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ORIGINAL ARTICLE

The influence of physical activity-induced energy expenditure on the variance in body weight change among individuals during a diet intervention

Tomoaki Matsuo[a,*], Tomohiro Okura[b,c], Yoshio Nakata[b,c], Noriko Yabushita[b], Shigeharu Numao[a], Hiroyuki Sasai[d], Kiyoji Tanaka[b,c]

[a] *Doctoral Program in Graduate School of Comprehensive Human Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan*
[b] *Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan*
[c] *Center for TARA (Tsukuba Advanced Research Alliance), University of Tsukuba, Japan*
[d] *Master's Program in Health and Physical Education, University of Tsukuba, Japan*

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KEYWORDS
Weight reduction;
Energy intake;
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Accelerometry sensor;
Weighed dietary record

Summary
Objective: We investigated the relationship between the variability in body weight change among individuals and diet restriction or physical activity during a 14-week intervention.
Design: A prospective clinical trial with a 14-week weight reduction intervention design. In total, 90 middle aged, Japanese, obese women enrolled as subjects.
Measurements: The outcome variable was the change in body weight during the intervention period. Other primary variables were total energy intake, carbohydrate intake, fat intake, protein intake, total energy expenditure (TEE), and activity energy expenditure (AEE). Diet intake was assessed by 3 days, weighed dietary records and dietary recall interviews. Physical activity was assessed by a uniaxial accelerometry sensor and a diary of exercise.
Results: Significant reductions were observed in body weight (−8.5 kg) as a result of intervention. When the subjects were assigned to three categories depending on AEE during intervention, the loss of body weight was significantly greater for subjects within the *upper* (−9.6 kg) AEE category than for those in the *middle* (−8.5 kg) or *lower* AEE (−7.5 kg) categories. In addition, a significant correlation ($r = 0.57$, $p < 0.0001$) was observed between a subject's AEE before and during the intervention. On the other hand, no significant correlation was observed between

* Corresponding author. Tel.: +81 29 853 2655; fax: +81 29 853 2986.
E-mail address: matsuo@stat.taiiku.tsukuba.ac.jp (T. Matsuo).

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body weight reduction and energy intake, indicating that strict diet restriction does not always result in a large weight loss.

Conclusion: Activity energy expenditure, not only through voluntary exercise but also through spontaneous, daily, physical activities can have a positive effect on reducing body weight.

Introduction

Obesity is closely associated with some major lifestyle-related disorders such as hypertension, dyslipidemia, type 2 diabetes, and cardiovascular disease [1,2]. In addition, the prevalence of obesity continues to increase in many countries [3]. In Japan, despite the fact that the Japanese population has only a 2–3% "obesity category" [4], defined by the World Health Organization as a body mass index (BMI) $\geqq 30\,kg/m^2$, the prevalence of lifestyle-related disorders are high ([5,6]). This suggests that, even with mild obesity, Japanese individuals are likely to develop lifestyle-related disorders. Therefore, the Japan Society for the Study of Obesity (JASSO) originally defined obesity as a BMI $\geqq 25\,kg/m^2$ [7]. On the other hand, it is well known that many of the risk factors related to obesity tend to improve after body weight reduction. Some studies [8–11] have indicated that coronary heart disease (CHD) risk factors such as intra-abdominal fat area, blood pressure, and biochemical variables (triglycerides, fasting plasma glucose, etc.) improve in response to weight reduction achieved through a strictly supervised weight reduction intervention.

However, although obese individuals may participate in the same intervention program for weight reduction, changes in the values of CHD risk factors tend to differ among individuals; there is a lot of variability in the mean values of the CHD risk factors [9–11]. Changes in these risk factors are known to be closely associated with the change in body weight [10,12]. The variances in CHD risk factors could be due to the difference in body weight reduction among individuals even when they participate in the same weight-reduction program. Therefore, if weight management instructors take into consideration the variability in body weight change among individuals, they could provide more effective prescriptions regarding physical activity and diet for obese individuals.

In general, the most important factor for reducing body weight is a balance between energy intake and energy expenditure [13]. Instructors of body weight reduction programs should indicate the proper diet and appropriate physical activity for their patients. Understandably, they cannot control everything in a participant's daily life, and as a result, it is inevitable that the change in energy balance would vary among individuals. The body weight might decrease resulting when energy expenditure exceeds energy intake. Diet restriction and increased physical activity could have a strong effect on creating such imbalances.

The effect of diet restriction and physical activity on changes in body weight may be the most important considerations when investigating the variance in body weight change among individuals. Actually, little is known about the relationship between variable body weight changes among individuals and diet restriction or physical activity.

Taking these factors into consideration, in this study we examined whether the variance in body weight change among individuals may be influenced by energy intake (diet restriction) or energy expenditure (physical activity).

Methods

Subjects

Subjects were recruited through advertisements in local newspapers, and 111 women were initially screened. We included individuals for our study who met at least one of following two criteria: (1) BMI $> 25\,kg/m^2$; or (2) intra-abdominal fat area at the level of the umbilicus $>100\,cm^2$, as determined by CT scan. None of the subjects had any cardiac disease. We also excluded individuals who were unable to perform all measurements for personal reasons. In total, 90 women, aged 30–65 years, with a baseline mean BMI of $27.8\,kg/m^2$ ($22.5–34.6\,kg/m^2$) (Table 1) were selected for the study. The aim and design of the study were explained to each subject before they gave their written informed consent. This study was approved by the Ethical Committee of the University of Tsukuba.

Table 1 Participants' descriptive characteristics and any changes in values during the intervention period

n = 90	Week 0	Week 10	Week 15	d
Age (year)	52.3 ± 7.1 (30–65)			
Height (cm)	156.3 ± 4.9 (144.1–167.9)			
Body weight (kg)	68.1 ± 8.4 (52.0–92.6)	62.9 ± 7.4 (49–86)	59.5 ± 7.6 (46.6–83.6)	−8.5 ± 2.4**
Body mass index (kg/m²)	27.8 ± 2.6 (22.5–34.6)	25.8 ± 2.3 (21.3–32.4)	24.4 ± 2.4 (20.1–31.8)	−3.4 ± 0.9**
Waist circumference (cm)	95.5 ± 8.1 (80.5–120.9)		87.1 ± 7.6 (71.4–114.3)	−8.4 ± 3.6**
Intra-abdominal fat area (cm²)	114.6 ± 40.6 (39.3–225.3)		84.9 ± 34.4 (27.1–188.1)	−30 ± 24**
Systolic blood pressure (mmHg)	132 ± 19 (96–218)		117 ± 16 (90–158)	−15 ± 13**
Diastolic blood pressure (mmHg)	90 ± 10 (64–113)		73 ± 10 (54–98)	−9 ± 8**
Total cholesterol (mg/dl)	226 ± 40 (130–367)		199 ± 33 (121–304)	−27 ± 26**
Triglycerides (mg/dl)	121 ± 92 (38–680)		74 ± 30 (31–183)	−47 ± 79**
High-density lipoprotein cholesterol (mg/dl)	60 ± 13 (35–89)		61 ± 12 (40–92)	1 ± 8
Low-density lipoprotein cholesterol (mg/dl)	142 ± 36 (75–282)		123 ± 30 (57–235)	−19 ± 25**
Fasting plasma glucose (mg/dl)	100 ± 20 (77–210)		91 ± 9 (73–120)	−9 ± 16**

Values are presented as the mean ± standard deviation and ranges. Week 0, before weight reduction program; week 10, at the beginning of the 10th week of the weight reduction program; week 15, after weight reduction program; d: (15 wk) − (0 wk).
** $p < 0.01$.

Anthropometric variables

Body weight was measured to the nearest 0.1 kg using a digital scale (TBF-215; Tanita, Tokyo, Japan), height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (TBF-215; Tanita, Tokyo, Japan), and BMI was calculated as weight (in kilograms) divided by height squared (meters squared). Waist circumference was measured to the nearest 0.1 cm at the level of the umbilicus with subjects in the standing position.

Abdominal adipose tissue area by CT

Intra-abdominal fat area (IFA) (cm^2) was measured at the level of the umbilicus (L4–L5) using CT scans (TSX-002A; Toshiba, Tokyo, Japan) performed on subjects in the supine position. The IFA was calculated using a computer software program (FatScan; N2 system, Osaka, Japan).

Blood pressure and biochemical assays of blood

Systolic and diastolic blood pressures (SBP and DBP) were taken from the right arm using a mercury manometer after the subjects rested at least 20 min in a sitting position. Cuff sizes were selected based on upper arm girth and length.

A blood sample of ~10 ml was drawn from each subject after an overnight fast. Serum total cholesterol (TC) and triglycerides (TG) were determined enzymatically, serum high-density lipoprotein-cholesterol (HDL-C) was measured by the heparin-manganese precipitation method and fasting plasma glucose (FPG) was assayed by a glucose oxidase method. Serum low-density lipoprotein-cholesterol (LDL-C) was estimated according to the equation of Friedewald et al. [14]: LDL-C = total cholesterol − (HLD-C + triglycerides/5).

Dietary assessments

Total energy intake (in kilocalories) and the amounts of each nutrient (carbohydrates, fat, and protein in grams) before and at the beginning of week 10 of the weight reduction program were assessed by both 3-day weighed dietary records (3-day WDR) and dietary recall interviews for each subject performed by one skilled dietician. The dietician explained how to complete the 3-day WDR in detail before the measurements. The subjects learned how to use a digital cooking scale for weighing food. The dietitian collected the recorded sheets from the 3-day WDR and codified the food items and food weights. Thereafter, the dietician

Table 2 Changes in energy intake and energy expenditure

$n = 90$	Week 0	Week 10	d
Total energy intake (kcal/d)	1867 ± 332 (1292–2816)	1195 ± 127 (943–1545)	−671 ± 325**
Carbohydrates intake (g/d)	259 ± 50 (119–410)	169 ± 24 (114–237)	−90 ± 49**
Fat intake (g/d)	57 ± 16 (30–97)	32 ± 8 (17–63)	−25 ± 17**
Protein intake (g/d)	70 ± 12 (41–101)	60 ± 9 (37–81)	−10 ± 14**
Total energy expenditure (kcal/d)	1916 ± 187 (1558–2365)	1855 ± 165 (1488–2262)	−61 ± 112**
Activity energy expenditure (kcal/d)	455 ± 111 (236–755)	474 ± 105 (246–823)	20 ± 100†
Total energy expenditure/body weight (kcal/kg d)	28.3 ± 2.2 (23.0–34.4)	29.7 ± 2.5 (23.9–37.1)	1.4 ± 1.8**
Activity energy expenditure/body weight (kcal/kg d)	6.7 ± 1.6 (3.7–11.1)	7.6 ± 1.8 (3.9–13.9)	0.9 ± 1.6**

Values are presented as the mean ± standard deviation and ranges. Week 0, before weight reduction program; week 10, at the beginning of the 10th week of the weight reduction program; *d*: (10 wk) − (0 wk).

** $p < 0.01$.

† $p < 0.1$.

interviewed each subject to elicit more information about the subject's food intake for the 3-day period. Based on the Standard Tables of Food Composition in Japan fourth revised edition [15], total energy intake along with carbohydrate, fat, and protein intakes were calculated (Table 2).

Energy expenditure assessments

The total energy expenditure (TEE) and activity energy expenditure (AEE) were assessed by both a uniaxial accelerometry sensor (Lifecorder; Suzuken Co. Ltd.) and a diary of exercise. Lifecorder can assess two types of AEE by activity level: energy expenditure of activities (EE_{Act}) and energy expenditure of minor activities ($EE_{minorAct}$) [16]. In this study, AEE was estimated as a total of these two EEs ($AEE = EE_{Act} + EE_{minorAct}$). Subjects wore the Lifecorder constantly (except while sleeping or bathing), for a 7-day period prior to the weight reduction program and for 7 days during week 10 of the weight reduction program. During these 7-day segments, the subjects were also instructed to keep a diary of their exercise. The diary consisted of the status of wearing the Lifecorder and detailed exercise information (i.e. type, time, and ratings of perceived exertion (RPE) [17]). For times of exceptional physical exertion (e.g. swimming), we estimated this exceptional EE by its metabolic equivalent (MET) from the diary entries. For these measurement periods, we calculated TEE and AEE from both the Lifecorder data and the diary entries.

Subjects were classified into the following three categories with regard to their AEE during the intervention: *upper*, the top 25% of participants in terms of activity level, $n = 23$, with a mean physical activity level (PAL) of 1.67 ± 0.08; *middle*, middle 50% of our participants, $n = 44$, PAL of 1.55 ± 0.04; *lower*, the least active 25% of our participants, $n = 23$, PAL of 1.45 ± 0.05.

Weight-reduction program

The weight reduction program in this study combined diet counseling with an exercise program. The diet counseling consisted of weekly lectures (90 min per lecture, 12 times in 14 weeks) and individual counseling by skilled dieticians. All subjects were instructed to consume a well-balanced 1200 kcal diet per day while also keeping a daily food diary in which they recorded in detail every food they ate during the 14-week intervention period. Skilled dieticians checked the subjects' energy intake (1200 kcal) and nutritional balance at every diet counseling class.

The exercise program consisted of some lectures on exercise (60 min per lecture, 3 times in 14 weeks) and supervised exercise sessions (90 min per session, 24 times in 14 weeks). To increase subjects' adherence to the weight reduction program during the intervention period, subjects were assigned to one of two groups taking personal lifestyles into account (occupations, daily schedules, etc.). Participants in group A ($n = 56$) took part in the supervised exercise sessions in addition to the lectures on exercise, while group B participants ($n = 34$) only attended the exercise lectures. However, all subjects could perform free exercise in the fitness gym where the weight reduction program was held so that they were not restricted from performing daily exercise on their own during the intervention period. Our intention was to not control the subjects' daily physical activity (either regular exercise or spontaneous physical activity).

The exercise lectures consisted of the instructions on the basics of exercise, such as the proper way of walking or how to prevent injury during exercise, and all subjects were instructed on how to increase their physical activity during the intervention period. The exercise sessions mainly consisted of aerobic exercise, such as aerobic dance, supervised by physical fitness trainers. Before and after the main exercise session (60 min per session), subjects performed stretching exercises as a warm up or cool down (15 min each).

Statistical analysis

Values are expressed as the mean ± standard deviation and as mean ± standard errors. Student paired *t*-tests were performed to test the significance of changes in variables measured before (week 0), during (week 10) or after (week 15) the intervention program. Differences among the three groups for AEE during the intervention were examined using one-way analysis of covariance (ANCOVA) with the baseline BMI representing the covariates, and Tukey–Kramer's post-hoc test applied when the difference was significant ($p < 0.05$) according to the ANCOVA results. The relationship between two measurement variables was assessed by Pearson's product moment correlation, with values <0.05 regarded as significant. The data were analyzed with the Statistical Analysis System (SAS), version 9.01 (SAS Institute Inc., Cary, NC, USA).

Results

Tables 1 and 2 show subjects' measurement variables from before (week 0), during (week 10) or after (week 15) the intervention program along with changes (*d*) in the measurement variables. Significant reductions were observed in body weight, BMI, waist circumference, IFA, SBP, DBP, TC, TG, LDL-C, FPG, total energy intake, carbohydrate intake, fat intake, protein intake, and TEE. Significant increases were observed in TEE/body-weight and AEE/body-weight.

Table 3 shows correlation coefficients and partial correlation coefficients between weight reduction during the intervention and other measurement variables. Significant correlations were observed between the reduction in body weight and 0 wk body weight, 0 wk BMI, 10 wk TEE, 10 wk AEE, delta (subtraction of 10 wk from 0 wk values) total energy

Table 3 Correlation analysis of body weight reduction with predictors

	Correlation coefficient vs. weight reduction	*p*	Partial correlation[a] coefficient vs. weight reduction	*p*
Baseline: week 0				
Body weight	0.45	<0.0001		
BMI	0.43	<0.0001		
During intervention: week 10				
Total energy intake	−0.01	0.96	0.06	0.56
Carbohydrates intake	−0.03	0.78	0.02	0.86
Fat intake	−0.02	0.85	0.02	0.87
Protein intake	−0.02	0.88	0.03	0.83
Total energy expenditure	0.43	<0.001	0.26	0.01
Activity energy expenditure	0.42	<0.001	0.39	<0.001
Delta: subtraction of "week 10" from "week 0"				
Total energy intake	0.27	0.01	0.20	0.06
Carbohydrates intake	0.25	0.02	0.18	0.09
Fat intake	0.17	0.08	0.12	0.27
Protein intake	0.06	0.58	0.03	0.75
Total energy expenditure	−0.18	0.09	−0.11	0.30
Activity energy expenditure	0.02	0.87	0.09	0.42

BMI, body mass index; week 0, before weight reduction program; week 10, at the beginning of the 10th week of the weight reduction program.
[a] Partial correlation coefficients adjusted for BMI.

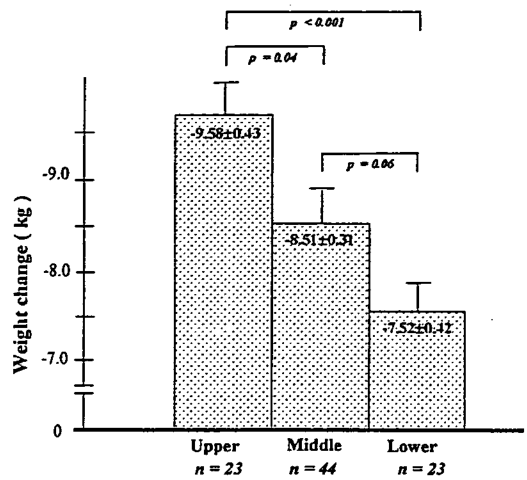

Figure 1 Comparison of weight changes across three categories of activity energy expenditure. Values are presented as the mean ± standard error. Values are adjusted for baseline BMI. *Upper*, upper level of activity energy expenditure (top 25%); *middle*, middle level of activity energy expenditure (middle 50%); *lower*, lower level of activity energy expenditure (least active 25%).

intake, and delta carbohydrate intake. The correlations of 10 wk TEE and 10 wk AEE with the body weight reduction were also significant when those were adjusted by the 0 wk BMI.

To investigate the relationship between the weight lost and the AEE during the intervention, subjects were assigned to three categories depending on their AEE during the intervention (*upper*: top 25% of participants, $n = 23$; *middle*: middle 50%, $n = 44$; *lower*: least active 25%, $n = 23$). Fig. 1 presents the results of the one-way ANCOVA. Reductions in body weight were compared among the three AEE categories and adjusted for the 0 wk BMI. The decrease in body weight was significantly greater for the subjects in the *upper* AEE classification than for the subjects in the *middle* or *lower* AEE groups. Fig. 2a compares the total energy intake and the intake of each nutrient (carbohydrates, fat, and protein) during the intervention among the three AEE categories adjusted for the 0 wk BMI. Furthermore, Fig. 2b shows the changes in total energy and nutrient intakes before and during intervention, adjusted for the 0 wk BMI, for each of the three AEE categories. No differences across the three AEE categories were observed in any of the analyses.

Fig. 3 shows the relationship between AEE/body-weight before intervention and AEE/body-weight during intervention. The correlation coefficient was 0.57 ($p < 0.0001$). Fig. 4 is a scatter plot showing the percentage of body weight lost relative to the restriction in total energy intake. There

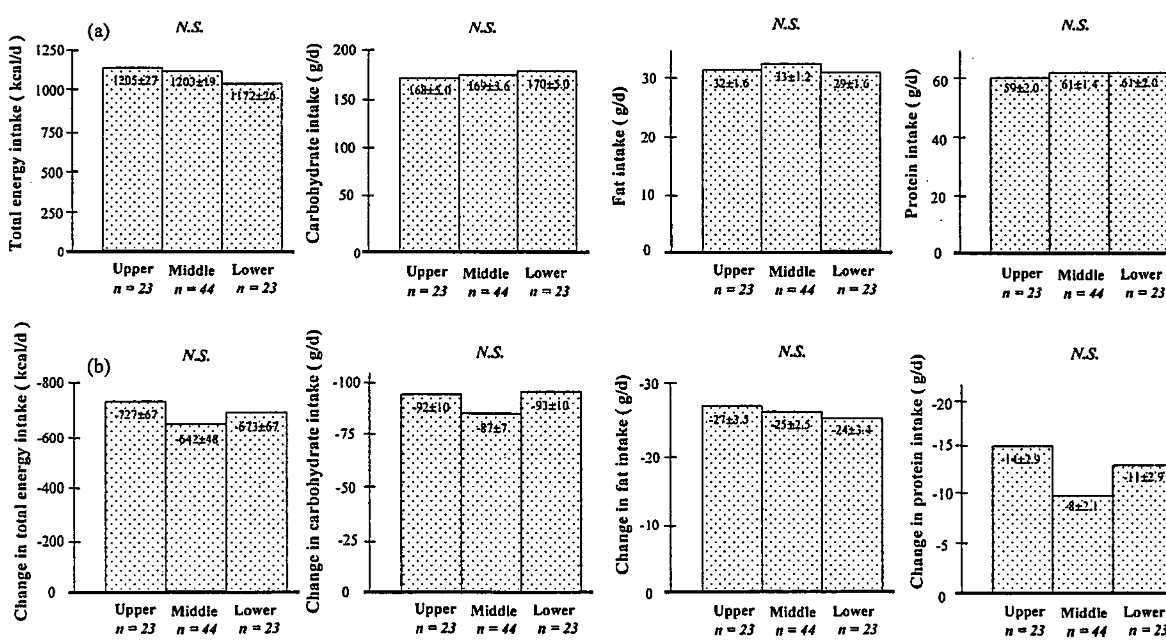

Figure 2 Comparison of the total energy, carbohydrate, fat and protein intakes during the program (a), and comparison of any changes in total energy, carbohydrate, fat and protein intakes (b) across the activity energy expenditure categories. Values are presented as the mean ± standard error. Values are adjusted for the baseline BMI. *Upper*, upper level of activity energy expenditure (top 25%); *middle*, middle level of activity energy expenditure (middle 50%); *lower*, lower level of activity energy expenditure (least active 25%), N.S., not significant.

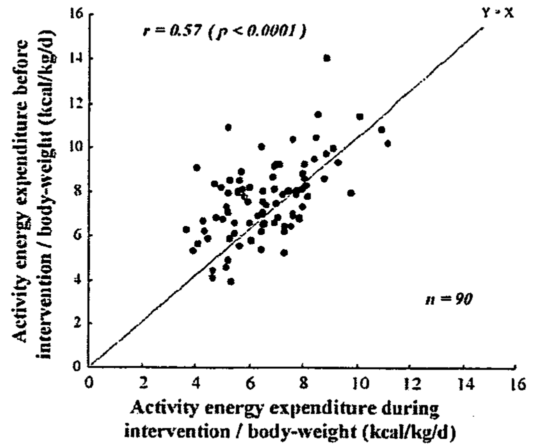

Figure 3 Scatter plot of the activity energy expenditure before and during intervention.

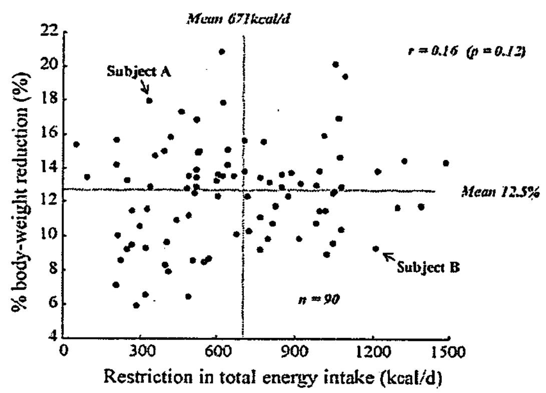

Figure 4 Scatter plot of restriction in total energy intake and % body weight reduction.

was no significant correlation between these two variables.

Discussion

There was no difference in AEE during the intervention between the two exercise groups (group A: 488.4 ± 111.1 kcal/d; group B: 451.5 ± 90.2 kcal/d; data not shown in the table). We considered two possible reasons why there was no difference between the groups: (1) subjects' diaries indicated that some of the group B subjects, which had no supervised exercise sessions, performed free exercise during the intervention period; (2) some studies [18,19] indicate that voluntary exercise has little impact on total energy expenditure and that spontaneous physical activity has become the major determinant of physical activity energy expenditure. In our study, AEE measured by Lifecorder included not only exercise sessions but also the subjects' daily physical activity; there were subjects whose daily physical activity placed them in the upper level of activity even though they were in group B. In this study, we regarded these two groups as one group.

The reduction in body weight was significantly greater for the subjects whose activity level placed them into the *upper* AEE than for subjects in the *middle* or *lower* AEE (Fig. 1), although there were no dietary differences among the categories (Fig. 2). These results indicate that AEE during the intervention influenced the amount of the body weight lost. Fig. 3 shows the relationship between AEE before and during intervention. A significant correlation ($r = 0.57$) was observed between those two variables, indicating that subjects who had a higher level of physical activity during the intervention also had a higher level of physical activity before the intervention. In other words, the subjects who fell into the *upper* AEE category had a more active lifestyle than the subjects within the *lower* AEE category. Although physical activity level during the intervention was related to the reduction in body weight (Fig. 1), this influence was probably due to spontaneous, daily, physical activities, and not due to voluntary exercise. That is, it depended on a subject's daily lifestyle.

Several studies [20—22] have indicated that higher level exercises are necessary to promote body weight reduction. However, we have shown the possibility that ordinary, daily, physical activity, under a strictly controlled diet, can help reduce body weight, and increasing physical activity in one's daily life could play an important role in a weight reduction program.

Levine et al. [23] reported that it is not variable exercise levels, but rather the variance in NEAT (non-exercise activity thermogenesis) that accounts for most of the variability in activity thermogenesis. NEAT is the energy expenditure from all physical activities other than volitional sporting-like exercise [23]. The change in NEAT is predictive of fat gain. Those who, with overfeeding, increase their NEAT the most, gain the least fat, while those who, with overfeeding, do not increase their NEAT, gain the most fat [24]. Our results are consistent with those studies.

On the other hand, it can be difficult to understand why diet restriction alone would not influence body weight reduction. Dunn et al. [25] indicated that dietary changes appeared to be more effective than increased physical activity for weight reduction. It was natural to assume that the dietary restriction in this study (mean decrease of −671 kcal/d) would play an important role in the subjects' loss of body weight. However, we could find no significant correlation between body weight reduction and diet restriction (i.e. restriction of total energy intake) in this study. Fig. 4 shows the scatter plot of diet restriction relative to body weight reduction. We can see that the reduction of subject A's body weight was greater than the mean value, yet this subject had a less restricted diet than the mean. In contrast, subject B, who lost less weight than the mean, actually had a greater diet restriction than the mean. It is possible that strict diet restriction alone does not always result in a large loss of body weight.

In this study, we investigated the influence of energy intake on body weight reduction in light of diet intake only, but this matter is not so simple. Factors such as digestion, assimilation, metabolism, and evacuation should all be considered in the study of weight loss. Unfortunately, such factors are too complicated to examine them all at this time. Furthermore, Leibel et al. [26] demonstrated that body weight change was not as simple as measuring the energy balance alone. For instance, genetic factors may come into play for people who lose weight easily or with difficulty. Several researchers [27,28] have already reported on the relationship between gene polymorphism and body weight reduction. More approaches to such genetic factors will be needed to solve this issue.

Our study did have two limitations. First, we measured a subject's energy intake during the intervention at just one time (week 10). This one set of intake records was probably not sufficient (3-day WDR) to represent a study period of 14 weeks. However, all subjects still kept food diaries during the 14-week intervention period. They had to record all consumed foods in detail including sugar (g) or oil (ml). Skilled dieticians checked the subjects' energy intakes through the daily diaries at least once every week. We considered most subjects' energy intakes to be approximately 1200 kcal/d during the intervention period, although there were some daily and individual variations.

Second, we assessed subjects' energy expenditures by a uniaxial accelerometry sensor (Lifecorder). Subjects took off the Lifecorder while sleeping or bathing. Since we could not measure EE during these periods, there may have been an underestimation of TEE and AEE. However, the device is becoming an extremely useful method for investigating energy expenditure for epidemiological research [29] because a strong correlation has been observed between Lifecorder readings and calorimetry [16] or the doubly labeled water method [30].

In conclusion, physical activity in daily life in conjunction with a controlled diet can have a positive effect on reducing body weight. This information may be helpful when prescribing a weight management program in obese or overweight women since it is difficult for health advisors to recommend high-intensity exercise programs for them. On the other hand, we did not find any significant correlation between diet restriction and body weight reduction. Our results also suggest that the response to diet restriction tends to differ for each subject. Additional research is needed to increase our understanding of the variability in body weight reduction among individuals so we can develop more effective body weight management programs in the future.

Conflict of interest

The authors of the "The influence of physical activity-induced energy expenditure on the variance in body weight change among individuals during a diet intervention" have no ownership of beneficial interests in such commercial entities, options or warrants to purchase stock or other equity interests, patent-licensing arrangements, or advisory and consulting positions with such commercial entities.

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日本骨粗鬆症学会 平成18年度 研究奨励賞

軽度要介護者の血中ビタミンDレベルの分布状況とビタミンD・乳酸カルシウム製剤補充による介護予防効果

－生活機能・身体機能と血中ビタミンDレベルとの関連より－

柳　久子・奥野純子・戸村成男
大蔵倫博・田中喜代次

はじめに

介護保険制度が施行され5年がたち，要介護認定者が約2倍に増加している。全体の約5割を占める要支援・要介護1の軽度要介護者は，サービスが開始された2年後には半数以上が重度化しており，サービスが利用者の状態改善につながっていないといわれている。平成13年国民生活基礎調査によると介護が必要となった主な疾患は骨折・転倒，関節疾患（リウマチ等）で27～28%を占めている。在宅の軽度要介護者の多くは，関節疾患などがあるために下肢機能の低下が目立ち，要介護の主要原因としてあげられている転倒・骨折の危険性が高い集団であり，これらを予防することが介護度悪化を予防できると推測される。

ビタミンDの欠乏は，骨粗鬆症の危険因子であり，高齢者のふらつきや転倒と関連があり[1,2]，筋力の低下[3]や高齢者の下肢機能低下[4]をもたらすといわれており，筋力低下は高齢者の転倒の主要な危険因子である[5]。ビタミンDの補充は，骨密度を増加させる作用の他に，転倒の予防やふらつき・つまずきを改善するという報告がある[1,2]。一方，効果がみられないという報告もあり[6]，ビタミンDの評価は一定ではない。

われわれはこれまで地域在住の軽度要介護者と特定高齢者を対象にビタミンD濃度と生活機能・身体機能との関連を研究してきたので報告する。

1 目　的

本研究では，①要支援・要介護1の高齢者・特定高齢者の血清ビタミンD濃度と身体機能との関連を横断調査により明らかにする。また，②ビタミンD・乳酸カルシウム製剤の補充による身体機能への効果を縦断的に検討することを目的とした。

2 方　法

1) 対　象

平成17・18年の6～9月に介護予防教室に参加した茨城県Y町（北緯36度）の地域在住の要支援・要介護1の65歳以上の高齢者（軽度者），特定高齢者レベルの者（基本チェックリストの「運動」の項目が3個以上該当）61名。

2) 調査方法

質問紙による面接聞き取り調査，体力測定，採血を行った。

①質問項目：属性，生活機能，ADL（Barthel index），転倒回数，つまずき・ふらつきの有無，外出回数，椅子やベッドからの起き上がり・立ち上がりなどを調査した。

②体力測定：歩行能力としてTimed Up & Go（TUG）・5m歩行，柔軟性として長座体前屈，バランス能力としてファンクショナルリーチ・開眼

Key words：軽度要介護者，ビタミンD，乳酸カルシウム製剤

筑波大学大学院人間総合科学研究科

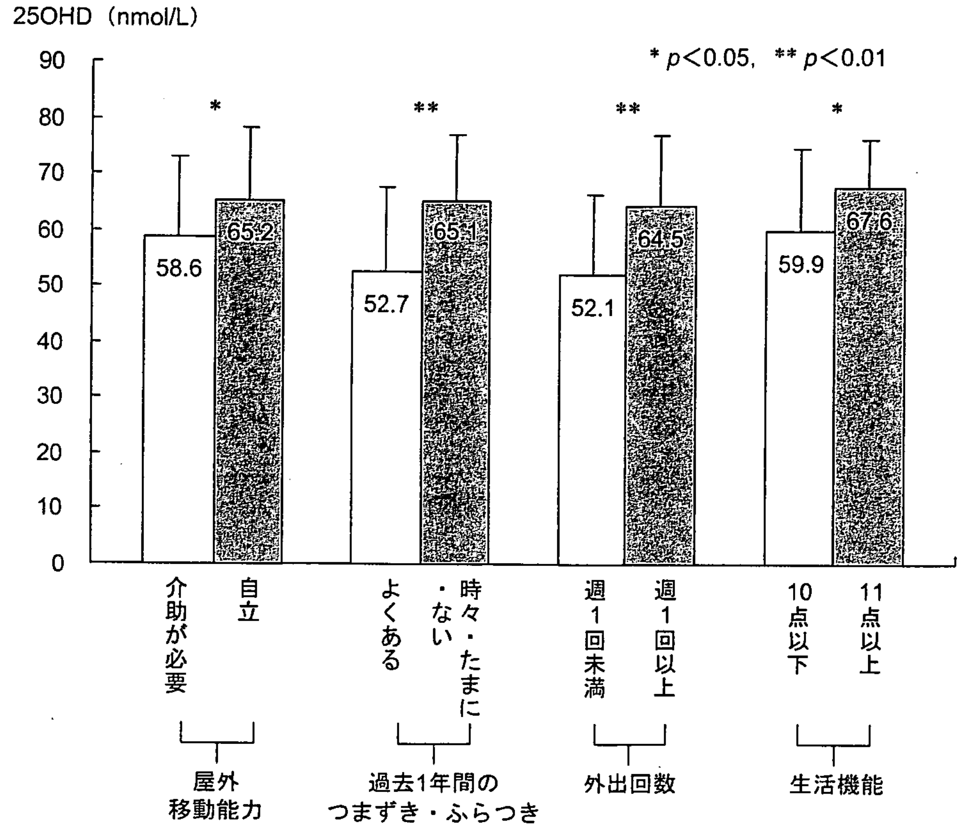

図1　血清ビタミンDと関連のある項目

片足立ち，筋力として握力・足関節背屈力を測定した。2回測定し平均値を用いた。測定が1回の者はその値を用いた。開眼片足立ち・握力・足関節背屈力は左右の平均値を用いた。

③血液データ：血清アルブミン，クレアチニン，カルシウム，intact PTH（iPTH）（ECLIA法），ビタミンD（25OHD）（RIA法）を測定した。

上記の調査は初回と3ヵ月目に実施した。

④介護予防教室：運動と栄養指導を合わせた包括的なプログラムよりなり，週1回約90分12回開催した。参加者のうち38名は介護予防教室へ参加し（運動群），残りの23名は介護予防について説明を受けただけである（コントロール群）。

⑤ビタミンD・乳酸カルシウム製剤の補充効果：運動群の希望者（n=17）にアルファカルシドール1μg/日および乳酸カルシウム4g/日を3ヵ月間投与した。服用1ヵ月目に採血を実施し副作用チェックを行った。

3）解析方法

2群間の比較には，連続変数の場合はt検定を，カテゴリー変数の場合はχ^2検定またはFisherの直接法により比較検討した。また，多重ロジスティック回帰分析により「過去1年間のつまずき・ふらつき」に影響する要因を検討した。ビタミンD製剤服用有無の影響は繰り返しのある2元配置分散分析により解析した。また，開始前後の連続変数の比較はpaired t-testを実施した。統計解析にはSPSS 12.0 J for Windowsを用い，$p<0.05$を有意差ありとした。

本研究は，筑波大学人間総合科学研究科の倫理委員会の承認を得，参加者には文章と口頭による説明を行い同意を得てから実施した。

3　結　　果

1）対象者の背景

参加者61名（運動群とコントロール群）の開始時の特性は，平均年齢：77.0±5.6歳（65～90歳），男性：18名（29.5%），Barthel index平均得点：89.7±10.3，過去1年間に転倒経験有：32名（52.5%），つまずき・ふらつき経験有：45名

表1　ビタミンD・乳酸カルシウム製剤服用有無による身体機能への効果－運動群において－

	ビタミンD非服用群 (n=16)	ビタミンD服用群 (n=16)	交互作用
25OHD (nmol/L)	59.9±11.9	65.1±14.8	
pre TUG (sec)	17.1±8.2[a]	23.4±8.0	
post TUG (sec)	14.4±7.9*	18.6±5.1**	
pre 5m 通常歩行 (sec)	8.2±3.3	8.5±2.8	
post 5m 通常歩行 (sec)	7.2±4.7	8.3±2.4	
pre 長座体前屈 (cm)	7.6±8.1	3.0±6.4	
post 長座体前屈 (cm)	6.6±11	3.7±6.7	
pre ファンクショナルリーチ (cm)	21.6±5.5	20.6±5.0	
post ファンクショナルリーチ (cm)	24.2±5	22.1±6.5	
pre 開眼片足立ち[b] (sec)	9.1±11.7	4.0±2.6	
post 開眼片足立ち (sec)	11.3±13.1	5.8±4.6*	
pre 握力[b] (kg)	17.7±8.7	19.6±7.4	
post 握力 (kg)	19.7±7.5	21.2±6.3	
pre 足関節背屈力[b] (kg)	8.7±2.1	10.7±3.6	#
post 足関節背屈力 (kg)	9.4±2.1	13.4±3.7**	

Values are Means±SD, TUG : Timed Up & Go, *$p<0.05$ vs pre, **$p<0.01$ vs pre,
a : $p<0.05$ vs ビタミンD服用群
b : 開眼片足立ち・握力・足関節背屈力は左右の平均値を用いた。
: $p<0.05$

(73.8%)，血清25OHD濃度（±SD）: 62.0±14.0 nmol/L（27.5～87.5），血清25OHD<50nmol/Lの割合: 18.0%，血清iPTH（±SD）: 48.7±22.5pg/mL（17.0～118.0）であった。年齢・性で調整後も，血清25OHDはiPTHと有意な負の相関(r=－0.38, p<0.01）を示していた。

2）血清25OHDと生活機能・身体機能との関連

一人で歩ける者は支えが必要な者に比し，週1回以上外出する者は未満の者に比し，生活活動能力指標総得点が11点以上の者は10点以下の者に比し，25OHD濃度は有意に高い値を示し，過去につまずき・ふらつきがよくあると回答した者はその他の者に比し，25OHD濃度が有意に低い値を示した（図1）。また，「つまずき・ふらつきがよくある」に影響する因子を年齢・性で調整した多重ロジスティック回帰分析を実施した結果，25OHD濃度が独立した影響因子であった（OR : 0.92, 95%信頼区間0.87～0.97）。

3）ビタミンD・乳酸カルシウム製剤服用有無と身体機能との関連

運動群（n=38）の希望者（n=17）にビタミンDとしてアルファカルシドール1μg/日と乳酸カルシウム4g/日を3ヵ月間投与し（服用群），希望しなかった者は非服用群（n=21）とした。開始時と3ヵ月目の身体測定データがある者を解析対象とし（服用群16名，非服用群16名），身体機能との関連を検討した（表1）。服用群と非服用群間で開始時の25OHD濃度，25OHD<50 nmol/Lの割合，TUG以外の体力測定値に有意差はみられなかった。開始時のTUGは服用群のほうが非服用群より有意に劣っていたが，3ヵ月目には有意差はみられなかった。1ヵ月目の採血で副作用があった者はいなかった。ビタミンD・乳酸カルシウム投与の効果を繰り返しのある2元配置分散分析を行った結果，足関節背屈力にビタミンD・乳酸カルシウム投与有無と時間との間に

交互作用がみられたが，その他の項目ではみられなかった。そこで，各群において，開始時と3ヵ月目の身体機能を比較検討した。服用群では，TUG・開眼片足立ち・足関節背屈力が有意に改善し，非服用群においてもTUGが有意に改善していた。さらに，開始時の25OHD濃度がどのように影響しているか各群で検討を試みたところ，50nmol/L以上の者は有意に改善していたが，50nmol/L未満の者は数が少なく統計的な解析は困難であったが，身体機能の改善はみられない傾向であった。

4 考　察

ビタミンDの欠乏は，転倒・骨折，下肢機能の低下や筋力の低下と関連があると報告されている。本研究の目的①として，起き上がり・立ち上がり・歩行能力の低下が特徴とされている地域在住の軽度要介護者と特定高齢者を対象に血清ビタミンD濃度とADL，身体機能との関連について横断的に検討した。屋外での移動能力として「支えが必要・一人で移動ができない者」，「過去1年間のつまずき・ふらつきがよくある者」，「外出回数が週1回未満の者」，「生活機能得点が10点以下の者」は，その他の者に比し，25OHD濃度は有意に低い値であり，また，25OHD濃度が1nmol/L上昇すると「つまずき・ふらつき」が8%低下することから，25OHD濃度は生活機能・歩行能力・バランス能力と関連があることが示唆された。

本研究の対象者の52.5%は過去1年間に1回以上の転倒経験があり，日本の地域在住高齢者の1年間の転倒発生率である約10〜20%[7]と比較すると，本研究対象者のほうが転倒歴のある者の割合が高かった。さらに，約74%が過去1年間につまずき・ふらつきの経験があり，将来，転倒・骨折へつながる可能性が非常に高い集団であることが推測されることから，軽度要介護者や特定高齢者の25OHD濃度を測定することは介護予防にとって重要と思われた。

ビタミンD補充の効果に関しては一致した見解はない[4,6]。さらに，日本の介護保険対象者へのビタミンD補充による介護予防効果に関する研究はほとんどみあたらない。本研究では目的②のビタミンDの補充効果について，介護予防教室に参加した運動群の希望者にビタミンDとしてアルファカルシドール1μg/日，乳酸カルシウム4g/日を3ヵ月間投与し（服用群），身体機能への効果を服用しなかった群（非服用群）と比較検討した。繰り返しのある2元配置分散分析により分析した結果，3ヵ月目の足関節背屈力は開始時に比し，ビタミンD・乳酸カルシウム製剤補充により有意に改善し，ビタミンD・乳酸カルシウムの補充は，脚筋力を改善し，将来の転倒を予防できる可能性が示唆された。しかし，その他の身体機能に関しては，ビタミンD・乳酸カルシウム製剤服用有無で身体機能に交互作用はみられなかった。そこで，各群で教室前後の身体機能を比較した結果，服用群ではTUG・開眼片足立ち・足関節背屈力が有意に改善した。さらに，非服用群でもTUGが有意に改善しており，その理由の一つとして，開始時の25OHD濃度が50nmol/L未満だと3年後には体力が低下しているという報告があるように[8]，開始時のビタミンD濃度がその後の体力に影響している可能性が考えられる。また，先のわれわれの横断研究により[9]，歩行能力やふらつきとの関連からビタミンD不足の閾値を25OHD＜50nmol/Lと設定した場合，各群とも開始時の25OHDが50nmol/L未満の者は教室終了時にも身体機能は改善しない傾向を示していた。開始時の25OHD濃度を少なくとも50nmol/L以上維持し，軽い運動を継続することで下肢筋力，歩行能力を維持改善できる可能性が推測されるが，本研究は対象者数が少ないことから，今後さらに対象者を増やし検討する必要がある。

結　論

地域在住の軽度要介護者・特定高齢者を対象とした場合，低い25OHD濃度は，「閉じこもり」高齢者，生活機能が低下している者，移動能力が劣っている者，バランス能力が低下している者と関連があった。また，運動の提供と同時にビタミンD・乳酸カルシウム製剤の補充は歩行能力・バ

ランス能力・下肢筋力を改善することが示唆された。

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ORIGINAL ARTICLE

Bone mineral density in post-menopausal female subjects is associated with serum antioxidant carotenoids

M. Sugiura · M. Nakamura · K. Ogawa · Y. Ikoma ·
F. Ando · M. Yano

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Abstract
Summary High intake of fruit and vegetables may reduce the risk of osteoporosis. Carotenoids exist in abundance in these foods. This study showed the association of bone mineral density with serum carotenoids. The findings suggest that β-cryptoxanthin and β-carotene might provide benefits to bone health in post-menopausal female subjects.
Introduction Antioxidant carotenoids are abundant in fruit and vegetables. Recent epidemiological studies show that high intakes of fruit and vegetables may reduce the risk of osteoporosis, but little is known about the association of bone mineral density (BMD) with serum carotenoids.
Methods A total of 699 subjects (222 males and 477 females) who had received health examinations in the town of Mikkabi, Shizuoka Prefecture, Japan, participated in the study. Radial BMD was measured using dual-energy X-ray absorptiometry (DXA). The associations of serum carotenoid concentrations with the radial BMD were evaluated cross-sectionally.
Results In male and pre-menopausal female subjects, the six serum carotenoids were not associated with the radial BMD. On the other hand, in post-menopausal female subjects, serum β-cryptoxanthin and β-carotene were weakly but positively correlated with the radial BMD. After adjustment for confounders, the odds ratio (OR) for the lowest quartile of BMD in the high groups (Q2–Q4) of serum β-cryptoxanthin against the lowest quartile (Q1) was 0.45 (95% confidence interval: 0.22–0.95) in post-menopausal female subjects. However, this association was not significant after further adjusting for intakes of minerals and vitamins.
Conclusions Antioxidant carotenoids, especially β-cryptoxanthin, significantly but partly associate with the radial BMD in post-menopausal female subjects.

Keywords Bone mineral density · Carotenoids · Fruit and vegetables · Post-menopausal female

M. Sugiura (✉) · K. Ogawa · Y. Ikoma · M. Yano
Research team for health benefit of fruit,
National Institute of Fruit Tree Science,
485-6 Shimizu-Okitsu-nakachou, Shizuoka-shi,
Shizuoka 424-0292, Japan
e-mail: msugiura@affrc.go.jp

M. Nakamura · F. Ando
Department of Epidemiology,
National Institute for Longevity Sciences,
Aichi, Japan

Introduction

Osteoporosis and related fractures are a major public health problem [1]. Osteoporosis is a chronic disease characterized by low bone density and microarchitectural disruption, leading to bone fragility and an increased susceptibility to fractures [2]. Nutrition is an important modifiable factor in the development and maintenance of bone health, and numerous studies on nutrition and bone health have been conducted [3, 4]. With regard to nutritional approaches to bone metabolism, calcium and vitamin D have been identified as important nutritional factors to maintain normal bone metabolism. Other nutrients, such as potassium, magnesium, zinc, copper, iron, vitamin C, and vitamin K, may also have beneficial effects. Furthermore, recent epidemiological studies have shown an association between fruit and vegetable intake with the bone mineral density (BMD) in both young and elderly subjects [5–10]. Fruit and vegetables are rich sources of nutrients for bone metabolism, such as potassium, magnesium, vitamin C and K, folate, and other constituents that may influence bone health, such as flavonoids. Therefore, the intake of these types of food might affect bone health.

Antioxidant carotenoids exist in abundance in fruit and vegetables, and its serum levels elevate with increasing fruit and vegetable intake [11–14]. Recent studies show that carotenoids have been known to contribute to the body's defense against reactive oxygen species [15, 16]. Oxidative stress is thought to play an important role in the development of several chronic diseases [17]. Therefore, antioxidant carotenoids may have a beneficial effect against oxidative stress-related chronic diseases. On the other hand, recent epidemiological studies have shown the relationship between oxidative stress and BMD or osteoporosis [18–21]. Although smoking is recognized as a major risk factor for osteoporotic fracture [22], Melhus et al. found that an insufficient dietary intake of antioxidant vitamins increased the risk of hip fracture in current smokers [18]. Furthermore, Maggio et al. reported a marked decrease in plasma antioxidants in aged osteoporotic women [20]. In this report, Maggio et al. demonstrated that plasma vitamins A, C, and E, and antioxidant enzyme activities in osteoporotic women were significantly lower than those in control subjects. Furthermore, very recently, they found that plasma levels of carotenoids and retinol in involutional osteoporotic women were significantly low compared with those in control subjects [23]. These results suggest that antioxidant carotenoids are beneficial micronutrients for the maintenance of normal bone metabolism. However, a nutritional epidemiological study about the association of BMD with serum antioxidant carotenoids has not been thoroughly studied.

The objective of this study was to investigate whether the BMD is higher in the presence of a high serum carotenoid concentration. The associations of six serum carotenoid concentrations, i.e., lutein, lycopene, α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin, with the radial BMD were evaluated cross-sectionally.

Subjects and methods

Subjects

In this survey, study subjects were recruited from participants in an annual health check-up program conducted by the local government of the town of Mikkabi, Shizuoka Prefecture, Japan in April 2005. Mikkabi is located in western Shizuoka, and about 40% of its residents work in agriculture. Fruit trees are the key industry in Mikkabi, which is an important producer of mandarin orange in Japan. A total of 1,891 males and females were subjects for the annual health check-up program. As results, 1,369 males and females (72.4% of total subjects), ranging in age from 30 to 70 years, had received the health check-up program. Participants were recruited for this study, and informed consent was obtained from 699 subjects (222 males and 477 females). The response rate was 51.1%. This study was approved by the ethics committee of the National Institute of Fruit Tree Science and the Hamamatsu University School of Medicine.

Serum carotenoid analysis

Blood samples were obtained in the morning after overnight fasting. Serum was separated from blood cells by centrifugation and stored at −80°C until analysis of the serum carotenoid concentrations. The concentrations of six serum carotenoids, lutein, lycopene, α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin, were analyzed by reverse-phase high-performance liquid chromatography (HPLC) using β-apo-8′-carotenal as an internal standard at the Laboratory of Public Health and Environmental Chemistry, Kyoto Biseibutsu Kenkyusho (Kyoto, Japan), as described previously [24].

Bone mineral density measurement

The radial BMD was measured using dual-energy X-ray absorptiometry (DXA) of each participant's non-dominant forearm with an osteometer (model DCS-600EX-III, ALOKA Co., LTD., Tokyo, Japan). This osteometer automatically measured the forearm length from the styloid process on the ulna, and DXA scan was automatically placed on the radial centered 1/3 of the forearm length. Calibration of the machine was performed daily, and quality assurance was performed by measuring the manufacturer's phantom. The CV of the radial BMD measurement was within 0.5%. In this study, the measurement of the radial BMD of each participant was performed by well-trained clinical technologist of the Seirei Preventive Health Care Center (Shizuoka, Japan).

Self-administered questionnaire

A self-administered questionnaire was used to collect information about a subject's history of osteoporosis, hormone use, and lifestyle, including tobacco use (current smoker, ex-smoker, or non-smoker), exercise (1+ times per week), regular alcohol intake (1+ times per 15 week), dietary supplement use (non-user, occasional-user, current-user), and dietary habits. Diet was assessed with a modified validated simple food-frequency questionnaire (FFQ) developed especially for the Japanese [25, 26]. In this FFQ, Wakai et al. selected a total of 97 foods and dishes through a two-step procedure: first by ranking food items according to the contribution to the population intake of energy and nutrients, and second by stepwise multiple regression analysis of individual food items as the independent variables and of total nutrient intake as the dependent variable. For simplicity, questions on portion sizes were not included except for a few selected food items; resulting in short time to complete the questionnaire. They validated this FFQ for food groups by referring to four 4-day dietary records (DRs), and correlation coefficients between FFQ and DRs were larger than 0.4 for most food groups. Information about alcohol consumption and the daily intake of 18 nutrients was estimated from the monthly food intake frequencies with either standard portion size (for most types of food) or subject-specified usual portion size (for rice, bread, and alcoholic and non-alcoholic beverages) using FFQ analysis software package for windows (Food Frequency Questionnaire System, System Supply Co., LTD., Kanagawa, Japan). This FFQ analysis software computes individual's foods and nutrients intake from FFQ data based on "Standard tables of food composition in Japan" [27, 28]. The intake of total energy, calcium, potassium, magnesium, and vitamins C, D, and E of each subjects was used in this report.

Statistical analysis

For this study, the following subjects were excluded from the data analysis: (1) those who reported a history of osteoporosis in the self-administered questionnaire (n=8); (2) those for whom the self-administered questionnaire datawere as incomplete (n=14); and (3) those for whom blood samples for serum-carotenoid analysis were not collected (n=1). As a result, a total of 222 male and 454 female subjects were included in further data analysis.

All subjects were categorized into three groups stratified by sex and menopausal status. Serum carotenoid concentrations and intake of vitamins C, D, and E were skewed toward the higher concentrations. These values were $\log_e$ (natural)-transformed to improve the normality of their distribution. Analysis of covariance adjusted for age followed by Bonferroni multiple comparisons was used to test between-group differences. All variables were presented as an original scale. The data are expressed as means (standard deviation), geometric mean (95% confidence interval), range, or percent.

The standard regression coefficients of serum carotenoid concentration with the radial BMD were calculated after adjusting for confounding factors by multiple linear regression analysis. In male and pre-menopausal female models, age, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and intake of total energy, calcium, magnesium, potassium, and vitamins C, D, and E were adjusted. In the post-menopausal female model, we adjusted further for years since menopause.

The multivariate adjusted mean of the radial BMD by the quartiles of the serum carotenoid concentration was calculated after adjusting for confounding factors. Differences in the multivariate adjusted mean of the radial BMD among each quartile of serum carotenoid concentration were tested by Bonferroni multiple comparison. The serum carotenoid concentrations were assigned to four categories, using the mean of the serum carotenoid concentrations in each quartile, and the associations among the radial BMD across four categories were assessed with a test for linear trends using linear regression.

Low radial BMD was defined as the lowest quartile of the value among study participants; i.e., equal to or less than 0.725 g/cm^2 in male subjects, equal to or less than 0.646 g/cm^2 in pre-menopausal female subjects, and equal to or less than 0.501 g/cm^2 in post-menopausal female subjects. To assess the relationship between the serum carotenoid concentrations with low radial BMD, logistic regression analyses were performed after adjusting for age, weight and height. Multivariable adjustment was further conducted to control for potential confounders. We did not adjust each carotenoid concentration in the multivariate models because Pearson's correlation analyses of serum carotenoid concentrations revealed significant positive correlations among all combinations of the six carotenoids.

The detection limit for the serum lycopene concentration for the method used in the study was 0.04 μg/mL (0.075 μmol/L), and the values below the limit of detection of the assay were marked as 0.03 μg/mL (0.056 μmol/L) in the analysis. All statistical analyses were performed using statistical software package for Windows (SPSS ver. 12.0J, SPSS Inc., Chicago, IL, USA) on personal computers.

Results

Clinical, biochemical and nutrient intake profiles of study subjects

Table 1 shows the characteristics of the study subjects stratified by sex and menopausal status. The radial BMD in pre- and post-menopausal female subjects was significantly lower than that in male subjects. In post-menopausal female subjects, the radial BMD was particularly low. Although the total energy intake including ethanol was significantly lower in pre- and post-female subjects than in male subjects, the total energy intake excluding ethanol did not differ among the three groups. The intake of calcium, potassium, and vitamins C, D, and E in post-menopausal female subjects were significantly higher than that in male subjects. The serum carotenoid concentrations in post-menopausal female subjects were significantly higher than those in male subjects. In comparison with those of pre-menopausal female subjects, the serum carotenoid concentrations of post-menopausal female subjects were not statistically but slightly higher, except for lycopene.

Table 1 Characteristics of the study subject stratified by sex or menopausal status[a]

	Male		Female					
			Pre-menopausal			Post-menopausal		
n	222		161			293		
Age (y)	56.1	(9.2)	44.1	(5.3)		60.2	(6.2)	
Body weight (kg)	165.8	(6.2)	156.0	(5.0)	g	152.0	(5.5)	g
Body height (cm)	65.5	(9.8)	54.6	(8.9)	g	51.9	(7.6)	g
Body mass index (kg/m²)	23.8	(2.9)	22.4	(3.7)	g	22.5	(3.0)	g
Total energy intake (MJ/day)								
Including ethanol	8.90	(2.04)	8.03	(1.83)	g	8.20	(2.01)	g
Excluding ethanol	8.31	(1.95)	7.96	(1.84)		8.15	(2.00)	
Calcium intake (mg/day)	517	(230)	566	(190)	f	651	(256)	g
Potassium intake (mg/day)	2448	(817)	2474	(799)		2910	(967)	g
Magnesium intake (mg/day)	265	(72)	242	(71)		281	(81)	
Vitamin C intake (mg/day)[b]	120	(112–129)	110	(102–119)		170	(161–179)	g
Vitamin D intake (μg/day)[b]	201	(184–219)	195	(177–215)		256	(238–276)	f
Vitamin E intake (mg/day)[b]	7.3	(7.0–7.6)	7.6	(7.2–8.0)		8.1	(7.8–8.4)	f
Bone mineral density (g/cm²)	0.771	(0.067)	0.677	(0.055)	g	0.561	(0.084)	d, g
Range		0.593–0.934		0.412–0.817			0.366–0.820	
Serum carotenoid concentrations (μmol/L)[b]								
Lutein	0.44	(0.42–0.46)	0.46	(0.44–0.48)	f	0.54	(0.51–0.56)	g
Lycopene	0.30	(0.28–0.32)	0.46	(0.43–0.49)	f	0.37	(0.35–0.39)	g
α-Carotene	0.12	(0.12–0.14)	0.19	(0.17–0.20)	g	0.21	(0.20–0.23)	g
β-Carotene	0.54	(0.50–0.59)	0.84	(0.77–0.91)	g	1.12	(1.06–1.18)	g
β-Cryptoxanthin	1.11	(0.98–1.25)	0.89	(0.79–1.01)		1.75	(1.61–1.90)	g
Zeaxanthin	0.19	(0.18–0.19)	0.19	(0.18–0.20)		0.20	(0.20–0.21)	e
Current tobacco use (%)	28.8		3.7			1.7		
Exercise habits (%)[c]	21.2		14.9			21.5		
Regular alcohol intake (%)[c]	59.9		19.9			11.0		
Current supplement use (%)	2.7		9.9			9.6		

[a] Data are mean (standard deviation), geometric mean (95% confidence interval), range, or percent
[b] These variables were represented as original scale after analysis by log (natural) transformed values
[c] >= 1 times/wk
[d] Significantly different vs. pre-menopausal female by analysis of covariance adjusted for age followed by Bonferroni multiple comparison test: [d] $P<0.001$
[e, f, g] Significantly different vs. male by analysis of covariance adjusted for age followed by Bonferroni multiple comparison test: [e] $P<0.05$, [f] $P<0.01$, [g] $P<0.001$

Multiple-linear regression analysis of the association of serum carotenoid with the radial BMD

In multiple linear regression analysis, after adjustment for age, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy intake, the radial BMD in male and pre-menopausal female subjects were not correlated with serum carotenoid concentrations (Table 2). On the other hand, in post-menopausal female subjects, the radial BMD was weakly but significantly correlated with serum β-carotene and β-cryptoxanthin concentrations after adjusting for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy intake (Table 2). After further adjusting for intake of calcium, magnesium, potassium and vitamins C, D, and E, a significant correlation was observed in β-cryptoxanthin. No other statistically significant correlations were observed.

Multivariate adjusted means of the radial BMD associated with the quartiles of each serum carotenoid concentrations

In post-menopausal female subjects, although no significant differences among each quartile were observed in all six serum carotenoids, the multivariate adjusted means of the radial BMD showed significant increasing trends under linearity with the quartiles of serum β-cryptoxanthin and β-carotene (Fig. 1). The results show the multivariate adjusted means of the radial BMD by quartiles of serum β-carotene