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The −3826 A→G Variant of the Uncoupling Protein-1 Gene Diminishes Postprandial Thermogenesis after a High Fat Meal in Healthy Boys

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This study investigated whether the −3826 A→G nucleotide variant of the uncoupling protein-1 (UCP1) gene is correlated with postprandial thermogenesis after a high fat meal in children. Healthy boys, aged 8–11 yr, were examined for resting energy expenditure and the thermic effect of a meal (TEM), which were measured by indirect calorimetry for 180 min after a high fat (70% fat, 20% carbohydrate, and 10% protein, providing 30% of the daily energy requirement) and a high carbohydrate meal (20% fat, 70% carbohydrate, and 10% protein). The sympatho-vagal activities were assessed by means of spectral analysis of the heart rate variability during the same period. Children were genotyped for UCP1 polymorphism by applying a PCR-restriction fragment length polymorphism using buccal samples. There was no reaction of sympathetic activity to the high carbohydrate meal in either the GG allele or the AA+AG group and no significant difference in TEM. However, after the high fat meal, sympathetic responses were found in both groups; further, the GG allele group showed significantly lower TEM than the AA+AG group. In conclusion, despite fat-induced sympathetic stimulation, GG allele carriers have a lowered capacity of TEM in response to fat intake, suggesting that such impaired UCP1-linked thermogenesis can have adverse effects on the regulation of body weight. (*J Clin Endocrinol Metab* 88: 5661–5667, 2003)

AN UPWARD TREND in childhood obesity has been observed internationally at an alarming rate (1). As body weight is maintained by precise physiological mechanisms, positive energy balance, even a small amount, will cause obesity over the long term (1). Certainly, genetic factors can influence a child's predisposition, and environmental factors are also linked to the childhood obesity epidemic (1). Regarding the environmental influences, excessive dietary fat intake is believed to be one of the etiological factors involved in the onset and/or development of childhood obesity (2). As a high fat diet promotes food consumption resulting from lower satiation (3) and reduced postprandial thermogenesis (4), a lower thermic effect of high fat diet may be a further causative factor for energy storing and fat gain in children who prefer to consume fat-rich foods.

Concerning the genetic factors, uncoupling protein-1 (UCP1) is considered a key moderator of the thermogenic function of brown adipose tissue (BAT) in humans (5). The thermogenic activity of BAT depends on its quantity, its UCP1 content, and the extent of stimulation by the sympathetic nervous system (SNS) (6). In children, anatomical studies demonstrated that there was a wide distribution of active BAT in all areas of the body during the first decade of human life (7); moreover, a higher UCP1 content in perirenal adipose tissue was found in children aged 18 months to 15 yr than in infants (5). The role of UCP1 during childhood in this phenomenon may be more important than that in adulthood in terms of quantitative as well as functional differences. To evaluate the effect of UCP1 on childhood obesity, therefore, it is meaningful to examine UCP1 polymorphism and the thermogenic response to perturbation, such as food intake in children.

With respect to lipid metabolism and SNS, fat oxidation depends largely on a sympathetic stimulus compared with protein and carbohydrate utilization (8). A recent human study (9) demonstrated that an infusion of lipid emulsion stimulates SNS activity. It was previously shown that the thermic effect of a meal (TEM) mediated by BAT is stimulated through high fat feeding (10). As the sympathetic branch of the system leads to UCP1 expression and contributes to increased TEM and lipolysis, diminished SNS activity can lead to the accumulation of fat in the body (11). Because of its role as a possible endogenous sympathetic stimulant, it would be of interest to investigate the contribution of intrinsic SNS activity to modulating the thermoregulatory system in BAT in children.

Accordingly, this study attempted to investigate whether the −3826 A→G nucleotide variant of the UCP1 gene is correlated with postprandial thermogenesis after a high fat meal in healthy boys.

Subjects and Methods

Subjects

The subjects were 22 healthy boys, aged 8–11 yr. Their health, medical history, physical activity level, diet, and daily lifestyle were determined through a careful interview with the boys and their parents, and none

Abbreviations: BAT, Brown adipose tissue; ECG, electrocardiogram; EE, energy expenditure; SNS, sympathetic nervous system; TEM, thermic effect of meal; UCP1, uncoupling protein-1.

had a medical history of any significant physical illness, including diabetes or other endocrine diseases. The boys were requested to keep to their usual diet for at least 2 wk before the study. The profiles of the boys 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 boys and their 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.

Experimental procedures

On the day before the test, food and drink was denied after 2200 h. The consumption of coffee, tea, and fat-rich food was not allowed the day before the test, and no sports activities were permitted that evening.

On the day of the test, each boy arrived at the laboratory at 0730 h in a fasting condition. After measuring height, body mass, and percentage of body fat, determined by a bioelectrical impedance analyzer (model TBF-534, Tanita Corp, Tokyo, Japan), each boy was equipped with electrocardiogram (ECG) electrodes and then rested for at least 30 min in a temperature- (24–25 C) and humidity-controlled environment. After the subject was given an explanation of the procedure and adjusted to the mask, CM_5 lead ECG and gas exchange parameters, using an open-circuit computerized indirect calorimeter (Aero monitor AE 280, Minato Medical Science, Tokyo, Japan), were continuously recorded while the boy remained seated in a comfortable chair. During the measurement, the boy rested quietly watching videotapes. The calorimeter was calibrated before each test with a reference gas mixture (15% O_2 and 5% CO_2). Continuous ventilatory volumes (VO_2, and VCO_2) were displayed on a computer at 15-sec intervals, and the mean value of each minute was recorded.

The test meal was served at 0830 h and was eaten within 15 min. The order of the meals given was randomly assigned. Postprandial energy expenditure (EE) was measured for 180 min (0900–1130 h), and ECG and gas samples were taken every 30 min for 6 min. During the test period, the subjects watched videotapes or read books quietly.

Genetic analysis

A noninvasive genotyping sampling method was implemented for collecting buccal mucosa cells by cotton wool swabs. DNA (0.2–2 μg/subject) was obtained using the phenol-extraction procedure. The *Bcl*I polymorphism of the UCP1 gene, which detects the A-G point mutation at position −3826 bp in the 5′-flanking domain, was determined by PCR-restriction fragment length polymorphism analysis according to the method described by Cassad-Doulcier *et al.* (12). The PCR primers were 5′-CTTGGGTAGTGACAAAGTAT-3′ (upstream) and 5′-CCAAAGGGTCAGATTTCTAC-3′ (downstream). Genomic DNA (100 ng) in a total volume of 20 μl was used for the PCR. PCR was performed by initial denaturation at 94 C for 5 min; 30 cycles at 94 C for 30 sec, 55 C for 30 sec, and 72 C for 30 sec; and a final extension at 72 C for 10 min. We then incubated 5 μl PCR product for 1 h with 10 U *Bcl*I 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 *Bcl*I is lost; therefore, the allele of this polymorphism corresponds to the 470 bp undigested band. The *Mva*I polymorphism of the β_3-adrenergic receptor gene, which detects the $Trp^{64}Arg$ mutation, was determined by PCR-restriction fragment length polymorphism analysis according to our previously reported method (13). The PCR primers were 5′-CCAATACCGCCAACACACCAGT-3′ (upstream) and 5′-AGGAGTCCCATCACCAGGTC-3′ (downstream), which flank the whole exon 1 of the β_3-adrenergic receptor gene. Genomic DNA (100 ng) in a total volume of 20 μl was used for the PCR. PCR was performed by initial denaturation at 94 C for 5 min; 30 cycle s at 94 C for 30 sec, 67 C for 30 sec, and 72 C for 30 sec; and a final extension at 72 C for 10 min. We then incubated 5 μl PCR product for 1 h with 10 U *Mva*I at 37 C in a final volume of 10 μl without further purification. The samples were 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 *Mva*I is lost; therefore, the allele of this polymorphism corresponds to the 158-bp undigested band.

Diet

The energy content of the meal corresponded to 30% of each boy's daily energy requirement, which was determined using reference values from the metabolism tables for Japanese children (14). A multiplication factor of 1.5 was used to account for the medium physical activity level of the subjects. The high carbohydrate (70% of energy as carbohydrate, 20% of energy as fat, and 10% of energy as protein) and high fat meal (20% of energy as carbohydrate, 70% of energy as fat, and 10% of energy as protein) were compiled using normal food items consumed for breakfast, which were prepared according to each boy's individual energy requirement, adjusted to the nearest 100 kJ (Table 1). The macronutrient composition of the meals (Table 2) was calculated using the Japanese food composition table (15).

Calculation of energy expenditure

The mean of the stable 12-min period was calculated for preprandial EE. The six periods of stable 6 min were averaged over the total 3-h thermogenic response. The TEM was calculated by subtracting the preprandial EE value (kilojoules per minute) from the averaged postprandial EE (kilojoules per minute), and this was multiplied by the duration of the postprandial period (180 min). The TEM was expressed as an

TABLE 1. Physical characteristics and the parameters of energy metabolism during pre- and postprandial periods in 22 healthy Japanese boys when classified in accordance with their genotype of the A→G nucleotide variant of UCP1

	GG allele (n = 9)	AA + AG (n = 13)	*P*
Age (yr)	8.9 (0.4)	9.2 (0.4)	0.64
Height (cm)	136.0 (1.8)	140.0 (2.4)	0.53
Body mass (kg)	36.0 (2.2)	36.5 (2.9)	0.88
BMI (kg/m^2)	19.5 (1.3)	19.0 (1.1)	0.78
Body fat (%)	26.4 (2.7)	21.2 (1.9)	0.13
Nutritional requirement (kJ/d)	8703 (566)	8608 (549)	0.90
Test meal energy (kJ)	2580 (35)	2516 (41)	0.83
High carbohydrate meal			
Preprandial EE (kJ/d)	7464 (366)	7187 (278)	0.56
TEM (kJ/3 h)	123 (17)	138 (14)	0.50
TEM (% preprandial EE)	1.6 (0.2)	2.0 (0.3)	0.27
TEM (% energy intake)	4.7 (0.5)	5.3 (0.5)	0.52
High fat meal			
Preprandial EE (kJ/d)	7525 (391)	7200 (231)	0.48
TEM (kJ/3 h)	102 (15)	138 (8)	0.047
TEM (% preprandial EE)	1.3 (0.2)	2.0 (0.1)	0.01
TEM (% energy intake)	3.9 (0.5)	5.3 (0.3)	0.045

Values represent means (SE). Nutritional requirements were recommended by the Ministry of Health, Labor, and Welfare in Japan. *P* values compare boys carrying wild-type (AA) and heterozygous (AG) alleles with boys carrying homozygous (GG) alleles for the polymorphism.

TABLE 2. Composition, energy content, and macronutrient composition of the test meals

Test meals	Weight (g)	Energy (kJ)	Protein (g)	Fat (g)	Carbohydrate (g)
High carbohydrate meal					
Boiled rice	215	1274	4.8	0.5	69.7
Vegetable stew	145	645	5.0	10.6	10.8
Egg	25	161	3.3	2.6	0.1
Margarine	1	26	0	0.7	0
Low-fat yogurt	50	140	1.2	0	8.0
Orange juice	200	343	0	0.1	21.2
Total	696	2589	1.0	14.5	109.8
Energy %			10.3	20.6	69.1
High fat meal					
Hamburger	103	1350	10.9	20.4	23.9
Egg	25	161	3.3	2.6	0.1
Butter fat	5	154	0.5	4.0	0
High-fat cream	60	864	0.9	21.5	1.5
Sugar	3	19	0	0	2.9
Water	200	0	0	0	0
Total	396	2548	15.6	48.5	28.4
Energy %			10.2	71.1	18.7

Subjects were fed in energy balance, with protein/carbohydrate/fat content of 10/70/20 and 10/20/70 percentage of energy, with fixed 30% of each boy's daily energy requirement.

absolute as well as a relative value and as the percent increase over the preprandial EE.

R-R spectral analysis procedure

Our R-R interval power spectral analysis procedures have been fully described previously (16–18) and required only minimal cooperation from the boys being tested because of the simple and noninvasive method. Briefly, the analog output of the ECG monitor (MEG-6100, Nihon Kohden, Tokyo, Japan) was digitized via a 13-bit analog to digital converter (HTB 410, Trans Era, Orem, UT) at a sampling rate of 1024 Hz. The digitized ECG signals were differentiated, and the resultant ECG QRS spikes and intervals of the impulses (R-R intervals) were stored sequentially on a hard disk for later analysis.

Before R-R spectral analysis was performed, the stored R-R interval data were displayed, aligned sequentially to obtain equally spaced samples with an effective sampling frequency of 2 Hz (19), and displayed on a computer screen for visual inspection. Then the direct current component and linear trend were eliminated by digital filtering for the bandpass between 0.03 and 0.5 Hz. The root mean square value of the R-R interval was calculated as representing the average amplitude. After passing through the Hamming-type data window, power spectral analysis by means of a fast Fourier transform was performed on a consecutive 512-sec time series of R-R interval data obtained during the test.

Identification of sympathetic and parasympathetic components

Before this study, we conducted a pharmacological blockade experiment on six young male subjects to confirm the validity of the heart rate variability power spectral analysis to quantify the sympathovagal activity and to examine the effects of the autonomic nervous system activities on regulating energy metabolism in humans (Fig. 1).

Figure 1 represents typical sets of raw R-R intervals and the corresponding power spectra obtained from a young male subject, who participated in our preliminary study, in the resting condition (A) and during the autonomic pharmacological blockade (B and C). After atropine administration, a parasympathetic muscarinic antagonist was iv injected. Heart rate variability was markedly reduced, and the high frequency components were almost entirely abolished, whereas the low frequency components were partly decreased (Fig. 1B). When propranolol, a β-adrenoceptor antagonist, was additionally injected, heart rate fluctuations were almost entirely abolished, leading to a metronome-like heart beat (Fig. 1C). When the autonomic nervous system was blocked completely by these pharmacological agents, the resting EE was significantly reduced (-1247 ± 200 kJ/d; $P < 0.01$; Fig. 1D) (20).

Based on our previous experiments (17, 18, 20), the spectral powers in the frequency domain were quantified by integrating the areas under the curves for the following respective band widths: low frequency (0.03 and 0.15 Hz), jointly mediated by both sympathetic and vagal activity; high frequency (0.15 and 0.5 Hz), solely reflecting vagal activity; total power (0.03 and 0.5 Hz), representing the overall autonomic nervous system activity, and the ratio of low to high frequency (SNS index), reflecting SNS activity.

During the resting and postprandial periods, an adult subject is usually requested to breathe in synchrony with a metronome 15 times/min (0.25 Hz) to ensure that respiratory-linked variations in heart rate do not overlap with low frequency components (<0.15 Hz) from other sources. It should be noted that according to our preliminary experiments as well as pediatric physiology (21), children's breathing frequency is generally higher than 9 times/min (>0.15 Hz). Thus, the children breathed without controlling their respiration rates during the ECG measurements.

Statistical analyses

All data are presented as the mean ± SE. All of the statistical analyses were performed with the Statistical Package of Social Science (SPSS for Windows, version 11.0, SPSS, Inc., Chicago, IL). Statistical differences among the groups in terms of time-course changes in postprandial energy expenditure and SNS responses were analyzed by two-way ANOVA with repeated measurements. All other group comparisons were made by *t* test. $P < 0.05$ was regarded as statistically significant.

Results

Distribution of genotypes

The distribution of the genotypes defined by the −3826 A→G polymorphism of UCP1 gene and the Trp/Arg^{64} variant of the β_3-adrenergic receptor gene is presented in Table 3. Previous genetic studies regarding the −3826 A→G polymorphism of UCP1 clearly indicated that GG homozygotes demonstrated an increased tendency to gain weight over time (22). Additionally, there is no Arg/Arg^{64} variant of the β_3-adrenergic receptor gene in homozygous carriers of the A→G UCP1 variant. Therefore, the boys were divided into two groups according to UCP1 genotype, GG allele (n = 9; all were homozygous carriers), and AA+AG (n = 13; five wild-type and eight heterozygous carriers) groups.

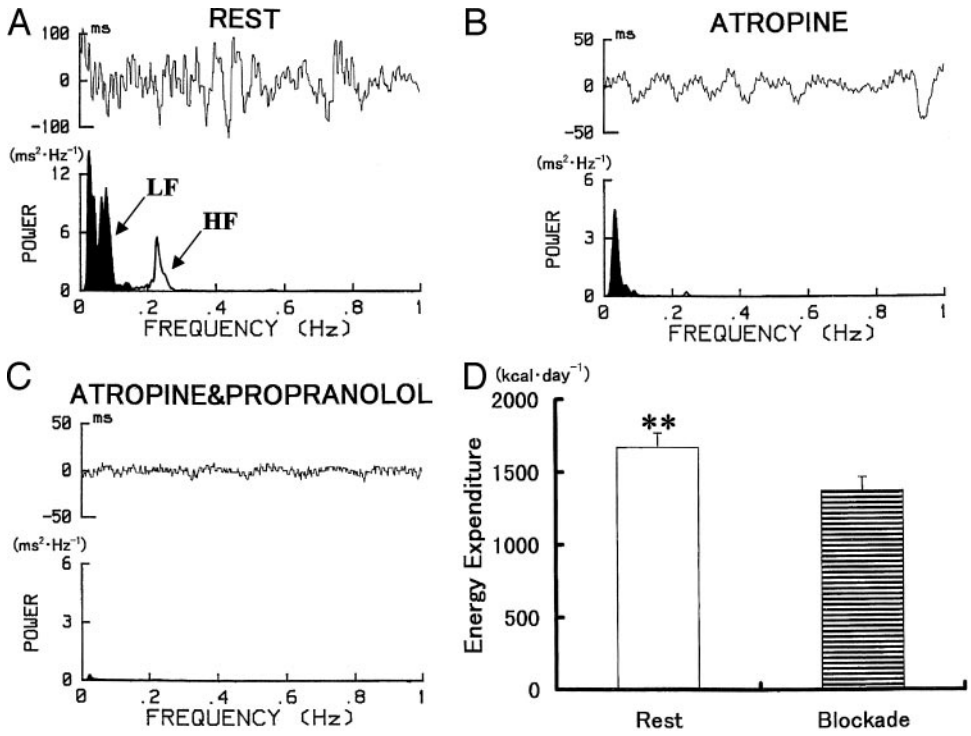

FIG. 1. Typical sets of ECG R-R interval changes and the corresponding power spectrum for a young male subject, a participant in our preliminary experiment, in the resting condition (A) and after the injection of parasympathetic muscarinic blocker, atropine (B), and a β-sympathetic blocker, propranolol (C). Comparison of EE in the resting condition and after complete autonomic blockade in six young male subjects (D). The data are expressed as the mean ± SE. **, $P < 0.01$. LF, Low frequency; HF, high frequency.

Meal-induced energy expenditure

Table 1 summarizes the physical characteristics and the parameters of energy metabolism during pre- and postprandial periods for the boys. When comparisons were made between the GG allele and AA+AG groups, there were no significant differences in physical parameters, calories of the test meal, or preprandial EE values of each test meal. Postprandial thermogenesis, expressed as TEM values, were not different between the groups after the high carbohydrate meal; however, the TEM of the high fat meal were significantly lower ($P < 0.05$) in the GG allele group than in the AA+AG group regardless of the expression, *i.e.* absolute or relative values (see Table 1).

Figure 2 shows the time-course changes in the net increase in postprandial EE after the high carbohydrate and high fat meals, expressed as a percentage above the preprandial EE value, in the two groups. There was no group difference in postprandial EE values after the high carbohydrate meal. In contrast, a significant group effect was found ($F = 7.98$; $P = 0.01$) in postprandial EE values after the high fat meal, suggesting that GG allele carriers possess a blunted metabolic response to the high fat meal.

SNS response

Figure 3 represents typical sets of raw R-R intervals and the corresponding power spectral data obtained from a boy during the pre- and postprandial periods of each test meal, respectively. According to the visual inspection, there were no changes in any components of the power spectrum from pre- to postprandial periods after the high carbohydrate meal. On the other hand, after the high fat meal, the low frequency component predominantly increased in the postprandial compared with the preprandial periods.

TABLE 3. Distribution of genotype defined by the −3826 A→G nucleotide variant of the UCP1 promoter gene and the β_3-adrenergic receptor (BAR) gene in 22 healthy Japanese boys

Trp/Arg[64] variant of BAR	A→G variant of the UCP1		
	AA	AG	GG
Trp/Trp	4 (18.2)	5 (22.7)	6 (27.4)
Trp/Arg	1 (4.5)	3 (13.6)	3 (13.6)
Arg/Arg	0 (0.0)	0 (0.0)	0 (0.0)

Values represent the number of subjects (percentage).

Figure 4 shows the time-course changes in the SNS index, representing SNS activity, after high carbohydrate and high fat feedings, respectively. There were no significant changes in the SNS index after the high carbohydrate meal in either group. However, after the high fat meal, the SNS index was rapidly increased compared with value during the preprandial periods in the GG allele group ($P < 0.05$, 30 min after *vs.* preprandial). A similar response occurred in the AA+AG group 60 min later, but the difference did not reach statistical significance ($P = 0.07$, 60 min after *vs.* preprandial).

Discussion

This study provided intriguing information regarding the potential contribution of UCP1 polymorphism as an endogenous disposition to blunt high fat-induced thermogenesis in human BAT. The main finding was that increased SNS activity was shown after high fat feeding regardless of genotype; however, TEM after a high fat meal was significantly lower in the GG allele carriers than in boys with AG and AA genotypes. The augmented SNS activity observed after high fat feeding may be an energy regulation system, burning abundant energy from excessive fat intake as heat production through UCP1-linked brown fat thermogenesis. Despite

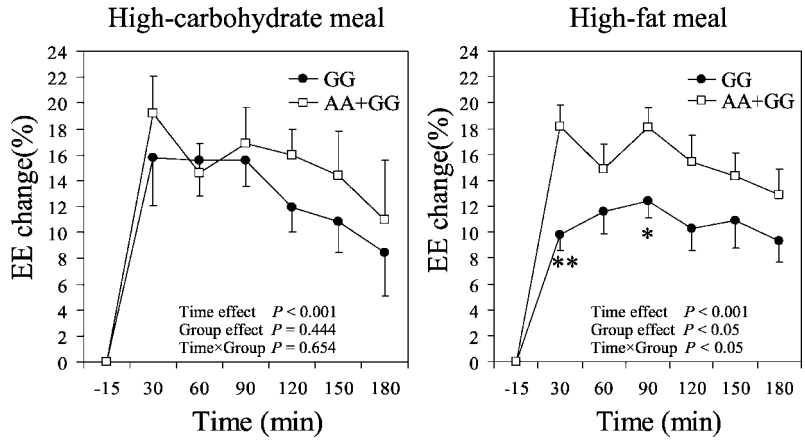

FIG. 2. Time course of postprandial EE after a high carbohydrate (*left*) and a high fat (*right*) meal, respectively, expressed as a percentage of the preprandial baseline in the two groups (●, GG allele; □, AA+AG). The results are expressed as the mean ± SE for each group. *, $P < 0.05$, GG allele *vs.* AA+AG group. Time effect, meal effect, and time × meal interaction were calculated by repeated ANOVA.

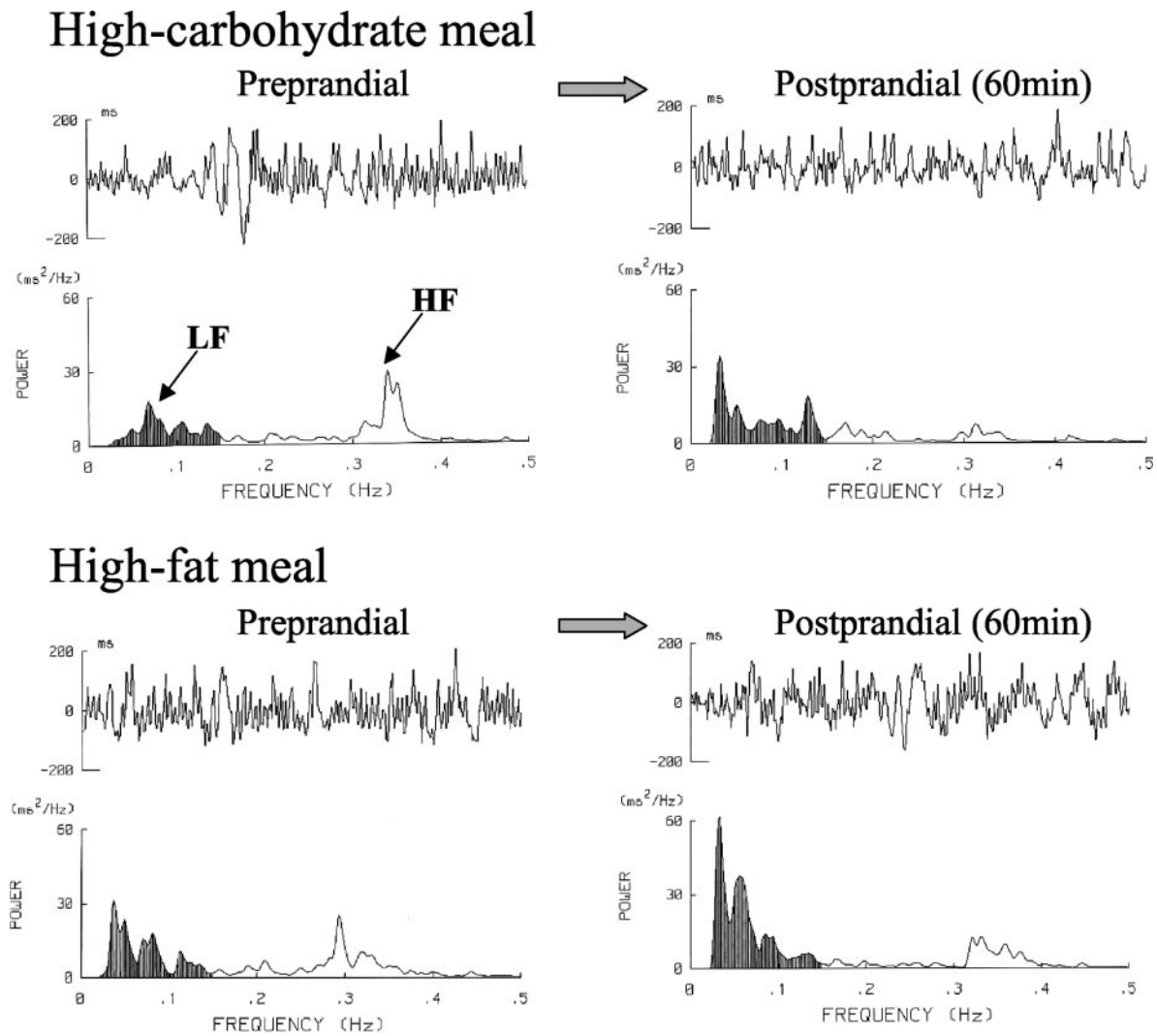

FIG. 3. Examples of ECG raw R-R interval and the corresponding power spectrum obtained from a same boy in the preprandial (*left*) and postprandial (*right*) periods after a high carbohydrate and a high fat meal, respectively. LF, Low frequency; HF, high frequency.

the SNS responsiveness to thermogenic stimuli such as high fat feeding, the magnitude of the increase in postprandial energy expenditure was lower in boys with the GG allele of UCP1, suggesting that such impaired thermoregulatory function may be caused by lower UCP1 mRNA expression levels due to the −3826 A→G polymorphism (23).

As a key contributor to the regulation of energy balance, mitochondrial UCP1 in BAT is generally assumed to play an

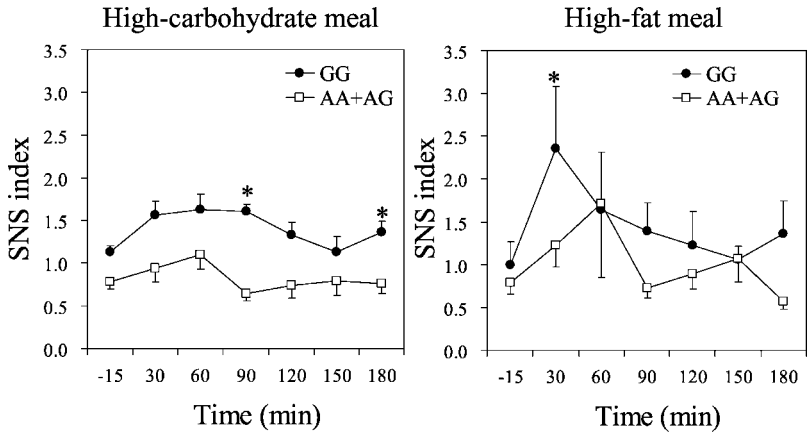

FIG. 4. Time-course changes in the SNS index (low to high frequency ratio) represent SNS activity after a high carbohydrate (*left*) and a high fat (*right*) meal in the two groups (●, GG allele; □, AA+AG), respectively. The results are expressed as the mean ± SE for each group. *, $P < 0.05$, postprandial *vs.* preprandial. Time effect, meal effect, and time × meal interaction were calculated by repeated ANOVA.

important role in body weight regulation in rodents and humans (6, 7, 23, 24). The relationship between UCP1 polymorphism and human obesity has been supported (22, 23). However, controversial results were obtained through extensive and large scale genetic studies in both child and adult populations (22). This may be partly attributable to the small proportion of individuals with the GG genotype of the UCP1 gene in Caucasian populations (22). Moreover, it is difficult to evaluate precisely the independent effect on the UCP1 gene mutation, because obesity is a multifactorial disorder with a wide variety of genetic backgrounds as well as behavioral and environmental influences.

Previous studies in Japanese adults (24, 25) revealed that the frequency rate of GG allele carriers accounted for over 20% of their subjects, indicating that the frequency rate of the GG homozygote of the UCP1 gene would be higher in Japanese than in Caucasian populations (22). As the distribution of active BAT appears during childhood (7, 26), the role of BAT as well as the function of the UCP1 gene have an impact on facultative energy expenditure for Japanese children. Moreover, previous human studies indicated that a low amount of BAT was associated with abdominal obesity in adult males (27), whereas abundant BAT induced by the stimulation of a cold environment was found among Finnish outdoor workers (28). Therefore, we assume that this study aimed at Japanese children with the GG allele of the UCP1 gene is adequate to investigate whether BAT dysfunction, depending on reducing UCP1 expression, affects thermogenic function associated with the onset or development of human obesity.

In animal studies it is well documented that the increased UCP1 gene expression in BAT is mediated through norepinephrine secretion from sympathetic nerve endings against various physiological conditions, such as cold exposure (6, 29) and high fat meal (10). In humans, it is uncertain whether UCP1 expression as well as SNS stimuli act in an identical way as in animal studies due to the lack of direct or other adequate methodology for evaluating the extent of UCP1 in BAT. Our recent study, using the power spectral analysis of heart rate variability, showed a significant SNS response to cold exposure in young lean female subjects (20). In addition, our present data further elucidated that high fat feeding stimulates SNS activity in boys, indicating that thermogenesis in BAT is induced by acute cold exposure and high fat feeding in humans as well. However, in the absence of any direct measurement of SNS activity to BAT in the human studies, caution needs to be used when interpreting these results.

The results of this study are based on a small number of children. Therefore, the data should be interpreted carefully, and moreover, further experiments, including acute cold exposure and longitudinal tracking studies, are needed to confirm the results of this study. During recent years, extensive progress has been achieved in understanding the mechanism for the thermoregulatory function of BAT. To the best of our knowledge, however, few studies have been performed to investigate the impact of the UCP1 gene mutation on thermogenesis during the early stage of life. For that reason this study provides valuable data regarding the potential association of UCP1 mutation and reduced thermogenesis, at least in lipid metabolism in humans.

In summary, we investigated whether the −3826 A→G nucleotide variant of the UCP1 gene is correlated with postprandial thermogenesis and the sympathetic response to a high fat meal in healthy boys. Despite fat-induced sympathetic acceleration, boys with the GG allele genotype had a lowered capacity of TEM to fat intake. Our findings suggest that such impaired UCP1-linked thermogenesis can have adverse effects on the regulation of body weight. In addition, our results further imply that children with the GG allele genotype of the UCP1 gene may easily become obese as a consequence of abundant fat intake over a long period of time.

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