Which cytokine is the most related to weight loss-induced decrease in arterial stiffness in overweight and obese men?

Hiroshi Kumagai^{1),2),3)}, Asako Zempo-Miyaki⁴⁾, Toru Yoshikawa^{3),5)}, Miki Eto⁶⁾, Rina So⁷⁾, Takehiko Tsujimoto⁸⁾, Takeshi Nishiyasu¹⁾, Kiyoji Tanaka¹⁾ and Seiji Maeda¹⁾

¹⁾ Faculty of Health and Sport Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

²⁾ Graduate School of Health and Sports Science, Juntendo University, Chiba, Japan

³⁾ Research Fellow of Japanese Society for the Promotion of Science, Tokyo, Japan

⁴⁾ Faculty of Sports and Health Sciences, Ryutsu Keizai University, Ryugasaki, Ibaraki, Japan

⁵⁾ Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

⁶⁾ Faculty of Human Sciences, Osaka University of Economics, Higashiyodogawa, Osaka, Japan

⁷⁾ Research Center for Overwork-Related Disorders, National Institute of Occupational Safety and Health, Kawasaki, Kanagawa, Japan

⁸⁾ Faculty of Human Sciences, Shimane University, Matsue, Shimane, Japan

Abstract. Obesity and increased arterial stiffness are risk factors for cardiovascular disease. A well-known characteristic of obesity is the chronic low-grade inflammatory state, and it causes elevation of arterial stiffness. Weight-loss reduces arterial stiffness and inflammatory level in obese individuals. However, it is unclear which inflammatory factor is most related to weight loss-induced decreases in arterial stiffness in overweight and obese men. Thus, the aim of this study was to determine which circulating cytokine level has the most effect on decreasing arterial stiffness after lifestyle modification. Twenty overweight and obese men completed a 12-week period of lifestyle modifications (combination of aerobic exercise training and dietary modification). We measured brachial-ankle pulse wave velocity (baPWV) as an index of arterial stiffness, and circulating cytokine levels using comprehensive analysis. After the 12-week lifestyle modifications, body mass was markedly decreased. Also, baPWV and the levels of several circulating cytokines significantly decreased after the lifestyle modifications. We observed a positive correlation between changes in baPWV and circulating interleukin-6 (IL-6) levels. Furthermore, multiple liner regression analysis revealed that change in baPWV was significantly associated with that in IL-6 levels after consideration of changes in systolic blood pressure and body mass index. These results suggest that for overweight and obese men, a 12-week period of lifestyle modifications-induced a decrease in circulating cytokine levels (especially IL-6 levels), leads to decreased baPWV.

Key words: Arterial stiffness, Circulating cytokine levels, Obesity, Lifestyle modifications

GLOBALLY, there have been an increasing number of obese individuals. Adipose tissue produces and secretes “adipokines”, which are known as inflammatory factors [1]. Circulating levels of inflammatory factors are consistently higher in obese people than in their normal-weight peers, because obesity carries higher body fat

mass [2-4]. Several previous studies have revealed that weight-loss is an effective way to reduce the levels of many types of inflammatory substances (*i.e.*, C-reactive protein [CRP], tumor necrosis factor alpha [TNF- α], interleukin-6 [IL-6], interleukin-1 beta [IL-1 β], monocyte chemoattractant protein 1 [MCP-1] etc.) in obese individuals [5-9]. Numerous types of inflammatory substances have been found and most of these levels are known to increase in obesity. Thus, weight loss is necessary to prevent increased inflammatory substances-induced disorders in obese population.

The obesity-induced high inflammatory state is associ-

Submitted Mar. 23, 2017; Accepted Sep. 6, 2017 as EJ17-0117

Released online in J-STAGE as advance publication Sep. 30, 2017

Correspondence to: Seiji Maeda, Ph.D., Faculty of Health and Sport Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8574, Japan

E-mail: maeda.seiji.gn@u.tsukuba.ac.jp

ated with various disorders, including insulin resistance, diabetes, and non-alcoholic fatty liver disease [3]. In particular, increasing in systemic inflammatory condition is known as a strong determining factor for elevated arterial stiffness, which is an independent predictor of future cardiovascular events [10]. It is known that arterial stiffness in obese people is higher compared to that of their age-matched normal-weight peers [11]. A previous study demonstrated that anti-inflammatory medication decreased arterial stiffness in rheumatoid patients who had high circulating levels of pro-inflammatory factors [12]. We and other research groups have shown that weight-loss caused by lifestyle modifications reduced arterial stiffness in obese individuals [13–16]. However, there have been no reports regarding obese individuals and the effect of weight-loss on the change in several types of inflammatory factors, as a mechanism for decreased arterial stiffness.

In this study, we hypothesized that lifestyle modifications reduce circulating cytokine levels, which in turn contribute to reducing the arterial stiffness in overweight and obese men. Therefore, the purpose of this study was to investigate whether lifestyle modifications-induced decreases in circulating cytokine levels are associated with decreased arterial stiffness, and if so, to determine which cytokine mainly affects the decrease in arterial stiffness. To examine our hypothesis, we performed comprehensive analyses of circulating cytokine levels and measured arterial stiffness in overweight and obese men, prior to and after a 12-week period of lifestyle modifications (*i.e.* combining dietary modification and aerobic exercise training).

Materials and Methods

Participants

At base line, total of 67 subjects participated in our study. During the 12-week intervention, 5 subjects were dropped out, and after the intervention, we excluded 10 subjects who had the history of cardiovascular disease and depression. In addition, we excluded 7 current smokers and 23 subjects who used medications (antihypertensive, antidiabetic, hypoglycemic, antidepressants, and antihyperuricemic). After excluded these subjects, we randomly selected 20 subjects into the comprehensive analysis of inflammation. Thus, a total of 20 overweight and obese men (age: 50 ± 6 yr; body mass index [BMI]: 29 ± 1 kg/m²) were analyzed in this study. Also, the present study was the sub-analysis of our previous

study [17] and subjects were overlapped to that study. This study was reviewed and approved by the institutional review board of the University of Tsukuba. All study procedures and potential risks were explained to the participants, and each participant provided written informed consent to participate in the study.

Experimental design

All overweight and obese men were examined prior to and after the 12-week period of lifestyle modifications (dietary modification and aerobic exercise training). All measurements were obtained after an overnight fast including abstinence from caffeine and alcohol, and none of the participants exercised on the previous day. The participants were studied under supine resting conditions in a quiet, temperature-controlled room (24–26°C). All measurements were performed after a rest period of at least 20 min.

Lifestyle modifications

Aerobic exercise training

The participants participated in an aerobic exercise class for up to 90 min/day, 3 times per week for 12 weeks as previously described [13, 14, 17, 18]. In addition, the participants were instructed to perform exercise training by themselves at least once each week. The exercise program included a 15–20 min warm-up session, followed by a walking and/or jogging session lasting approximately 40–60 min, and concluded with a 15–20 min cool-down session.

Exercise intensity was set so that it increased a participant's heart rate to between 60% and 85% of his maximum heart rate. Exercise intensity was monitored by using a heart rate monitor (Polar RS400™; Polar Electro Oy, Kempele, Finland) and an activity monitor with a uniaxial accelerometer (Kenz Lifecorder GS; Suzuken Co, Ltd, Nagoya, Japan). Total daily steps were determined by using the uniaxial accelerometer every day, beginning from 2 weeks prior to the intervention period and throughout the 12-week program.

Dietary modifications

Participants were placed on a dietary restriction of 1,680 kcal per day, which was maintained through a nutritionally balanced diet restriction program consisting of 12 weekly lectures, as previously described [13, 14, 16, 17]. During the 12-week intervention period, the participants maintained food diaries that were monitored by dietitians who provided recommendations at the weekly lectures. Individual counseling was provided after these

classes to assist the participants in adhering to the calorie consumption guidelines. At baseline and at week 12, the participants maintained daily food intake records for 3 days. A dietician used the food intake records to estimate the total daily energy intake by using Excel Eiyō-Kun version 4 software (Kenpakusya, Tokyo, Japan).

Anthropometric measurements

Anthropometric measurements were made prior to and after the 12-week lifestyle modifications. Body mass was measured once to the nearest 0.1 kg by using a digital scale (WB-150; TANITA, Tokyo, Japan), and height was measured once to the nearest 0.1 cm by using a wall-mounted stadiometer (YG-200; Yagami, Nagoya, Japan). Body mass and height were measured prior to participants' morning meal and while the participants were barefoot and dressed only in their underwear. BMI values were calculated by dividing the weight (kg) measurement by the square of the height (m). Body fat percentage was measured by using bioelectrical impedance (HBF-301; OMRON). Waist circumference was directly measured on the skin at the level of the umbilicus in a standing position. Duplicate waist circumference measurements were recorded to the nearest 0.1 cm.

Blood biochemistry and cytokines

We collected blood samples from each participant prior to and after the program. Each blood sample was placed in a serum separator tube, clotted for 2 h and then centrifuged at 3,000 rpm for 15 min at 4°C. The serum obtained was stored at –80°C until assay. The levels of serum IL-1 receptor antagonist (IL-1ra), interleukin-4 (IL-4), interleukin-5 (IL-5), IL-6, interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-17 (IL-17), eotaxin, fibroblast growth factor (FGF) basic, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon- γ (IFN- γ), interferon-inducible protein 10 (IP-10), MCP-1, macrophage inflammatory protein 1 α (MIP-1 α), macrophage inflammatory protein 1 β (MIP-1 β), TNF- α , and vascular endothelial growth factor (VEGF) were measured by using the Bio-Plex 200 system [19]. Serum concentrations of triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, insulin, and homeostasis model assessment of insulin resistance (HOMA-IR), and plasma concentration of glucose were determined by using standard enzymatic techniques.

Peak oxygen uptake (VO_{2peak})

Prior to and after the 12-week period of lifestyle modifications, peak oxygen uptake (VO_{2peak}) was determined during a graded exercise test by using a cycling ergometer (828E; Monark, Stockholm, Sweden). After a 2-min warm-up at 30 W, the participants started with a workload of 15 W per min until they felt exhausted or reached 85% of the age-predicted maximal heart rate. Participants' individual VO_{2peak} was calculated by using regression analyses of the slopes of CO_2 production, O_2 uptake, and minute ventilation plots. Pulmonary ventilation and gas exchange were also measured, breath-by-breath, by using an online data acquisition system (Oxycon Alpha System; Mijnhardt, Breda, The Netherlands).

Blood pressure and heart rate

The systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) in the supine position were recorded from the right arm by using a semi-automated device (Form PWV/ABI; Colin Medical Technology, Aichi, Japan). Heart rate was simultaneously measured by electrocardiogram (form PWV/ABI).

Brachial-ankle pulse wave velocity

Duplicate measurements of baPWV were obtained by using a modified oscillometric pressure-sensor method as described in the previous study [20]. Briefly, bilateral brachial and post-tibial arterial pressure waveforms were stored for 10 s by using extremity cuffs that were connected to an oscillometric sensor and wrapped onto both arms and ankles, and inflated at a low pressure.

Statistical analysis

The Shapiro-Wilk tests were used to assess the normality of all parameters. Paired Student's *t*-test and Wilcoxon signed-rank test were used to assess the changes between data prior to and after the 12-week period of lifestyle modifications. Possible associations between changes in characteristics and circulating cytokine levels were assessed by using Pearson's correlation analysis and Spearman's rank correlation coefficient. Also, we applied the multiple regression analysis to determine the effects of the changes in IL-6, SBP, and BMI on that in baPWV. All data were expressed as mean $\pm$ standard deviation (SD). Values of $p < 0.05$ were considered statistically significant.

Results

Table 1 shows the characteristics of the overweight and obese men prior to and after the 12-week period of lifestyle modifications. Body mass, BMI, waist circumference, and body fat percentage markedly decreased, and SBP, DBP, MAP, heart rate, triglyceride, total cholesterol, LDL cholesterol, insulin, HOMA-IR and total energy intake also significantly decreased after the 12-week period of lifestyle modifications compared to values prior to the program. Also, HDL cholesterol, the relative value of VO_{2peak} (divided by weight) and the number of steps significantly increased. While, fasting blood glucose and absolute value of VO_{2peak} (not divided by weight) did not change significantly. Table 2 shows the circulating cytokine levels prior to and after the lifestyle modifications. The levels of IL-4, IL-5, IL-6, IL-9, IL-12, IL-17, G-CSF, MCP-1, MIP-1 β , and VEGF were significantly decreased after the lifestyle modifications and others did not change significantly. Of note, the baPWV was significantly lower after the 12-week program (Fig. 1) and the effect size was 0.87, which means relatively large effect. Table 3 shows the relationship between changes in characteristics and inflammatory markers which were significantly changed before and after the intervention. We found significant correlations between changes in serum IL-6 levels and that in baPWV ($r_s = 0.58$, $p < 0.05$), serum insulin levels ($r_s = 0.50$, $p < 0.05$), and HOMA-IR ($r_s = 0.48$, $p < 0.05$). In addition, we found significant associations between changes in serum IL-9 levels and waist circumference ($r = 0.49$, $p < 0.05$), body fat percentage ($r = 0.56$, $p < 0.05$), HOMA-IR ($r = 0.46$, $p < 0.05$), HDL cholesterol levels ($r = -0.64$, $p < 0.05$), and steps ($r = -0.60$, $p < 0.05$). Also, changes in serum IL-17 levels were significant correlated with steps ($r = -0.54$, $p < 0.05$), and changes in serum VEGF levels were significantly correlated with VO_{2peak} ($r_s = -0.50$, $p < 0.05$). Multiple regression analysis has revealed that changes in baPWV was significantly associated with that in IL-6 ($\beta = 0.50$, $p < 0.05$), while changes in systolic blood pressure ($\beta = 0.26$, $p = 0.24$) and changes in BMI ($\beta = -0.15$, $p = 0.45$) were not associated with that in baPWV.

Discussion

In this study, we investigated the effect of a 12-week period of lifestyle modifications on circulating cytokine levels by conducting comprehensive analyses of the rela-

Table 1 Characteristics of overweight and obese men before and after the lifestyle modification

	Before	After
Age (years)	50 $\pm$ 10	—
Height (cm)	172.0 $\pm$ 5.7	—
Body mass (kg)	85.1 $\pm$ 5.6	72.5 $\pm$ 4.7**
Body mass index (kg/m ²)	28.8 $\pm$ 1.4	24.5 $\pm$ 1.4**
Waist circumference (cm)	99.2 $\pm$ 4.8	86.2 $\pm$ 5.9**
Body fat percentage (%)	28.5 $\pm$ 3.5	22.9 $\pm$ 4.8**
Triglyceride (mg/dL)	167 $\pm$ 126	76 $\pm$ 29**
Total cholesterol (mg/dL)	209 $\pm$ 37	187 $\pm$ 36**
HDL cholesterol (mg/dL)	52 $\pm$ 12	56 $\pm$ 13*
LDL cholesterol (mg/dL)	127 $\pm$ 27	110 $\pm$ 22**
Fasting blood glucose (mg/dL)	91 $\pm$ 9	87 $\pm$ 6
Insulin (μ U/dL)	7.2 $\pm$ 3.7	3.8 $\pm$ 1.7**
HOMA-IR	1.6 $\pm$ 0.9	0.8 $\pm$ 0.4**
VO_{2peak} (mL/min)	2,577 $\pm$ 506	2,715 $\pm$ 389
VO_{2peak} (mL/min/kg)	30.3 $\pm$ 5.9	37.7 $\pm$ 6.4**
Steps (steps/day) ^a	7,564 $\pm$ 1,701	11,943 $\pm$ 2,768**
Energy intake (kcal/day) ^a	2,182 $\pm$ 640	1,398 $\pm$ 236**
SBP (mmHg)	140 $\pm$ 17	120 $\pm$ 12**
DBP (mmHg)	88 $\pm$ 11	75 $\pm$ 11**
MAP (mmHg)	107 $\pm$ 15	92 $\pm$ 12**
Heart rate (beat/min)	62 $\pm$ 7	53 $\pm$ 7**

Date are expressed as mean $\pm$ SD.

Significant difference vs. before lifestyle modification, ** $p < 0.01$, * $p < 0.05$.

HDL: high-density lipoprotein; LDL: low-density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; baPWV: brachial-ankle pulse wave velocity.

^a Data available in 19 individuals.

tionship between changes in circulating cytokine levels and baPWV. We observed that baPWV and the levels of some cytokines (*i.e.* IL-6, IL-12, MCP-1, VEGF) significantly decreased after the 12-week period of lifestyle modifications for overweight and obese men. Furthermore, we observed a positive correlation between changes in circulating IL-6 levels and baPWV. These results suggest that the 12-week period of lifestyle modifications for overweight and obese men induced a decrease in circulating cytokine levels (especially IL-6), leading to decreased baPWV.

Table 2 Circulating cytokine levels before and after the lifestyle modification

	Before	After
IL-1ra (pg/mL)	213.5 ± 141.4	194.6 ± 145.8
IL-4 (pg/mL)	3.7 ± 1.4	3.2 ± 1.0*
IL-5 (pg/mL)	13.0 ± 5.6	10.3 ± 3.8*
IL-6 (pg/mL)	9.8 ± 5.5	8.0 ± 3.3*
IL-7 (pg/mL)	11.4 ± 5.6	9.6 ± 4.1
IL-8 (pg/mL)	25.7 ± 8.8	23.4 ± 7.2
IL-9 (pg/mL)	18.0 ± 9.1	15.8 ± 9.5*
IL-10 (pg/mL)	16.0 ± 10.2	12.6 ± 5.8
IL-12 (pg/mL)	53.2 ± 29.0	40.6 ± 23.3*
IL-13 (pg/mL)	20.1 ± 11.9	16.0 ± 7.9
IL-17 (pg/mL)	107.8 ± 59.8	83.7 ± 48.2*
Eotaxin (pg/mL)	94.5 ± 109.1	69.0 ± 45.1
FGF basic (pg/mL) ^a	60.5 ± 24.0	51.6 ± 19.6
G-CSF (pg/mL)	90.0 ± 44.9	70.3 ± 31.0*
GM-CSF (pg/mL)	37.7 ± 18.8	31.6 ± 15.1
IFN- γ (pg/mL)	118.0 ± 55.7	106.0 ± 45.9
IP-10 (pg/mL)	1,007.0 ± 399.5	1,028.8 ± 542.3
MCP-1 (pg/mL)	43.4 ± 19.7	32.3 ± 13.2*
MIP-1 α (pg/mL)	6.8 ± 2.6	6.2 ± 2.2
MIP-1 β (pg/mL)	158.9 ± 64.8	138.5 ± 60.2*
TNF- α (pg/mL)	41.7 ± 18.6	36.9 ± 15.6
VEGF (pg/mL)	67.9 ± 41.8	48.9 ± 30.3*

Date are expressed as mean $\pm$ SD.

Significant difference vs. before lifestyle modification, * $p < 0.05$.

IL-1ra, IL-1 receptor antagonist; FGF basic, fibroblast growth factor basic; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN- γ , interferon- γ ; IP-10, interferon-inducible protein 10; MCP-1, monocyte chemoattractant protein 1; MIP-1 α , macrophage inflammatory protein 1 α ; MIP-1 β , macrophage inflammatory protein 1 β ; TNF- α , Tumor Necrosis Factor- α ; VEGF, vascular endothelial growth factor.

^a Data available in 18 individuals.

In this study, we have observed that several inflammatory factors including IL-4, IL-5, IL-6, IL-9, IL-12, IL-17, G-CSF, MCP-1, MIP-1 β , and VEGF, decreased after the 12-week period of lifestyle modification. All of these factors are known to be adipokines, and it has been reported that their circulating levels are higher in obese individuals than in their normal weight peers [3, 4,

Fig. 1 baPWV before and after the 12-week lifestyle modification. Data are expressed as mean $\pm$ SD.

21-24]. A previous study demonstrated that the circulating level of IL-8, IL-9, MCP-1, MIP-1 β , and VEGF decreased after weight-loss due to bariatric surgery in severely obese patients, but IL-6 circulating levels did not decrease [22]. On the other hand, it was reported that weight-loss induced by dietary restriction and habitual exercise decreased levels of IL-6 and MCP-1 [5]. These data suggest that the method of achieving weight-loss produces a different effect on the balance of inflammatory factors. Our study here showed for the first time that weight-loss after lifestyle modification decreases circulating levels of IL-5, IL-9, and IL-17 in obese men. Moreover, circulating IL-9 levels significantly correlated with indexes of abdominal obesity, such as waist circumference and percentage of body fat. One of the characteristics of obesity is a chronic systemic inflammatory status, which suggests that high a level of adipokines reflects systemic inflammation. Therefore, in our study here, we hypothesized that a decrease in fat mass caused a decrease in circulating inflammatory substances in obese individuals.

In this study, we demonstrated that arterial stiffness decreased in obese men after a 12-week period of dietary modification and exercise training. This finding is also supported by our previous publications as well as those from another research group [13-15]. In obese people, increased circulating cholesterol, triglycerides and insulin are associated with elevated arterial stiffness. Although 12-week lifestyle modification significantly decreased these factors, we did not found significant associations between changes in baPWV and total cho-

Table 3 Correlations (*r*) between changes in characteristics and that in inflammatory markers.

	IL-4	IL-5	IL-6	IL-9	IL-12	IL-17	G-CSF	MCP-1	MIP1- β	VEGF
Body mass (kg)	0.24	0.24	<i>0.26</i>	0.39	0.17	0.10	0.15	<i>0.32</i>	0.28	<i>0.27</i>
Body mass index (kg/m ²)	0.16	0.116	<i>0.11</i>	0.40	0.14	0.02	0.09	<i>0.25</i>	0.25	<i>0.19</i>
Waist circumference (cm)	0.18	0.22	<i>-0.05</i>	0.49*	0.31	0.20	0.21	<i>0.07</i>	-0.04	<i>0.15</i>
Body fat percentage (%)	0.19	0.29	<i>0.15</i>	0.56*	0.07	0.23	0.31	<i>0.29</i>	-0.02	<i>0.18</i>
Triglyceride (mg/dL)	<i>0.26</i>	<i>0.26</i>	<i>0.27</i>	<i>0.21</i>	<i>0.04</i>	<i>0.20</i>	<i>0.25</i>	<i>0.38</i>	<i>0.27</i>	<i>0.03</i>
Total cholesterol (mg/dL)	0.36	0.32	<i>0.25</i>	-0.05	0.13	0.25	0.24	<i>0.17</i>	0.40	<i>0.05</i>
HDL cholesterol (mg/dL)	-0.21	-0.34	<i>0.08</i>	-0.64*	-0.05	-0.35	-0.44	<i>-0.15</i>	0.13	<i>-0.04</i>
LDL cholesterol (mg/dL)	<i>0.27</i>	<i>0.19</i>	<i>-0.06</i>	<i>0.10</i>	<i>0.39</i>	<i>0.34</i>	<i>0.20</i>	<i>-0.14</i>	<i>0.11</i>	<i>0.25</i>
Insulin (μ U/dL)	0.35	0.42	<i>0.50*</i>	0.44	0.25	0.37	0.38	<i>0.23</i>	0.27	<i>0.31</i>
HOMA-IR	0.39	0.47*	<i>0.48*</i>	0.46*	0.28	0.41	0.42	<i>0.24</i>	0.23	<i>0.32</i>
VO _{2peak} (mL/min/kg)	-0.26	-0.20	<i>0.04</i>	-0.42	-0.26	-0.27	-0.18	<i>0.09</i>	0.04	<i>-0.50*</i>
Steps (steps/day)	-0.39	-0.42	<i>-0.29</i>	-0.60*	-0.26	-0.54*	-0.45	<i>-0.35</i>	-0.33	<i>-0.20</i>
Energy intake (kcal/day)	<i>0.15</i>	<i>0.04</i>	<i>0.12</i>	<i>-0.04</i>	<i>-0.13</i>	<i>-0.05</i>	<i>-0.01</i>	<i>0.06</i>	<i>0.27</i>	<i>0.14</i>
SBP (mmHg)	0.41	0.30	<i>0.45*</i>	-0.01	0.06	0.35	0.15	<i>0.17</i>	0.15	<i>0.36</i>
DBP (mmHg)	0.21	0.19	<i>-0.01</i>	-0.10	-0.08	0.24	-0.01	<i>-0.22</i>	0.05	<i>0.29</i>
MAP (mmHg)	0.26	0.17	<i>0.23</i>	-0.22	-0.03	0.13	-0.05	<i>-0.01</i>	0.03	<i>0.26</i>
Heart rate (beat/min)	0.28	0.30	<i>0.09</i>	0.21	0.04	0.37	0.37	<i>0.38</i>	0.42	<i>0.05</i>
baPWV (cm)	0.30	0.31	<i>0.58*</i>	0.04	0.33	0.33	0.29	<i>0.37</i>	0.23	<i>0.11</i>

HDL: high-density lipoprotein; LDL: low-density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; baPWV: brachial-ankle pulse wave velocity; IL-4: interleukin-4; IL-5: interleukin-5; IL-6: interleukin-6; IL-9: interleukin-9; IL-12: interleukin-12; IL-17: interleukin-17; G-CSF: granulocyte-colony stimulating factor; GM-CSF: granulocyte macrophage colony-stimulating factor; MCP-1: monocyte chemoattractant protein 1; MIP1- β : macrophage inflammatory protein 1 β ; VEGF: vascular endothelial growth factor.

* $p < 0.05$

Italic means Spearman's rank correlation coefficient.

lesterol ($r = 0.19$, $p = 0.41$), LDL cholesterol ($r = 0.16$, $p = 0.50$), triglycerides ($r = -0.15$, $p = 0.51$), insulin ($r = 0.20$, $p = 0.40$). Thus, the effects of these factors on decrease in arterial stiffness may be relatively small. On the other hand, we found a significant positive correlation between changes in baPWV and that in circulating IL-6 levels before and after the lifestyle modification, but other inflammation factors were not significantly correlated with baPWV. In addition, multiple regression analysis revealed that this relationship was independent of the change in systolic blood pressure and BMI. Thus, decrease in circulating IL-6 levels are associated with decrease in arterial stiffness. It is known that IL-6 plays multiple roles: stimulating CRP production in liver, activation of macrophages, increasing platelet adhesion molecules, and stimulating proliferation of smooth muscle

cells; all of these processes are known to be induced in cardiovascular diseases [25-27]. Moreover, it is well known that circulating IL-6 levels critically elevate during an attack of acute myocardial infarction [28], and increase in circulating IL-6 levels are associated with increase in arterial stiffness [29]. Taken together, IL-6 may participated in the mechanisms of decreasing arterial stiffness in obese individuals after lifestyle modification.

IL-6, produced and released from skeletal muscle as "myokine", has beneficial effects on metabolism [30-33]. Previous studies have reported that acute aerobic exercise increased circulating IL-6 levels [34, 35], which may be associated with increased insulin sensitivity after acute aerobic exercise. On the other hand, circulating IL-6 levels are elevated in obesity and patients with type

2 diabetes [36-38], and chronic elevation in circulating IL-6 levels have a negative effect on metabolism [39]. Thus, although acute increase in circulating IL-6 levels have beneficial effects on metabolism, chronic increase in circulating IL-6 levels have negative effects on metabolism like obesity and type 2 diabetes. In this study, our subjects were overweight and obese, and 12-week lifestyle modification decreased basal circulating IL-6 levels. In addition, changes in circulating IL-6 levels were significantly correlated with that in circulating insulin levels and HOMA-IR. Taken together, in our overweight and obese subjects, IL-6 has negative effects on metabolism, and 12-week lifestyle modification decrease circulating IL-6 levels, insulin levels and HOMA-IR.

A previous study that investigated the effect of a 16-week period of dietary restriction on circulating levels of MIP-1 β , G-CSF, and VEGF found that the levels did not change significantly [6]. Compared with the results of our study here, the degree of weight-loss was smaller in the previous study, which may have caused a different effect on the inflammatory condition. On the other hand, our study did not show any change in the levels of TNF- α , a well-known inflammatory factor. Actually, some previous studies demonstrated that the effect of weight-loss on circulating TNF- α levels have induced definitely different result, increase, decrease, or unchanged [5, 7-9]. Bruun *et al.* showed that adipose TNF- α levels decreased after a 15-week period of dietary restriction and habitual exercise intervention, but the circulating TNF- α levels did not decrease [5]. In one of their other studies, it was demonstrated that weight-loss achieved through an 8-week period of dietary restriction (average of 12 kg weight loss) did not decrease circulating TNF- α levels. However, they observed a significant decrease in TNF- α levels after a further 8 weeks and the participants' body mass was maintained [7]. In another study, the authors demonstrated that dietary restriction decreased body mass by 19 kg, and also significantly decreased circulating TNF- α levels after a 24-week intervention period [8]. In our study here, we selected the method of weight-loss for obese individuals to consist of a well-

nutrient balanced dietary restriction and moderate intensity exercise habituation. The method of training is also one of the determinants of TNF- α levels, because contradictory levels of circulating TNF- α were observed after exercise training [5, 7-9]. Therefore, we suggest that differences in the use of the term "intervention", and/or the methods of exercise training used may account for the contradictory results in systemic inflammatory condition reported by different studies.

We demonstrated that several inflammatory factors: IL-4, IL-5, IL-6, IL-9, IL-12, IL-17, G-CSF, MCP-1, MIP-1 β , and VEGF, decreased in obese individuals after the 12-week period of lifestyle modification in our study here. Inflammation is known to be a strong inductive factor of an obesity-related increase in arterial stiffness. We observed that a 12-week period of lifestyle modification for obese men significantly decreased: body weight, the circulating levels of several inflammatory factors, and arterial stiffness. Furthermore, we demonstrated a positive significant relationship between the change in IL-6 and arterial stiffness. This relationship was independent of blood pressure and BMI. Taken together, our study suggests that lifestyle modifications for overweight and obese men induce a decrease in circulating cytokine levels (especially IL-6), leading to decreased arterial stiffness. However, the sample size of the present study was too small to conclude our results (*e.g.*, relationship between arterial stiffness and circulating cytokine levels, statistical methods, *etc.*). Thus, further large study will be needed to clarify these relations.

Acknowledgements

H.K. and T.Y. were recipients of a Grant-in-Aid for JSPS Fellow from the Japan Society for Promotion of Science.

Conflicts of interest

The authors have no conflicts of interest to disclose related to this manuscript.

References

1. Berg AH, Scherer PE (2005) Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 96: 939-949.
2. Hotamisligil GS (2006) Inflammation and metabolic disorders. *Nature* 444: 860-867.
3. Ferrante AW Jr (2007) Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. *J Intern Med* 262: 408-414.
4. Kondo T, Kobayashi I, Murakami M (2006) Effect of

- exercise on circulating adipokine levels in obese young women. *Endocr J* 53: 189–195.
5. Bruun JM, Helge JW, Richelsen B, Stallknecht B (2006) Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. *Am J Physiol Endocrinol Metab* 290: E961–E967.
 6. Rizkalla SW, Prifti E, Cotillard A, Pelloux V, Rouault C, *et al.* (2012) Differential effects of macronutrient content in 2 energy-restricted diets on cardiovascular risk factors and adipose tissue cell size in moderately obese individuals: a randomized controlled trial. *Am J Clin Nutr* 95: 49–63.
 7. Bruun JM, Pedersen SB, Kristensen K, Richelsen B (2002) Opposite regulation of interleukin-8 and tumor necrosis factor- α by weight loss. *Obes Res* 10: 499–506.
 8. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, *et al.* (2003) Regulation of adiponectin by adipose tissue-derived cytokines: *in vivo* and *in vitro* investigations in humans. *Am J Physiol Endocrinol Metab* 285: E527–E533.
 9. Hayase H, Nomura S, Abe T, Izawa T (2002) Relation between fat distribution and several plasma adipokines after exercise training in premenopausal and postmenopausal women. *J Physiol Anthropol* 21: 105–113.
 10. Mahmud A, Feely J (2005) Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension* 46: 1118–1122.
 11. Miyaki A, Maeda S, Choi Y, Akazawa N, Eto M, *et al.* (2013) Association of plasma pentraxin 3 with arterial stiffness in overweight and obese individuals. *Am J Hypertens* 26: 1250–1255.
 12. Mäki-Petäjä KM, Hall FC, Booth AD, Wallace SM, Yasmin, *et al.* (2006) Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor- α therapy. *Circulation* 114: 1185–1192.
 13. Kumagai H, Miyaki A, Higashino R, Akazawa N, Choi Y, *et al.* (2014) Lifestyle modification-induced increase in serum testosterone and SHBG decreases arterial stiffness in overweight and obese men. *Artery Res* 8: 80–87.
 14. Maeda S, Miyaki A, Kumagai H, Eto M, So R, *et al.* (2013) Lifestyle modification decreases arterial stiffness and plasma asymmetric dimethylarginine level in overweight and obese men. *Coron Artery Dis* 7: 583–588.
 15. Goldberg Y, Boaz M, Matas Z, Goldberg I, Shargorodsky M (2009) Weight loss induced by nutritional and exercise intervention decreases arterial stiffness in obese subjects. *Clin Nutr* 28: 21–25.
 16. Miyaki A, Maeda S, Yoshizawa M, Misono M, Saito Y, *et al.* (2009) Effect of weight reduction with dietary intervention on arterial distensibility and endothelial function in obese men. *Angiology* 60: 351–357.
 17. Kumagai H, Zempo-Miyaki A, Yoshikawa T, Tsujimoto T, Tanaka K, *et al.* (2015) Lifestyle modification increases serum testosterone levels and decrease central blood pressure in overweight and obese men. *Endcr J* 62: 423–430.
 18. Miyaki A, Maeda S, Yoshizawa M, Misono M, Saito Y, *et al.* (2009) Effect of habitual aerobic exercise on body weight and arterial function in overweight and obese men. *Am J Cardiol* 104: 823–828.
 19. Gingras V, Vigneault J, Weisnagel SJ, Tchernof A, Robitaille J (2013) Accelerometry-measured physical activity and inflammation after gestational diabetes. *Med Sci Sports Exerc* 45: 1307–1312.
 20. Sugawara J, Hayashi K, Yokoi T, Cortez-Cooper MY, DeVan AE, *et al.* (2005) Brachial-ankle pulse wave velocity: an index of central arterial stiffness? *J Hum Hypertens* 19: 401–406.
 21. Strissel KJ, DeFuria J, Shaul ME, Bennett G, Greenberg AS, *et al.* (2010) T-cell recruitment and Th1 polarization in adipose tissue during diet-induced obesity in C57BL/6 mice. *Obesity (Silver Spring)* 18: 1918–1925.
 22. Dalmas E, Rouault C, Abdenmour M, Rovere C, Rizkalla S, *et al.* (2011) Variations in circulating inflammatory factors are related to changes in calorie and carbohydrate intakes early in the course of surgery-induced weight reduction. *Am J Clin Nutr* 94: 450–458.
 23. Tarantino G, Costantini S, Finelli C, Capone F, Guerriero E, *et al.* (2014) Is serum interleukin-17 associated with early atherosclerosis in obese patients? *J Transl Med* 12: 214.
 24. Schmidt FM, Weschenfelder J, Sander C, Minkwitz J, Thormann J, *et al.* (2015) Inflammatory cytokines in general and central obesity and modulating effects of physical activity. *PLoS One* 10: e0121971.
 25. Bassuk SS, Rifai N, Ridker PM (2004) High sensitivity C-reactive protein: clinical importance. *Curr Probl Cardiol* 29: 439–493.
 26. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S (2005) Adipokines: molecular links between obesity and atherosclerosis. *Am J Physiol Heart Circ Physiol* 288: H2031–H2041.
 27. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V (2000) Inflammation, obesity, stress and coronary heart disease: Is interleukin-6 the link? *Atherosclerosis* 148: 209–214.
 28. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH (2000) Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 101: 1767–1772.
 29. Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C, Ioakeimidis N, *et al.* (2005) Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. *Circulation* 112: 2193–2200.

30. Febbraio MA, Pedersen BK (2005) Contraction-induced myokine production and release: Is skeletal muscle an endocrine organ? *Exerc Sport Sci Rev* 33: 114–119.
31. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, *et al.* (2003) Searching for the exercise factor: Is IL-6 a candidate? *J Muscle Res Cell Motil* 24: 113–119.
32. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, *et al.* (2004) The metabolic role of IL-6 produced during exercise: Is IL-6 an exercise factor? *Proc Nutr Soc* 63: 263–267.
33. Petersen AM, Pedersen BK (2005) The anti-inflammatory effect of exercise. *J Appl Physiol* 98: 1154–1162.
34. Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, *et al.* (2001) Cytokine changes after a marathon race. *J Appl Physiol* 91: 109–114.
35. Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, Henson DA, Butterworth DE, *et al.* (1997) Carbohydrate and the cytokine response to 2.5 h of running. *J Appl Physiol* 82: 1662–1667.
36. Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, *et al.* (2000) Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab* 85: 3338–3342.
37. Carey AL, Bruce CR, Sacchetti M, Anderson MJ, Olson D, *et al.* (2004) Interleukin-6 and tumor necrosis factor- α are not increased in patients with type 2 diabetes: evidence that plasma IL-6 is related to fat mass and not insulin responsiveness. *Diabetologia* 47: 1029–1037.
38. Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, *et al.* (2001) Circulating interleukin-6 in relation to adiposity, insulin action, insulin secretion. *Obes Res* 9: 414–417.
39. Lazar MA (2005) How obesity causes diabetes: not a tall tale. *Science* 307: 373–375.