Note

The Frequency of Fish-Eating Could Negatively Associate with Visceral Adiposity in Those Who Eat Moderately

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(Received January 19, 2015)

Summary Visceral fat accumulation is regarded as one of the major phenotypes of metabolic syndrome. There have not been enough data on the relationship between the fish-eating habit and visceral adiposity. A total of 94 male participants received abdominal CT for the measurement of the visceral fat area (VFA), serum sampling for the fatty acid composition and questionnaires about their life-style. We divided the participants into two groups: whether they ate their fill (group F, $n=70$) or they ate in moderation (group M, $n=24$). Step-wise multiple linear regression analyses showed that usual alcohol consumption and lower daily physical activity in group F, and infrequent fish-eating and frequent fat-rich deserts in group M were the significant positive correlates with the VFA. The serum eicosapentaenoic acid (EPA)/arachidonic acid (AA) ratio showed significant correlation with the frequency of fish-eating in both groups. Interestingly, in group M, the serum EPA/AA ratio negatively correlated with the VFA, while it failed in group F. In conclusion, the present data suggest that the fish-eating habit might negatively associate with visceral fat accumulation only in those who are moderate in eating in the general population.

Key Words visceral adiposity, fish-eating, EPA/AA ratio

Management of obesity is an important clinical subject because it is considered to be linked with various diseases. Some researchers regard visceral adiposity as the major pathology in metabolic syndrome (MetS) (1). A recent prospective study has shown that visceral adiposity is associated with incident cardiovascular disease (2).

Fatty acid composition has been thought to be important for the prevention of cardiovascular diseases. A low value for the eicosapentaenoic acid (EPA)/arachidonic acid (AA) ratio or relatively low intake of $n-3$ polyunsaturated fatty acids (PUFAs) has been reported to be related to various cardiovascular diseases (3–5). Furthermore, the Japanese fish-eating habit has been reported to reduce the onset of diabetes mellitus (6). In an experimental report, mice fed with fish-oil showed a reduction in visceral fat accumulation (7).

These data suggest that the fish-eating habit could affect MetS or visceral adiposity through the increase in serum $n-3$ PUFAs. Although many studies have found a relationship of desaturase activities and $n-3$ PUFAs with visceral adiposity (8, 9), there have been fewer reports on the effectiveness of the fish-eating habit on visceral obesity. In the present study, we aimed to examine whether the fish-eating habit was associated with the extent of visceral adiposity in the general population in Japan.

Materials and Methods

Subjects. Among the participants who visited the Health Care Center of Kinki Central Hospital for the purpose of a comprehensive medical examination from August 2012 to December 2012, 94 male participants agreed to undergo blood sampling tests for serum fatty acid composition. All of them underwent abdominal CT scan for the measurement of body fat composition voluntarily. During that term, the total number of males who came to our institute was 763, and those who received the fat scan was 106, indicating that 88.7% of the participants agreed to join the present study. The present study was approved by the ethical committee in Kinki Central Hospital and carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki).

Questionnaire about the life-style and the fish-eating habit. All of the participants filled in a self-administered questionnaire about their life-style and the fish-eating habit. The items to be answered “yes” or “no” are shown in Table 1a. The multiple choice questionnaire about the frequency of exercise and the fish-eating frequency is shown in Table 1b. Then the fish-eating frequency was divided into three degrees (the frequency of the fish-eating is less than 3 times per week (group 0: G0), 3 times or 4 times per week (G1), and 5 times or more per week (G2)) according to a previous study, with some modifications (10).

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Table 1a. Questionnaire answered by yes or no.

Q1.	Do you prefer strong seasoning?
Q2.	Do you prefer to add soy sauce or salt to a dish?
Q3.	Do you have soup together with noodles?
Q4.	Do you consume dairy products at least once a day?
Q5.	Do you eat your fill?
Q6.	Do you have a meat dish at least twice a week?
Q7.	Do you eat fatty meat or the chicken skin?
Q8.	Do you eat eggs at least three times a week?
Q9.	Do you use butter at least once a week?
Q10.	Do you have cakes at least twice a week?
Q11.	Do you prefer low-fat milk?
Q12.	Do you eat anything else between meals every day?
Q13.	Do you consume canned coffee or carbonated drinks every day?
Q14.	Do you have meals regularly every day?
Q15.	Do you eat fruit every day?
Q16.	Do you consume soybean products every day?
Q17.	Do you have more than one vegetable dish?
Q18.	Do you consume enough fiber through your diet?
Q19.	Do you have breakfast every morning?
Q20.	Do you have a smoking habit?
Q21.	Do you have a habit of drinking alcohol every day?

Blood sampling and measurement of fatty acid composition. All patients underwent in the morning after a fasting period of more than 8 h. Serum fatty acid composition was determined by the gas-chromatograph method and performed by SRL, Inc., Tokyo, Japan.

Measurement of the body fat composition. Abdominal fat composition was determined by the standard method using the abdominal CT with a specific computer program for the fat area measurement (11). Briefly, a cross-sectional image at the umbilicus level was obtained by abdominal CT (Toshiba, Inc., Tokyo, Japan), and it was further processed for the fat composition analysis.

Statistical analysis. Data are shown as means $\pm$ SE. Comparisons of two groups were performed with Student's *t*-test for the continuous variables and the Chi-square test with Fisher's exact test for the categorical data. In the stepwise multiple linear regression analysis, parameters with an *F* value >4.0 were subsequently entered into the regression analysis as independent variables. Comparisons among multiple groups were performed by one-way analysis of variance (ANOVA) with Bonferroni correction.

All of the statistic analyses were performed by using the StatView computer program (Version 5.0). The *p* values <0.05 were regarded as statistically significant.

Results

The general characteristics of the participants are shown in Table 2. The mean age was 55.4 y old, and the average waist circumference (WC) and body mass index (BMI) were 88.8 cm and 25.4 kg/m², respectively. The average visceral fat area (VFA) was 134.2 cm². These data indicate that the participants showed features of metabolic syndrome. The average EPA/AA ratio was 0.41, which was comparable to our recent report on the

Table 1b. Questionnaire answered by selecting an item.

Q22.	The frequency of the exercise is ① seldom, ② not so usual, ③ usual, ④ frequent
Q23.	The frequency of the fish eating is a. few, b. once–3 times/month, c. once–twice/week, d. three–4 times/week, e. five–6 times/week, f. once/day, g. more than twice/day Then the fish-eating frequency was divided into three groups as follows: G0: less than 3 times a week (a–c) G1: three times or more, and less than 5 times a week (d) G2: five times or more a week (e–g)

general population (12).

In order to search for the life-style factors that are significantly correlated with visceral adiposity, we performed univariable regression analysis. In the analysis, the VFA was used as a dependent variable and the items on the questionnaire were used as independent parameters. We did not find a significant relationship between the VFA and the frequency of fish-eating.

There was a report that *n*-3 PUFAs were effective to improve insulin resistance when they were administered with energy restriction (13). We divided the participants into two groups: whether they ate their fill (group F, *n*=70) or they ate in moderation (group M, *n*=24). By single regression analysis, the frequency of fish-eating and frequent fat-rich dessert-eating were suggested to be related with VFA in group M (*r*=−0.457 and 0.546, *p*=0.028 and 0.007, respectively), and the usual alcohol consumption and the lower daily physical activity were indicated to be related with VFA in group F (*r*=0.288 and −0.264, *p*=0.016 and 0.028, respectively) (Table 3). In group M, stepwise multivariable regression analysis revealed that the frequency of the fish-eating and the frequent fat-rich desserts were the independent determinants for the VFA (Table 3). On the other hand, in group F, the usual alcohol consumption and the lower daily physical activity were the independent determinants for the VFA (Table 3). Table 2 shows the data about the general characteristics of each fish-eating frequency group (G0, 1 and 2) in group M and group F. The VFA of the G2 was significantly lower than that of the G0 in group M, but not in group F (Table 2). Figure 1 shows the relationship between the fish-eating frequency and the VFA. We found a negative correlation of VFA with the fish-eating frequency in group M; however, it was not observed in group F. When we compared the meat-eating frequencies, we did not see any significant tendencies in meat-eating with the fish-eating frequencies in group M or F.

Next, we examined the relationship between the VFA and the serum EPA/AA ratio. As shown in Fig. 2a, we found a significant tendency for the VFA to be negatively correlated with the serum EPA/AA ratio in group M. However, in group F, this phenomenon was not observed. Importantly, in both groups, the fish-eating

Table 2. The general characteristics of the participants.

	Group M (n=24)				Group F (n=70)			
	Total	G0 (n=5)	G1 (n=9)	G2 (n=10)	Total	G0 (n=30)	G1 (n=19)	G2 (n=21)
All (n=94)								
Age (y old)	55.4±0.9	58.1±1.8	55.8±2.6	61.1±3.4	54.5±1.0	53.3±1.2	52.3±2.2	58.1±1.8 ^{§#}
Height (cm)	169.7±0.6	169.9±1.1	168.9±1.5	168.6±1.8	169.6±0.7	170.4±1.1	169.2±1.3	168.8±1.2
Weight (kg)	73.3±1.1	70.4±1.9	69.7±2.3 [§]	66.2±2.7 [§]	74.4±1.3	76.8±2.1	72.3±1.6	72.7±2.7
WC (cm)	88.8±0.8	85.8±1.5	85.6±1.8 [§]	82.3±2.0 [§]	89.8±1.0 [§]	91.0±1.6	88.6±1.4	89.3±2.0
BMI (kg/m ²)	25.4±0.3	24.3±0.6	24.4±1.8	23.3±1.0	25.8±0.4 [§]	26.4±0.6	25.2±0.4	25.4±0.8
VFA (cm ²)	134.2±5.3	122.3±9.8	130.6±13.4	99.0±14.2 [§]	138.3±6.2	146.1±9.1	124.0±10.9	140.1±12.5
SFA (cm ²)	145.5±5.9	123.1±10.8	112.9±14.4 [§]	102.8±15.0 [§]	153.2±6.8 [§]	160.6±10.9	135.2±7.7	158.8±14.7
SBP (mmHg)	127.8±1.8	127.9±3.5	120.7±2.6 [§]	128.3±6.8	127.7±2.1	130.5±4.0	126.4±3.0	124.9±2.9
DBP (mmHg)	80.4±1.2	81.1±2.2	78.3±1.4 [§]	79.3±4.1	80.1±1.5	82.8±2.8	77.7±2.4	78.3±2.1
HBP	18/94	6/24	1/9	3/10	12/70	4/30	1/19	7/21
DL	18/94	3/24	1/9	2/10	15/70	10/30	1/19	4/21
DM	5/94	0/24	0/9	0/10	5/70	4/30	1/19	0/21
TG (mg/dL)	143.0±7.8	140.6±20.7	186.3±39.5	98.2±10.4	143.8±7.8	152.7±12.0	122.2±11.4	150.7±16.0
T-cho (mmHg)	207.9±3.7	208.7±8.7	209.1±14.3	208.4±12.7	207.7±4.1	209.2±5.9	212.1±8.0	201.4±7.8
HDL (mg/dL)	56.6±1.4	59.6±3.3	55.9±6.4	66.6±4.4	55.6±1.5	55.5±2.1	54.5±2.3	56.8±3.4
LDL (mg/dL)	133.5±3.3	131.3±7.6	131.2±11.7	129.0±12.2	134.2±3.7	134.6±5.3	141.8±7.0	126.7±7.4
FBS (mg/dL)	105.5±2.2	103.2±2.6	98.7±2.7	105.3±2.4	106.3±2.9	111.6±6.1	96.4±1.5 [§]	107.8±3.2
HbA1c (%)	5.4±0.1	5.3±0.1	5.1±0.4	5.3±0.1	5.4±0.1	5.6±0.2	5.1±0.1 [§]	5.4±0.1
HOMA-IR	1.8±0.1	1.7±0.3	1.9±0.6	1.6±0.3	1.9±0.2	2.1±0.3	1.3±0.2 [§]	1.9±0.2
hs-CRP (mg/L)	0.119±0.020	0.186±0.060	0.215±0.107	0.170±0.109	0.097±0.017	0.101±0.022	0.115±0.052	0.076±0.014
DGLA (mg/L)	38.4±1.1	34.8±2.3	35.6±4.1	32.6±3.0	39.6±1.3	43.9±1.8	37.6±2.3	35.3±2.3 [§]
AA (mg/L)	167.5±4.1	156.8±7.1	154.8±14.9	162.2±9.3	171.2±4.9	180.2±8.4	166.1±8.6	163.0±8.0
EPA (mg/L)	66.8±4.2	74.4±8.5	60.0±11.3	100.3±13.8 ^{§#}	64.2±4.8	55.2±6.1	59.6±7.2	81.3±11.2 ^{§#}
DHA (mg/L)	128.9±4.7	134.2±8.9	137.7±15.8	149.2±9.9 [§]	127.1±5.6	117.9±8.1	119.0±5.4	147.6±13.1 ^{§#}
EPA/AA ratio	0.41±0.02	0.47±0.05	0.38±0.06	0.62±0.08 ^{§#}	0.39±0.03	0.31±0.03	0.37±0.05	0.51±0.06 ^{§#}

WC, waist circumference; BMI, body mass index; VFA, visceral fat area; SFA, subcutaneous fat area; SBP, systolic blood pressure; DBP, diastolic blood pressure; HBP, high blood pressure; DL, dyslipidemia; DM, diabetes mellitus; TG, triglyceride; HDL, high-density lipoprotein; FBS, fasting blood sugar; Hb, hemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; CRP, C-reactive protein; DGLA, dihomo gamma linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Comparisons between two groups were performed with Student's *t*-test for the continuous variables and the Chi-square test with Fisher's exact test for the categorical data, and comparisons among multiple groups were done by one-way analysis of variance (ANOVA) with Bonferroni correction.

*indicates $p<0.05$ vs. group M in comparing two groups, § indicates $p<0.05$ vs. G0 and # indicates $p<0.05$ vs. G1 by the multiple comparison among G0–2.

Table 3. Statics for the questionnaire.

	Group M					Group F				
	Yes/total	Single regression analysis	Stepwise multiple linear regression analysis			Yes/total	Single regression analysis	Stepwise multiple linear regression analysis		
			β	t	p			β	t	p
Q1	0/24	NS				11/70	NS			
Q2	5/24	NS				15/70	NS			
Q3	8/24	NS				40/70	NS			
Q4	21/24	NS				55/70	NS			
Q5	0/24	NA				70/70	NA			
Q6	10/24	NS				43/70	NS			
Q7	10/24	NS				46/70	NS			
Q8	13/24	NS				42/70	NS			
Q9	8/24	NS				32/70	NS			
Q10	9/24	<0.05	0.465	2.847	0.010	40/70	NS			
Q11	12/24	NS				17/70 [†]	NS			
Q12	2/24	NS				24/70 [†]	NS			
Q13	5/24	NS				24/70	NS			
Q14	18/24	NS				48/70	NS			
Q15	14/24	NS				28/70	NS			
Q16	12/24	NS				27/70	NS			
Q17	14/24	NS				14/70 [†]	NS			
Q18	12/24	NS				18/70 [†]	NS			
Q19	21/24	NS				65/70	NS			
Q20	2/24	NS				4/70	NS			
Q21	9/24	NS				21/70	<0.05	0.277	2.448	0.017
Q22	①/②/③/④ 0/6/13/5	NS				①/②/③/④ 12/23/30/5	<0.05	-0.251	-2.222	0.030
Q23	G0/G1/G2 5/9/10	<0.05	-0.44	-2.698	0.014	G0/G1/G2 30/19/21	NS			

NS, not significant; NA, not assessed.

β indicates standardized partial regression coefficient.

[†]indicates $p < 0.05$ compared with group M by Fisher's exact test.

The adjusted R-square of the stepwise multiple linear regression analysis was 0.433 for group M, and 0.120 for group F.

frequency and the serum EPA/AA ratio showed positive correlations (Table 2 and Fig. 2b).

Discussion

In the present study, we found that a simple division, that subjects ate their fill or ate in moderation, might be a key behavior in their life-style which determined the effectiveness of the fish-eating-habit in the prevention against visceral adiposity. We found a significant relationship between visceral adiposity and the fish-eating-habit only in those who were moderate in eating.

Recently, Inoue et al. have demonstrated a relationship between visceral adiposity and the serum EPA/AA ratio among the outpatients of their clinic (14). In their study, more than 80% of the participants were patients with diabetes. In general, patients with diabetes are advised to limit their habitual energy intake. It is possible to regard them as people who are moderate in eating. From this viewpoint, the data that their serum EPA/AA ratio is negatively correlated with VFA is compatible to ours although energy restriction was not specifically

mentioned in their cohort.

Very little is known about the underlying mechanisms for the phenomenon that the fish-eating habit and high serum EPA/AA level are negatively associated with visceral adiposity in those under energy restriction. A report (15) showed that energy restriction can cause changes in the secretome. In line with this concept, we speculate that there might be some interactions among secreted factors triggered by energy restriction and the obesity-regulating signals. One candidate circulating factor is adiponectin, an endogenous stimulator of AMP-activated protein kinase (AMPK) (16). It has been reported that energy restriction results in the activation of Sirtuin1 (SIRT1) (17). SIRT1 has an anti-metabolic-disorder action, including the induction of adiponectin and the activation of AMPK and peroxisome proliferator-activated receptor- α (PPAR- α), leading to fatty-acid oxidation (18). Energy restriction-induced adiponectin has been reported to be involved in revascularization in an animal model (19) and it has been reported that adiponectin is also induced by fish-eating (20). Because of

Fig. 1. The relationship between the fish-eating frequency and VFA. G0: the frequency of fish-eating is less than 3 times per week, G1: the frequency of fish-eating is 3 times or 4 times per week, G2: the frequency of fish-eating is 5 times or more per week. Comparisons among groups were done by one-way analysis of variance (ANOVA) with Bonferroni correction. * indicates $p < 0.05$ compared with G0.

Fig. 2. The relationship between VFA and the serum EPA/AA ratio (a) and the relationship between the fish-eating frequency and the serum EPA/AA ratio (b). G0: the frequency of fish-eating is less than 3 times per week, G1: the frequency of fish-eating is 3 times or 4 times per week, G2: the frequency of fish-eating is 5 times or more per week. NS indicates not significant. * indicates $p < 0.05$ compared with G0 and G1.

frequent fish-eating, those who are moderate in eating might reach a sufficient serum concentration of adiponectin and/or reach sufficient activation of AMPK for the prevention of visceral adiposity.

In addition to the secreted proteins, energy restriction can result in the induction of microRNAs (miRNA) which are also found in the serum (21). In this regard, it is of great interest to search for the differences in the secretome of proteins and/or miRNAs among various life-styles.

There are some limitations in this study. First of all, we did not have precise data about the habitual energy

intakes of the participants. In particular, it is of great importance to see the differences in the energy intake among G0, G1 and G3 in the M or F group. When we compared the BMI among the G0–3 groups in group M or F, we did not find statistically significant differences in BMI (Table 2). BMI might be an indicator for obesity or energy imbalances. On the other hand, we found a significant difference in the WC between G0 and G3 only in the moderate eating group (Table 2), not in the full eating group, which was consistent with the data on VFA. The waist circumference well represents the visceral adiposity. Of course, these data are not enough to con-

clude the equality in the energy intakes among groups, and still there are some possibilities of the differences in energy intakes among the different fish-eating frequency groups. All together, we would like to speculate that the *n*-3 PUFAs could change the body fat composition in the moderately eating group, although we could not exclude the possibility of differences in the energy intake. It is necessary to investigate the food-intake habit together with the habitual energy intake and physical activities, in order to elucidate the precise relationship between *n*-3 PUFAs and visceral adiposity. The second limitation is that the participants were those who wanted to undergo the fat-scan in their comprehensive medical examination. It indicates that the participants might have tendencies to have some concerns about their life-style or health. That might be the reason why the participants in the present study had tendencies toward MetS. Third, because the present study was carried out in a single center, the number of participants was relatively small. We think that the results of the present study should be examined and be confirmed in larger cohorts.

In conclusion, we found that the frequency of fish-eating might negatively associate with visceral adiposity in those who are moderate in eating.

REFERENCES

- 1) Matsuzawa Y, Funahashi T, Nakamura T. 2011. The concept of metabolic syndrome: Contribution of visceral fat accumulation and molecular mechanism. *J Atheroscler Thromb* **18**: 629–639.
- 2) Britton KA, Massaro JM, Murabito JM, Kreger BE, Hoffmann U, Fox CS. 2013. Body fat distribution, incident cardiovascular disease, cancer, and all-cause mortality. *J Am Coll Cardiol* **62**: 921–925.
- 3) Wang C, Harris SW, Chung M, Lichtenstein HA, Balk ME, Kupelnick B, Jordan SH, Lau J. 2006. *n*-3 Fatty acids from fish or fish-oil supplements, but not α -linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr* **84**: 5–17.
- 4) Lavie JC, Milani VR, Mehra RM, Ventura OH. 2009. Omega-3 polyunsaturated fatty acids and cardiovascular diseases. *J Am Coll Cardiol* **54**: 585–594.
- 5) Oshima Y, Niki K, Hiramoto Y, Morimoto S, Takami H, Izumi M. 2013. Serum eicosapentaenoic acid/arachidonic acid ratio is low in patients with pulmonary thromboembolism. *J Nutr Sci Vitaminol* **59**: 474–477.
- 6) Nanri A, Mizoue T, Noda M, Takahashi Y, Matsushita Y, Poudel-Tandukar K, Kato M, Oba S, Inoue M, Tsugane S. 2011. Fish intake and type 2 diabetes in Japanese men and women: the Japanese Public Health Center-based Prospective Study. *Am J Clin Nutr* **94**: 884–891.
- 7) Arai T, Kim H-J, Chiba H, Matsumoto A. 2009. Anti-obesity effect of fish oil and fish oil-fenofibrate combination in female KK mice. *J Atheroscler Thromb* **16**: 674–683.
- 8) Warensjo E, Ohrvall M, Vessby B. 2006. Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women. *Nutr Metab Cardiovasc Dis* **16**: 128–136.
- 9) Kawashima A, Sugawara S, Okita M, Akahane T, Fukui K, Hashiuchi M, Kataoka C, Tsukamoto I. 2009. Plasma fatty acid composition, estimated desaturase activities, and intakes of energy and nutrient in Japanese men with abdominal obesity or metabolic syndrome. *J Nutr Sci Vitaminol* **55**: 400–406.
- 10) Belin RJ, Greenland P, Martin L, Oberman A, Tinker L, Robinson J, Larson J, Van Horn L, Lloyd-Jones D. 2011. Fish intake and the risk of incident heart failure: the Women's Health Initiative. *Circ Heart Fail* **4**: 404–413.
- 11) Yoshizumi T, Nakamura T, Yamane M, Islam AH, Menju M, Yamasaki K, Arai T, Kotani K, Funahashi T, Yamashita S, Matsuzawa Y. 1999. Abdominal fat: standardized technique for measurement at CT. *Radiology* **211**: 283–286.
- 12) Oshima Y, Niki K, Hiramoto Y, Morimoto S, Takami H, Saito H, Koga M, Izumi M. 2012. Analyses on the serum fatty acid composition of participants in comprehensive medical examination. *Med J Kinki Cent Hosp* **33**: 19–24.
- 13) Ramel A, Martinez A, Kiely M, Morais G, Bandarra NM, Thorsdottir I. 2008. Beneficial effects of long-chain *n*-3 fatty acids included in an energy-restricted diet on insulin resistance in overweight and obese European young adults. *Diabetologia* **51**: 1261–1268.
- 14) Inoue K, Kishida K, Hirata A, Funahashi T, Shimomura I. 2013. Low serum eicosapentaenoic acid/arachidonic acid ratio in male subjects with visceral obesity. *Nutr Metab (Lond)* **10**: 25.
- 15) Csiszar A, Sosnowska D, Tucsek Z, Gautam T, Toth P, Losonczy G, Colman RJ, Weindruch R, Anderson RM, Sonntag WE, Ungvari Z. 2013. Circulating factors induced by caloric restriction in the nonhuman primate macaca mulatta activate angiogenic processes in endothelial cells. *J Gerontol A Biol Sci Med Sci* **68**: 235–249.
- 16) Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. 2002. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* **8**: 1288–1295.
- 17) Gillum MP, Erion DM, Shulman GI. 2011. Sirtuin-1 regulation of mammalian metabolism. *Trends Mol Med* **17**: 8–13.
- 18) Libert SaG L. 2013. Metabolic and neuropsychiatric effects of calorie restriction and sirtuins. *Annu Rev Physiol* **75**: 669–684.
- 19) Kondo M, Shibata R, Miura R, Shimano M, Kondo K, Li P, Ohashi T, Kihara S, Maeda N, Walsh K, Ouchi N, Murohara T. 2009. Caloric restriction stimulates revascularization in response to ischemia via adiponectin-mediated activation of endothelial nitric-oxide synthase. *J Biol Chem* **284**: 1718–1724.
- 20) Kondo K, Morino K, Nishio Y, Kondo M, Fuke T, Ugi S, Iwakawa H, Kashiwagi A, Maegawa H. 2010. Effect of a fish-based diet on the serum adiponectin concentration in young, non-obese, healthy Japanese subjects. *J Atheroscler Thromb* **17**: 628–637.
- 21) Csiszar A, Gautam T, Sosnowska D, Tarantini S, Banki E, Tucsek Z, Losonczy G, Koller A, Reglodi D, Giles CB, Wren JD, Sonntag WE, Ungvari Z. 2014. Caloric restriction confers persistent anti-oxidative, pro-angiogenic, and anti-inflammatory effects and promotes anti-aging miRNA expression profile in cerebrovascular endothelial cells of aged rats. *Am J Physiol Heart Circ Physiol* **307**: H292–H306.