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Relationship between high-density lipoprotein cholesterol levels and endothelial function in women: a cross-sectional study

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ABSTRACT

Objectives The purpose of this study was to evaluate the relationship between high-density lipoprotein cholesterol (HDL-C) levels and endothelial function in women.

Design Cross-sectional study.

Setting 22 university hospitals and affiliated clinics in Japan.

Participants 1719 Japanese women aged 17–90 years who were not receiving lipid-lowering therapy.

Measures We evaluated flow-mediated vasodilation (FMD) and serum levels of HDL-C. All participants were divided into four groups by HDL-C level: low HDL-C (<40 mg/dL), moderate HDL-C (40–59 mg/dL), high HDL-C (60–79 md/dL) and extremely high HDL-C (≥80 mg/dL).

Results Univariate regression analysis revealed a significant relationship between FMD and HDL-C (r=0.12, p<0.001). FMD values were significantly smaller in the low HDL-C group (5.2%±3.8%) and moderate HDL-C group (5.2%±3.8%) than in the extremely high HDL-C group (6.7%±3.4%) (p=0.024 and p=0.003, respectively), while there was no significant difference in FMD between the high HDL-C group and the extremely high HDL-C group. Multiple logistic regression analysis did not show a significant association between HDL-C levels and FMD.

Conclusions Endothelial function increased in relation to HDL-C levels. However, there was no association of HDL-C levels with endothelial function after adjustment of traditional cardiovascular risk factors in women.

Trial registration number UMIN000012950; Results.

Strengths and limitations of this study

- ► Our study included a large number of women who were not receiving lipid-lowering medicine and who underwent a flow-mediated vasodilation test.
- ► Our study shows the relationship between high-density lipoprotein cholesterol levels and endothelial dysfunction in women.
- ► Owing to a lack of data, we did not perform detailed evaluation of menopausal status, including age at menopause and duration of menopause, and hormone therapy use in our study subjects.
- ► There may exist residual confounding for this cross-sectional analysis.

INTRODUCTION

Several observational studies have shown that high-density lipoprotein cholesterol (HDL-C) concentration is inversely associated with coronary heart disease.[1–3] Serum HDL-C levels are also inversely associated with the risk of ischaemic stroke.[4 5]

It is well known that the atheroprotective effect of HDL is largely due to reverse cholesterol transport, which is transportation of excess cholesterol in the peripheral tissues to the liver. Moreover, HDL induces endothelial nitric oxide synthase (eNOS) activation and stimulates nitric oxide (NO) release in human endothelial cells.[6 7] In addition to reverse cholesterol transport and eNOS activation, HDL has been suggested to have the ability to inhibit low-density lipoprotein (LDL) oxidation, reduce inflammatory activation, attenuate endothelial cell apoptosis and stimulate endothelial repair processes.[8–11]

Recent studies have suggested that the atheroprotective features of HDL can be lost and become proatherogenic or dysfunctional in certain situations.[12–14] Hirata *et al*[15] reported that extremely high levels of HDL-C were significantly associated with increased risk of atherosclerotic cardiovascular disease mortality. Several studies have shown that a high HDL-C concentration is not always protective in postmenopausal or elderly woman.[16–18] Large randomised clinical trials have shown that raising HDL-C levels using cholesteryl ester transfer protein (CETP) inhibitors did not prevent cardiovascular

events.[19–21] Some studies have shown that HDL function rather than HDL-C levels was associated with cardiovascular events.[22 23] These studies have suggested that modulation of HDL-C levels alone is not a sufficient therapeutic target to prevent cardiovascular events.

Endothelial dysfunction and injury are established as a key early step of an atherosclerotic lesion and lead to cardiovascular disease progression.[24 25] Measurement of flow-mediated vasodilation (FMD) of the brachial artery has been used for assessment of endothelial function in clinical practice. FMD has been shown to be a significant predictor of cardiovascular events independent of cardiovascular risk factors.[26 27]

A few studies have demonstrated a protective effect of HDL-C on endothelial function. However, evidence regarding the relationship between HDL-C and endothelial function is very limited.[28 29] We previously reported that an association of HDL-C levels with endothelial dysfunction was found in men in a community-based study.[30] In men, high levels of HDL-C were independently associated with endothelial dysfunction. In the present study, we evaluated the relationship between HDL-C levels and endothelial function assessed by FMD in women not receiving lipid-lowering therapy.

METHODS

Subjects

A total of 10 247 Japanese subjects were enrolled from the FMD-Japan registry (n=7385) and from the Hiroshima University Vascular Function (HUVF) registry (n=2862). The study design of FMD-J is publicly available.[31] In the HUVF registry, 2862 subjects who underwent a health check-up at Hiroshima University Hospital between August 2007 and August 2016 were enrolled. From these subjects, 2565 women were recruited (the remaining 7682 subjects were males). Subjects without information on HDL-C level (n=171) and subjects with unclear images of the brachial artery interfaces (n=1) were excluded. In order to eliminate the effect of treatment, subjects who were receiving lipid-lowering agents (eg, statins, proprotein convertase subtilisin/kexin type 9 inhibitors, ezetimibe, fibrates, bile acid sequestrants, eicosapentaenoic acid and niacin) (n=674) were also excluded. Finally, 1719 subjects were enrolled in this study (online supplementary figure 1). The age range of the subjects was 17–90 years. Diabetes mellitus was defined according to the American Diabetes Association recommendations.[32] Hypertension was defined as treatment with oral antihypertensive agents or systolic blood pressure of more than 140 mm Hg and/or diastolic blood pressure of more than 90 mm Hg without medication. Dyslipidaemia was defined according to the third report of the National Cholesterol Education Program.[33] Smokers were defined as those who were current smokers. Coronary heart disease included angina pectoris, myocardial infarction and unstable angina. Cerebrovascular disease included haemorrhagic stroke, ischaemic stroke and transient ischaemic attack. Cardiovascular disease was defined as coronary heart disease and cerebrovascular disease. Framingham risk score was calculated by points of risk factors: age, total cholesterol level, HDL-C level, systolic blood pressure, diabetes mellitus and smoking status.[34]

All of the participants were divided into four groups according to the definitions used in a previous study in Japan: low HDL-C (<40 mg/dL), moderate HDL-C (40–59 mg/dL), high HDL-C (60–79 md/dL) and extremely high HDL-C (≥80 mg/dL).[35–37] Reduced HDL-C is defined as HDL-C <40 mg/dL in WHO and Japanese criteria for metabolic syndrome.[38 39] The Framingham Study has shown that increased plasma HDL-C (>60 mg/dL) is a negative risk factor for cardiovascular disease.[40]

Informed consent for participation in the study was obtained from all participants.

Study protocol

The subjects were instructed to fast overnight for at least 12 hours and avoid smoking, alcohol, caffeine and antioxidant vitamins on the day of the examination.[30 31] Measurement of FMD was performed while each subject was in the supine position in a dark, quiet, air-conditioned room. Venous blood samples were obtained from the deep antecubital vein. FMD assessments were conducted in a blinded manner.

Measurement of FMD

The protocol for measurement of FMD has been previously described.[30 31] In the brachial artery, vascular response to reactive hyperaemia was assessed as an index of endothelial function. An occlusion cuff was wrapped around the forearm. We evaluated FMD using high-resolution ultrasonography and computer-assisted analysis software (UNEXEF18G, UNEX Co, Nagoya, Japan). This software is an automated edge detection system for measurement of brachial artery diameter. A longitudinal image of the brachial artery (5–10 cm above the elbow) was scanned. The transducer was held at the point at which the clearest B-mode image of the anterior and posterior intimal interfaces can be obtained. Gain was set to obtain optimal images of the arterial lumen wall interface. When the tracking gate was placed on the brachial arterial lumen, the diameter was automatically tracked. After the baseline longitudinal image had been acquired, the occlusion cuff was inflated for 5 min (50 mm Hg over systolic pressure). We recorded the longitudinal image of the artery until 3–5 min after cuff deflation. FMD was automatically calculated as the maximal percentage change in vessel diameter from the baseline value. The correlation coefficient between FMD analysed at the core laboratory and participant institutions was 0.84 (p<0.001). In our laboratory, the coefficient of variation for FMD was 10.1%.

Patient and public involvement

Patients and the public were not involved in the design or planning of the study.

Statistical analysis

All reported probability values were two sided. Continuous variables were summarised as mean±SD or medians (IQR). Comparison of variables among two or more groups by differences in the HDL-C levels was performed using the Wilcoxon test or Kruskal-Wallis test. Categorical values such as medications and medical histories were compared by means of the χ^2 test. We preformed Tukey's post hoc test to compare the differences in FMD between groups. To identify independent variables associated with a lower quartile of FMD (<3.8%), multiple logistic regression analysis was performed. In model 2, we adjusted for age, and in model 3, age, body mass index (BMI), hypertension, diabetes and current smoker were entered into the multiple logistic regression analysis. Receiver operating characteristic (ROC) analysis was performed to determine the cut-off value of HDL-C at which FMD would be lower than 3.8% (lower quartile). The subjects were divided into two groups according to the optimal cut-off value of HDL-C (56 mg/dL). We generated a set of matched cases (subjects with HDL-C <56 mg/dL) and controls (subjects with HDL-C ≥56 mg/dL) using propensity score analysis. A logistic regression model was used to estimate the propensity of HDL-C <56 mg/dL based on variables associated with HDL-C, including age, BMI, presence of hypertension and diabetes, triglycerides, low-density lipoprotein cholesterol, use of antihypertensive drugs (yes or no) and use of antihyperglycaemic drugs (yes or no). We created two well-matched groups based on clinical characteristics with these propensity scores using a calliper width of 0.2 SD of the logit of the propensity score, and we compared the prevalences of endothelial dysfunction (defined as less than the lowest quartile of FMD in all participants). The data were processed using JMP V.13.0 software and Stata V.15. A probability value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

The baseline characteristics of the 1719 subjects are shown in table 1. Seven hundred and forty-one subjects (43.1%) had hypertension, 629 (36.6%) had dyslipidaemia, 145 (8.4%) had diabetes mellitus, 59 (3.4%) had previous cardiovascular disease and 133 (7.8%) were current smokers.

Relationship between HDL-C and endothelial function

Univariate regression analysis revealed that HDL-C significantly correlated with FMD (r=0.12, p<0.001) as shown in figure 1. FMD values were 5.2%±3.8% in the low HDL-C group, 5.9%±3.7% in the moderate HDL-C group, 6.4%±3.6% in the high HDL-C group and 6.7%±3.4% in the extremely high HDL-C group. FMD values were significantly smaller in the low HDL-C group and moderate HDL-C group than in the extremely high HDL-C group (p=0.024 and p=0.003, respectively; figure 2). When the extremely high HDL-C group was divided into an HDL-C of 80–99 mg/dL group and an HDL-C of ≥100 mg/dL group, FMD values were better in the HDL-C of ≥100 mg/dL group than in the HDL-C of 80–99 mg/dL group (online supplementary figure 2 and online supplementary table 1). A lower quartile of FMD was less than 3.8% in this population. Multiple logistic regression analysis revealed that low HDL-C was not independently associated with a lower quartile of FMD after adjustment for age, BMI, presence of hypertension, diabetes and smokers (OR 1.13, 95% CI 0.58 to 2.20; p=0.715; table 2).

We next categorised subjects into four groups according to the quartiles of HDL-C. Baseline characteristics are summarised in online supplementary table 2. FMD values were 5.5%±3.7% in the low HDL-C group (quartile 1), 6.5%±3.7% in the moderate HDL-C group (quartile 2), 6.4%±3.6% in the high HDL-C group (quartile 3) and 6.7%±3.4% in the extremely high HDL-C group (quartile 4). FMD values were significantly smaller in the low HDL-C group than in the moderate HDL-C group, high HDL-C group and extremely high HDL-C group (p<0.001, p=0.002 and p<0.001, respectively; online supplementary figure 3). There were no significant differences in FMD between the moderate HDL-C group, high HDL-C group and extremely high HDL-C group. Multiple logistic regression analysis revealed that low HDL-C was not independently associated with a lower quartile of FMD (online supplementary table 3).

Results of ROC curve analysis to assess the sensitivity and specificity of HDL-C for predicting a lower quartile of FMD (<3.8%) are shown in online supplementary figure 4. Area under the curve value of the ROC curve was 0.58, and the optimal cut-off value of HDL-C was 56 mg/dL. We divided the subjects into two groups according to the optimal cut-off value of HDL-C (56 mg/dL). FMD was significantly smaller in the lower group than the other group (p<0.001). Baseline characteristics of subjects are summarised in (online supplementary table 4). There were significant differences between the two groups except for smokers and previous cardiovascular disease. We next evaluated the association between HDL-C and FMD in the propensity score-matched population. There were significant differences in total cholesterol, HDL-C and Framingham risk score. After matching for confounding factors, there were no significant differences in the values of FMD between the two groups (p=0.581; online supplementary table 5).

DISCUSSION

In the present study, there was a positive relationship between HDL-C levels and FMD in women who did not receive lipid-lowering therapy. The low HDL-C group (<40 mg/dL) showed the highest OR for a lower quartile of FMD (<3.8%). Although the OR was highest in the low HDL-C group, statistical significance was diminished after adjustment for traditional cardiovascular risk factors. There was no significant difference in the values of FMD between the two groups divided by the optimal

Table 1 Clinical characteristics of the subjects on the basis of HDL-C

Variables	Total (n=1719)	Low <40 mg/dL (n=53)	Moderate 40–59 mg/dL (n=548)	High 60–79 mg/dL (n=771)	Extremely high ≥80 mg/dL (n=347)	P value for rend
Age, year	52.6±14	56.5±16	54.2±15	51.8±14	51.3±13	<0.001
Body mass index, kg/m^2	22.3±3.7	24.3±4.7	23.7±4.2	21.9±3.3	20.4±2.5	<0.001
Systolic blood pressure, mm Hg	123.2±19	127.6±21	127.6±20	121.6±18	119.2±19	<0.001
Diastolic blood pressure, mm Hg	76±12	77±12	77±12	75±12	74±11	<0.001
Heart rate, bpm	67±11	72±13	68±10	67±12	65±11	<0.001
Total cholesterol, mg/dL	206±36	184±41	198±36	206±35	220±32	<0.001
Triglycerides, mg/dL	99 (60 to 121)	164 (113 to 196)	124 (76 to 155)	89 (57 to 109)	71 (48 to 85)	<0.001
HDL-C, mg/dL	67±16	35±3	52±5	69±6	91±10	<0.001
LDL-C, mg/dL	120±32	116±40	123±32	120±32	116±30	0.018
Glucose, mg/dL	98±22	113±36	102±23	96±19	94±18	<0.001
Medications, n (%)						
Antihypertensive therapy	535 (31.1)	21 (39.6)	233 (42.5)	209 (27.1)	72 (20.8)	<0.001
Antihyperglycaemic therapy	82 (4.8)	9 (17.0)	42 (7.7)	20 (2.6)	11 (3.2)	<0.001
Framingham risk score, %	5.4±5.3	10.6±7.9	8.1±6.5	3.9±3.7	3.7±3.6	<0.001
Medical history, n (%)						
Hypertension	741 (43.1)	34 (64.2)	314 (57.3)	290 (37.6)	103 (29.7)	<0.001
Dyslipidaemia	629 (36.6)	53 (100.0)	242 (44.2)	251 (32.6)	83 (23.9)	<0.001
Diabetes mellitus	145 (8.4)	14 (26.4)	69 (12.6)	40 (5.2)	22 (6.3)	<0.001
Smokers	133 (7.8)	4 (7.6)	49 (9.0)	56 (7.3)	24 (6.9)	0.630
Previous cardiovascular disease	59 (3.4)	4 (7.6)	20 (3.7)	27 (3.5)	8 (2.3)	0.248
FMD, %	6.3±3.6	5.2±3.8	5.9±3.7	6.4±3.6	6.7±3.4	<0.001

Data are presented as mean±SD or median (IQR).
FMD, flow-mediated vasodilation; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

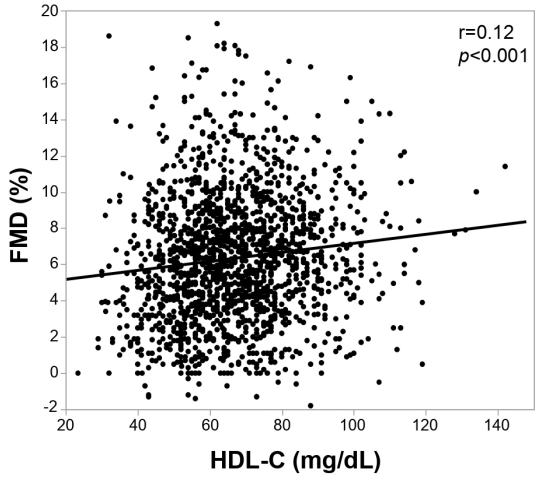

Figure 1 Scatter plots show the relationship between flow-mediated vasodilation (FMD) and high-density lipoprotein cholesterol (HDL-C).

cut-off value of HDL-C (56 mg/dL) after matching for confounding factors. These findings suggest that there is no association of HDL-C levels with endothelial function in women after adjustment of traditional cardiovascular risk factors.

Previous studies have shown that HDL-C concentration was inversely associated with coronary heart disease and ischaemic stroke.[1–5] However, the precise mechanism by which low HDL-C causes cardiovascular complications remains unclear. Low HDL-C levels are often seen in patients with hypertension, hypertriglyceridaemia, insulin resistance and obesity. Indeed, in the present study, the values of triglycerides and glucose, age, BMI, prevalence of hypertension and prevalence of diabetes mellitus were higher in the low HDL-C group than in the other groups. It is possible that these confounding factors reflect endothelial function in subjects with low HDL-C. Low HDL-C and metabolic disorders coexist and may influence each other, leading to impairment of endothelial function.

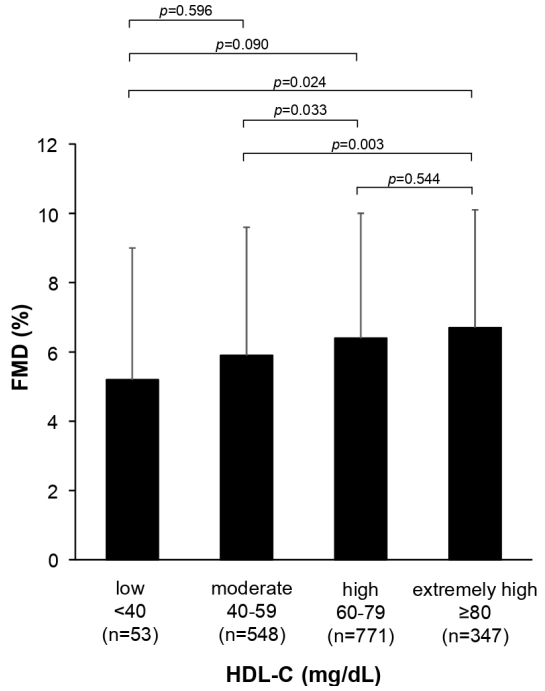

Figure 2 Bar graphs show values of flow-mediated vasodilation (FMD) in the groups of low levels of high-density lipoprotein cholesterol (HDL-C), moderate levels of HDL-C, high levels of HDL-C and extremely high levels of HDL-C. The error bars indicate SD.

In the WHO criteria for metabolic syndrome, reduced HDL-C is defined as HDL-C <40 mg/dL.[38] It was shown in the Framingham Study that the increased plasma HDL-C (>60 mg/dL) is a negative risk factor for cardiovascular disease.[40] In the present study, ROC analysis was performed to determine the cut-off value of HDL-C at which FMD would be lower than 3.8% (a lower quartile of FMD). The cut-off value derived from the results of ROC analysis was HDL-C <56 mg/dL. However, the area under the curve value was very small (0.58), indicating poor discriminatory power, and the HDL-C cut-off value of <56 mg/dL therefore has weak support.

The present study showed that FMD was significantly greater in subjects with extremely high HDL-C than in subjects with low HDL-C. However, extremely high HDL-C was not significantly associated with better endothelial function after adjustment for cardiovascular risk factors. Recent pharmacological studies have suggested that modulation of HDL-C levels alone is not a sufficient therapeutic target to prevent cardiovascular events. In large clinical trials, efforts to raise HDL-C levels by inhibition of CETP did not decrease cardiovascular outcomes despite increases in HDL-C.[19–21] In our study, although the extremely high HDL-C group had a lower Framingham risk score than the scores in the other groups, extremely high HDL-C was not significantly associated with better endothelial function after adjustment for cardiovascular risk factors. These findings suggest that high HDL-C does not necessarily lead to good endothelial function even in individuals with low cardiovascular risk.

We previously reported that extremely high levels of HDL-C were independently associated with endothelial dysfunction in men who were not receiving lipid-lowering therapy.[30] Interestingly, it is likely that there is a significant difference in the relationships between extremely high levels of HDL-C and endothelial function in men and women. In the present study, we divided the extremely high HDL-C group into an HDL-C of 80–99 mg/dL group and an HDL-C of ≥100 mg/dL group. Endothelial function was greater in the HDL-C of ≥100 mg/dL group than in the HDL-C of 80–99 mg/dL group in women. Possible reasons for the opposite results concerning the relationship between HDL-C levels and endothelial function in men and women may be differences in CETP activity and levels of oestradiol. It has been reported that CETP deficiency is one of the major causes of increased HDL-C levels in Japan.[41 42] Some single nucleotide polymorphisms (SNPs) in *CETP* had a differential effect on HDL levels and cardiovascular disease in men and women.[43 44] A haplotype consisting of the *rs5883T* and *rs9930761C* SNPs in *CETP* was shown to be associated with increased risk of progression to cardiovascular disease in men but not in women.[43] Although gender differences in HDL function have not been elucidated, endogenous oestrogen presumably protects against atherosclerosis. It has been

Table 2 Multivariate analysis of the relationship between FMD and HDL-C

	Model 1		Model 2		Model 3	
Variables	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
HDL-C, mg/dL						
<40	1.36 (0.76 to 2.44)	0.303	1.19 (0.62 to 2.31)	0.598	1.13 (0.58 to 2.20)	0.715
40–59	1 (reference)		1 (reference)		1 (reference)	
60–79	0.62 (0.48 to 0.80)	<0.001	0.70 (0.53 to 0.92)	0.011	0.84 (0.63 to 1.12)	0.228
≥80	0.50 (0.36 to 0.69)	<0.001	0.59 (0.41 to 0.83)	0.003	0.73 (0.49 to 1.07)	0.108

Lower quartile of FMD indicate less than 3.8%.
Model 1: unadjusted model.
Model 2: adjusted for age.
Model 3: adjusted for age, BMI, the presence of hypertension, diabetes and current smoking.
FMD, flow-mediated vasodilation; HDL-C, high-density lipoprotein cholesterol.

suggested that HDL and oestradiol interact to stimulate eNOS activity and production of NO. HDL isolated from women stimulated eNOS activity, whereas HDL isolated from men had minimal activity for stimulation of eNOS activity.[45] Oestradiol may have an impact on the effects of HDL on endothelial function. In addition to genetic variants and levels of oestradiol, environmental factors such as smoking, alcohol consumption, coffee intake and exercise contribute to the difference in the role of HDL-C in endothelial function in men and women.

Our results suggest that the circulating level of HDL-C lacks usefulness as an independent marker of endothelial function in women. It is well known that HDL inhibits atherogenesis through cholesterol efflux from peripheral tissues, activation of eNOS, inhibition of LDL oxidation, prevention of endothelial cell apoptosis and stimulation of endothelial repair processes.[6–11] Several studies have provided evidence that the effects of HDL on endothelial function are highly heterogeneous.[46] Antiatherogenic effects of HDL are impaired in patients with diabetes mellitus, coronary artery disease and chronic kidney disease.[47 48] Although the precise mechanism by which endothelial effects of HDL are altered in patients with increased cardiovascular risk remains to be elucidated, it has been shown that an inflammatory response can cause alterations of HDL-C protein and lipid cargo.[46 48] It has been reported that isolated HDL-C from type 2 diabetes patients had reduced ability to stimulate eNOS activity (−40%, p<0.001) and to prevent nuclear factor kappa B (NF-κB) activation stimulated by tumour necrosis factor alpha in human microvascular endothelial cells (−20%, p<0.001).[49] Recently, HDL function, especially cholesterol efflux capacity, has been used as an indicator of the relationship between HDL-C and cardiovascular disease.[22 23] We should pay attention to HDL function rather than circulating levels of HDL-C.

There are some limitations in this study. First, we did not perform detailed evaluation of menopausal status, including age at menopause and duration of menopause, and hormone therapy use in our study subjects. Several studies have shown that a high level of HDL-C is not always cardioprotective in postmenopausal women.[16 17] It has been proposed that a decrease in oestrogen levels during the menopausal transition may lead to chronic inflammation.[50] This systematic inflammation could potentially convert HDL-C to a dysfunctional form. In the present study, there was limited information on the precise menopausal status and hormone therapy use. To evaluate the association between endothelial function and HDL-C levels over the menopausal transition, it is necessary to consider differences in HDL-C and FMD before and after menopause in the same subjects. Second, we were not able to evaluate lifestyles including alcohol drinking, detailed smoking status, diet and regular exercise, since there was limited information on lifestyles in our database. It has been shown that there is a positive gradient of HDL-C levels with alcohol consumption.[51] Although the relationship between endothelial function and alcohol intake in women remains unclear, the amount of alcohol drinking might have an impact on the relationship between HDL-C and endothelial function. In addition, in the statistical analysis, consideration was only given to whether the subject was currently a smoker. Whether the subject is a current smoker as well as the frequency and number of cigarettes, such as pack years, are important when considering their effects on endothelial function. Finally, unfortunately, HDL-C functions were not evaluated. Further studies are needed to confirm the effects of HDL functions on endothelial function in a large clinical trial.

In conclusion, endothelial function increased in relation to HDL-C levels in women. However, there was no association of HDL-C levels with endothelial function in women after adjustment of traditional cardiovascular risk factors.

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