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Correlations between Oxidative Stress and Blood Lipids Are Stronger in Men than Women

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Abstract: Oxidative stress is one cause of atherosclerosis that makes it a lifestyle-related disease. Oxidized low-density lipoprotein (OxLDL) was previously found to be related to oxidative stress, measured using the diacron-reactive oxygen metabolites (d-ROMs) test and showed a negative correlation between biological antioxidant potential (BAP) test results and triglycerides (TG). In addition, large gender differences exist among vascular disorders caused by arteriosclerosis. However, such gender differences and their correlation with oxidative stress and blood lipids have not been clarified. In this study, gender differences in correlations between oxidative stress and blood lipids as factors in the development of atherosclerosis was addressed. Subjects were 149 individuals who underwent medical examinations conducted in Ashikaga Teishin Clinic in Tochigi, Japan (98 males and 51 females). A strong positive correlation was observed between d-ROMs test results and OxLDL in men ($R = 0.480$, $P < 0.0001$), but no correlation was seen in women. A strong negative correlation between BAP test results and TG was also noted in men ($R = -0.571$, $P < 0.0001$), and a moderate negative correlation was detected in women ($R = -0.344$, $P = 0.0133$). A positive correlation between d-ROMs tests and OxLDL was seen in women under 50 years of age ($R = 0.399$, $P = 0.0393$), but this correlation was not present in women who were 50 years of age or older ($R = -0.00656$, $P = 0.976$). Correlations between oxidative stress and OxLDL and between antioxidant potential and TG in men were more prominent than in women. This finding suggests that decreasing oxidative stress in the blood to prevent atherosclerosis is more important for men.

Key words: oxidative stress, diacron-reactive oxygen metabolites (d-ROMs) test, biological antioxidant potential (BAP) test, oxidized low-density lipoprotein (OxLDL), gender difference

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Introduction

The gender difference in vascular disorders caused by arteriosclerosis is significant[1], and the risk of coronary artery disease is largely related to gender and age. According to the Framingham Study, the incidence of cardiovascular disease gradually increases with age in men. However, it remains extremely low in women under 50 years of age before significantly increasing after menopause. At 65 years of age or above, the incidence in men and women is similar[2]. In women, estrogen affects lipid metabolism and lowers low-density lipoprotein (LDL) by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity[3] and enhancing LDL receptor expression[4]. Further, estrogen promotes synthesis of apolipoprotein A-I, a major constituent of high-density lipoprotein (HDL), and enhances the production of HDL[5].

Oxidative stress, an important factor in atherosclerosis, is caused by pathological states such as hypertension[6], diabetes[7], and dislipidemia[8]. LDL is changed to oxidized (Ox) LDL by oxidative stress in the blood, and OxLDL is thus an important marker for development of atherosclerosis[8]. OxLDL is taken up by macrophages via the scavenger receptor, which leads to the appearance of foam cells. These cholesterol-filled macrophages create the initial lesions in atherosclerosis[9]. Peroxide in atherosclerotic lesions causes inflammation and cytokine release from vascular wall cells and macrophages. Atherosclerosis is caused by the disturbance and growth of vascular wall cells and the accumulation of substances such as lipids[10].

Recently, simple methods for measuring oxidative stress and antioxidant potential, the diacron-reactive oxygen metabolites (d-ROMs) test and the biological antioxidant potential (BAP) test, have become available[11]. The d-ROMs test is a colorimetric determination of hydroperoxide levels in the blood caused by reactive oxygen and free radicals and is an all-inclusive measure of levels of oxidative stress *in vivo*[11]. The BAP test measures the ability of reducing agents to donate electrons to reactive oxygen and free radicals to stop oxidative reactions[11]. Previously, OxLDL was shown to be related to oxidative stress and a negative correlation was shown to exist between antioxidant potential and triglycerides (TG)[12].

Small dense LDL is important, in addition to OxLDL, in atherosclerosis[13]. Small dense LDL more easily passes through cell membranes, has lower affinity for the LDL receptor, and undergoes slower catabolism than large LDL. These characteristics give it a primary role in the induction of atherosclerosis[14]. The LDL / HDL ratio is an independent predictor of myocardial infarction[15]. However, gender differences in the relationship between oxidative stress and blood lipids have not been clarified. In this study, gender differences in the correlation between oxidative stress and blood lipids were investigated as a factor in the development of atherosclerosis.

Materials and methods

Study population

Subjects included 149 individuals who underwent medical examination (April 5, 2006 to June 30, 2006) conducted in Ashikaga Teishin Clinic in Tochigi, Japan (98 males and 51 females). The mean age of subjects was 44.11±13.7 years (median 43 years: range 20-68 years). This

study was approved by the Ethics Committee of the Ashikaga Teishin Clinic. This study presents new analyses performed using the same subjects as in a previously published paper[12].

Data collection

Age and glycohemoglobin A1c (HbA1c), OxLDL, LDL, TG, apolipoprotein B (ApoB), total cholesterol (TC), and HDL were recorded. 4HNE (hydroxy-nonenal)-LDL was quantified to represent OxLDL using ELISA (Mitsubishi Petrochemical, Tokyo). For small dense LDL, a positive correlation is observed between LDL / ApoB ratio and LDL diameter[16], thus, a negative correlation exists between small dense LDL and LDL / ApoB ratio. Correlations between oxidative stress and LDL / ApoB ratio were generated. In addition, LDL / HDL ratio and OxLDL / HDL ratio were assessed as markers for atherosclerosis.

Blood serum samples were stored at −80°C until centrifugation to separate serum. d-ROMs tests were performed using N-diethyl-paraphenylenediamine (WISMERLL, Tokyo) as a measure of oxidative stress. BAP tests were performed using $FeCl_3$ to measure antioxidant potential[12].

Statistical analysis

Subjects were divided by gender and analyzed separately. Gender groups were subdivided into individuals less than 50 years of age and individuals 50 years of age or older. This subdivision reflects the cyclical effects of female sex hormones (e.g., estrogen) prior to menopause. Women 50 years of age or older are mostly postmenopausal and less affected by female hormones. Regression analyses were used to characterize correlations (OriginPro. Lightstone Co., Tokyo). Comparisons between groups used the Mann-Whitney U test (ystat2013. Xls, Tokyo) and are shown as means±standard deviation. $P < 0.05$ was considered significant.

Results

No difference between men and women was observed for LDL ($P = 0.801$). A significant difference between men and women in OxLDL was seen ($P = 0.041$). Further, a significant difference was found in LDL / HDL ($P = 0.038$) and OxLDL / HDL ($P < 0.001$) ratios. Another significant gender difference was detected in LDL / ApoB ratio as an indicator of small dense LDL ($P = 0.002$), and in TG ($P = 0.002$) and HDL ($P < 0.001$). No gender difference in d-ROMs test ($P = 0.776$) and BAP test ($P = 0.552$) results was identified. However, in d-ROMs test by women, a significant difference was noted between those under 50 and 50 and over ($P = 0.010$) (Table 1).

Correlation between plasma lipids and oxidative stress included a strong positive correlation between d-ROMs test results and OxLDL in men ($R = 0.480$, $P < 0.001$) (Fig. 1A), but no such correlation in women ($R = 0.241$, $P = 0.089$) (Fig. 1B). Further, a strong positive correlations between d-ROMs test results and OxLDL in men under 50 and 50 and over [($R = 0.435$, $P = 0.001$) (Fig. 2A) and ($R = 0.545$, $P < 0.001$) (Fig. 2B), respectively]. A weaker positive correlation between d-ROMs test results and OxLDL was found in women under 50 ($R = 0.399$, $P = 0.039$) (Fig. 2C), and no such correlation was observed in women 50 and over ($R = -0.007$, $P = 0.976$) (Fig. 2D).

Table 1. Comparisons between groups by age and gender

		Total	Men	Women	P
Total	LDL (mg/dl)		115.0±27.4	117.7±25.9	0.801
Age≤50		110.3±25.2	110.2±28.0	110.5±18.6	0.928
Age>50		123.2±27.6	121.7±25.6	125.8±31.1	0.697
P		0.004	0.045	0.042	
Total	OxLDL(U/ml)		1.76±0.45	1.63±0.44	0.041
Age≤50		1.66±0.46	1.72±0.48	1.55±0.41	0.100
Age>50		1.78±0.43	1.82±0.40	1.71±0.47	0.314
P		0.035	0.100	0.166	
Total	LDL/HDL		2.12±0.76	1.82±0.53	0.038
Age≤50		1.94±0.74	2.05±0.80	1.70±0.53	0.053
Age>50		2.13±0.65	2.22±0.72	1.96±0.50	0.308
P		0.036	0.176	0.052	
Total	OxLDL/HDL		0.033±0.01	0.025±0.01	< 0.001
Age≤50		0.030±0.01	0.032±0.01	0.024±0.01	0.005
Age>50		0.031±0.01	0.034±0.01	0.027±0.08	0.031
P		0.157	0.348	0.137	
Total	LDL/ApoB		1.16±0.20	1.26±0.19	0.002
Age≤50		1.18±0.18	1.15±0.18	1.25±0.17	0.019
Age>50		1.21±0.22	1.18±0.22	1.27±0.21	0.033
P		0.127	0.234	0.341	
Total	TG (mg/dl)		130.6±88.1	93.8±42.5	0.002
Age≤50		108.8±61.8	120.4±68.3	84.2±35.1	0.006
Age>50		130.0±93.8	144.8±110.1	104.7±48.7	0.076
P		0.066	0.241	0.055	
Total	HDL(mg/dl)		57.6±13.2	66.9±11.5	< 0.001
Age≤50		60.6±12.9	57.2±12.3	67.9±11.7	0.001
Age>50		61.0±14.2	58.1±14.6	65.8±12.2	0.027
P		0.943	0.850	0.464	
Total	d-ROMs (U.CARR)		292.9±64.4	301.9±74.1	0.776
Age≤50		284.6±69.0	286.6±67.6	280.4±72.9	0.510
Age>50		301.7±64.9	301.6±60.2	326.1±70.8	0.274
P		0.005	0.114	0.010	
Total	BAP (μmol/l)		3627.2±341.5	3611.3±281.2	0.552
Age≤50		3651.0±303.6	3654.2±319.6	3644.2±272.5	0.600
Age>50		3584.0±345.8	3589.8±374.6	3574.2±297.7	0.904
P		0.153	0.254	0.391	
Total	ApoB (mg/dl)		100.1±22.2	94.7±21.5	0.070
Age≤50		94.5±21.5	96.5±21.5	90.3±21.1	0.083
Age>50		103.2±22.2	105.2±22.6	99.7±21.7	0.500
P		0.007	0.079	0.022	
Total	TC (mg/dl)		198.8±29.4	203.4±28.0	0.451
Age≤50		192.7±25.5	191.5±28.8	195.3±16.5	0.480
Age>50		210.2±30.7	208.8±27.9	212.5±35.6	0.742
P		0.006	0.005	0.045	
Total	HbA1c (%)		5.33±0.62	5.15±0.44	0.114
Age≤50		5.06±0.52	5.12±0.59	4.93±0.29	0.315
Age>50		5.54±0.51	5.62±0.53	5.40±0.45	0.076
P		< 0.001	< 0.001	< 0.001	

Comparisons between groups by gender and by age. Men, n=98 (median 41.5 years: range 20-65 years); Women, n=51 (median 47.0 years: range 23-68 years), those under 50 years of age, n=84; those 50 years of age or older, n=62; men under 50 years of age, n=57 (median 31.0 years: range 20-49 years); men 50 years of age or older, n=41 (median 56.0 years: range 51–65 years); women under 50 years of age, n=27 (median 38.5 years: range 23-47 years); women 50 years of age or older, n=24 (median 60.0 years: range 51-68 years).

Comparisons between groups used the Mann-Whitney U test and means ± standard deviation are shown. $P < 0.05$ was considered significant.

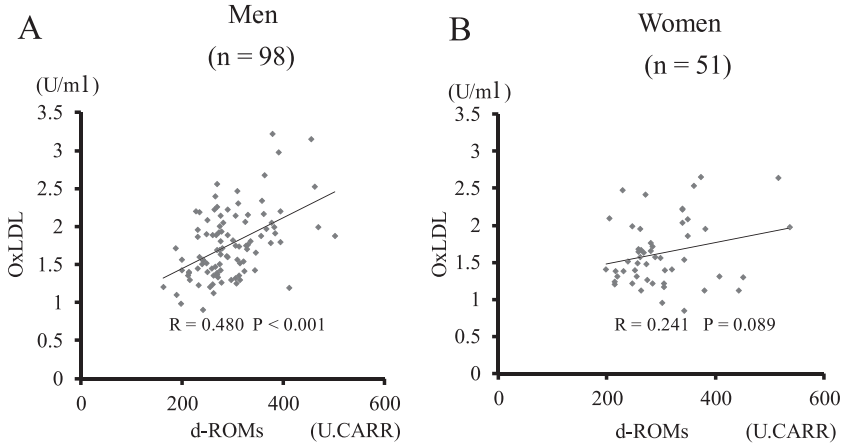

Fig. 1. Correlations between oxidative stress and OxLDL
A : Scatter plot of diacron-reactive oxygen metabolites (d-ROMs) test results and oxidized low-density lipoprotein (OxLDL) in men. R = 0.480, P < 0.001
B : Scatter plot of d-ROMs test results and OxLDL in women. R = 0.241, P = 0.089

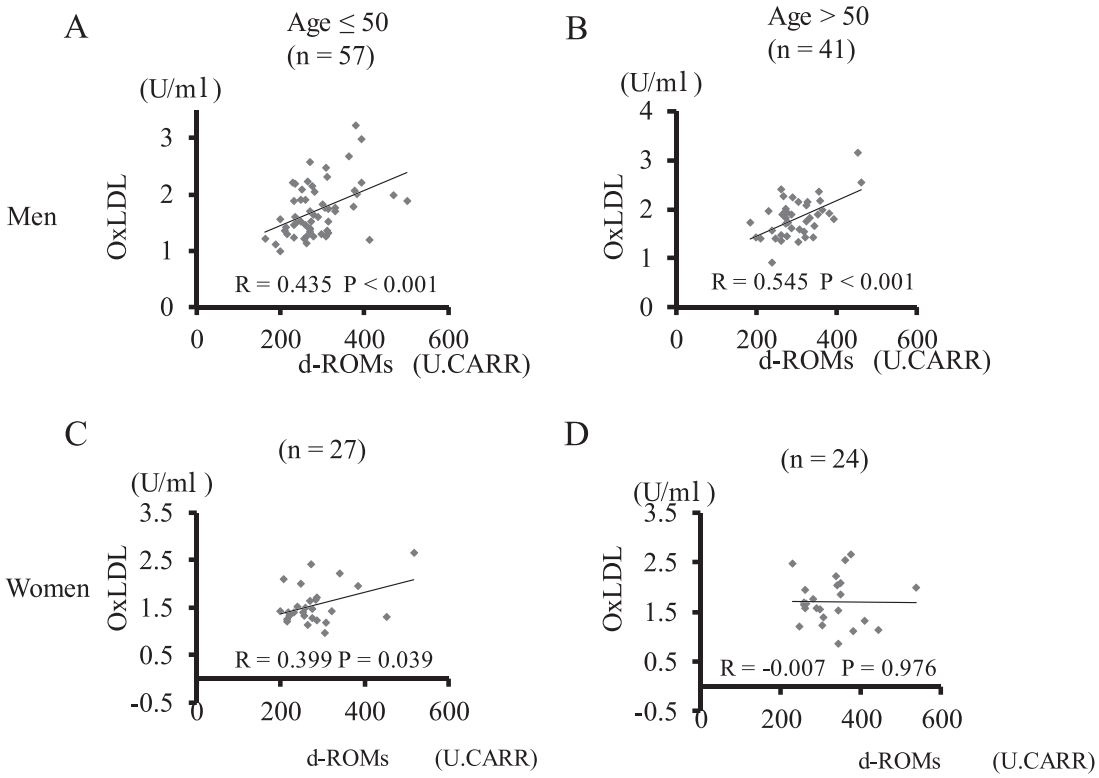

Fig. 2. Correlations between oxidative stress and OxLDL
A : Scatter plot of diacron-reactive oxygen metabolites (d-ROMs) test results and oxidized low-density lipoprotein (OxLDL) in men under 50 years of age. R = 0.435, P < 0.001
B : Scatter plot of d-ROMs test results and OxLDL in men 50 years of age or older. R = 0.545, P < 0.001
C : Scatter plot of d-ROMs test results and OxLDL in women under 50 years of age. R = 0.399, P = 0.039
D : Scatter plot of d-ROMs test results and OxLDL in women 50 years of age or older. R = −0.007, P = 0.976

A strong negative correlation between BAP test results and TG in men (R = −0.571, P < 0.001) (Fig. 3A), and a weaker negative correlation in women (R = −0.344, P = 0.013) (Fig. 3B) were observed. Further, strong negative correlations were found between BAP test results and TG in men under 50 and 50 and over years of age [(R = −0.613, P < 0.001) (Fig. 4A) (R = −0.544, P < 0.001) (Fig. 4B), respectively]. However, no such correlation was found in women under 50 (R = −0.003, P = 0.987) (Fig. 4C), but a negative correlation was found for women 50 and over (R = −0.572, P = 0.004) (Fig. 4D).

Small dense LDL was evaluated using LDL / ApoB ratios. A negative correlation between d-ROMs test results and LDL / ApoB ratio was found in both men (R = −0.252, P = 0.012) (Fig. 5A) and women (R = −0.307, P = 0.028) (Fig. 5B). No such correlation was observed in men under 50 years of age (R = −0.126, P = 0.349) (Fig. 6A). A negative correlation between d-ROMs test results and LDL / ApoB ratio was seen in men 50 years of age or older (R = –0.444, P = 0.004) (Fig. 6B), and was weaker than the correlation between d-ROMs test results and OxLDL. A similar negative correlation was observed in women under 50 years of age (R = −0.524, P = 0.005) (Fig. 6C), and was stronger than the correlation between d-ROMs test results and OxLDL. No correlation was observed for women 50 or older (R = −0.184, P = 0.389) (Fig. 6D).

A positive correlation between d-ROMs test results and OxLDL / HDL ratios was found for men under 50 and 50 and over years of age, (R = 0.392, P = 0.003 and (R = 0.525, P < 0.001, respectively). A negative correlation between BAP test results and OxLDL / HDL ratio was seen in men under 50 (R = −0.458, P < 0.001) (Table 2).

No correlations between OxLDL and small dense LDL were observed for men or women (R = −0.002, P = 0.988 and = 0.160, P = 0.263, respectively), in men or women under 50 (R = 0.163, P = 0.225 and R = 0.101, P = 0.616, respectively) or in men or women 50 and over (R = –0.239, P = 0.133 and R = 0.188, P = 0.378, respectively) (Table 3).

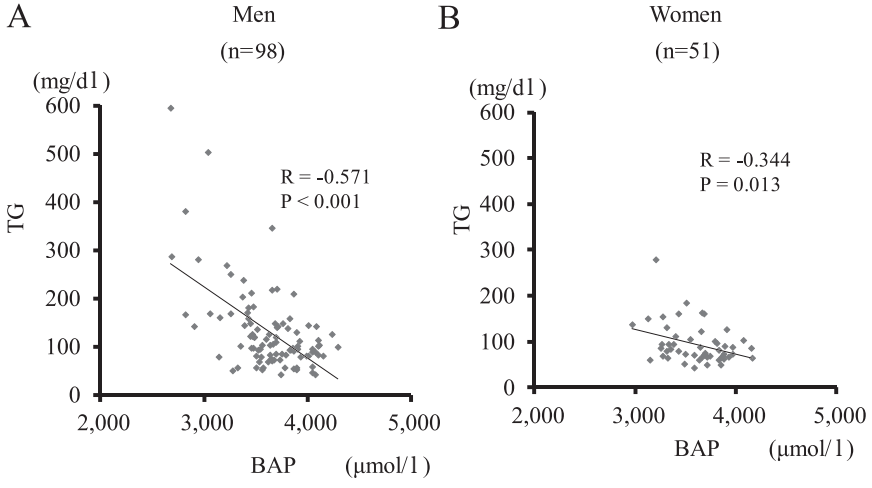

Fig. 3. Correlations between antioxidant potential and TG
A : Scatter plot of biological antioxidant potential (BAP) test results and triglycerides (TG) in men. R = −0.571, P < 0.001
B : Scatter plot of BAP test results and TG in women. R = −0.344, P = 0.013

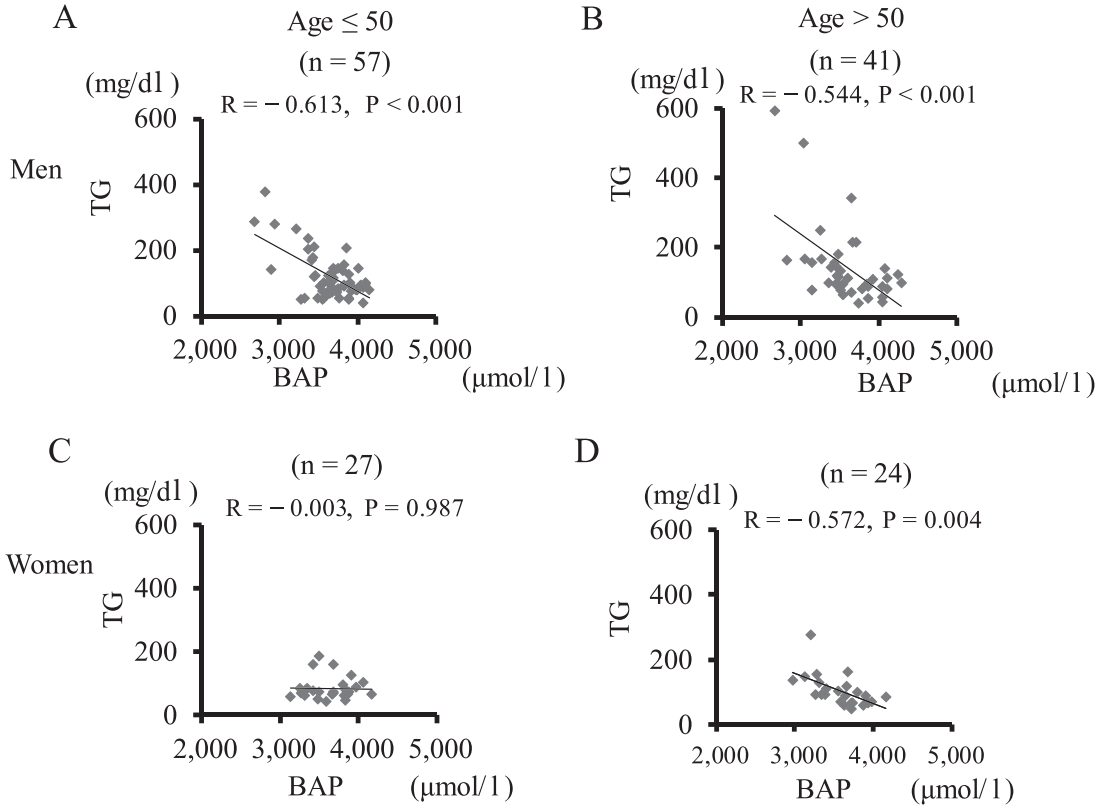

Fig. 4. Correlations between antioxidant potential and TG

A : Scatter plot of biological antioxidant potential (BAP) test results and triglycerides (TG) in men under 50 years of age. R = −0.613, P < 0.001

B : Scatter plot of BAP test results and TG in men 50 years of age or older. R = −0.544, P < 0.001

C : Scatter plot of BAP test results and TG in women under 50 years of age. R = −0.00329, P = 0.987

D : Scatter plot of BAP test results and TG in women 50 years of age or older. R = −0.572, P = 0.004

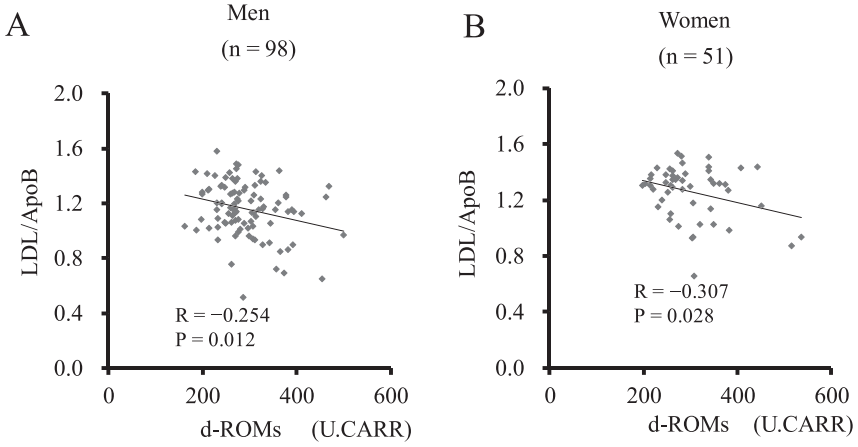

Fig. 5. Correlations between oxidative stress and LDL / ApoB ratio

A : Scatter plot of diacron-reactive oxygen metabolites (d-ROMs) test results and LDL / ApoB ratio in men. R = −0.254, P = 0.012

B : Scatter plot of d-ROMs test results and LDL / ApoB ratio in women. R = −0.307, P = 0.028

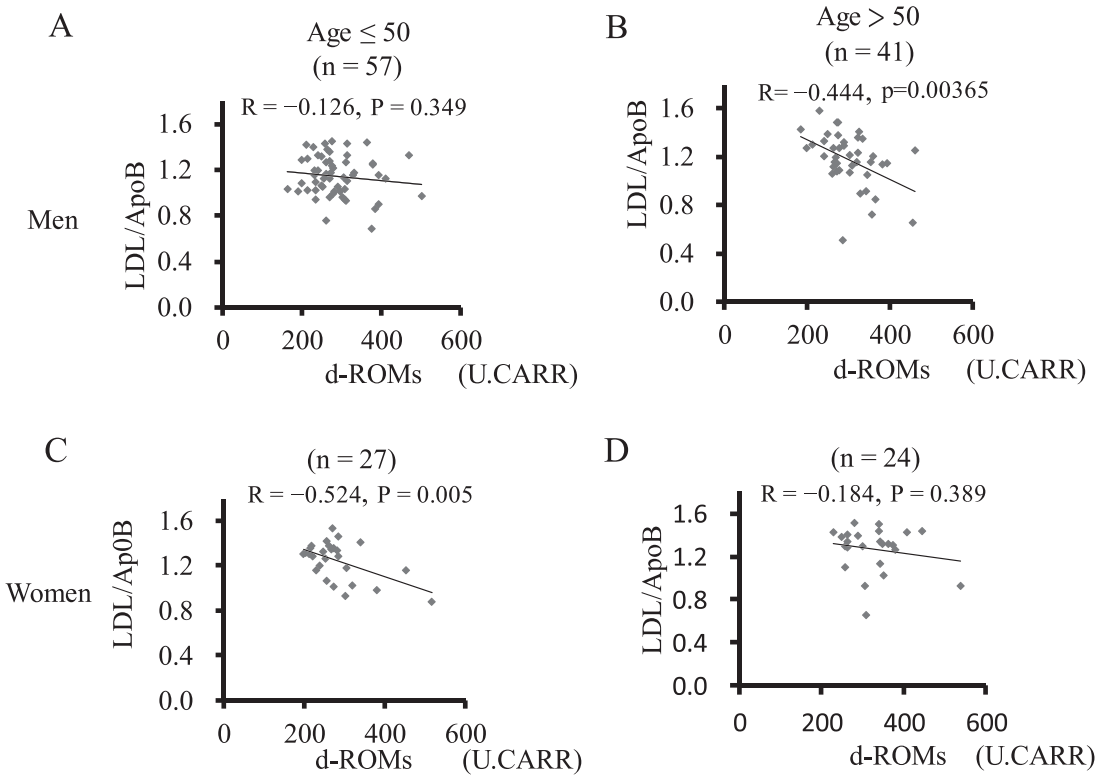

Fig. 6. Correlations between oxidative stress and LDL / ApoB ratio

A : Scatter plot of diacron-reactive oxygen metabolites (d-ROMs) test results and LDL / ApoB ratio in men under 50 years of age. R = −0.126, P = 0.349

B : Scatter plot of d-ROMs test results and LDL / ApoB ratio in men 50 years of age or older. R = −0.444, P = 0.004

C : Scatter plot of d-ROMs test results and LDL / ApoB ratio in women under 50 years of age. R = −0.524, P = 0.005

D : Scatter plot of d-ROMs test results and LDL / ApoB ratio in women 50 years of age or older. R = −0.184, P = 0.389

Table 2. Correlations between oxidative stress and OxLDL / HDL

	d-ROMs and OxLDL / HDL	BAP and OxLDL / HDL
Men		
Total	R = 0.443, P < 0.001	R = −0.336, P < 0.001
Age ≤ 50	R = 0.392, P < 0.001	R = −0.458, P < 0.001
Age > 50	R = 0.525, P < 0.001	R = −0.177, P = 0.270
Women		
Total	R = 0.375, P = 0.007	R = 0.025, P = 0.860
Age ≤ 50	R = 0.472, P = 0.013	R = 0.226, P = 0.258
Age > 50	R = 0.189, P = 0.377	R = −0.166, P = 0.437

Correlations between oxidative stress and OxLDL / HDL ratio in men and women and in age groups under 50 years of age and 50 years of age or older.

Table 3. Correlations between OxLDL and LDL / ApoB ratio

	Men	Women
Total	R = −0.002, P = 0.988	R = 0.160, P = 0.263
Age ≤ 50	R = 0.163, P = 0.225	R = 0.101, P = 0.616
Age > 50	R = −0.239, P = 0.133	R = 0.188, P = 0.378

Correlations between OxLDL and the LDL / ApoB ratio in men and women, and in age groups under 50 years of age and 50 years of age or older.

Discussion

Recently, coronary risk assessment for factors such as gender, age, smoking, serum cholesterol, and systolic blood pressure have been used to manage target values of dyslipidemia[17]. According to NIPPON DATA80, mortality from ischemic heart disease is extremely low for women under 50 years of age (<0.5%)[1]. In this study, significant differences between men and women in OxLDL, LDL / ApoB ratio, OxLDL / HDL ratio, LDL / HDL ratio, TG, and HDL were found. Estrogen increases nitric oxide production in endothelial cells and inhibits proliferation of vessel wall cells[18]. No difference between men and women were observed in LDL and d-ROMs test results, but a significant difference was seen with OxLDL. Possibly, when estrogen is suppressed in women, LDL is oxidized to LDL by oxidative stress. No difference in LDL between men and women was seen, but a significant difference in LDL / ApoB ratios as an indicator of small dense LDL was seen. Estrogen may suppress the change of LDL to small dense LDL, which more effectively induces atherosclerosis[14].

In this study, a strong positive correlation was found between d-ROMs test results and OxLDL in men, but no correlation was observed in women. Also, a negative correlation was seen between BAP test results and TG in both men and women, but it was stronger in men. Likewise, a similar correlation was seen between d-ROMs test results and small dense LDL in both men and women, indicating that small dense LDL, as well as OxLDL, are markers of oxidative stress. The correlation between OxLDL and small dense LDL in men was stronger than that in women. A positive correlation between oxidative stress and OxLDL / HDL was also observed in both men and women, and the correlation was stronger in women.

Correlation analysis between oxidative stress and lipids in plasma was positive for d-ROMs test results and OxLDL in women under 50 years of age, but the correlation was weaker than the correlation between d-ROMs test results and small dense LDL. In men, the opposite was found, where the OxLDL, d-ROMs test correlation was stronger. These results suggest that estrogen can suppress the oxidation of small dense LDL to OxLDL.

In women 50 years of age or older, no correlations were observed between OxLDL or small dense LDL and d-ROMs test results. In women, a positive correlation was found for d-ROMs test results with age, but no correlation with OxLDL. In women, factors other than estrogen were involved in the change of LDL to OxLDL by oxidative stress.

Recently, an increase in smoking rates among young Japanese women has been reported[19]. After smoking, substances present in tobacco are absorbed into the bloodstream and oxidize LDL, thus promoting the progression of atherosclerosis[20]. However, coronary artery mortality in women under 50 years of age is less than 0.5% regardless of smoking behavior[1]. Possibly, smoking promotes the progression of atherosclerosis in women under 50 years of age, but in women 50 years of age or older, no correlation between oxidative stress and OxLDL was seen, even after menopause. Further, exercise is increasing among the elderly[21], and moderate exercise is effective in preventing oxidative stress[22]. Possibly, increased exercise also helps retard the progression of atherosclerosis after menopause.

Correlations among d-ROMs test results and small dense LDL and OxLDL / HDL ratios in men 50 years of age and OxLDL was similar in the two age groups. Many men 50 years of age or older with lifestyle-related diseases receive treatment. The occurrence of oxidative stress may be prevented or reduced by treatment for diabetes[23], hypertension[24], and dyslipidemia[25]. Such treatment could affect correlations between d-ROMs test results and OxLDL.

No correlation is observed between BAP test results and TG in women under 50 years of age, and a negative correlation is found in women 50 or over. Estrogen inhibits fat accumulation by controlling the activity and expression of lipoprotein lipase[26], and in women under 50, study results suggest that estrogen suppresses the increase in TG even when antioxidant potential is low. A negative correlation between BAP tests and TG in women 50 years and older exists and it is possible that antioxidant potential measurements will help postmenopausal women alter their diet to improve TG levels. In study subjects, medication and dietary supplement use for lifestyle-related illnesses were unknown, and it is possible that drugs and supplements might have affected outcomes.

Effects of statin therapy differ between men and women[27, 28], and the present study may provide an explanation. Correlations between oxidative stress and OxLDL and between antioxidant potential and TG in men were stronger than those in women. For correlations between lipids and oxidative stress, estrogen may affect oxidation of LDL to OxLDL or to small dense LDL. For men, decreasing oxidative stress in the blood may be more important than for women to prevent atherosclerosis.

Conflict of Interest

No authors have any potential conflicts of interest relevant to this article.

References

1) NIPPON DATA80 Research Group. Risk assessment chart for death from cardiovascular disease based on a 19-year follow up study of a Japanese representative population. *Circ J*. 2006;**70**:1249–1255.
2) Kannel WB, Hjortland MC, Mcnamara PM, *et al*. Menopause and risk of cardiovascular disease: the Framingham study. *Ann Intern Med*. 1976;**85**:447–452.
3) Trapani L, Pallottini V. Age-related hypercholesterolemia and HMG-CoA reductase dysregulation: sex does matter (a-gender perspective). *Curr Gerontol Geriatr Res*. 2010:420139. (accessed 2019 Dec 20) Available from: https://

www.ncbi.nlm.nih.gov/pmc/articles/PMC2863156/pdf/CGGR2010-420139.pdf

4) Bruring JC, Linqohr P, Gillete J, *et al*. Estrogen receptor-alpha and Sp1 interact in the induction of the low density lipoprotein-receptor. *J Steroid Biochem Mol Biol*. 2003;**86**:113-121.
5) Lamon-Fava S, Micherone D. Regulation of apoA-1 gene expression: mechanism of action of estrogen and genistein. *J Lipid Res*. 2004;**45**:106-112.
6) Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res*. 2000;**86**:494-501.
7) Nishikawa T, Edelstein D, Yamagishi S, *et al*. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. 2000;**404**:787-790.
8) Nishi K, Itabe H, Uno M, *et al*. Oxidized LDL in carotid plaques and plasma associates with plaque instability. A*rterioscler Thromb Vasc Biol*. 2002;**22**:1649-1654.
9) Steinberg D. Oxidative modification of LDL and atherogenesis. *Circulation*. 1997;**95**:1062-1071.
10) McMurray HF, Parthasarathy S, Steinberg D. Oxidatively modified low density lipoprotein is a chemoattractant for human T lymphocytes. *J Clin Invest*. 1993;**92**:1004-1008.
11) Fukui T, Yamauchi K, Maruyama M, *et al*. Significance of measuring oxidative stress in lifestyle-related diseases from the viewpoint of correlation between d-ROMs and BAP in Japanese subjects. *Hypertens Res*. 2011;**34**:1041-1045.
12) Kawakami T, Yura A, Ogawa K, *et al*. The correlation between oxidative stress and lipids in blood. *J Showa Univ Soc*. 2014;**74**:403-412. (in Japanese).
13) St-Pierre AC, Cantin B, Dagenais GR, *et al*. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men. *Arterioscler Thromb Vasc Biol*. 2005;**25**:553-559.
14) Packard CJ, Demant T, Stewart JP, *et al*. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *J Lipid Res*. 2000;**41**:305-318.
15) Yokokawa H, Yasumura S, Tanno K, *et al*. Serum low-density lipoprotein to high-density lipoprotein ratio as a predictor of future acute myocardial infarction among men in a 2.7-year cohort study of a Japanese northern rural population. *J Atheroscler Thromb*. 2011;**18**:89-98.
16) Hirano T, Ito Y, Yoshino G. Measurement of small dense low-density lipoprotein particles. *J Atheroscler Thromb*. 2005;**12**:67-72.
17) Japan atherosclerosis society (JAS). Guidelines for the prevention of atherosclerotic cardiovascular diseases in Japanese-2012 version.
18) Hisamoto K, Ohmichi M, Kurachi H, *et al*. Estrogen induces the Akt dependent activation of endothelial nitric-oxide synthase in vascular endothelial cells. *J Biol Chem*. 2001;**276**:3459-3467.
19) Sakuta T, Abe Y, Sakuta A. Rapid increase in juvenile smokers in Japan. *Int Med J*. 2001;**8**:65.
20) Yamaguchi Y, Matsuno S, Kagota S, *et al*. Oxidants in cigarette smoke extract modify low-density lipoprotein in the plasma and facilitate atherogenesis in the aorta of Watanabe heritable hyperlipidemic rabbits. *Atherosclerosis*. 2001;**156**:109-117.
21) Ministry of Health, Labour and Welfare. The National Health and Nutrition Survey in Japan, 2012 version.
22) Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*. 2003;**189**:41-54.
23) Yang HC, Deleuze S, Zuo Y, *et al*. The PPARgamma agonist pioglitazone ameliorates aging-related progressive renal injury. *J Am Soc Nephrol*. 2009;**20**:2380-2388.
24) Onozato ML, Tojo A, Kobayashi N, *et al*. Dual blockade of aldosterone and angiotensin II additively suppresses TGF-beta and NADPH oxidase in the hypertensive kidney. *Nephrol Dial Transplant*. 2007;**22**:1314-1322.
25) Kostapanos MS, Spyrou AT, Tellis CC, *et al*. Ezetimibe treatment lowers indicators of oxidative stress in hypercholesterolemic subjects with high oxidative stress. *Lipids*. 2011;**46**:341-348.
26) Homma H, Kurachi H, Nishino Y, *et al*. Estrogen suppresses transcription of lipoprotein lipase gene. Existence of a unique estrogen response element on the lipoprotein lipase promoter. *J Biol Chem*. 2000;**275**:11404-11411.

27) Mizuno K, Nakaya N, Ohashi Y, *et al*. Usefulness of pravastatin in primary prevention of cardiovascular events in women: analysis of the Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA study). *Circulation*. 2008;**117**:494–502. (accessed 2019 Dec 20) Available from: https://doi.org/10.1161/CIRCULATIONAHA.106.671826

28) Gutierrez J, Ramirez G, Rundek T, *et al*. Statin therapy in the prevention of recurrent cardiovascular events: a sex-based meta-analysis. *Arch Intern Med*. 2012;**172**:909–919. (accessed 2019 Dec 20) Available from: https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/1195535

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