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 \emph{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

\textit{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\(^\text{12}\).

**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\(^\text{16}\), 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 \(-80^\circ C\) 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\(^\text{12}\).

**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).
| Total     | LDL (mg/dl) | Men              | Women             | P     |
|-----------|-------------|------------------|-------------------|-------|
| Age ≤ 50  | 110.3±25.2  | 115.0±27.4       | 117.7±25.9        | 0.801 |
| 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) |                  |                   |       |
| Age ≤ 50  | 1.76±0.45   | 1.72±0.48        | 1.63±0.44         | 0.041 |
| Age > 50  | 1.82±0.40   | 1.81±0.47        | 1.71±0.47         | 0.314 |
| P         | 0.035       | 0.100            | 0.166             |       |
| Total     | LDL/HDL     |                  |                   |       |
| Age ≤ 50  | 2.12±0.76   | 1.82±0.53        | 2.05±0.80         | 0.038 |
| Age > 50  | 2.22±0.72   | 1.96±0.50        | 2.00±0.70         | 0.308 |
| P         | 0.036       | 0.176            | 0.052             |       |
| Total     | OxLDL/HDL   |                  |                   | < 0.001|
| Age ≤ 50  | 0.030±0.01  | 0.033±0.01       | 0.025±0.01        | < 0.001|
| 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    |                  |                   |       |
| Age ≤ 50  | 1.16±0.20   | 1.15±0.18        | 1.26±0.19         | 0.002 |
| Age > 50  | 1.18±0.22   | 1.18±0.22        | 1.27±0.21         | 0.033 |
| P         | 0.127       | 0.234            | 0.341             |       |
| Total     | TG (mg/dl)  |                  |                   |       |
| Age ≤ 50  | 130.6±88.1  | 120.4±68.3       | 93.8±42.5         | 0.002 |
| 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) |                  |                   |       |
| Age ≤ 50  | 576±13.2    | 572±12.3         | 66.9±11.5         | < 0.001|
| Age > 50  | 610±14.2    | 581±14.6         | 65.8±12.2         | 0.027 |
| P         | 0.943       | 0.850            | 0.464             |       |
| Total     | d-ROMs (U/CARR) |          |                   |       |
| Age ≤ 50  | 292.9±64.4  | 286.6±67.6       | 301.9±74.1        | 0.776 |
| Age > 50  | 301.7±64.9  | 301.6±60.2       | 280.4±72.9        | 0.510 |
| P         | 0.005       | 0.114            | 0.011             |       |
| Total     | BAP (µmol/l)|                  |                   |       |
| Age ≤ 50  | 3627.2±341.5| 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)|                  |                   |       |
| Age ≤ 50  | 94.5±21.5   | 96.5±21.5        | 94.7±21.5         | 0.070 |
| Age > 50  | 103.2±22.2  | 105.2±22.6       | 99.7±21.7         | 0.083 |
| P         | 0.007       | 0.079            | 0.022             |       |
| Total     | TC (mg/dl)  |                  |                   |       |
| 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 (%)   |                  |                   |       |
| 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 age and gender. 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; median 50 years of age or older, n=57 (median 31.0 years; range 20-49 years); median 50 years of age or older, n=41 (median 56.0 years; range 51-65 years); median 50 years of age or older, n=27 (median 38.5 years; range 23-47 years); median 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.
Oxidative Stress and Blood Lipids in Men and Women

**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).

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**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. \(R = -0.344, P = 0.013\)
Oxidative Stress and Blood Lipids in Men and Women

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. \( R = -0.444, P = 0.00365 \)
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 \( \leq 50 \)    | \( R = 0.392, P < 0.001 \) | \( R = -0.458, P < 0.001 \) |
| 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 \( \leq 50 \)    | \( R = 0.472, P = 0.013 \) | \( R = 0.226, P = 0.258 \) |
| 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.
Oxidative Stress and Blood Lipids in Men and Women

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\textsuperscript{17}. According to NIPPON DATA80, mortality from ischemic heart disease is extremely low for women under 50 years of age (\textless 0.5\%). 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\textsuperscript{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\textsuperscript{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.

| Table 3. Correlations between OxLDL and LDL / ApoB ratio |
|-----------------------------------|
|                                   |
| **Men**                           |
| Total R = -0.002, P = 0.988       |
| Age \leq 50 R = 0.163, P = 0.225  |
| Age \geq 50 R = -0.239, P = 0.133 |
| **Women**                         |
| Total R = 0.160, P = 0.263        |
| Age \leq 50 R = 0.101, P = 0.616  |
| Age \geq 50 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.
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.

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