もしも運動中に人が倒れたら

BLS（1 次救命処置）

―素早く質の高い応急処置は予後を向上させます。

―救命の連鎖（通報、心肺蘇生、除細動、病院で二次救命処置）がつながることが重要です。

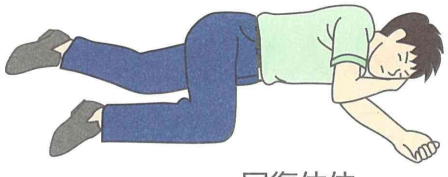
安全確認
自分、周囲、傷病者

反応確認
「大丈夫ですか？」呼びかけ

反応あったら

反応なし

気道確保
応援・救急隊を待つ
回復体位を考慮する



回復体位

119 番、AED
他の救助者がいれば依頼

呼吸あったら

呼吸確認
胸～腹、10 秒

呼吸なし、または死戦期呼吸

Compression 胸骨圧迫
Airway 気道確保
Breathing 人工呼吸

- ただちに胸骨圧迫を開始する
- 救急隊に引き継ぐまで、または呼吸や目的のある仕草が出るまで

事故が起きたら、慌てず迅速に

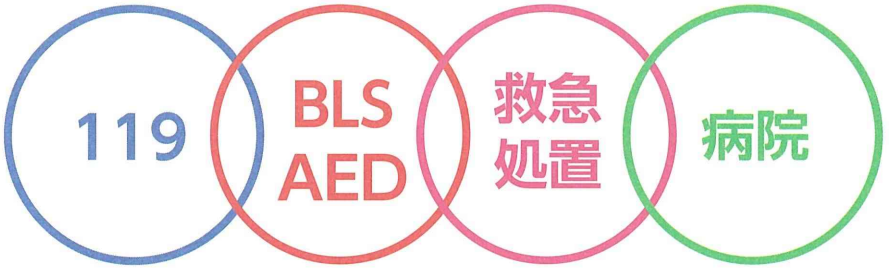
- ☑ 意識・呼吸・脈・ケガなどを確認
- ☑ 意識・呼吸がおかしい、強い胸痛または強い頭痛と冷や汗がある場合はすぐに **119 番通報**を
- ☑ **人**や**救急物品**を集めましょう
- ☑ 必要があれば救急隊、家族に連絡を
- ☑ 事故後は事故の経緯や対応を記録しましょう

救急要請は 119 番通報

- ☑ 施設住所＿＿＿＿＿＿＿＿
- ☑ 施設名＿＿＿＿＿＿＿＿
- ☑ 施設電話番号＿＿＿＿＿＿
- ☑ 事故状況の説明＿＿＿＿＿＿
- ☑ 通報者の名前＿＿＿＿＿＿

楽しく安全に健康づくりをしましょう

- ☑ 自分に合った**運動強度**を守る
- ☑ 年に一度は**メディカルチェック**を受ける
- ☑ もしもの時のために**救命処置**を身につけておく
  - **AED** で助かる命があります
  - **救命の連鎖**を迅速につなげることが重要です



救命の連鎖

研究成果の刊行に関する一覧(平成23年度)

原著

発表者氏名	論文タイトル名	発表雑誌名
A Muramoto, K Tsushita, A Kato, N Ozaki, M Tabata, M Endo, Y Oike and Y Oiso	Angiopoietin-like protein 2 sensitively responds to weight reduction induced by lifestyle intervention on overweight Japanese men.	Nutrition and Diabetes (2011) 1, e 20; doi :10.1038/nutd.2011.16
A Ozeki, A Muramoto, Y Tanmatsu, T Kishimoto, A Shinozaki and K Tsushita.	Dose Maintenance of Body Shape Contribute to Health in Middle-Aged Women?-Relationship between Changes in Body Shape During Over thirty Years and Laboratory Findings-	Anti-Aging Medicine 8 (5) : 53-59, 2011
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T. Hanioka, M. Ojima M. Nakamura	Effects of Smoking and Smoking Cessation and Smoking Cessation Intervention	Periodontal Diseases - A Clinician's Guide 107-128、2012

取材記事

雑誌・新聞等	記事タイトル名	発表日等
医薬経済	特定保健指導に医療費節減効果あり	2011.05.15
健康づくり	地域・職域の課題・ニーズに即した戦略的取り組みを	2011.07
肥満と糖尿病 座談会	特集「予備群と特定健診」座談会	10（4）512-529, 2011
メタボレター （産経新聞）	日本での特定保健指導の成果	Vol.2 2011
メタボレター （産経新聞）	こんなメタボ対策で 血圧もコントロール	Vol.3 2011
読売新聞	脱メタボ４人に１人	2012.02.28 夕刊
中日新聞（東京新聞）	内臓脂肪、血管の炎症治まる（３か月～半年、運動や食改善）	2012.03.13 朝刊

関連報告書

全国労働衛生団体連合会	特定保健指導の効果に関する特別調査結果報告書	津下一代、臼田多佳夫、澤田典子、遠藤恵子、平野幸子、秋元順子	2011

厚生労働省検討会への資料提供

保険局	第１回保険者による健診・保健指導等に関する検討会	生活習慣病予防活動・疾病管理による健康指標に及ぼす効果と医療費適正化効果に関する研究	2011.04.25
健康局	第１回 健診・保健指導の在り方に関する検討会	特定健診・特定保健指導に関する研究・調査	2011.12.07
	第３回 健診・保健指導の在り方に関する検討会	非肥満者で高血糖、脂質異常症、高血圧症を有する者への対応について	2012.02.06

Citation: Nutrition and Diabetes (2011) 1, e20; doi:10.1038/nutd.2011.16
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www.nature.com/nutd

ORIGINAL ARTICLE

Angiopoietin-like protein 2 sensitively responds to weight reduction induced by lifestyle intervention on overweight Japanese men

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Objective: Overexpression of Angiopoietin-like protein 2 (Angptl2) in obese adipose tissues promotes adipose tissue inflammation and its-related metabolic abnormalities. In a comparative study with adiponectin, we investigated whether alterations in serum Angptl2 concentrations reflect the effect of lifestyle intervention on weight loss and improved metabolic parameters in overweight subjects.
Methods: A total of 154 Japanese men (age, 40.9 ± 5.1 years; body mass index, 26.9 ± 3.6 kg m^{-2}; abdominal circumference, 94.1 ± 8.9 cm) underwent a 3-month lifestyle intervention and underwent follow-up for 3 months thereafter.
Results: Decreased serum Angptl2 levels, but not increased serum adiponectin levels, were immediately apparent at the end of 3-month lifestyle intervention. Angptl2 levels continued to decrease for 3 months in parallel with body weight loss and improvement in metabolic indicators. In subjects showing ⩾6% weight reduction, markedly reduced Angptl2 levels were detected at the end of 3-month intervention, whereas increased adiponectin levels were detected 3 months after the end of intervention. Multivariate analysis revealed changes in serum Angptl2 levels associated with changes in triglycerides (TGs), aspartate aminotransferase and alanine aminotransferase. In contrast, changes in serum adiponectin levels were associated with altered high-density lipoprotein cholesterol (HDL-C) and fasting plasma glucose levels.
Conclusion: A 3-month lifestyle intervention promoted weight reduction and improved glucose and lipid metabolism, an effect maintained 3 months later. Notably, our findings indicate that decreased Angptl2 levels are a good indicator of reduced visceral fat and metabolic improvement at early stages of lifestyle intervention. Thus, Angptl2 reflects adiposity and might be a key protein to regulate inflammation and TG metabolism, whereas adiponectin levels could reflect improved glucose and HDL-C metabolism.
Nutrition and Diabetes (2011) **1**, e20; doi:10.1038/nutd.2011.16; published online 7 November 2011

Keywords: metabolic syndrome; lifestyle intervention; body weight reduction; angiopoietin-like protein 2; adiponectin

Introduction

Obesity is a pandemic medical and social problem that increases lifestyle-related diseases, such as cardiovascular disease, type 2 diabetes, hypertension, dyslipidemia and cancer, all of which result in increased mortality.[1–5] Therefore, antagonizing weight gain is critical to decrease occurrence of these diseases. Recent reports demonstrate that weight loss has ameliorating effects on type 2 diabetes, hypertension or dyslipidemia, independent of differences in race, sex, age, intervention method and intervention period.[6–9]

Recently, the concept has emerged that obesity-related inflammation is associated with high risk of type 2 diabetes and cardiovascular diseases.[10–15] Circulating levels of C-reactive protein (CRP), fibrinogen and some adipose tissue-derived cytokines, all associated with inflammation, are decreased by body weight reduction, an occurrence associated with improved insulin resistance.[6,16–19]

More recently, we revealed that circulating levels of angiopoietin-like protein 2 (Angptl2), which is a stress responsive adipose tissues-secreted protein, were higher than

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normal in cases of obesity in human and mice, particularly in cases with visceral fat accumulation, leading to chronic adipose tissue inflammation and subsequent development or progression of insulin resistance and metabolic syndrome.[20–22] We observed accumulation of fat in the liver and skeletal muscle was mild in Angptl2 knockout mice compared with wild-type mice, and Angptl2 deletion ameliorated adipose tissue inflammation.[20] We also observed significant decreases in circulating Angptl2 concentrations in obese diabetic men following treatment with the PPARγ agonist pioglitazone, and the percent decrease in Angptl2 levels was positively correlated with the percent decreases in visceral fat area. These findings suggest that visceral fat is a likely primary source of circulating Angptl2 and that levels of that factor are significantly correlated with systemic insulin resistance and inflammation.[20–22]

But it remains unknown whether Angptl2 could respond to weight reduction and its-related metabolic abnormalities by lifestyle intervention, and if so, we'd like to know the difference between angptl2 and adiponectin.

Thus, the aim of the present study was to investigate whether Angptl2 levels reflect weight reduction, the degree of weight reduction and obesity-related metabolic abnormalities. For comparison, we monitored the influence of weight reduction on adiponectin levels, which reportedly increase in the circulation with weight loss and serve as a biomarker to assess the improvement of obesity and its-related metabolic abnormalities.[6–9]

Materials and methods

Subjects

'Overweight subjects' were recruited to participate in a lifestyle intervention program through newspaper or website advertising. We defined the subjects with a body mass index (BMI) $\geqslant 25.0\,kg\,m^{-2}$ or an abdominal circumference $\geqslant 85\,cm$ as 'overweight subject' in this study, because this criteria is established as an adequate risk for categorizing 'obesity disease' in Japan in relation to obesity-related complications in Japan.[23] All subjects gave written consent after having received verbal and written information about this study. The Ethical Review Board of the Aichi Health Plaza Comprehensive Health Science Center approved the study procedures.

Study design

Subjects underwent lifestyle intervention for 3 months and then were observed without intervention for 3 months thereafter. At the beginning of intervention, and at the 3- and 6-month time points, a questionnaire about lifestyle, anthropometric measurements, blood pressure (BP) measurements and blood tests were performed. The questionnaire contains smoking status, drinking habit and exercise habit. For anthropometric measurements, height, weight, abdominal circumference and body fat percentage (% fat) were measured. For blood tests, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), serum insulin (insulin), aspartate aminotransferase (AST), alanine aminotransferase (ALT), high-sensitive CRP (hs-CRP), Angptl2 and adiponectin levels were measured.

Lifestyle intervention

Subjects received detailed results of an examination that they underwent at the beginning of intervention and attended a lecture regarding the association of obesity and health problems and the benefits of weight reduction. That lecture included illustrations and graphs depicting the relationship of lifestyle on metabolic syndrome-related conditions. Subjects were then advised by support staff (including a public health nurse, nutritionist and health exercise trainer) to set their own behavioral targets. Then, for 3 months, subjects received lifestyle improvement support for the purpose of weight reduction. Actually, subjects were instructed in proper meal preparation for the energy-balance or received support in the form of exercise training. We instructed them by interview or supported them by e-mail once or twice a month, depending on subjects' living conditions.

Anthropometric measurements

Weight was measured with the subjects wearing light clothing and barefoot. BMI was calculated as weight (kg) divided by height (m^{-2}). Body fat percentage was determined using the bioelectrical impedance analysis (BIA) method (TBF-102; Tanita Corporation, Tokyo, Japan). Abdominal circumference was measured at the level of the umbilicus (horizontal to the ground). Systolic and diastolic blood pressure was measured using an automatic sphygmomanometer (SunTech Medical, Morrisville, NC, USA).

Biochemical analysis

Blood samples were taken after overnight fasting around at 0900 hours. LDL-C was measured using the direct method (Determiner L, LDL-C, Kyowa Medex Co., Ltd, Tokyo, Japan). HbA1c (Japan Diabetes Society) was measured using the latex agglutination method with a commercial test kit (Kyowa Medex Co., Ltd, Tokyo, Japan). The HbA1c defined by the National Glycohemoglobin Standardization Program, which is the internationally used HbA1c, is expressed by adding 0.4% to the HbA1c (JDS).[24] The HbA1c data are shown by HbA1c (National Glycohemoglobin Standardization Program). Insulin was measured using an enzyme immunoassay with a commercial test kit (Eiken Chemical Co, Ltd, Tokyo, Japan). Homeostasis model assessment of the insulin resistance index (HOMA-IR) was calculated using a

method described elsewhere.[25] There is a good correlation between HOMA-IR and glucose infusion rate obtained by the euglycemic-hyperinsulinemic clamp method.[26] Given the combination of accuracy and ease of testing, we use HOMA-IR as an index of insulin resistance. Hs-CRP levels were measured using the latex agglutination method with a commercial test kit (N-assay LA, CRP-T, Nittobo, Tokyo, Japan). Adiponectin was measured by ELISA using a test kit (Adiponectin ELISA kit; Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan).

Angptl2 was measured by ELISA as previously reported.[20,27] In brief, the K2-1A1 mouse monoclonal antibody was fixed to 96-well plates. After 10-fold dilution, serum samples were immobilized on plates for 1 h at 37 °C, followed by washing with PBS containing 0.05% Tween20 (PBST) and addition of horseradish peroxidase-conjugated K1-12A4 mouse monoclonal antibody. After 1 h of incubation at 4 °C, plates were washed with PBS containing 0.05% Tween20, and a tetramethylbenzidine detection reagent was added to the wells. After 30 min, the reaction was stopped by addition of an equal amount of 1 N H_2SO_4, and absorbance was measured at 450 nm. We confirmed the validity of this ELISA system through the following experiments. Absorbance at 450 nm increased linearly with human Angptl2 calibrators of 50–350 pg per assay with the least detectable concentration of 50 pg (correlation coefficient >0.99). In all three intra-assay determinations of the same samples showed coefficients of variation less than 5% at all Angptl2 concentrations tested. The mean ± s.d. concentrations measured (and coefficient of variation) were: sample 1, 1.76 ± 0.05 ng ml^{-1} (2.9%); sample 2, 0.43 ± 0.01 ng ml^{-1} (3.2%); and sample 3, 0.14 ± 0.01 ng ml^{-1} (3.3%). The three inter-assay determinations of the same serum gave coefficients of variation of less than 10%: sample 1, 1.82 ± 0.09 ng ml^{-1} (5.2%); sample 2, 0.44 ± 0.02 ng ml^{-1} (4.6%); and sample 3, 0.142 ± 0.01 ng ml^{-1} (5.0%). Plasma concentrations of other factors were determined using standard clinical biochemistry methods.

Statistical analysis

To assess the effectiveness of lifestyle intervention, changes in population characteristics at the beginning (0), end of the 3-month intervention or end of the 6-month program (3-month intervention plus 3-month observation) were assessed with the Wilcoxon signed rank test.

We used partial correlation analysis to examine association between baseline Angptl2 or adiponectin levels and baseline values of each laboratory measurement. We also analyzed the association between changes in Angptl2 or adiponectin levels and changes in each laboratory parameter, from the beginning of the intervention to 3 and then 6 months later. We tested baseline data and changes for normal distribution by Kolmogorov–Smirnov method. From this result, we logarithmically transformed TG, IRI, HOMA-IR, AST, ALT, hs-CRP at baseline and changes in hs-CRP at 3 and 6 months. Multiple linear regression analysis was conducted for each change in BP, lipid metabolism, glucose metabolism and liver function from the beginning of the intervention to 3 and 6 months, later as target variables, and values highly correlated with each variation as explanatory variables. Furthermore, subjects were grouped per 2% weight reduction at 3 or 6 months after the beginning of intervention. Changes in laboratory data values were analyzed by analysis of variance or the Kruskal–Wallis test (for changes in diastolic blood pressure and HbA1c after 3 months). Statistically significant changes were compared using multiple comparison by the Bonferroni method.

We used the statistics software PASW Statistics Base 18.0 (SPSS, Tokyo, Japan) and expressed results as means ± s.d. or s.e.m. Differences at the level of $P<0.05$ were considered statistically significant.

Results

Baseline characteristics of subjects

A total of 154 men were enrolled. The average age was 40.9 ± 5.1 years and BMI was 26.9 ± 3.6 kg m^{-2} (Table 1). Patients with an endocrine disorder or those undergoing drug treatment for diabetes, hypertension or dyslipidemia were excluded. Clinical and laboratory examination to characterize baseline parameters before lifestyle intervention revealed one patient with diabetes, which required therapy. During the 3-month lifestyle intervention, seven subjects dropped out of the study because of traffic accidents or occupational reasons. During the subsequent 3-month follow-up, 11 of the remaining 146 subjects could not be followed for various reasons, such as occupational issues or lack of motivation. Finally, 135 subjects who could be followed for all 6 months of the program were analyzed (continuation rate of 87.7%) (Figure 1).

At the baseline, correlation analysis adjusted by age showed a significant positive correlation of Angptl2 levels with adiposity, as estimated by BMI, abdominal circumference and fat mass (Table 2a). In contrast, baseline adiponectin levels showed an inverse correlation with adiposity (Table 2a).

Correlation analysis adjusted by age and BMI to compare baseline Angptl2 levels with clinical and laboratory data showed a significant positive correlation of Angptl2 with diastolic Blood Pressure, TG, Insulin and HOMA-IR and a significant inverse correlation of Angptl2 with HDL-C (Table 2b). By contrast, baseline adiponectin levels were inversely correlated with TG, Insulin, HOMA-IR, AST, ALT and hs-CRP, and showed a significant positive correlation with HDL-C. Baseline Angptl2 and adiponectin levels showed an inverse relationship trend ($P=0.056$) (Table 2b). When we added smoking status, drinking habit and exercise habit as control variables, association between Angptl2 and adiponectin at baseline was significant ($r=-0.215$, $P=0.014$).

Table 1 Characteristics of the 135 subjects at 0 (baseline), 3 and 6 months after initiation of the intervention

	Baseline	*3 months*	P-*value*	*6 months*	P-*value*
Age	40.9 ± 5.1				
Weight (kg)	79.4 ± 11.8	77.0 ± 11.5	<0.001	76.4 ± 11.6	<0.001
BMI (kg m^{-2})	26.9 ± 3.6	26.1 ± 3.6	<0.001	25.9 ± 3.6	<0.001
Abdominal circumference (cm)	94.1 ± 8.9	91.4 ± 9.0	<0.001	90.5 ± 9.5	<0.001
% fat (%)	26.6 ± 5.4	24.6 ± 4.7	<0.001	24.4 ± 4.8	<0.001
Fat mass (kg)	21.6 ± 7.5	19.4 ± 6.7	<0.001	19.1 ± 6.8	<0.001
SBP (mm Hg)	121.6 ± 12.4	121.9 ± 11.9	0.523	121.7 ± 12.3	0.992
DBP (mm Hg)	72.4 ± 10.3	76.6 ± 9.9	<0.001	75.4 ± 10.6	<0.001
TG (mg per 100 ml)	149.8 ± 114.2	122.6 ± 71.7	<0.001	136.2 ± 116.3	0.009
HDL-C (mg per 100 ml)	58.4 ± 13.2	55.5 ± 12.1	<0.001	57.1 ± 12.8	0.051
LDL-C (mg per 100 ml)	134.8 ± 35.3	125.1 ± 31.0	<0.001	126.7 ± 33.2	<0.001
LDL-C/HDL-C	2.43 ± 0.84	2.39 ± 0.86	0.166	2.35 ± 0.89	0.155
FPG (mg per 100 ml)	99.9 ± 12.0	96.3 ± 9.7	<0.001	96.3 ± 9.9	<0.001
HbA1c (%)	5.38 ± 0.45	5.20 ± 0.36	<0.001	5.22 ± 0.35	<0.001
Insulin (mcU ml-1)	10.12 ± 9.17	7.37 ± 5.14	<0.001	8.09 ± 5.74	<0.001
HOMA-IR	2.57 ± 2.71	1.79 ± 1.37	<0.001	1.96 ± 1.49	<0.001
AST (IU l^{-1})	25.5 ± 9.0	22.2 ± 8.5	<0.001	23.0 ± 8.7	0.005
ALT (IU l^{-1}	37.1 ± 21.9	28.6 ± 15.9	<0.001	30.0 ± 20.2	<0.001
Hs-CRP (mg per 100 ml)	0.086 ± 0.156	0.086 ± 0.125	0.907	0.090 ± 0.120	0.811
Angptl2 (ng ml^{-1})	3.02 ± 1.18	2.79 ± 1.11	0.001	2.65 ± 1.08	<0.001
Adiponectin (mcg ml^{-1})	6.59 ± 3.78	6.22 ± 3.94	<0.001	6.45 ± 3.93	0.108

Abbreviations: Angptl2, angiopoietin-like protein 2; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of the insulin resistance index; Hs-CRP, high sensitive C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglyceride. Data are presented as means ± s.d. Statistical differences of clinical and laboratory data at the 3- and 6-month time points compared with baseline values were examined using the Wilcoxon signed rank test.

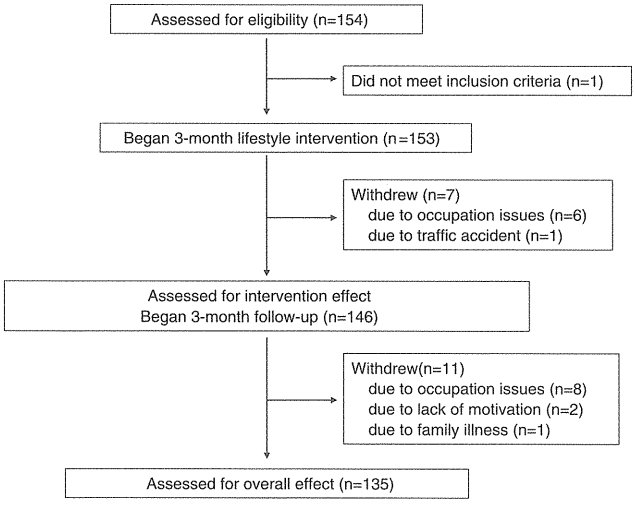

Figure 1 Flow of participants through the intervention and follow-up study.

Changes in anthropometric and biochemical parameters after 3 and 6 months

As shown in Table 1, subjects' weight at 3 and 6 months after the beginning of lifestyle intervention was decreased significantly compared with baseline values (reduced by 2.4 ± 2. 5 kg and 2.9 ± 3.5 kg, respectively). In addition, BMI, abdominal circumference, % fat, fat mass, TG, LDL-C, FPG, HbA1c, insulin, HOMA-IR, AST and ALT levels were

Table 2a Correlations of Angptl2 and adiponectin levels with anthropometric measurements at baseline levels

	Angptl2		*Adiponectin*	
	r	P	r	P
BMI	0.257	0.003	−0.242	0.005
Abdominal circumference	0.262	0.002	−0.321	<0.001
Fat mass	0.268	0.002	−0.277	<0.001

Abbreviations: Angptl2, angiopoietin-like protein 2; BMI, body mass index. Analysis was performed and adjusted by age. *r* and *P* indicate correlation coefficients and *P*-values, respectively.

significantly reduced, indicating that the 3-month lifestyle intervention significantly reduced adiposity and ameliorated glucose and lipid metabolism. There was no significant change in hs-CRP levels at 3 and 6 months after the beginning of lifestyle intervention compared with those observed at baseline. Serum Angptl2 levels were significantly decreased from 3.02 ± 1.18 to 2.79 ± 1.11 ng ml^{-1} (vs baseline value, $P=0.001$) 3 months after the beginning of intervention, and significantly decreased to 2.65 ± 1.08 ng ml^{-1} (vs baseline value, $P<0.001$) at the 6-month time point. Although serum adiponectin levels were expected to increase based on previous reports,[6–9] they decreased from 6.59 ± 3.78 μg ml^{-1} to 6.22 ± 3.94 μg ml^{-1} (vs baseline value, $P=0.001$) by the 3-month time point. At the 6-month time point, serum adiponectin levels were significantly increased

compared with those at the 3-month time point ($P=0.006$), however, they were not higher than baseline values (vs baseline value, $P=0.108$).

Table 2b Correlations of Angptl2 and adiponectin levels with blood pressure and laboratory data at baseline levels

	Angptl2		*Adiponectin*	
	r	P	r	P
SBP	0.096	0.271	−0.151	0.084
DBP	0.247	0.004	−0.149	0.087
Log TG	0.255	0.003	−0.291	0.001
HDL-C	−0.424	<0.001	0.299	<0.001
LDL-C	0.070	0.421	−0.112	0.200
FPG	0.085	0.331	−0.069	0.432
HbA1c	0.039	0.657	−0.108	0.217
Log Insulin	0.386	<0.001	−0.235	0.006
Log HOMA-IR	0.368	<0.001	−0.232	0.007
Log AST	0.100	0.250	−0.193	0.026
Log ALT	0.058	0.506	−0.240	0.005
Log Hs-CRP	0.093	0.287	−0.278	0.001
Angptl2	—	—	−0.166	0.056
Adiponectin	−0.166	0.056	—	—

Abbreviations: Angptl2, angiopoietin-like protein 2; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of the insulin resistance index; Hs-CRP, high sensitive C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglyceride. Analysis was performed and adjusted by age and BMI. *r* and *P* indicate correlation coefficients and *P*-values, respectively.

Changes in serum Angptl2 levels showed a significant positive correlation with changes in BMI, abdominal circumference and fat mass immediately after the intervention, whereas those in serum adiponectin levels showed an inverse correlation with BMI and fat mass values, but not with abdominal circumference (Table 3). By the 6-month time point, changes in serum Angptl2 levels continued to show a significant positive correlation with BMI, abdominal circumference and fat mass values, whereas serum adiponectin levels were inversely correlated with these parameters (Table 3).

Regarding changes in clinical parameters, decreases in serum Angptl2 levels showed a significant positive correlation with TG, AST, ALT and hs-CRP immediately after the intervention, whereas those in serum adiponectin levels showed a positive correlation with HDL-C and LDL-C. At the end of the program, changes in serum Angptl2 levels showed a significant positive correlation with TG, AST and hs-CRP values, and an inverse correlation with HDL-C, whereas serum adiponectin levels showed a positive correlation with HDL-C values and an inverse correlation with FPG (Table 3). Notably, we observed an inverse correlation between Angptl2 and adiponectin levels after 6 months, but not after 3 months ($r=-0.186$, $P=0.032$). Similar associations were observed after 3 months and after 6 months when we added smoking status, drinking habit and exercise habit as control variables ($r=0.070$, $P=0.432$ and $r=-0.203$, $P=0.020$, respectively).

Table 3 Correlations between changes in Angptl2 (left) and adiponectin (right) levels and changes in adiposity, blood pressure and laboratory data at 3 and 6 months after beginning the intervention relative to baseline data, which was estimated before the intervention began

From baseline	*ΔAngptl2*				*ΔAdiponectin*			
	To 3 months		*To 6 months*		*To 3 months*		*To 6 months*	
	r	P	r	P	r	P	r	P
ΔAdiposity								
ΔBMI	0.370	<0.001	0.362	<0.001	−0.190	0.028	−0.190	<0.001
ΔAbdominal circumference	0.265	0.002	0.311	<0.001	−0.017	0.843	−0.017	0.003
ΔFat mass	0.254	0.003	0.353	<0.001	−0.209	0.015	−0.209	<0.001
ΔClinical and laboratory data								
ΔSBP	0.078	0.372	0.021	0.812	0.071	0.415	−0.066	0.451
ΔDBP	0.165	0.057	0.119	0.172	0.106	0.226	0.044	0.612
ΔTG	0.360	<0.001	0.194	0.025	0.102	0.241	0.080	0.359
ΔHDL-C	−0.055	0.526	−0.196	0.024	0.203	0.019	0.369	<0.001
ΔLDL-C	−0.114	0.193	−0.100	0.250	0.179	0.039	0.145	0.096
ΔFPG	0.021	0.813	0.157	0.072	−0.132	0.130	−0.273	0.001
ΔHbA1c	0.082	0.350	0.107	0.221	0.134	0.123	0.045	0.610
ΔInsulin	−0.086	0.324	−0.037	0.676	0.040	0.649	0.081	0.355
ΔHOMA-IR	−0.109	0.213	−0.044	0.615	0.024	0.782	0.070	0.425
ΔAST	0.272	0.002	0.195	0.024	0.110	0.208	−0.066	0.454
ΔALT	0.216	0.012	0.161	0.064	0.118	0.176	−0.032	0.718
ΔLog Hs-CRP	0.241	0.005	0.257	0.003	−0.062	0.480	−0.021	0.809
ΔAngptl2	—	—	—	—	0.068	0.436	−0.186	0.032
ΔAdiponectin	0.068	0.436	−0.186	0.032	—	—	—	—

Abbreviations: Angptl2, angiopoietin-like protein 2; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of the insulin resistance index; Hs-CRP, high sensitive C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglyceride. Analysis was performed and adjusted by age (for adiposity) and BMI (for clinical and laboratory data). *r* and *P* indicate correlation coefficients and *P*-values, respectively.

Table 4 Multiple linear regression analysis to examine changes in clinical data mediated by the intervention

Target variable	*Explanatory variable*											
	ΔBMI		*ΔAbdominal Circumference*		*ΔFat mass*		*ΔLog Hs- CRP*		*ΔAngptl2*		*ΔAdiponectin*	
	β	P	*β*	P	*β*	P	*β*	P	*β*	P	*β*	P
From baseline to 3months												
ΔDBP	0.023	0.872	0.151	0.223	−0.127	0.234	−0.079	0.379	0.197	0.04	0.056	0.53
ΔTG	−0.103	0.431	0.07	0.54	0.17	0.084	−0.195	0.019	0.42	<0.001	0.077	0.348
ΔHDL-C	−0.167	0.238	0.075	0.542	0.073	0.492	−0.041	0.649	−0.066	0.486	0.208	0.02
ΔLDL-C	0.531	<0.001	−0.175	0.135	0.045	0.655	0.032	0.701	−0.141	0.117	0.189	0.025
ΔAST	0.044	0.74	0.085	0.464	0.178	0.075	−0.009	0.919	0.268	0.003	0.111	0.183
ΔALT	0.116	0.384	−0.039	0.739	0.241	0.017	−0.034	0.682	0.213	0.019	0.144	0.086
From baseline to 6months												
ΔTG	−0.079	0.642	0.168	0.272	0.088	0.455	−0.015	0.868	0.221	0.023	0.129	0.177
ΔHDL-C	−0.185	0.24	0.148	0.294	−0.048	0.659	−0.047	0.566	−0.123	0.166	0.338	<0.001
ΔFPG	0.176	0.277	−0.104	0.474	0.103	0.358	0.028	0.744	0.09	0.326	−0.257	0.005

Abbreviations: Angptl2, angiopoietin-like protein 2; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of the insulin resistance index; Hs-CRP, high sensitive C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglyceride. Target variables: changes in SBP, DBP, TG, HDL-C, LDL-C, FPG, HbA1c, insulin, HOMA-IR, AST and ALT levels from intervention onset to 3 and 6 months. Explanatory variables: changes in BMI, abdominal circumference, fat mass, log hs-CRP, Angptl2 and adiponectin levels at 3- and 6-month time points.

We next performed multiple linear regression analysis to examine which changes contributed to improvement of clinical data. As shown in Table 4, changes in serum Angptl2 concentrations were positively correlated with diastolic blood pressure, TG, AST and ALT values, whereas changes in serum adiponectin concentrations were positively correlated with HDL-C values at 3 months. At the end of the program (6 months), changes in TG were positively correlated with serum Angptl2 concentrations, whereas changes in HDL-C were positively correlated with serum adiponectin concentrations, while FPG changes were inversely correlated with adiponectin levels.

Weight reduction rates and changes in laboratory parameters

At 3 and 6 months after the beginning of the intervention, subjects were grouped by the degree of weight reduction rate into 5 groups: a weight gain group (3 months; $n=19$, 6 months; $n=23$), the 0 to <2% weight reduction group, which was designated as the unchanged control group for the following analysis (3 months; $n=35$, 6 months; $n=32$), the 2 to <4% weight reduction group (3 months; $n=41$, 6 months; $n=26$), the 4 to <6% weight reduction group (3 months; $n=20$, 6 months; $n=17$ and the 6% or more weight reduction group (3 months; $n=20$, 6 months; $n=37$).

At the 3-month time point, one-way analysis revealed significant differences in alteration in LDL-C, FPG, AST and ALT among the 5 groups (Figure 2a). Multiple comparison analysis revealed significant decreases in LDL-C in the 6% or more weight reduction group and significant decreases in ALT in the 4 to <6% weight reduction group compared with the unchanged control group. At the 6-month time point, one-way analysis revealed significant differences in alteration of HDL-C, LDL-C, FPG, HbA1c, AST and ALT among the 5 groups (Figure 2b). Multiple comparison analysis showed significant decreases in LDL-C in the 6% or more weight reduction group and decreases in AST and ALT in the 4–<6% group and 6% or more compared with the unchanged control group.

One-way analysis also revealed significant differences in alteration of serum Angptl2 levels among the 5 groups at both 3- and 6-month time points (Figures 2c and d), whereas significant alterations in serum adiponectin concentration were seen only at the 6 month time point. Serum Angptl2 concentrations tended to decrease with increased percentage of weight reduction, and the 6% or more weight reduction group showed a significant decrease relative to the unchanged control group at both the 3- and 6-month time points (Figures 2c and d). By contrast, serum adiponectin concentrations tended to increase with increased percentage of weight reduction, and the 6% or more weight reduction group showed a significant increase compared with the unchanged control group at 6 months after the beginning of the intervention, a change that was not observed at the 3-month time point (Figures 2c and d).

Discussion

We conducted a 3-month lifestyle intervention for men who were overweight and observed maintenance of weight reduction for another 3 months thereafter. Significant weight reduction was obtained, improved lipid and glucose metabolism and lowered plasma liver enzymes were observed immediately after the end of the 3-month lifestyle intervention. Moreover, improvement was maintained until the 6-month time point. Notably, decreased serum Angptl2

levels were seen immediately after the end of 3-month lifestyle intervention and continued for the entire 6-month period. This finding was significant in the 6% or more weight reduction group. By contrast, an expected increase in serum adiponectin levels was observed only in subjects with ⩾6% weight reduction at the 6-month time point.

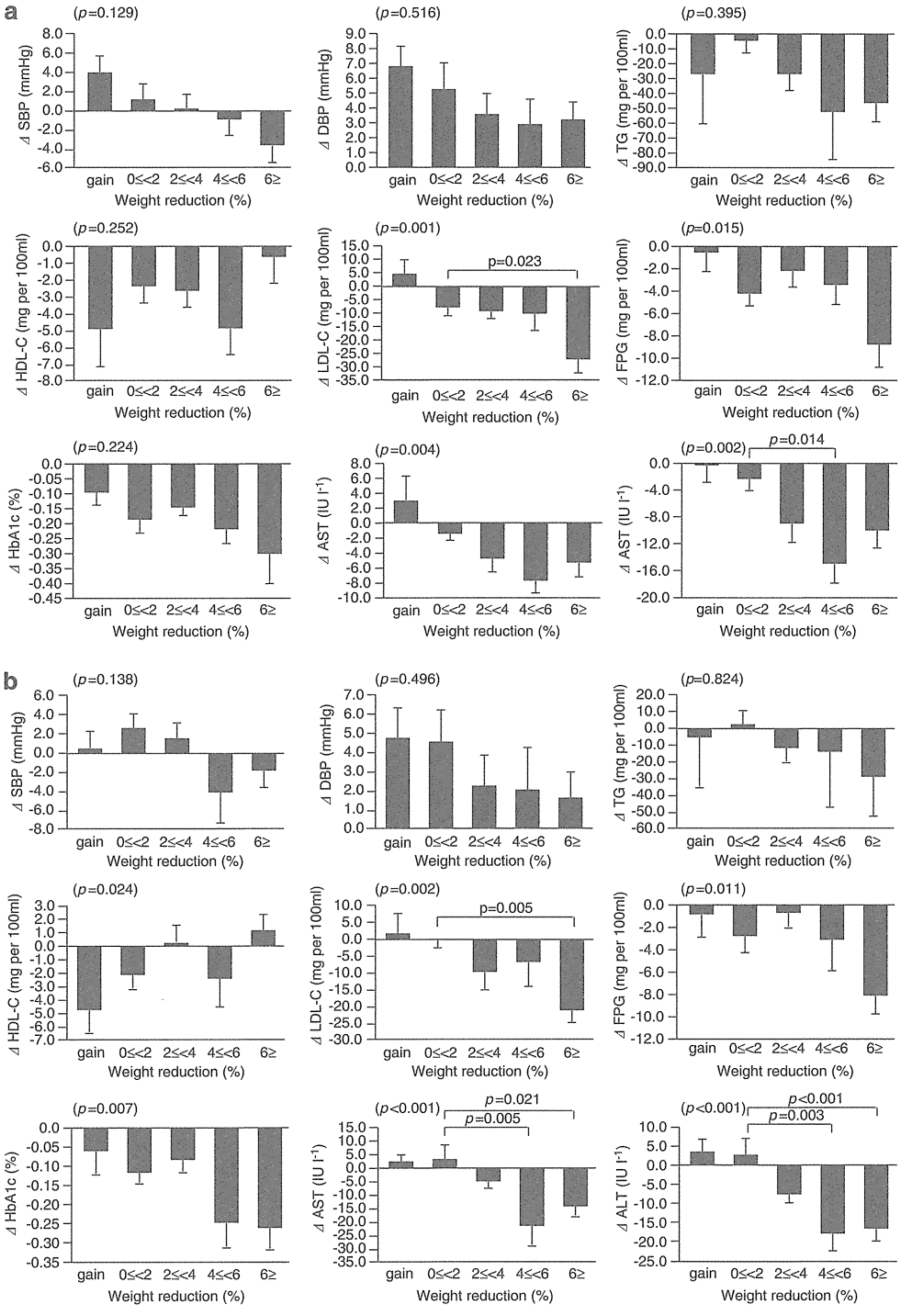

Figure 2 Changes in BP, laboratory parameters related to lipid and glucose metabolism and liver function, and serum Angptl2 and adiponectin levels in each weight reduction group. (**a**, **b**) Changes are evaluated at 3 (**a**) and 6 (**b**) months after beginning the intervention. (**c**, **d**) Changes in serum Angptl2 and adiponectin at 3 (**c**) and 6 (**d**) months after beginning the intervention. Vertical axes indicate changes in laboratory data, and the horizontal axes indicate percent weight reduction. Data were analyzed by one-way analysis of variance analysis of variance and compared between groups by multiple comparisons using the Bonferroni method. The Kruskal–Wallis test was used to evaluate changes in DBP and HbA1c at the 3-month point (**a**). Data are presented as means ± s.e.m.

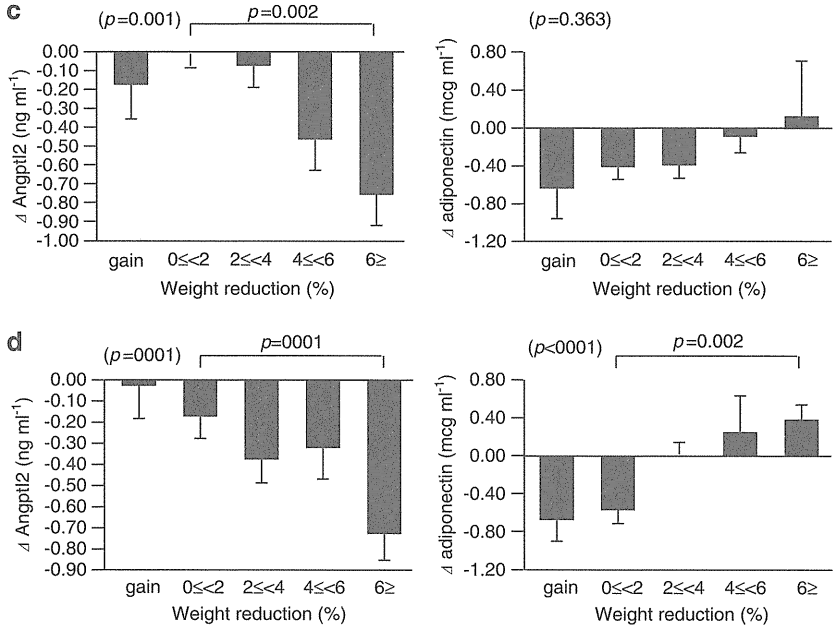

Figure 2 Continued.

In previously reported intervention studies, such as the Diabetes Prevention Program,[28] the Finnish Diabetes Prevention Study[29] and the Malmo feasibility study,[30] longer 6- or 12-month interventions supported on a one-to-one basis by case managers were undertaken. By comparison, our intervention was mild, because only one or a few case workers were available to the subjects for 3 months. However, it is noteworthy that our intervention resulted in significant weight reduction and moreover, improvement was maintained for another 3 months after the end of the first 3-month intervention.

Many previous studies have shown that circulating adiponectin concentrations are inversely correlated with adiposity and BMI.[6–9] Decreased adiponectin was found in cases of visceral fat accumulation, and, conversely, weight reduction promoted adiponectin increases.[6–9] Here, significantly increased serum adiponectin levels were not detected until 6 months after the intervention was started, whereas loss of body weight and adiposity as estimated by BMI, abdominal circumference, percent fat and fat mass were detected at 3 months after the beginning of intervention. These facts might mean adiponectin responds to weight reduction slowly, so early changes in labolatroy data might not be induced by adiponectin.

Recently, the multimeric adiponectin is considered the active form and is better correlated with metabolic parameters. Bobbert *et al.*[7] reported that weight reduction brought increased quantities of high molecular weight isoforms of adiponectin, but total adiponectin showed no change. On the other hand, some studies have shown increased total adiponectin by lifestyle intervention.[6,8] In this study, we measured total adiponectin, so further studies will be needed for the multimeric adiponectin.

When we examined the association between weight reduction percentage and adiponectin levels, adiponectin began to increase in the >6% weight reduction group after 3 months and in the >4% weight reduction groups after 6 months; however, significant adiponectin increases were restricted to the >6% weight reduction groups after 6 months. Thus, greater weight reduction over longer follow-up periods are required to detect increases in circulating adiponectin after an intervention is initiated. Nonetheless, changes in HDL-C were positively correlated with serum adiponectin concentrations, and changes in FPG and adiposity as estimated by BMI, abdominal circumference and fat mass showed an inverse relationship with adiponectin levels. Overall, our observations suggest that adiponectin might not be a highly sensitive marker of improved metabolism at early time points when substantial weight loss is not yet apparent, but rather may be a good marker of metabolic improvement in the late phase based on normalization of adipocytes following significant weight loss. This idea is consistent with the idea that adiponectin is produced from only adipocytes, and its production from enlarged and/or inflammatory adipocytes seen in obesity is significantly decreased.[6–9]

Increased Angptl2 levels owing to visceral fat accumulation cause chronic inflammation and subsequent metabolic disturbance.[20–22] We found that Angptl2 levels tended to decrease immediately in subjects showing 2% or more weight reduction and continued until 3 months after the intervention. Angptl2 gradually decreased with increased

weight reduction, and those decreases in the 6% or more weight reduction group were significant compared with the unchanged control group, indicative of an early effect of weight reduction on improved metabolism. We speculate that differences in changes of circulating levels of Angptl2 and adiponectin after the intervention are due to the types of cells expressing each factor: Angptl2 is produced by adipocytes and other cell types, such as vascular endothelia cells and monocyte/macrophages, while adiponectin expression is restricted to adipocytes. Angptl2 level changes showed a significant positive correlation with changes in adiposity and levels of hs-CRP, TG, AST and ALT and a significant inverse correlation with HDL-C and adiponectin levels. By contrast, adiponectin levels showed a significant positive correlation with changes in HDL-C and a significant inverse correlation with the changes in FPG, adiposity and Angptl2. Interestingly, Angptl2 and adiponectin levels were inversely correlated. It is noteworthy that changes in Angptl2 levels are closely associated with changes in inflammation, TG metabolism and ALT, whereas changes in adiponectin are associated with glucose metabolism.

There are several limitations to this study. We used BIA method to determine body fat percentage. Some studies showed a good relationship between BIA and dual-energy X-ray absorptiometory,[31] whereas others indicate that the BIA method lacked accuracy.[32] Further studies are required to evaluate Angptl2 as an appropriate marker of amelioration of obesity and its-related metabolic disturbances. In particular, it is important to determine whether changes of Angptl2 observed here apply over longer follow-up periods and to a wider population of subjects, such as females, or individuals with severe obesity or with metabolic disease.

In conclusion, we showed that a 3-month lifestyle intervention induced weight reduction and improved glucose and lipid metabolism, changes that continued for 3 months thereafter. Our findings indicate that decreased Angptl2 levels are a good indicator of reduced visceral fat and metabolic improvement at early stages of lifestyle intervention. Thus, Angptl2 reflects adiposity and might be a key protein to regulate inflammation and TG metabolism, whereas adiponectin levels could reflect improved glucose and HDL-C metabolism.

Conflict of interest

The authors declare no conflict of interest.

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Original Article

Does Maintenance of Body Shape Contribute to Health in Middle-Aged Women? -Relationship Between Changes in Body Shape During Over Thirty Years and Laboratory Findings-

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Abstract

Body weight gain or obesity has been reported to increase the risk of developing lifestyle-related diseases. However, few longitudinal studies have examined the relationship between body shape change and health, and the few that do exist used body shapes reported by the subjects.

We investigated whether or not maintaining a "youthful" body shape helps prevent the development of lifestyle-related diseases in healthy women. Changes in body shape in a group of healthy women were evaluated over 30 years starting from sometime in their 20s by visually judging pictures of the subjects and using body shape vectors. Subjects were classified into two groups: a group of 12 (mean age: 44.9±5.1 years) who were judged to have maintained their youthful body shape (maintained group) and a group of 16 (mean age: 47.5±6.1 years) who were judged not to have maintained their shape (non-maintained group). Body size and composition measurements were then compared with biochemical markers that measure the risk of developing lifestyle-related diseases. Mean body weight in the maintained group was 4.0 kg less than in the non-maintained group, while body fat mass was 4.6 kg less. In addition, the maintained group showed better scores for biochemical and pulse wave velocity tests.

Taken together, our results suggest that women who maintain their youthful body shape into their later years have a reduced risk of the lifestyle-related diseases that frequently accompany age.

KEY WORDS: middle-aged women, body shape, aging, lifestyle-related disease, PWV

Introduction

According to the 2008 National Health and Nutrition Survey in Japan, the percentage of obese Japanese women has steadily decreased since 2000 [1], a trend attributed more to women's efforts to maintain their youthful body shape than to prevent disease [2,3], as even those with normal body shapes aspire for further weight loss to counter typical body shape changes that accompany pregnancy, menopause, and aging [4,5].

While several studies have investigated the relationship between weight change, obesity, and visceral fat with age and the risk of developing lifestyle-related diseases [6-9], the effect of maintaining one's youthful body shape (in terms of appearance) on reducing such risks has been given significantly less attention, particularly in healthy women.

We therefore investigated the relationship between body-shape changes evaluated objectively by body shape vectors and risk of developing lifestyle-related diseases in generally healthy middle-aged women over a 30-year period.

Subjects

The Human Science Research Center at the Wacoal Corp. (Kyoto-city, Kyoto) has performed continuous physical measurements on female subjects since 1964, including 101 subjects who have been studied over 30 years (third to sixth decade of life).

The 101 initial subjects were classified into two groups by visually judging their body shape using standardized pictures of the subjects (described further in Methods below) and the body shape vector method proposed by Kurokawa *et al.*[10] Based on these results, the subjects were divided into two groups: one consisting of 25 subjects who had maintained their youthful body shapes (maintained group) and 76 who did not (non-maintained group). Subjects in both groups were then asked to undergo physical measurements and laboratory tests to assess risk of developing lifestyle-related diseases on scheduled dates. Twenty-eight subjects ultimately agreed (mean age: 46.4±5.7 years): 12 from the maintained group (mean age: 44.9±5.1 years) and 16 from the non-maintained group (mean age: 47.5±6.1 years) (***Fig. 1***).

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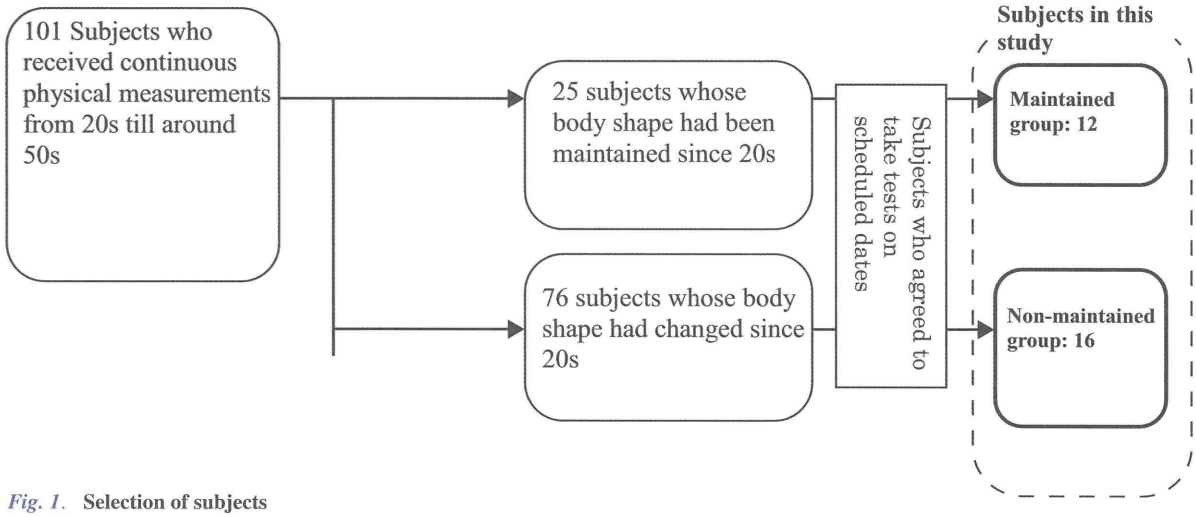

Fig. 1. **Selection of subjects**

Methods

Evaluating and classifying body shape

Visual judgment of body shape was performed using standardized pictures of the subjects in their undergarments taken from their front, diagonal, side, and back in the standing position, with their ears and eyes on the same horizontal line as determined by Martin's anthropometry. Classification of body shape as maintained or non-maintained was based on measuring whether or not the bust was sagging, the waist had vanished, the hips were flattened or sagging, the entire abdomen protruded, and a backward tilt and abdominal protrusion were evident. Body shape evaluations were made by 5 researchers who each evaluate 500-1000 people annually and were capable of performing Martin's anthropometry with a measurement error of less than 5 mm.

The front and side view body shape vectors for the earliest and latest age recorded for each subject were obtained via Kurokawa's method [10]. To obtain body shape vectors, the vertical or y-axis was set at the jugular notch of the superior margin of the sternum for both the front and side views, while the horizontal or x-axis was set at the jugular notch of the superior margin of the sternum for the front view and at the midaxillary middle point and the inferior margin of the pubic symphysis for the side view. Differences in the vector elemental values between the two ages corresponding to the five aforementioned criteria were calculated by the methods described in ***Table 1*** and ***Fig. 2***. The rank sum of the five items was obtained to which the subjects were ranked in ascending order. Using this order and the results from the pictures, the subjects were divided into two groups.

Measuring body size and body composition

The abdominal circumference of each subject was measured at the umbilical level in the standing position with light exhalation based on the abdominal circumference measurement method for metabolic syndrome diagnostic criteria described in the Standardized Program for Health Checkups and Health Consultation Program (final version) [11]. Body fat percentage, muscle mass of the trunk and limbs, and body fat mass were measured via dual-energy X-ray absorptiometry (DXA) using QDR-1500A (HOLOGIC, Inc., Bedford, MA, USA). Bone mineral density (BMD) was measured at lumbar vertebrae 2-4 via DXA and compared with the young-adult-matched (YAM) value and the average value of the same age group. In addition, bone mineral densities of the entire body as well as 10 separate body parts (left and right arms, left and right ribs, thoracic and lumbar vertebrae, pelvis, left and right legs, head) were evaluated via DXA total body scan.

Clinical laboratory tests

Biochemical analyses were conducted to quantify lifestyle-related disease markers such as total cholesterol (TC), triglyceride (TG), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), fasting plasma glucose (FPG), uric acid (UA), hemoglobin A1c (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γGTP). Blood pressure and pulse wave velocity (PWV) were measured using a BP-203RPEII (Omron Colin, Bunkyo-ku, Tokyo). For PWV, the brachial-ankle PWV (baPWV) and ankle-brachial index (ABI) were studied.

Table 1 ***Body shape evaluation criteria by macroscopic observation of pictures and body shape vectors***

	Visual judgment evaluation criteria	Body shape vector measurements (See Fig. 2 for body shape vectors)
Bust	Sagging	Jugular notch of the sternum – nipple height
Abdomen	Protruding	Side view: coordinates (1), (4), (7), and (11)
Waist	Vanished	Front view: coordinates (5) and (13)
Hips	Flattened and/or sagging	• The most protruding point of the hip curve – deepest point of the back curve. • Hip height
Posture	Backwards tilt and protruding abdomen	Side view: (tilt) most protruding point of the back curve – most protruding point of the hip curve; (protrusion) most protruding point of the abdomen – jugular notch of the superior margin of the sternum

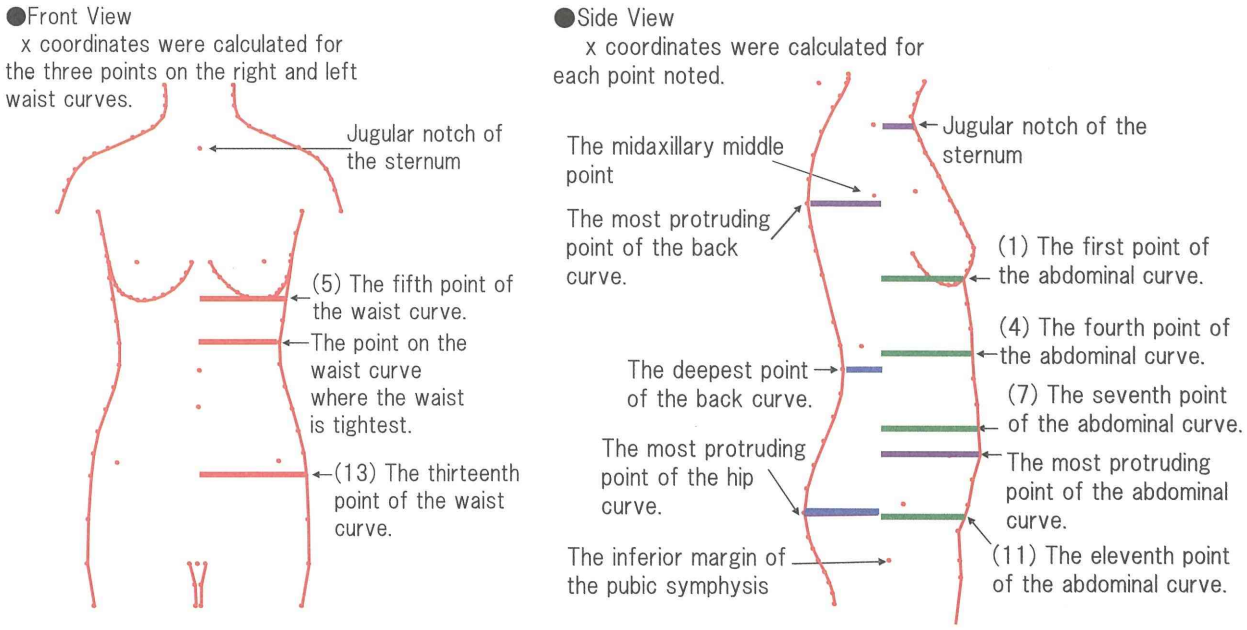

Fig. 2. **Equence of points represented by the body shape vector of Kurokawa *et al.* and the points used for the evaluation criteria.**

Lifestyle assessment

We asked subjects to fill in a questionnaire about daily exercise and dietary habit. We assessed the amount of energy intake and the ratio of fat intake by using a semi-quantitative food frequency questionnaire.

Ethical considerations

The Human Science Research Center at Wacoal Corp. has been recruiting subjects since 1964 by advertising in popular media. At enrollment, subjects were informed both verbally and in writing of the purpose and disclosure of the measured data, the method of measurements, details of the monitors' activities, management of the collected data, and the extent of publication and confidentiality. Written consent was obtained from all participating subjects. Procedures for enrollment of monitors, physical measurements, regulations regarding publication of pictures, and all other regulatory matters were approved by the Ethics Review Committee at Wacoal Corp.

Subjects who volunteered to receive laboratory tests associated with lifestyle-related diseases were informed verbally and in writing of the purpose of using their laboratory data and the details of the examinations. Subjects were further informed that the use of laboratory data in the study depended on their discretion and that no benefits would be lost in the case of refusal, privacy would always be protected, and the laboratory data would be used only for the purpose of this study. Again, written consent was given by all participants. The use of laboratory data was approved by the Ethics Review Committee of Comprehensive Health Science Center, Aichi Health Promotion Foundation.

Statistical analyses

All data are presented as mean ± standard deviation. The Mann-Whitney test was used for between-group data comparisons. Statistical analyses were performed with SPSS14.0 J for Windows. The level of significance was less than 5%.

Results

Physical size and body composition data

Physical size and body composition at baseline and present are given in ***Table 2***. There was no significant difference in both body mass index (BMI) and body weight between groups at baseline.

In the maintained group, no significant BMI and body weight change was observed, whereas BMI changed significantly in the non-maintained group from 19.4 ± 1.3 kg/m^2 to 21.8 ± 2.4 kg/m^2 and body weight gain was 5.9±6.7 kg.

Mean BMI at present and body fat mass as measured by DXA were found to be significantly lower in the maintained group than in the non-maintained group. BMI categories as defined by the Japan Society for the Study of Obesity [12] showed that most subjects had normal weight in both groups (83.3% in the maintained group and 81.3% in the non-maintained group).

Regarding body fat percentages, 11 subjects (91.7%) had values within normal range in the maintained group, while in the non-maintained group, 6 subjects (31.3%) had values within normal range. With regards to fat mass by body part, consistent with the overall body fat percentage, these values were significantly lower in both the arms and trunk of the maintained group (***Table 3***).

When compared with YAM values, the mean BMD in the maintained and non-maintained groups were 94.5%±10.3% and 96.0%±10.6%, respectively. No statistically significant differences in BMD were noted between groups or by body part. Upon excluding post-menopausal subjects from analysis, however, BMD in the pelvis was found to be significantly lower in the maintained group (***Table 4***).

Values associated with lifestyle-related diseases

While the mean values for all biochemical results were within the normal range for both groups, the values for TC and TG were significantly higher in the non-maintained group than in the maintained group (***Table 5***).

Respective mean values of baPWV, which indicates the

Table 2 Physical size and body composition at approximately 50 years old

	Maintained group (n=12)		Non-maintained group (n=16)	
	Baseline	present	Baseline	present
Height (cm)	159.7 ± 3.1	159.8 ± 2.9	157.1 ± 5.8	157.1 ± 6.0
Body weight (kg)	50.9 ± 4.1	49.8 ± 3.2	48.0 ± 4.8	53.9 ± 6.8 **
BMI (kg/m^2)	20.0 ± 1.5	19.5 ± 1.4	19.4 ± 1.3	21.8 ± 2.4 ** ††
Abdominal circumference (cm)	—	69.4 ± 4.2	—	78.7 ± 7.1 †††
Body fat percentage (%)	—	24.6 ± 4.4	—	31.1 ± 3.6 †††
Bone mineral mass (kg)	—	2.0 ± 0.2	—	1.9 ± 0.3
Muscle mass (kg)	—	35.9 ± 2.8	—	35.4 ± 4.0
Fat mass (kg)	—	12.4 ± 2.5	—	17.0 ± 3.5 ††

BMI, body mass index, —, data was not obtained.
Values presented as mean ± standard deviation
Non-parametric test (Wilcoxon signed-rank test) $^{**}p<0.01$ compared with baseline(20-29y) within the same group
Non-parametric test (Mann-Whitney test) $^{††}p<0.01$, $^{†††}p<0.001$ between groups at present

Table 3 Fat mass (g) by body part at approximately 50 years old

	Maintained group (n=12)	Non-maintained group (n=16)
Left arm	675.4 ± 190.4	968.5 ± 213.8 **
Right arm	646.7 ± 193.1	928.0 ± 181.4 **
Body trunk	4891.6 ± 1370.2	7803.9 ± 1913.9 ***
Left leg	2670.4 ± 596.3	3193.7 ± 942.1
Right leg	2625.0 ± 573.0	3238.9 ± 951.2
Head	884.2 ± 63.8	882.6 ± 59.0

Values presented as mean ± standard deviation
Non-parametric test (Mann-Whitney test)
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

Table 4 Bone mineral density (g/cm^2) by body part at approximately 50 years old (excluding post-menopause subjects)

Body part	Maintenance group (n=11)	Non-maintenance group (n=12)
Left arm	0.676 ± 0.047	0.675 ± 0.043
Right arm	0.696 ± 0.044	0.698 ± 0.055
Left ribs	0.599 ± 0.068	0.595 ± 0.064
Right ribs	0.590 ± 0.056	0.593 ± 0.074
Thoracic vertebrae	0.831 ± 0.114	0.841 ± 0.095
Lumbar vertebrae	0.986 ± 0.122	1.050 ± 0.113
Pelvis	1.059 ± 0.103	1.172 ± 0.011 *
Left leg	1.096 ± 0.105	1.077 ± 0.083
Right leg	1.089 ± 0.101	1.082 ± 0.091
Head	2.240 ± 0.314	2.380 ± 0.241

Values presented as mean ± standard deviation
Non-parametric test (Mann-Whitney test)
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

Table 5 Laboratory values associated with lifestyle-related diseases

	Maintenance group (n=12)	Non-maintenance group (n=16)
SBP (mmHg)	107.4 ± 13.4	110.7 ± 12.4
DBP (mmHg)	60.9 ± 10.0	66.7 ± 9.0
TC (mg/dl)	186.3 ± 30.2	208.4 ± 26.1 *
TG (mg/dl)	54.9 ± 15.4	92.7 ± 45.3 **
HDL-C (mg/dl)	76.8 ± 10.3	75.0 ± 13.9
LDL-C (mg/dl)	101.9 ± 32.3	119.8 ± 23.0
FPG (mg/dl)	89.7 ± 6.3	93.4 ± 7.4
HbA1c (%)	4.8 ± 0.2	4.8 ± 0.3
Uric acid (mg/dl)	3.8 ± 0.7	4.3 ± 1.0
AST (IU/l)	18.7 ± 2.8	18.3 ± 6.7
ALT (IU /l)	16.2 ± 5.5	17.4 ± 16.6
γ-GTP (IU/ l)	13.6 ± 2.9	19.9 ± 15.2
Hemoglobin (g/dl)	12.6 ± 0.8	12.6 ± 1.4
Albumin (g/dl)	4.4 ± 0.2	4.4 ± 0.3

SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; UA, uric acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGTP, γ-glutamyl transpeptidase
Values presented as mean ± standard deviation
Non-parametric test (Mann-Whitney test)
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

degree of atherosclerosis from the upper limbs to the foot joints, including the heart, were 1113.8±79.3 and 1106.6±86.4 cm/sec for the left and right sides in the maintained group and 1255.9±147.9 and 1243.4±114.2 cm/sec for the left and right sides in the non-maintained group. The differences in each side between groups were significant (*Fig. 3*).

Respective mean values of ABI, which indicates arterial stenosis/occlusion in the lower limbs, was 1.19±0.07 and 1.20±0.07 for the left and right sides, respectively, of the maintained group and 1.12±0.07 and 1.14±0.09 for the left and right sides, respectively, of the non-maintained group. The differences in each side between groups were also significant (*Fig. 4*).

Mean blood vessel age, estimated from the subject's age and baPWV using the method described by Yamashina *et al.*[13], was determined to be 44.9±5.1 years in the maintained group and 50.3±6.5 years in the non-maintained group. In addition to being a significant difference, the blood vessel age in the non-maintained group exceeded the actual age of the subjects.

The result of lifestyle assessment

Subjects who take regular exercise were 33.3% in the maintained group and 12.5% in the non-maintained group. Energy intake was 2070.1±336.9 kcal/day and 2037.3±293.8 kcal/day, and the ratio of fat intake was 34.1±4.1% and 33.3±5.3% respectively. There was no significant difference between groups in energy and fat intake.

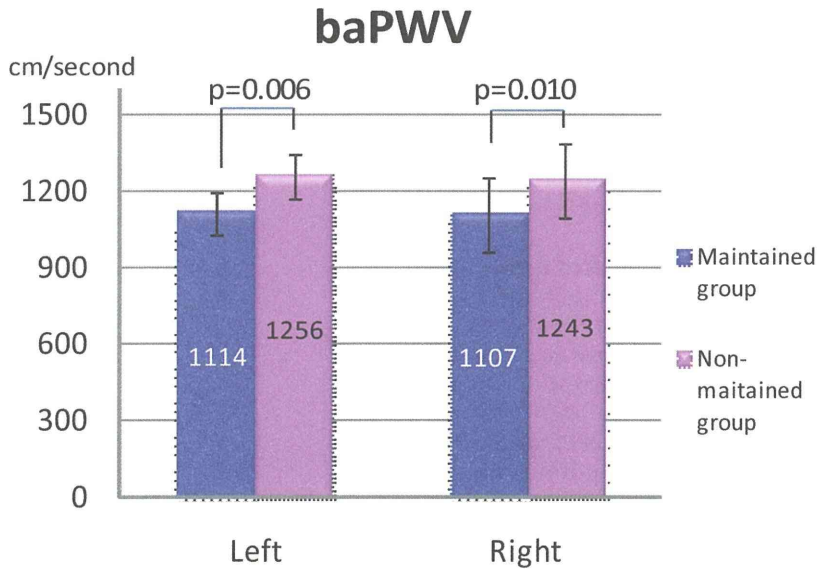

Fig. 3. **Pulse wave velocity: brachial-ankle PWV (baPWV)**

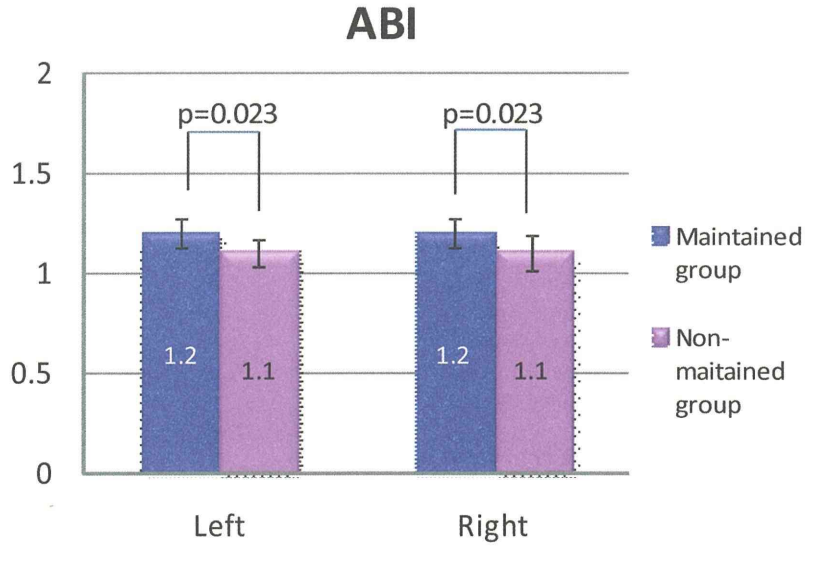

Fig. 4. **Pulse wave velocity: ankle-brachila index (ABI)**