Table 2 Daily physical activity at baseline

Variables	Total (n = 230)	Men (n = 111)	Women (n = 119)
No. steps	7815 ± 3211	7601 ± 3300	8015 ± 3127
PAEE (kcal)	258 ± 115	271 ± 127	246 ± 102*
Adjusted PAEE (METs·h/wk)	3.09 ± 1.38	3.02 ± 1.43	3.15 ± 1.35
Time spent in light PA (%)	77.2 ± 12.2	76.1 ± 12.2	78.2 ± 12.2
Time spent in moderate PA (%)	21.5 ± 11.0	23.0 ± 11.9	20.0 ± 9.9*
Time spent in vigorous PA (%)	1.1 ± 1.4	0.9 ± 1.1	1.2 ± 1.5
Time spent in sedentary activity (min)	381 ± 230	436 ± 247	324 ± 188*

PAEE, physical-activity-related energy expenditure; METs, metabolic equivalents; PA, physical activity
*: $p < 0.05$ vs. men

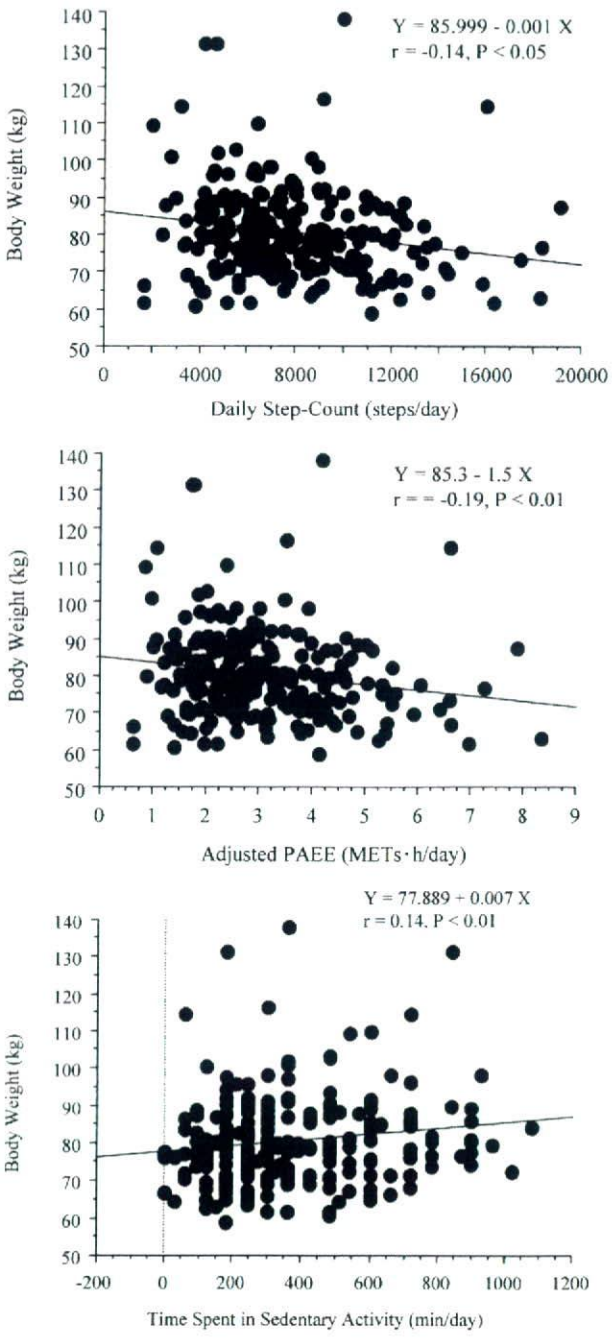

Fig. 1. Relationships between body weight and daily step-count (upper), adjusted physical activity-related energy expenditure (middle), and time spent in sedentary activity (bottom).

are notably lower in both men and women compared to the prevalence calculated using the International Diabetes Federation definition 14 based on waist circumference for Japanese (men: 77.6%, women: 72.3%), whereas only the values for women are lower using the American Heart Association/National Heart, Lung, and Blood Institute definition (men: 51.7%, women: 72.3%).[15]

The physical activity properties at baseline (*i.e.*, daily step-count, PAEE, adjusted PAEE, and time spent in light, moderate, and vigorous physical activity) are shown in ***Table 2***. The daily PAEE was significantly larger in men as compared with women. The time spent in moderate physical activity was longer in men than in women. In contrast, the time spent in sedentary activity in women was significantly shorter than that in men. There were no significant differences in other physical activity parameters between men and women. Although the association between occupation and PAEE was examined, there were no significant differences among the occupational categories (data not shown).

In all subjects, the daily step-count was closely related to the daily PAEE ($r=0.92$, $P<0.001$) and adjusted PAEE ($r=0.99$, $P<0.001$). The daily step-count was positively associated with the time spent in moderate physical activity ($r=0.35$, $P<0.001$), but negatively associated with time spent in light physical activity ($r=-0.30$, $P<0.001$). BMI was negatively correlated with the daily step-count ($r=-0.13$, $P<0.05$) and adjusted PAEE ($r=-0.14$, $P<0.05$). Moreover, body weight was negatively correlated to the daily step-count ($r=-0.19$, $P<0.01$, ***Figure 1, top***) and adjusted PAEE ($r=-0.18$, $P<0.01$, ***Figure 1, middle***). Visceral fat area was negatively and significantly correlated to the daily step-count ($r=-0.14$, $P<0.05$, ***Figure 2, top***) and adjusted PAEE ($r=-0.15$, $P<0.05$, ***Figure 2, bottom***). Abdominal

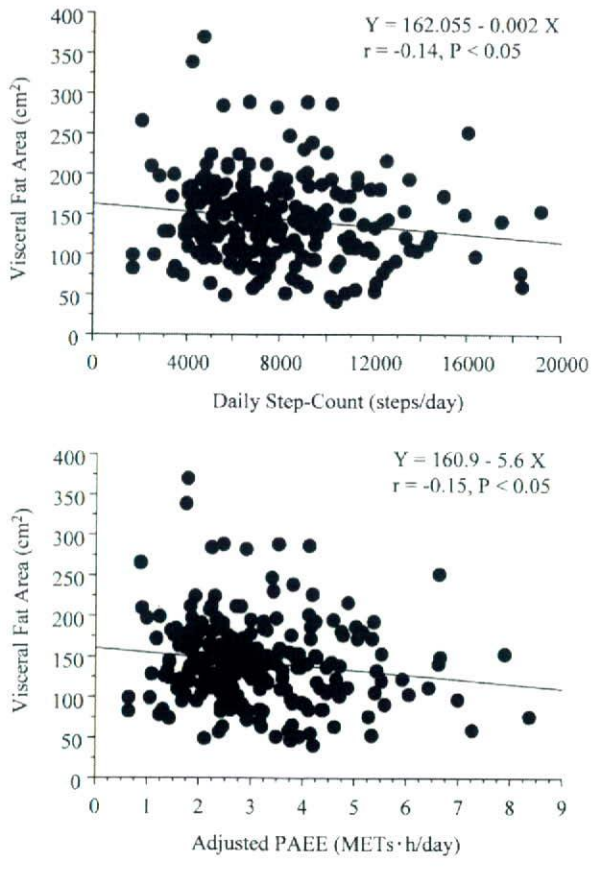

Fig. 2. Relationships between visceral fat area and daily step-count (upper), adjusted physical activity-related energy expenditure (middle), and time spent in sedentary activity (bottom).

circumference as a surrogate measurement of abdominal obesity was negatively and significantly related to the daily step-count ($r=-0.14$, $P<0.05$) and adjusted PAEE ($r=-0.16$, $P<0.05$). However, body weight had positive and significant correlations with daily PAEE ($r=0.15$, $P<0.05$) and the time spent in sedentary activity ($r=0.14$, $P<0.05$, ***Figure 1, bottom***). If all activities were weight-bearing, the PAEE would only be expected to be directly related to body weight.

Stepwise regression analysis showed that the daily step-count could be adopted as an independent variable for BMI and body weight, and adjusted PAEE could be adopted as an independent variable for visceral fat area and abdominal circumference.

Discussion

The main findings of this descriptive study were as follows. First, the mean daily step-count was 7,815 steps in all SCOP subjects, with no difference between men (7,601 steps) and women (8,015 steps). Second, the adjusted PAEE for body weight was 3.09 METs·h/day in all subjects, and there was no sex-related difference. The adjusted PAEE was somewhat smaller than the reference values for the quantity of physical activity for primary prevention of lifestyle-related diseases (3.3 METs·h/day) established by the Ministry of Health, Labour, and Welfare of Japan.[16] Third, the amount of physical activity (daily step-count and adjusted PAEE) was significantly and negatively related to body size (body weight and BMI) and abdominal fat (visceral fat area and abdominal circumference) in the pooled subjects, although the correlation coefficients were weak ($r=-0.1$ to -0.2).

Average daily step-count in Japanese men is generally greater than that in Japanese women as assessed by a national health and nutrition survey.[17] In the present study, the daily step-count in female subjects was about 1,400 steps/day greater than that in male participants. The unexpectedly higher daily step-count in the female subjects may be related to their slower walking speed and shorter stride than the male subjects. In fact, the time spent in moderate physical activity (brisk walking) by women was significantly shorter than that by men, and the time spent in light physical activity (slow walking) tended to be longer in women as compared with men.

In 2006 the Ministry of Health, Labour, and Welfare reexamined the recommended quantity of exercise for primary prevention of lifestyle-related diseases (originally proposed in 1989) and set reference values for the quantity of physical activity and exercise for Japanese people between the ages of 20 and 69 years. Specifically, for individuals who intend to promote health mainly through physical activity, walking 8,000 to 10,000 steps/day (23 METs·h/week) was set as the target daily amount of physical activity.[16] In the present study, the daily step-count and adjusted PAEE for body weight were 7,815 steps/day and 3.09 METs·h/day, respectively, which were somewhat lower than the reference values described above.

Several previous studies from the USA and UK indicated that daily step-counts in overweight and obese adults are lower than those in normal-weight peers.[18,19] The present study showed that adjusted PAEE and daily step-count were significantly and negatively correlated with visceral fat and abdominal circumference in the pooled overweight and obesity subjects. This is the first evidence that the amount of physical activity is partly associated with not only systemic obesity but also abdominal obesity. Furthermore, in accordance with the results of stepwise regression analysis, although daily step-count was an independent predictor of weight and BMI, adjusted PAEE was an independent predictor of abdominal obesity, *i.e.*, visceral fat area and abdominal circumference. As adjusted PAEE is determined by the duration and intensity of physical activity, accumulation of abdominal fat may be associated with not only the duration but also the intensity of physical activity. We should emphasize that the relationships between amount of physical activity and obesity variables were weak ($r=-0.1$ to -0.2). This implies that factors other than physical inactivity (*e.g.*, overeating) may strongly contribute to obesity in the SCOP subjects. To clarify the cause of obesity in SCOP subjects, the results from the uniaxial accelerometer should be compared with the responses to dietary history questionnaires.

Increasing physical activity and reducing caloric intake are indispensable for the improvement of excess weight and obesity. SCOP is a randomized control crossover study aiming to reduce visceral fat of overweight and obese subjects by interventions of physical activity and diet. Our systematic review suggested that an increase in adjusted PAEE at 10 METs·h/week (1.38 METs·h/day) is necessary to reduce visceral fat of overweight and obese subjects. The increase in daily step-count corresponds to an increase of almost 3,000 steps/day as compared with the baseline. Therefore, all SCOP subjects receive physical activity modification education so that their daily step-count increases gradually by 3,000 steps/day, and it is necessary to set the mean value of action targets for 11,000 steps/day and 4.5 METs·h/day.

The validity and reliability of the uniaxial accelerometer have been established.[6,7,10] One methodological limitation, however, is that a uniaxial accelerometer cannot measure very light physical activity (<1.8 METs).[7] Daily life includes a great deal of very light physical activity, and very light PAEE occupies more than the half of total PAEE. Therefore, we should emphasize that the PAEE obtained in the present study was not total PAEE but PAEE at 2METs intensity or more. Moreover, the cross-sectional study design is another limitation of the present study. The results of the present cross-sectional study must be confirmed prospectively with exercise intervention studies in future.

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References

1) Levine JA, Eberhardt NL, Jensen MD. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science 283:212-214, 1999.
2) Ravussin E, Bogardus C. Energy balance and weight regulation: genetics versus environment. Br J Nutr 83:S17-S20, 2000.
3) Weinsier RL, Hunter GR, Heini AF, et al. The etiology of obesity: relative contribution of metabolic factors, diet, and physical activity. Am J Med 105:145-150, 1998.
4) Ebina N, Shimada M, Tanaka H. Comparative study of total energy expenditure in Japanese men using doubly labeled water method against activity record, heart rate monitoring, and accelerometer methods. Jpn J Phys Fitness Sports Med 51:151-164, 2002. (in Japanese with English abstract)
5) Schutz Y, Ravussin E, Diethelm R, et al. Spontaneous physical activity measured by radar in obese and control subject studied in a respiration chamber. Int J Obes 6:23-28, 1982.
6) Suzuki I, Kawakami N, Shimizu H. Accuracy of calorie counter method to assess daily energy expenditure and physical activities in athletes and nonathletes. J Sports Med Phys Fitness 37:131-136, 1997.
7) Kumahara H, Schutz Y, Ayabe M, et al. The use of uniaxial accelerometry for the assessment of physical-activity-related energy expenditure: a validation study against whole-body indirect calorimetry. Br J Nutr 91:235-243, 2004.
8) Ohkawara K, Tanaka S, Miyachi M, et al. A dose–response relation between aerobic exercise and visceral fat reduction: systematic review of clinical trials. Int J Obes (Lond) 31:1786-1797, 2007.
9) Watanabe S, Morita A, Aiba N, et al. Study Design of the Saku Control Obesity Program (SCOP). Anti-Aging Med 4:70-74, 2007.
10) Schneider PL, Crouter SE, Lukajic O, et al. Accuracy and reliability of 10 pedometers for measuring steps over a 400-m walk. Med Sci Sports Exerc 35:779-784, 2003.
11) American College of Sports Medicine, ACSM's Guideline for Exercise Testing and Prescription, 7th ed. Lippincott Williams & Willkins, Phyiladelphia, 272-314, 2006.
12) Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 35:381-395, 2003.
13) Yoshizumi T, Nakamura T, Yamane M, et al. Abdominal fat: standardized technique for measurement at CT. Radiology 211:283-286, 1999.
14) Alberti KG, Zimmet P, Shaw J. Metabolic syndrome: a new world-wide definition—a consensus statement from the International Diabetes Federation. Diabet Med 23:469-480, 2006.
15) Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 112:2735-2752, 2005.
16) The Ministry of Health, Labour and Welfare, Japan. Exercise and Physical Activity Reference Quantity for Health Promotion 2006 (EPARQ2006) -Physical Activity, Exercise, and Physical Fitness-. Available at http://www.mhlw.go.jp/bunya/kenkou/undou02/pdf/data.pdf, 2006. (in Japanese)
17) The Ministry of Health, Labour and Welfare, Japan. National Health and Nutrition Survey 2006. Available at http://www.mhlw.go.jp/houdou/2006/05/h0508-1a.html, 2006. (in Japanese)
18) Clemes SA, Griffiths PL, Hamilton SL. Four-week pedometer-determined activity patterns in normal weight and overweight UK adults. Int J Obes (Lond) 31:261-266, 2007.
19) Chan CB, Spangler E, Valcour J, et al. Cross-sectional relationship of pedometer-determined ambulatory activity to indicators of health. Obes Res 11:1563-1570, 2003.

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Original Article

Accuracy of Predictive Equations for Basal Metabolic Rate and Contribution of Abdominal Fat Distribution to Basal Metabolic Rate in Obese Japanese People

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Abstract

BACKGROUND: Large errors may occur when predicting basal metabolic rate (BMR) based on physical characteristics in obese people. In addition, the contribution of abdominal visceral fat to BMR remains controversial. This study examined the accuracy of several predictive equations for BMR and the contribution of abdominal fat distribution to BMR in obese Japanese participants in the Saku Control Obesity Program (SCOP).

METHODS: BMR was determined using a mask and Douglas bag in adult males (n = 12) and females (n = 11). We measured abdominal subcutaneous and visceral fat areas using computerized tomography.

RESULTS: All the equations, with the exception of Bernstein's, overestimated BMR in obese males. Some equations, including the Japan-Dietary Reference Intakes and the Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University equations, overestimated BMR in obese females, while the Harris-Benedict and Henry equations provided relatively accurate predictions of BMR in obese females. We found no correlation between abdominal visceral fat area and BMR when adjusted for sex, fat-free mass, and abdominal subcutaneous fat area (partial r =−0.022). Abdominal subcutaneous fat area correlated significantly with BMR when adjusted for sex, fat-free mass, and abdominal visceral fat area (partial r = 0.732), although this correlation was no longer significant after adjustment for total fat mass (partial r = 0.266).

CONCLUSIONS: In obese Japanese subjects, most the predictive equations overestimated BMR in males, whereas some equations were relatively accurate for females. Our findings indicate abdominal fat distribution may not be independently related with BMR.

KEY WORDS: Basal metabolic rate, obese, predictive equation, abdominal visceral fat

Introduction

Basal metabolic rate (BMR) constitutes the largest component of total energy expenditure in the majority of people. Because BMR can be predicted from simple anthropometric measurements, it is often used to estimate total energy expenditure.

Many equations have been developed for estimating basal or sleeping metabolic rates based on anthropometric measurements, age, and sex.[1,2] These equations can be helpful when actual metabolic measurements are not available. It has been shown in Caucasians, however, that BMR is considerably more difficult to predict in obese than in normal-weight subjects.[2-5] Studies have found that predictive equations overestimate BMR and/or that large prediction errors may occur in obese subjects.[2-5] In addition, most of the equations currently available apply only to Caucasians. The validity of the predictive equations has not been examined in obese Japanese subjects, despite several studies showing that some of the predictive equations are not applicable to nonwhite populations.[6-9]

In addition, the contribution of abdominal fat distribution to BMR remains controversial. Some studies have shown a relationship between abdominal visceral fat (AVF) area and BMR or resting metabolic rate,[10-14] whereas others have not.[15-17] To our knowledge, no study has examined these relationships in Japanese subjects using computerized tomography (CT), with the exception of Okura et al, who investigated the relationship in healthy elderly subjects.[14] They reported that adjusted resting energy expenditure correlated inversely with AVF but not with abdominal subcutaneous fat (ASF). While significant, this relationship with AVF was relatively weak (r = −0.131).

In the present study, we examined the validity of predictive equations for BMR in obese Japanese men and women. The contribution of abdominal fat distribution, as measured by CT, to BMR was also examined.

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Methods

Subjects

The subjects in the study were 50- to 54-year-old obese subjects (12 males and 12 females) residing in Saku city. They were randomly selected from among the participants in the Saku Control Obesity Program (SCOP), the details of which are described elsewhere in this supplement.[18,19] The measurements of BMR for one of the female subjects failed; therefore, data for 12 males and 11 females were used in the present study.

The study protocol was approved by the Ethics Committee of the National Institute of Health and Nutrition and the Ethics Committee of Saku Central Hospital. The study protocol was explained to the subjects prior to enrollment, and all subjects provided their informed consent.

Basal Metabolic Rate

The subjects reported to the hospital for the series of measurements at approximately 8 am on the study day. BMR was measured in the supine position and in the post-absorptive state (12 hours or longer after the last meal). The temperature in the room was controlled at 24–26°C. The measurement was performed using a mask and Douglas bag for 20 minutes with 1 minute of intermission. The volume of expired air was measured with a certified dry gas meter (Shinagawa DC-5, Tokyo, Japan). The expired air was sampled and the O_2 and CO_2 concentrations were measured using a gas analyzer (Arco System, AR-1, Kashiwa, Japan) with a galvanic O_2 sensor and an infrared CO_2 sensor. For each of the consecutive measurements, the gas analyzer was calibrated initially using atmospheric air. The values of O_2 consumption and CO_2 production were expressed under standard temperature, pressure, and dry air conditions. BMR was estimated from O_2 consumption and CO_2 production using Weir's equation.[20]

Anthropometric Measurements

Body weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm using a stadiometer. The measurements were performed in light clothing and underwear. Body mass index (BMI) was calculated as weight (kg) divided by square of height (m^2). Percentage body fat was evaluated by the bioelectric impedance method (Tanita, BF-220, Tokyo, Japan).

To assess ASF and AVF levels, a CT scan was performed at the level of the umbilicus, with the subject in the supine position. ASF and AVF areas were determined using commercially available software (Fat Scan; N2 System Corp., Osaka, Japan).[21] The attenuation range of CT numbers for ASF was set as the mean ± 3 standard deviation (SD).

Predictive Equations of Basal Metabolic Rate

The predictive equations of Japan-Dietary Reference Intakes (DRI),[22] Harris and Benedict,[23] the Food and Agriculture Organization of the United Nations/World Health Organization /United Nations University (FAO/WHO/UNU),[24] Henry,[25] Owen,[26,27] Mifflin,[28] and Bernstein[29] were evaluated (*Table 1*). For the Japan-DRI equations, the Ministry of Health and Welfare proposed adjusting for body weight.[30] Therefore, the equations including this adjustment were also examined. For the FAO/WHO/UNU equations, those using body weight only are often used. However, in the present study, equations using body weight and height were also examined.

Statistical Analyses

The results are presented as the mean ± SD. The % difference of the prediction error was calculated as the residual divided by the measured value for each subject. The relationship between measured and predicted values of BMR and anthropometric measurements was examined using Pearson's correlation. Sex was treated as a binomial variable (0 for males, 1 for females) and was adjusted for in the partial correlation analysis. Adjustment for age was not performed because the range was small (50–54 years). Statistical significance was set at $p < 0.05$ for all predictors. The statistical analyses were performed using SPSS® for Windows (version 14.0; SPSS Inc., Chicago, IL, USA).

Table 1 Predictive equations for basal metabolic rate used in the present study

Predictive equations (kcal/day)	Males	Females
Japan-DRI [22]	weight × 21.5	weight × 20.7
Japan-DRI with adjustment for body weight [22,30]	[weight + (10.8 - 0.173 × weight)] × 21.5	[weight + (10.8 - 0.172 × weight)] × 20.7
Harris and Benedict [23]	66 + (13.7 × weight) + (5.0 × height) − (6.8 × age)	665 + (9.6 × weight) + (1.8 × height) − (4.7 × age)]
FAO/WHO/UNU (body weight) [24]	879 + (11.6 × weight)	829 + (8.7 × weight)
FAO/WHO/UNU (body weight and height) [24]	901 + (11.3 × weight) + (16.0 × height/100)	865 + (8.7 × weight) − (25.0 × height/100)
Henry [25]	[(59.2 × weight + 2480)]/4.184	[(40.7 × weight + 2900)]/4.184
Owen [26,27]	879 + (10.20 × weight)	795 + (7.18 × weight)
Mifflin [28]	5 + (9.99 × weight) + (6.25 × height) − (4.92 × age)	−161 + (9.99 × weight) + (6.25 × height) − (4.92 × age)
Bernstein [29]	−1032 + (11.0 × weight) + (10.2 × height) − (5.8 × age)	844 + (7.48 × weight) + (0.42 × height) − (3.0 × age)

weight: kg, height: cm, age: year.
Predictive equations for 50 to 54-yr-old obese subjects were used.

Results

The physical characteristics of the subjects are summarized in *Table 2*. There was a similar degree of correlation between the measured and predicted values of BMR for the various predictive equations (r = 0.839–0.859). The relationships between measured and predicted BMR based on the Japan-DRI, DRI-adjusted, Harris-Benedict, and Bernstein equations are shown in *Figures 1 and 2*. In obese males, the majority of equations overestimated BMR, particularly for those with lower BMR (*Figure 1*), whereas Bernstein's equation significantly underestimated BMR (*Table 3*). In particular, the Japan-DRI and FAO/WHO/UNU equations overestimated BMR to the greatest extent. The Mifflin equation provided a better prediction of BMR, while the equation overestimated BMR. In obese females, the Japan-DRI and FAO/WHO/UNU equations overestimated BMR, whereas the Harris-Benedict and Henry equations provided a relatively accurate prediction of BMR (*Figure 2*). In both sexes, adjustment for body weight in the Japan-DRI equations attenuated the overestimation of BMR, although the value still remained too high. In addition, the FAO/WHO/UNU equations with or without body height provided almost identical values. The SD of the % difference of the predicted BMR was comparable between the various equations.

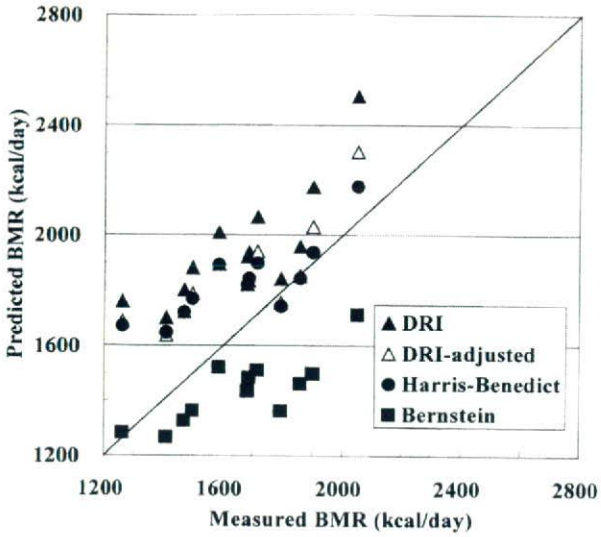

Fig. 1. Relationships between measured and predicted basal metabolic rate (BMR) in obese males. DRI: Japan-Dietary Reference Intakes.

Following adjustment for sex and fat-free mass, BMR correlated significantly with ASF (r = 0.806, p < 0.001) and AVF (r = 0.493, p < 0.05). When sex, fat-free mass, and ASF were adjusted for, BMR was not correlated with AVF (r = −0.022, n.s.). The relationships between AVF and residual of BMR adjusted for sex, fat-free mass, and ASF is shown in *Figure 3*. On the other hand, BMR correlated significantly with ASF when adjusted for sex, fat-free mass, and AVF (r = 0.732, p < 0.001); however, this correlation was no longer significant after additional adjustment for total fat mass (r = 0.266, n.s.).

Table 2 Physical characteristics of subjects

	Mean± SD	Range
Males		
Age (year)	52 ± 1	50.0 - 54.0
Body height (cm)	172.8 ± 3.9	168.8 - 179.2
Body weight (kg)	91.3 ± 10.0	79.1 - 116.5
Body mass index (kg/m^2)	30.6 ± 3.3	27.7 - 39.2
Percentage of body fat (%)	28.3 ± 4.9	23.4 - 39.2
Abdominal subcutaneous fat area (cm^2)	272 ± 80	163 - 436
Abdominal visceral fat area (cm^2)	165 ± 51	98 - 289
Females		
Age (year)	53 ± 2	50.0 - 54.0
Body height (cm)	158.6 ± 5.8	152.0 - 169.3
Body weight (kg)	82.5 ± 12.2	69.3 - 109.7
Body mass index (kg/m^2)	32.7 ± 3.8	28.4 - 40.0
Percentage of body fat (%)	44.3 ± 7.0	34.7 - 62.5
Abdominal subcutaneous fat area (cm^2)	383 ± 96	250 - 619
Abdominal visceral fat area (cm^2)	140 ± 57	84 - 266

SD: standard deviation

Table 3 Measured and predicted basal metabolic rate in obese people

	Values(kcal/day)		%difference(%)	
	Mean± SD	Range	Mean± SD	Range
Males				
Measured	1659 ± 226	1262 - 2051		
Predicted				
Japan-DRI [22]	1963 ± 216	1701 - 2505	19.2 ± 9.8	2.7 - 39.6
Japan-DRI with adjustment for body weight [22,30]	1856 ± 178	1639 - 2304	12.8 ± 9.7	-2.1 - 33.8
Harris and Benedict [23]	1831 ± 142	1648 - 2178	11.4 ± 10.0	-2.9 - 32.5
FAO/WHO/UNU (body weight) [24]	1938 ± 116	1797 - 2230	18.2 ± 12.0	4.2 - 45.0
FAO/WHO/UNU (body weight and height) [24]	1961 ± 113	1822 - 2245	19.6 ± 12.3	5.4 - 46.9
Henry [25]	1885 ± 142	1712 - 2241	14.8 ± 10.7	0.7 - 38.8
Owen [26,27]	1811 ± 102	1686 - 2067	10.5 ± 11.4	-2.7 - 35.9
Mifflin [28]	1744 ± 108	1601 - 1996	6.3 ± 10.3	-6.4 - 28.2
Bernstein [29]	1436 ± 125	1267 - 1713	-12.7 ± 7.4	-24.0 - 1.7
Females				
Measured	1477 ± 210	1192 - 1895		
Predicted				
Japan-DRI [22]	1709 ± 253	1435 - 2271	15.8 ± 7.2	-1.8 - 24.5
Japan-DRI with adjustment for body weight [22,30]	1599 ± 210	1372 - 2064	8.6 ± 6.6	-6.2 - 18.5
Harris and Benedict [23]	1496 ± 126	1367 - 1777	2.1 ± 8.0	-7.5 - 15.7
FAO/WHO/UNU (body weight) [24]	1547 ± 106	1432 - 1783	5.8 ± 9.2	-5.9 - 21.9
FAO/WHO/UNU (body weight and height) [24]	1543 ± 106	1430 - 1778	5.6 ± 9.2	-6.2 - 21.6
Henry [25]	1496 ± 119	1367 - 1760	2.2 ± 8.2	-7.1 - 16.7
Owen [26,27]	1388 ± 88	1293 - 1583	-5.0 ± 8.6	-16.5 - 9.9
Mifflin [28]	1396 ± 149	1240 - 1719	-4.9 ± 7.0	-15.0 - 5.9
Bernstein [29]	1370 ± 94	1273 - 1581	-6.3 ± 8.1	-16.6 - 7.7

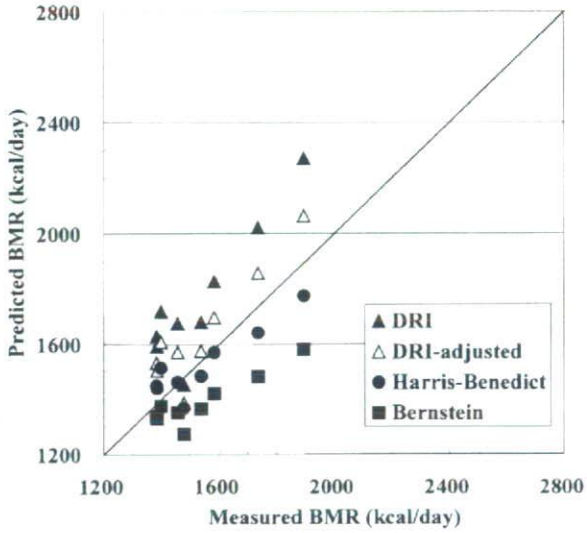

Fig. 2. Relationships between measured and predicted basal metabolic rate (BMR) in obese females. DRI: Japan-Dietary Reference Intakes.

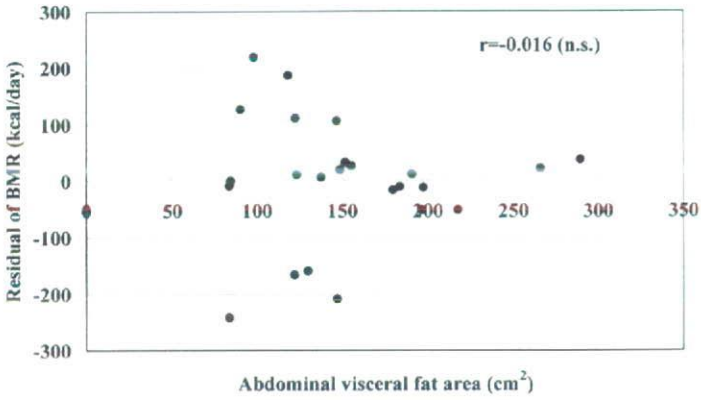

Fig. 3. Relationships between AVF and residual of BMR adjusted for sex, fat-free mass, and ASF.

Discussion

This study examined the accuracy of predictive equations for BMR in obese Japanese people. The findings indicate that many of the equations, including the Japan-DRI and FAO/WHO/UNU equations, overestimate BMR, particularly in obese males. Similar results have been reported in many studies on Caucasians. However, several of the equations provided accurate estimates of BMR, mainly in obese females. Among the equations, the Mifflin equation in males, and the Harris-Benedict and Henry equations in females, provided a better accurate prediction of BMR.

The Japan-DRI equations are simple multiples of body weight (21.5 × body weight for males, 20.7 × body weight for females). The other equations include an intercept in addition to a term for body weight and in some cases terms for body height and age as well. Considering these differences, it is understandable that the Japan-DRI equations overestimate BMR in obese subjects of both sexes. To improve this, a term for adjustment of body weight was provided for each sex.[30] While this term reduced the overestimation of BMR, a large overestimation of BMR (12.8 ± 9.7 % for males and 8.6 ± 6.6 % for females) still remained. The suggested values for adjustment of body weight were obtained in rather lean Japanese subjects, with fat-free mass contributing considerably more to BMR than fat mass. Therefore, a low fat-free mass relative to body weight (indicated as a high percentage of body fat) may explain the overestimation, even after additional adjustment for body weight.[1] In contrast, Bernstein's equation, which was developed for obese Caucasians, underestimated BMR, especially in obese males.

The other equations incorporate an intercept, and some of them have terms for body height and age. Thus, it is expected that the terms adjust BMR for body composition to some degree, similar to BMI. However, the FAO/WHO/UNU equations that include terms for body weight and height provided comparable values to those with a term for body weight only. Considering the mean values of % difference, inclusion of terms for body height and/or age is not likely to improve the prediction of BMR in obese people, whereas the existence of an intercept or a curvilinear term would be expected to improve the predictive ability of the equation.

With the exception of the Japan-DRI equation, the predictive equations did not overestimate BMR to a large extent in obese females. This sex difference was not observed in previous studies. Female subjects in the present study had slightly higher BMI than male subjects, and their percentage body fat was also greater. While these differences should have been associated with overestimation of BMR considering the result of the present study, this can not explain the sex difference. Thus, the reason for the observed sex difference in the present study remains unclear.

The SD values of the % difference ranged from 7.4% to 12.3% in obese males and 6.6% to 9.2% in obese females. Previous studies reported that the interindividual coefficient of variation was about 8-13% in healthy people,[31,32] although interindividual variability of sleeping metabolic rate was less, at least for Japanese subjects.[9] The values calculated in the present study were within this range, but different from those of a previous study of obese subjects.[3] A possible reason for this difference may have been the uniformity of body composition, although the range of percentage body fat in the present study was large in both sexes, suggesting that this was unlikely to be the reason.

It remains controversial whether AVF is related to BMR.[10-17] Because AVF is related to sex, fat mass, and ASF, it is necessary to adjust for these variables in order to examine the relationship between AVF and BMR. In the present study, we adjusted not only for sex and fat-free mass but also for ASF to clarify the independent contribution of abdominal fat distribution. As a result, ASF but not AVF correlated independently with BMR after adjustment for sex and fat-free mass. However, this significant correlation disappeared after additional adjustment for fat mass, indicating that the independent correlation between ASF and BMR may actually reflect the relationship between fat mass and BMR. Adipose tissue has a small but definite contribution to BMR, while ASF and fat mass are correlated with each other, particularly in obese people, who have a large fat mass. If the relationship between ASF and BMR reflects the relationship between fat mass and BMR, this implies that abdominal fat distribution is not associated with BMR in obese Japanese people.

One of the limitations of the present study was the relatively small sample size. However, to obtain values of % difference when using predictive equations of BMR, the sample size used should provide relatively stable results. In the present study, normal-weight subjects were not included. To clarify the characteristics of obese subjects, this may be another problem. In

addition, the analysis evaluating the contribution of abdominal fat distribution was performed in all subjects with adjustment for the effect of sex. As a consequence, comparison with the results of earlier studies could not be undertaken. Some of these previous studies reported the results for separate age categories, sex, and menopausal status.

In conclusion, the majority of the predictive equations overestimated BMR in obese Japanese males, whereas some equations were relatively accurate for obese females. In obese people, overestimated BMR may lead to overestimation of total energy expenditure. Caution is therefore needed when selecting predictive equations of BMR for obese Japanese people. Our results indicate abdominal fat distribution was not independently related to BMR.

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References

1) Cunningham JJ. Body composition as a determinant of energy expenditure: a synthetic review and a proposed general predictive equation. Am J Clin Nutr 54:963-969, 1991.
2) Frankenfield D, Roth-Yousey L, Compher C. Comparison of predictive equations for resting metabolic rate in healthy nonobese and obese adults: a systematic review. J Am Diet Assoc 105:775-789, 2005.
3) Foster GD, McGuckin BG. Estimating resting energy expenditure in obesity. Obes Res 9(Suppl.5):S367-S372, 2001.
4) Siervo M, Boschi V, Falconi C. Which REE prediction equation should we use in normal-weight, overweight and obese women? Clin Nutr 22:193-204, 2003.
5) Frankenfield DC, Rowe WA, Smith JS, et al. Validation of several established equations for resting metabolic rate in obese and nonobese people. J Am Diet Assoc 103:1152-1159, 2003.
6) Case KO, Brahler CJ, Heiss C. Resting energy expenditures in Asian women measured by indirect calorimetry are lower than expenditures calculated from prediction equations. J Am Diet Assoc 97:1288-1292, 1997.
7) Liu HY, Lu YF, Chen WJ. Predictive equations for basal metabolic rate in Chinese adults: a cross-validation study. J Am Diet Assoc 95:1403-1408, 1995.
8) Yamamura C, Kashiwazaki H. Factors affecting the post-absorptive resting metabolic rate of Japanese subjects: reanalysis based on published data. Jpn J Nutr Diet 60:75-83, 2002.
9) Ganpule AA, Tanaka S, Ishikawa-Takata K, et al. Interindividual variability in sleeping metabolic rate in Japanese subjects. Eur J Clin Nutr 61:1256-1261, 2007.
10) Leenen R, van der Kooy K, Deurenberg P, et al. Visceral fat accumulation in obese subjects: relation to energy expenditure and response to weight loss. Am J Physiol 263:E913-E919, 1992.
11) Busetto L, Perini P, Giantin V, et al. Relationship between energy expenditure and visceral fat accumulation in obese women submitted to adjustable silicone gastric banding (ASGB). Int J Obes Relat Metab Disord 19:227-233, 1995.
12) Armellini F, Zamboni M, Mino A, et al. Postabsorptive resting metabolic rate and thermic effect of food in relation to body composition and adipose tissue distribution. Metabolism 49:6-10, 2000.
13) Sharp TA, Bell ML, Grunwald GK, et al. Differences in resting metabolic rate between white and African-American young adults. Obes Res 10:726-732, 2002.
14) Okura T, Koda M, Ando F, et al. Relationships of resting energy expenditure with body fat distribution and abdominal fatness in Japanese population. J Physiol Anthropol Appl Human Sci 22:47-52, 2003.
15) Armellini F, Robbi R, Zamboni M, et al. Resting metabolic rate, body-fat distribution, and visceral fat in obese women. Am J Clin Nutr 56:981-987, 1992.
16) Nicklas BJ, Goldberg AP, Bunyard LB, Poehlman ET. Visceral adiposity is associated with increased lipid oxidation in obese, postmenopausal women. Am J Clin Nutr 62:918-922, 1995.
17) Macor C, Ruggeri A, Mazzonetto P, et al. Visceral adipose tissue impairs insulin secretion and insulin sensitivity but not energy expenditure in obesity. Metabolism 46:123-129, 1997.
18) Watanabe S, Morita A, Aiba N, et al. Study design of the Saku Control Obesity Program (SCOP). Anti-Aging Med 4:70-73, 2007.
19) Morita A, Ohmori Y, Suzuki N, et al. Anthropometric and clinical findings in obese Japanese: the Saku Control Obesity Program (SCOP). Anti-Aging Med 5:13-16, 2008.
20) Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 109:1-9, 1949.
21) Yoshizumi T, Nakamura T, Yamane M, et al. Abdominal fat: standardized technique for measurement at CT. Radiology 211:283-286, 1999.
22) Ministry of Health, Labour and Welfare. Japan Dietary Reference Intakes for Japanese, 2005. Daiichi shuppan: Tokyo, 29, 2005. (in Japanese)
23) Harris JA, Benedict GF. A Biometric Study of Basal Metabolism in Man. Publication no. 297. Carnegie Institute, Washington DC, 1919.
24) FAO/WHO/UNU. Energy and protein requirements: report of a joint FAO/WHO/UNU expert consultation. WHO Tech Rep Ser 724:1-206, 1985.
25) Henry CJ. Basal metabolic rate studies in humans: measurement and development of new equations. Public Health Nutr 8:1133-1152, 2005.
26) Owen OE, Holup JL, D'Alessio DA, et al. A reappraisal of the caloric requirements of men. Am J Clin Nutr 46:875-885, 1987.
27) Owen OE, Kavle E, Owen RS, et al. A reappraisal of caloric requirements in healthy women. Am J Clin Nutr 44:1-19, 1986.
28) Mifflin MD, St Jeor ST, Hill LA, et al. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 51:241-247, 1990.
29) Bernstein RS, Thornton JC, Yang MU, et al. Prediction of the resting metabolic rate in obese patients. Am J Clin Nutr 37:595-602, 1983.
30) Ministry of Health and Welfare, Japan. Recommended Dietary Allowances for the Japanese, revision in 1975. Ministry of Health and Welfare, Tokyo, 23-48, 1975. (in Japanese)
31) Shetty PS, Henry CJ, Black AE, et al. Energy requirements of adults: an update on basal metabolic rates (BMRs) and physical activity levels (PALs). Eur J Clin Nutr 50:S11-S23, 1996.
32) Muller MJ, Bosy-Westphal A, Klaus S, et al. World Health Organization equations have shortcomings for predicting resting energy expenditure in persons from a modern, affluent population: generation of a new reference standard from a retrospective analysis of a German database of resting energy expenditure. Am J Clin Nutr 80:1379-1390, 2004.

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Original Article

DNA polymorphism of obese people in Saku Control Obesity Program (SCOP)

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Abstract

BACKGROUND: Various genes related to fat-, sugar- or energy-metabolism are suspected that their polymorphisms (SNPs) are susceptible to development of obesity and other metabolic syndromes. Although many lines of evidences are accumulated, inspections as to their relation are still insufficient. Here, we assembled 235 obese people in Saku Control Obesity Program (SCOP), and are going to investigate the association between those SNPs, body mass index (BMI) and other clinical parameters.

METHODS: 235 subjects, whose BMI belonged in upper quintile (over 28.3), are recruited from examinees for periodic medical checkup in the Health Dock of Saku Central Hospital (Nagano, Japan). Genotyping for the SNPs were conducted using the PCR-PFLP method from peripheral venous blood. The frequencies of the SNPs in UCP1 (-3826A/G), UCP2 (Ala55Val), UCP3 (-55C/T), PPARγ2 (Pro12Ala), adiponectin (Ile164Thr), leptin receptor (LEPR, Arg109Lys), calpain 10 (SNP43), β2AR (Arg16Gly, Gln27Glu), β3AR (Trp64Arg) were analyzed and associations between those SNPs, body mass index (BMI) and other clinical parameters were investigated. The frequency was compared with those in HapMap Project or in Nansei Cohort..

RESULTS: Frequencies of above SNPs in SCOP were not different from those of healthy people in Nansei Cohort or HapMapProject, except for adiponectin Ile164Thr. SNPs of UCPs or β2, β3AR genes, often reported their association with BMI, were not confirmed in obese subjects in SCOP. We found significant association in the LEPR Arg109Lys in woman; Lys/Lys minor homozygotes had higher levels of leptin, TNF-α, C-peptide, insulin, triglyceride and fasting serum glucose, and also, the genotype showed a trend of higher values of HbA1c, BMI, body fat mass and waist circumference.

CONCLUSION: Adipocytokines, such as leptin and adiponectin, may play an important role in the development of metabolic syndrome, and they would be influenced by polymorphism of UCP or β2, β3AR genes.

KEY WORDS: SNPs, lipokine, Obesity, Epidemiology, Intervention study

Introduction

Metabolic syndrome is defined as a combination of disorders that increase one's risk for cardiovascular disease and diabetes mellitus type 2. Particularly, visceral obesity is shown to predispose to these syndromes. Although lifestyle is important, there is considerable evidence that genetic factors also have a significant role in its pathogenesis.[1)]

Various genes related to fat-, sugar- or energy-metabolism are suspected that their polymorphisms (SNPs) are susceptible to develop obesity and other metabolic syndrome. Uncoupling protein (UCP) 1, 2 or 3, adrenergic receptor (AR) β2 or β3, peroxisome proliferator-activated receptor (PPAR) γ2, adiponectin, leptin receptor (LEPR), calpain 10 are comprised in these genes. Although many lines of evidences are accumulating, inspections as to their relation are still insufficient.

Here, we assembled 235 obesity people in Saku Contorol Obestity Program (SCOP), and are going to investigate the association between those SNPs, body mass index (BMI) and other clinical parameters. Genotyping for their SNPs were conducted using the PCR-PFLP method from peripheral blood after taking written informed consent. The frequencies of the SNPs in UCP1 (-3826A/G), UCP2 (Ala55Val), UCP3 (-55C/T), PPARγ2 (Pro12Ala), adiponectin (Ile164Thr), leptin receptor (LEPR, Arg 109 Lys), calpain 10 (SNP43), β2AR (Arg16Gly, Gln27Glu), β3AR (Trp64Arg) were compared with those of healthy people published in HapMap Project or those previously analyzed in Nansei Cohort (n=422, BMI=23.3).

HapMap is a public database of common variation in the human genome exhibited by The International HapMap Consortium.[2)] The map includes information about more than one million SNPs obtained in 269 DNA samples from four populations; Yoruba in Ibadan, Nigeria; Utah, USA; Beijing, China; Tokyo, Japan. Some SNPs were also analyzed in Nansei Cohort. Total 422 healthy people were recruited from the group in 2001 and 2002, who received periodic medical checkup in the Nansei-cho Hospital (Mie Prefecture, Japan).

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Method

· Subjects

Japanese obese subjects aged 40-64 years old with a BMI greater than 28.3 were selected from people who had undergone a medical checkup in the Saku Central Hospital. They were asked to participate in the intervention program for weight loss named Saku Control Obesity Program (SCOP). The participants had an anthropometric and clinical examination (height, weight, body fat percentage, waist circumference, visceral fat area, and biochemical markers of blood and urine) and were assessed for present illness, physical activity and dietary habit at the start of this program. Details are described in elsewhere.[3,4]

· Genotyping and Statistical Analysis

DNA was purified from subjects' blood using QIAamp DNA blood 96 kit (Qiagen). All the SNPs were genotyped by a polymerase chain reaction (PCR) followed by digestion by restriction enzyme (PCR-RFLP method) *(Table 1)*. 80 ~ 200 bp fragments containing objective SNP were amplified in a 20 μl reaction mixture including 50 mM Tris-HCl, pH9.0, 20 mM ammonium sulfate, 0.7 mM $MgCl_2$, 0.2 mM 4dNTPs, 1.25 units Taq Pol (ToYoBo), genomic DNA and 12.5 pmol of each primer (with/without 1 or 2 bases mismatch). PCR was conducted as follows: 10 min at 94°C as initial denature, 35 cycles of 2 min at 94°C, 2 min at 52°C, 1 min at 72°C, and 10 min at 72°C as final extension. PCR product was digested with each restriction enzyme (NEB or Fermentus) and subjected to electrophoresis in Spreadex EL300 gel (Elchrom Scientific) at 55°C.

The database was made in EXCEL file and converted to SPSS database. Genotype frequencies were compared using chi-square test, and analysis of variance (ANOVA) was conducted for detection of association between genotype frequencies and clinical parameters. These statistical analyses were done by SPSS ver14.0. If ANOVA was significant, Bonferroni test was also performed.

Results

Frequencies of SNPs in SCOP were listed in *Table 2*. There was no statistically significant difference among SNP frequencies in those of Nansei Cohort or HapMapProject. Adiponectin Ile164Thr heterozygotes, however, were significantly more frequent in obese subjects in SCOP than those reported by Kondo et al. As for the SNP frequencies between in men and women showed that UCP1-3826 G/G minor homozygotes were more frequent in woman than that in man (P=0.070), suggesting that the homozygotes were susceptible to obesity in woman *(Table 3)*.

We investigated the association among the SNPs, contents of adipocytokines, clinical parameters and plasma biochemical markers. Comparison of adipocytokine contents sorted out for every SNPs revealed that, in PPARγ2 12Pro/Ala heterozygotes (woman), plasma C-peptide (P=0.004) and insulin level (P=0.008) were higher than Pro/Pro major homozygotes *(Table 4)*. Adiponectin 164Ile/Thr heterozygotes had lower adiponectin blood concentration than the major Ile/Ile homozygotes both in men (P=0.072) and women (P=0.016). These results coincide with the report of Kondo et al.[9], in which subjects with 164Thr allele had lower adiponectin concentration in blood.

Leptin receptor (LEPR) 109Lys/Lys minor homozygotes had significantly higher leptin (P=0.002), TNF-α (P=0.046), C-peptide (P=0.018) and insulin level (P=0.064) than Arg/Lys hetero or Arg/Arg major homozygotes only in woman. In calpain10 SNP43G/A heterozygotes (woman), free fatty acid was higher than the G/G major homozygotes (P=0.040). β3AR Trp64 allele had trend in higher C-peptide (P=0.009) and insulin contents (P=0.053) than Arg allele in man, which was contrary to results supposed from the accepted hypothesis. Heterozygotes (Trp/Arg) had the intermediate contents.

Comparison of BMI and other biochemical parameters sorted out for every SNPs revealed that UCP1 -3826G/G homozygotes had elevated total- (P=0.046) and LDL- choesterol level (P=0.063) than other genotypes in woman *(Table 5)*. In PPARγ2 12Pro/Ala heterozygotes (woman), triglyceride level were higher than Pro/Pro major homozygotes (P=0.052), which is converse with general hypothesis. Adiponectin 164Ile/Thr heterozygotes

Table 1 Genotyping of metabolic syndrome-related genes by PCR-RFLP

gene	SNP	dbSNP	amplified region (Bold letters indicate polymorphysm.)	restriction enz.	generated frag (bp)
UCP1	-3826A/G	rs1800592	5'- CACAAAGAAGAAGCAGAGAGG ... ... **T/C** GATCA ... ... CTCTCATTAGCCACCACTGG -3' (Shihara et al.)[5]	Bcl I	277 151+126(A)
UCP2	Ala55Val (C/T)	rs660339	5'- TCAAGGGCCAGTGTTTGGAG ... ... **C/T** CAGCGCCCAGTACCGCGGT -3'	BstN I	146→128+18(C)
UCP3	-55C>T	rs1800849	5'- ACCCCAAGTCAAGAGGACTG ... ... **C/T** GTGTGTATAAGACCAGTGCAA -3'	Sma I Dde I	153→131+22(C) 153→129+24(T)
PPARγ2	Pro12Ala (C→G)	rs1801282	5'- TCTGGGAGATTCTCCTATTGAC **C/G** ... ... CTCCTGTAGTTGTCTTCCAG -3'	Hae III Hha I	154→20+134C) 154→22+132(G)
adiponectine	Ile164Thr (T→C)	Not Found	5'- GCTGTACTACTTTGCCTACCACA **T/C** ... ... CTATGCTCTTCACCTATGATCA -3'	Dpn II	96→21+75(T)
LEPR	Arg 109 Lys (G>A)	rs1137100	5'- TTTCCACTGTTGCTTTCGGAG ... ... **A/G** GACATTTGTTTCAACAGTAAATTCTT -3'	Ava II	95→26+69(G)
calpain10	SNP43 (G→A)	rs3792267	5'- ACGCTTGCTGCGAAGTAAGGC **G/A** ... ... GACCATGGGAATCAGAGAGG -3'	Mlu I Nsi I	146→18+128(G) 146→22+124(A)
β2AR	Arg16Gly (A/G) Gln27Glu (C→G)	rs1042713 rs1042714	5'- GCCTTCTTGCTGGCACCCAAT **A/G** ... ... GTTTGGCAATGTGCTGGTCAT -3' 5'- GCCTTCTTGCTGGCACCCAAT ... ... GCAG **C/G** ... ... GTTTGGCAATGTGCTGGTCAT -3'	Nco I Fnu4H I	120→18+102(G) 120→52+68(C)
β3AR	Trp64Arg (T→C)	rs4994	5'- GCAGCTGCCCCTTTAAGCG ... ... CC **T/C** GG ... ... GGTGATGGGACTCCTGG -3'	Nci I	213,125, 148→69+79(C)

Table 2 Genotype frequencies of metabolic syndrome-related genes

gene	SNP	cohort	n	genotype			HWP	allele frequency	P-value of SCOP compared with
				major homo	hetero	minor homo			
UCP1	-3826A/G	SCOP	233	52 (22.3)	127 (54.5)	54 (23.2)	0.17	0.496 : 0.504	
		Nansei	422	117 (27.7)	206 (48.8)	99 (23.5)	0.65	0.521 : 0.479	0.264
		HapMap-JPT		Not Found	(Not Analyzed)				
		Shihara et al. (Japanese men) [5]	349	87 (24.9)	191 (54.7)	71 (20.3)	0.07	0.523 : 0.477	0.631
UCP2	Ala55Val (C/T)	SCOP	232	61 (26.3)	108 (46.6)	63 (27.2)	0.29	0.496 : 0.504	
		Nansei	422	116 (27.5)	204 (48.3)	102 (24.2)	0.51	0.517 : 0.483	0.702
		HapMap-JPT	44	11 (25.0)	26 (59.1)	7 (15.9)	0.20	0.545 : 0.455	0.216
		JBIC	1500					0.500 : 0.500	0.611
		Shiinoki et al. (Japanese) [6]	120	28 (23.3)	71 (59.2)	21 (17.5)	0.04	0.529 : 0.471	0.054
UCP3	-55C>T	SCOP	232	108 (46.6)	104 (44.8)	20 (8.6)	0.47	0.690 : 0.310	
		Nansei	422	194 (46.0)	179 (42.4)	49 (11.6)	0.43	0.672 : 0.328	0.477
		HapMap-JPT	44	21 (47.7)	21 (47.7)	2 (4.5)	0.25	0.716 : 0.284	0.654
		JBIC	1478	Not Applicable				0.675 : 0.325	0.664
		Liu et al (Caucasian) [7]	1873	Not Applicable				0.735 : 0.265	0.096
PPARγ2	Pro12Ala (C→G)	SCOP	232	215 (92.7)	17 (7.3)	0	0.56	0.963 : 0.037	
		Nansei	422	392 (92.9)	30 (7.1)	0	0.45	0.964 : 0.036	0.918
		HapMap-JPT	44	39 (88.6)	5 (11.4)	0	0.69	0.943 : 0.057	0.365
		Mori et al. (Japanese men) [8]	215	203 (94.4)	11 (5.1)	1 (0.5)	0.06	0.970 : 0.030	0.370
adiponectine	Ile164Thr (T→C)	SCOP	232	225 (97.0)	7 (3.0)	0	0.82	0.985 : 0.015	
		Kondo et al. (Japanese) [9]	452	450 (99.6)	2 (0.4)	0	0.96	0.998 : 0.002	0.005
LEPR	Arg109Lys (G>A)	SCOP	232	142 (61.2)	77 (33.2)	13 (5.6)	0.55	0.778 : 0.222	
		HapMap-JPT	44	27 (61.4)	16 (36.4)	1 (2.3)	0.44	0.795 : 0.205	0.631
		Park et al. (Korean) [10]	680	458 (67.4)	200 (29.4)	22 (3.2)	0.92	0.821 : 0.179	0.115
		Matsuoka et al.(Japanese) [11]	68	Not Applicable				0.765 : 0.235	0.913
calpain10	SNP43 (G→A)	SCOP	232	212 (91.4)	20 (8.6)	0	0.49	0.957 : 0.043	
		Nansei	247	226 (91.5)	21 (8.5)	0	0.49	0.957 : 0.043	0.963
		HapMap-JPT	45	37 (82.2)	8 (17.8)	0	0.51	0.911 : 0.089	0.062
		Horikawa et al. (Japanese) [12]	172	Not Applicable				0.950 : 0.05	0.712
β2AR	Arg16Gly (A/G)	SCOP	233	51 (21.9)	115 (49.4)	67 (28.8)	0.90	0.466 : 0.534	
		HapMap-JPT	44	6 (13.6)	22 (50.0)	16 (36.4)	1.00	0.386 : 0.614	0.379
		Yamada et al. (Japanese) [13]	450	113 (25.1)	224 (49.8)	113 (25.1)	0.92	0.500 : 0.500	0.486
		Hayakawa et al.(Japanese men) [14]	210	57 (27.1)	104 (495)	49 (23.3)	0.91	0.519 : 0.481	0.288
β2AR	Gln27Glu (C→G)	SCOP	233	195 (83.7)	37 (15.9)	1 (0.4)	0.59	0.916 : 0.084	
		HapMap-JPT	44	38 (86.4)	5 (11.4)	10 (2.3)	0.15	0.921 : 0.079	0.321
		Yamada et al. (Japanese) [13]	450	389 (86.4)	59 (131)	2 (0.4)	0.88	0.930 : 0.070	0.614
		Hayakawa et al. (Japanese men) [14]	210	188 (895)	22 (10.5)	0	0.42	0.948 : 0.052	0.153
β3-AR	Trp64Arg (T→C)	SCOP	233	165 (70.8)	61 (26.2)	7 (3.0)	0.64	0.839 : 0.161	
		Nansei	422	290 (68.7)	115 (27.3)	17 (4.0)	0.20	0.823 : 0.177	0.745
		JBIC	1064	Not Applicable				0.821 : 0.179	0.596
		Shihara et al. (Japanese men) [5]	349	262 (75.1)	84 (24.1)	3 (0.9)	0.18	0.871 : 0.129	0.114

HWP : Hardy Weinberg Plot
JBIC : Japanese Biological Informatics Consortium's data
P-values are given by the chi-square test.

Table 3 Genotype frequencies of metabolic syndrome-related genes (male/female)

gene	genotype	Male		Female		p
		n	%	n	%	
UCP1	A/A	29	25.2	23	19.7	0.070
(-3826A/G)	A/G	67	58.3	60	51.3	
	G/G	19	16.5	34	29.1	
UCP2	Ala/Ala	34	29.6	27	23.1	0.355
(Ala55Val)	Ala/Val	54	47.0	54	46.2	
	Val/Val	27	23.5	36	30.8	
UCP3	C/C	58	50.4	50	42.7	0.464
(-55C/T)	C/T	47	40.9	57	48.7	
	T/T	10	8.7	10	8.5	
PPARγ2	Pro/Pro	105	91.3	110	94.0	0.428
(Pro12Ala)	Pro/Ala	10	8.7	7	6.0	
adiponectin	Ile/Ile	111	96.5	114	97.4	0.684
(Ile164Thr)	Ile/Thr	4	3.5	3	2.6	
LEPR	Arg/Arg	65	56.5	77	65.8	0.345
(Arg109Lys)	Lys/Arg	43	37.4	34	29.1	
	Lys/Lys	7	6.1	6	5.1	
calpain10	G/G	102	88.7	110	94.0	0.149
(SNP43 G→A)	G/A	13	11.3	7	6.0	
β2AR	Arg/Arg	23	20.0	28	23.9	0.687
(Arg16Gly)	Arg/Gly	60	52.2	55	47.0	
	Gly/Gly	32	27.8	34	29.1	
β2AR	Gln/Gln	97	84.3	97	82.9	0.604
(Gln27Glu)	Gln/Glu	18	15.7	19	16.2	
	Glu/Glu	0	0.0	1	0.9	
β3AR	Trp/Trp	78	67.8	87	74.4	0.363
(Trp64Arg)	Trp/Arg	32	27.8	28	23.9	
	Arg/Arg	5	4.3	2	1.7	

P-values are given by the chi-square test.

had higher total- (P=0.015) and LDL-cholesterol (P=0.040) than the major Ile/Ile homozygotes in man *(Table 5)*, although in woman, these association were insignificant. LEPR 109Lys/Lys minor homozygotes had significantly higher triglycerid level in woman (P=0.088). Also, their fasting blood sugar level was higher than that of Arg/Arg major homozygotes (P=0.054). To the contrary, in man, Lys/Lys homozygotes had the reduced waist circumference than the other (P=0.033). In calpain10 SNP43G/G major homozygotes (woman), triglyceride was significantly higher level than the G/A heterozygotes (P=0.042), in agreement with negative association in the level of HDL cholesterol (P=0.012). In some genotypes which is reportedly preposition to obesity or diabetes (PPARγ2 Pro/Pro in both sexes, adiponectin 164Ile/Thr in both sexes, LEPR 109Lys/Lys in woman, β3AR Arg/Arg in woman), C-reactive protein (CRP) showed higher trend than other genotypes.

The difference among LEPR Arg109Lys SNP was further tested with Bonferroni adjustment *(Table 6)*. Acutally, leptin level, C-peptide level and BMI were significantly higher in the Lys/Lys homozygotes than in other genotypes in women.

Table 4 Genotypes of metabolic syndrome-related genes and adipocytokines

gene	genotype	Male	fFA	leptin	TNF-α	adiponectin	C-peptide	insulin	Female	fFA	leptin	TNF-α	adiponectin	C-peptide	insulin
UCP1	A/A	29	0.50 ± 0.20	7.68 ± 4.97	1.19 ± 0.40	2.88 ± 2.09	2.79 ± 1.36	11.95 ± 12.55	23	0.50 ± 0.21	21.55 ± 10.72	1.11 ± 0.34	4.88 ± 2.99	2.85 ± 1.46	11.52 ± 7.99
	A/G	67	0.51 ± 0.18	8.62 ± 6.26	1.34 ± 0.55	2.87 ± 1.72	2.83 ± 1.16	11.70 ± 8.43	60	0.56 ± 0.21	22.53 ± 10.92	1.25 ± 0.43	5.68 ± 2.89	2.45 ± 0.72	10.94 ± 5.54
	G/G	19	0.50 ± 0.18	7.56 ± 4.16	1.25 ± 0.46	2.35 ± 1.44	3.04 ± 1.44	13.79 ± 11.21	34	0.62 ± 0.22	19.11 ± 11.84	1.24 ± 0.48	5.43 ± 3.43	2.50 ± 0.76	11.25 ± 5.07
UCP2	Ala/Ala	34	0.54 ± 0.20	8.21 ± 5.63	1.21 ± 0.51	2.59 ± 1.33	2.84 ± 1.09	11.05 ± 7 13	27	0.60 ± 0.23	22.91 ± 14.12	1.16 ± 0.47	6.11 ± 3.56	2.42 ± 0.64	9.68 ± 3.80
	Ala/Val	54	0.48 ± 0.18	7.93 ± 6.17	1.30 ± 0.50	2.73 ± 1.95	2.77 ± 1.33	12.40 ± 11.20	54	0.55 ± 0.20	21.06 ± 10.47	1.24 ± 0.42	5.48 ± 3.00	2.51 ± 0.72	10.79 ± 4.75
	Val/Val	27	0.50 ± 0.16	8.75 ± 4.54	1.36 ± 0.51	3.17 ± 1.91	3.04 ± 1.32	12.87 ± 10.82	36	0.57 ± 0.23	19.92 ± 9.55	1.19 ± 0.42	5.02 ± 2.88	2.63 ± 1.32	12.49 ± 8.19
UCP3	C/C	58	0.51 ± 0.20	8.16 ± 5.13	1.28 ± 0.52	2.56 ± 1.54	2.96 ± 1.35	12.78 ± 11.49	50	0.57 ± 0.19	20.88 ± 10.99	1.22 ± 0.43	5.88 ± 3.43	2.42 ± 0.75	10.43 ± 4.97
	C/T	47	0.51 ± 0.17	7.94 ± 6.13	1.27 ± 0.46	2.93 ± 2.05	2.75 ± 1.16	11.42 ± 8.49	57	0.57 ± 0.24	21.37 ± 11.67	1.19 ± 0.42	5.33 ± 2.93	2.64 ± 1.07	11.51 ± 6.65
	T/T	10	0.48 ± 0.20	9.74 ± 6.30	1.39 ± 0.66	3.52 ± 1.50	2.71 ± 1.15	11.46 ± 7.67	10	0.55 ± 0.20	21.10 ± 8.79	1.26 ± 0.50	4.44 ± 2.02	2.34 ± 0.78	11.36 ± 5.42
PPARγ2	Pro/Pro	105	0.51 ± 0.18	8.26 ± 5.74	1.28 ± 0.51	2.81 ± 1.83	2.87 ± 1.26	12.28 ± 10.22	110	0.57 ± 0.21	21.14 ± 11.18	1.21 ± 0.43	5.52 ± 3.16	2.46 ± 0.78	10.69 ± 5.16
	Pro/Ala	10	0.45 ± 0.19	7.64 ± 4.56	1.33 ± 0.48	2.58 ± 1.10	2.71 ± 1.29	10.34 ± 7.58	7	0.57 ± 0.24	21.16 ± 10.61	1.14 ± 0.35	4.89 ± 1.90	3.50 ± 2.08 p= 0.004	16.86 ± 12.14 p= 0.008
adiponectin	Ile/Ile	111	0.50 ± 0.19	8.09 ± 5.49	1.28 ± 0.50	2.84 ± 1.78	2.85 ± 1.27	12.10 ± 10.17	114	0.57 ± 0.22	21.32 ± 11.18	1.21 ± 0.43	5.59 ± 3.06	2.52 ± 0.93	11.09 ± 5.95
	Ile/Thr	4	0.53 ± 0.13	11.45 ± 9.25	1.40 ± 0.81	1.23 ± 0.26 p= 0.072	2.86 ± 0.63	12.45 ± 4.02	3	0.61 ± 0.13	14.37 ± 3.47	0.97 ± 0.21	1.27 ± 0.25 p= 0.016	2.44 ± 0.34	9.50 ± 1.66
LEPR	Arg/Arg	65	0.50 ± 0.19	7.74 ± 4.60	1.37 ± 0.53	2.74 ± 1.71	2.87 ± 1.29	12.40 ± 11.13	77	0.56 ± 0.21	20.47 ± 9.75	1.14 ± 0.42	5.37 ± 3.32	2.48 ± 0.81	11.04 ± 5.35
	Lys/Arg	43	0.51 ± 0.18	9.20 ± 7.18	1.17 ± 0.45	2.90 ± 1.95	2.87 ± 1.28	12.04 ± 8.91	34	0.61 ± 0.22	19.93 ± 10.80	1.32 ± 0.41	5.84 ± 2.82	2.43 ± 0.75	10.15 ± 4.87
	Lys/Lys	7	0.54 ± 0.18	6.44 ± 1.60	1.21 ± 0.49	2.57 ± 1.20	2.61 ± 0.72	9.85 ± 4.48	6	0.51 ± 0.20	36.93 ± 18.59 p= 0.002	1.48 ± 0.44 p= 0.046	4.92 ± 0.66	3.57 ± 2.21 p= 0.018	16.28 ± 13.25 p= 0.064
caipain10	G/G	102	0.51 ± 0.19	8.20 ± 5.74	1.28 ± 0.49	2.82 ± 1.82	2.86 ± 1.27	12.06 ± 10.19	110	0.56 ± 0.21	21.41 ± 11.18	1.21 ± 0.43	5.37 ± 3.03	2.55 ± 0.94	11.14 ± 5.91
	G/A	13	0.46 ± 0.16	8.28 ± 4.90	1.36 ± 0.63	2.51 ± 1.35	2.84 ± 1.17	12.51 ± 8.81	7	0.72 ± 0.21 p= 0.040	17.90 ± 10.14	1.17 ± 0.50	6.86 ± 3.72	2.20 ± 0.73	9.96 ± 5.68
β2AR Arg16Gly	Arg/Arg	23	0.48 ± 0.14	7.24 ± 4.52	1.34 ± 0.61	2.87 ± 1.78	2.78 ± 0.98	10.81 ± 7.08	28	0.57 ± 0.26	21.12 ± 11.99	1.16 ± 0.42	6.09 ± 3.88	2.72 ± 1.32	12.46 ± 8.02
	Arg/Gly	60	0.50 ± 0.19	8.83 ± 6.85	1.33 ± 0.52	2.83 ± 1.78	2.89 ± 1.36	12.51 ± 11.47	55	0.57 ± 0.20	21.42 ± 9.87	1.18 ± 0.38	5.54 ± 2.71	2.53 ± 0.84	11.17 ± 5.57
	Gly/Gly	32	0.54 ± 0.19	7.73 ± 3.30	1.17 ± 0.36	2.65 ± 1.80	2.85 ± 1.26	12.30 ± 9.00	34	0.56 ± 0.19	21.40 ± 12.67	1.32 ± 0.51	4.78 ± 2.78	2.43 ± 0.61	10.02 ± 4.13
β2AR Gln27Glu	Gln/Gln	97	0.51 ± 0.19	8.11 ± 5.88	1.25 ± 0.50	2.91 ± 1.82	2.83 ± 1.26	12.28 ± 10.42	97	0.56 ± 0.22	21.19 ± 11.26	1.19 ± 0.40	5.50 ± 3.14	2.57 ± 0.96	11.22 ± 6.10
	Gln/Glu	18	0.46 ± 0.13	8.70 ± 4.11	1.46 ± 0.49	2.14 ± 1.36 p= 0.093	3.01 ± 1.22	11.22 ± 7.62	19	0.60 ± 0.17	22.32 ± 11.16	1.33 ± 0.57	5.05 ± 2.66	2.45 ± 0.71	10.83 ± 5.21
	Glu/Glu	0							1	0.49	17.90	1.30	8.20	2.06	10.20
β3AR	Trp/Trp	78	0.48 ± 0.17	8.88 ± 6.28	1.33 ± 0.55	2.61 ± 1.69	3.10 ± 1.40	13.65 ± 11.55	87	0.57 ± 0.22	20.40 ± 9.98	1.22 ± 0.42	5.34 ± 3.13	2.57 ± 0.99	11.44 ± 6.36
	Trp/Arg	32	0.56 ± 0.20	6.96 ± 3.71	1.19 ± 0.37	3.24 ± 1.99	2.35 ± 0.65	9.08 ± 4.13	28	0.57 ± 0.20	23.23 ± 13.43	1.19 ± 0.48	5.82 ± 2.99	2.50 ± 0.70	10.44 ± 4.36
	Arg/Arg	5	0.51 ± 0.22	5.68 ± 2.75	1.18 ± 0.54	2.66 ± 1.17	2.28 ± 0.55 p= 0.009	7.54 ± 3.35 p= 0.053	2	0.37 ± 0.01	35.85 ± 20.29 p= 0.090	1.20 ± 0.42	5.00 ± 0.14	2.31 ± 1.25	8.10 ± 5.80

Table 5a Genotypes of metabolic syndrome-related genes and plasma biochemical markers (Male)

gene	genotype	Male	Total-Cho	HDL-Cho	LDL-Cho	TG	HbA1c	Fasting Glc	CRP	BMI	body fat %	Waist Circumf.
UCP1	A/A	29	204.2 ± 34.2	51.38 ± 10.06	119.8 ± 32.7	165.2 ± 77.9	5.65 ± 0.78	108.0 ± 18.3	0.15 ± 0.18	29.57 ± 2.12	27.51 ± 3.83	100.9 ± 6.6
	A/G	67	205.2 ± 27.2	49.13 ± 9.51	118.7 ± 33.1	187.0 ± 142.9	5.91 ± 1.10	113.5 ± 28.7	0.20 ± 0.35	30.82 ± 4.18	29.80 ± 4.50	102.1 ± 10.1
	G/G	19	200.6 ± 19.9	49.74 ± 9.94	121.5 ± 24.1	147.0 ± 76.5	5.72 ± 0.73	111.4 ± 18.7	0.14 ± 0.08	30.45 ± 2.63	28.75 ± 4.64 p= 0.065	100.3 ± 6.1
UCP2	Ala/Ala	34	203.4 ± 23.6	49.74 ± 10.27	114.0 ± 33.1	198.4 ± 169.5	6.02 ± 1.02	115.1 ± 29.1	0.14 ± 0.15	30.93 ± 4.14	29.05 ± 4.11	102.0 ± 10.3
	Ala/Val	54	203.9 ± 32.3	49.94 ± 9.06	122.9 ± 32.3	155.5 ± 84.3	5.74 ± 1.06	111.0 ± 24.6	0.19 ± 0.37	29.87 ± 3.04	28.34 ± 4.41	99.9 ± 7.8
	Val/Val	27	205.6 ± 24.3	49.59 ± 10.48	119.3 ± 27.4	184.1 ± 104.5	5.70 ± 0.68	109.0 ± 19.6	0.18 ± 0.20	30.99 ± 3.66	30.44 ± 4.70	104.0 ± 8.0
UCP3	C/C	58	203.8 ± 27.6	49.64 ± 9.66	118.4 ± 34.6	179.4 ± 142.9	5.95 ± 0.96	114.8 ± 26.5	0.15 ± 0.15	30.62 ± 3.50	29.08 ± 4.59	101.5 ± 8.4
	C/T	47	205.1 ± 29.4	48.91 ± 9.66	120.4 ± 30.3	179.1 ± 97.2	5.58 ± 0.65	107.0 ± 16.7	0.19 ± 0.39	30.16 ± 3.23	29.15 ± 3.83	101.1 ± 8.8
	T/T	10	201.6 ± 25.1	54.90 ± 9.26	121.0 ± 15.3	129.1 ± 55.8	6.09 ± 1.87 p= 0.097	116.0 ± 42.2	0.25 ± 0.28	30.75 ± 5.31	28.35 ± 6.38	103.6 ± 10.5
PPARγ2	Pro/Pro	105	203.7 ± 29.0	49.80 ± 9.53	118.9 ± 32.5	175.6 ± 124.7	5.82 ± 1.01	112.2 ± 25.5	0.18 ± 0.29	30.51 ± 3.67	29.13 ± 4.48	101.6 ± 9.0
	Pro/Ala	10	208.9 ± 12.6	49.80 ± 11.76	125.4 ± 16.4	167.7 ± 59.9	5.67 ± 0.43	107.2 ± 17.0	0.12 ± 0.05	29.76 ± 1.88	28.09 ± 4.09	101.0 ± 5.8
adiponectin	Ile/Ile	111	203.0 ± 26.6	50.06 ± 9.68	118.3 ± 30.7	173.3 ± 121.1	5.83 ± 0.98	111.9 ± 25.2	0.15 ± 0.15	30.39 ± 3.50	28.94 ± 4.46	101.3 ± 8.6
	Ile/Thr	4	237.3 ± 46.5 p= 0.015	42.50 ± 7.00	151.0 ± 38.7 p= 0.040	218.0 ± 96.9	5.35 ± 0.45	107.8 ± 11.9	0.80 ± 1.27 p< 0.001	31.78 ± 5.15	31.88 ± 2.56	107.2 ± 10.3

Table 5a

gene	genotype	Male	Total-Cho	HDL-Cho	LDL-Cho	TG	HbA1c	Fasting Glc	CRP	BMI	body fat %	Waist Circumf.
LEPR	Arg/Arg	65	203.1 ± 29.1	48.86 ± 8.91	117.8 ± 32.1	182.1 ± 131.3	5.88 ± 1.09	112.0 ± 28.9	0.18 ± 0.35	29.99 ± 2.67	29.08 ± 4.00	100.2 ± 6.9
	Lys/Arg	43	205.3 ± 27.9	51.60 ± 10.57	121.7 ± 30.7	160.0 ± 93.6	5.71 ± 0.82	111.6 ± 19.4	0.17 ± 0.16	31.14 ± 4.69	29.26 ± 5.22	104.2 ± 10.9
	Lys/Lys	7	207.7 ± 19.1	47.43 ± 10.56	120.4 ± 33.5	199.6 ± 163.8	5.83 ± 0.62	110.6 ± 14.2	0.16 ± 0.11	30.33 ± 2.02	27.39 ± 2.78	97.9 ± 4.8 p= 0.033
caipain10	G/G	102	205.0 ± 27.4	50.61 ± 9.77	120.0 ± 31.4	172.2 ± 124.8	5.78 ± 0.92	111.4 ± 21.8	0.18 ± 0.30	30.38 ± 3.58	28.84 ± 4.26	101.4 ± 8.9
	G/A	13	198.0 ± 32.4	43.46 ± 6.19 p= 0.012	115.3 ± 32.9	195.8 ± 76.8	6.05 ± 1.34	114.5 ± 43.2	0.13 ± 0.11	30.90 ± 3.43	30.60 ± 5.57	102.5 ± 7.3
β2AR Arg16Gly	Arg/Arg	23	200.6 ± 27.3	46.04 ± 10.26	114.7 ± 43.2	199.3 ± 207.7	5.73 ± 0.89	110.1 ± 18.7	0.20 ± 0.21	30.41 ± 4.37	29.12 ± 5.17	100.7 ± 9.5
	Arg/Gly	60	203.5 ± 24.8	50.40 ± 9.26	120.0 ± 27.2	166.1 ± 86.3	5.81 ± 0.93	111.0 ± 25.2	0.18 ± 0.35	30.49 ± 3.91	29.22 ± 4.52	101.4 ± 9.8
	Gly/Gly	32	207.9 ± 33.9	51.38 ± 9.64	121.9 ± 29.5	173.8 ± 88.5	5.88 ± 1.12	114.3 ± 28.4	0.15 ± 0.13	30.38 ± 1.92	28.66 ± 3.77	102.2 ± 5.6
β2AR Gln27Glu	Gln/Gln	97	203.6 ± 28.2	49.44 ± 9.48	119.2 ± 32.5	174.9 ± 124.7	5.74 ± 0.81	110.4 ± 22.2	0.18 ± 0.30	30.48 ± 3.80	28.97 ± 4.60	101.5 ± 9.3
	Gln/Glu	18	207.3 ± 27.1	51.72 ± 10.79	120.8 ± 26.0	174.6 ± 96.0	6.20 ± 1.57 p= 0.064	119.1 ± 36.0	0.15 ± 0.10	30.24 ± 1.68	29.48 ± 3.40	101.5 ± 4.5
	Glu/Glu	0										
β3AR	Trp/Trp	78	204.6 ± 28.7	48.31 ± 9.94	121.2 ± 30.7	175.6 ± 96.3	5.86 ± 1.06	113.0 ± 26.0	0.20 ± 0.33	30.71 ± 3.72	29.30 ± 4.49	102.1 ± 9.2
	Trp/Arg	32	203.9 ± 26.9	52.94 ± 7.91	114.5 ± 34.9	182.2 ± 171.7	5.67 ± 0.77	109.1 ± 23.4	0.11 ± 0.07	30.10 ± 3.21	28.73 ± 4.27	101.3 ± 7.1
	Arg/Arg	5	199.8 ± 28.4	53.00 ± 12.17 p= 0.055	123.6 ± 17.7	116.6 ± 26.6	5.94 ± 0.68	109.8 ± 16.7	0.15 ± 0.09	28.40 ± 2.18	26.92 ± 4.80	94.0 ± 7.9

Table 5b Genotypes of metabolic syndrome-related genes and plasma biochemical markers (Female)

gene	genotype	Female	Total-Cho	HDL-Cho	LDL-Cho	TG	HbA1c	Fasting Glc	CRP	BMI	body fat %	Waist Circumf.
UCP1	A/A	23	209.3 ± 36.2	57.48 ± 10.72	122.8 ± 33.0	145.4 ± 75.2	5.96 ± 1.64	110.6 ± 19.4	0.17 ± 0.15	31.49 ± 3.82	41.92 ± 6.14	104.7 ± 9.7
	A/G	60	211.2 ± 35.8	55.63 ± 11.85	127.8 ± 30.2	139.1 ± 82.1	5.81 ± 0.92	111.6 ± 28.8	0.19 ± 0.20	31.24 ± 2.98	40.43 ± 5.07	104.0 ± 8.2
	G/G	34	230.6 ± 45.7 p= 0.046	55.59 ± 11.90	142.1 ± 39.2 p= 0.063	164.8 ± 73.0	6.01 ± 1.13	114.6 ± 27.7	0.17 ± 0.15	30.63 ± 2.97	40.39 ± 5.66	102.7 ± 7.8
UCP2	Ala/Ala	27	216.1 ± 40.1	58.19 ± 11.86	133.5 ± 36.0	122.0 ± 50.2	5.97 ± 1.41	116.8 ± 32.1	0.14 ± 0.13	31.77 ± 2.93	41.01 ± 4.38	103.8 ± 8.1
	Ala/Val	54	211.9 ± 36.0	54.44 ± 10.69	127.7 ± 28.4	148.9 ± 77.9	5.91 ± 1.13	111.2 ± 23.1	0.18 ± 0.19	30.64 ± 2.90	40.12 ± 4.90	103.3 ± 7.8
	Val/Val	36	223.8 ± 44.6	56.64 ± 12.60	134.1 ± 40.5	165.5 ± 92.0 p= 0.090	5.81 ± 0.96	110.5 ± 27.7	0.20 ± 0.18	31.32 ± 3.59	41.38 ± 6.83	104.4 ± 9.5
UCP3	C/C	50	213.4 ± 44.0	55.50 ± 11.77	128.1 ± 36.5	149.0 ± 74.5	5.91 ± 1.35	110.4 ± 23.3	0.17 ± 0.16	31.07 ± 2.71	40.75 ± 5.17	103.3 ± 8.0
	C/T	57	220.5 ± 36.8	56.35 ± 11.77	134.7 ± 33.6	146.9 ± 82.6	5.89 ± 0.92	113.8 ± 25.0	0.18 ± 0.18	31.19 ± 3.59	40.83 ± 5.75	104.0 ± 8.8
	T/T	10	209.7 ± 34.6	56.30 ± 10.49	124.1 ± 23.0	147.0 ± 80.7	5.90 ± 1.29	113.2 ± 47.8	0.21 ± 0.23	30.87 ± 2.67	39.82 ± 5.52	104.4 ± 8.2
PPARγ2	Pro/Pro	110	215.2 ± 40.4	55.87 ± 11.55	130.5 ± 34.6	144.3 ± 76.0	5.88 ± 1.16	112.3 ± 27.0	0.18 ± 0.18	31.13 ± 3.07	40.64 ± 5.15	103.7 ± 8.0
	Pro/Ala	7	236.7 ± 20.7	57.71 ± 12.75	138.6 ± 24.7	203.4 ± 99.3 p= 0.052	6.09 ± 0.96	112.6 ± 22.8	0.14 ± 0.13	30.87 ± 4.47	41.79 ± 9.50	104.9 ± 13.6
adiponectin	Ile/Ile	114	216.7 ± 40.0	56.19 ± 11.63	130.9 ± 34.4	147.8 ± 79.2	5.89 ± 1.16	112.4 ± 27.0	0.17 ± 0.18	31.11 ± 3.18	40.67 ± 5.46	103.8 ± 8.3
	Ile/Thr	3	209.7 ± 30.9	48.00 ± 6.24	132.3 ± 22.6	147.3 ± 41.2	6.20 ± 0.20	107.3 ± 6.0	0.31 ± 0.04	31.00 ± 1.15	42.17 ± 5.56	103.9 ± 11.3
LEPR	Arg/Arg	77	215.6 ± 34.8	55.53 ± 11.74	131.7 ± 31.7	142.1 ± 72.5	5.81 ± 1.02	108.1 ± 18.9	0.16 ± 0.15	31.17 ± 3.17	40.60 ± 5.38	103.6 ± 8.7
	Lys/Arg	34	219.5 ± 50.7	57.12 ± 11.59	132.7 ± 41.3	148.9 ± 70.0	6.03 ± 1.30	119.6 ± 34.9	0.19 ± 0.17	30.47 ± 2.63	40.51 ± 4.69	102.9 ± 6.3
	Lys/Lys	6	210.7 ± 34.6	55.33 ± 10.84	112.3 ± 5.8	215.2 ± 155.8 p= 0.088	6.32 ± 1.74	125.0 ± 47.5 p= 0.054	0.31 ± 0.40	33.95 ± 4.34 p= 0.041	43.25 ± 9.79	110.4 ± 11.9
caipain10	G/G	110	215.7 ± 40.5	55.31 ± 11.23	130.1 ± 34.3	151.5 ± 79.1	5.90 ± 1.16	112.6 ± 27.2	0.18 ± 0.18	31.06 ± 3.20	40.63 ± 5.56	103.6 ± 8.4
	G/A	7	229.1 ± 22.5	66.57 ± 12.78 p= 0.012	144.7 ± 29.7	89.6 ± 30.7 p= 0.042	5.89 ± 0.90	107.6 ± 17.5	0.13 ± 0.09	31.86 ± 2.15	41.96 ± 3.20	107.1 ± 7.5
β2AR Arg16Gly	Arg/Arg	28	218.1 ± 33.5	57.79 ± 12.44	134.5 ± 30.4	128.9 ± 59.4	5.59 ± 0.62	108.8 ± 27.1	0.15 ± 0.11	31.03 ± 3.25	40.27 ± 6.18	102.9 ± 9.7
	Arg/Gly	55	223.4 ± 44.3	56.95 ± 11.99	135.6 ± 37.5	154.5 ± 81.5	5.87 ± 1.05	109.3 ± 17.1	0.19 ± 0.17	30.96 ± 3.25	41.22 ± 5.67	103.3 ± 8.5
	Gly/Gly	34	204.1 ± 34.3 p= 0.081	52.94 ± 9.79	120.6 ± 29.7	152.6 ± 86.1	6.20 ± 1.52	120.1 ± 36.6	0.19 ± 0.22	31.42 ± 2.95	40.25 ± 4.43	105.3 ± 6.8
β2AR Gln27Glu	Gln/Gln	97	218.0 ± 40.8	56.45 ± 11.96	132.4 ± 35.7	146.0 ± 75.3	5.87 ± 1.13	112.1 ± 27.4	0.18 ± 0.19	30.99 ± 3.23	40.66 ± 5.52	103.6 ± 8.6
	Gln/Glu	19	209.8 ± 34.6	54.16 ± 9.45	124.7 ± 25.3	154.4 ± 95.3	6.05 ± 1.28	113.5 ± 24.3	0.15 ± 0.10	31.77 ± 2.78	40.84 ± 5.35	105.0 ± 7.2
	Glu/Glu	1	196.0	45.00	111.0	198.0	5.50	112.0	0.30	30.80	42.90	97.6
β3AR	Trp/Trp	87	218.3 ± 42.4	55.76 ± 10.49	133.0 ± 35.8	147.6 ± 74.4	5.92 ± 1.22	111.5 ± 26.9	0.16 ± 0.15	31.08 ± 3.34	40.53 ± 5.64	103.7 ± 8.7
	Trp/Arg	28	213.7 ± 30.6	56.61 ± 14.99	126.4 ± 28.8	153.3 ± 91.4	5.87 ± 0.95	115.9 ± 26.9	0.19 ± 0.16	31.20 ± 2.65	41.54 ± 4.89	103.9 ± 7.5
	Arg/Arg	2	179.0 ± 4.2	57.00 ± 2.83	106.0 ± 4.2	80.0 ± 15.6	5.35 ± 0.07	98.0 ± 11.3	0.57± 0.77 p= 0.005	31.10 ± 0.71	37.15 ± 3.89	103.8 ± 7.4

Table 6 Significance in LEPR Arg109Lys SNP with ANOVA or Bonferroni adjustment

LEPR(Female)		leptin	TNF-α	C-peptid	insulin	TG	Fasting Glc	BMI
ANOVA		**0.002**	**0.046**	**0.018**	0.064	0.088	0.054	**0.041**
Bonferroni								
Arg/Arg	Lys/Arg	1.000	0.164	1.000	1.000	1.000	0.108	0.812
Arg/Arg	Lys/Lys	**0.001**	0.192	**0.021**	0.124	0.084	0.394	0.108
Lys/Arg	Lys/Lys	**0.001**	1.000	**0.015**	0.058	0.166	1.000	**0.037**

P-values less than 0.05 are shown in bold.

Discussion

Association of BMI and other clinical parameters with genotypes of UCPs or β2, β3AR genes are reported in many studies. In obese subjects in SCOP, however, only a few of these associations were confirmed. On the contrary, some converse results were obtained; for example, Arg/Arg minor homozygote of β3AR showed lower C-peptide (P=0.009) and insulin (P=0.053), and higher HDL cholesterol level (P=0.055) in men. Plausibly, comparison with non-obese people in Saku cohort (case-control study) may explain these disagreements.

Leptin is an adipocyte-specific hormone, and regulates adipose-tissue mass via hypothalamus. The leptin receptor is found in many tissues including the hypothalamus, and has a single transmembrane domain, which is common with the cytokine receptor family. It is easily inferred from the important role of LEPR that the dysfunction resulted from SNPs may predispose to obesity and other metabolic syndromes. Several reports suggest the association between their SNPs and these diseases, however definite statement cannot be made as yet.

We showed significant association in the LEPR Arg109Lys, which is resided in exon 4 of the gene; Lys/Lys minor homozygotes (woman) had higher levels of leptin, TNF-α, C-peptide or insulin, compared with other genotypes; and in the homozygotes, triglyceride and fasting serum glucose were significantly higher, and also, the genotype showed a trend of higher values of HbA1c, BMI, body fat mass and waist circumference.

Associations described above were observed only in woman *(Table 4)*. Similarly, Rosmond et al.[15] reported that Arg/Arg homozygotes had lower leptin concentration, and probably as a result, lower BMI and abdominal sagittal diameter, as well as lower blood pressure, but that was in men. Wauters et al.[16] found associations of Lys109Arg with fasting glucose and oral glucose tolerance test in post- and pre-menopausal woman with impaired glucose tolerance. The fact that in females leptin levels are higher than males suggests that estrogens have effect on leptin secretion. Probably, combination of increased secretion of leptin by estrogens with dysfunction of leptin receptor predispose to obesity and other metabolic syndromes. However, the mechanism as to the development in detail remains to be elucidated.

A (Lys) allele of Lys109Arg is the major type in European (A:G=0.658:0.342, HapMap-CEU) or Sub-Saharan African (A:G=0.883:0.117, HapMap-YRI). On the contrary, the SNP frequencies are reversed in Asian; G (Arg) allele is the major type in Asian (A:G=0.205:0.795, HapMap-JPT; A:G=0.144:0.856, HapMap-HCB). The frequency in SCOP was A:G=0.222:0.778, and also in Korean, Arg was a major allele.[10] This racial difference may influence their distinct susceptibility to obesity and other metabolic diseases.

Also, we demonstrated that adiponectin 164Ile/Thr heterozygotes had truly lower plasma adiponectin level than the major Ile/Ile homozygotes both in men and women. These results are consistent with the report by Kondo et al.[9] The heterozygotes had higher total cholesterol (P=0.015) and LDL-cholesterol (P=0.040) than the major Ile/Ile homozygotes in man, although in woman, these association were insignificant. Besides, C-reactive protein (CRP) showed higher trend in the heterozygotes than another genotype (P<0.001). The results coincided with the report by Mita et al. [17]; higher CRP is the marker of early-stage type 2 diabetes mellitus in Japanese patients.

Imbalance between the adiponectin and leptin would influence and lead to insulin resistance, which cause type 2 diabetes mellitus. [18] Accordingly, adipocytokines, such as leptin and adiponectin, may play an important role in the development of diabetes mellitus. Here, we showed that SNPs of leptin receptor and adiponectin genes are remarkable for the development of metabolic syndromes as well as UCP or β2, β3AR genes.

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References

1) Bell CG, Walley AJ, Froguel P. The genetics of human obesity. Nature Rev Genet 6:221-234, 2005.
2) The International HapMap Consortium. A haplotype map of the human genome. Nature 437:1299-1320, 2005.
3) Watanabe S, Morita A, Aiba N, et al. Study design of the Saku Control Obesity Program (SCOP). Anti-Aging Med 4:70-73, 2007.
4) Morita A, Ohmori Y, Suzuki N, et al. Anthropometric and clinical findings in obese people in Saku Control Obesity Program (SCOP). Anti-Aging Med. (in press)
5) Shihara N, Yasuda K, Moritani T, et al. Synergistic effect of polymorphisms of uncoupling protein 1 and β3-adrenergic receptor genes on autonomic nervous system activity. Int J Obes 25:761-766, 2001.
6) Shiinoki T, Suehiro T, Ikeda Y, et al. Screening for variants of the uncoupling protein 2 gene in Japanese patients with non-insulin dependent diabetes mellitus. Metabolism 48:581-584, 1999.
7) Liu Y-J, Liu P-Y, Long J, et al. Linkage and association analysis of the UCP3 gene with obesity phenotypes in Caucasian families. Physiol Genomics 22:197-203, 2005.
8) Mori Y, Kim-Motoyama H, Katakura T, et al. Effect of the Pro12Ala variant of the human peroxisome proliferator-activated receptor gamma gene on adiposity, fat distribution, and insulin sensitivity in Japanese men. Biochem Biophys Res Commun 251:195-198, 1998.
9) Kondo H, Shimomura I, Matsukawa Y, et al. Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. Diabetes 51:2325-2328, 2002.
10) Park KS, Shin HD, Park BL, et al. Polymorphisms in the leptin receptor (LEPR)- putative association with obesity and T2DM. J Hum Genet 51:85-81, 2006.
11) Matsuoka N, Ogawa Y, Hosoda K, et al. Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. Diabetologia 40:1204-1210, 1997.
12) Horikawa Y, Oda N, Yu L, et al. Genetic variations in calpain-10 gene are not a major factor in the occurrence of type 2 diabetes in Japanese. J Clin Endocrinol Metab 88:244-247, 2003.
13) Yamada K, Ishiyama-Shigemoto S, Ichikawa F, et al. Polymorphism in the 5'-leader cistron of the β2-adrenergic receptor gene associated with obesity and type 2 diabetes. J Clin Endocrinol Metab 84:1754-1757, 1999.
14) Hayakawa T, Nagai Y, Kahara T, et al. Gln27Glu and Arg16Gly polymorphisms of the β2-adrenergic receptor gene are not associated with obesity in Japanese men. Metabolism 49:1215-1218, 2000.
15) Rosmond R, Chagnon YC, Holm G, et al. Hypertension in obesity and the leptin receptor gene locus. J Clin Endocrinol Metab 85: 3126-3131, 2000.
16) Wauters M, Martens I, Rankinen T, et al. Leptin receptor polymorphisms are associated with insulin in obese woman with impaired glucose tolerance. J Clin Endocrinol Metab 86:3227-3231, 2001.
17) Mita T, Watada H, Uchino H, et al. Association of C-reactive protein with early-stage carotid atherosclerosis in Japanese patients with early-state type 2 diabetes mellitus. Endocr J 53:693-698, 2006.
18) Inoue M, Yano M, Yamakado M, et al. Relationship between the adiponectin-leptin ratio and parameters of insulin resistance in subjects without hyperglycemia. Metabolism 55:1284-54, 2006.

APPENDIX

APPENDIX 2

Apendix Table 1 Basic characteristics of subjects by group*

	Intervention group (n=119)	Control group (n=116)	P-value***
Age (years)	53.7 ± 6.7	53.3 ± 6.4	0.687
Sex (% women)	50.4	50.9	0.946
Body height (cm)	161.6 ± 8.5	162.0 ± 8.8	0.757
Body weight (kg)	79.7 ± 9.9	81.7 ± 13.9	0.194
Body mass index (kg/m^2)	30.5 ± 2.9	31.1 ± 3.8	0.196
Change of body weight from 20 years old (kg)**	17.4 ± 8.7	20.3 ± 8.8	0.013
Intentional of dietary change (%)			
No	60.2	60.9	
Yes (within 1y)	18.6	20.0	0.979
Yes (within 3y)	9.3	8.7	
Yes (more than 3y ago)	11.9	10.4	
Current dietary couseling (%)			
No	83.2	82.8	0.929
Yes	16.8	17.2	
Rate of eating (%)			
Very fast	26.9	26.7	
Relatively fast	45.4	48.3	
Medium	21.0	18.1	0.957
Relatively slow	5.0	4.3	
Very slow	1.7	2.6	
Fat prefarence (%)			
Pork fat and beef fat			
like and usually eat	23.5	15.5	
Neither like nor dislike	41.2	48.3	0.272
dislike and don' t eat	35.3	36.2	
Poultry skin			
like and usually eat	18.5	19.0	
Neither like nor dislike	37.0	44.8	0.386
dislike and don' t eat	44.5	36.2	

*Values are mean±standard deviation or percent.

** Present body weight minus body weight at 20 years old (n=232; 118 intervention group

*** Significant difference between randomized groups.

Appendix Table 2 Basic characteristics of men and women by group*

	Men			Women		
	Intervention group (n=59)	Control group (n=57)	P-value***	Intervention group (n=60)	Control group (n=59)	P-value***
Age (years)	52.9 ± 6.8	52.8 ± 6.4	0.909	54.4 ± 6.6	53.8 ± 6.3	0.641
Body height (cm)	168.0 ± 5.9	168.9 ± 5.7	0.400	155.4 ± 5.5	155.3 ± 5.5	0.936
Body weight (kg)	84.2 ± 8.4	88.6 ± 14.2	0.045	75.2 ± 9.2	75.1 ± 9.9	0.950
Body mass index (kg/m^2)	29.8 ± 2.3	31.0 ± 4.4	0.071	31.1 ± 3.2	31.1 ± 3.1	0.936
Change of body weight from 20 years old (kg)**	16.4 ± 7.7	19.3 ± 9.4	0.082	18.3 ± 9.5	21.2 ± 8.2	0.082
Intentional of dietary change (%)						
No	55.9	63.2		64.4	58.6	
Yes (within 1y)	23.7	17.5	0.756	13.6	22.4	0.614
Yes (within 3y)	8.5	10.5		10.2	6.9	
Yes (more than 3y ago)	11.9	8.8		11.9	12.1	
Current dietary couseling (%)						
No	86.4	84.2	0.734	80.0	81.4	0.851
Yes	13.6	15.8		20.0	18.6	
Rate of eating (%)						
Very fast	33.9	35.1		20.0	18.6	
Relatively fast	44.1	40.4		46.7	55.9	
Medium	20.3	19.3	0.822	21.7	17.0	0.855
Relatively slow	1.7	3.5		8.3	5.1	
Very slow	0.0	1.8		3.3	3.4	
Fat prefarence (%)						
Pork fat and beef fat						
like and usually eat	33.9	22.8		13.3	8.5	
Neither like nor dislike	39.0	59.7	0.084	43.3	37.3	0.441
dislike and don' t eat	27.1	17.5		43.3	54.2	
Poultry skin						
like and usually eat	23.7	24.6		13.3	13.6	
Neither like nor dislike	44.1	47.4	0.886	30.0	42.4	0.333
dislike and don' t eat	32.2	28.1		56.7	44.1	

*Values are mean±standard deviation or percent.

** Present body weight minus body weight at 20 years old (n=232; 58 intervention and 54 control group in men, 60 intervention and 60 control group in women).

*** Significant difference between sexes.

Appendix Table 3 Basic characteristics of subjects according to quartile of body mass index (BMI)*

	Quartiles of BMI				
	1 (n=58)	2 (n=59)	3 (n=59)	4 (n=59)	P-value***
Body mass index (kg/m^2)	27.6 ± 0.8	29.3 ± 0.4	30.9 ± 0.6	35.2 ± 3.5	
Age (years)	54.1 ± 6.5	53.8 ± 6.3	54.2 ± 6.6	51.8 ± 6.6	0.166
Sex (% women)	44.8	44.1	52.5	61.0	0.219
Body height (cm)	162.8 ± 8.0	163.5 ± 9.4	160.5 ± 8.3	160.4 ± 8.6	0.105
Body weight (kg)	73.4 ± 7.2	78.6 ± 8.9	79.7 ± 8.0	91.0 ± 15.0	<.0001
Change of body weight from 20 years old (kg)**	15.4 ± 5.4	17.2 ± 6.6	22.8 ± 15.1	25.9 ± 10.7	<.0001
Intentional of dietary change (%)					
No	43.1	61.0	67.2	70.7	
Yes (within 1y)	37.9	17.0	13.8	8.6	
Yes (within 3y)	8.6	8.5	10.3	8.6	0.017
Yes (more than 3y ago)	10.3	13.6	8.6	12.1	
Current dietary couseling (%)					
No	77.6	83.1	86.4	84.8	
Yes	22.4	17.0	13.6	15.3	0.609
Rate of eating (%)					
Very fast	22.4	33.9	20.3	30.5	
Relatively fast	43.1	44.1	49.2	50.9	
Medium	29.3	17.0	25.4	6.8	0.029
Relatively slow	3.5	5.1	0.0	10.2	
Very slow	1.7	0.0	5.1	1.7	
Fat prefarence (%)					
Pork fat and beef fat					
like and usually eat	15.5	22.0	15.3	25.4	
Neither like nor dislike	43.1	39.0	54.2	42.4	0.500
dislike and don' t eat	41.4	39.0	30.5	32.2	
Poultry skin					
like and usually eat	13.8	20.3	17.0	23.7	
Neither like nor dislike	41.4	33.9	45.8	42.4	0.641
dislike and don' t eat	44.8	45.8	37.3	33.9	

*Values are mean±standard deviation or percent.
** Present body weight minus body weight at 20 years old (n=232; 112 men and 120 women
*** Significant difference between BMI categories.

Appendix Table 4 Mean ± standard eviation (SD) of energy and nutrient intakes estimated by a self-administered diet history questionnaire (DHQ) among 235 subjects*

	Crude model		Density model	
Energy	kcal /day	2456 ±839		
Protein	g/day	90.4 ±30.7	% energy	14.9 ±2.5
Fat	g/day	73.2 ±35.2	% energy	26.4 ±6.6
Total fatty acid	g/day	63.9 ±31.4	% energy	23.0 ±6.0
Saturated fatty acid	g/day	20.3 ±9.5	% energy	7.4 ±1.9
Monounsaturated fatty acid	g/day	26.2 ±14.1	% energy	9.3 ±2.9
Polyunsaturated fatty acid	g/day	17.1 ±8.5	% energy	6.2 ±1.7
Cholesterol	mg/day	393 ±181	mg/1000 kcal	159 ±54
Alcohol	g/day	17.2 ±35.1	% energy	4.2 ±7.6
Sodium	mg/day	5473 ±2150	mg/1000 kcal	2271 ±622
Potassium	mg/day	3276 ±1087	mg/1000 kcal	1371 ±305
Calcium	mg/day	924 ±288	mg/1000 kcal	395 ±118
Magnesium	mg/day	345 ±110	mg/1000 kcal	144 ±30
Phosphorus	mg/day	1460 ±460	mg/1000 kcal	607 ±109
Iron	mg/day	8.7 ±3.1	mg/1000 kcal	3.6 ±0.9
Zinc	mg/day	10.2 ±3.4	mg/1000 kcal	4.2 ±0.6
Copper	mg/day	1.4 ±0.5	mg/1000 kcal	0.6 ±0.1
Retinol	μg/day	424 ±495	μg/1000 kcal	169 ±166
Carotene	μg/day	3494 ±2397	μg/1000 kcal	1480 ±962
Vitamin D	μg/day	11.5 ±6.8	μg/1000 kcal	4.7 ±2.3
Thiamin	mg/day	1.1 ±0.5	mg/1000 kcal	0.5 ±0.1
Riboflavin	mg/day	2.1 ±0.6	mg/1000 kcal	0.9 ±0.2
Niacin	mg/day	20.9 ±9.2	mg/1000 kcal	8.5 ±2.2
Vitamin C	mg/day	122 ±77	mg/1000 kcal	51 ±28
Soluble dietary fiber	g/day	3.6 ±1.7	g/1000 kcal	1.5 ±0.6
Insoluble dietary fiber	g/day	10.8 ±4.4	g/1000 kcal	4.5 ±1.5
Total dietary fiber	g/day	15.0 ±6.2	g/1000 kcal	6.3 ±2.1

* Values are expresssed as mean±standard deviation.