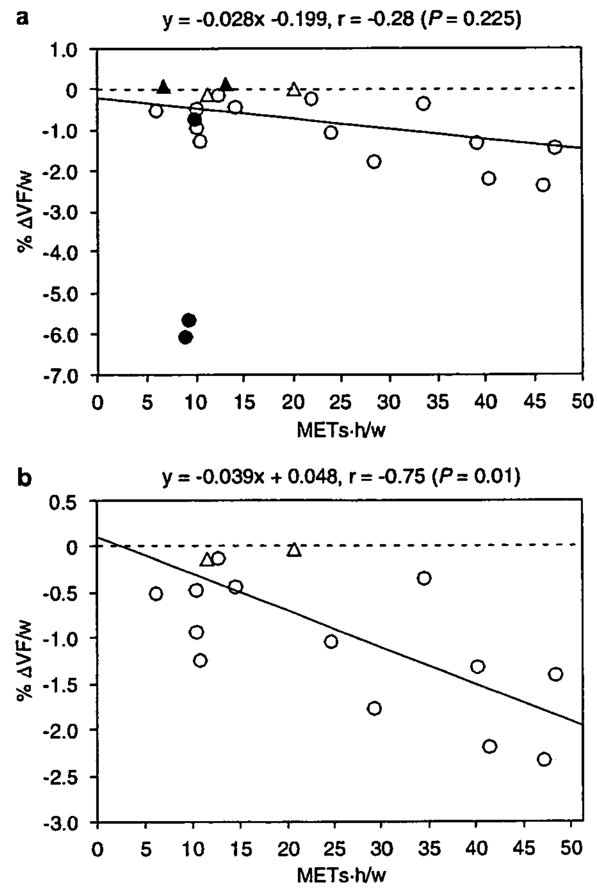

Figure 1 Relations between METs · h/w and %ΔVF/w during interventions in the all selected groups (a) and the groups without metabolic-related disorder subjects (b). Abbreviations: METs · h/w, ∑(metabolic equivalents × hour) per week; %ΔVF/w, percentage of visceral fat change per week; *r*, Pearson's correlation coefficient weighted for the number of subjects in each group; ○, the no metabolic-related disorder group with a significant visceral fat reduction ($P<0.05$); △, the no metabolic-related disorder group without a significant visceral fat reduction ($P<0.05$); ●, the metabolic-related disorder group with a significant visceral fat reduction ($P<0.05$); ▲, the metabolic-related disorder group without a significant visceral fat reduction ($P<0.05$).

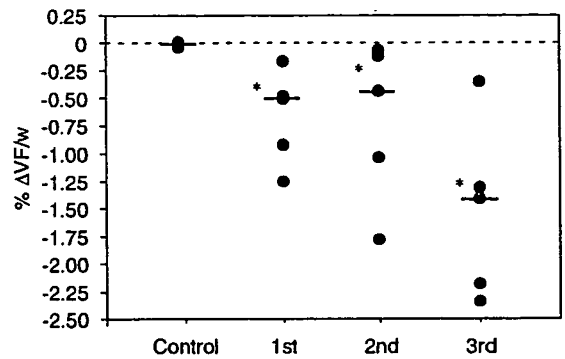

Figure 2 Comparison of mean %ΔVF/w between a control group and exercise groups divided into tertiles by METs · h/w amount in the groups without metabolic-related disorder groups. Ranges of METs · h/w in each categorized group were 5.9–11.4 (1st), 12.3–28.5 (2nd), 33.4–47.1 (3rd). Side bar means median in each group. Statistically significant difference between the groups were observed ($P=0.003$). * A significant difference was found in comparison with the control group using the *post hoc* test ($P<0.05$). Abbreviations: %ΔVF/w, percentage of visceral fat change per week; METs · h/w, ∑(metabolic equivalents × hour) per week.

physical activity and total or regional fat reduction. As a result, even though some literatures were added for the analysis, whether physical activity was associated with reductions in abdominal fat in a dose–response manner was still unclear. Kay and Fiatarone Singh[10] also reviewed the beneficial influence of physical activity on visceral fat reduction, but dose–response data were not examined. These previous reviews did not include studies involving large amounts of exercise. In our analysis, some additional studies, especially three studies with values of 35 METs · h/w or more,[21,22,29] were included in addition to papers used by previously published reviews. Furthermore, the amount of aerobic exercise undertaken during the intervention was expressed as METs · h/w, because METs · h could adjust the EE for each subject's weight. As a result, there was no relationship between METs · h/w and %ΔVF in the 21 groups from 16 studies including the metabolic-related disorder subjects. However, in subjects without metabolic-related disorders, we found a dose–response relationship between aerobic exercise and visceral fat reduction. Indeed, if obese subjects without metabolic-related disorders practiced aerobic exercise, the degree of visceral fat reduction could be directly attributed to the aerobic exercise amount. For example, if an obese person without metabolic-related disorders tries to reduce 10% of his VF amount in 10 weeks, instructors should prescribe about 27 METs · h/w, because 27 METs · h/w corresponds to 1% of ΔVF/w. Thus, our findings could be used to affect decisions on the amount of aerobic exercise recommended for visceral fat reduction in obese people.

In the selected studies, six groups from four studies consisted of metabolic-related disorder subjects. Results from the metabolic-related disorder subjects were contradictory. Two groups with type 2 diabetes[9,38] clearly exhibited a significant visceral fat reduction, although these results may have been exaggerated by the shortest-term intervention (8 weeks) in the selected studies. Two groups with dyslipidemia[24] did not significantly reduce visceral fat, while the group with type 2 diabetes reported by Giannopoulou *et al.*[16] was close to the regression line for identifying a dose–response relationship. Kelly and Simoneau[39] showed that the capacity of fat oxidation during aerobic exercise in individuals with type 2 diabetes was lower than that for healthy individuals. However, several other investigators did not find any significant difference in fat oxidation capacity between subjects with or without type 2 diabetes.[40,41] Furthermore, Raguso *et al.*[42] observed that fat oxidation during aerobic exercise in the group with type 1 diabetes was higher than that of the control group. These studies were conducted

Table 3 Mean METs · h/w and %ΔVF/w, and correlate coefficients between METs · h/w and %ΔVF/w during interventions in the groups categorized by intervention duration or gender

Groups	Intervention duration		Gender	
	≤16 week	>16 week	Women only	Men only
From all the selected groups				
Number of groups	10	11	7	6
Number of subjects	183	399	168	98
METs · h/w	23.5±17.1	17.1±9.1	23.1±13.0	27.6±17.7
%ΔVF/week	−2.22±2.00	−0.41±0.55	−0.90±0.86	−1.83±1.98
r (P value)	−0.06 (0.877)	−0.34 (0.302)	−0.89 (0.007)	−0.05 (0.931)
From the groups without metabolic-related disordered subjects				
Number of groups	7	8	6	5
Number of subjects	154	271	157	90
METs · h/w	29.5±17.2	18.2±9.8	25.3±12.7	31.3±17.1
%ΔVF/w	−1.40±0.67	−0.55±0.58	−0.93±0.94	−1.07±0.73
r (P value)	−0.81 (0.027)	−0.36 (0.378)	−0.93 (0.008)	−0.71 (0.184)

Abbreviations: METs · h/w, ∑(metabolic equivalents × hour) per week; r, Pearson's correlate coefficient; %ΔVF/w, percentage of visceral fat change per week. r values were weighted for the number of subjects in each group.

under conditions where the subjects with or without diabetes had fasted.[39–42] Thus, visceral fat reduction in the metabolic-related disorder subjects could be due to more complex mechanisms. Therefore, formulation of a dose–response relationship between aerobic exercise and visceral fat reduction has to take into account the separation of subjects with and without metabolic-related disorders.

How much exercise is needed for significant visceral fat reduction?

It is important to suggest a lower limit for the quantity of aerobic exercise required for significant visceral fat reduction. In our selected groups, METs · h/w values ranged from 5.9 to 47.1. Except for the lowest METs · h/w obtained from Miyatake *et al.*[20] in which the subjects were instructed to increase the number of steps walked every day for 1 year, significant visceral fat reduction was observed from about 10 METs · h/w.[16,18,25] Thus, at least 10 METs · h/w is required for significant visceral fat reduction by aerobic exercise, such as brisk walking, light jogging or stationary ergometer usage. For the purpose of weight or body fat loss, the American College of Sports Medicine (ACSM) recommends obese individuals to engage in moderately intense physical activity for minimum 150 min/w, and preferably more than 200–300 min/w.[43] The minimum value in this recommendation nearly equals to 10 METs · h/w when performing moderate physical activities such as brisk walking. In the present study, we divided the aerobic exercise groups into tertiles by their METs · h/w amount to determine the boundary of obvious visceral fat reduction. As a result, each exercise group category had a higher visceral fat reduction than the control group. However, there was no significant difference between %ΔVF/w values in the three exercise categories. This result may be due to an insufficient number of groups. The median of %ΔVF/w in the 3rd tertile exercise group was 40.2, which was much higher than that of the 1st and 2nd tertile exercise groups. That is to say, approximately 40 METs · h/w or more may be required to reduce visceral fat solely by aerobic exercise, such as brisk walking, light jogging or stationary ergometer usage. Forty METs · h/w equates to approximately 3780 kcal/w for a person with 90 kg body weight. Although this value is slightly lower than the ACSM's recommendation corresponding to a minimum 4500 kcal/w for combined exercise and diet with intakes of not lower than 1200 kcal/d, this results in an energy deficiency of approximately 500–1000 kcal/d, which could be hard for obese people with low physical fitness to practice continuously. Therefore, for an individual's prescription for visceral fat reduction, recommendations that balance diet and exercise should be examined in another research.

Influences of intervention duration or gender on the dose–response relationship

Ross and Janssen[13] revealed that an increase in physical activity is positively associated with a reduction in total fat in a dose–response manner in short-term interventions (≤16 week), but not in long-term interventions (≤26 week). In the review by Kay and Fiatarone Singh,[10] there was no relation between change in abdominal fat and intervention duration. In the present study, EE by aerobic exercise was positively correlated with visceral fat reduction in the short-term (≤16 wk) studies when the metabolic-related disorder groups were discounted. Ross and Janssen[13] suggested that in long-term exercise studies, it is difficult to complete a weight loss of an expected volume from expended energy consumption, although it is not clear which factors, such as the adherence to interventions, or over-reporting of exercise amount, influenced the results. Our results support this trend with

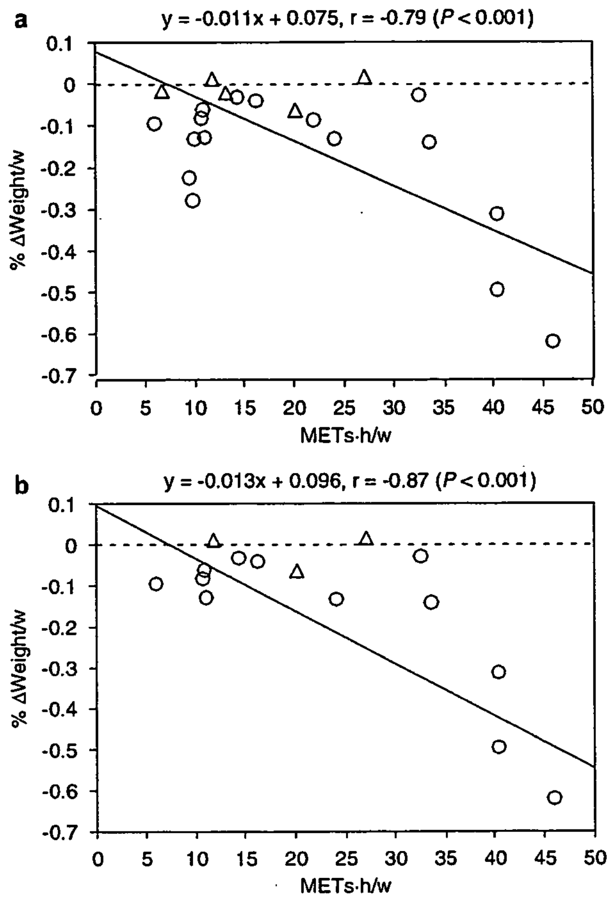

Figure 3 Relations between METs·h/w and %ΔWeight/w during interventions in the all selected groups (a) and after excluding the groups with metabolic-related disorder subjects (b). Abbreviations: METs·h/w, ∑(metabolic equivalents × hour) per week; %ΔWeight/w, percentage of weight change per week; *r*, Pearson's correlate coefficient; ○, the group with a significant visceral fat reduction ($P<0.05$); Δ, the group without a significant visceral fat reduction ($P<0.05$). The groups without a weight loss intentionally were excluded for these analysis.

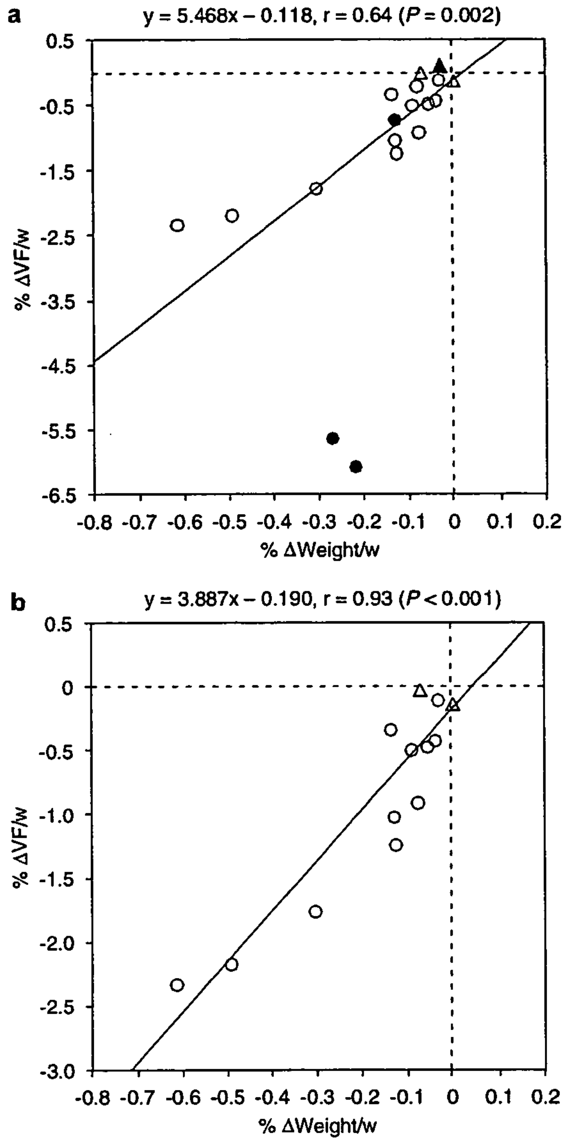

Figure 4 Relations between %ΔVF/w and %ΔWeight/w during interventions in the all selected groups (a) and after excluding the groups with metabolic-related disorder subjects (b). Abbreviations: %ΔVF/w, percentage of visceral fat change per week; %ΔWeight/w, percentage of weight change per week; *r*, Pearson's correlation coefficient weighted for the number of subjects in each group; ○, the no metabolic-related disorder group with a significant visceral fat reduction ($P<0.05$); Δ, the no metabolic-related disorder group without a significant visceral fat reduction ($P<0.05$); ●, the metabolic-related disorder group with a significant visceral fat reduction ($P<0.05$); ▲, the metabolic-related disorder group without a significant visceral fat reduction ($P<0.05$).

respect to visceral fat reduction. That is, if subjects can complete the instructed exercise volume, short-term interventions could be more efficient than long-term interventions for weekly visceral fat reduction. Generally, if participants do not quickly observe the benefits of a weight-loss program, their motivation for continuing the regimen is reduced.[44,45] Accordingly, for significant visceral fat reduction, obese people should initially practice a relatively high volume of aerobic exercise, which can then be reduced to a manageable amount that they can practice for the long term.

In the present study, a significant relationship between METs·h/w and %ΔVF was observed in women-only groups, with and without the metabolic-related disorder subjects, while there was no significant relationship in the men-only groups. The limited number of studies was insufficient to determine the influence of gender on the dose–response relationship. However, it is difficult to compare differences of the amount of visceral fat reduction by aerobic exercise between men and women, as women generally store a greater total fat mass relative to body weight than men.[46] Also, body fat distribution is different between men and women as men tend to have more central obesity than women.[47] Initial values of visceral fat could contribute to the amount of visceral fat lost during intervention. If these biases between men and women were excluded, that is, if the absolute amount of total and visceral fat were matched between men and women, then the relative obesity levels for each gender would be much different. It is likely that gender, as well as intervention duration, could be factors in the differences in rate of visceral fat reduction per week.

Relationship between visceral fat reduction and weight reduction
Weight reduction during interventions could be seen solely as the result of fat mass reduction, because fat-free mass reduction accounts for only a small part of weight reduction.[38] Visceral fat volume is about 10–20% of total fat volume[48,49] and reduction of the subcutaneous fat volume largely reflects weight reduction. In a limited number of selected studies, METs·h/w and %ΔWeight/w had a significant correlation in both the groups with and without metabolic-related disorders. Therefore, metabolic-related disorders, especially type 2 diabetes, may have a small impact on a dose-relation between weight loss and aerobic exercise during intervention compared to the amount of visceral fat reduction.

On the other hand, our results indicate a significant relationship between %ΔWeight/w and %ΔVF/w, especially in the subjects without metabolic-related disorders. We can say that %ΔVF/w corresponds to four to five times %ΔWeight/w when obese people practice aerobic exercise. However, previous studies suggest that visceral fat is used more quickly as an energy resource than subcutaneous fat during aerobic exercise-induced weight loss.[50] In our analysis, the intercept of the regression line between %ΔWeight/w and %ΔVF/w in the subjects without metabolic-related disorders was significantly different from zero. Although the trend showed that the more weight was lost, the more visceral fat was reduced, a significant reduction of visceral fat, which occupies less than 5% of body weight,[48,49] may also occur without a significant weight reduction with aerobic exercise. In fact, this phenomenon was reported by studies that examined whether or not visceral fat was reduced by aerobic exercise, if energy intake corresponding to the EE value by prescribed aerobic exercise was added to the baseline. Such an adjustment in the calculation did not lead to a significant weight reduction.[22,29] Generally, it is difficult for obese people to reduce weight largely by practicing exercise alone, compared to diet.[8] Therefore, exercise is inclined to be optional with a diet therapy for weight loss. However, even if insufficient weight loss does occurs, visceral fat could be reduced by doing aerobic exercise, a prescription supported by recent studies.[16,22] These results provide evidence of the usefulness of aerobic exercise for visceral fat reduction.

There are a number of limitations in the present study. The number of selected studies, especially those which measured EE for the prescribed exercises, were still insufficient for defining a clear aerobic exercise amount that resulted in significant visceral fat reduction. Additionally, the influence of several factors, such as metabolic-related disorders, gender and intervention duration, on visceral fat reduction remains unclear. Most of trials in the selected studies had applied brisk walking, light jogging and stationary ergometer, so whether or not other types of activities could lead to a similar result cannot be clarified from this study. Furthermore, while the present study investigated visceral fat reduction, studies with visceral fat gain should also be included in the analyses.

In conclusion, data collected from selected studies suggested that aerobic exercise as a weight loss intervention has a dose–response relationship with visceral fat reduction in obese subjects, excluding groups with metabolic-related disorders. Additionally, visceral fat reduction is significantly related to weight reduction during aerobic exercise intervention, although a significant visceral fat reduction may also occur without significant weight loss. Furthermore, for significant visceral fat reduction, at least 10 METs·h/w of aerobic exercise is required. However, since the number of selected studies was still insufficient, further studies are required.

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Relationship between Blood Adipocytokines and Resting Energy Expenditure in Young and Elderly Women

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Summary It has been demonstrated in a previous study that resting energy expenditure (REE) is associated with adiponectin levels in the blood. However, body composition was not taken into consideration in that study. The purpose of the present study was to again investigate the relationship between blood adipocytokines and REE, adjusted by body composition, in both young and elderly women. REE and blood adipocytokines were measured in 115 young (age: 22.3±2.1 y, BMI: 21.3±1.9 kg/m^2) and 71 elderly (63.4±6.5 y, 22.9±2.3 kg/m^2) women. Dual energy X-ray absorptiometry was used to measure percent body fat. Fat mass and fat free mass (FFM) were calculated. REE (kcal/d and kcal/kg BW/d) was lower in elderly women than in young women, but no significant difference was observed in REE, expressed as kcal/kg FFM/d, between the two groups. Although elderly women had a higher percent body fat and higher serum leptin concentrations than young women, plasma adiponectin concentrations did not differ between young and elderly women. In elderly women, REE (kcal/d) was significantly and inversely correlated with plasma adiponectin concentration ($r=-0.386$, $p<0.001$), but REE expressed per kilogram of BW or FFM was not significantly correlated. Furthermore, no significant correlation was observed between REE (kcal/d) and concentrations of plasma adiponectin or serum leptin, after adjusting for potential confounders such as body composition and hormones, in either age group. These results suggest that adipocytokines do not influence REE in adult women.
Key Words resting energy expenditure, adiponectin, leptin, age, female adults

Resting energy expenditure (REE) accounts for 60 to 80% of total daily energy expenditure and is the basis for estimating energy requirements. In the field of energy metabolism, early investigators showed intense interest in establishing the factors contributing to REE (*1–3*).

Recent progress has shown that adipocytokines, which are bioactive substances secreted from adipocytes (*4, 5*), play an important role in regulation of basal metabolic rate (*6*). In particular, much attention is paid to adiponectin, which is suggested to play a role in improving insulin resistance and protecting against arteriosclerosis (*7–9*). In addition, rodent studies suggest that energy expenditure is regulated by adiponectin (*6, 10–13*). Further, Ruige et al. (*14*) recently demonstrated that a low resting metabolic rate (RMR; kcal/d) is strongly and inversely associated with high adiponectin levels in overweight or obese humans. Together these studies suggest that there is a relationship between adiponectin and REE.

It is well known that body mass, especially fat free mass (FFM), is a useful parameter for estimating REE (*15–17*). Fat mass (FM) is also an important predictor of REE in elderly people even though it is low-metabolic-rate tissue (*17–21*). However, in a previous study conducted to investigate the relationship between adiponectin and REE, body composition (FFM and FM) was not taken into consideration (*14*). It is therefore necessary to assess the effect of adiponectin on REE after adjusting for the confounding influence of body composition.

Therefore, we re-evaluated the relationship between blood adipocytokines and REE adjusted by body compo-

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sition in young and elderly women. We did not find any significant relationship between plasma adiponectin concentration and REE adjusted by body composition in either age group.

MATERIALS AND METHODS

Subjects. One hundred and fifteen young women (22.3±2.1 y) and 71 elderly women (63.4±6.5 y), who were at least 3 y (13.5±7.5 y) post-menopause, were recruited for the study. Women who had BMI (kg/m^2) which fell outside the range BMI<18.5, BMI>30 were excluded. There were 4 young and 17 elderly subjects who were overweight (25≤BMI<30). Included participants were not using any medications, including estrogen-replacement drugs. All subjects were informed of the purpose and possible risks of the study and then provided written informed consent, as approved by the Ethical Committee at the National Institute of Health and Nutrition in Japan.

Study protocol and indirect measurement of REE. Participants came to the National Institute of Health and Nutrition in the early morning. They were asked to minimize any walking prior to their laboratory visit for the REE measurement. REE was measured directly by open-circuit indirect calorimetry. Measurements were performed between 0700 and 0900 h in a room at constant temperature (23–25°C). After entering the laboratory, subjects rested in the supine position for at least 30 min, and wore a Hans-Rudolph full face mask (Hans Rudolph Inc., Kansas City, MO, USA). Two samples of expired air were collected in Douglas bags over each of two 10 min periods, and the mean of the two values was used for analysis. For young subjects, all measurements were made during the follicular phase of the menstrual cycle.

An oxygen and carbon dioxide analyzer (Arco-1000A, Arco System, Japan) was used to analyze the rate of oxygen consumption and carbon dioxide production. The volume of expired air was determined using a dry gas volume meter (DC-5, Shinagawa, Japan) and converted to standard temperature, pressure and dry gas (STPD). Gas exchange results were converted to REE (kcal/d) using Weir's equation (22).

Body composition analysis. Body weight (BW) was measured to the nearest 0.1 kg using an electronic scale (Inner Scan BC-600, TANITA Co., Japan), and height (Ht) was measured to the nearest 0.1 cm using a stadiometer (YL-65, YAGAMI Inc., Japan). Body mass index (BMI) was calculated by dividing BW in kilograms by the Ht in meters squared (kg/m^2). The percentage of whole body fat (% body fat) was measured using dual energy X-ray absorptiometry (Hologic QDR-4500 DXA Scanner, Hologic Inc., Waltham, MA, USA). Manufacturer's software version 11.2 for Windows was used to analyze the % body fat. FFM and FM were calculated by BW and % body fat.

Blood samples. Venous blood samples (fasting for at least 12 h) were collected for measurements of serum glucose, glycosylated hemoglobin (HbA_{1c}), total cholesterol, HDL-cholesterol, triglycerides, estradiol (E_2), total triiodothyronine (T_3), leptin, and plasma adiponectin. Serum and plasma samples were stored at −80°C for subsequent analysis. All blood parameters were analyzed by SRL, Inc. (Tokyo, Japan) and Mitsubishi Chemical Medience Corporation (Tokyo, Japan).

Statistical analysis. The data were presented as mean±standard deviation (SD). Statistical analyses were carried out with the Sigma Stat 2.03 (Systat Software Inc., California, USA). Statistical analysis was performed using the Student's *t*-test for parametric variables and the Mann-Whitney rank sum test for non-parametric variables to determine differences between young and elderly women. To determine the associations between REE and adipocytokines, partial correlation coefficients were used after adjusting for the potential confounding influence of FM (kg), FFM (kg), E_2 (pg/mL), and T_3 (ng/dL). For all the statistical analyses, the level of significance was defined as a p value of less than 0.05.

RESULTS

Table 1 presents comparisons of characteristics, whole body composition, and blood biochemical profiles. Ht was significantly lower in the elderly women than in young women. However, no significant difference in BW was noted between the two groups. The elderly women had significantly higher levels of % body fat and FM, and lower levels of FFM than the young group.

REE (kcal/d and kcal/kg BW/d) in the elderly group was significantly lower than in the young group (Table 2). When REE is expressed in terms of kcal/kg FFM/d, however, no significant difference in REE was found between the two groups. No significant difference in plasma adiponectin concentration was noted between the two groups, whereas serum leptin concentration was significantly higher in elderly women than in young women.

There was no significant relationship observed between REE (kcal/d, kcal/kg BW/d and kcal/kg FFM/d) and plasma adiponectin concentration in young women (Fig. 1, a-1–a-3). In contrast, in elderly women, REE (kcal/d) was significantly and inversely correlated with plasma adiponectin concentration ($r=-0.386$, $p<0.001$, Fig. 1, b-1), but REE expressed per kilogram BW and FFM were not significantly correlated (Fig. 1, b-2, b-3). Significant and inverse relationships between REE adjusted by BW and concentrations of serum leptin were observed in the two groups (young: $r=-0.318$, elderly: $r=-0.426$, $p<0.001$, respectively, Fig. 2, a-2, b-2). When REE was expressed relative to FFM, however, no significant relationships were obtained for either group (Fig. 2, a-3, b-3).

A partial correlation coefficient calculated after adjusting for the confounding influence of FM (kg), FFM (kg), E_2 (pg/mL), and T_3 (ng/dL) did not demonstrate any significant relationship between REE and concentrations of plasma adiponectin and serum leptin (Table 3).

Table 1. Physical and biochemical characteristics in young and elderly women.

	Young (n=115)		Elderly (n=71)	
Age (y)	22.3±2.1	(19.1–29.5)	63.4±6.5†	(50.2–77.0)
Ht (cm)	161.3±6.7	(142.2–181.0)	153.8±5.2*	(141.3–164.7)
BW (kg)	55.4±6.5	(41.7–73.7)	54.2±6.0	(41.4–72.2)
BMI (kg/m^2)	21.3±1.9	(18.5–26.6)	22.9±2.3*	(19.4–28.9)
% body fat	24.0±4.4	(14.3–35.7)	30.2±4.8*	(18.6–38.9)
FM (kg)	13.3±3.0	(7.5–24.8)	16.5±4.0*	(8.2–26.7)
FFM (kg)	42.1±5.5	(31.4–57.5)	37.7±3.5†	(28.2–38.9)
Glucose (mg/dL)	87±5	(69–100)	94±8†	(78–115)
HbA$_{1c}$ (%)	4.8±0.3	(3.9–5.7)	5.1±0.3†	(4.6–6.0)
Total cholesterol (mg/dL)	178±26	(121–249)	216±27*	(158–282)
HDL-cholesterol (mg/dL)	69±13	(40–100)	66±15	(34–107)
Triglycerides (mg/dL)	60±24	(25–182)	93±44†	(34–280)
E$_2$ (pg/mL)	75±60	(10–295)	11±3†	(10–29)
T$_3$ (ng/dL)	108±16	(61–150)	112±18	(80–160)

Values are means±SD (range; minimum–maximum), Ht: height, BW: body weight, BMI: body mass index, FM: fat mass, FFM: fat free mass, HbA$_{1c}$: glycosylated hemoglobin, E$_2$: estradiol, T$_3$: total triiodothyronine, *$p<0.001$ vs. young group (Student's t-test), †$p<0.001$ vs. young group (Mann-Whitney rank sum test).

Table 2. Resting energy expenditure and adipocytokines in young and elderly women.

	Young (n=115)		Elderly (n=71)	
REE (kcal/d)	1,190±154	(830–1,622)	1,085±109†	(913–1,459)
(kcal/kg BW/d)	21.5±1.9	(17.8–27.5)	20.1±1.9*	(16.2–25.1)
(kcal/kg FFM/d)	28.4±2.3	(24.0–33.7)	28.9±2.4	(23.5–35.7)
Adiponectin (μg/mL)	9.9±3.8	(2.9–22.1)	9.9±4.1	(2.6–20.8)
Leptin (ng/mL)	6.1±3.1	(1.1–19.8)	7.8±4.4††	(1.5–25.7)

Values are means±SD (range; minimum–maximum), REE: resting energy expenditure, *$p<0.001$ vs. young group (Student's t-test), †$p<0.001$ and ††$p<0.05$ vs. young group (Mann-Whitney rank sum test).

DISCUSSION

The present study demonstrates that plasma adiponectin and serum leptin concentrations are not associated with REE in either young or elderly women when confounding factors, such as FM and FFM, are taken into account.

Elderly women recruited in this study were at least 3 y post-menopause. Although no significant difference in BW was noted between the young and elderly women, the elderly women had significantly higher levels of % body fat and FM, and lower levels of FFM than the young group (Table 1). These results are consistent with previous studies, the results of which indicated that adipose tissue mass is controlled by steroid hormones and that menopause is also associated with increased body mass accompanied by elevated adiposity in females (*23, 24*).

In the present cross-sectional study, REE (kcal/d and kcal/kg BW/d) in the elderly group was significantly lower than in the young group. However, when REE was expressed per kilogram of FFM, no significant difference was observed between the two groups (Table 2). This evidence suggests that the specific metabolic rate per FFM does not decline with advancing age, and that REE is regulated mainly by the mass of the tissue-organs with both lower and higher metabolic rates, including skeletal muscle, intestinal organs and residuals in adults (*25–28*).

It is generally well known that thyroid function is associated with REE (*29*). In addition, previous studies in premenopausal women (*30, 31*) have shown that REE is lower during the early follicular phase of menstrual cycle, when E$_2$ is low, than in the midluteal phase. The present investigation demonstrated a significant relationship between serum T$_3$ concentration and REE in terms of kcal/kg FFM/d in all subjects (young: r=0.493, elderly: r=0.385, all subjects: r=0.456, $p<0.001$, respectively). In the young group but not the elderly group, serum E$_2$ concentration significantly correlated with REE (kcal/kg FFM/d), which was measured during the follicular phase of the menstrual cycle (r=0.222, $p<0.05$). These results suggest that concentrations of serum T$_3$ and E$_2$ may have a role in the regulation of REE in adult women.

Previous animal studies demonstrated that adiponec-

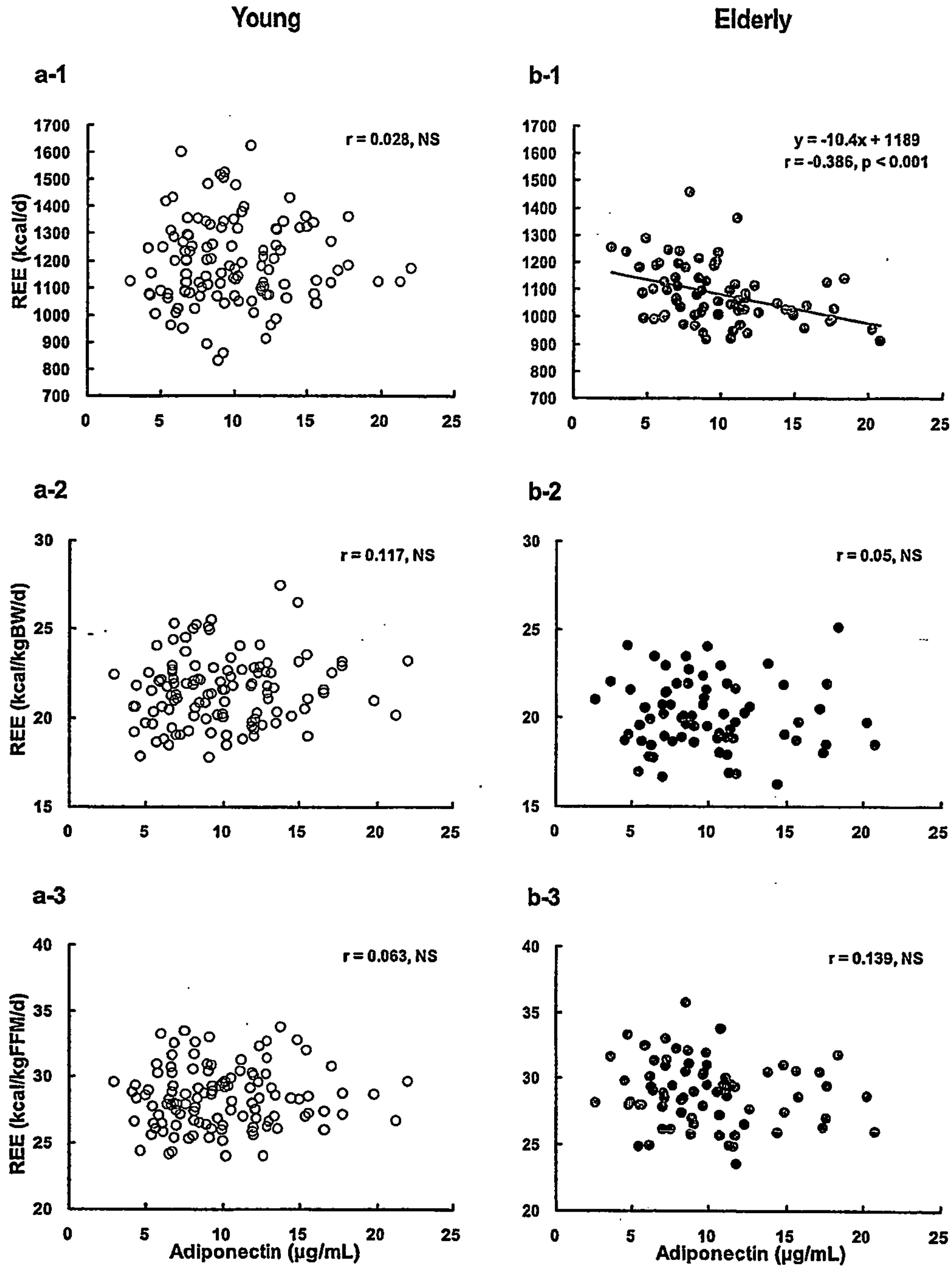

Fig. 1. Relationship between REE (kcal/d, kcal/kg BW/d, kcal/kg FFM/d) and adiponectin.

tin increases β-oxidation through AMP-kinase activation, suggesting that adiponectin plays crucial and central roles in the regulation of energy expenditure (*32*). In contrast, we demonstrate that a significant inverse link exists between plasma adiponectin concentrations and REE in elderly women ($r=-0.386$, $p<0.001$, Fig. 1, b-1). This is consistent with the results observed in overweight and obese males and females (*14*). These results might provide the possibility that protection by adiponectin against obesity-related disorders is especially important for human subjects with low RMR, and it is tempting to suggest plasma adiponectin as a valuable predictor for RMR, or vice versa.

We previously demonstrated that both FFM and FM are important predictors of REE in elderly people (*20*, *21*). Thus, the inverse association between REE (kcal/d) and concentrations of plasma adiponectin in the present investigation in the elderly group may reflect spurious correlations. Therefore, for the purpose of determining the precise relationship between plasma adiponectin concentrations and REE, we adopted a partial correlation coefficient after adjusting for confounding influences of FM (kg), FFM (kg), E_2 (pg/mL), and T_3 (ng/dL). As a consequence, we have demonstrated that REE is not significantly correlated with plasma adiponectin concentrations (Table 3). These results suggest that adiponectin is not involved in the regulation of REE in young or elderly women.

Jørgensen et al. (*33*) demonstrated that serum leptin was a strong positive determinant for RMR in men. However, in their study, no adequate adjustment of RMR was made for either FFM or FM. In the present

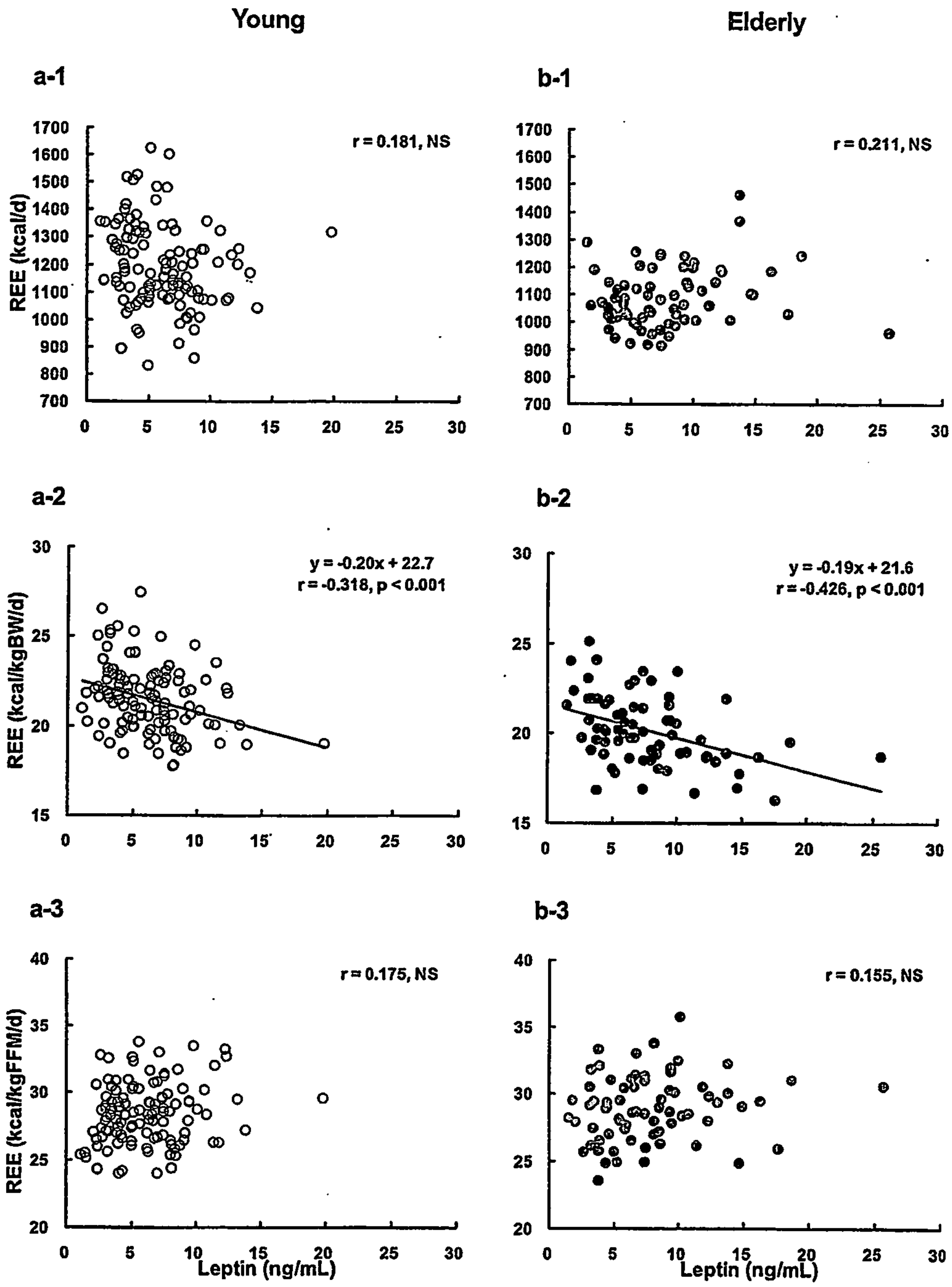

Fig. 2. Relationship between REE (kcal/d, kcal/kg BW/d, kcal/kg FFM/d) and leptin.

Table 3. Partial correlation coefficient to REE in female subjects.

	Young (n=115)		Elderly (n=71)	
Variable	β	p	β	p
REE (kcal/d)				
Adiponectin (μg/mL)	0.138	0.148	−0.200	0.104
Leptin (ng/mL)	−0.006	0.947	0.067	0.588

β: partial correlation coefficient: controlling for FM (kg), FFM (kg), E_2 (pg/mL), and T_3 (ng/dL).

investigation, no significant relationship between leptin and REE adjusted by body composition was observed (Table 3). This result is consistent with the report of Neuhäuser-Berthold et al. (*34*), suggesting that leptin might not have a significant role in the regulation of REE.

Our investigation has a few limitations. First, we did not test middle-aged (30–49 y) adults. Second, we did not include male subjects. Third, although adipocytokines such as adiponectin and leptin were related to the levels of body fat mass, we did not observe for lean and obese subjects. Future studies are needed to investigate this association in lean and obese adults and middle-aged adults.

In conclusion, the present investigation provides evidence to suggest that adipocytokines, such as adiponectin and leptin, do not influence REE in adult women.

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Experimental Physiology

Resistance training in men is associated with increased arterial stiffness and blood pressure but does not adversely affect endothelial function as measured by arterial reactivity to the cold pressor test

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Resistance training is a popular mode of exercise, but may result in stiffening of the central arteries. Changes in carotid artery diameter were determined using the cold pressor test (CPT), which results in production of nitric oxide via sympathetic activation and is one of the novel methods available for assessing endothelial function in the carotid artery. To investigate the effect of resistance training on endothelial function, we designed a cross-sectional study of carotid arterial vasoreactivity to CPT in men participating in regular resistance training with increased carotid arterial stiffness compared with age-matched control subjects. Twelve resistance-trained middle-aged men (age 38.7 ± 1.7 years) and 17 age-matched control subjects (age 36.8 ± 1.2 years) were studied. The direction and magnitude of changes in carotid artery diameter were measured by B-mode ultrasonography during sympathetic stress induced by submersion of the foot in ice slush for 90 s. Carotid arterial β-stiffness index, and systolic and mean arterial blood pressure were higher (7.7 ± 0.7 *versus* 6.0 ± 0.4 arbitrary units, 116 ± 2 *versus* 131 ± 4 mmHg and 86 ± 2 *versus* 95 ± 2 mmHg, respectively, all $P < 0.05$) in the resistance training group compared with control subjects. There were, however, no significant differences in the amount or percentage change in carotid artery diameter in CPT between the two groups (resistance training group, 0.33 ± 0.07 mm and 5.2 ± 1.1%; control group, 0.37 ± 0.06 mm and 5.8 ± 0.9%, respectively). These findings suggest that while carotid arterial stiffening and higher blood pressure are observed in regular resistance-trained men, these are not associated with abnormalities in carotid arterial vasoreactivity to sympathetic stimulus, which implies intact endothelial function.

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Resistance training is a popular form of exercise, and has become an integral component of exercise recommendations endorsed by a number of national health organizations (American College of Sports Medicine Position Stand, 1998; Pollock *et al.* 2000). Resistance training has favourable effects on the musculoskeletal system, thereby contributing to maintenance of functional capacity and prevention of sarcopenia and osteoporosis. In contrast, resistance training may be associated with reduction of compliance and increases in arterial stiffness in the central elastic artery (carotid artery; Bertovic *et al.* 1999; Miyachi *et al.* 2003, 2004; Cortez-Cooper *et al.* 2005; Kawano *et al.* 2006).

Increased arterial stiffness and reduced arterial compliance may be associated with endothelial dysfunction (Lind *et al.* 1999; Cheung *et al.* 2002; Nakamura *et al.* 2004). Indeed, impaired endothelial function and arterial stiffening are induced with advancing age and in the presence of cardiovascular diseases (Zeiher *et al.* 1989; O'Rourke, 1990; Taddei *et al.* 1995; Tanaka *et al.* 2000; Najjar *et al.* 2005). Therefore,

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Table 1. Subject characteristics

	Control	Resistance trained
Number of subjects	17	12
Age (years)	36.8 ± 1.2	38.7 ± 1.7
Height (cm)	171.0 ± 1.2	171.0 ± 1.8
Body weight (kg)	71.9 ± 1.9	74.9 ± 2.1
Percentage body fat (%)	19.4 ± 1.2	12.3 ± 0.9*
Total cholesterol (mmol l^{-1})	5.0 ± 0.2	4.7 ± 0.2
HDL cholesterol (mmol l^{-1})	1.3 ± 0.1	1.6 ± 0.1*
Plasma glucose (mmol l^{-1})	5.0 ± 0.1	5.1 ± 0.1
Triglycerides (mmol l^{-1})	1.5 ± 0.3	0.9 ± 0.1
Resting heart rate (beats min^{-1})	58 ± 2	56 ± 2
Maximal heart rate (beats min^{-1})	186 ± 3	183 ± 4
$\dot{V}_{O_2max}$ (l min^{-1})	2.7 ± 0.1	2.8 ± 0.1
$\dot{V}_{O_2max}$/body weight (ml kg^{-1} min^{-1})	37.7 ± 1.4	36.9 ± 1.3
Leg extension power (W)	1719 ± 91	2293 ± 155*
Handgrip (kg)	45.6 ± 1.6	51.0 ± 2.0*

Data are means ± S.E.M.; $\dot{V}_{O_2max}$, maximal oxygen consumption. *$P < 0.05$ *versus* control subjects.

impaired endothelial function is thought to be one of the physiological mechanisms underlying the reduction in carotid arterial compliance with resistance training. In this context, we hypothesized that resistance training would cause impairment of endothelial function in the carotid artery.

Local endothelial function in humans can be estimated by flow-mediated dilatation (Corretti *et al.* 2002) and/or vasoreactivity in response to medication with acetylcholine, etc. (Ludmer *et al.* 1986). Since it is difficult to determine endothelial function of the carotid artery in healthy humans using these methods, the cold pressor test (CPT), which results in production of nitric oxide (NO) via sympathetic activation (Nase & Boegehold, 1996; Tousoulis *et al.* 1997) is one of the novel methods (Rubenfire *et al.* 2000; Lavi *et al.* 2006) available for assessing endothelial function in the carotid artery.

To evaluate our hypothesis, we designed a cross-sectional study in which carotid arterial vasoreactivity to receptor-mediated sympathetic cold stimulus in regular resistance-trained men with reduced carotid arterial compliance was compared with age-matched sedentary control subjects.

Methods

Subjects

A total of 29 healthy men, 28–49 years of age, participated in the present study (Table 1). The sedentary subjects were recruited through various forms of advertisement and had not participated in a regular exercise programme for at least the previous 2 years. The resistance-trained men were recruited from various fitness clubs and had been performing vigorous resistance training for > 10 years. All resistance-trained men had been performing moderate- to high-intensity 'full-body' resistance exercise involving large muscle groups. To better isolate the effects of resistance exercise training, those who had been concurrently performing regular aerobic exercise (i.e. 'cross-training') were excluded from the study. All subjects were normotensive (< 140/90 mmHg), non-obese and free of overt chronic diseases as assessed by medical history, physical examination and complete blood chemistry and haematological evaluation. Candidates who smoked in the past 4 years, were taking medications, had ever used anabolic steroids or other performance-enhancing drugs, or who had significant femoral intima–media thickening (< 1.1 mm), plaque formation and/or other characteristics of atherosclerosis [ankle–brachial index (ABI) < 0.9] were excluded. All subjects gave their written, informed consent to participation in this study. All procedures were reviewed and approved by the Human Research Committee of the National Institute of Health and Nutrition.

Measurements

Before testing, subjects abstained from caffeine and fasted for at least 4 h (a 12 h overnight fast was used for determination of metabolic risk factors). All measurements were performed under comfortable laboratory conditions in the morning. Tests of resistance-trained men were conducted 20–24 h after their last exercise training session to avoid the immediate (acute) effects of exercise, but they were still considered to be in their normal (i.e. habitually exercising) physiological state.

Body composition

Body composition was determined using dual-energy X-ray absorptiometry (DEXA; model DPX-IQ, Lunar

Radiation) with subjects in the supine position. Measurement of fat mass using DEXA has been well validated against other standards (Haarbo *et al.* 1991).

Carotid arterial intima–media thickness (IMT)

Carotid artery IMT was measured from the images obtained using a SonoSite 180 PLUS ultrasound system (SonoSite, Bothell, WA, USA) equipped with a high-resolution linear-array broad-band transducer as previously described (Miyachi *et al.* 2004). Ultrasound images were analysed using image analysis software (NIH Image 1.63, Bethesda, MD, USA). At least 10 measurements of IMT were taken at each segment, and the mean values were used for analysis. This technique has excellent day-to-day reproducibility (coefficient of variation, $3 \pm 1\%$) for the carotid IMT.

Carotid arterial compliance

A combination of ultrasound imaging of the pulsatile common carotid artery with simultaneous applanation of tonometrically obtained arterial pressure from the contralateral carotid artery permits non-invasive determination of arterial compliance (Tanaka *et al.* 2000). The carotid artery diameter was measured from images obtained using an ultrasound system (Sonosite, Bothell, WA, USA) equipped with a high-resolution linear-array transducer. A longitudinal image of the cephalic portion of the common carotid artery was acquired 1–2 cm proximal to the carotid bulb. All image analyses were performed by the same investigator who was blinded to the group assignments.

Pressure waveforms and amplitudes were obtained from the common carotid artery with a pencil-type probe incorporating a high-fidelity strain-gauge transducer (SPT-301; Millar Instruments, Houston, TX, USA; Kelly *et al.* 1989; Tanaka *et al.* 2000). Since baseline levels of blood pressure are subjected to hold-down force, the pressure signal obtained by tonometry was calibrated by equating the carotid mean arterial and diastolic BP to the brachial artery value (Tanaka *et al.* 2000; Miyachi *et al.* 2004). In addition to arterial compliance (Van Merode *et al.* 1988), we also calculated the β-stiffness index, which provides an index of arterial compliance adjusted for distending pressure (Hirai *et al.* 1989). The arterial compliance and the β-stiffness index were calculated using the following equations:

$$\text{arterial compliance} = \frac{[(D_1 - D_0/D_0)]}{2\,(P_1 - P_0)} \times \pi \times D_0^2$$

and

$$\beta - \text{Stiffness index} = \frac{\ln\,(P_1/P_0)}{[(D_1 - D_0)\,/D_0]}$$

where D_1 and D_0 are the maximal and minimal diameters, and P_1 and P_0 are the highest and lowest blood pressures, respectively. The day-to-day coefficients of variation were 2 ± 1, 7 ± 3 and $5 \pm 2\%$ for the carotid artery diameter, pulse pressure and arterial compliance, respectively.

Cold pressor test

The CPT was performed by submersion of the right foot up to the ankle in ice slush for 90 s, a modification of the method published previously (Corretti *et al.* 1995*b*; Rubenfire *et al.* 2000). The foot was chosen to maximize the haemodynamic and sympathetic responses (Seals, 1990). Subjects were instructed to avoid breath-holding, muscle contractions and Valsalva's manoeuvre. Measurements of carotid arterial geometry were obtained before (baseline) and for 10 s during CPT. The day-to-day coefficient of variation for the change in carotid arterial diameter response to CPT was $4 \pm 1\%$.

Maximal oxygen uptake

We measured maximal oxygen consumption ($\dot{V}_{O_2max}$) during incremental cycle ergometer exercise (Miyachi *et al.* 2001). Oxygen consumption (coefficient of variation, $4 \pm 1\%$), heart rate and ratings of perceived exertion were measured throughout the protocol (Miyachi *et al.* 2001).

Metabolic risk factors for coronary heart disease

To screen for the presence of coronary heart disease, concentrations of fasting serum lipids and plasma glucose were determined with enzymatic techniques (Tanaka *et al.* 2000).

Arterial blood pressure at rest

Chronic levels of arterial blood pressure at rest were measured with a semi-automated device (Form PWV/ABI; Colin Medical, Komaki, Japan) over the brachial and dorsalis pedis arteries. Recordings were made in triplicate with subjects in the supine position (Miyachi *et al.* 2005).

Muscle strength

Leg extension power was determined using a dynamometer (Anaero Press 3500; Combi Wellness, Tokyo, Japan) in the sitting position. The subjects were fastened with a seat belt to a chair. In the starting position, the feet were placed on a sliding plate with the knee angle adjusted to 90 deg. Subjects were advised to vigorously extend their legs. Five trials were performed at 15 s intervals and the average of the two highest recorded power outputs (in W) was taken as the definitive measurement (Yoshiga *et al.* 2002).

Table 2. Cardiovascular measures

	Control	Resistance trained
Brachial systolic BP (mmHg)	116 ± 2	131 ± 4*
Brachial mean BP (mmHg)	86 ± 2	95 ± 3*
Brachial diastolic BP (mmHg)	71 ± 2	74 ± 3
Brachial PP (mmHg)	45 ± 1	57 ± 2*
Carotid systolic BP (mmHg)	104 ± 2	123 ± 5*
Carotid PP (mmHg)	33 ± 2	48 ± 4*
Carotid artery diameter (mm)	6.4 ± 0.1	6.2 ± 0.1
Carotid artery IMT (mm)	0.64 ± 0.02	0.65 ± 0.03

Data are means ± S.E.M.; BP, blood pressure; PP, pulse pressure; IMT, intima–media thickness. *$P < 0.05$ *versus* control subjects.

Handgrip strength of the right arm was measured with a hand-held dynamometer, with the subject standing and the arms extended by their sides. The subjects then gripped the dynamometer as strongly as possible for 3 s without pressing the instrument against their body or bending at the elbow, and values (in kg) were recorded as the averages of two trials.

Statistics

Statistical analyses were performed using statistical software (StatView, SAS, Cary, NC, USA). All data are presented as means ± S.E.M. Mean differences between resistance-trained and control men were examined using Student's unpaired t test. Analysis of covariance (ANCOVA) was used to test for differences in carotid arterial compliance and β-stiffness index between resistance-trained men and control subjects, with mean arterial blood pressure as a covariate.

Statistical significance was set *a priori* at $P < 0.05$ for all comparisons.

Results

Subject characteristics are presented in Table 1. Body fat was lower in the resistance-trained men compared with the control subjects. Although all metabolic risk factors were well within clinically normal levels in both groups, high-density lipoprotein (HDL) cholesterol levels were higher in resistance-trained men compared with control subjects. Muscle strength, assessed by leg extension power and handgrip strength, was higher in resistance-trained men than in the control subjects. There were no significant differences in other parameters between the two groups.

Table 2 shows cardiovascular measures. With the exception of diastolic blood pressure in the brachial artery, blood pressure parameters of brachial and carotid arteries were higher in resistance-trained men compared with control subjects. Ankle–brachial index was lower in resistance-trained men than control subjects. There were no significant differences in the diameter or IMT in the carotid artery between the two groups.

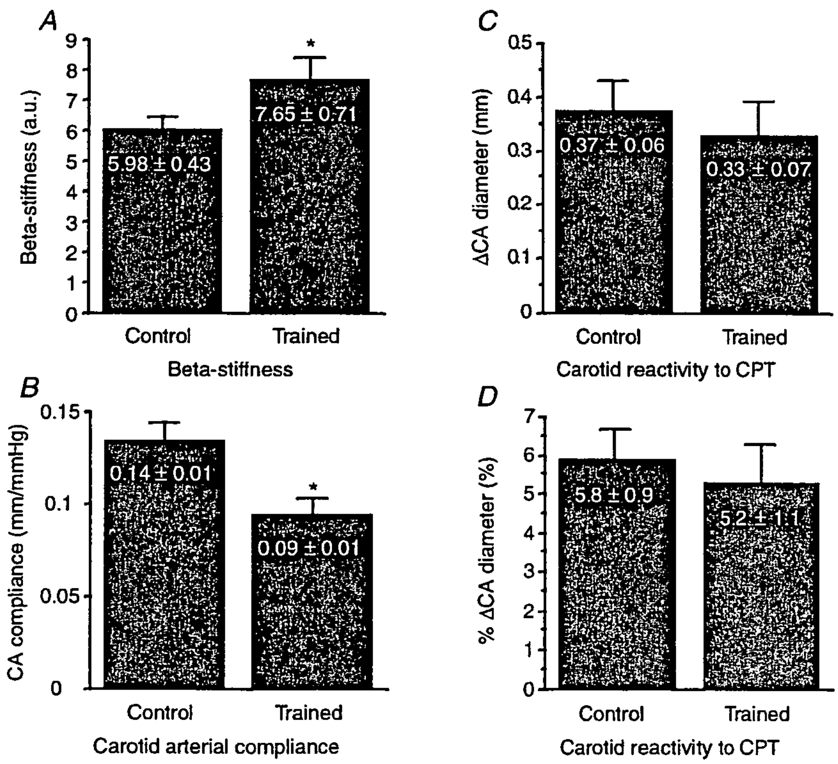

Figure 1. Carotid arterial β-stiffness index (*A*), carotid arterial (CA) compliance (*B*), and amount (*C*) and percentage change (*D*) in carotid artery diameter in response to CPT in resistance-trained men and control subjects

Values are means ± S.E.M. *$P < 0.05$ *versus* control subjects.

Carotid arterial β-stiffness (Fig. 1*A*) was higher and compliance (Fig. 1*B*) was lower in resistance-trained men compared with control subjects. There were no significant differences in the amount (Fig. 1*C*) or percentage change (Fig. 1*D*) of carotid artery diameter in response to CPT between resistance-trained men and control subjects. The differences in carotid arterial compliance and β-stiffness index between resistance-trained men and control subjects disappeared after normalizing carotid arterial compliance and β-stiffness index relative to mean arterial blood pressure (ANCOVA; $P = 0.081$ and $P = 0.101$, respectively).

Discussion

The results of the present study indicated that, although the carotid arterial compliance was lower in resistance-trained men compared with age-matched control subjects, there were no significant differences in the amount or percentage change of carotid arterial diameter in CPT between resistance training and control groups. In contrast to our original hypothesis, these findings suggest that while regular resistance training can increase carotid arterial stiffness, this is not associated with abnormalities of carotid arterial vasoreactivity to sympathetic physiological stress induced by cold.

The endothelial function of conduit arteries is one of the vascular functions, and has been identified as a primary target of injury from mechanical forces and processes that increase cardiovascular risk, such as hypertension (Moyna & Thompson, 2004). Owing to the clinical and functional importance of health of the endothelium, we examined the impact of resistance training on endothelial function. As a primary approach to resolve this issue, we performed a cross-sectional study. To isolate the effects of resistance training as much as possible, resistance-trained men and control subjects were carefully matched for age, height, body weight, aerobic capacity and metabolic risk factors. Although subjects were recruited carefully, as described in the Methods, blood pressure in resistance-trained men was higher than that in the control subjects. As a result, we found a 30% reduction in central arterial compliance in resistance-trained men compared with control subjects. These results are consistent with those of a previous cross-sectional study (Bertovic *et al.* 1999). Differences in carotid arterial compliance and β-stiffness index between resistance-trained men and control subjects were affected after normalizing carotid arterial compliance and β-stiffness index relative to mean arterial blood pressure. Given this association between blood pressure and arterial compliance, higher blood pressure may lead to lower arterial compliance in resistance-trained men than in control subjects due to equation using arterial distensibility and blood pressure. However, we feel that the higher blood pressure in resistance-trained men may be induced by greater arterial stiffening associated with the resistance training. Nevertheless, despite the higher arterial stiffness and blood pressure in resistance-trained men than in control subjects, there was no difference in carotid arterial vasoreactivity to CPT between the two groups.

The response of conduit arteries to systemic cold may be the result of the balance between adrenergic vasoconstriction and vasodilatation, with the latter being mediated by endothelial function (Nabel *et al.* 1988; Zeiher *et al.* 1989; Vita *et al.* 1992; Corretti *et al.* 1995*a*). The normal coronary vasodilator response to CPT can be blocked by competitive inhibition of L-arginine, a substrate for NO synthase (Tousoulis *et al.* 1997), and L-arginine can normalize the vasoconstrictor response to CPT in coronary artery disease (Gellman *et al.* 1996). In addition, both endogenous NO and exogenously administered NO donors suppress sympathetic outflow at the prejunctional level, and NO may exert a tonic influence on the discharge of sympathetic efferents (Zanzinger *et al.* 1994; Nase & Boegehold, 1996). Therefore, the endothelial function, via NO, may play an important role in changing the conduit artery diameter response to sympathetic stimulation by the CPT. We first examined the impact of resistance training with arterial stiffening on endothelial function of the carotid artery using CPT, and found that there were no significant differences in the amount or percentage change in carotid arterial diameter in response to CPT between resistance-trained men and control subjects. Our results were consistent with those of a previous study, which demonstrated that resistance training did not affect endothelial function in the peripheral muscular artery evaluated by flow-mediated dilation (FMD) (Rakobowchuk *et al.* 2005). These findings are consistent with the posit that regular resistance training may protect against the adverse effects of resistance load associated hypertension by preserving arterial endothelial function (Jurva *et al.* 2006).

The results of the present study indicated that carotid arterial compliance in resistance-trained men was lower than that in control subjects, and blood pressure was significantly higher in resistance-trained men compared with control men. In contrast, HDL cholesterol level was higher in resistance-trained men than in control subjects, and there were no differences in other lipid profiles or IMT between the two groups. Considering the relationships between reduction in arterial compliance and impaired endothelial function, hypertrophied IMT or abnormal lipid profile with advancing age and/or the presence of cardiovascular disease (Zeiher *et al.* 1989; O'Rourke, 1990; Taddei *et al.* 1995; Tanaka *et al.* 2000; Najjar *et al.* 2005), the decrease in carotid arterial compliance induced by resistance training may be different from vascular alterations seen in ageing or in the presence of cardiovascular disease. Arterial compliance is affected by endothelial function as well as by sympathetic vascular tone, arterial calcification, elastin-to-collagen ratio and IMT, and correlates with clinical parameters, such as aerobic capacity, age, blood pressure, body fat, waist circumference and lipids (Nichols & O'Rourke, 1998; Tanaka *et al.* 2000). The degree to which these other factors affect the relationship between training-associated decrease in arterial compliance independent of endothelial function will require further studies in a larger cohort.

Rubenfire *et al.* (2000) reported that the direction and magnitude of the change in carotid artery diameter in response to CPT are altered based on the presence of risk factors and coronary disease independent of IMT. The carotid artery vasoreactivity to CPT may have a valuable role in coronary risk assessment and in predicting response to therapy. The present study revealed that there were no significant differences in carotid arterial vasoreactivity to CPT and IMT between resistance-trained men and control subjects, suggesting that regular resistance training may not affect at least two of the cardiovascular disease risk factors. In addition, HDL cholesterol, leg extension power and handgrip strength were higher in resistance-trained men than in control subjects. Given these functional and physiological benefits of resistance training, we should emphasize that the practice of resistance training should not be discouraged.

Limitations

Endothelial function assessed by FMD should optimally be adjusted by shear stress, shear rate or blood flow velocity (Pyke & Tschakovsky, 2005; Rakobowchuk *et al.* 2005). However, it is technically difficult to determine the blood velocity or shear stress during the relatively short period (90 s) of CPT used in our study. Further, in contrast to the occlusion release technique for assessing brachial endothelial function, the carotid artery vasoreactivity to CPT is a complex interaction between clinical, adrenergic nerve and hormonal responses and endothelial function.

Conclusion

The results of the present study showed that regular resistance training is associated with reduction of central arterial compliance as measured using a combination of ultrasound images and applanation tonometry. However, there were no differences in carotid arterial vasoreactivity to CPT between resistance-trained men and sedentary control subjects. These findings suggest that while carotid arterial stiffening and higher blood pressure are observed in regular resistance-trained men, they are not associated with impaired vasoreactivity to sympathetic stimulus, which implies intact endothelial function. Nevertheless, the results of the present cross-sectional study must be confirmed in future prospective exercise intervention studies.

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