1) 動脈硬化関連因子が好中球の活性酸素種産生量に及ぼす影響

Influence of atherosclerosis-related biomarkers on neutrophil basal reactive oxygen species production in the general population

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Introduction

Cardiovascular diseases (CVD), including myocardial infarction, angina, and stroke are the leading causes of death worldwide[1]. According to the WHO, approximately one-third of worldwide mortality in 2012 was attributed to CVD[2]. In addition, one-fourth of the causes of long-term care is caused by CVD in Japan[3]. Arteriosclerosis is the pathological process that causes CVD. Therefore, arteriosclerosis can be considered as the maximum risk factor for life and quality of life (QOL), and its prevention and protection are important issues.

Dyslipidemia, diabetes, and hypertension are major risk factors for arteriosclerosis. Many studies have reported on how these diseases increase the daily production of reactive oxygen species (ROS) and cause oxidative stress, which result in the impairment of blood vessel function[4-6]. Additionally, obesity is associated with these diseases and is an independent risk factor for CVD. However, no previous studies have shown how rise in obesity and serum lipid, blood glucose, or blood pressure levels influence the daily ROS production in healthy adults. Despite increases in the daily oxidative stress levels, the mechanisms by which these are achieved still remain unclear. Therefore, adequate health promotion strategies cannot be implemented, as objective measures of oxidative stress for the suppression of arteriosclerosis in healthy people are still unclear.

While ROS are produced by almost all cells, white blood cells, especially neutrophils, produce a large amount of ROS [7] as a part of reactions against foreign bodies. However, they continuously produce ROS even in the absence of a foreign body in a process known as basal ROS production. Basal ROS production by neutrophils increases the oxidative stress and may promote atherosclerosis [8][9]. Nevertheless, while previous studies have focused on ROS production as a reaction against foreign bodies, there is only a limited number of studies on basal ROS production. Therefore, in order to elucidate the mechanisms of initial arteriosclerosis progression, it is important to investigate the relationship between

atherosclerosis-related biomarkers (i.e., obesity levels, serum lipid levels, blood glucose levels and blood pressure) and basal ROS production in healthy subjects.

Few studies have measured basal ROS production with only several dozen of samples in the past. There are other studies that had similar sample sizes as the present study [10] however, we are the first research that collected and analyzed whole blood. In addition, arteriosclerosis is a typical multifactorial disease, caused by various related factors. Therefore, in such studies, epidemiological studies that consider the influence of various factors are more important than experimental studies [11]. The present study consisted approximately 1000 adults aged between 20 and 80 years from the general population, and the influence of atherosclerosis-related biomarkers on neutrophil basal ROS production was evaluated from their basal ROS production in whole blood samples.

Investigation Method

Participants

Eight hundred and nine male and female participants were included in the 2011 Iwaki Health Promotion Project. This project included participants living in the Iwaki region of Hirosaki City in the Aomori Prefecture in northern Japan. The purpose of this project was to maintain and promote physical and mental health of the local community in order to prevent lifestyle-related diseases and prolong their lifespans. Approval for the study was obtained from the Ethics Committee of the Hirosaki University School of Medicine, and all subjects provided their written informed consent prior to the research project.

A total of 378 participants (142 males and 236 females) were enrolled in the present study. Participants with diabetes mellitus (diagnosed by a medical doctor), malignant tumors, immune disorders, ischemic heart disease, cerebral infarction, or those who were currently taking immunosuppressive agent, hypolipidemic agent, antidiabetic drug or hormones, and

those who have missing values or measurement items were excluded from the study. Those with triglyceride levels of ≥ 400 mg/dL, low-density lipoprotein (LDL) cholesterol level of ≥ 140 mg/dL, high-density-lipoprotein (HDL) cholesterol level of ≤ 40 mg/dL, HbA1c level of ≥ 6.1% and fasting blood glucose level of ≥ 125 mg/dL were also excluded.

Lifestyle habits and physical measurements

Self-reported questionnaires were sent to subjects prior to the investigation and were collected after reviewing the answers during personal interviews on the day of the study. In the questionnaire, subjects were asked about their age, gender, current and past illnesses, menopause status, medication histories, smoking habits, alcohol use and exercise habits. Body mass index [BMI, weight (kg)/height $(cm)^2$] and waist circumference were calculated and measured as an index of obesity. In addition, systolic blood pressure, diastolic blood pressure and brachial-ankle pulse wave velocity (baPWV) were measured to assess arterial stiffness.

Blood parameters

Blood samples were collected peripherally after a period of fasting in the early morning. Neutrophil counts were measured using an automated blood cell analyzer (SE9000; Sysmex, Kobe, Japan). The measurements of total and HDL cholesterol, blood glucose and HbA1c levels were consigned to the Mitsubishi Chemical Medience after serum was separated from whole blood by centrifugation. Total cholesterol and HDL cholesterol levels were measured using enzymatic methods. LDL cholesterol was calculated using Friedewald formula (LDL cholesterol = Total cholesterol − HDL cholesterol − Triglyceride/5). Blood glucose level was measured according to the established methods adopted by the Japan Diabetes Society (JDS). The National Glycohemoglobin Standardization Program (NGSP) value conversion expression stipulated by the Japan Diabetes Society was then calculated accordingly (NGSP

value = 1.02 × JDS value (%) + 0.25%). We performed analyses in this study using the JDS value.

ROS generation in peripheral blood neutrophils

Neutrophil functions and basal, nonstimulated ROS production were measured with FAC-Scan (Becton Dickinson, San Jose, CA, USA) using the two-color method. ROS production was measured using the ROS-reacting fluorescent agent, hydroethidine (HE; Polyscience Inc., Warrington, PA, USA). In brief, 44 μL of 8 μm hydroethidine (Polyscience) was added to 200 μL aliquots of heparinized whole blood and then incubated at 37°C for 5 min. After incubation, 1 mL of a hemolytic agent was added to each sample and mixed well. After confirming hemolysis of red blood cells, 250 μL of fixative (Polyscience) was added to the samples, and the solution was allowed to stand for 5 min. The samples were then washed twice in phosphate-buffered saline containing sodium azide, followed by the addition of 50 μL of 5% paraformaldehyde.

Using flow cytometry, neutrophils were irradiated with a 488-nm laser beam generated from a 15-mW argon laser with forward- and side-scattering emission, which was simultaneously recorded. Green fluorescence generated from FITC was detected through a 530-nm filter, and orange fluorescence generated from HE was detected through a 585-nm filter. Fluorescence intensity was measured as the value of neutrophils per 10,000 screened from the forward- and side-scattering emission for each sample. Cumulative fluorescence intensity (CFI), i.e., sum of the values of fluorescence intensity (FI) multiplied by the number of positive cells per 10,000 (‱), was used as a quantitative index.

Statistical analysis

Statistical analyses were carried out after participants were divided into two groups on the

basis of gender. The relationship between neutrophil functions and the atherosclerosis-related biomarkers, (i.e., BMI and waist circumference, and total cholesterol, LDL cholesterol, HDL cholesterol, fasting blood glucose and HbA1c levels as well as systolic and diastolic blood pressures and baPWV) were analyzed using multiple regression analysis. The statistical models were adjusted for age, BMI, cigarette smoking, alcohol use, exercise frequency, hypotensive drug intake and menopause status.

Furthermore, we categorized subjects into five groups according to HDL cholesterol levels (40–54 mg/dL, 55–69 mg/dL, 70–84 mg/dL, 85–100 mg/dL, and >100 mg/dL) and compared the total basal ROS production between groups using an analysis of covariance (ANCOVA). We then corrected the values for age, BMI, smoking, alcohol use, exercise habits, hypotensive drug intake and menopause status, and used the Bonferroni method for multiple comparisons. Data analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 J statistical software (SPSS Inc., Chicago, IL, USA). The differences were considered statistically significant when $p < 0.05$.

Results

Blood biochemical values and physical characteristics of participants and their lifestyle habits are listed in Table 1. The average age was 54.9 ± 14.4 for male and 52.9 ± 14.3 for female participants. Average BMI, waist circumference, blood pressure, and baPWV were both significantly higher in male than in female participants. Although smoking and drinking habits were more common in male participants, gender differences in exercise habits were not observed. Postmenopausal female patients comprised 66.2% of the study population. Blood biochemical values are listed in Table 2. The average HDL cholesterol level was significantly higher in female participants than male participants, while the average fasting blood glucose level was significantly higher in male than female patients. Although the average basal ROS

production per active cell was significantly higher in women, we did not identify significant differences in total basal ROS production.

Influence of atherosclerosis-related biomarkers on neutrophil basal ROS production

Multiple regression analysis for male participants revealed the absence of correlation between neutrophil function and atherosclerosis-related biomarkers among males (Table 3). On the other hand, a positive correlation between total basal ROS production and basal ROS production per active cell for both total cholesterol and HDL cholesterol levels (for total basal ROS production: total cholesterol p=0.002, HDL cholesterol $p < 0.001$; for basal ROS production per active cell: total cholesterol p=0.021, HDL cholesterol p=0.016) was demonstrated among female participants. A positive correlation was also revealed between basal ROS production per active cell and fasting blood glucose levels (p=0.03) (Table 4).

For the analysis of covariance, total basal ROS production was significantly higher in the group with HDL cholesterol levels of 100 mg/dL or more than in other groups (HDL cholesterol levels > 100 mg/dL: 40–54 mg/dL p=0.001, 55–69 mg/dL p=0.008, 70–84 mg/dL P=0.02) (Figure 1).

Discussion

This is the first epidemiological study on the association between basal neutrophil ROS production and obesity, serum lipid, blood glucose and blood pressure levels, among healthy subjects.

In this study, no significant relationship was detected between obesity levels and basal ROS production. Although previous studies have shown elevated neutrophil count [12)13)] and increased neutrophil activity among obese patients [14)15)], there have been very few reports on basal ROS production in obese subjects with adequate sample sizes. In addition, while there

have been reports linking obesity and ROS production among neutrophils [16], another report failed to show a relationship [17], highlighting the diversity in opinions. In those studies reporting neutrophil hyperactivity in obese individuals, the study populations consisted of highly obese subjects (BMI $\geqq$30kg/m^2 or the average BMI of 30~35 kg/m^2) [14-16]. In our study, because the average BMI was low (22.9±2.7 kg/m^2 in male and 21.9±3.1 kg/m^2 in female) and more than 98% of calculated BMI were $\leq$30 kg/m^2, we believe that correlation between obesity and neutrophil function was not easily demonstrated.

Previous studies have reported positive correlations between basal ROS production in neutrophils and LDL-C levels [18-21]. In all of these studies, except those conducted in vivo [19][20], there were small sample sizes, and neutrophil function was not evaluated using whole blood. The mechanism proposed involves an increase in the Ox-LDL levels generated by LDL increase ROS production of neutrophils [22][23]. However, since subjects with LDL-C $\geq$ 140 mg/dL were excluded from the study, generation of oxLDL was suppressed and no correlation was demonstrated between LDL-C and basal ROS production of neutrophils.

It has been reported that HDL-C also reduces basal ROS production of neutrophils [19][24][25], while some studies that have reported the absence of an association between the two [26]. In these studies, only the (19) basal ROS production was measured, but separating neutrophils had used in this study as previously described. On the other hand, even with HDL-C concentrations of up to 100 mg/dL, no correlation was demonstrated with ROS production in our study. ROS production was higher with HDL-C concentrations of 100 mg/dL or more in women compared to that in other groups. HDL-C also has antioxidant, anti-inflammatory properties [27-29]. However, it has recently been recognized that the presence of dysfunctional HDL can enhance inflammation [30-32]. In addition, when HDL-C levels are very high, the possibility of increased production of dysfunctional HDL-C also increases [33]. Patients with very high HDL-C have an increased risk of atherosclerosis and cardiovascular disease [34-36]. In

addition, Dysfunctional HDL-C reported increases ROS production significantly compared with normal HDL-C by activating NADPH oxidase [37]. Therefore, if HDL-C is more than 100 mg/dL in women, it also increases basal ROS production and subsequent oxidative stress. Although positive correlation was observed between total cholesterol and basal ROS production this was likely due to the effect of HDL cholesterol as one of the fractions of total cholesterol.

A study investigating the relationship between blood glucose levels and basal ROS production of neutrophil in non-diabetics has demonstrated positive correlation between blood glucose, HbA1c and basal ROS production of neutrophils [38]. Although our study has shown a similar result, there was no correlation shown between ROS production and HbA1c. As this factor, it was conceived the impact of the choice of subject. While Saito et al. excluded only diabetics with HbA1c≥6.1% [38], we also excluded persons with fasting blood glucose levels ≥125 mg/dL. Therefore, correlation is difficult to demonstrate between HbA1c and basal ROS production of neutrophil in patients who are in a healthier state. However, given the correlation tendency between blood glucose and basal ROS production of neutrophil shown in this study, blood glucose management is important in oxidative stress.

In this study, an association between baPWV, which is significantly related to blood pressure and reflects the degree of arteriosclerosis, and basal ROS production of neutrophil, has not demonstrated. No previous studies have evaluated the neutrophil basal ROS production in whole blood as well as the relationship between ROS production in immune cells [10)39)40)]. On the other hand, significant differences in ROS production between patients with hypertension and without hypertension was not observed. No correlation between ROS production of neutrophil and mean arterial pressure (which reflects the degree of arteriosclerosis) has been reported [40]. Because hypertension and arteriosclerosis are phenomena caused by oxidative stress and are multi-factor in nature, correlation was difficult

to demonstrate during basal ROS production of neutrophils.

In this study, a significant association of the relationship between the arteriosclerosis-related factors and basal ROS production of neutrophil was only seen in female participants. A limitation of this study is that clear reasons for the gender differences observed could not be ascertained because of the absence of sex hormones level measurements. Previous studies have suggested neutrophil function activation by the female hormone [41]. In addition, the relationship between glucose metabolism and neutrophils basal ROS production was only seen in women [38]. On the other hand, there were only four male participants with HDL-C of 100 mg/dL or more in this study. Therefore, we considered that a significant correlation between HDL-C and basal ROS production of neutrophil was only observed in female participants.

Conclusion

We investigated the relationship between atherosclerosis-related biomarkers and basal ROS production of neutrophils among healthy subjects with normal levels of arteriosclerosis-related biomarkers. Based on our results, we believe that elevated HDL-C and blood glucose levels in female subjects increase oxidative stress by enhancing the basal ROS production of neutrophils. In addition, when HDL-C exceeds 100 mg/dL, basal ROS production of neutrophils increased significantly, which was considered to be disadvantageous for biological functions. Thus, strict control of glycemic and HDL cholesterol levels are extremely important in females. Also, HDL-C levels exceeding 100 mg/dL are considered detrimental to health.

References

1. Taylor F, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G, et al. Statins for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 2013;1:CD004816.
2. The top 10 causes of death. [Updated May 2014; cited 6 November] Available from: http://www.who.int/mediacentre/factsheets/fs310/en/
3. Kubota Katsu. Mortality of Long-Term Care Recipients in Japan: The journal of the Association of Insurance Medicine of Japan 2012;110: 211-6
4. Singh U, Jialal I. oxidative stress and atherosclerosis. Pathophysiology 2006;13:129-42.
5. Jane A. Leopold, and Joseph Loscalzo. Oxidative mechanisms and atherothrombotic cardiovascular disease. Drug Discov Today Ther Strateg 2008;5:5-13.
6. Bahorun T, Soobratte MA, Luximon-Ramma V, Aruoma OI. FREE RADICALS AND ANTIOXIDENTS IN CARDIOVASCULAR HEALTH AND DISEASE. Internet Journal of Medical Update 2006;1:25-41.
7. Roos D, van Bruggen R, Meischl C. Oxidative killing of microbes by neutrophils. Microbes Infect 2003; 5:1307-15.
8. Matés JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 2000;153:83-104.
9. Fialkow L, Wang Y, Downey GP. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. Free Radic Biol Med 2007;42:153-64.
10. Kenichi Y, Kensaku M, Munehiro N, Junichi Y. Oxidative Stress in Leukocytes Is a Possible Link Between Blood Pressure, Blood Glucose, and C-Reacting Protein. Hypertension 2002;39: 777-80.
11. Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children

and young adults. The Bogalusa Heart Study. N Engl J Med 1998;338:1650-6.

12. Dixon JB, O'Brien PE. Obesity and the white blood cell count: changes with sustained weight loss. Obes Surg 2006;16:251-7.

13. Herishanu Y, Rogowski O, Polliack A, Marilus R. Leukocytosis in obese individuals: possible link in patients with unexplained persistent neutrophilia. Eur J Haematol 2006;76:516-20.

14. Nijhuis J, Rensen SS, Slaats Y, van Dielen FM, Buurman WA, Greve JW. Neutrophil activation in morbid obesity, chronic activation of acute inflammation. Obesity 2009;17:2014-8.

15. Trellakis S, Rydleuskaya A, Fischer C, Canbay A, Tagay S, Scherag A, Bruderek K, Schuler PJ, Brandau S. Low adiponectin, high levels of apoptosis and increased peripheral blood neutrophil activity in healthy obese subjects. Obes Facts 2012;5:305-18.

16. Zielińska-Przyjemska M, Olejnik A, Dobrowolska-Zachwieja A, Grajek W. In vitro effects of beetroot juice and chips on oxidative metabolism and apoptosis in neutrophils from obese individuals. Phytother Res 2009;23:49-55.

17. Guoda Pilkauskaite, Skaidrius Miliauskas, and Raimundas Sakalauskas. Reactive Oxygen Species Production in Peripheral Blood Neutrophils of Obstructive Sleep Apnea Patients. The Scientific World Journal 2013;2013:421763.

18. Couderc R, Bonneau C, Tissot M, Bailleul S, Roch-Arveiller M, Giroud JP. Effects of plasma lipoproteins on the production of superoxide anion by human polymorphonuclear leukocytes in vitro. Biofactors 1997;6:157-63.

19. Fernanda B. Araujo, Decio S. Barbosa, Chang Y. Hsin, Raul C. Maranhao, Dulcineia S.P. Abdalla. Evaluation of oxidative stress in patients with hyperlipidemia. Atherosclerosis 1995;117:61-71.

20. Mazor R, Shurtz-Swirski R, Farah R, Kristal B, Shapiro G, Dorlechter F, et al. Primed

polymorphonuclear leukocytes constitute a possible link between inflammation and oxidative stress in hyperlipidemic patients. Atherosclerosis 2008;197:937-43

21. Sugano R, Matsuoka H, Haramaki N, Umei H, Murase E, Fukami K, et al. Polymorphonuclear leukocytes may impair endothelial function: results of crossover randomized study of lipid-lowering therapies. Arterioscler Thromb Vasc Biol 2005;25:1262-7.
22. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752-61.
23. Ryouta Maeba, Akiko Maruyama, Osamu Tarutani, Nobuo Ueta, Hiroyuki Shimasak. Oxidized low-density lipoprotein induces the production of superoxide by neutrophils. FEBS Letters 1995;377(3)27: 309-312(4)
24. Kopprasch S, Pietzsch J, Graessler J. The protective effects of HDL and its constituents against neutrophil respiratory burst activation by hypochlorite-oxidized LDL. Molecular and Cellular Biochemistry 2004;258:121-7.
25. Fatiha Tabet, Gilles Lambert, Luisa F. Cuesta Torres, Liming Hou, Irene Sotirchos, et al. Lipid-Free Apolipoprotein A-I and Discoidal Reconstituted High-Density Lipoprotein Differentially Inhibit Glucose-Induced Oxidative Stress in Human Macrophages. Arterioscler Thromb Vasc Biol 2011;31:1192-200.
26. Blackburn WD Jr, Dohlman JG, Venkatachalapathi YV, Pillion DJ, Koopman WJ, Segrest JP, et al. Apolipoprotein A-I decreases neutrophil degranulation and superoxide production. J Lipid Res 1991;32:1911-8.
27. Murphy AJ, Westerterp M, Yvan-Charvet L, Tall AR. Anti-atherogenic mechanisms of high density lipoprotein: effects on myeloid cells. Biochim Biophys Acta 2012;1821:513-21

28. Sakuma N, Saeki T, Ito T, Hibino T, Suzuki S, Kunimatsu M, et al. HDL2 can inhibit further oxidative modification of partially oxidized LDL. J Atheroscler Thromb. 2010;17:229-34
29. Umemoto T, Han CY, Mitra P, Averill MM, Tang C, Goodspeed L, et al. Apolipoprotein AI and high-density lipoprotein have anti-inflammatory effects on adipocytes via cholesterol transporters: ATP-binding cassette A-1, ATP-binding cassette G-1, and scavenger receptor B-1. Circ Res 2013;112:1345-54.
30. Cogny A, Paul JL, Atger V, Soni T, Moatti N. Structural changes of high-density-lipoprotein apolipoproteins following incubation with human polymorphonuclear cells. Eur J Biochem 1994;222:965-73
31. Smith JD. Dysfunctional HDL as a diagnostic and therapeutic target. Arterioscler Thromb Vasc Biol 2010;30:151-5
32. Ansell BJ, Watson KE, Fogelman AM, Navab M, Fonarow GC High-density lipoprotein function recent advances. J Am Coll Cardiol 2005;46:1792-8.
33. Ansell BJ, Navab M, Hama S, Kamranpour N, Fonarow G, Hough G, et al. Inflammatory/Antiinflammatory Properties of High-Density Lipoprotein Distinguish Patients From Control Subjects Better Than High-Density Lipoprotein Cholesterol Levels and Are Favorably Affected by Simvastatin Treatment. Circulation 2003;108:2751-6
34. Hirano K, Yamashita S, Nakajima N, Arai T, Maruyama T, Yoshida Y, et al. Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. Arterioscler Thromb Vasc Biol 1997;17:1053-9.
35. Nagano M, Yamashita S, Hirano K, Takano M, Maruyama T, Ishihara M, et al. Molecular mechanisms of cholesteryl ester transfer protein deficiency in Japanese. J Atheroscler Thromb 2004;11:110-21.

36. van der Steeg WA, Holme I, Boekholdt SM, Larsen ML, Lindahl C, Stroes ES, et al. High-Density Lipoprotein Cholesterol, High-Density Lipoprotein Particle Size, and Apolipoprotein A-I: Significance for Cardiovascular Risk. J Am Coll Cardiol 2008;51:634-42
37. Wang Y, Ji L, Jiang R, Zheng L, Liu D. Oxidized High-Density Lipoprotein Induces the Proliferation and Migration of Vascular Smooth Muscle Cells by Promoting the Production of ROS. J Atheroscler Thromb 2014;21:204-16.
38. Saito Y, Takahashi I, Iwane K, Okubo N, Nishimura M, Matsuzaka M, et al. The influence of blood glucose on neutrophil function in individuals without diabetes. Luminescence 2013; 28:569-73
39. Fortuño A, Oliván S, Beloqui O, San José G, Moreno MU, Díez J, et al. Association of increased phagocytic NADPH oxidase-dependent superoxide production with diminished nitric oxide generation in essential hypertension. J Hypertens 2004;22:2169-75.
40. Kristal B, Shurtz-Swirski R, Chezar J, Manaster J, Levy R, Shapiro G, et al. Participation of peripheral polymorphonuclear leukocytes in the oxidative stress and inflammation in patients with essential hypertension. Am J Hypertens 1998;11:921-8.
41. Chiang K, Parthasarathy S, Santanam N. Estrogen, neutrophils and oxidation. Life Sci 2004;75:2425-38

Table 1. Characteristics of the participants

	male n=142	female n=236	
Age, years	54.9±14.4	52.9±14.3	
BMI, kg/m^2	22.9±2.7	21.9±3.1	**
Waist circumference, cm	82.2±7.49	79.4±9.46	**
systolic blood pressure , mmHg	133.5±18.5	127.8±19.5	**
diastolic blood pressure , mmHg	78.1±12	74.7±13	*
baPWV, cm/s	1560.6±373.2	1422.3±353.7	**
Smoker %	30.1	11.4	**
Alcohol drinker %	76.8	31.4	**
Exercise habits (1≤/week), %	32.4	25.0	
Menopause, %	-	66.2	

Table 1. Characteristics of the participants

Data are expressed as mean ± standard deviation.

BMI, body mass index; ba PWV, brachial-ankle pulse wave velocity.

*p<0.05 vs the opposite sex. **p<0.01 vs the opposite sex.

Table 2. Characteristics of the participants

	male n=142	female n=236	
total cholesterol, mg/dL	192.2±22.8	196.8±24.2	
LDL cholesterol, mg/dL	108.6±20.3	110.9±20.1	
HDL cholesterol, mg/dL	62.9±14.8	70.7±16.1	*
fasting blood glucose, mg/dL	88.2±10.4	85.2±9.5	*
HbA1c, % (JDS)	5.2±0.3	5.2±0.3	
HbA1c, %(NGSP)	5.6±0.3	5.5±0.3	
Total Basal ROS production, CFI	3885.6±3763.5	4508.7±4231.2	
Basal ROS production per active cell, FI	38.6±13.1	43.3±16.8	*
Basal ROS production proportion, ‰	101.6±102.2	100.5±79.5	

Table 2. Characteristics of the participants

Data are expressed as mean ± standard deviation.

LDL, Low density lipoprotein; HDL, High density lipoprotein; JDS, Japan Diabetes Society; NGSP, National Glycohemoglobin Standardization Program; ROS, reactive oxygen species. CFI, cumulative fluorescence intensity; FI, fluorescence intensity.

*p<0.05 vs the opposite sex. **p<0.01 vs the opposite sex.

Table 3.
Multiple regression analysis with neutrophil basal ROS production (male)

Objective variables	Explanatory variables	β -coefficient	P-values	R^2
Total Basal ROS production	BMI	-0.060	0.480	0.065
	Waist circumference	-0.075	0.377	0.067
	total cholesterol	-0.124	0.142	0.080
	LDL cholesterol	-0.043	0.629	0.067
	HDL cholesterol	-0.115	0.209	0.076
	fasting blood glucose	0.053	0.589	0.067
	HbA1c	0.001	0.987	0.065
	systolic blood pressure	0.062	0.527	0.068
	diastolic blood pressure	0.015	0.864	0.065
	ba PWV	0.058	0.664	0.067
Basal ROS production per active cell	BMI	-0.076	0.372	0.045
	Waist circumference	-0.078	0.358	0.046
	total cholesterol	-0.100	0.245	0.055
	LDL cholesterol	0.012	0.890	0.046
	HDL cholesterol	-0.080	0.389	0.051
	fasting blood glucose	-0.030	0.766	0.046
	HbA1c	-0.089	0.330	0.052
	systolic blood pressure	-0.057	0.567	0.048
	diastolic blood pressure	-0.100	0.256	0.055
	ba PWV	0.088	0.477	0.049
Basal ROS production proportion	BMI	0.006	0.942	0.046
	Waist circumference	-0.005	0.957	0.046
	total cholesterol	-0.074	0.387	0.052
	LDL cholesterol	-0.016	0.856	0.047
	HDL cholesterol	-0.109	0.237	0.056
	fasting blood glucose	0.058	0.561	0.049
	HbA1c	0.055	0.546	0.049
	systolic blood pressure	0.114	0.253	0.056
	diastolic blood pressure	0.097	0.269	0.055
	ba PWV	0.048	0.698	0.048

Table 3.Multiple regression analysis with neutrophil basal ROS production (male)

There was no correlation between neutrophil function and atherosclerosis-related biomarkers among males.

ROS, reactive oxygen species; BMI, body mass index; LDL, Low densty lipoprotein; HDL, High density lipoprotein; baPWV, brachial-ankle pulse wave velocity.

Table 4.
Multiple regression analysis with neutrophil basal ROS production (female)

Objective variables	Explanatory variables	β -coefficient	P-values	R^2
Total Basal ROS production	BMI	-0.021	0.763	0.046
	Waist circumference	0.014	0.839	0.045
	total cholesterol	0.214	0.002	0.084
	LDL cholesterol	0.085	0.244	0.050
	HDL cholesterol	0.257	0.000	0.098
	fasting blood glucose	0.126	0.089	0.057
	HbA1c	0.088	0.209	0.051
	systolic blood pressure	0.037	0.651	0.046
	diastolic blood pressure	0.031	0.665	0.046
	ba PWV	-0.073	0.492	0.048
Basal ROS production per active cell	BMI	-0.077	0.273	0.028
	Waist circumference	-0.019	0.791	0.023
	total cholesterol	0.089	0.207	0.035
	LDL cholesterol	0.066	0.376	0.032
	HDL cholesterol	0.060	0.411	0.031
	fasting blood glucose	-0.004	0.958	0.028
	HbA1c	-0.055	0.435	0.031
	systolic blood pressure	-0.040	0.631	0.029
	diastolic blood pressure	0.093	0.872	0.028
	ba PWV	-0.050	0.624	0.029
Basal ROS production proportion	BMI	0.021	0.764	0.052
	Waist circumference	0.048	0.488	0.054
	total cholesterol	0.160	0.021	0.074
	LDL cholesterol	0.100	0.171	0.060
	HDL cholesterol	0.172	0.016	0.076
	fasting blood glucose	0.157	0.033	0.071
	HbA1c	0.122	0.081	0.065
	systolic blood pressure	0.081	0.320	0.056
	diastolic blood pressure	0.046	0.516	0.054
	ba PWV	-0.003	0.975	0.052

Table 4. Multiple regression analysis with neutrophil basal ROS production (female)

A positive correlation between total basal ROS production and basal ROS production per active cell for both total cholesterol and HDL cholesterol levels (for total basal ROS production: total cholesterol p=0.002, HDL cholesterol $p < 0.001$; for basal ROS production per active cell: total cholesterol p=0.021, HDL cholesterol p=0.016) was demonstrated among female participants. A positive correlation was also revealed between basal ROS production per active cell and fasting blood glucose levels (p=0.03)

ROS, reactive oxygen species; BMI, body mass index; LDL, Low density lipoprotein; HDL, High density lipoprotein; baPWV, brachial-ankle pulse wave velocity.

Figure 1. Covariance analysis of HDL-C levels and basal ROS production in females

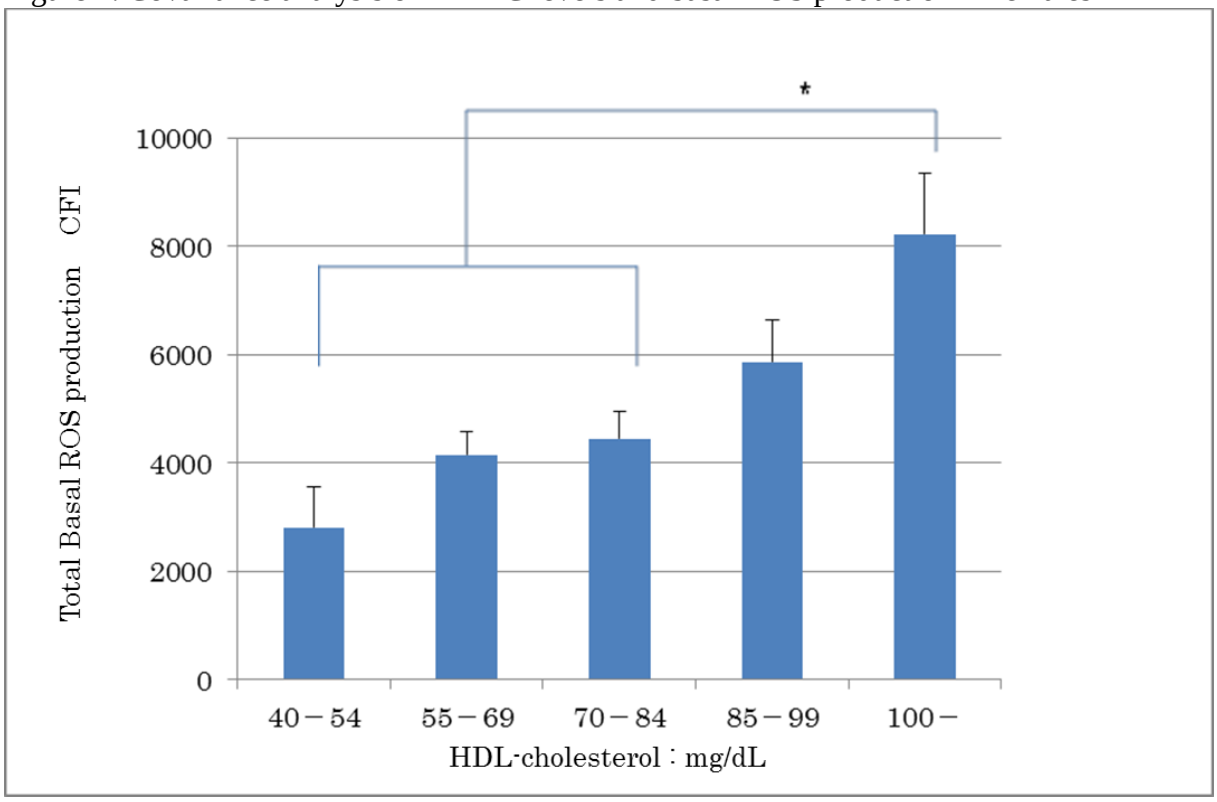

Figure 1. Covariance analysis of HDL-C levels and basal ROS production in females

Total basal ROS production was significantly higher in the group with HDL cholesterol levels of >100 mg/dL compared to other groups. (* = $p < 0.05$)

ROS, reactive oxygen species; CFI, cumulative fluorescence intensity.

抄録

今回我々は、健常人において動脈硬化関連因子が好中球由来の酸化ストレスに及ぼす影響を調査した。調査対象は、20 歳以上の一般住民 809 名であり、生活習慣（喫煙、飲酒、運動習慣）、動脈硬化関連因子（肥満度、脂質、血糖値、血圧）、平常時（非異物反応時）の好中球活性酸素腫（ROS）産生量について調査を行った。その結果、女性において、HDL コレステロールが 100mg/dL を超える群や正常範囲内であっても空腹時血糖が高い者では、平常時の好中球 ROS 産生量が高かった。したがって、好中球由来の酸化ストレスを抑制するためには、正常範囲内であっても空腹時血糖の上昇を抑制すること、HDL は約 100mg/dL を上限閾値とする管理が重要である可能性が考えられた。

キーワード

動脈硬化関連因子、好中球、活性酸素腫、一般住民、疫学研究

Abstract

We investigated the effects of arteriosclerosis-related factors on the oxidative stress derived from neutrophil in healthy adults. Subjects were 809 males and females from the general population who were over 20 years old. Life style parameters (smoking, alcohol consumption and exercise habits), arteriosclerosis-related factors (obesity level, cholesterol level, blood glucose level and blood pressure) and basal neutrophil (ie. not stimulated) reactive oxygen species (ROS) production were measured. As a result, female subjects with HDL cholesterol level of 100mg/dL or higher, or those with high normal blood glucose level showed higher basal ROS production from neutrophil. Therefore, in order to suppress the oxidative stress derived from neutrophils, it is important to maintain a strict control of glycemic level and the control of HDL cholesterol levels to less than 100mg/dL.

Key words

atherosclerosis-related biomarkers, neutrophil, reactive oxygen species, general population, epidemiological research