Moderate Oxidative Stress and High Antioxidative Activity Are Associated with Steatosis in Japanese Males

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Abstract

Background and aim: Steatosis is an increasingly common problem worldwide, accompanying increasing obesity. Recently, it has been suggested that oxidative stress plays an important role in development of fatty liver disease. We carried out an epidemiological study to clarify the role of oxidative stress and antioxidative activity in steatosis.

Methods: This study was conducted with 184 male workers who had received their annual health checkup. Steatosis was confirmed using ultrasonography. Oxidative stress and antioxidative activity were assessed using the dROM test and the BAP test, respectively.

Results: Steatosis was confirmed in 59 subjects (29.7%) by ultrasonography. There was no significant difference between cases and controls in BAP levels (2229.0 $\mu\text{mol/L}$ vs. 2194.3 $\mu\text{mol/L}$, $p = 0.83$). The steatosis group showed significantly lower dROM levels than the control group (332.7 U. CARR vs. 316.8 U. CARR, $p < 0.05$). In addition, we carried out logistic regression analysis to assess the combination between dROM levels and BAP levels. Subjects with high dROM levels and high BAP levels had 74% lower risk for steatosis than subjects with low dROM levels and high BAP levels.

Conclusions: Our results suggested that moderate oxidative stress and high antioxidative activity was associated with decreased steatosis risk in Japanese males. Clin Trans Sci 2013; Volume 6: 45–49

Keywords: epidemiology, liver disease, obesity

Introduction

Reactive oxygen species (ROS) are continuously produced and eliminated by living organisms. However, an imbalance between ROS generation and elimination leads to enhanced ROS levels, a state called “oxidative stress.” Oxidative stress is a serious cause of cell damage associated with initiation and development of several diseases,¹ aging,² and obesity.³

Steatosis is an increasingly common problem worldwide, accompanying increasing obesity. Recently, nonalcoholic fatty liver disease (NAFLD) has become a major cause of chronic liver disease,⁴ coronary heart disease, and atherosclerosis.⁵

The most widely accepted explanation for nonalcoholic steatohepatitis (NASH) pathogenesis is the two hit theory.⁶ According to this hypothesis, the first hit leads to conversion of normal liver to steatosis. Then the second hit, increases in oxidative stress, pro-inflammatory cytokines and endotoxemia, leads to the development of NASH. Therefore, oxidative stress may play an important role in the development of fatty liver disease. It has been reported that subjects with fatty liver disease have elevated levels of plasma biomarkers of inflammation,⁷ endothelial dysfunction,^{8,9} and diabetes mellitus.^{10,11} However, much of the evidence relating to the role of oxidative stress in fatty liver disease is derived from animal and clinical studies. The results in many of these studies have been conflicting.^{12–14} Moreover, no study has reported the combination between oxidative stress and antioxidative activity. The purpose of this epidemiological study is to investigate the role of oxidative stress and antioxidant activity in steatosis.

Methods

The study subjects were 199 male workers, who had received annual health checkups at clinics from 2008 to 2009. Subjects who agreed to participate in this study were interviewed after informed consent had been obtained. We excluded subjects with

cancer and hepatitis ($n = 3$), with cancer ($n = 4$), with hepatitis ($n = 4$), high sensitivity C-reactive protein (hsCRP) levels ≥ 10000 ng/mL ($n = 2$) and those for whom diacron reactive oxygen metabolites (dROM) data was unavailable ($n = 2$). There were no subjects with cirrhosis. This left a final total of 184 Japanese males. The diagnosis of steatosis was based on the brightness of the liver on ultrasonography in comparison with the kidney, vascular blurring of the hepatic vein trunk, and deep attenuation in the right hepatic lobe. This study was approved by the ethics committee of Fukuoka University.

Measurements

Serum samples were collected at the annual health checkups, and were immediately used for biochemical analyses and oxidative stress profiling. Each subject completed a questionnaire covering his clinical history in addition to smoking status, drinking status, and physical activity. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice using a standard mercury sphygmomanometer with the cuff on the right arm and the subjects in a sitting position. The body mass index (BMI) was calculated by the following formula: Weight (kg)/height² (m). Waist circumference (cm) was measured at the midpoint between the lower costal edge and upper iliac crest following a normal expiration. Serum total cholesterol (TC) was measured by the cholesterol dehydrogenase UV method. High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by the direct method. Triglyceride was measured by the enzymatic method. Hemoglobin A1c (HbA1c) was measured by latex agglutination. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ -glutamyltranspeptidase (GGT) were measured using the Japan Society of Clinical Chemistry (JSCC) consensus method. White blood cell counts were done using an automatic analyzer. Fasting

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	Controls	(n = 125)	Cases	(n = 59)	p value
Age (years) mean (SD)	53.9	(7.7)	52.4	(8.3)	0.23
BMI (kg/m ²) mean (SD)	23.1	(2.4)	26.2	(3.6)	<0.001
Systolic blood pressure (mmHg) mean (SD)	124.1	(15.5)	127.1	(16.5)	0.23
Diastolic blood pressure (mmHg) mean (SD)	80.0	(10.5)	82.2	(10.8)	0.18
Waist circumference (cm) mean (SD)	83.4	(7.0)	92.6	(9.5)	<0.001
Smoking rate n (%)	68	(54.4)	19	(32.2)	0.09
Drinking status n (%)					0.66
7 days/week	53	(42.4)	21	(35.6)	
1–6 days/week	44	(35.2)	24	(40.7)	
Nondrinker	28	(22.4)	14	(23.7)	
Hypertension n (%)	39	(31.2)	24	(40.7)	0.66
Diabetes mellitus n (%)	12	(10.8)	6	(5.9)	0.90
Metabolic syndrome n (%)	11	(8.8)	18	(30.5)	<0.001

BMI = body mass index. p value by unpaired *t*-test for continuous variables and χ^2 test for categorical variables. Statistical significance is expressed in bold type.

Table 1. Basic characteristics of study subjects.

blood glucose was measured by the Hexokinase UV-Method. High sensitivity C reactive protein (hsCRP) levels were measured by the nephelometry method using a commercial kit (Siemens Healthcare Diagnostics K.K., Tokyo, Japan). Insulin levels were measured by the chemiluminescence enzyme immunoassay (CLEIA) method using a commercial kit (Fujirebio Inc. Tokyo, Japan). Homeostasis model assessment-Insulin Resistance (HOMA-IR) was calculated according to the formula: insulin ($\mu\text{U/mL}$) $\times$ fasting glucose (mmol/L) / 22.5. The dROM test, which provides a marker for oxidative stress of blood samples, was applied. This method makes it possible to estimate the total amount of hydroperoxide present in a 20 μL sample. The results of the dROM test are expressed in arbitrary units called “Carratelli units” (U. CARR), where 1 U. CARR corresponds to 0.08 mg/dL of H_2O_2 . Antioxidant abilities were measured with the bioantioxidant potency (BAP) test. The underlying principle of this test is similar to that of the well-known FRAP test, which measures the ferric reducing ability of plasma.¹⁵ The BAP test and dROM test are conducted using a specially designed photometer in conjunction with the FRAS4 system (Health & Diagnostic Ltd. Co., Parma, Italy). We defined hypertension as a SBP of 140+ mmHg and/or a DBP of 90+ mmHg and/or taking antihypertensive medication, and diabetes mellitus as HbA1c of 6.5+% and/or having treatment for diabetes mellitus with insulin or oral agents. Metabolic syndrome was diagnosed by the Japanese criteria.

Statistical methods

For continuous variables, results are presented as mean $\pm$ standard deviation (SD) or median (minimum, maximum), and differences between the two groups were evaluated with an unpaired *t*-test. Because the distribution of hsCRP levels was skewed, log transformation was used, which yielded more normally distributed data. Categorical variables are presented as frequency counts, and intergroup comparisons were tested for statistical significance using a χ^2 test. Associations between fatty liver and hsCRP, HOMA-IR, dROM, or BAP and combinations of dROM and BAP were analyzed by logistic regression analysis.

Results are presented as odds ratios (OR) together with their 95% confidence intervals. A *p* values less than 0.05 were considered to be statistically significant. All analyses were conducted using the Statistical Analysis System (SAS, Version 9.1 for WINDOWS; Cary, NC, USA).

Results

Basic characteristics are summarized in *Table 1*. Fifty-nine subjects (29.7%) showed a steatosis in the ultrasonic examination. Subjects with steatosis had significantly higher BMI, waist circumference and prevalence of metabolic syndrome. There was no significant difference between cases and controls in regard to SBP, DBP, smoking status, drinking status, and prevalence of diabetes mellitus or hypertension.

The biochemical characteristics are presented in *Table 2*. AST, ALT, GGT, TC, triglyceride, LDL-C, and insulin were significantly higher in the subjects with steatosis than in those with normal liver. In contrast, HDL-C levels in the subjects with steatosis were lower than in those with normal liver. Serum hsCRP levels in the steatosis group were higher than in the control group, but there was no significant difference (850.9 ng/mL vs. 1073.8 ng/mL, *p* = 0.31). HOMA-IR in the steatosis group was significantly higher than in the control group (2.6 vs. 1.4, *p* < 0.001). There was no significant difference between cases and controls in regard to BAP levels (2229.0 $\mu\text{mol/L}$ vs. 2194.3 $\mu\text{mol/L}$, *p* = 0.83). The steatosis group showed significantly lower dROM levels than the control group (332.7 U. CARR vs. 316.8 U. CARR, *p* < 0.05).

We conducted logistic regression analysis to assess the relationship between steatosis and dROM levels, BAP levels, HOMA-IR, or hsCRP levels (*Table 3*). dROM levels, BAP levels, HOMA-IR, and hsCRP levels were divided into quartiles using the following cutoff points: dROM levels 288.5, 319.5, and 357.0 U. CARR; BAP levels 1995.5, 2176.5, and 2426.5 $\mu\text{mol/L}$; hsCRP levels 246.0, 436.5, and 918.5 ng/mL; HOMA-IR 0.87, 1.37, and 2.61.

Compared to subjects in the lowest quartile, the odds ratio of subjects in the highest quartile of dROM levels had a significantly

	Controls (n = 125)		Cases (n = 59)		p value
	Means	(SD)	Means	(SD)	
AST (IU/L)	22.1	(5.9)	27.2	(8.1)	<0.001
ALT (IU/L)	22.9	(10.8)	36.4	(10.2)	<0.001
GGT (IU/L)	45.8	(43.2)	66.2	(88.1)	0.10
Total cholestel levels (mg/dL)	202.7	(29.3)	209.1	(29.1)	0.18
Triglyceride (mg/dL)	122.6	(93.5)	170.9	(149.8)	<0.05
HDL cholesterol levels (mg/dL)	57.2	(13.1)	50.3	(12.2)	<0.01
LDL cholesterol levels (mg/dL)	120.3	(26.1)	129.2	(25.3)	<0.05
Hemoglobin A1c (%)	5.4	(0.8)	5.6	(0.8)	0.13
White blood cell counts (per μ L)	5709.6	(1608.9)	6306.8	(1425.3)	<0.05
Insulin (μ U/mL)	5.2	(3.2)	9.5	(5.4)	<0.001
Fasting blood glucose (mg/dL)	104.8	(21.6)	109.9	(26.5)	0.16
HOMA-IR	1.4	(0.9)	2.6	(2.1)	<0.001
HsCRP (ng/mL)	850.9	(1393.9)	1073.8	(1363.9)	0.31
BAP (μ mol/L)	2229.0	(331.7)	2194.3	(424.2)	0.83
dROM (U. CARR)	332.7	(60.5)	316.8	(52.4)	<0.05

ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; GGT = γ -glutamyltranspeptidase; HOMA-IR = Homeostasis model assessment-Insulin Resistance; HsCRP = high sensitivity C-reactive protein. p value by unpaired t-test. Statistical significance is expressed in bold type.

Table 2. Biochemical characteristics of the study subjects.

	Controls		Cases		OR	Crude	OR	Adjusted*
	n	(%)	n	(%)		95%CI		95%CI
HOMA-IR								
≤0.87	42	(33.6)	4	(6.8)	1.00	(reference)	1.00	(reference)
0.87< ≤1.37	39	(31.2)	7	(11.9)	1.89	(0.51–6.94)	1.60	(0.41–6.23)
1.37< ≤2.61	27	(21.6)	19	(32.2)	7.39	(2.27–24.09)	4.41	(1.25–15.54)
2.61<	17	(13.6)	29	(49.2)	17.91	(5.46–58.73) p for trend < 0.001	6.31	(1.68–23.79) p for trend < 0.01
HsCRP level (ng/mL)								
≤246.0	34	(27.2)	12	(20.3)	1.00	(reference)	1.00	(reference)
246.0< ≤436.5	34	(27.2)	12	(20.3)	1.00	(0.39–2.54)	0.88	(0.31–2.55)
436.5< ≤918.5	31	(24.8)	15	(25.4)	1.37	(0.56–3.38)	0.96	(0.33–2.82)
918.5<	26	(20.8)	20	(33.9)	2.18	(0.91–5.25) p for trend = 0.31	2.04	(0.72–5.80) p for trend = 0.21
BAP level (pmol/L)								
2426.5≤	27	(21.6)	19	(32.2)	1.00	(reference)	1.00	(reference)
2426.5> ≥2176.5	31	(24.8)	15	(25.4)	0.57	(0.23–1.46)	0.71	(0.29–2.58)
2176.5> ≥1995.5	36	(28.8)	10	(17.0)	1.00	(0.42–2.39)	1.04	(0.50–4.04)
1995.5>	31	(24.8)	15	(25.4)	1.45	(0.62–3.41) p for trend = 0.55	2.12	(0.88–6.79) p for trend = 0.35
dROM level (U. CARR)								
≤288.5	27	(21.6)	19	(32.2)	1.00	(reference)	1.00	(reference)
288.5< ≤319.5	30	(24.0)	16	(27.1)	0.76	(0.33–1.76)	0.94	(0.37–2.63)
319.5< ≤357.0	32	(25.6)	14	(23.7)	0.62	(0.26–1.47)	0.85	(0.27–1.99)
357.0<	36	28.8	10	(17.0)	0.40	(0.16–0.99) p for trend = 0.09	0.45	(0.13–1.08) p for trend = 0.28
*Adjusted for age (continuous), BMI (continuous), smoking status (categorical), drinking status (categorical), physical activity (categorical). Statistical significance is expressed in bold type.								

*Adjusted for age (continuous), BMI (continuous), smoking status (categorical), drinking status (categorical), physical activity (categorical). Statistical significance is expressed in bold type.

Table 3. Crude and adjusted odds ratios of steatosis for HOMA-IR, hsCRP levels, dROM levels, and BAP levels.

	Controls		Cases		Crude		Adjusted*	
	n	%	n	%	OR	95% CI	OR	95% CI
Low-dROM • High-BAP	26	(20.8)	17	(28.8)	1.00	(reference)	1.00	(reference)
Low-dROM • Low-BAP	31	(24.8)	18	(30.5)	1.13	(0.49–2.62)	1.15	(0.40–3.28)
High-dROM • Low-BAP	27	(21.6)	16	(27.1)	1.02	(0.44–2.38)	1.23	(0.44–3.40)
High-dROM • High-BAP	41	(32.8)	8	(13.6)	0.34	(0.13–0.87)	0.26	(0.08–0.78)

* Adjusted for age (continuous), BMI (continuous), smoking status (categorical), drinking status (categorical), physical activity (categorical).

Table 4. Combination between dROM levels and BAP levels on steatosis.

decreased risk for steatosis. However, after adjusting for age, BMI, smoking status, drinking status, and physical activity, the odds ratio was slightly attenuated (adjusted OR 0.45, 95% CI 0.13–1.08). Subjects in the lowest quartile of BAP levels had 2.12 times higher risk than those in the highest quartile, but the association was not significant (adjusted OR 2.12, 95% CI 0.88–6.79). In addition, we carried out logistic regression analysis to assess impact of the combination between dROM levels and BAP levels on steatosis (Table 4). BAP levels and dROM levels were divided at the median. Subjects with high dROM levels and high BAP levels had 74% lower risk for steatosis than subjects with low dROM levels and high BAP levels. This association remained significant when adjusted for potential confounding factors including age, BMI, smoking status, drinking status, and physical activity (adjusted OR 0.24, 95%CI 0.08–0.78).

Discussion

This study was conducted to assess the association between steatosis and oxidative stress or antioxidant activity. Moreover, this is one of the first studies to examine the combination between oxidative stress and antioxidant activity on steatosis in healthy males. This study indicates that subjects with steatosis have higher hsCRP levels and HOMA-IR than those with normal liver. Although there are no significant differences in hsCRP levels, our results regarding hsCRP and HOMA-IR were consistent with those of previous studies.^{16,17} Several animal and clinical studies have demonstrated increased oxidative stress markers in patients with NAFLD or NASH. Previous clinical studies indicated that oxidative stress markers are significantly increased among patients with NAFLD compared with healthy controls.^{13,14} We estimated oxidative stress and antioxidant activity using the dROM test and the BAP test. These tests are novel, fast methods for the detection of oxidative stress and antioxidative activity in blood. In conclusion, means of BAP levels showed no significant difference between cases and controls. On the other hand, it was significant that means of dROM levels were lower in the cases than in the controls. Moreover, logistic regression analysis showed that the lowest antioxidant activity group had just over twice the risk for steatosis compared with the highest antioxidant activity group. Surprisingly, subjects with the highest oxidative stress had 55% lower risk of steatosis than those with the lowest oxidative stress.

Oxidative stress is an imbalance between the production and the elimination of oxidant species. Therefore, we confirmed an combination between oxidative stress and antioxidant activity. This analysis revealed that subjects with high oxidative stress and high antioxidant activity had 76% decreased risk for steatosis than subjects with low oxidative stress and high antioxidant activity.

According to a previous report,¹⁸ the normal range of dROM, measured in a large population of healthy controls, is 200–300 U. CARR. The levels between 300 and 400 U. CARR. are defined as moderate oxidative stress. Means of dROM levels in our study were marginally higher than those in the previous study.¹⁸ We excluded subjects with severe disorders, for example, cancer and so on. Almost all our cases had mild to moderate steatosis. Therefore, our subjects may have been relatively healthy male workers and tolerant of some stresses. Moreover, when we divided the cases into three groups according to steatosis severity (mild, moderate, and severe), the severe group had lower BAP levels and dROM levels than the other groups, but there were no significant differences (data not shown). Increased fat accumulation in the liver may affect the balance between oxidative stress and antioxidative activity. Alternatively, our results might be caused by hormesis or adaptive response.¹⁹ Hormesis is a phenomenon in which agents which are harmful in high doses or over long periods actually produce beneficial effects, including lifespan extension, when used at low doses or over short periods. Recent studies reported that low doses of radiation and short-term exposure to heat and oxygen can produce beneficial effects. In general, low-dose stresses induce hormesis. ROS are essential for the normal functioning of organisms. Moderate oxidative stress and high antioxidant activity may represent the best balance for the organism.

This study has some limitations. We could not carry out liver biopsies, because it is impractical to conduct them at health checkups. Although some markers of oxidative stress and antioxidant activity including thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) are well-established, we measured only dROM levels and BAP levels. Because our study included only men, our results cannot necessarily be extrapolated to women. It has been reported that oxidative stress is affected not only by lifestyle but also by psychological factors.²⁰ Therefore, unmeasured potential variables related to oxidative stress may confound an association between oxidative stress and fatty liver. Ours is a cross-sectional study, thus the temporal relationship between oxidative stress and development of steatosis cannot be inferred. Further analyses of the relationship between oxidative stress and steatosis are needed to test the validity of our results and to overcome the above-mentioned limitations.

Conclusion

In conclusion, our study demonstrates the associations between oxidative stress and antioxidant activity on steatosis. Elevated HOMA-IR and hsCRP levels were associated with increased steatosis risk. Subjects with the highest BAP had higher risk than those with the lowest BAP. Surprisingly, subjects with the

highest dROM levels were associated with decreased steatosis risk compared with those with the lowest dROM levels. Moreover, subjects with high dROM and BAP levels had about a quarter the risk of those with low dROM levels and high BAP levels. Our results suggest that moderate oxidative stress and high antioxidative activity is associated with decreased steatosis risk in Japanese males.

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Conflicts of interest

The authors reported no conflict of interest. The authors alone are responsible for content and writing of the paper.

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