Impact of Aging and β_3 -Adrenergic-Receptor Polymorphism on Thermic and Sympathetic Responses to a High-Fat Meal

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Summary The present study was designed to investigate the effect of aging and β_3 -adrenergic-receptor (β_3 -AR) polymorphism on the thermic effect of meal (TEM) and sympathetic nervous system (SNS) response to a high-fat meal in 13 boys, 12 young men, and 11 middle-aged men. SNS activity was assessed via power spectral analysis of heart rate variability. Significantly higher very-low-frequency (VLF) components associated with thermogenic SNS activity and energy expenditure per lean body mass (EE_{LBM}) were observed in boys during the pre- and postprandial periods. There were no significant differences in VLF and EE_{LBM} in the preprandial period between the young and middle-aged men. After feeding, however, the middle-aged men showed a significantly lower TEM (% test-meal energy) and VLF compared to the young men. A multiple regression analysis revealed that age was the only significant variable contributing to both TEM and VLF, but β_3 -AR polymorphism and percentage of body fat were not statistically significant. In conclusion, age likely has a greater influence on TEM and SNS thermoregulation than genetic factors such as β_3 -AR polymorphism, suggesting that this age-related decrease in thermogenic response may be involved in the development of obesity among middle-aged men.

Key Words Age, β_3 -AR polymorphism, thermic effect of meal, high-fat meal, sympathetic nervous system

The β_3 -adrenergic receptor (β_3 -AR) is predominantly expressed in brown and white adipose tissues, regulating lipolysis and thermogenesis (1, 2). Therefore, reduced β_3 -adrenergic sensitivity can lead to lower thermogenesis and consequently cause an elevated level of body fat storage.

It is commonly believed that reduced β_3 -AR function is caused by genetic and physiological factors. Concerning genetic factors, Trp/Arg⁶⁴ polymorphism of the β_3 -AR gene has been considered to be associated with the early development of Type-2 diabetes mellitus (3, 4), insulin resistance (4, 5), increased body weight (6–9), and resistance to weight loss (6, 10, 11). With respect to physiological factors, advancing age is associated with alterations in β -adrenergic sensitivity (12–14), density (13), and post-receptor signaling (15, 16). According to these findings, β_3 -AR polymorphism and advancing age may diminish β -adrenergically-induced thermogenesis, which is mediated by sympathetic nervous system (SNS) activity (17), and consequently plays a predisposing role in the development of obesity among middle-aged or older males (18).

On the other hand, a human study has revealed direct effects of plasma fatty acid concentrations on the

excitation of sympathetic nervous system (SNS) activity (19). Moreover, in our recent study (20) using iso-energetic high-fat and high-carbohydrate meals, greater thermoregulatory SNS activity (20–22) as well as fat oxidation were observed after the high-fat meal.

Accordingly, the aim of the present study was to investigate the impact of aging and β_3 -AR polymorphism on postprandial thermogenesis and thermoregulatory SNS activity via power spectral analysis of heart rate variability (HRV) in response to a high-fat meal, which induced postprandial SNS acceleration (19, 20) in healthy male subjects of various age groups.

METHODS

Subjects. The experiments were performed on 36 healthy and non-obese subjects including 13 boys (6–11 y), 12 young men (22–28 y), and 11 middle-aged men (41–53 y). Young male subjects were recruited from our campus. Boys and middle-aged men (their fathers) were recruited through two public elementary schools in the city of Kyoto, Japan. The medical history, physical activity level, and dietary habit were determined through an interview with the subjects, and parents in the case of the boys. None had a medical history of any significant physical illnesses, including obesity, diabetes, or other endocrine diseases. The weight of adult subjects had been stable for at least 1 y, and none

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Table 1. Subject characteristics.

	Boy (n=13)	Young (n=12)	Middle (n=11)
Age (y)	8.7 (0.3)	22.8 (0.2) ^a	45.8 (1.4) ^{a,b}
Height (cm)	137.2 (1.8)	174.2 (2.4) ^a	172.9 (1.8) ^a
Body mass (kg)	30.9 (1.0)	65.3 (1.9) ^a	73.3 (2.4) ^{a,b}
BMI (kg/m ²)	16.4 (0.3)	21.5 (0.4) ^a	24.5 (0.7) ^{a,b}
Body fat (%)	18.2 (1.0)	18.5 (1.1) ^a	24.4 (1.4) ^{a,b}
LBM (kg)	25.2 (0.6)	53.2 (1.4) ^a	55.3 (1.7) ^a
Nutritional requirement (MJ/d)	7.5 (0.2)	9.8 (0.3) ^a	10.3 (0.3) ^a
Test meal energy (MJ)	2.5 (0.1)	3.3 (0.1) ^a	3.4 (0.1) ^a
Preprandial EE			
Resting EE (kJ/min)	4.9 (0.2)	6.2 (0.2) ^a	7.0 (0.2) ^{a,b}
Resting EE (MJ/d)	7.0 (0.2)	9.0 (0.3) ^a	10.1 (0.3) ^{a,b}
Resting EE (kJ/LBM)	278 (6.1)	168 (3.5) ^a	184 (0.2) ^a
Postprandial EE			
TEM (kJ/3 h)	130 (12)	136 (12)	119 (12)
TEM (% preprandial EE)	1.9 (0.2)	1.5 (0.1)	1.2 (0.1) ^a
TEM (% test meal energy)	5.2 (0.5)	4.2 (0.3)	3.5 (0.3) ^a
TEM (kJ/LBM)	5.2 (0.5)	2.6 (0.2) ^a	2.1 (0.2) ^a

Values represent means (SE). Nutritional requirements were recommended by the Ministry of Health, Labor, and Welfare, Japan. LBM, lean body mass. EE, energy expenditure. TEM, thermic effect of meal.

^a $p < 0.05$ young or middle vs. boy group. ^b $p < 0.05$ middle vs. young group (by one-way ANOVA combined with post hoc Tukey test).

Table 2. Distribution of genotype defined by the β_3 -adrenergic receptor gene in the subjects.

	Boy (n=13)	Young (n=12)	Middle (n=11)
Trp/Arg ⁶⁴ mutation of β_3 -AR			
Trp/Trp	9 (69.2)	8 (66.7)	9 (81.8)
Trp/Arg	4 (30.8)	4 (33.3)	1 (9.1)
Arg/Arg	0 (0.0)	0 (0.0)	1 (9.1)
Trp/Arg ⁶⁴ mutation of β_3 -AR			
Arg allele frequency	0.15	0.17	0.14

Values represent n (%). AR, adrenergic receptor.

were athletes or smokers. Subjects were requested to avoid any medication and keep to their usual diet for at least 2 wk before the test. Descriptive characteristics of the subjects are presented in Table 1. The experimental procedures were approved by the Institutional Review Board of Kyoto University Graduate School and were in accordance with the Helsinki Declaration. All subjects and parents were carefully informed about the purpose and potential risks of the test, and all gave their written informed consent to participate in the study.

The distribution of the genotypes. The distribution of the genotypes defined by the Trp/Arg⁶⁴ polymorphism of the β_3 -AR gene is presented in Table 2. Values of the allelic frequency of the mutation are 0.15 (boys), 0.17 (young men), and 0.15 (middle-aged men), respectively. Despite the small amount of β_3 -AR mutation in middle-aged subjects, no significant difference of allelic frequency rate was observed among the three groups. Therefore, we conducted multiple regression analysis using these data.

Experimental procedure. On the day before the test, all consumption of food and drink had to cease before

10 PM. The consumption of coffee, tea, and alcohol was not allowed the day before the test, and no sports activities were permitted that evening.

On the day of the test, each subject arrived at the laboratory at 7:30 AM in a fasted condition. After measurements of height, body mass and percentage of body fat were determined using a bioelectrical impedance analyzer (Model TBF-534, Tanita Corp., Tokyo, Japan), the subject was equipped with electrocardiogram electrodes and then rested for at least 30 min in a temperature (24–25°C) and humidity-controlled environment. After the subject had received an explanation of the procedure and adjusted his mask, CM₅ lead ECG and gas exchange parameters were recorded using an open-circuit computerized indirect calorimeter (Aero monitor AE 280, Minato Medical Science, Tokyo, Japan) while the subject remained seated in a comfortable chair. The calorimeter was calibrated before each test with a reference gas mixture (15% O₂ and 5% CO₂). Continuous ventilatory volumes, $\dot{V}O_2$ and $\dot{V}CO_2$ were displayed on a computer at 15-s intervals and the mean value for each minute was printed out.

The test meal was served at 8:30 AM and eaten within 15 min. Postprandial energy expenditure (EE) was measured for 180 min (until 11:30 AM), and gas samples were taken for a period of 6 min every 30 min. During the test period, the subjects remained seated.

Genetic analysis. A noninvasive genotype sampling method has been implemented for collecting buccal mucosa cells using cytobrushes. After the phenol-extraction procedure, 0.2 to 2 μg of DNA per subject was obtained. The *MvaI* polymorphism of the β_3 -AR gene, which detects Trp/Arg⁶⁴ mutation, was determined using PCR-restriction fragment length polymorphism analysis according to our previously reported method (4). The PCR primers were 5'-CCAATACGCCA-ACACACAGT-3' (upstream) and 5'-AGGAGTCCCATC-ACCAGGTC-3' (downstream), which flank the whole exon 1 of the β_3 -AR gene. Genomic DNA (100 ng) in a total volume of 20 μL was used for PCR. Polymerase chain reaction was performed by initial denaturation at 94°C for 5 min, 30 cycles at 94°C for 30 s, 67°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 10 min. We then incubated 5 μL of the PCR product for 1 h with 10 U of *MvaI* at 37°C in a final volume of 10 μL without further purification. The samples were then run on a 3.0% agarose gel, stained with ethidium bromide, and analyzed under UV light. In the presence of the polymorphism, the restriction site for *MvaI* is lost; therefore, the allele of this polymorphism corresponds to the 158 bp undigested band.

Diet. The energy content of the meal corresponded to one-third of each subject's daily energy requirement, determined using reference values from metabolism tables for Japanese (23). A multiplication factor of 1.5 was used to account for the median physical activity level of the subjects. A high-fat meal (20% of energy as carbohydrate, 70% as fat, and 10% as protein, with fixed 80 $\text{kJ}\cdot\text{wt}^{-1}$ [boys] and 50 $\text{kJ}\cdot\text{wt}^{-1}$ [men]) was compiled using the same normal food items as applied in our previous experiment (24) and consumed for breakfast. It was prepared according to each subject's individual energy requirement, and adjusted to the nearest 100 kJ (Table 1). The macronutrient composition of the meals was calculated using the Japanese food composition table (25).

Calculation of energy expenditure. The mean of a stable 12-min period was calculated for preprandial energy expenditure (EE). Six periods of 6 min were averaged over the total 3-h thermogenic response. The thermic effect of the meal (TEM) was calculated by subtracting the preprandial EE value ($\text{kJ}\cdot\text{min}^{-1}$) from the averaged postprandial EE ($\text{kJ}\cdot\text{min}^{-1}$), and this was multiplied by the duration of the postprandial period (180 min). The TEM was expressed as an absolute as well as a relative value and the percentage increase over the preprandial EE.

R-R spectral analysis procedure. Our R-R interval power spectral analysis procedures have been previously described (20–22, 24, 26). Briefly, the ECG data obtained from the CM₅ lead was digitized via a 13-bit analog-to-digital converter (HTB 410; Trans Era, Utah,

USA) at a sampling rate of 1,024 Hz. Then, the digitized R-R interval time series was aligned in a 2 Hz sequence for power spectral analysis. The direct current component and linear trend were completely eliminated by digital filtering for the band-pass between 0.007 and 0.5 Hz. The high-pass filtering at 0.007 Hz was chosen to include the frequency components associated with the thermogenic function of the autonomic nervous system (ANS) (21, 22). After passing through a Hamming-type data window, power spectral analysis using a fast Fourier transform was performed on a consecutive 1,024-s time series of R-R interval data obtained during the test.

We analyzed the following: the very-low-frequency (0.007–0.035 Hz, VLF) component, reflecting SNS activity related to energy metabolic regulation (21, 22); the low-frequency (0.035–0.15 Hz, LF) component, jointly regulated by both the SNS and parasympathetic nervous system (PNS) activity; the high-frequency (0.15–0.5 Hz, HF) component, solely reflecting PNS activity; and total power (0.007–0.5 Hz, TP), representing overall ANS activity, achieved by integrating the spectrum for the respective band width. During the measurement, adult subjects were requested to breathe in synchrony with a metronome set at 15 times/ min^{-1} (0.25 Hz) to ensure that respiratory-linked variations in the heart rate did not overlap with low-frequency components (<0.15 Hz) from other sources. According to our previous experiments (20), there was no difference in ventilatory volumes and $\dot{V}\text{O}_2$ between the synchronic breathing with a metronome and normal breathing. The boys breathed without controlling their respiration rate during ECG measurement, because the breathing frequency of children is generally higher than 9 times/ min^{-1} (>0.15 Hz) (27).

Statistical analyses. All data are presented as the mean $\pm$ SE. All of the statistical analyses were performed with the Statistical Package for Social Science (SPSS for Windows, version 11.0, Inc., Chicago, IL, USA). All other group comparisons were made with one-way ANOVA using a post hoc Tukey test for subsequent pairwise comparisons. Multiple regression analysis was used to evaluate the impact of aging, percentage of body fat, and mutation of the β_3 -AR gene (as explanatory variables) on TEM or VLF, an index of thermoregulatory SNS activity (response variables). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Sympathetic response

Figure 1 represents typical sets of raw R-R intervals (top of each figure) and the corresponding power spectral data (bottom of each figure) obtained from subjects during the pre- (left) and postprandial right (periods), respectively. According to visual inspection, middle-aged men possessed remarkably reduced ranges of R-R variability as well as all frequency components of the power spectrum compared to the boys and young men in the preprandial period. While the VLF component of the HRV, associated with thermoregulatory SNS activ-

Fig. 1 A typical set of computer-aided ECG raw R-R intervals and the corresponding power spectrum obtained from boys (top), young men (middle), and middle-aged men (bottom) in the pre- (left) and postprandial (right) periods, respectively.

ity, increased in the young men after the high-fat meal, it decreased in the middle-aged men. The boys possessed a remarkably greater range of R-R variability and larger VLF component during the preprandial period than the young and middle-aged men, and maintained a similar level of VLF component from the pre- to postprandial periods.

Metabolic responses

Table 1 summarizes the physical characteristics and parameters of energy metabolism during the pre- and postprandial periods for the subjects. Although there were no obese subjects ($\text{BMI} > 30$), the middle-aged men had significantly higher BMI ($p < 0.001$) and percent body fat compared with the young men and boys ($p < 0.01$). Nutritional requirements and test meal energy were slightly, but not significantly, higher in the middle-aged men than in the young men due to their greater body weight. Absolute values of preprandial

energy expenditure (EE) were significantly higher in the middle-aged men than in the young men. However, the relative value of preprandial EE (divided by lean body mass [LBM]) was not significantly different between young and middle-aged men. Postprandial thermogenesis, expressed as an absolute value of the TEM, was not significantly different among the three groups; however, relative values of the TEM expressed as a percentage of the preprandial EE ($p < 0.01$, vs. boys, $p = 0.08$, vs. young) and percentage of test meal energy ($p < 0.05$, vs. boys) were lower in the middle-aged men.

Figure 2 shows the EE and VLF values during the pre- and postprandial periods among the three groups. In the preprandial condition, the values of EE (divided by LBM) and VLF were significantly higher in the boys, and no significant difference was found between the young and middle-aged men. After consuming the high-fat meal, however, the middle-aged men had a

Fig. 2. The energy expenditure (EE) and VLF in the pre- and postprandial periods. The preprandial EE (values per lean body mass, top left), the TEM (percentage of test meal energy, top right), and absolute values of VLF at rest (bottom left) and mean values after the meal (bottom right) in the three groups. Analyses were performed using by one-way ANOVA combined with post hoc Tukey test..

Table 3. Multiple regression analysis with preprandial EE as the response variable (transformed by the square root) in all subjects.

Explanatory variable	Regression coefficient (95% CI)	p value
Preprandial EE (kJ/d)	R=0.79	<0.001
Age	68.8 (41.1–96.4)	<0.001
Percentage of body fat	58.7 (–32.0–149.5)	0.197
Trp/Arg ⁶⁴ mutation	–236.6 (–1035.8–562.6)	0.551
Preprandial EE (kJ/LBM)	R=0.71	<0.001
Age	–2.82 (–3.9––1.8)	0.014
Percentage of body fat	3.40 (–0.04–6.84)	0.053
Trp/Arg ⁶⁴ mutation	–7.45 (–37.7–22.9)	0.620

CI, coefficient interval.

Table 4. Multiple regression analysis with TEM as the response variable (transformed by the square root) in all subjects.

Explanatory variable	Regression coefficient (95% CI)	p value
TEM (% test meal energy)	R=0.47	0.048
Age	–0.043 (–0.082––0.005)	0.029
Percentage of body fat	–0.020 (–0.154––0.114)	0.962
Trp/Arg ⁶⁴ mutation	–0.160 (–1.023––1.344)	0.474
TEM (% preprandial EE)	R=0.51	0.022
Age	–0.018 (–0.030––0.004)	0.014
Percentage of body fat	–0.011 (–0.045–0.047)	0.960
Trp/Arg ⁶⁴ mutation	–0.158 (–0.246–0.561)	0.432
TEM/LBM	R=0.68	<0.001
Age	–0.084 (–0.036––0.006)	<0.001
Percentage of body fat	0.054 (–0.066–0.174)	0.367
Trp/Arg ⁶⁴ mutation	–0.075 (–0.936–1.181)	0.815

CI, coefficient interval.

slightly diminished TEM (percentage of test meal energy, $p=0.08$), as well as a significantly lower VLF (mean value after meal, $p<0.05$) than the young men.

Influence of age, body fat, and β_3 -AR polymorphism on EE

Multiple-regression analysis with age, percent body

fat, and β_3 -AR polymorphism as independent variables affecting preprandial EE showed a significant negative association between age and absolute value ($p<0.001$), as well as relative values of preprandial EE ($p<0.001$) (Table 3). Furthermore, age was a significant variable

Table 5. Multiple regression analysis with VLF as the response variable (transformed by the square root) in all subjects.

Explanatory variable	Regression coefficient (95% CI)	<i>p</i> value
Preprandial VLF (rest)	<i>R</i> =0.59	0.003
Age	-13.7 (-26.5--0.98)	0.036
Percentage of body fat	-20.6 (-62.5--21.2)	0.323
Trp/Arg ⁶⁴ mutation	358.9 (-9.8-727.7)	0.056
Postprandial VLF (mean)	<i>R</i> =0.78	<0.001
Age	-0.030 (-0.075--0.045)	<0.001
Percentage of body fat	-0.035 (-0.028--0.071)	0.100
Trp/Arg ⁶⁴ mutation	0.010 (-0.365-0.385)	0.957

CI, coefficient interval.

contributing to the variances in the relative TEM values, expressed as percent of energy intake ($p<0.05$), percent preprandial EE ($p=0.014$), and TEM divided by LBM ($p<0.001$) (Table 4).

Influence of age, body fat, and β_3 -AR polymorphism on VLF

Age was a major variable contributing to the variance in the pre- ($p<0.05$) and postprandial ($p<0.001$) VLF values. β_3 -AR polymorphism tended to contribute to the variance in preprandial VLF ($p=0.056$), but was not statistically significant (Table 5). Although percentage of body fat was higher in middle-aged men than the young men and boys, no correlation between TEM and body fat was found.

DISCUSSION

The present results show that aging creates a possible endogenous disposition to blunted metabolic and sympathetic responses to high-fat feeding, and may have a greater influence on energy balance through a diminished TEM than genetic factors such as β_3 -AR polymorphism. This suggests that an age-related β_3 -AR malfunction may have a greater impact on postprandial thermogenesis than on the extent of β_3 -AR polymorphism.

According to previous studies (4, 5, 10, 11), the frequency of Trp/Arg⁶⁴ mutation of the β_3 -AR gene in the Japanese population (0.17–0.23) is higher than in Caucasian population (28–30), and ranks after that of Canadian Eskimos (0.38) (31) and Pima Indians (0.31) (28). Therefore, β_3 -AR polymorphism is considered to be an important factor in the obesity development of Japanese. Moreover, Kurokawa et al. (9) performed a meta-analysis and demonstrated that the mean difference in BMI (Trp/Arg⁶⁴ carriers vs. noncarriers) was +0.26 kg/m². Despite the small difference in BMI, this β_3 -AR polymorphism observed in carriers may contribute to weight gain in Japanese.

Regarding the role of β_3 -AR polymorphism in obesity, the mechanism of this metabolic effect has been considered to be due to the stimulation of β_3 -AR via SNS, which activates adenylate cyclase, thus raising intracellular concentrations of cyclic AMP and resulting in augmented lipolysis and thermogenesis (32). Moreover, an animal study (33) using transgenic mice indicated that synergetic interactions between decreased brown

adipose tissue (BAT) and the adoption of a Western diet can cause marked obesity. In the present study, however, we failed to detect a relationship between TEM and β_3 -AR polymorphism. Possible reasons for this lack of relationship include: 1) the test meal size we employed was within the normal range of calories (i.e., not overfed), 2) the postprandial thermogenesis induced by fat was lower than other macronutrients such as protein and carbohydrates, and/or 3) the age-related diminished function of β_3 -AR may have a greater impact than the existence of β_3 -AR polymorphism.

When focusing on the association between aging and metabolism, multiple regression analyses with age, percent body fat, and β_3 -AR polymorphism as independent explanation variables suggested that only age significantly contributed to preprandial energy expenditure and postprandial thermogenesis, strongly indicating that reduced resting energy expenditure and postprandial thermogenesis are a consequence of aging alone. Previous animal studies elicited results showing that the β_3 -AR mRNA expression in BAT was higher during the initial stage of life and gradually decreased in adult and aged animals (34). Additionally, alterations in β_3 -AR density (13), sensitivity (12–14), and postreceptor defects (15, 16) were reported for aging humans, which may be a cause of human obesity. Moreover, anatomical observations suggest that the amount of BAT and its functioning capacity decrease with age in humans (13). Since our results of a blunted thermogenic response with advancing age are likely to correspond with the aforementioned research, it is quite reasonable to suggest that an age-related malfunction of energy homeostasis may contribute to reduced postprandial thermogenesis. However, it should be noted that the results of this study are based on a small number of subjects with β_3 -AR mutation. Therefore, the data should be interpreted carefully, and further experiments including obese subjects are needed to confirm the results of this study.

With respect to the sympathetic effect on postprandial thermogenesis, results from the multiple regression analysis imply that the subjects with a mutation of the β_3 -AR gene are more likely to possess higher preprandial VLF, an index of thermoregulatory SNS activity, as well as lower postprandial VLF. Although the reason for

alteration to VLF is uncertain at the present time, it may be related to the diminished β_3 -adrenergic sensitivity due to the mutation. Previous human studies (12, 14, 18) have further substantiated the above observed phenomenon amongst elderly populations. Elderly people have been shown to exhibit increased resting SNS activity and decreased response to stimulation by a test meal (18) or drugs (12, 14). Potential reasons for these alterations in SNS activity with advancing age are considered to be due to a diminution of β_3 -AR density (13) and its sensitivity (12, 14, 34), and the fact that higher SNS activity may compensate for the impaired β -adrenergic sensitivity of the tissues. Moreover, part of the reason for the lower postprandial SNS activity, as well as the blunted SNS response to the high-fat meal, may be due to a malfunction of the SNS because of the regularly higher basal SNS activity observed in the obese groups. Although the methodology (i.e., experimental procedures, selection of subjects and measurements for SNS) differed between the studies mentioned, our observations agree with those of previous investigators (12, 14, 18). In addition, the present results reinforce the notion that altered SNS activity, as well as diminished TEM, may be significant symptoms reflecting impairments in the regulation of energy homeostasis with advancing age.

In conclusion, we investigated whether aging and β_3 -AR polymorphism were associated with decreases in TEM and SNS activity following a high-fat meal in non-obese and healthy males of various ages. The present results indicate that age may have a greater influence on TEM and thermoregulatory SNS activity. These age-related changes in β_3 -AR may cause a blunted thermogenic response due to a reduction of energy metabolism, thus contributing to an increase in body fat storage with advancing aging.

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