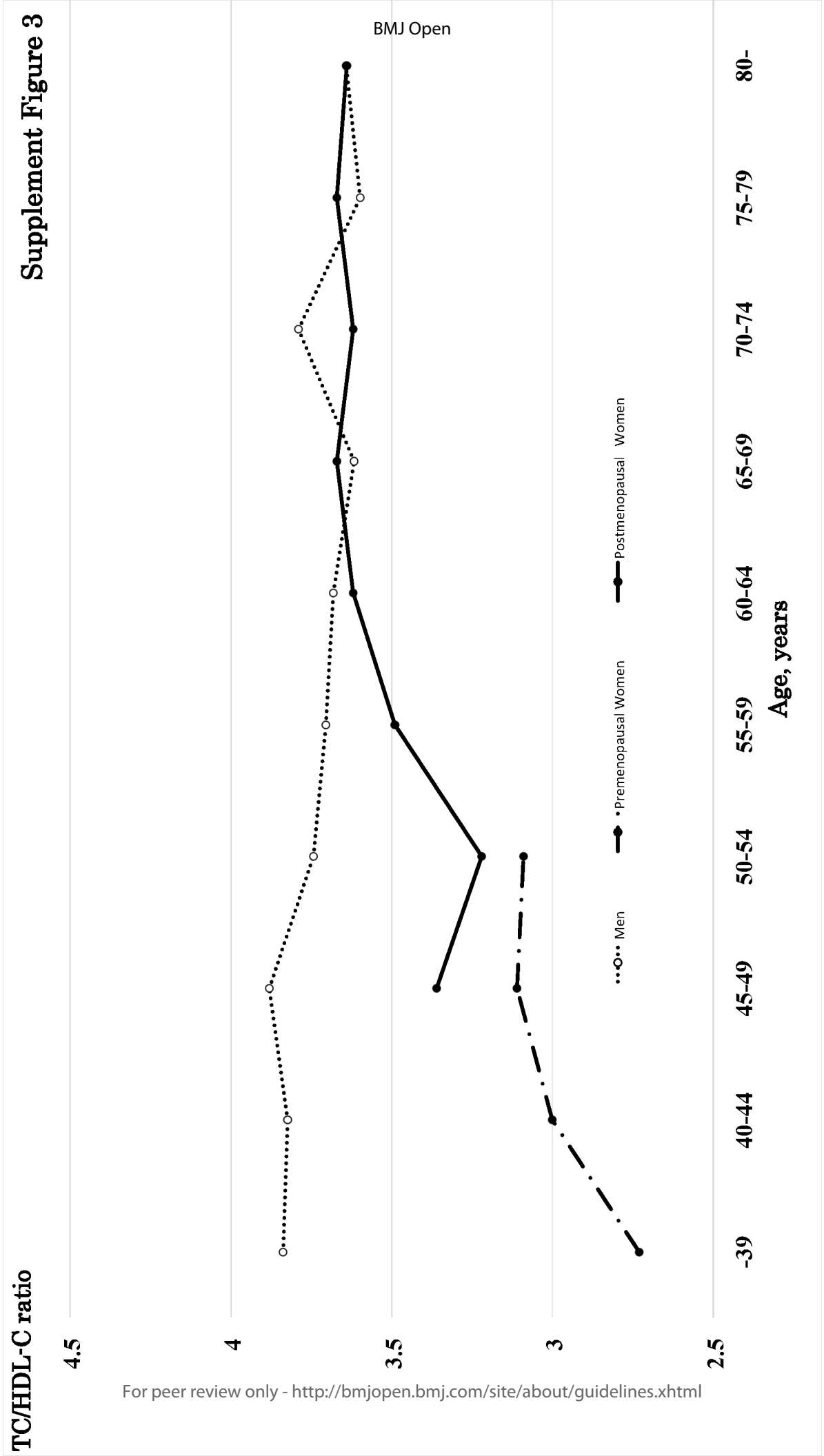
254x142mm (72 x 72 DPI)

Supplement Figure 1

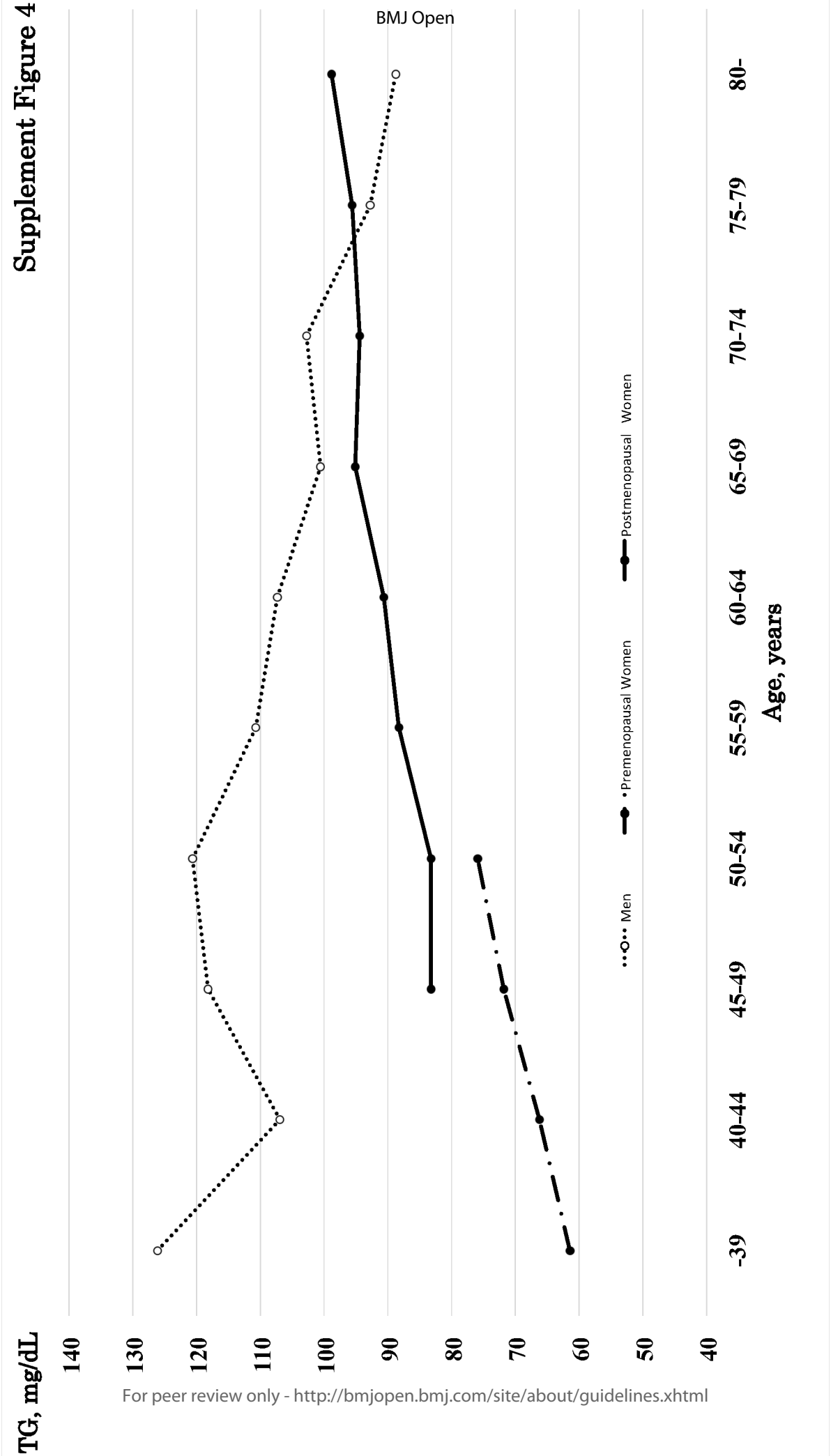


Supplement Figure 2

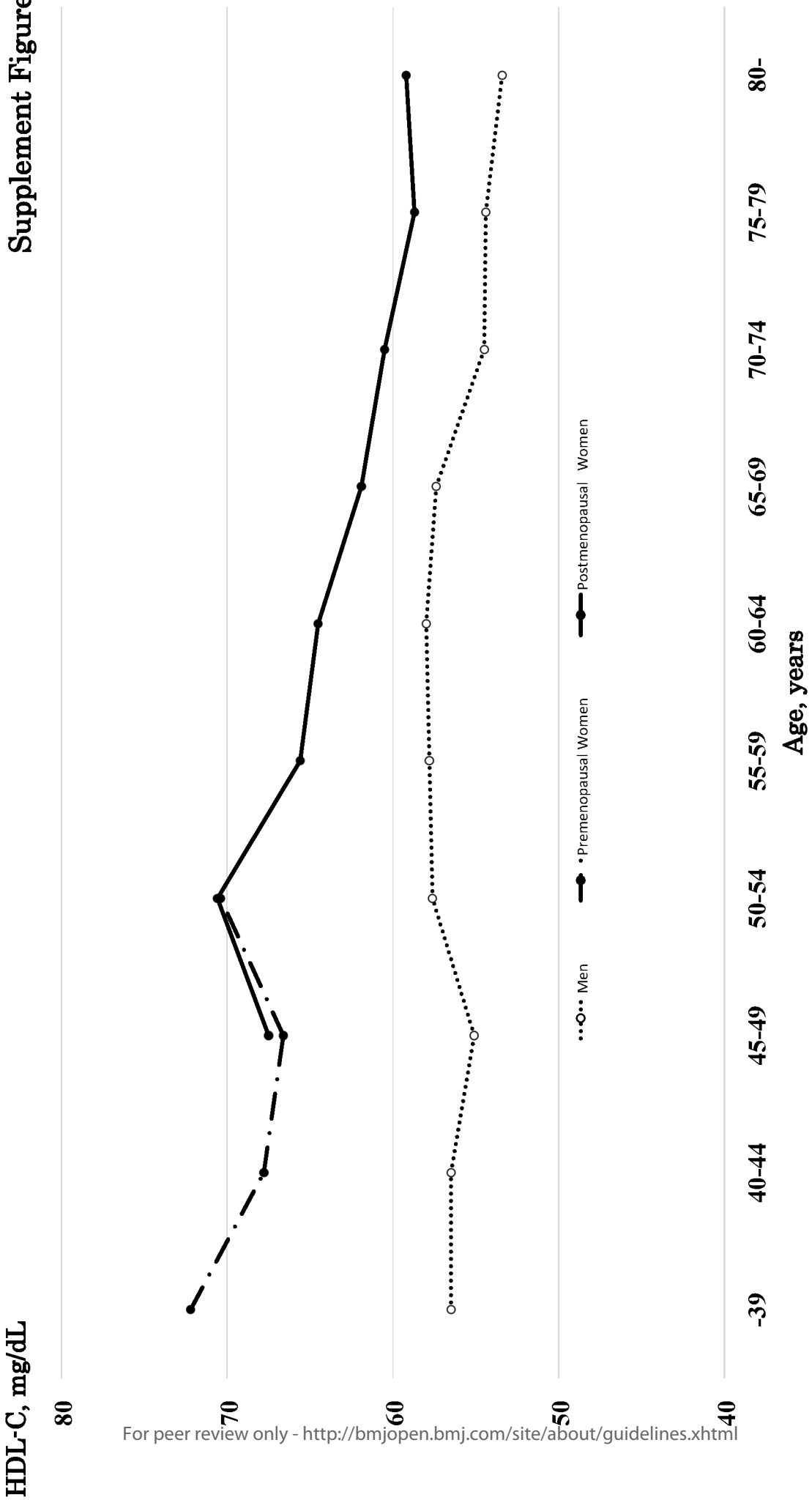



Supplement Figure 3
TC/HDL-C ratio
4.5
4
3.5
3
2.5
-39
40-44
45-49
50-54
55-59
60-64
65-69
70-74
75-79
80-
Age, years
Men
Premenopausal Women
Postmenopausal Women

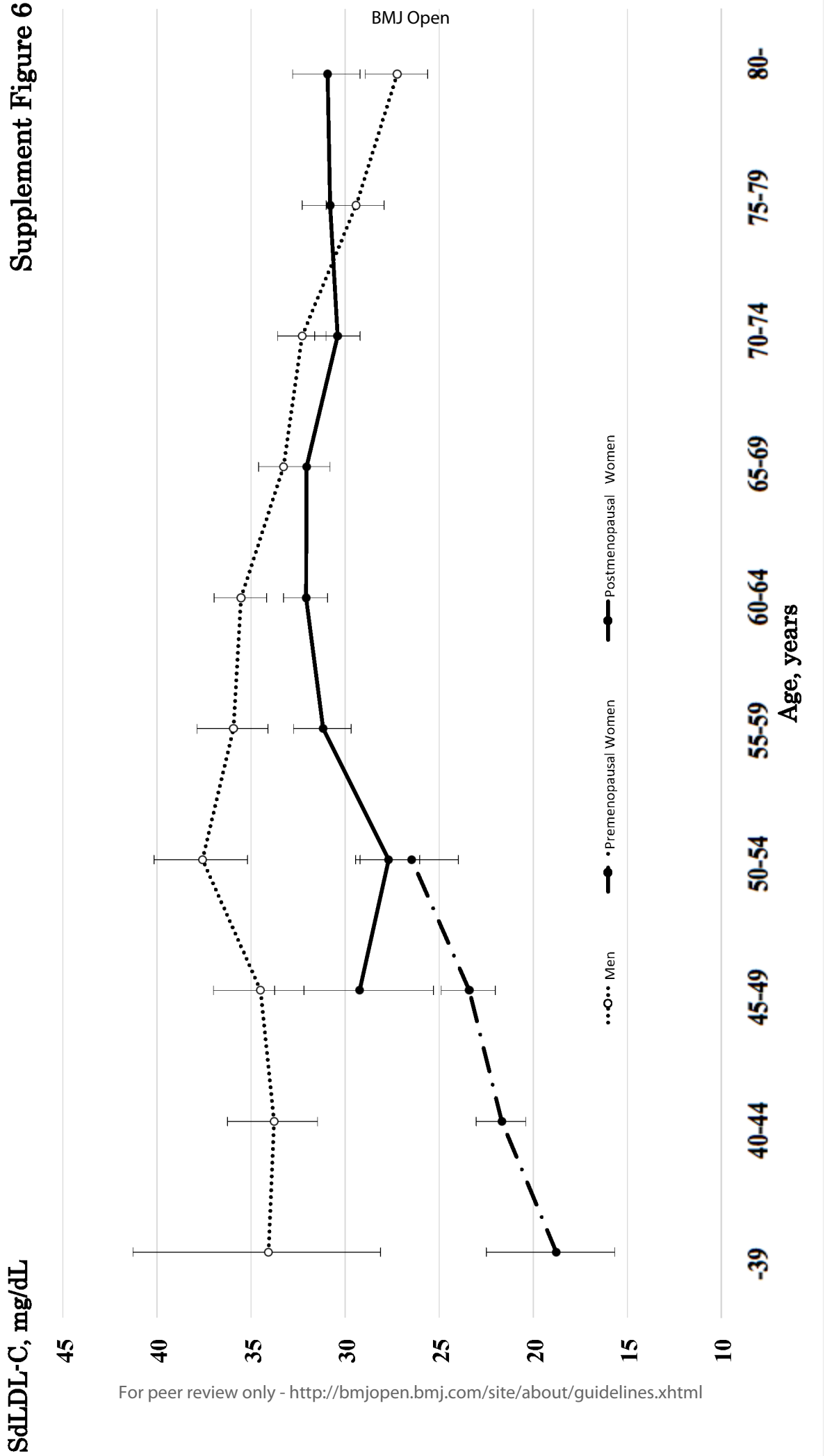
Supplement Figure 4



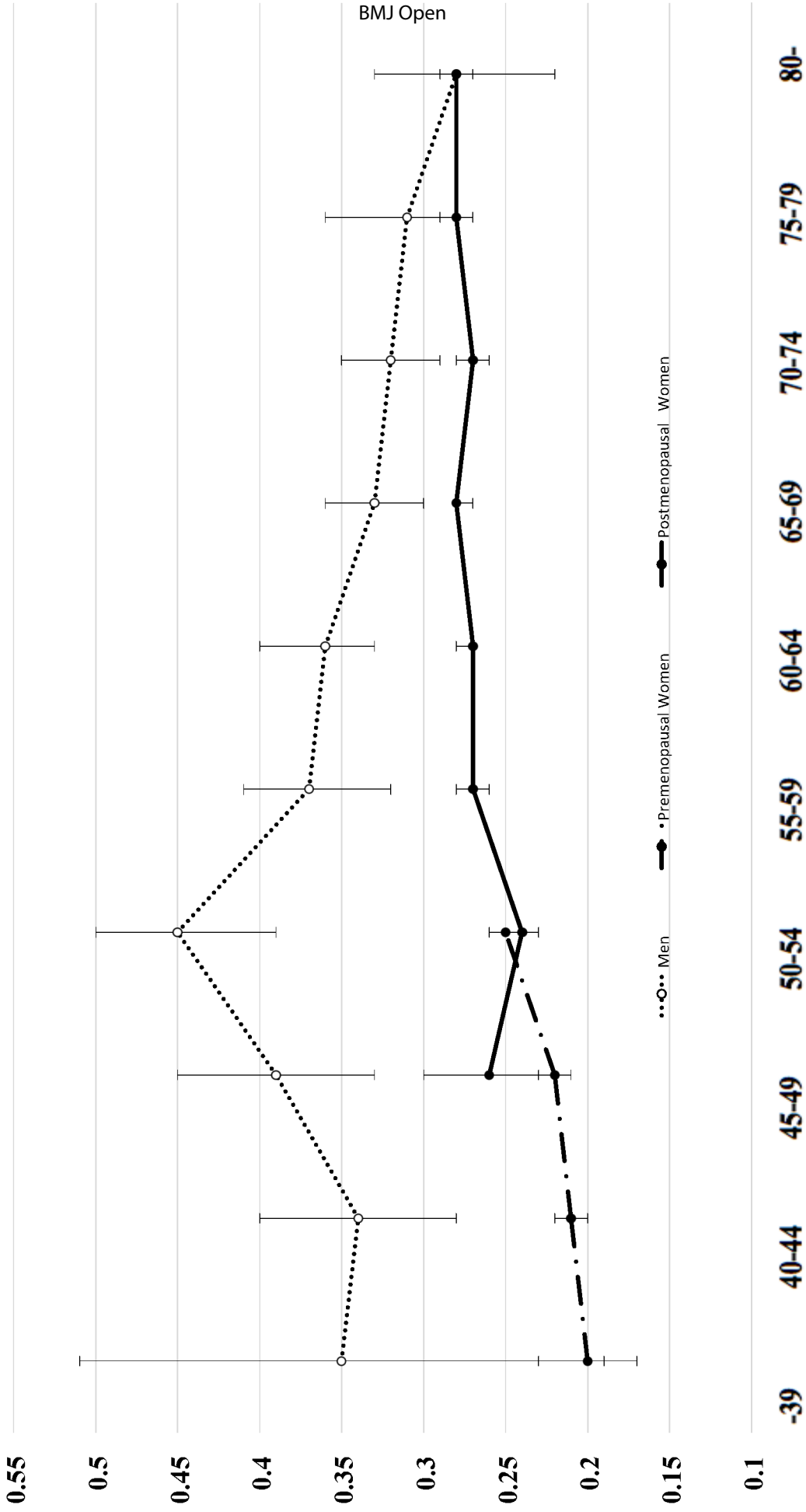
Supplement Figure 5



Supplement Figure 6



Supplement Figure 7



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	1, 3, 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6, 7*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how loss to follow-up was addressed	-
		(*e*) Describe any sensitivity analyses	-
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6, 8
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-10
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	Report numbers of outcome events or summary measures over time	-
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-16
		(*b*) Report category boundaries when continuous variables were categorized	8-16
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-16
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-16
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20-21

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.