Original Article

Effect of β_3-Adrenergic Receptor Gene Polymorphism on Body Weight Change in Middle-Aged, Overweight Women

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Abstract

Objectives: To examine the effects of β_3-adrenergic receptor gene polymorphism on body weight change during a weight reduction program for middle-aged, overweight women with careful consideration of their energy intake and expenditure.

Methods: Design: Intervention study of weight reduction for 12 weeks in a community setting. **Subjects:** Eighty overweight middle-aged women who completed the individualized lifestyle modification program. **Measurements:** β_3-adrenergic receptor gene polymorphism was identified by polymerase chain reaction and consecutive restriction fragment-length polymorphism analysis. Anthropometrical parameters, lifestyle factors, blood lipid and glucose levels, physical activity level and energy intake were measured before and at the end of the program.

Results: The numbers of subjects with the Trp64Trp, Trp64Arg, and Arg64Arg genotypes were 45, 30 and 5, respectively. Baseline characteristics among subjects with the Trp64Trp, Trp64Arg and Arg64Arg alleles did not differ. After 12 weeks, the subjects with the 64Arg allele had significantly smaller decrease in body weight and energy intake than those without the 64Arg allele. The change of other clinical characteristics did not differ between the two groups. After adjusting for the %change of energy intake, the %change of body weight did not differ between the two groups.

Conclusion: The 64Arg allele of the β_3-AR gene is not likely to be the factor determining the difficulty in losing body weight in Japanese middle-aged, overweight women. Lifestyle factors, such as the decrease in energy intake, might mask the effect of the 64Arg allele on body weight loss. Specific considerations for the management of energy intake would be needed to promote body weight loss for those with the 64Arg allele.

Key words: β_3-adrenergic receptor gene, polymorphism, body weight change, energy intake, physical activity

Introduction

The β_3-adrenergic receptor (β_3-AR) gene is established as the principal mediator of thermogenesis in brown adipose tissue and lipolysis in white adipose tissue both in animals and humans (1–6). First, Walston et al. (7) found that the single-nucleotide polymorphism of the gene encoding this receptor was often observed in Pima Indians, and they suggested that the polymorphism is one of the main causes of the high prevalence of obesity in this population. Since then, the correlations of this polymorphism with obesity, and obesity-related disorders have been intensively investigated.

The β_3-AR gene polymorphism of the human β_3-AR gene, characterized by the substitution of arginine for tryptophan at position 64 (64Arg allele), has been reported to be positively associated with body weight (BW) gain (8, 9), obesity (10, 11), and obesity-related metabolic syndrome (12–14). However, there are also reports that showed no significant correlation of the 64Arg allele with BW gain and metabolic disorders (15–18). These inconsistent results of observational studies could be attributed mainly to the wide variations in the criteria used to select patients (19, 20), and other confounding factors such as energy intake, physical activity (PA) level and/or other lifestyle

factors. Marti et al. (21) suggested that the PA level attenuated the obesity risk of the 64Arg allele.

An intervention study concerning the influence of the 64Arg allele on BW loss is required to resolve these controversial results of observational studies. Previous intervention studies showed that there is no significant difference in the magnitude of BW loss between those with and without the 64Arg allele (19, 22–26). However, other studies showed that subjects with the 64Arg allele had difficulty in losing BW (27–29), or had difficulty in losing visceral fat (24) or abdominal adipose tissue (30) during BW reduction programs. These discrepancies among investigations might be explained by lifestyle factors including energy intake and expenditure. Dietary intake and PA level may interact with genetic factors and may mask genotype influences. Nevertheless, in the majority of studies, careful consideration was not made on the overall lifestyle factors such as daily energy intake or PA including exercise that might contribute to BW change in study subjects.

Although this polymorphism is more common among Japanese than Caucasians and blacks (7, 8), there have been only a few studies concerning the effects of the 64Arg allele on BW loss conducted in community settings in Japan. Community-based BW reduction programs have been prevalent, and if the 64Arg allele could influence BW loss, we would need specific considerations for those with the 64Arg allele.

The purpose of this study was to assess the relationship between the magnitude of BW loss and β3-AR gene polymorphism in middle-aged Japanese women with careful consideration of energy intake, energy expenditure and other lifestyle factors. The change in percent body fat, waist circumference, serum lipid level and plasma glucose level were also examined.

Methods

The subjects were 80 women (range of age: 40 to 69 years old) who were recruited to join a 12-week lifestyle modification BW reduction program held in Tokyo, Japan, and completed the program between 1999 and 2001.

Recruitment to the program was conducted through ward newsletters. The main purpose of this program was to modify cardiovascular risk factors (such as hypertension, dyslipidemia and hyperglycemia) by reducing more than 5% of BW through the 12-week individualized lifestyle modification program. The participants underwent weekly workshops in which health professionals, registered dieticians and exercise experts offered individualized counseling on energy intake reduction. Sixty-minute aerobic exercise of moderate intensity was held once a week. Participants were also instructed to wear a pedometer and to increase their daily number of walking steps up to 10 thousand steps per day.

Measurements

The presence of the 64Arg allele in the β3-AR gene was examined in peripheral blood leukocyte DNA by polymerase chain reaction (PCR) and consecutive restriction fragment-length polymorphism (RFLP) screening. DNA was extracted from peripheral blood leucocytes using a commercially available DNA extraction kit (Dr. Gentle, Takara Biomedicals, Otsu, Japan). PCR was carried out using 50 to 100 ng of genomic DNA from leukocytes as a template with a primer (upstream, 5'-CGCCCAATACCGCCAACAC-3'; downstream, 5'-CCAC-CAGGAGTCCCATCACC-3') under the same conditions used by Clement et al. (8). PCR products were digested with endonuclease Bst NI, and were separated according to the method of Kadowaki et al. (31).

The following study variables were measured 1 week before and at the 12th week of the program.

BW and height were measured to the nearest 0.1 kg and 0.1 cm respectively, with the subjects in light clothing and without shoes in the fasting state in the morning. Body mass index (BMI: BW (kg) over height (m) squared) was used as relative BW.

Tricep (the midpoint between the acromion and olecranon) and subscapula (lower perpendicular point of the subscapula) skinfold thicknesses (mm) were measured on the right side of the body in a natural standing position using an Eiken caliper skinfold thickness meter. Whole-body density for the Japanese was estimated by the sum of skinfold thicknesses at two points (32). Density was applied to Brozek's equation for calculating percent (%) body fat (33).

Waist circumference (cm) was measured at the level of the navel using a steel tape with the subjects in a standing position.

The number of walking steps per day was continuously recorded using a pedometer (EC-200, Yamasa Company, Japan) during the entire period of participation in the program. Participants were instructed to wear a pedometer all day except during sleeping time. The mean numbers of daily walking steps for 1 week before and at the 12th week of the program were calculated.

Information on habitual exercise, smoking, alcohol intake and the history of illness was collected from medical records.

Energy intake (kcal per day) was calculated from the dietary records of food intake for two consecutive days, 1 week before the program and again at the end of the program. Two registered dieticians instructed each subject on how to record detailed descriptions of all foods and beverages consumed (ingredients and cooking methods). The registered dieticians checked the dietary records and personally clarified any ambiguous information to ensure the completeness of records, and confirmed the actual portion sizes. Energy intake and nutritional intake were calculated using the Standard Tables of Food Composition in Japan (34).

Blood samples were collected from the anterior cubital vein in the overnight fasting state, and serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG) and fasting plasma glucose (FPG) levels were immediately analyzed in the Foundation of Yobo Igaku Kyoukai (Tokyo, Japan). TC, HDL-C, TG and FPG levels were determined by enzymatic methods using an automatic analyzer (Nippon Denshi BM-12, Japan). Quality control for blood testing was performed every day using pooled standard blood samples. The coefficients of variation (CV) of TC, HDL-C, TG and FPG levels obtained using this measurement system were 1.81, 0.64, 1.47 and 1.36 respectively. Low-density lipoprotein cholesterol (LDL-C) level was calculated using the Friedewald formula (35).

This study was conducted in accordance with the ethical principles stated in The Declaration of Helsinki (by the World

Medical Association). The autonomy of the participants was fully respected, and written information including the purpose of the study, use and application of the study, assurance of their right to refuse, benefits and risks, and the security of personal information was provided to each participant. All participants signed the form of consent to participate in this study.

Table 1 Baseline characteristics of subjects with or without 64Arg allele

	Without 64Arg allele	With 64Arg allele	
n	45	35	
Age [years]	50.1±6.5	51.2±7.0	ns[A]
Body weight at 20y old [kg]	49.9±5.0	49.8±5.0	ns[A]
Postmenopause (%)[a]	20 (44.4)	18 (51.4)	ns[B]
Habitual exercisers [2] (%)[b]	8 (17.8)	4 (11.4)	ns[B]
Smokers (%)[c]	4 (8.9)	3 (8.6)	ns[B]
Alcohol drinkers (%)[d]	21 (46.7)	12 (34.3)	ns[B]
Number of steps [/day]	7910.8±2961.0	7162.3±2385.3	ns[A]
Energy intake [kcal/day]	1933.2±292.7	1888.6±402.0	ns[A]
Height [cm]	154.6±3.8	153.9±5.8	ns[A]
Body weight [kg]	65.1±7.3	64.0±9.4	ns[A]
BMI [kg/m²]	27.2±2.8	26.9±3.0	ns[A]
Fat [%]	37.2±4.9	36.7±4.5	ns[A]
Waist [cm]	90.8±8.0	88.8±8.4	ns[A]
Total cholesterol [mg/dL]	224.3±37.0	238.1±42.2	ns[A]
HDL-C [mg/dL]	62.8±15.3	64.2±16.9	ns[A]
LDL-C [mg/dL]	136.2±33.2	149.9±39.7	ns[A]
Triglyceride [mg/dL]	115.3±87.7	112.3±60.6	ns[A]
Plasma glucose [mg/dL]	98.3±30.6	96.5±14.5	ns[A]

Data are means±SD or number (proportion).
[a] Postmenopause: no menstruation for more than 12 months when their age was over 45 years old, or surgical menopause with clear history of both oophorectomy. [b] Habitual exercisers: subjects who engaged in aerobic exercise with moderate intensity, more than 30 minutes at one time, and more than 2 times a week, regularly. [c] Smokers: subjects who smoke currently. [d] Alcohol drinkers: subjects who drink more than once a week, regularly. [A] Test of Welch: ns, no significance. [B] Chi-square tests: ns, no significance.

Statistical analysis

Data are shown as means±SD or least squares means±SE. Baseline characteristics among the subjects with β₃-AR gene polymorphism (Trp64Trp, Trp64Arg and Arg64Arg) were compared using ANOVA or the chi-square test. The numbers of subjects with the Trp/Trp, Trp/Arg, and Arg/Arg genotypes were 45, 30 and 5, respectively. There were no significant differences in any baseline characteristics among three groups. Therefore, the subjects were divided into two groups on the basis of their β₃-AR gene polymorphism (without the 64Arg allele: Trp/Trp, and with the 64Arg allele: Trp/Arg and Arg/Arg), and baseline characteristics were compared using Welch's test and the chi-square test.

To compare changes after the program with respect to baseline values obtained before the program, percent (%) change (proportion of magnitude of change to the baseline value) was used. The %change in each group was compared using the t-test. When comparing the differences in %change between the subjects with and without the 64Arg allele, the t-test was used. When comparing the differences in %change between the subjects with and without the 64Arg allele, the analysis of covariance was applied after adjustment for %changes of energy intake and the number of walking steps. All analyses were two-tailed and a p-value <0.05 was considered statistically significant. All statistical analyses were performed using the SAS (Release 9.1; SAS Institute Inc., Cary, NC, USA) computer program.

Results

Table 1 shows the comparison of baseline characteristics between the subjects with and without the 64Arg allele.

The frequency of the 64Arg allele among the study subjects was 0.25. There were no significant differences in age, BW at 20 years old, the proportions of postmenopausal women, habitual exercisers, smokers and alcohol drinkers, height, BW, BMI, %body fat, waist circumference, blood lipid level and glucose level between the two groups.

The clinical characteristics of the subjects with and with-

Table 2 Changes in clinical characteristics after the program in subjects with and without 64Arg allele

	Without 64Arg allele				With 64Arg allele				P[A]	P[B]
	After	%change[a]	P[a]	%change[b]	After	%change[b]	P[a]	%change[b]		
Energy intake [kcal/day]	1562.2±268.5	−18.1±14.9	**	—	1690.1±337.5	−8.3±19.4	*	—	*	
Number of steps [/day]	10356.0±2554.0	48.4±65.5	**	—	9646.2±2635.9	42.2±36.2	**	—	ns	
Body weight [kg]	61.5±6.4	−5.3±3.0	**	−5.2±0.4	61.3±9.1	−4.1±2.4	**	−4.4±0.5	*	ns
BMI [kg/m²]	25.8±2.5	−5.4±3.0	**	−5.2±0.4	25.8±3.0	−4.1±2.3	**	−4.4±0.5	*	ns
Fat [%]	33.8±4.9	−8.9±7.8	**	−8.5±1.2	33.6±5.7	−8.5±8.4	**	−9.0±1.4	ns	ns
Waist [cm]	85.1±7.6	−6.2±5.1	**	−6.2±0.8	84.9±8.1	−4.3±4.7	**	−4.3±0.9	ns	ns
Total cholesterol [mg/dL]	205.4±36.1	−7.6±13.1	**	−6.5±1.9	219.7±41.4	−7.6±12.8	**	−8.9±2.2	ns	ns
HDL-C [mg/dL]	64.8±14.4	4.7±14.9	*	5.3±2.1	65.1±14.7	3.0±13.0	ns	2.2±2.4	ns	ns
LDL-C [mg/dL]	126.2±32.0	−5.8±16.6	*	−4.8±2.8	136.7±36.8	−6.6±20.7	ns	−7.8±3.8	ns	ns
Triglyceride [mg/dL]	73.7±40.5	−17.7±43.1	**	−14.2±8.4	97.4±56.2	1.2±71.8	ns	−3.3±9.6	ns	ns
Plasma glucose [mg/dL]	93.3±11.7	−2.3±13.0	ns	−2.5±1.8	93.6±11.5	−2.1±10.0	ns	−1.8±2.1	ns	ns

Data are means±SD. %change[a]: proportion of magnitude of change after the program with respect to baseline value. %change[b] (least squared mean±SE): proportion of magnitude of change after adjusting for %change of energy intake and number of walking steps. P[a]: Significance of %change[a] in each group (t-test). P[A]: comparison of %change[a] between two groups (t-test). P[B]: comparison of %change[b] after adjusting for %change of energy intake and number of walking steps between two groups (analysis of covariance): *: p<0.05, **: p<0.01, ns: no significance.

out the 64Arg allele after the weight reduction program are shown in Table 2.

All of the mean values after the program, except for serum TG level, did not significantly differ between the two groups. The mean TG level after the program was significantly lower in the subjects without the 64Arg allele ($p<0.05$).

The %changes of energy intake, number of steps, BW, BMI, %body fat, waist circumference and TC level were significant in each group. However, the %change of FPG level was not significant in each group. However, the %changes of HDL-C, LDL-C and TG levels were significant in subjects without the 64Arg allele, but not in subjects with the 64Arg allele.

The results of comparison of the magnitude of %change between the subjects with and without the 64Arg allele showed that the former showed significantly smaller changes in energy intake, BW and BMI than the latter. However, the magnitudes of the %changes of the number of steps, %body fat, waist circumference, and TC, HDL-C, LDL-C, TG and FPG levels did not differ between the two groups.

After adjustment for the %changes of energy intake and the number of steps by analysis of covariance, the magnitude of the %change of BW or BMI did not differ between the two groups. Even when only adjustment for the %change of energy intake was considered, the magnitude of the %change of BW or BMI did not significantly differ between the two groups.

Discussion

In this study, we examined the effects of β₃-AR gene polymorphism on the response to a 12-week community-based weight reduction program for middle-aged, overweight women. Before the program, subjects with and without the 64Arg allele genotype showed similar BW, BMI and levels of lifestyle-related behavioral factors. During the 12-week lifestyle modification, BW, the number of daily walking steps and energy intake significantly changed in each group. However, subjects with the 64Arg allele had significantly smaller decreases in BW and energy intake compared with subjects without the 64Arg allele. After adjustment for the %change of energy intake during the program, the magnitude of %change of BW did not differ between the two groups. This result meant that the difference in change in BW between the two groups was mainly influenced by the change in energy intake.

To date, several intervention studies of the effect of the β₃-AR genotype on the response to BW reduction programs have been reported. Studies conducted in Finland (22), France (23) and Germany (25) could not elucidate the effect of β₃-AR gene polymorphism on the response to the BW reduction programs. In contrast, studies conducted in Japan (27–29) showed that subjects with the 64Arg allele have smaller decreases in BW after 12-week BW reduction programs than those without the 64Arg allele, although the baseline BMI showed no difference between those with and without the 64Arg allele.

The different responses to BW reduction program in studies concerning β₃-AR gene polymorphism seemed to depend on the frequency of the 64Arg allele among the subjects. In those studies, which could not elucidate the effect

of β₃-AR gene polymorphism on the magnitude of BW loss, the frequency of the 64Arg allele was very low (Finland (22), 0.09; France (23), 0.08 and Germany (25), 0.11). The frequency of the 64Arg allele has a marked ethnic difference (20), and these inconsistent results could be attributed to the wide variations in the 64Arg allele frequency and/or interactions with different genetic backgrounds or lifestyles of the study subjects. Therefore, the comparison of study results among the same ethnic and gender groups would be necessary (15).

In the present study, the frequency of the 64Arg allele was 0.25, and this allele frequency was similar to those (0.19 to 0.23) that were reported among Japanese women in other studies (27–29). In the present study, subjects with the 64Arg allele had significantly smaller decreases in BW after the 12-week BW reduction program than those without the 64Arg allele as in other Japanese studies (27–29). However, a significant difference in change of energy intake was observed between subjects with and without the 64Arg allele.

BW change is influenced by lifestyle factors such as daily energy intake or daily PA levels. Therefore, the amount of energy intake determined from the dietary record and energy expenditure determined from the record of the number of steps taken daily were carefully evaluated in this study. The result was reanalyzed after adjusting for the change in energy intake and number of steps, and there was no difference in the magnitude of %change of body weight between the two groups. Dietary intake and/or PA levels may interact with genetic factors and may mask genotype influences. This result meant that β₃-AR gene is not likely to be a factor determining the difficulty of losing body weight in overweight women. Further study is required to test this hypothesis.

Baseline BW and BMI were not different between the subjects with and without the 64Arg allele in this study as in other Japanese studies (27–29). Two meta-analyses of the correlation of β₃-AR gene polymorphism with BMI led to different conclusions. One showed no significant correlation of β₃-AR gene polymorphism with BMI (36). The other showed significant correlation of β₃-AR gene polymorphism with BMI, but the mean difference in BMI was only 0.3 kg/m² between the subjects with and without the 64Arg allele (9). In longitudinal observational studies (17, 18) conducted in Japan, the effects of β₃-AR gene polymorphism on BW and serum lipid level or on BW alone with a 4 year and 10 year follow-up were investigated. However, β₃-AR gene polymorphism did not show any effect on these parameters. Baseline BMI would be the cumulative effect of long-term BW change. The effect of β₃-AR gene polymorphism on long-term BW change could be modified by lifestyle factors. Several behavioral factors such as diet, PA and exercise could cumulatively modify long-term BW change as well as other gene polymorphism yet to be identified.

Prior studies suggested that β₃-AR polymorphism seemed to influence body fat distribution and metabolic abnormalities, and those with the 64Arg allele showed less improvement in waist and/or visceral fat, lipid and glucose profiles whether the difference of BW change was observed or not (24, 28–30). In the present study, the improvement of waist circumference and TC level did not differ between the two groups. The %changes of HDL-C and TG levels were not significant between the two

groups. However, HDL-C and TG levels significantly improved in subjects without the 64Arg allele, but not in those with the 64Arg allele. β₃-AR gene is almost exclusively expressed in adipose tissue, and subjects carrying the 64Arg allele have an impaired β₃-AR-induced lipolysis (1–6). β₃-AR gene could be responsible for lipid regulation as well as lipolysis of body fat (30).

The limitations of this study were that we did not include other associated genes, such as the UCP gene family or other β sub-AR genes, as suggested by previous studies (18, 25, 30). Further studies are needed to take into account other associated genes, sufficient sample size, and lifestyle factors, simultaneously. The merits of this study were that it included a population with a high prevalence rate of gene polymorphism in

a community setting and we carefully evaluated lifestyle factors during the study period.

In conclusion, the 64Arg allele of the β₃-AR gene is not likely to be the factor determining the difficulty in losing BW in Japanese middle-aged, overweight women, as found with dietary energy intake and energy expenditure carefully evaluated. This is the first study that examined the effects of β₃-AR gene polymorphism on BW loss by giving careful consideration on energy intake and energy expenditure in community-residing Japanese women. Specific considerations for the modification of lifestyle, such as the management of energy intake to promote BW loss for those with the 64Arg allele would be needed.

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