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# 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

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1 [TITLE] Effects of Age, Gender, and Menopausal status on Small Dense  
2 Low-Density Lipoprotein Cholesterol and Low-Density Lipoprotein Cholesterol  
3 Fractions; A Population-Based Study  
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30 menopause  
31

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38

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

For peer review only

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6 43 **ABSTRACT**

7 44 **Objectives:** Small dense low-density lipoprotein cholesterol (sdLDL-C) might be a  
8 45 better cardiovascular disease (CVD) indicator than low-density lipoprotein cholesterol  
9 46 (LDL-C); however, details regarding its epidemiology remain elusive. The present study  
10 47 aimed at evaluating the effect of age, gender, and menopausal status on sdLDL-C  
11 48 levels and sdLDL-C/LDL-C ratio in the Japanese population.  
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17 50 **Design:** This was a cross-sectional study.  
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20 51  
21 52 **Setting:** 13 rural districts in Japan, 2010-2017  
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24 53  
24 54 **Participants:** This study included 5,208 participants (2,397 men and 2,811 women),  
25 55 who underwent the health mass screening that was conducted in accordance with the  
26 56 medical care system for the elderly and obtained informed consent for this study.  
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29 57  
30 58 **Results:** In men, the sdLDL-C levels and sdLDL-C/LDL-C ratio increased during  
31 59 younger adulthood, peaked at 50–54 years, and then decreased. In women, relatively  
32 60 regular increasing trends of sdLDL-C level and sdLDL-C/LDL-C ratio until approximately  
33 61 65 years, followed by a downward or plateaued trend. The crossover of sdLDL-C levels for  
34 62 the genders occurred at 70–74 years, but the crossover of sdLDL-C/LDL-C ratio could  
35 63 not be observed. Standardized sdLDL-C levels and sdLDL-C/LDL-C ratio in 50-year old  
36 64 men, premenopausal women, and postmenopausal women were 26.6, 22.7, and 27.4  
37 65 mg/dL and 0.24, 0.15, and 0.23, respectively. The differences between premenopausal  
38 66 and postmenopausal women were significant ( $P<0.001$ ).  
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47 68 **Conclusions:** SdLDL-C and sdLDL-C/LDL-C ratios showed different distributions by  
48 69 age, gender, and menopausal status with trends different from other lipids. A  
49 70 subgroup-specific approach would be necessary to implement sdLDL-C for CVD  
50 71 prevention strategies, fully considering age-related trends, gender differences, and  
51 72 menopausal status.  
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55 73 (248 words / within 300 words)  
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6 **75 Strengths and limitations of this study**

7 76 1. To the best of our knowledge, the present study is the first to demonstrate the effects  
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9 77 of age, gender, and menopausal status on the sdLDL-C and sdLDL-C/LDL-C ratio.

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11 78 2. This study is based on a large representative sample from Japanese general  
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13 79 population.

14 80 3. Serum lipid markers were measured by the standardized program proposed by the  
15  
16 81 Clinical and Laboratory Standards Institute.

17 82 4. It is unclear whether our results of sdLDL-C would be valid for other populations.

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19 83 5. This study did not control for several confounding factors, such as diet, life activity,  
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21 84 socioeconomic status, and genetic factors.

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## 87 INTRODUCTION

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

99  
100 However, almost all of the current analytical strategies might be not able to adjust  
101 accurately the interaction between age and sdLDL-C due to the association between  
102 the lipid factors and age, which might follow a curvilinear model. Few studies have  
103 evaluated how age is associated with sdLDL-C and sdLDL-C/LDL-C ratio over a wide  
104 age range and distinguished the effects of menopause and gender on sdLDL-C and  
105 sdLDL-C fraction from those of aging.<sup>10,11</sup>

106  
107 Diet composition, which is affected by aging, is associated with blood cholesterol and  
108 the absorption, synthesis, and metabolism per se of fat and lipoproteins change with  
109 age.<sup>12,13</sup> Another study showed Asian age-related trends of traditional lipid profiles  
110 displayed roughly an increasing trend, followed by a decreasing one at the middle-aged  
111 stage.<sup>14,15</sup> Meanwhile, sdLDL-C has been regulated by more complex mechanisms than  
112 regulating traditional lipids and might be plateaued or increased even at the  
113 middle-aged by changed metabolic functions with aging influencing sdLDL-C  
114 synthesis.<sup>5,7,12,16,17</sup> Furthermore, the detailed multiple mechanisms of metabolizing  
115 sdLDLs are unknown in the real-world, population-based setting and the age-related  
116 trend of sdLDL-C might be different from the sdLDL-C/LDL-C ratio. In other words, the  
117 ability to generate sdLDL-C from LDL-C might be different among each generation,  
118 gender, and menopausal status. Therefore, we evaluated the effect of age, gender, and



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6 119 menopausal status on sdLDL-C and sdLDL-C/LDL-C ratio in Japanese general  
7 120 population.  
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## 12 123 **METHODS**

### 14 124 ***Population***

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17 125 The Jichi Medical School (JMS)-II Cohort Study is a prospective, population-based  
18 126 cohort study of the risk factors of atherosclerosis and CVD in the Japanese general  
19 127 population. A total of 6,436 individuals participated in this study. Details of the methods  
20 128 of enrollment have been reported previously.<sup>18,19</sup> In brief, from April 2010 through  
21 129 December 2017, this study evaluated Japanese individuals who were residents of 13  
22 130 rural districts in Japan, Shimotsuke, Kakara, Sue, Omori, Kamiichi, Wara, Takasu,  
23 131 Onabi, Nakatsu, Yame, Miwa, Ueno, and Saji areas. Local government offices in each  
24 132 community issued invitations to eligible residents for the mass CVD screening, and  
25 133 personal invitations were also sent to all potential participants by mail. All the  
26 134 participants in the present study provided written informed consent prior to inclusion.  
27 135 The study protocol and data analysis plan were approved by the institutional review  
28 136 board of Jichi Medical School (Tochigi, Japan, IRB No. G09-39 [G17-64 revised]).  
29 137 We excluded individuals as follows: 1) taking lipid-lowering agents or  
30 138 anti-hyperglycemia agents (n = 1,073); 2) the use of hormone replacement therapy (n =  
31 139 96); and 3) the data such as age, gender status, menopausal status, and sdLDL-C were  
32 140 not available (n = 73).  
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### 45 142 ***Measurements***

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48 143 A central committee, composed of the chief medical officers of all 13 participating  
49 144 districts, developed a detailed manual for data collection. Body weight was recorded  
50 145 with the subjects clothed. Height was measured with stockinged feet. Body mass index  
51 146 (BMI) was calculated as weight (kg) / height (m<sup>2</sup>). Blood samples were taken after  
52 147 overnight fasting. TC was measured via a cholesterol dehydrogenase-ultraviolet  
53 148 method. Triglycerides (TG) was measured using an enzymatic method. LDL-C and  
54 149 high-density lipoprotein cholesterol (HDL-C) were measured by direct methods.  
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6 150 SdLDL-C level was directly and selectively measured using a commercial kit (sdLDL-EX  
7 151 from Denka Seiken, Tokyo, Japan). An external laboratory (SRL, Tokyo, Japan)  
8 152 measured the serum lipid markers. The markers were measured by the standardized  
9 153 program proposed by the Clinical and Laboratory Standards Institute. The nonHDL-C  
10 154 was calculated by subtracting HDL-C from TC. Information about medical history,  
11 155 lifestyle, and menopausal status were obtained with a self-reported questionnaire.  
12 156 Smoking status was classified as smoking, former smoking, or never-smoking.  
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### 19 158 **Statistical analysis**

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21 159 Baseline characteristics were summarized as mean  $\pm$  standard deviation (SD) for  
22 160 normally distributed continuous variables and numbers and percentages for categorical  
23 161 variables. SdLDL-C and TG were highly skewed; these data were expressed as the  
24 162 median and interquartile range and transformed into natural logarithms before statistical  
25 163 analysis.

26  
27 164 The one-way analysis of variance (ANOVA) was used for comparison among three  
28 165 groups, and differences were tested via post hoc pairwise comparison (Bonferroni). To  
29 166 explore the age-related trend in sdLDL-C and sdLDL-C/LDL-C ratio with age, geometric  
30 167 means or means and 95 percent confidence intervals for each variable in 5-year age  
31 168 ranges were derived and plotted against age range in each of the three groups.

32  
33 169 Among the three groups, correlations between age and each parameter were assessed  
34 170 using multiple linear regression analysis. The agreement between the estimated  
35 171 sdLDL-C and sdLDL-C/LDL-C ratio and measured ones was assessed by Pearson's  
36 172 correlation coefficient. To evaluate the effect of menopausal status on sdLDL-C and  
37 173 sdLDL-C/LDL-C ratio, using the beta value of each variable from the analysis in the  
38 174 premenopausal and postmenopausal group, data were standardized to a nominal 50  
39 175 years of menopausal age, never smoking and zero alcohol for participants with normal  
40 176 weight (BMI 18.5-22.0). All statistical analyses were performed using SPSS version 22  
41 177 (IBM, Chicago, IL, USA), and statistical significance was defined as  $P < 0.05$ .

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### 54 179 **Patient and public involvement**

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56 180 Participants of this study or members of the public were not directly and personally  
57 181 involved with study design, data provision, analysis and publication of the study.  
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182 **RESULTS**183 **Baseline characteristics**

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

194 Table 1 Baseline characteristics

	<i>Group 1 (G1)</i>	<i>Group 2 (G2)</i>	<i>Group 3 (G3)</i>	<i>P</i>	<i>P</i>	<i>P</i>
	<b>Men</b>	<b>Premenopausal</b>	<b>Postmenopausal</b>	<i>G1 vs G2</i>	<i>G1 vs G3</i>	<i>G2 vs G3</i>
	<b>(n=2,397)</b>	<b>Women (n=517)</b>	<b>Women (n=2,294)</b>			
Age, years	64.1 $\pm$ 11.2	45.1 $\pm$ 4.2	66.5 $\pm$ 8.8	<0.001	<0.001	<0.001
BMI, kg/m <sup>2</sup>	23.3 $\pm$ 3.0	22.3 $\pm$ 3.6	22.5 $\pm$ 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 $\pm$ 17.8	90.9 $\pm$ 9.4	96.3 $\pm$ 12.3	<0.001	<0.001	<0.001
TC, mg/dL	198.7 $\pm$ 32.9	199.2 $\pm$ 31.2	215.4 $\pm$ 31.6	1.000	<0.001	<0.001
LDL-C, mg/dL	115.2 $\pm$ 29.6	114.2 $\pm$ 28.5	126.7 $\pm$ 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 $\pm$ 13.8	67.8 $\pm$ 14.7	62.8 $\pm$ 14.9	<0.001	<0.001	<0.001
Non HDL-C, mg/dL	142.4 $\pm$ 32.6	131.4 $\pm$ 31.2	152.5 $\pm$ 31.3	<0.001	<0.001	<0.001
TC/HDL-C	3.7 $\pm$ 1.0	3.1 $\pm$ 0.8	3.6 $\pm$ 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 $\pm$ 0.14	0.22 $\pm$ 0.08	0.29 $\pm$ 0.12	<0.001	<0.001	<0.001

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6 195 Data are expressed as mean±standard deviation (SD), %, and median (25th  
7 196 percentile, 75th percentile). P-values were assessed in one-way analysis of variance  
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9 197 (ANOVA) and post hoc pairwise comparison (Bonferroni). BMI=body mass index; TC=  
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11 198 total cholesterol; LDL-C= low-density lipoprotein cholesterol; TGs= triglycerides;  
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13 199 HDL-C=high-density lipoprotein cholesterol; non HDL-C= non high-density  
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15 200 lipoprotein cholesterol; sdLDL-C=small dense low-density lipoprotein cholesterol.

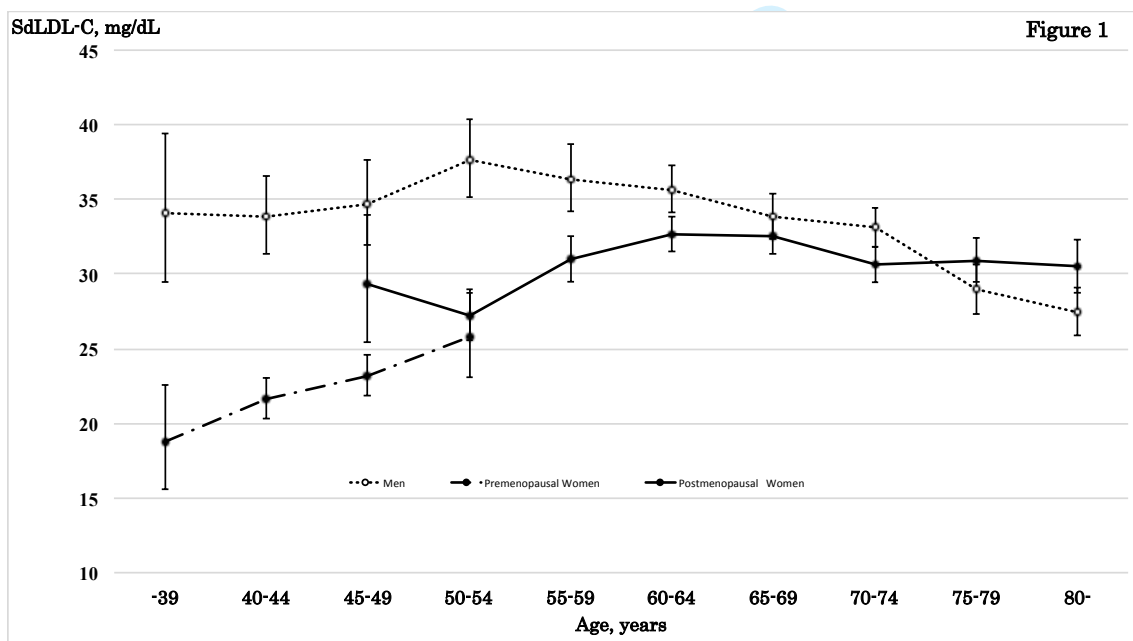
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### 202 **sdLDL-C trends in 5-year age groups**

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

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

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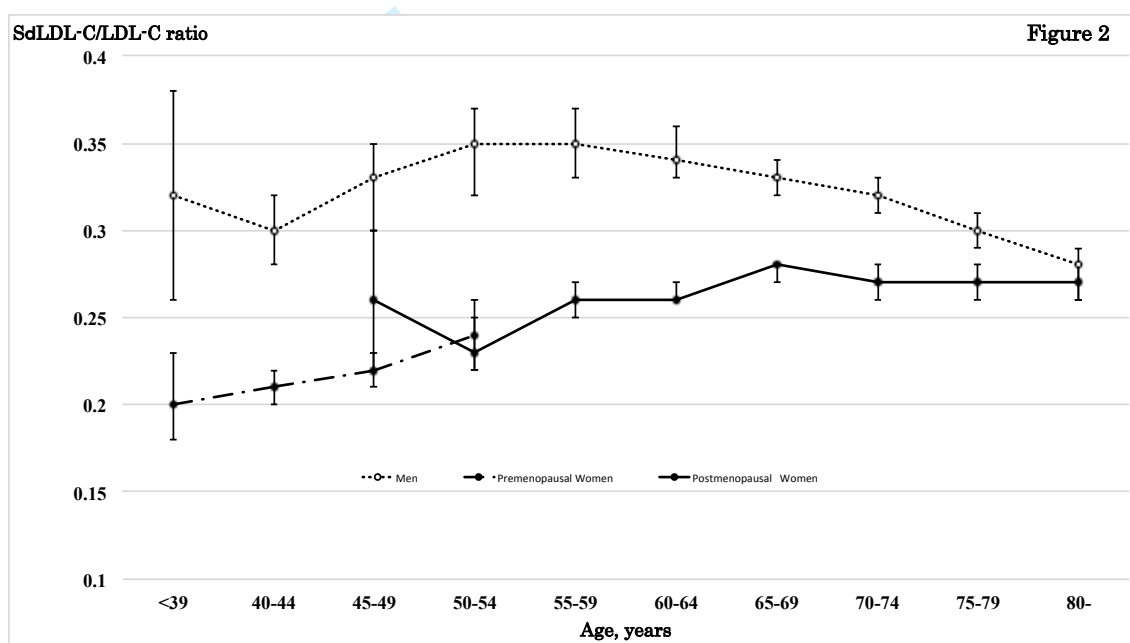


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217 ***sdLDL-C/LDL-C ratio trends in 5-year age groups***

218 SdLDL-C/LDL-C ratio in men increased from 0.30 in 40-44-year-olds to a maximum of  
 219 0.35 in 50-54-year-olds, plateaued in those of 55-59 years, and then decreased from  
 220 0.34 in those of 60-64 years to 0.28 in those of 80 ≤ years (Figure 2). For women, these  
 221 values increased from 0.20 in those < 39 years to a maximum of 0.28 in those of 65-69  
 222 years and plateaued after 70 ≤ years (with mean levels of 0.27). SdLDL-C/LDL-C ratio  
 223 in men was higher than those in women for all age groups and the crossover of  
 224 sdLDL-C/LDL-C ratio for the genders did not occur.

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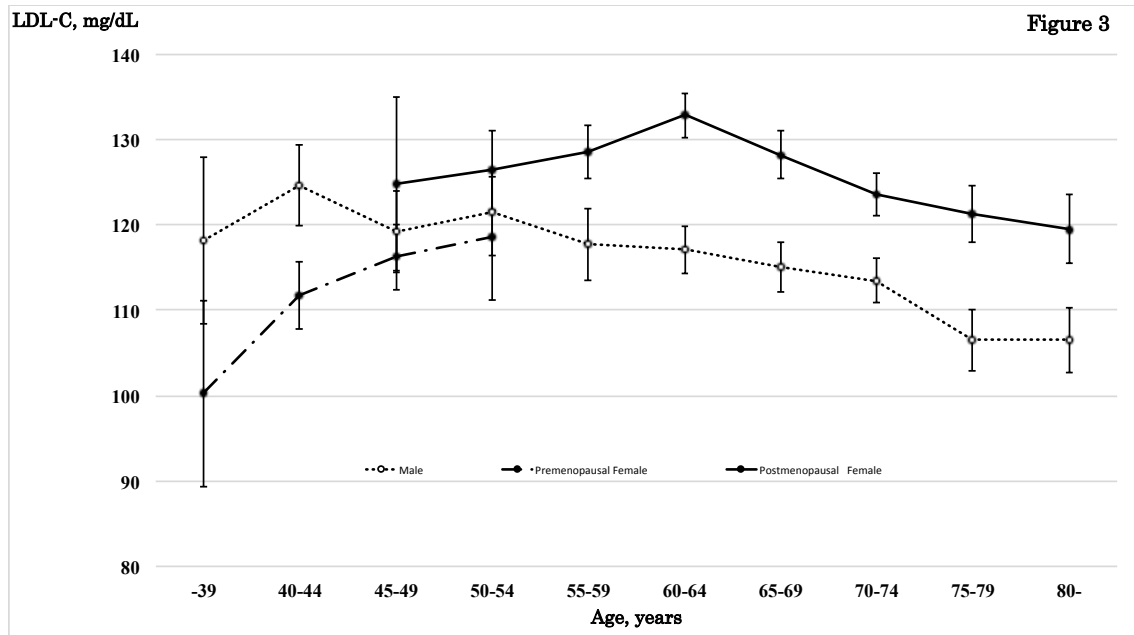
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228 ***Trends in other lipoproteins (LDL-C, total cholesterol, TG, HDL-C, and total***  
 229 ***cholesterol/HDL-C ratio) in 5-year age groups***

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

237 (Supplementary Figure 5).

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

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

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

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255

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

Variable	$\beta$	SE	P
<b>Men <math>\leq 54</math>, n=475; mean <math>\pm</math> SD, 46.7 <math>\pm</math> 4.9 years, Pearson's r= 0.320 (P&lt;0.001)</b>			
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
<b>Men <math>\geq 55</math>, n=1,922; 68.4 <math>\pm</math> 7.6 years, Pearson's r= 0.316 (P&lt;0.001)</b>			
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
<b>Women (Premenopausal), n=517; 45.1 <math>\pm</math> 4.2 years, Pearson's r=0.330 (P&lt;0.001)</b>			
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
<b>Women <math>\leq 64</math> years (Postmenopausal), n=978; 58.3 <math>\pm</math> 4.5 years, Pearson's r=0.261 (P&lt;0.001)</b>			
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
<b>Women 65≥ years (Postmenopausal), n=1,316; 72.6±5.7 year olds, Pearson's r=0.228 (P&lt;0.001)</b>			
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

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

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262 As shown in Table 3, the beta values of age for sdLDL-C/LDL-C ratio in men ≤ 54 years,  
 263 55-59 years, and 60 ≥ years, were 0.003, 0.004, and -0.002, respectively. In women,  
 264 the beta values of age for sdLDL-C/LDL-C ratio in premenopausal women,  
 265 postmenopausal women ≤ 69 years, and 70 ≥ years were 0.001, 0.002, and 0.000,  
 266 respectively. The association between sdLDL-C/LDL-C and age was not significantly  
 267 associated with men 55-59 years, premenopausal women, and postmenopausal  
 268 women 70 ≥ years.

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

Variable	$\beta$	SE	P
<b>Men ≤54 years, n=475; mean±SD, 46.7±4.9 year olds, Pearson's r= 0.320 (P&lt;0.001)</b>			
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
<b>Men 55-59 years, n=245; 57.2±1.4 years, Pearson's r= 0.222 (P&lt;0.001)</b>			
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
<b>Men 60≥ years, n=1,677; 70.0±6.8 years, Pearson's r= 0.272 (P&lt;0.001)</b>			
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
<b>Women (Premenopausal), n=517; 45.1±4.2 years, Pearson's r=0.313 (P&lt;0.001)</b>			
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
<b>Women≤69 years (Postmenopausal), n=1,434; 61.0±5.5 years, Pearson's r=0.264 (P&lt;0.001)</b>			
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
<b>Women 70<math>\geq</math> years (Postmenopausal), n=860; 75.6<math>\pm</math>4.6 year olds, Pearson's r=0.167 (P&lt;0.001)</b>			
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	0.102
Drinker	-0.004	0.006	0.501

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

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 278 Considering the beta value of each variable, 50-year old standardized sdLDL-C levels in  
 279 men, premenopausal women, and postmenopausal women were 26.6 mg / dL (95 %  
 280 CI; 26.4-26.9 mg / dL), 22.7 mg / dL (95 % CI; 22.5-22.9 mg / dL), and 27.4 mg / dL  
 281 (95 % CI; 27.3-27.5 mg/dL), respectively. Standardized sdLDL-C/LDL-C ratio in men,  
 282 premenopausal women, and postmenopausal women were 0.242 (95 % CI;  
 283 0.240-0.244), 0.154 (95 % CI; 0.153-0.156), and 0.227 (95 % CI; 0.224-0.230),  
 284 respectively. These differences between premenopausal women and postmenopausal  
 285 women were significant (Bonferroni analysis,  $P < 0.001$ ).

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## 287 DISCUSSION

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

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6 293 cholesterol profiles. The reason for this gender difference might be related to the  
7 294 mechanism of hypertriglyceridemia in postmenopausal women, which induced small  
8 295 LDL particles.<sup>20-22</sup> There were age or gender-related differences in the ability to  
9 296 generate sdLDL-C from LDL-C. This ability in men was higher than that in women for all  
10 297 age groups or standardized groups, which is identical to the fact that atherosclerosis is  
11 298 more common in men than in women, considering sdLDL-C is a highly atherogenic  
12 299 factor.

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18 300 Our study showed three important results. First, age showed partial correlation trends  
19 301 with sdLDL-C levels and sdLDL-C/LDL-C ratio and non-linear trends between age and  
20 302 sdLDL-C and sdLDL-C/LDL-C ratio were found in both men and women. Therefore,  
21 303 using the sdLDL-C and sdLDL-C/LDL-C ratio, the definition of CVD risk assessment  
22 304 and the adaption of the lipid-lowering therapy should fully consider age-related trends  
23 305 and gender differences.

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29 307 Second, menopausal status was an additional determinant of increasing sdLDL-C and  
30 308 sdLDL-C/LDL-C ratio. Many factors such as excess adiposity, free fatty acids,  
31 309 apo-lipoproteins, and action of lipoprotein lipase activity and cholesterol ester transfer  
32 310 protein affected multiple and complex mechanisms regulating sdLDL.<sup>12,16,17</sup> In  
33 311 postmenopausal women, the decrease of plasma estrogen levels plays a significant role  
34 312 in reducing the clearance of LDL particles via LDL receptor and increasing TG and the  
35 313 number of smaller LDL particles.<sup>23</sup> This hormone change was related to the process of  
36 314 regulating sdLDL-C but there was little evidence available on the association between  
37 315 menopausal status and sdLDL-C or sdLDL-C/LDL-C ratio in a real-world, population  
38 316 setting.<sup>24</sup> Our results showed that sdLDL-C in postmenopausal women was 0.8 or 3.9  
39 317 mg / dL higher than men or premenopausal women in the standardized analysis.

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49 319 Finally, the relationships between age-related trends in sdLDL-C and sdLDL-C/LDL-C  
50 320 ratio and gender were different from traditional lipid factors, such as LDL-C. The  
51 321 crossover of LDL-C for the genders occurred in middle-aged patients. On the contrary,  
52 322 the crossover of sdLDL-C occurred between 70-74 years and the sdLDL-C/LDL-C ratio  
53 323 did not occur. Rather than LDL-C, the results of the sdLDL-C and sdLDL-C/LDL-C ratio  
54 324 might reflect the fact that, for all age groups, men have more susceptible to CVD than  
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6 325 women, even with the narrowing gap of risk for CVD in postmenopausal women.<sup>25</sup>

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9 327 Our findings suggest that a subgroup-specific approach is required to develop efficient  
10 328 CVD prevention strategies using the sdLDL-C and sdLDL-C/LDL-C ratio.

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16 331 **Limitations**

18 332 Our study has several limitations. First, age-related trends and levels of traditional lipid  
19 333 factors were almost similar to National Health and Nutrition Survey in Japan and our  
20 334 age-related trends of these factors were also similar to the trends of the Korean and  
21 335 Chinese Singaporeans population.<sup>14,15</sup> But the trends of the US population or healthy  
22 336 Caucasian<sup>26,27</sup> were not similar. Especially in healthy Caucasian patients aged  $\geq 70$   
23 337 years, the trends for TC, LDL-C, and nonHDL-C differed from our observed trends and  
24 338 continuously increased. Although our results could not identify the mechanism, there  
25 339 might be racial differences. Therefore, it is unclear whether our results of sdLDL-C  
26 340 would be valid for these populations. Second, compared with mean lipid levels of the  
27 341 Korean population from KNHANES, Japanese men showed higher mean TC, LDL-C,  
28 342 and HDL levels (TC 199 mg / dL; LDL-C 115 mg / dL; HDL-C 56 mg / dL) compared to  
29 343 Korean men (TC 183 mg / dL; LDL-C 106 mg / dL; HDL 50 mg / dL), and Japanese  
30 344 women also showed higher mean levels (TC 212 mg / dL; LDL-C 124 mg / dL; HDL-C  
31 345 64 mg / dL) than Korean women (TC 188 mg / dL; LDL-C 111 mg / dL; HDL-C 55  
32 346 mg / dL). The reason for the difference in the lipoprotein profile between Japanese and  
33 347 Korean populations might be due to genetics and environmental factors. It is also  
34 348 unknown whether these factors might affect sdLDL-C levels and sdLDL-C/LDL-C ratio  
35 349 because sdLDLs are regulated through complex mechanisms. Third, we did not control  
36 350 for the effects of diet, life activity, socioeconomic status, and genetic factors, which  
37 351 might be associated with changes in lipid metabolism.<sup>28,29,30</sup>

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54 353 **CONCLUSION**

56 354 SdLDL-C and sdLDL-C/LDL-C ratio are differently distributed by age, gender, and  
57 355 menopausal status. Our findings suggest that a subgroup-specific approach is required

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6 356 to develop efficient CVD prevention strategies using the sdLDL-C and sdLDL-C/LDL-C  
7 357 ratio.  
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10 359 **List of abbreviations**

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12 360 sdLDL-C: small dense low-density lipoprotein cholesterol; CVD: cardiovascular disease;

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14 361 LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; LDL-C: low-density

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16 362 lipoprotein cholesterol; TGs :triglycerides; HDL-C :high-density lipoprotein cholesterol:

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18 363 nonHDL-C: non-high-density lipoprotein cholesterol; LNsdLDL-C: log-transformed small

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20 364 dense low-density lipoprotein cholesterol; JMS: Jichi Medical School; ANOVA; analysis

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6 368 **DECLARATIONS**

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16 374 **Ethics approval and consent to participate**

17 375 All procedures performed in studies involving human participants were in accordance  
18 376 with the ethical standards of the institutional and/or national research committee and  
19 377 with the 1964 Helsinki declaration and its later amendments or comparable ethical  
20 378 standards. All the participants included in the present study provided written informed  
21 379 consent prior to inclusion, and this study was approved by the Institutional Review  
22 380 Board of Jichi Medical School (Tochigi, Japan, IRB No. G09-39 [G17-64 revised]).

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29 382 **AUTHOR CONTRIBUTIONS**

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31 383 All authors have participated in the research and designed the study; TI and SI  
32 384 performed the statistics analysis; TI contributed to the drafting of the manuscript. All  
33 385 authors read and approved the final manuscript.

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38 387 **Consent for publication**

39 388 All the participants included in the present study provided written informed consent for  
40 389 publication.

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45 391 **Competing interests**

46 392 The authors declare they have no conflict of interest with respect to this research study  
47 393 and paper.

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6 508 **FIGURE LEGENDS**  
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8 509 **Figure 1. Geometric mean and 95% confidence interval of sdLDL-C for age,**  
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11 **gender, and menopausal status**  
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15 512 **Figure 2. Mean and 95% confidence interval of sdLDL-C/LDL-C ratio for age,**  
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17 **gender, and menopausal status**  
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20 515 **Figure 3. Mean and 95% confidence interval of LDL-C for age, gender, and**  
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22 **menopausal status**  
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25 518 **Supplementary Material**

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27 519 **Supplementary Figure 1. Mean of total cholesterol for age, gender, and**  
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29 **menopausal status**  
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32 522 **Supplementary Figure 2. Mean of non-high-density lipoprotein cholesterol for age,**  
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34 **gender, and menopausal status**  
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37 525 **Supplementary Figure 3. Mean of total cholesterol / high-density lipoprotein**  
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39 **cholesterol ratio for age, gender, and menopausal status**  
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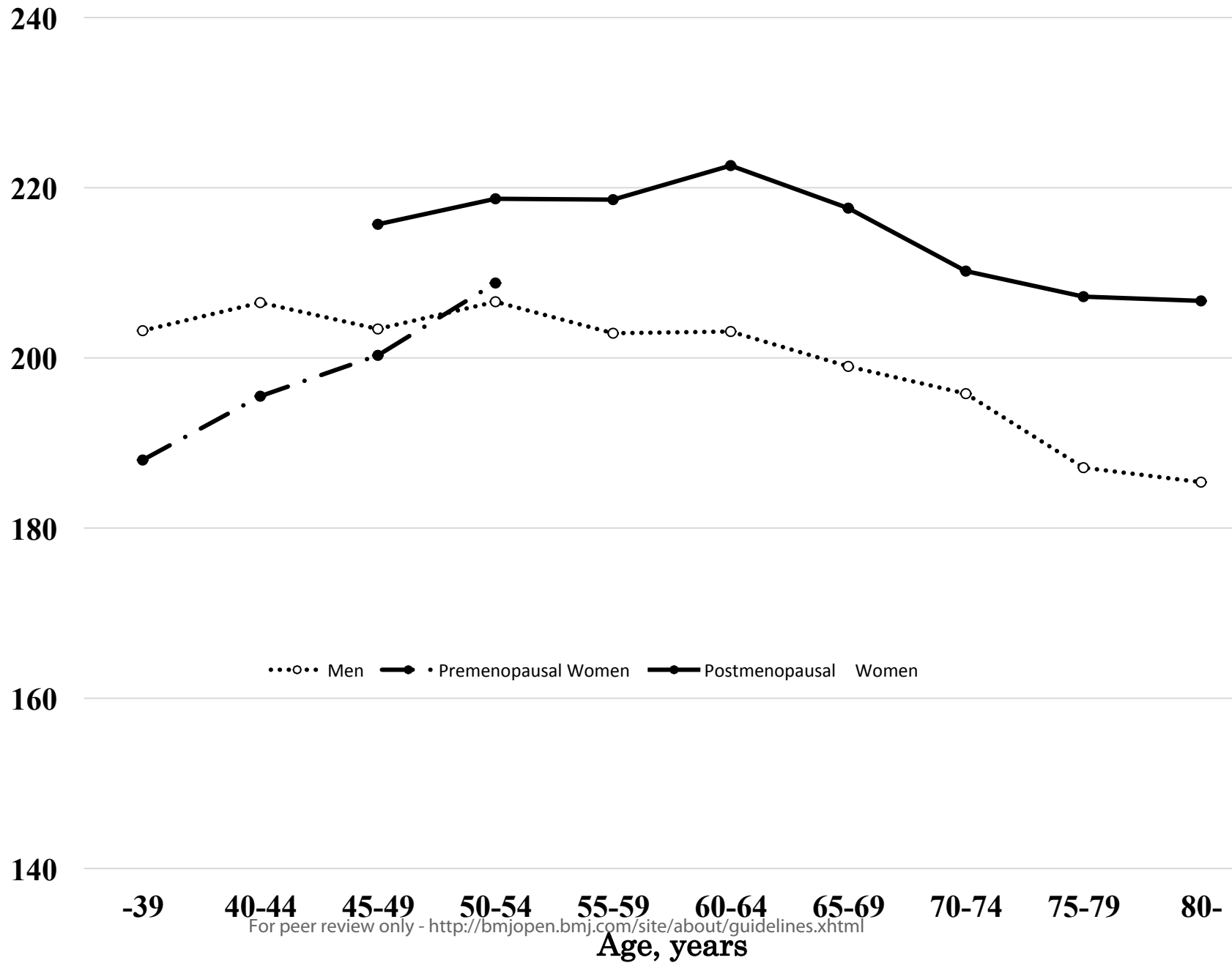
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44 529 **Supplementary Figure 4. Geometric mean of triglycerides for age, gender, and**  
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46 **menopausal status**  
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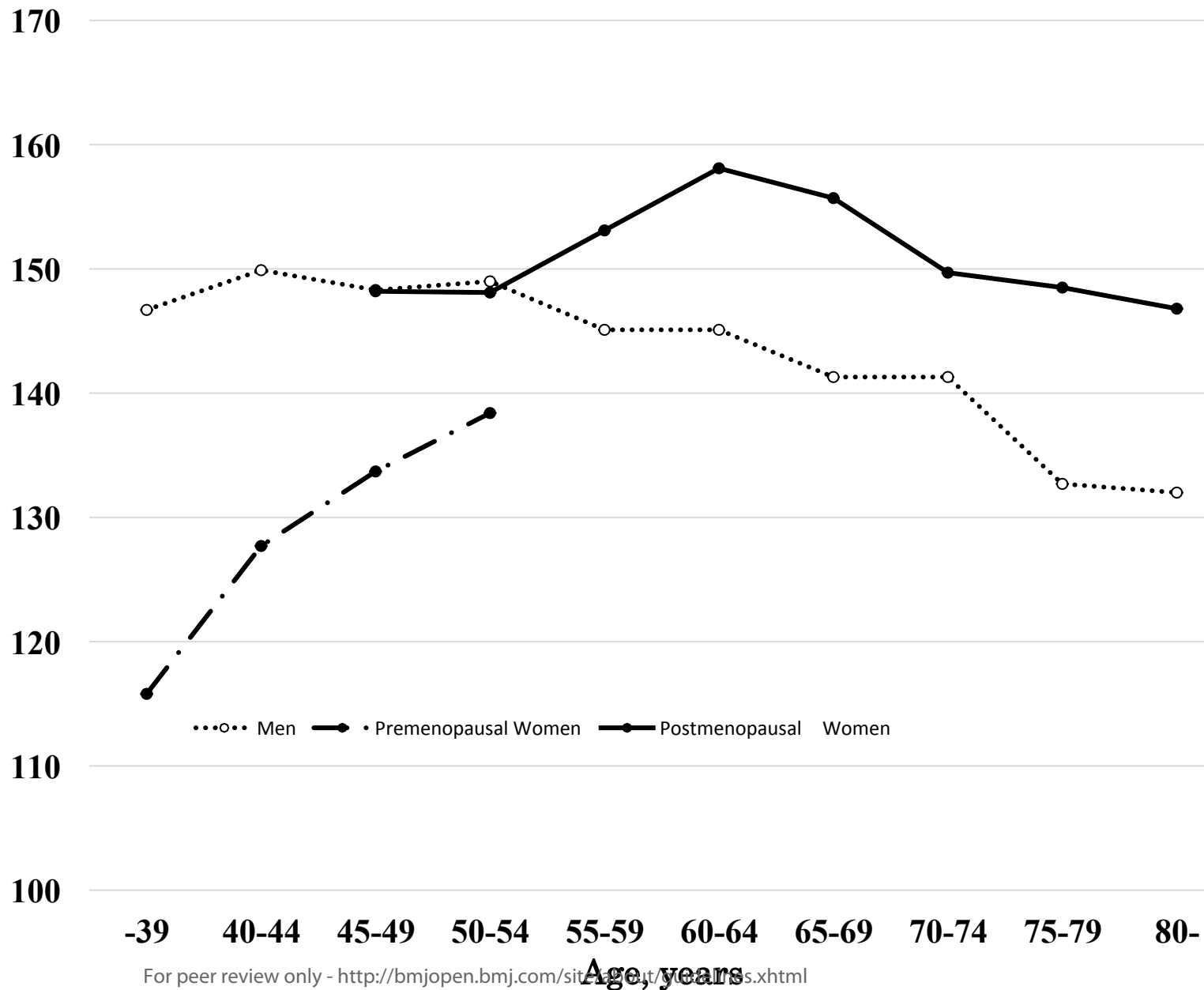
49 532 **Supplementary Figure 5. Mean of high-density lipoprotein cholesterol for age,**  
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51 **gender, and menopausal status**  
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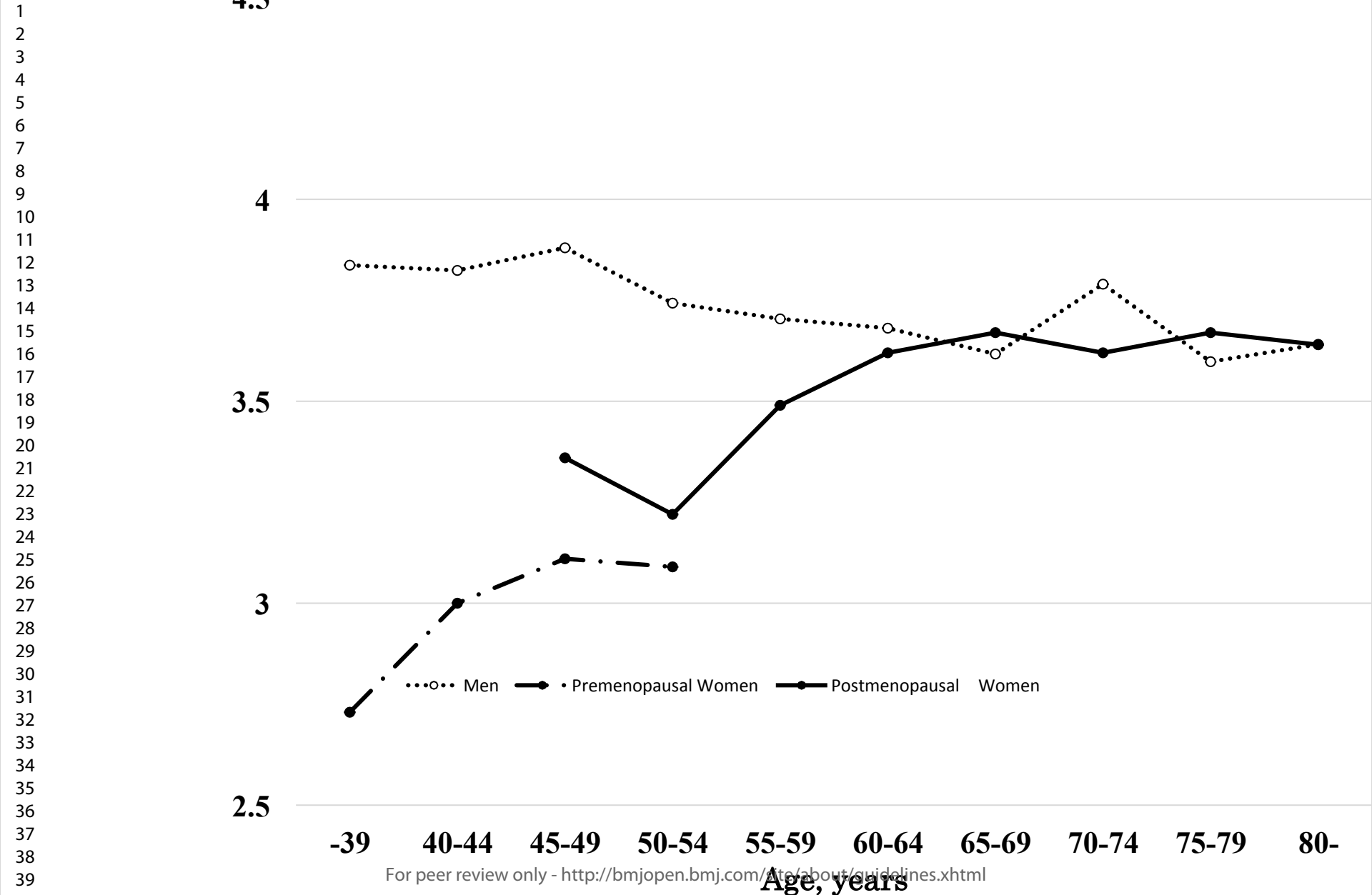
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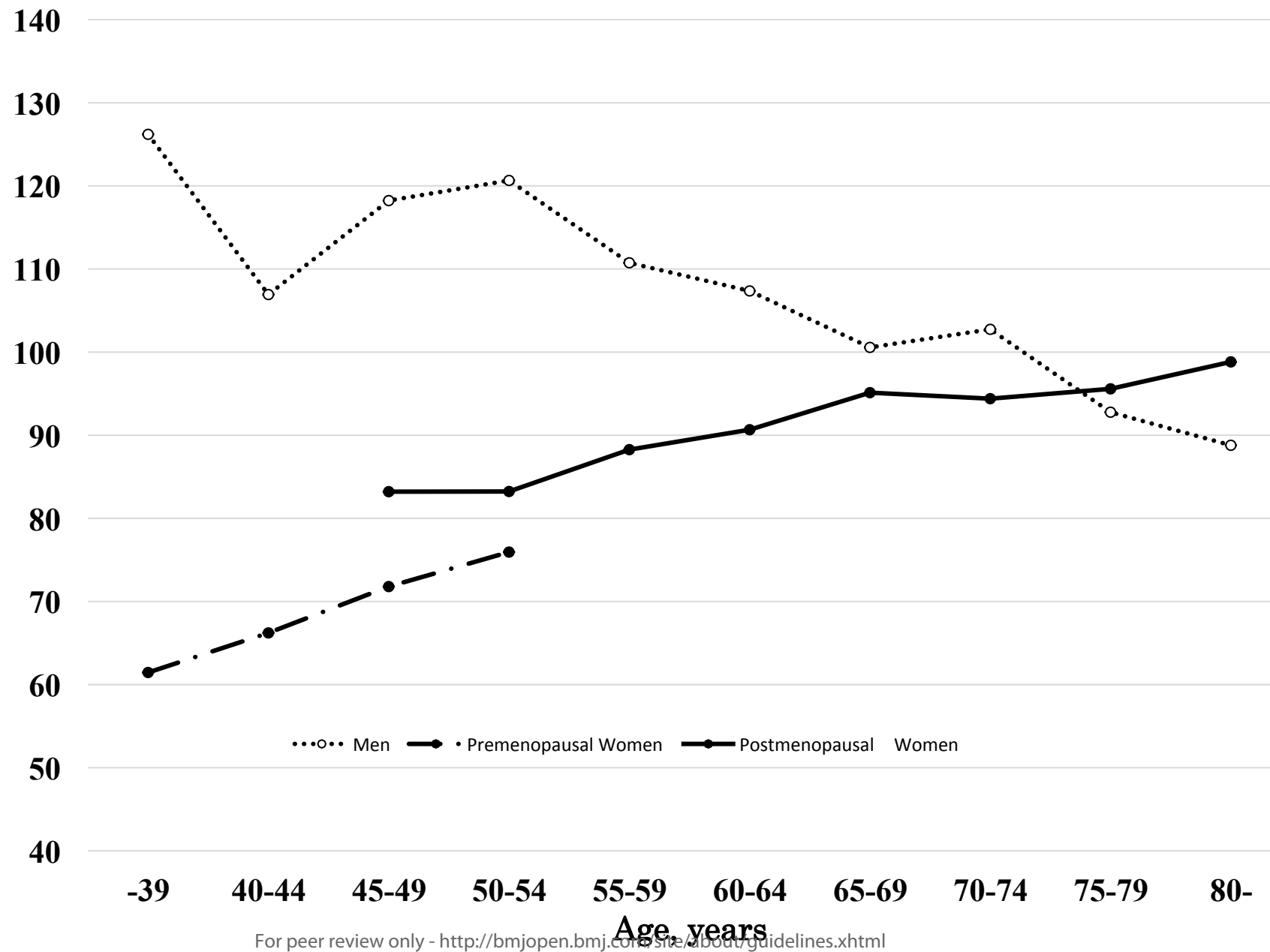


Men Premenopausal Women Postmenopausal Women

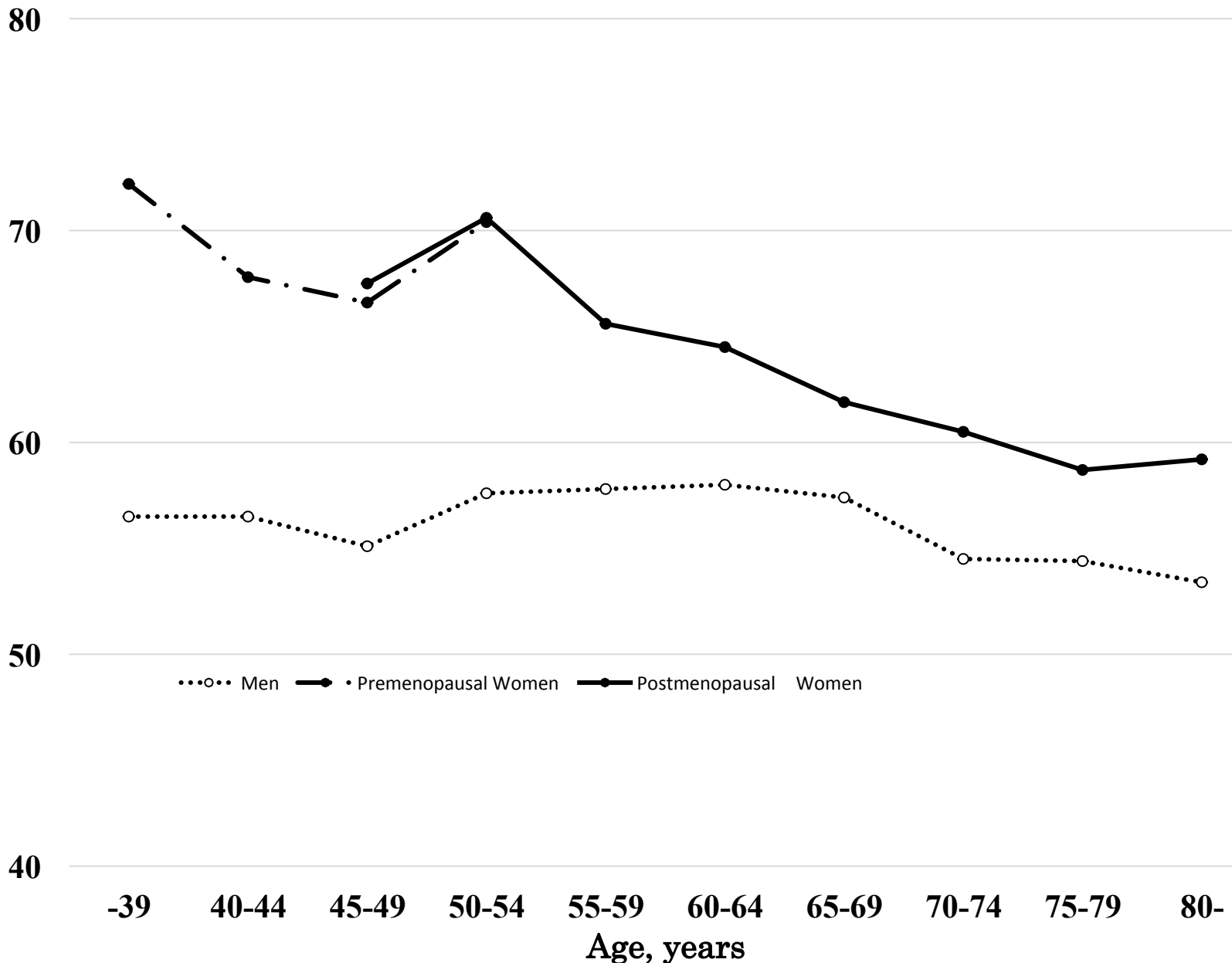




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Men Premenopausal Women Postmenopausal Women

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

Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	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
<b>Introduction</b>			
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
<b>Methods</b>			
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	-
<b>Results</b>			

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
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	12-16
<b>Limitations</b>			
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
<b>Other information</b>			
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](http://www.strobe-statement.org).

# BMJ Open

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

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<b>Primary Subject Heading</b>:	Epidemiology
Secondary Subject Heading:	Public health, Global health, Epidemiology, Cardiovascular medicine
Keywords:	CARDIOLOGY, Heart failure < CARDIOLOGY, Ischaemic heart disease < CARDIOLOGY, INTERNAL MEDICINE

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6 1 **[TITLE] The Association between Age, Gender, Menopausal Status, and Small**  
7 **Dense Low-Density Lipoprotein Cholesterol; A Cross-Sectional Study**  
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9 3

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36 26

37 27 **Keywords:** small dense low-density lipoprotein cholesterol, small dense low-density  
38 28 lipoprotein cholesterol / low-density lipoprotein cholesterol ratio, age, gender,  
39 29 menopause  
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41 31 **Total word count:** 3024 (abstract: 279, without keywords; main text: 2745, from the  
42 32 introduction until the conclusion)  
43 33

44 34 **Number of Tables:** 3

45 35 **Figures:** 3

46 36 **Supplementary materials:** 7  
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48 38 **AUTHOR CONTRIBUTIONS**  
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6 39 TI, YN, YS, and SI have participated in the research and designed the study; TI and SI  
7 40 performed the statistics analysis; TI contributed to the drafting of the manuscript. YN,  
8 41 YS, and SI provided feedback on the manuscript, and all authors read and approved the  
9 42 final manuscript.  
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6 43 **ABSTRACT**

7 44 **Objectives:** Small dense low-density lipoprotein cholesterol (sdLDL-C) might be a  
8 45 better cardiovascular disease (CVD) indicator than low-density lipoprotein cholesterol  
9 46 (LDL-C); however, details regarding its epidemiology remain elusive. The present study  
10 47 aimed at evaluating the association between the demographic factors, such as age,  
11 48 gender, and menopausal status, and sdLDL-C levels and sdLDL-C/LDL-C ratio in the  
12 49 Japanese population.

13 50  
14 51 **Design:** This was a cross-sectional study.

15 52  
16 53 **Setting:** 13 rural districts in Japan, 2010-2017

17 54  
18 55 **Participants:** This study included 5,208 participants (2,397 men and 2,811 women),  
19 56 who underwent the health mass screening that was conducted in accordance with the  
20 57 medical care system for the elderly and obtained informed consent for this study.

21 58  
22 59 **Results:** In total, 517 premenopausal women (mean age  $\pm$  SD, 45.1  $\pm$  4.2 years), 2,294  
23 60 postmenopausal women (66.5  $\pm$  8.8 years) and 2,397 men (64.1  $\pm$  11.2 years) were  
24 61 analyzed. In men, the sdLDL-C levels and sdLDL-C/LDL-C ratio increased during  
25 62 younger adulthood, peaked (36.4 mg/dL, 0.35) at 50–54 years, and then decreased. In  
26 63 women, relatively regular increasing trends of sdLDL-C level and sdLDL-C/LDL-C ratio  
27 64 until approximately 65 years (32.7 mg/dL, 0.28), followed by a downward or plateaued  
28 65 trend. Given the beta value of age, body mass index, fasting glucose, and smoking and  
29 66 drinking status by multiple linear regression analysis, standardized sdLDL-C levels and  
30 67 sdLDL-C/LDL-C ratio in 50-year old men, premenopausal women, and postmenopausal  
31 68 women were 26.6, 22.7, and 27.4 mg/dL and 0.24, 0.15, and 0.23, respectively. The  
32 69 differences between premenopausal and postmenopausal women were significant  
33 70 ( $P < 0.001$ ).

34 71  
35 72 **Conclusions:** SdLDL-C and sdLDL-C/LDL-C ratios showed different distributions by  
36 73 age, gender, and menopausal status. A subgroup-specific approach would be  
37 74 necessary to implement sdLDL-C for CVD prevention strategies, fully considering  
38 75 age-related trends, gender differences, and menopausal status.



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6 **77 Strengths and limitations of this study**

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8 78 1. To the best of our knowledge, the present study is the first to demonstrate the  
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10 79 association between age, gender, and menopausal status on the sdLDL-C and  
11  
12 80 sdLDL-C/LDL-C ratio.

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14 81 2. This study is based on a large representative sample from Japanese general  
15  
16 82 population.

17  
18 83 3. Serum lipid markers were measured by the standardized program proposed by the  
19  
20 84 Clinical and Laboratory Standards Institute.

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22 85 4. It is unclear whether our results of sdLDL-C would be valid for other populations.

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24 86 5. This study did not control for several confounding factors, such as diet, life activity,  
25  
26 87 socioeconomic status, and genetic factors.

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## 90 INTRODUCTION

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

104  
105 However, almost all of the current analytical strategies might be not able to adjust  
106 accurately the interaction between age and sdLDL-C. Few studies have evaluated how  
107 age is associated with sdLDL-C and sdLDL-C/LDL-C ratio over a wide age range and  
108 distinguished the effects of menopause and gender on sdLDL-C and sdLDL-C fraction  
109 from those of aging.<sup>10,11</sup>

110  
111 Diet composition, which is affected by aging, is associated with blood cholesterol and  
112 the absorption, synthesis, and metabolism per se of fat and lipoproteins change with  
113 age.<sup>12,13</sup> Another study showed Asian age-related trends of traditional lipid profiles  
114 displayed roughly an increasing trend, followed by a decreasing one at the middle-aged  
115 stage.<sup>14,15</sup> Meanwhile, sdLDL-C has been regulated by more complex mechanisms than  
116 regulating traditional lipids and might be plateaued or increased even at the  
117 middle-aged by changed metabolic functions with aging influencing sdLDL-C  
118 synthesis.<sup>5,7,12,16,17</sup> Furthermore, the detailed multiple mechanisms of metabolizing  
119 sdLDLs are unknown in the real-world, population-based setting and the age-related  
120 trend of sdLDL-C might be different from the sdLDL-C/LDL-C ratio. In other words, the  
121 ability to generate sdLDL-C from LDL-C might be different among each generation,

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6 122 gender, and menopausal status. Therefore, we evaluated the association between the  
7 123 demographic factors, such as age, gender, and menopausal status, and sdLDL-C and  
8 124 sdLDL-C/LDL-C ratio in Japanese general population.  
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## 14 127 **METHODS**

### 17 128 ***Population***

19 129 The present cross-sectional study was conducted as part of the Jichi Medical School  
20 130 (JMS)-II Cohort Study, a population-based cohort study of the risk factors of  
21 131 atherosclerosis and CVD in the Japanese general population. A total of 6,436  
22 132 individuals participated in this study. Details of the methods of enrollment have been  
23 133 reported previously.<sup>18,19</sup> In brief, from April 2010 through December 2017, this study  
24 134 evaluated Japanese individuals who were residents of 13 rural districts in Japan,  
25 135 Shimotsuke, Kakara, Sue, Omori, Kamiichi, Wara, Takasu, Onabi, Nakatsu, Yame,  
26 136 Miwa, Ueno, and Saji areas. Local government offices in each community issued  
27 137 invitations to eligible residents for the mass CVD screening, and personal invitations  
28 138 were also sent to all potential participants by mail. All the participants in the present  
29 139 study provided written informed consent prior to inclusion. The study protocol and data  
30 140 analysis plan were approved by the institutional review board of Jichi Medical School  
31 141 (Tochigi, Japan, IRB No. G09-39 [G17-64 revised]).

32 142 We excluded individuals as follows: 1) taking lipid-lowering agents or  
33 143 anti-hyperglycemia agents (n = 1,073); 2) the use of hormone replacement therapy (n =  
34 144 96); and 3) the data such as age, gender status, menopausal status, and sdLDL-C were  
35 145 not available (n = 73).  
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### 50 147 ***Measurements***

51 148 A central committee, composed of the chief medical officers of all 13 participating  
52 149 districts, developed a detailed manual for data collection. Body weight was recorded  
53 150 with the subjects clothed. Height was measured with stockings feet. Body mass index  
54 151 (BMI) was calculated as weight (kg) / height (m<sup>2</sup>). Blood samples were taken after  
55 152 overnight fasting. TC was measured via a cholesterol dehydrogenase-ultraviolet  
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6 153 method. Triglycerides (TG) was measured using an enzymatic method. LDL-C and  
7 154 high-density lipoprotein cholesterol (HDL-C) were measured by direct methods using a  
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9 155 commercial kit (Cholestest from Sekisui Medical, Tokyo, Japan). SdLDL-C level was  
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11 156 directly and selectively measured using a commercial kit (sdLDL-EX from Denka  
12  
13 157 Seiken, Tokyo, Japan). An external laboratory (SRL, Tokyo, Japan) measured the  
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15 158 serum lipid markers. The markers were measured by the standardized program  
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17 159 proposed by the Clinical and Laboratory Standards Institute. The nonHDL-C was  
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19 160 calculated by subtracting HDL-C from TC. Information about medical history, lifestyle,  
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21 161 and menopausal status were obtained with a self-reported questionnaire. Smoking  
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23 162 status was classified as smoking, former smoking, or never-smoking.  
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163

#### 164 **Statistical analysis**

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26 165 Baseline characteristics were summarized as mean  $\pm$  standard deviation (SD) for  
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28 166 normally distributed continuous variables and numbers and percentages for categorical  
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30 167 variables. SdLDL-C and TG were highly skewed; these data were expressed as the  
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32 168 median and interquartile range and transformed into natural logarithms before statistical  
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34 169 analysis. The participants were divided into three groups (men, premenopausal women,  
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36 170 and postmenopausal women) according to gender and menopausal status.

37 171 The one-way analysis of variance (ANOVA) was used for comparison among three  
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39 172 groups, and differences were tested via post hoc pairwise comparison (Bonferroni). To  
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41 173 explore the age-related trend in sdLDL-C and sdLDL-C/LDL-C ratio with age, geometric  
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43 174 means or means and 95 percent confidence intervals for each variable in 5-year age  
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45 175 ranges were derived and plotted against age range in each of the three groups.

46 176 Among the three groups, correlations between age and each parameter were assessed  
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48 177 using multiple linear regression analysis. Considering the beta value of age, body mass  
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50 178 index, fasting glucose, and smoking and drinking status, we calculated the estimated  
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52 179 sdLDL-C and sdLDL-C/LDL-C ratio. The agreement between the estimated sdLDL-C  
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54 180 and sdLDL-C/LDL-C ratio and measured ones was assessed by Pearson's correlation  
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56 181 coefficient. To evaluate the effect of menopausal status on sdLDL-C and  
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58 182 sdLDL-C/LDL-C ratio, using the beta value of each variable from the analysis in the  
59  
60 183 premenopausal and postmenopausal group, data were standardized to a nominal 50  
184 years of menopausal age, never smoking and zero alcohol for participants with normal

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

187

### 188 ***Patient and public involvement***

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

## 191 **RESULTS**

### 192 ***Baseline characteristics***

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

201

202

203 Table 1 Baseline characteristics

	<i>Group 1 (G1)</i>	<i>Group2 (G2)</i>	<i>Group3 (G3)</i>	<i>P</i>	<i>P</i>	<i>P</i>
	Men	Premenopausal	Postmenopausal	<i>P</i>	<i>P</i>	<i>P</i>
	(n=2,397)	Women (n=517)	Women (n=2,294)	<i>G1 vs</i>	<i>G1 vs</i>	<i>G2 vs</i>
				<i>G2</i>	<i>G3</i>	<i>G3</i>
Age, years	64.1 $\pm$ 11.2	45.1 $\pm$ 4.2	66.5 $\pm$ 8.8	<0.001	<0.001	<0.001
BMI, kg/m <sup>2</sup>	23.3 $\pm$ 3.0	22.3 $\pm$ 3.6	22.5 $\pm$ 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

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

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### 211 ***sdLDL-C trends in 5-year age groups***

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

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6 214 plotted against gender.

7 215 For men, the level of sdLDL-C increased from 34.1 mg / dL in those < 39 years to a  
8 216 maximum of 37.7 mg / dL in those of 50-54 years, followed by decreasing from 36.4  
9 217 mg / dL in those of 55-59 years to 27.4 mg / dL in those of 80 ≤ years (Figure 1). For  
10 218 women, a relatively regular increasing trend of the sdLDL-C level was found up to 60-64  
11 219 year-olds. After 65 years, a downward trend was fitted. The maximum of the sdLDL-C  
12 220 level of women was 32.7 mg / dL. Moreover, sdLDL-C levels in men were higher than  
13 221 those in women for all age groups younger than 70-74-year-olds but exceeded those in  
14 222 women after the age of 75-79 years.  
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#### 226 ***sdLDL-C/LDL-C ratio trends in 5-year age groups***

227 SdLDL-C/LDL-C ratio in men increased from 0.30 in 40-44-year-olds to a maximum of  
228 0.35 in 50-54-year-olds, plateaued in those of 55-59 years, and then decreased from  
229 0.34 in those of 60-64 years to 0.28 in those of 80 ≤ years (Figure 2). For women, these  
230 values increased from 0.20 in those < 39 years to a maximum of 0.28 in those of 65-69  
231 years and plateaued after 70 ≤ years (with mean levels of 0.27). SdLDL-C/LDL-C ratio  
232 in men was higher than those in women for all age groups and the crossover of  
233 sdLDL-C/LDL-C ratio for the genders did not occur.  
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#### 237 ***Trends in other lipoproteins (LDL-C, total cholesterol, TG, HDL-C, and total 238 cholesterol/HDL-C ratio) in 5-year age groups***

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



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

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

256 As shown in Table 2, among men, age was positively and negatively associated with  
 257 log-transformed small dense low-density lipoprotein cholesterol (LNsdLDL-C) levels in  
 258 those  $\leq 54$  years and  $\geq 55$  years. Among premenopausal women, postmenopausal  
 259 women  $\leq 64$  years, and postmenopausal women  $65 \geq$  years, age was positively,  
 260 positively, and negatively associated with LNsdLDL-C levels. But the association  
 261 between LNsdLDL-C and age was not significantly associated with men  $\leq 54$  years.

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

Variable	$\beta$	SE	P
<b>Men <math>\leq 54</math>, n=475; mean <math>\pm</math> SD, 46.7 <math>\pm</math> 4.9 years, Pearson's <math>r = 0.320</math> (P&lt;0.001)</b>			
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
<b>Men <math>\geq 55</math>, n=1,922; 68.4 <math>\pm</math> 7.6 years, Pearson's <math>r = 0.316</math> (P&lt;0.001)</b>			
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
<b>Women (Premenopausal), n=517; 45.1±4.2 years, Pearson's r=0.330 (P&lt;0.001)</b>			
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
<b>Women ≤64 years (Postmenopausal), n=978; 58.3±4.5 years, Pearson's r=0.261 (P&lt;0.001)</b>			
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
<b>Women 65≥ years (Postmenopausal), n=1,316; 72.6±5.7 year olds, Pearson's r=0.228 (P&lt;0.001)</b>			
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

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

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270 As shown in Table 3, age in men  $\leq$  54 years, 55-59 years, and 60  $\geq$  years, was  
 271 positively, positively, and negatively associated with sdLDL-C/LDL-C ratio. In women,  
 272 age in premenopausal women, postmenopausal women  $\leq$  69 years was positively  
 273 associated with sdLDL-C/LDL-C ratio, whereas age in postmenopausal women 70  $\geq$   
 274 years was not significantly associated sdLDL-C/LDL-C ratio. The association between  
 275 sdLDL-C/LDL-C and age was not significantly associated with men 55-59 years,  
 276 premenopausal women, and postmenopausal women 70  $\geq$  years.

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

Variable	$\beta$	SE	P
<b>Men <math>\leq</math>54 years, n=475; mean <math>\pm</math> SD, 46.7 <math>\pm</math> 4.9 year olds, Pearson's r= 0.320 (P&lt;0.001)</b>			
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
<b>Men 55-59 years, n=245; 57.2 <math>\pm</math> 1.4 years, Pearson's r= 0.222 (P&lt;0.001)</b>			
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
<b>Men 60 <math>\geq</math> years, n=1,677; 70.0 <math>\pm</math> 6.8 years, Pearson's r= 0.272 (P&lt;0.001)</b>			
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
<b>Women (Premenopausal), n=517; 45.1±4.2 years, Pearson's r=0.313 (P&lt;0.001)</b>			
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
<b>Women ≤69 years (Postmenopausal), n=1,434; 61.0±5.5 years, Pearson's r=0.264 (P&lt;0.001)</b>			
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
<b>Women 70≥ years (Postmenopausal), n=860; 75.6±4.6 year olds, Pearson's r=0.167 (P&lt;0.001)</b>			
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	0.102
Drinker	-0.004	0.006	0.501

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

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286 Considering the beta value of each variable, 50-year old standardized sdLDL-C levels in  
287 men, premenopausal women, and postmenopausal women were 26.6 mg / dL (95 %  
288 CI; 26.4-26.9 mg / dL), 22.7 mg / dL (95 % CI; 22.5-22.9 mg / dL), and 27.4 mg / dL  
289 (95 % CI; 27.3-27.5 mg/dL), respectively. Standardized sdLDL-C/LDL-C ratio in men,  
290 premenopausal women, and postmenopausal women were 0.24 (95 % CI; 0.24-0.24),  
291 0.15 (95 % CI; 0.15-0.16), and 0.23 (95 % CI; 0.22-0.23), respectively. These  
292 differences between premenopausal women and postmenopausal women were  
293 significant (Bonferroni analysis,  $P < 0.001$ ).

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## 295 DISCUSSION

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

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

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

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6 317 apo-lipoproteins, and action of lipoprotein lipase activity and cholesterol ester transfer  
7 318 protein affected multiple and complex mechanisms regulating sdLDL.<sup>12,16,17</sup> In  
8 319 postmenopausal women, the decrease of plasma estrogen levels plays a significant role  
9 320 in reducing the clearance of LDL particles via LDL receptor and increasing TG and the  
10 321 number of smaller LDL particles.<sup>23</sup> This hormone change was related to the process of  
11 322 regulating sdLDL-C but there was little evidence available on the association between  
12 323 menopausal status and sdLDL-C or sdLDL-C/LDL-C ratio in a real-world, population  
13 324 setting.<sup>24</sup> Our results showed that sdLDL-C in postmenopausal women was 0.8 or 3.9  
14 325 mg / dL higher than men or premenopausal women in the standardized analysis.  
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23 327 Finally, the relationships between age-related trends in sdLDL-C and sdLDL-C/LDL-C  
24 328 ratio and gender were different from traditional lipid factors, such as LDL-C. The  
25 329 crossover of LDL-C for the genders occurred in middle-aged patients. On the contrary,  
26 330 the crossover of sdLDL-C occurred between 70-74 years and the sdLDL-C/LDL-C ratio  
27 331 did not occur. Rather than LDL-C, the results of the sdLDL-C and sdLDL-C/LDL-C ratio  
28 332 might reflect the fact that, for all age groups, men have more susceptible to CVD than  
29 333 women, even with the narrowing gap of risk for CVD in postmenopausal women.<sup>25</sup>  
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35 335 Our findings suggest that a subgroup-specific approach is required to develop efficient  
36 336 CVD prevention strategies using the sdLDL-C and sdLDL-C/LDL-C ratio.  
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### 43 339 **Limitations**

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45 340 Our study has several limitations. First, age-related trends and levels of traditional lipid  
46 341 factors were almost similar to National Health and Nutrition Survey in Japan and our  
47 342 age-related trends of these factors were also similar to the trends of the Korean and  
48 343 Chinese Singaporeans population.<sup>14,15</sup> But the trends of the US population or healthy  
49 344 Caucasian<sup>26,27</sup> were not similar. Especially in healthy Caucasian patients aged  $\geq 70$   
50 345 years, the trends for TC, LDL-C, and nonHDL-C differed from our observed trends and  
51 346 continuously increased. Although our results could not identify the mechanism, there  
52 347 might be racial differences. Therefore, it is unclear whether our results of sdLDL-C  
53 348 would be valid for these populations. Second, compared with mean lipid levels of the  
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6 349 Korean population from KNHANES, Japanese men showed higher mean TC, LDL-C,  
7 350 and HDL levels (TC 199 mg / dL; LDL-C 115 mg / dL; HDL-C 56 mg / dL) compared to  
8 351 Korean men (TC 183 mg / dL; LDL-C 106 mg / dL; HDL 50 mg / dL), and Japanese  
9 352 women also showed higher mean levels (TC 212 mg / dL; LDL-C 124 mg / dL; HDL-C  
10 353 64 mg / dL) than Korean women (TC 188 mg / dL; LDL-C 111 mg / dL; HDL-C 55  
11 354 mg / dL). The reason for the difference in the lipoprotein profile between Japanese and  
12 355 Korean populations might be due to genetics and environmental factors. It is also  
13 356 unknown whether these factors might affect sdLDL-C levels and sdLDL-C/LDL-C ratio  
14 357 because sdLDLs are regulated through complex mechanisms. Third, we did not control  
15 358 for the effects of diet, life activity, socioeconomic status, and genetic factors, which  
16 359 might be associated with changes in lipid metabolism.<sup>28,29,30</sup> Fourth, there might be  
17 360 several biases. Selection bias might come from potential  
18 361 non-representativeness of the study population, which was rural dwelling. There  
19 362 might be information bias and data misclassification due to error in  
20 363 measurement of the lipid parameters. Fifth, as shown in the supplementary  
21 364 figure 6 and 7, the results regarding the association between demographic  
22 365 factors and sdLDL-C and sdLDL-C/LDL-C ratio remained the same in 6,282  
23 366 participants including patients taking lipid-lowering therapy. SdLDL-C/LDL-C  
24 367 ratio in men including patients taking lipid-lowering therapy was higher than in  
25 368 men excluding these patients (0.45 vs 0.35). Our assessment was limited in  
26 369 terms of this difference, because data regarding type and dose of medications  
27 370 for dyslipidemia were not available. We need to validate the association in  
28 371 patients taking lipid-lowering therapy in another cohort. Finally, our study could  
29 372 not evaluate the association between the demographic factors and other lipid  
30 373 markers, such as Lp(a) and oxidized LDL-C. Lp(a) was a significant risk factor  
31 374 for cardiovascular disorders and to be in the spotlight due to a novel therapy  
32 375 using antisense oligonucleotides. These lipid markers should be discussed in  
33 376 further study.<sup>31</sup>

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## 378 **CONCLUSION**

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

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6 381 to develop efficient CVD prevention strategies using the sdLDL-C and sdLDL-C/LDL-C  
7 382 ratio.  
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10 384 **List of abbreviations**

11 385 sdLDL-C: small dense low-density lipoprotein cholesterol; CVD: cardiovascular disease;

12 386 LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; LDL-C: low-density

13 387 lipoprotein cholesterol; TGs :triglycerides; HDL-C :high-density lipoprotein cholesterol:

14 388 nonHDL-C: non-high-density lipoprotein cholesterol; LNsdLDL-C: log-transformed small

15 389 dense low-density lipoprotein cholesterol; JMS: Jichi Medical School; ANOVA; analysis

16 390 of variance

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15 399 **Ethics approval and consent to participate**

17 400 All procedures performed in studies involving human participants were in accordance  
18 401 with the ethical standards of the institutional and/or national research committee and  
19 402 with the 1964 Helsinki declaration and its later amendments or comparable ethical  
20 403 standards. All the participants included in the present study provided written informed  
21 404 consent prior to inclusion, and this study was approved by the Institutional Review  
22 405 Board of Jichi Medical School (Tochigi, Japan, IRB No. G09-39 [G17-64 revised]).

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29 407 **Data sharing statement**

30 408 Data are available upon reasonable request.

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35 410 **Author contributions**

36 411 TI, YN, YS, and SI have participated in the research and designed the study; TI and SI  
37 412 performed the statistics analysis; TI contributed to the drafting of the manuscript. YN,  
38 413 YS, and SI provided feedback on the manuscript, and all authors read and approved the  
39 414 final manuscript.

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45 416 **Consent for publication**

46 417 All the participants included in the present study provided written informed consent for  
47 418 publication.

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52 420 **Competing interests**

53 421 The authors declare they have no conflict of interest with respect to this research study  
54 422 and paper.

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6 **541 FIGURE LEGENDS**

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8 **542 Figure 1. Geometric mean and 95% confidence interval of sdLDL-C for age,**  
9 **543 gender, and menopausal status**

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14 **544**

15 **545 Figure 2. Mean and 95% confidence interval of sdLDL-C/LDL-C ratio for age,**  
16 **546 gender, and menopausal status**

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20 **548 Figure 3. Mean and 95% confidence interval of LDL-C for age, gender, and**  
21 **549 menopausal status**

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25 **551 Supplementary Material**

26 **552 Supplementary Figure 1. Mean of total cholesterol for age, gender, and**  
27 **553 menopausal status**

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31 **555 Supplementary Figure 2. Mean of non-high-density lipoprotein cholesterol for age,**  
32 **556 gender, and menopausal status**

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36 **558 Supplementary Figure 3. Mean of total cholesterol / high-density lipoprotein**  
37 **559 cholesterol ratio for age, gender, and menopausal status**

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42 **562 Supplementary Figure 4. Geometric mean of triglycerides for age, gender, and**  
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47 **565 Supplementary Figure 5. Mean of high-density lipoprotein cholesterol for age,**  
48 **566 gender, and menopausal status**

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52 **568 Supplementary Figure 6. Geometric mean and 95% confidence interval of**  
53 **569 sdLDL-C for age, gender, and menopausal status**

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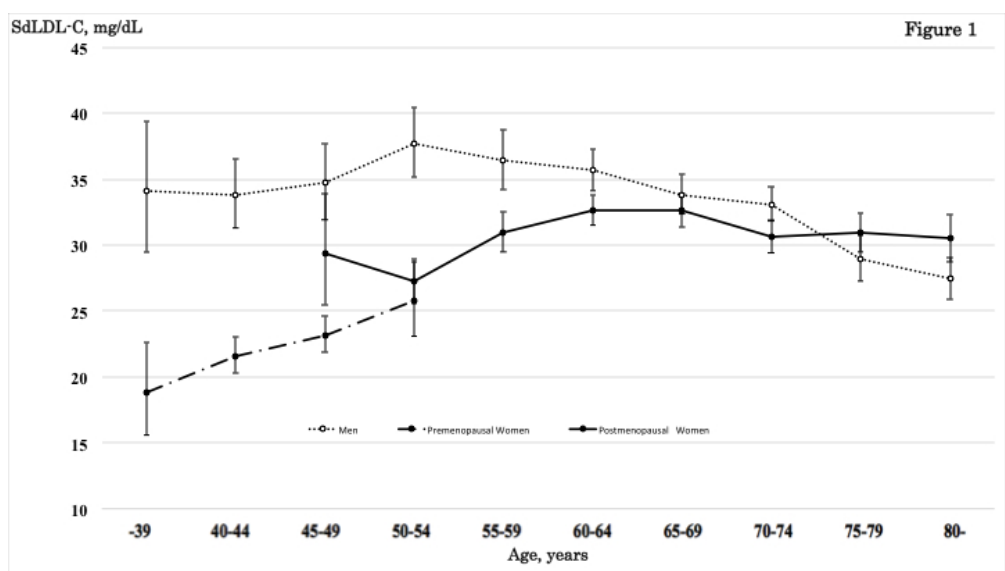
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8 571 **Supplementary Figure 7. Mean and 95% confidence interval of sdLDL-C/LDL-C**

9 572 **ratio for age, gender, and menopausal status**  
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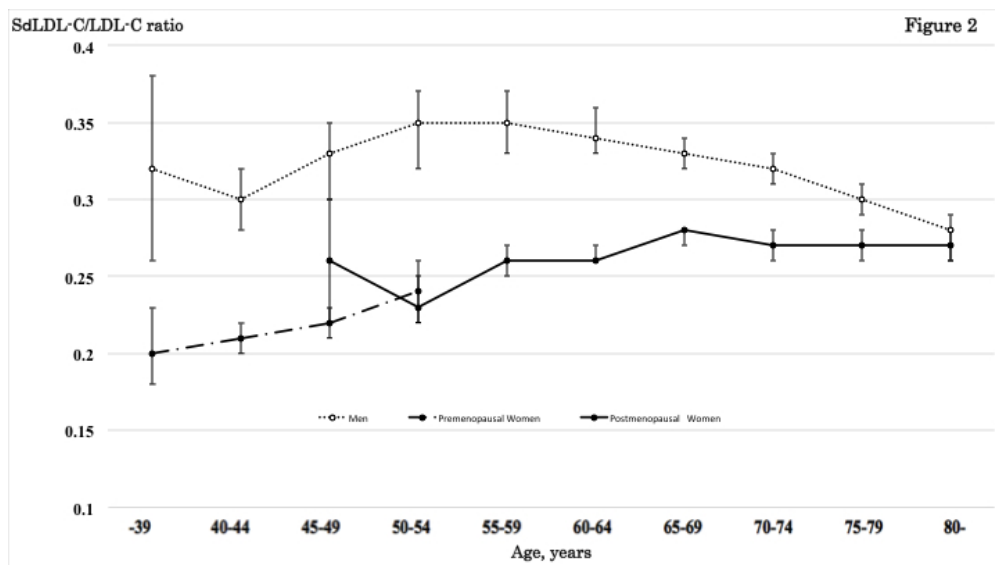


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Geometric mean and 95% confidence interval of sdLDL-C for age, gender, and menopausal status

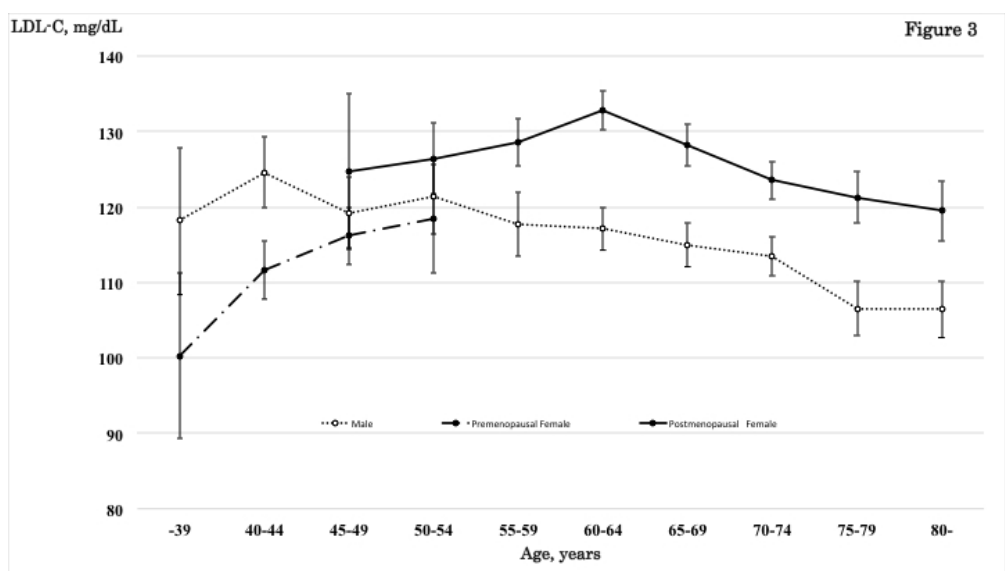
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Mean and 95% confidence interval of sdLDL-C/LDL-C ratio for age, gender, and menopausal status

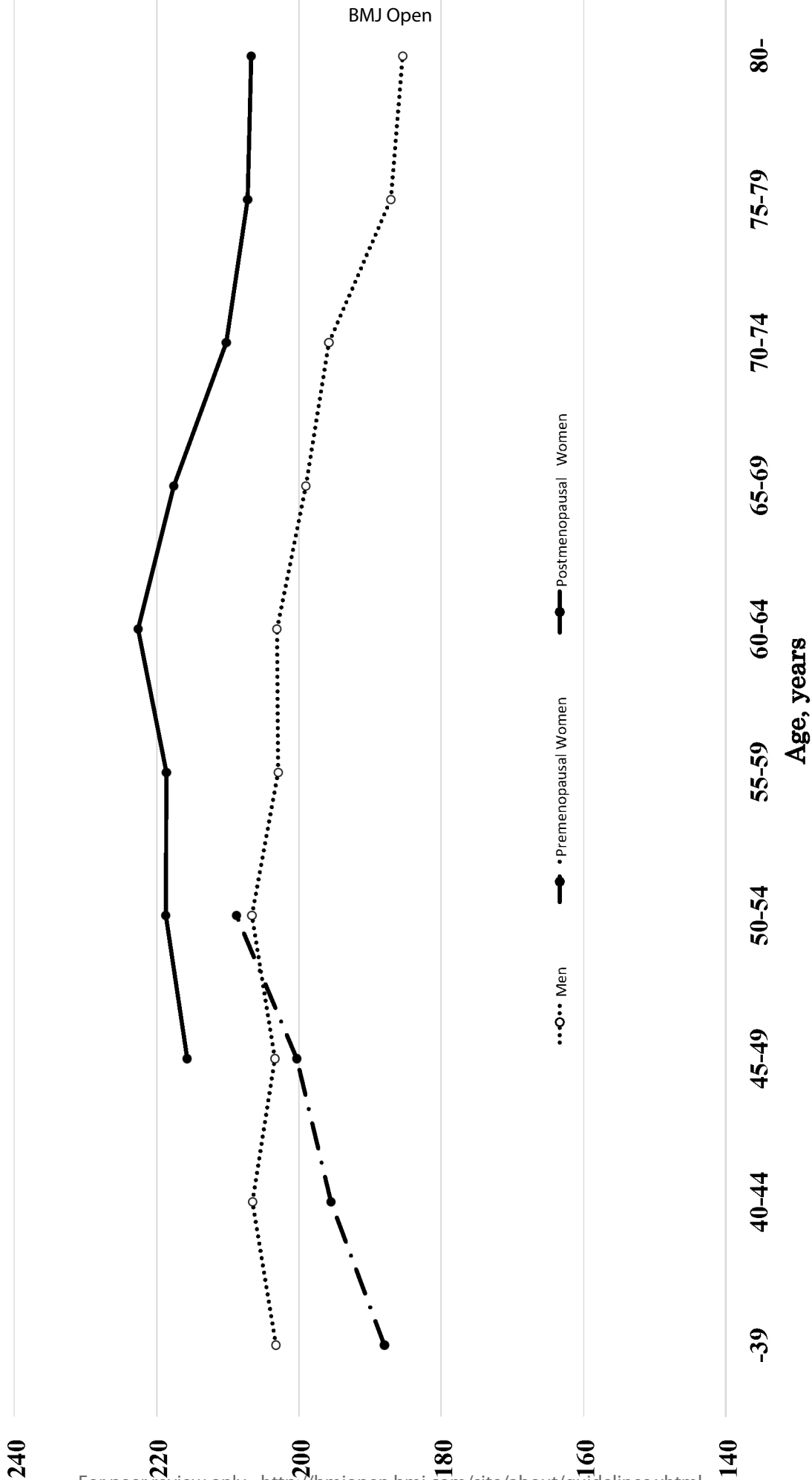
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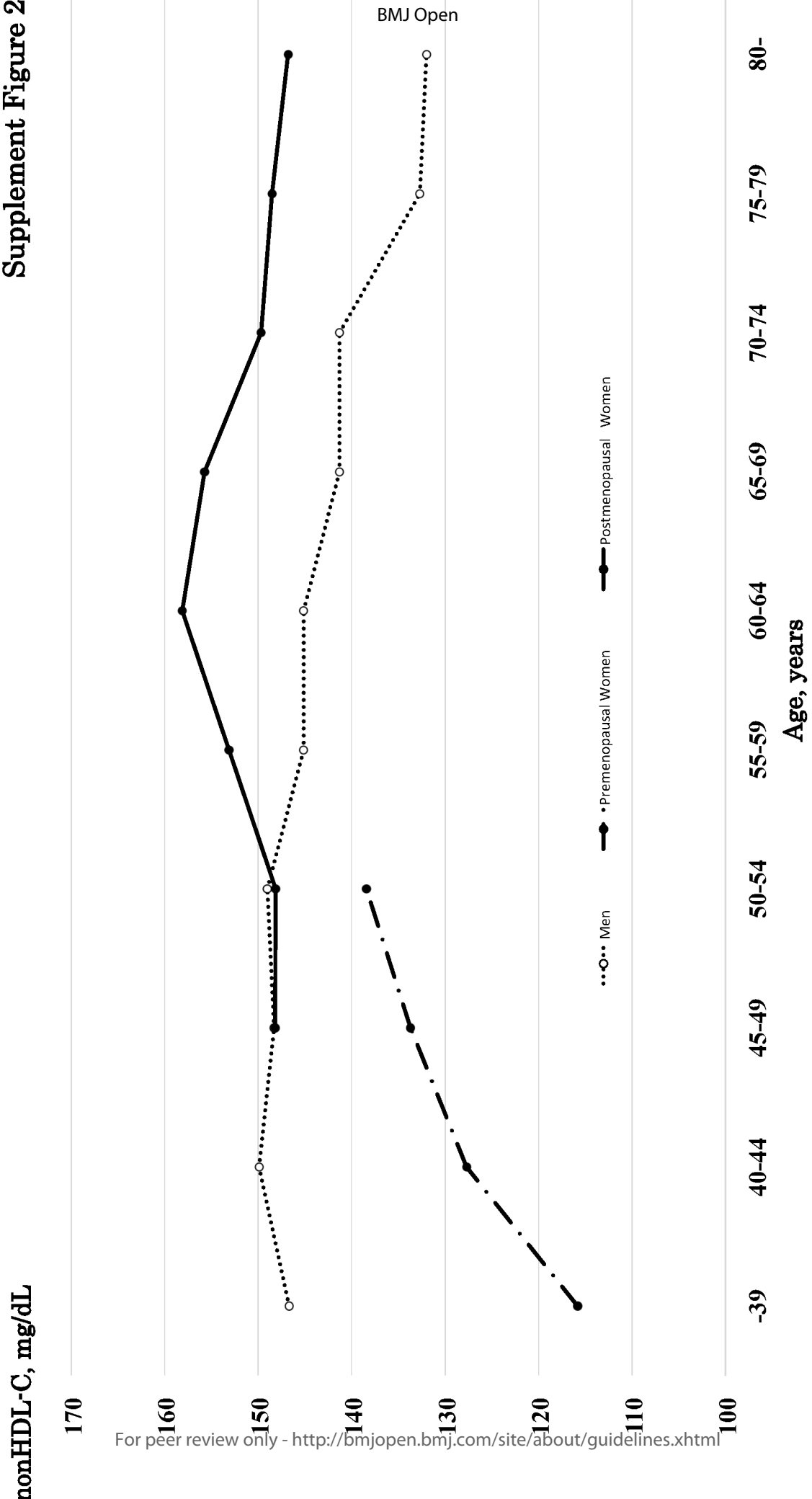


Mean and 95% confidence interval of LDL-C for age, gender, and menopausal status

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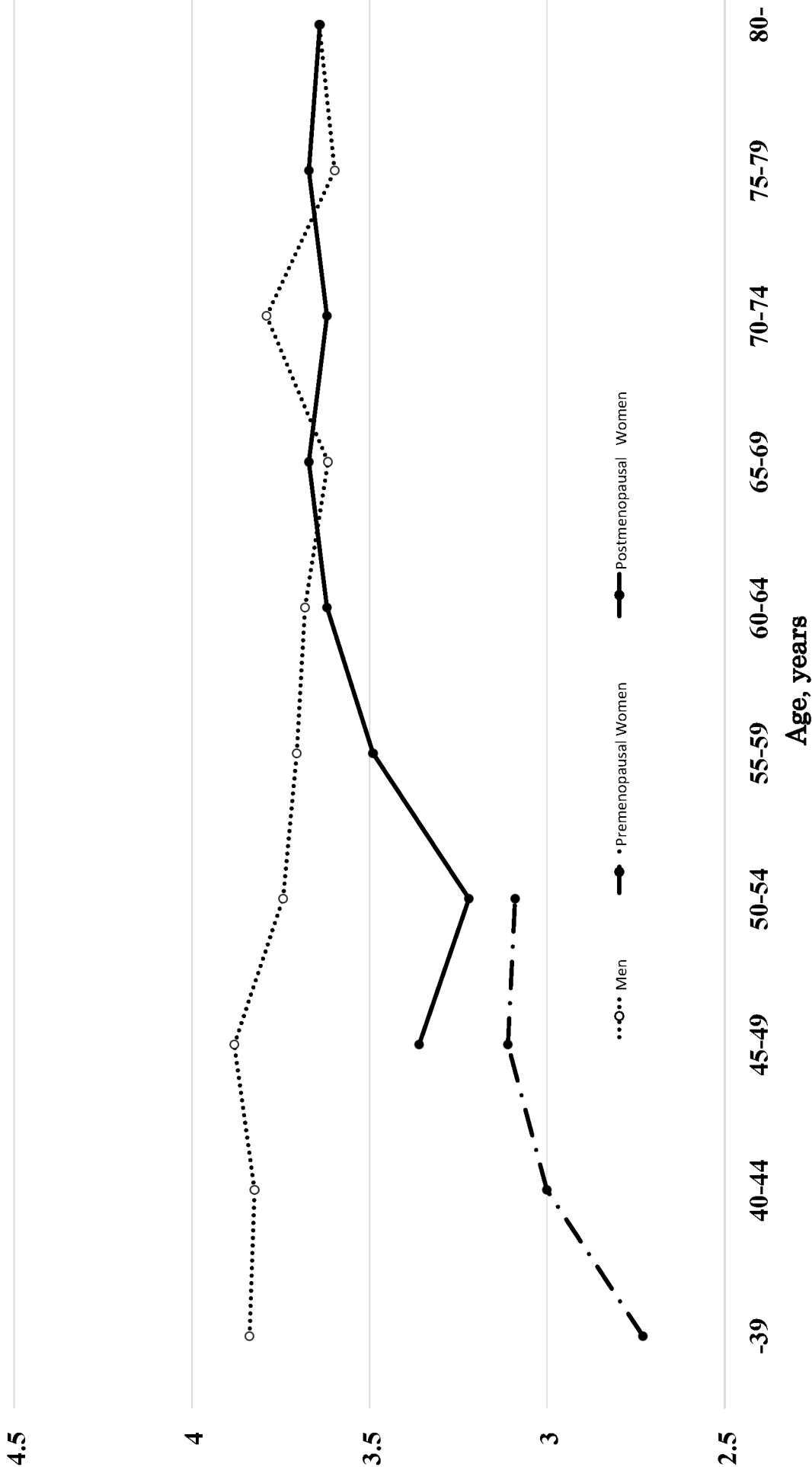


Supplement Figure 2

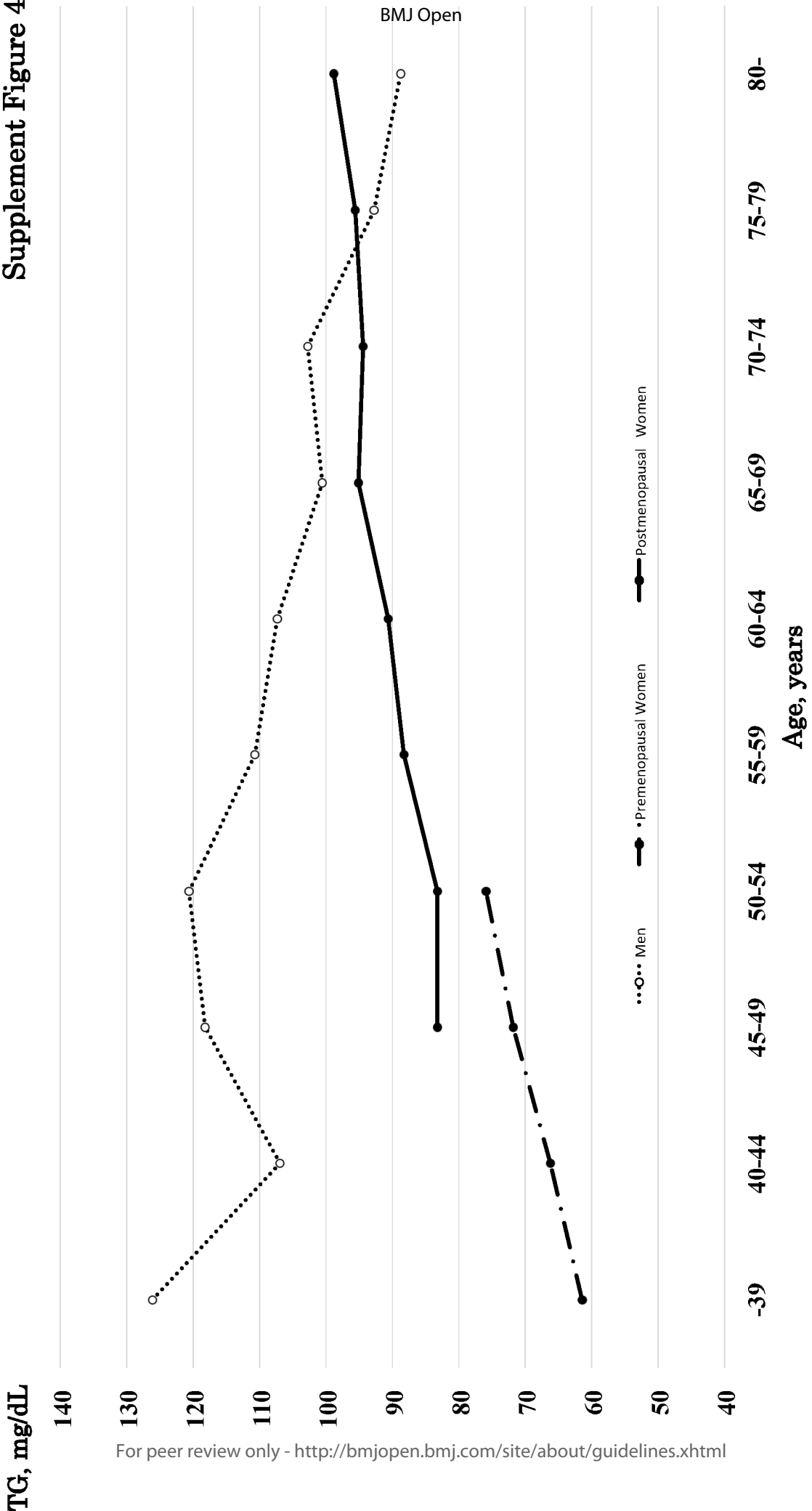


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TC/HDL-C ratio



Supplement Figure 4

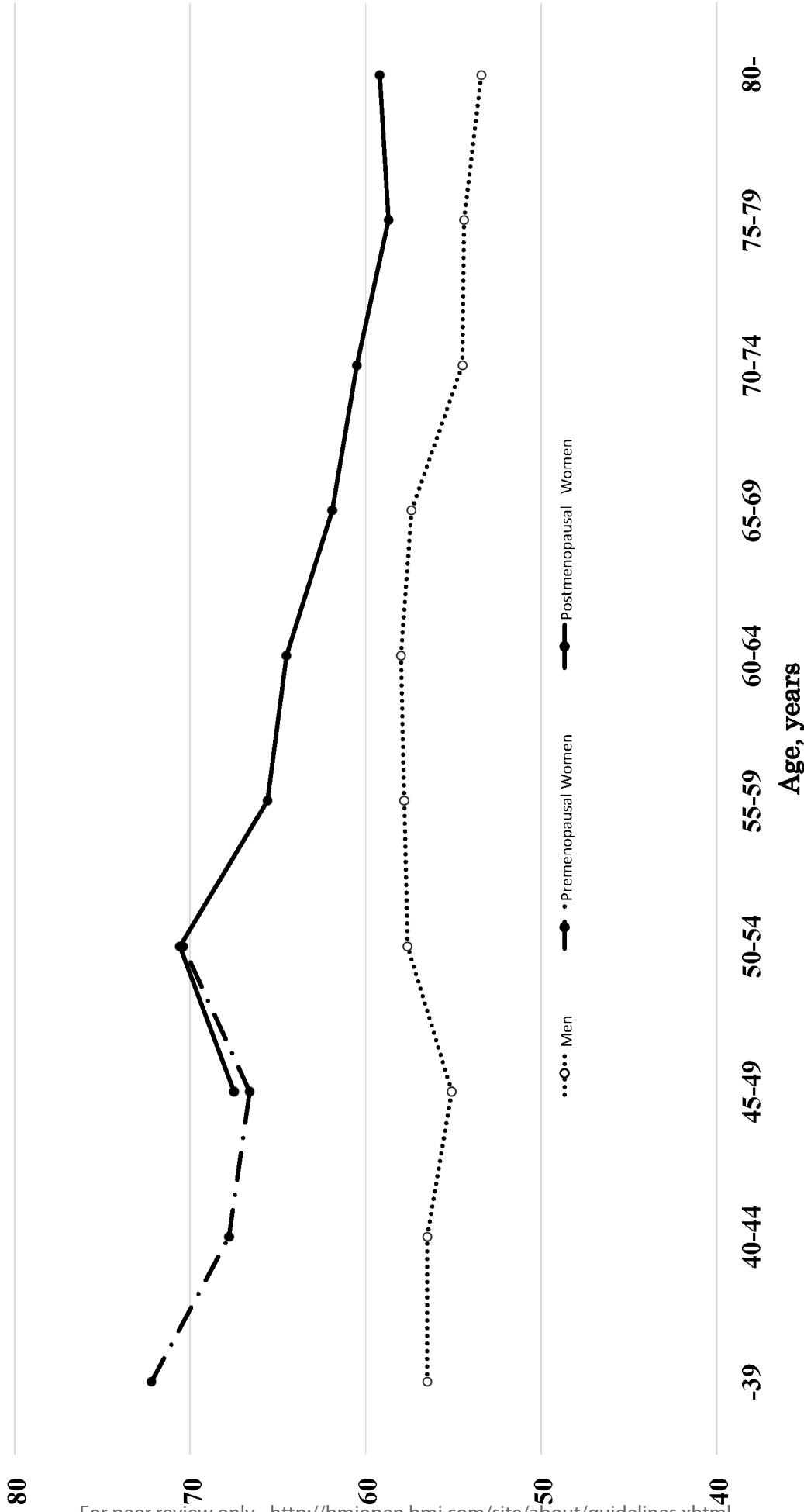


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Supplement Figure 5

HDL-C, mg/dL

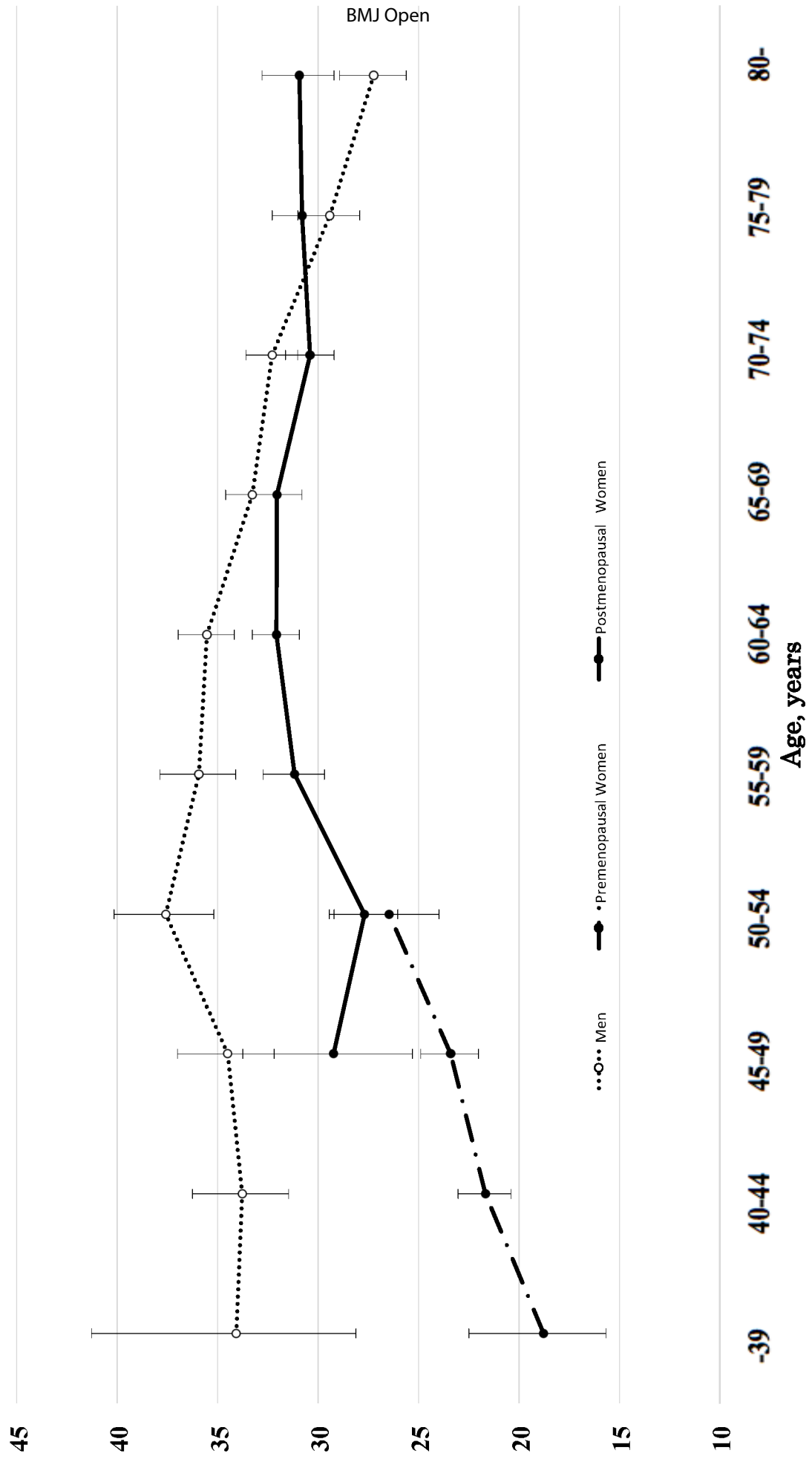
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Supplement Figure 6

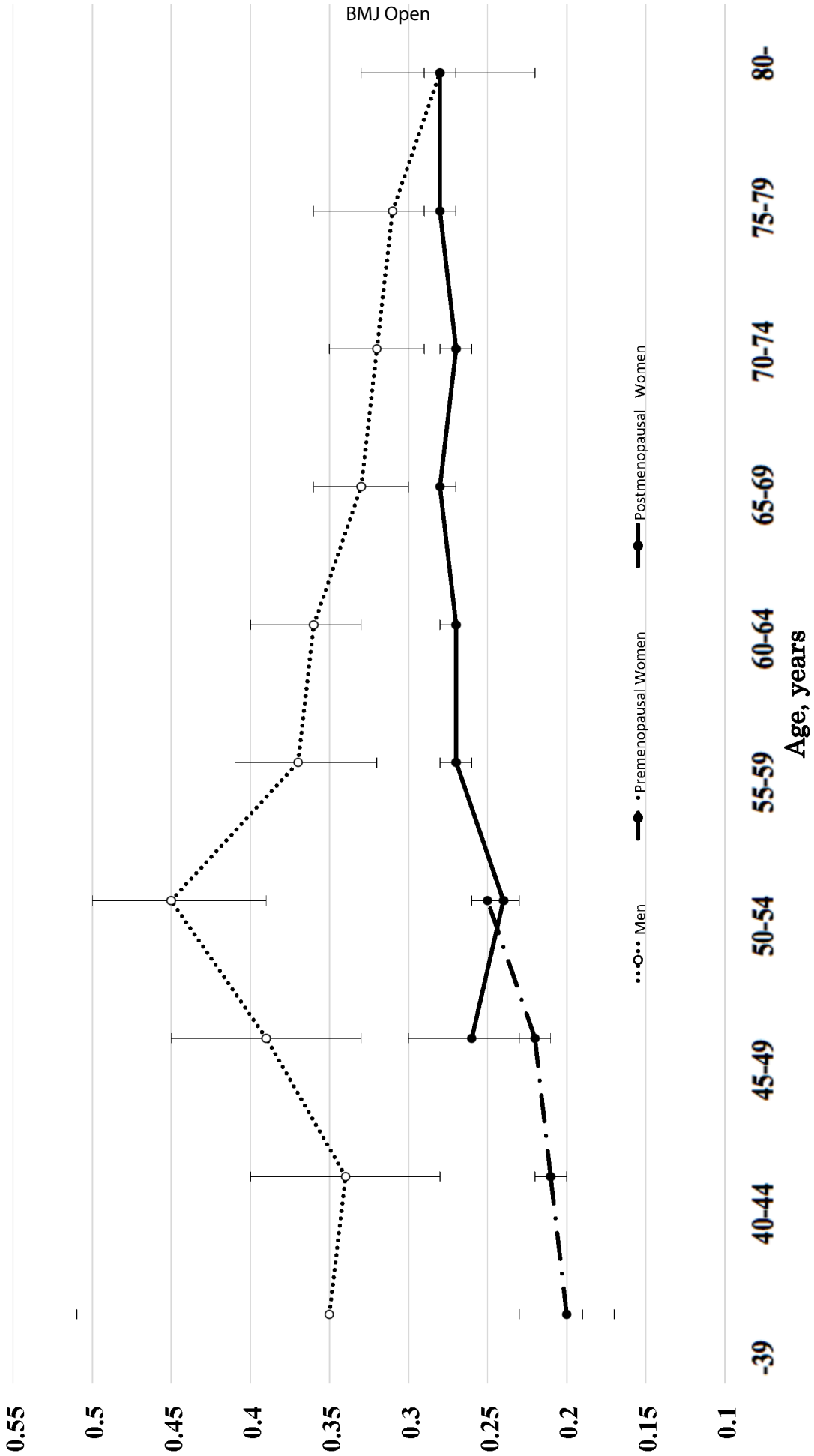
SdLDL-C, mg/dL



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Supplement Figure 7

SdLDL-C/LDL-C ratio



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

Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	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
<b>Introduction</b>			
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
<b>Methods</b>			
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	-
<b>Results</b>			

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
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	12-16
<b>Limitations</b>			
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
<b>Other information</b>			
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](http://www.strobe-statement.org).