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The effect of a high-carbohydrate meal on postprandial thermogenesis and sympathetic nervous system activity in boys with a recent onset of obesity

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Abstract

The purpose of the present study was to investigate the thermic effect of food (TEF) and sympathetic nervous system (SNS) activity in obese boys. Ten obese (9.2 ± 0.4 years) and 13 lean boys (8.8 ± 0.4 years) were examined for energy expenditure and fat oxidation measured via indirect calorimetry for 3 hours after a high-carbohydrate (HC; 70% carbohydrate, 20% fat, and 10% protein) or a high-fat (HF; 20% carbohydrate, 70% fat, and 10% protein) meal served on 2 different days at random. The activity of the SNS was assessed by means of a power spectral analysis of the heart rate variability. The TEF, expressed as a percentage of the consumed energy, was significantly lower in obese boys than in lean boys after the HC meal; however, such a difference was not observed after the HF meal. Multiple regression analysis revealed that obesity was a significant variable contributing to the variances in the TEF induced by the HC meal. Moreover, after the HC meal, the boys with a recent onset of obesity (duration, <3 years) manifested a lower TEF as well as a reduced very low frequency component of the heart rate variability, an index of thermoregulatory SNS functions, compared with the remaining obese and lean boys. In conclusion, obese boys possessed normal metabolic and sympathetic responses to the HF meal but showed a diminished thermogenic response to the HC meal, especially during the early phase of obesity.

1. Introduction

Because the thermic effect of food (TEF) is a loss of energy for the body, diminished TEF is considered to be a contributing factor causing the onset or development of childhood obesity because of a long-term positive energy balance [1]. Previous pediatric studies concerning the role of the TEF have mainly been conducted with regard to the following aspects: TEF and nutrient composition, especially the extent of fat intake [2]; differences in the TEF induced by a standardized meal [3-6] or sucrose load [7] between obese and nonobese children; the specific macronutrient use in obese children of carbohydrate [8], fat [9], and protein [10]. Considering the nutrient composition, Maffeis et al [2] reported that a high-fat (HF) meal induced a lower postprandial thermogenesis than an isoenergetic, isoproteic low-fat meal served to children, but no metabolic difference was found between obese and nonobese children. The authors [9] also provided evident data using isotope tracers that postprandial exogenous fat oxidation was positively related to children's fat mass, indicating that obese children might possess a capacity for enhanced fat oxidation when their body is exposed to a high amount of dietary fat. On the other hand, some studies using normal food items [3], mixed liquid meal [4-6], or sucrose [7] reported that the TEF was reduced more in obese children than in nonobese children, but the extent of the decline found in these studies was inconsistent because of differences in the proportion of nutrient composition, degree and/or duration of obesity, and other experimental settings. It is still unclear whether diets with very different proportions of fat or carbohydrates affect postprandial thermogenesis and the sympathetic nervous system (SNS) response in obese children.

Accordingly, the aim of the present study was to explore the TEF and thermoregulatory SNS activity in response to a high-carbohydrate (HC) meal and a HF meal in obese boys, with special reference to the duration of obesity.

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2. Methods

2.1. Subjects

Ten obese and thirteen lean boys aged 6 to 11years were recruited from 2 elementary schools in the city of Kyoto, Japan. Boys were defined as obese when their body weight was 120% above their ideal body weight corresponding to the same height and sex [11]. The general characteristics of the boys are presented in Table 1. Their health, medical history, physical activity level, diet, and daily lifestyle were determined through a careful interview with the boys and their parents. All children were healthy, free of disease, and not taking any medication that affected energy expenditure (EE). Based on our previous study [12] and that of Molnár et al [3], the obese boys were categorized into subgroups (less or greater than a 3-year history of obesity). The experimental procedures were approved by the institutional review board of the Kyoto University Graduate School and were in accordance with the revised Helsinki Declaration of 1983. All boys and their parents were carefully informed about the test, and all gave their written informed consent to participate in the study.

Table 1
General characteristics and metabolic profile of the study group

	Lean (n = 13)	Obese (n = 10)
Age (y)	8.8 (0.4)	9.2 (0.4)
Height (cm)	136.6 (1.8)	137.1 (2.3)
Body mass (kg)	30.9 (1.0)	44.1 (2.5)*
Body mass index (kg/m^2)	16.5 (0.4)	23.3 (0.8)*
Body fat (%)	18.8 (1.4)	29.7 (1.6)*
Trp^{64}Arg variant of the β_3-AR gene arg allele frequency	0.15	0.15
−3826A→G variant of the UCP1 gene allele frequency	0.58	0.65
Nutritional requirement (kJ/d)	7403 (126)	7898 (261)**
Test meal energy (kJ)	2510 (34)	2782 (82)**
HC meal		
Preprandial EE (kJ/d)	6899 (221)	7764 (342)**
TEF (kJ per 3 hours)	132 (11)	99 (15)
TEF (% energy intake)	5.2 (0.4)	3.7 (0.5)**
Fat intake (g)	13.5 (0.1)	14.3 (0.3)**
Postprandial fat oxidation (g)	10.9 (1.4)	12.3 (1.7)
Fat oxidation/fat intake (%)	80.9 (10.5)	86.4 (12.5)
Carbohydrate intake (g)	105.0 (1.4)	116.4 (3.5)*
Postprandial carbohydrate oxidation (g)	33.8 (2.9)	36.1 (3.8)
Carbohydrate oxidation/ carbohydrate intake (%)	32.1 (2.6)	30.7 (2.8)
HF meal		
Preprandial EE (kJ/d)	7053 (230)	7717 (332)
TEF (kJ per 3 hours)	116 (15)	106 (9)
TEF (% energy intake)	4.5 (0.6)	3.9 (0.3)
Fat intake (g)	47.3 (0.4)	50.1 (0.9)**
Postprandial fat oxidation (g)	14.9 (1.3)	18.1 (1.2)
Fat oxidation/fat intake (%)	31.5 (2.7)	36.5 (2.6)
Carbohydrate intake (g)	30.0 (0.4)	33.3 (1.0)*
Postprandial carbohydrate oxidation (g)	24.0 (2.3)	21.8 (3.7)
Carbohydrate oxidation/ carbohydrate intake (%)	80.0 (7.8)	64.1 (9.4)

N = 23. Data are expressed as mean (±SE). Nutritional requirements were recommended by the Ministry of Health, Labor, and Welfare of Japan.

* $P < .001$ vs lean boys, by unpaired t test.

** $P < .05$ vs lean boys, by unpaired t test.

2.2. Experimental procedure

Before the experiment was carried out, nutritional assessment by means of a daily dietary record method was performed in all boys. The amount of energy consumption was 9155 ± 996 kJ/d (mean ± SE) in obese boys and 8464 ± 238 kJ/d in lean boys. The carbohydrate proportion (%) to total daily energy intake was 59.2% ± 1.6% (range, 50.3%-66.5%) in obese boys and 57.1% ± 1.4% (range, 50.0%-65.0%) in lean boys. None of boys were taking a hypoenergetic diet. To ensure similar baseline measures, the boys and the mothers were requested to maintain their usual diet for at least 2 weeks before the test. Moreover, on the day before the test, the consumption of coffee and tea was not allowed, and all consumption of food and drink had to cease before 10:00 PM. The diet that the boys consumed in the 24-hour period preceding testing was checked by a registered dietitian through an interview with each boy's mother at our laboratory on the testing day. To avoid an effect on metabolic data, all boys did not perform any intensive physical activity on the day immediately before the test.

On the day of the test, each boy arrived at the laboratory at 07:30 AM in a fasted condition. After measurements of height, body mass, and percentage of body fat determined using a bioelectrical impedance analyzer (Model TBF-534, Tanita Corp, Tokyo, Japan), subjects were equipped with electrocardiogram (ECG) electrodes and then rested for at least 30 minutes in a temperature-controlled (24°C-25°C) room. After their respiratory masks have been adjusted, CM_5 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 subjects remained seated in a comfortable chair. The calorimeter was calibrated before each test with a reference gas mixture (15% O_2 and 5% CO_2). Continuous ventilatory volumes, oxygen uptake (VO_2) and carbon dioxide production (VCO_2), were displayed on a computer at 15-second intervals, and the mean value for each minute was printed out. The test meal was served at 08:30 AM and eaten within 15 minutes. Postprandial EE was measured for 3 hours (until 11:30 AM), and gas samples were taken for a 6-minute period every 30 minutes (24-minute interval) over the 3-hour period. During the test period, subjects remained seated quietly watching video tapes or reading books.

2.3. Diet

Two menus were served on 2 different days in a random order with an interval of 1 to 6 days in between. The energy

Table 2
Nutrient composition of the test meals

Test meals	Weight (g)	Energy (kJ)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)
HC meal						
Boiled rice	215	1274	4.8	0.5	69.7	0.6
Vegetable stew	145	645	5.0	10.6	10.8	1.7
Egg	25	161	3.3	2.6	0.1	0
Margarine	1	26	0	0.7	0	0
Low-fat yogurt	50	140	1.2	0	8.0	0
Orange juice	200	343	0	0.1	21.2	0
Total	696	2589	1.0	14.5	109.8	2.3
		En %:	10.3	20.6	69.1	
HF meal						
Hamburger	103	1350	10.9	20.4	23.9	0.7
Egg	25	161	3.3	2.6	0.1	0
Butter fat	5	154	0.5	4.0	0	0
HF cream	60	864	0.9	21.5	1.5	0
Sugar	3	19	0	0	2.9	0
Water	200	0	0	0	0	0
Total	396	2548	15.6	48.5	28.4	0.7
		En %:	10.2	71.1	18.7	

Subjects were fed in energy balance, with a 10:70:20 ratio of protein/carbohydrate/fat and a 10:20:70 percentage of energy, with fixed 80 kJ per kilogram of actual body mass in lean boys and 80 kJ per kilogram of ideal body mass in obese boys.

content of the meal was standardized individually: 80 kJ per kilogram of actual body mass (30.9 ± 1.0 kg) in lean boys and 80 kJ per kilogram of ideal body mass (33.2 ± 1.6 kg) [11] in obese boys, which corresponded to about one third of each boy's daily energy requirement (lean, 33.9% ± 0.3%; obese, 35.2% ± 0.5%), determined by using reference values from metabolism tables for Japanese children [13]. Ideal body mass was defined as a mean value for age- and sex-specific reference data obtained from the Statistical Survey on School Health Condition in Japan [11]. The HC meal (70% carbohydrate, 20% fat, and 10% protein) and the HF meal (20% carbohydrate, 70% fat, and 10% protein) were compiled using normal foods consumed for breakfast, which were prepared according to each boy's individual energy requirement, adjusted to the nearest 200 kJ (Table 1). The macronutrient composition and dietary fiber of the meals (Table 2) were calculated using the Japanese food composition table [14]. It should be noted that the contents of dietary fiber of both test meals were relatively low (2.3 g for the HC meal and 0.7 g for the HF meal) because previous studies [15,16] demonstrated that a high-fiber (8-26 g) meal can decrease the TEF as compared with a low-fiber (4-6 g) meal.

2.4. Calculation of EE

The mean of a stable 12-minute period was calculated for preprandial EE. Six periods of 6 minutes were used to calculate a total of 3 hours of thermogenic response. The nonprotein respiratory quotient (RQ) was determined from V_{O_2} and V_{CO_2} after subtraction of protein oxidation. The EE, carbohydrate, and fat oxidation rates were calculated using the tables of Lusk [17]. The TEF was calculated as the postprandial increase in EE above the preprandial EE values. The TEF for the 3-hour period were calculated as the area under the curve using the trapezoidal rule. The TEF was expressed in absolute as well as relative values and as a percentage increase over the preprandial EE.

2.5. The R-R spectral analysis procedure

Our R-R interval power spectral analysis procedures have been fully described elsewhere [18-23]. Briefly, the ECG data were obtained from the CM_5 lead and digitized via a 13-bit analog-to-digital converter (HTB 410, Trans Era, Utah) at a sampling rate of 1024 Hz. Then, the digitized R-R interval time series was aligned in a 2-Hz sequence [24] for power spectral analysis. The direct current component and linear trend were eliminated by digital filtering for the

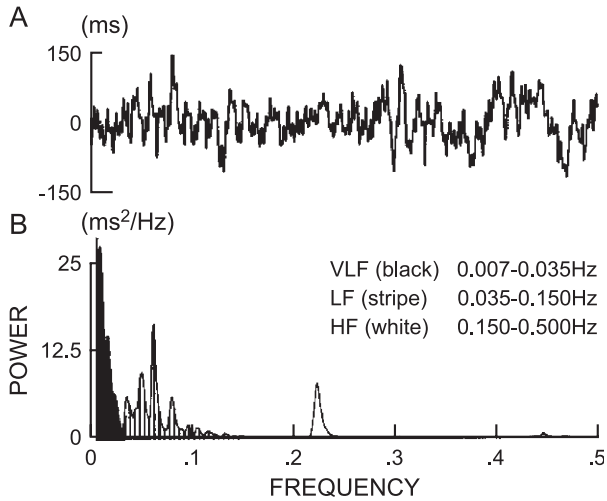

Fig. 1. A typical set of computer-aided ECG R-R interval power spectral analysis results: raw R-R interval changes (A) and the corresponding power spectrum, from which various ANS activity components are derived (B) [18,21,22].

bandpass between 0.007 and 0.5 Hz. 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) [22,23]. After passage through the Hamming-type data window, power spectral analysis using a fast Fourier transform was performed on a consecutive 1024-second series of R-R interval data obtained during the test.

To evaluate activity of the ANS, we analyzed a very low frequency (VLF) component (0.007-0.035 Hz) reflecting SNS activity related to energy metabolic regulation [22,23], a low frequency component (0.035-0.15 Hz) jointly regulated by both SNS and parasympathetic nervous system activities, and a high frequency component (0.15-0.5 Hz) that solely reflected parasympathetic nervous system activity by integrating the spectrum for the respective bandwidth. Typical sets of raw R-R intervals (top) and the corresponding power spectrum (bottom) are illustrated in Fig. 1.

2.6. Genetic analysis

A noninvasive genotyping 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 *Bcl*É polymorphism of the UCP1 gene, which detects the A-G point variant at position −3826 base pair (bp) in the 5′ -flanking domain, was determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis according to the method proposed by Cassard-Doulcier et al [25]. The PCR primers were 5′ -CTTGGGTAGTGACAAAG-TAT-3′ (upstream) and 5′ -CCAAAGGGTCAGATTTC-TAC-3′ (downstream). 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 minutes, 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. We then incubated 5 μL of the PCR product for 1 hour with 10 U of *Bcl*É 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*É 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 (AR) gene, which detects the $Trp^{64}Arg$ variant, was determined using PCR-restriction fragment length polymorphism analysis according to our previously reported method [26]. The PCR primers were 5′ -CCAATACCGCCAACA-CACCAGT-3′ (upstream) and 5′ -AGGAGTCCCATCAC-CAGGTC-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 minutes, 30 cycle s at 94°C for 30 seconds, 67°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. We then incubated 5 μL of the PCR product for 1 hour with 10 U of *Mva*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 *Mva*I is lost; therefore, the allele of this polymorphism corresponds to the 158 bp undigested band.

2.7. Statistical analysis

All data are presented as the mean ± SE. All of the statistical analyses were performed with the Statistical Package for Social Science (SPSS for Windows, version 11.0, Inc, Chicago, Ill). A statistical difference between the lean and obese groups in terms of time course changes in the postprandial EE was analyzed by means of a 1-way analysis of variance (ANOVA) with repeated measurements. A multiple regression analysis was applied to evaluate the impact of the variant of the UCP1 gene and the β_3-AR gene and the impact of obesity (as explanatory variables) on the TEF, postprandial fat oxidation, and postprandial carbohydrate oxidation (as response variables). The Student unpaired t test was used to compare the differences of the measurements between the lean and obese groups. Paired t test was used to compare the differences between preprandial and postprandial measurements. A group comparison among 3 groups (lean, obese >3 years, and obese <3 years) was made with ANOVA using post hoc Tukey's test for subsequent pairwise comparisons. P values lower than .05 were regarded as statistically significant.

3. Results

Table 1 summarizes the physical characteristics and the parameters of energy metabolism during preprandial and postprandial periods for the obese and lean groups. There was no significant difference in age, height, allele frequency of the $Trp^{64}Arg$ variant of the β_3-AR gene, and allele frequency of the −3826 A→G variant of the UCP1 gene. As expected, body mass, body mass index, and percentage of body fat were significantly higher ($P < .001$) in the obese group than in the lean group. The values of nutritional requirement, test meal energy, and preprandial EE before the HC meal were also higher ($P < .05$) in the obese group than in the lean group.

3.1. Metabolic responses

3.1.1. Respiratory values

The time courses of postprandial V_{O_2}, V_{CO_2}, and RQ values after each test meal are presented in Table 3. After adjusting to lean body mass (LBM), the obese group showed a significantly smaller net increase in V_{O_2} (lean, 1.25 ± 0.02 L; obese, 1.15 ± 0.20 L; $P < .01$) and in V_{CO_2}

Table 3
Time course of V_{O_2}, V_{CO_2}, and RQ after the HC and HF meals in the lean and obese groups

	Preprandial	Postprandial						Net increase (L/3 hours)	Net increase (L/3 hours per lean body mass)
		30 min	60 min	90 min	120 min	150 min	180 min		
HC meal									
V_{O_2} (L/min)									
Lean (n = 13)	0.24	0.28[a]	0.27[a]	0.27[a]	0.27[a]	0.27[a]	0.26[b]	6.25	1.25[c]
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.54)	(0.02)
Obese (n = 10)	0.27	0.30[d]	0.29[b]	0.30[d]	0.29[b]	0.29[d]	0.29[b]	4.58	1.15
	(0.01)	(0.02)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.74)	(0.20)
V_{CO_2} (L/min)									
Lean (n = 13)	0.20	0.25[a]	0.24[a]	0.24[a]	0.24[a]	0.23[a]	0.23[b]	6.29[b]	1.26[c]
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.57)	(0.03)
Obese (n = 10)	0.23	0.26[b]	0.25[b]	0.26[d]	0.26[d]	0.25[b]	0.25[b]	5.06	1.16
	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(1.09)	(0.03)
RQ									
Lean (n = 13)	0.86	0.87[b]	0.89[b]	0.89	0.87	0.86	0.86		
	(0.20)	(0.17)	(0.14)	(0.18)	(0.12)	(0.20)	(0.17)		
Obese (n =10)	0.85	0.87	0.88	0.87	0.87	0.87	0.88		
	(0.16)	(0.19)	(0.17)	(0.15)	(0.16)	(0.19)	(0.18)		
HF meal									
V_{O_2} (L/min)									
Lean (n = 13)	0.24	0.28[a]	0.27[a]	0.28[a]	0.28[a]	0.28[a]	0.28[a]	6.04	1.24
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.79)	(0.03)
Obese (n = 10)	0.26	0.29[d]	0.30[a]	0.30[a]	0.30[a]	0.29[d]	0.30[a]	5.63	1.19
	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.48)	(0.02)
V_{CO_2} (L/min)									
Lean (n = 13)	0.21	0.24[a]	0.24[a]	0.24[a]	0.23[a]	0.22[b]	0.22[b]	3.76	1.15[c]
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.62)	(0.03)
Obese (n = 10)	0.23	0.24[b]	0.25[d]	0.25[d]	0.25[d]	0.23	0.24[b]	2.50	1.08
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.61)	(0.02)
RQ									
Lean (n = 13)	0.86	0.85	0.86	0.84	0.82[d]	0.80[d]	0.79[a]		
	(0.16)	(0.12)	(0.15)	(0.16)	(0.15)	(0.14)	(0.15)		
Obese (n = 10)	0.85	0.82[d]	0.82[b]	0.82[d]	0.81[d]	0.78[a]	0.79[a]		
	(0.16)	(0.15)	(0.18)	(0.15)	(0.18)	(0.14)	(0.11)		

Data are expressed mean (±SE). Net increase of V_{O_2} and V_{CO_2} was calculated as the area under the curve.
[a] $P < .001$, preprandial vs postprandial, by paired t test.
[b] $P < .05$, preprandial vs postprandial, by paired t test.
[c] $P < .05$, lean vs obese, by unpaired t test.
[d] $P < .01$, preprandial vs postprandial, by paired t test.
[e] $P < .01$, lean vs obese, by unpaired t test.

(lean, 1.26 ± 0.03 L; obese, 1.16 ± 0.03 L; $P < .05$) after the HC meal. After the HF meal, the net increase in V_{O_2} was not statistically different between the 2 groups (lean, 1.24 ± 0.03 L; obese, 1.19 ± 0.02 L); however, the net increase in V_{CO_2} was significantly lower in the obese group than in the lean group (lean, 1.15 ± 0.03 L; obese, 1.08 ± 0.02 L; $P < .05$). In addition, the RQ values after the HF meal were significantly lower ($P < .01$) in the obese group whereas no significant change was observed after the HC meal. A lower V_{CO_2} level as well as decreased RQ values after the HF meal as shown in obese boys may be induced by predominant fat use.

3.1.2. Thermic effect of meals

The TEF, expressed as a relative value (% energy intake), was significantly lower ($P < .05$) in the obese group than in the lean group after the HC meal; however, such a difference was not observed after the HF meal (Table 1).

Fig. 2 shows the time course changes in the net increase in postprandial EE after the HC and HF meals, expressed as a percentage above the preprandial EE value in the 2 groups. There was no group difference in postprandial EE values after the HF meal. In contrast, a significant group effect was found ($F = 5.12$; $P < .001$) in postprandial EE values after the HC meal. Multiple regression analysis showed that obesity was a significant variable contributing to the variances in the TEF induced by the HC meal (Table 4), suggesting that obese boys may possess a blunted metabolic response to the HC meal.

3.1.3. Fat oxidation

Postprandial fat oxidation, expressed as absolute and relative values after each test meal, is presented in Table 1.

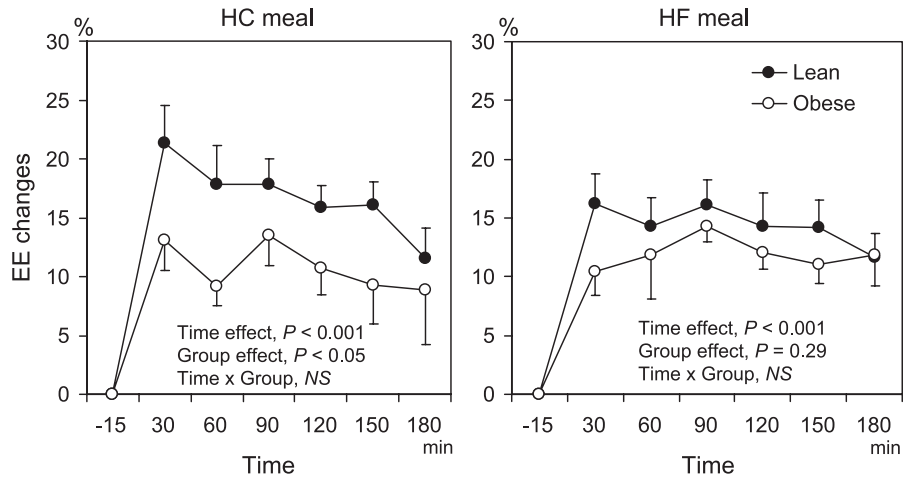

Fig. 2. Time course of postprandial EE after the HC meal (left) and the HF meal (right). Filled circle indicates lean; open circle, obese. The data are expressed as the mean ± SE for each group. Time effect, group effect, and time × group interaction are calculated by repeated ANOVA.

Although the obese group seemed to have a higher rate of fat oxidation after both meals, the change was not statistically significant. In addition, as shown in Table 4, multiple regression analysis showed that obesity was not a significant variable contributing to the variances in the fat oxidation induced by each test meal. Furthermore, even focusing on the duration of obesity, no group difference was found in fat oxidation values (% intake) between the boys with long (≥3 years; n=5) and short durations of obesity (<3 years; n=5) after the HC meal (51.1% ± 1.6% vs 49.0% ± 1.0%) as well as the HF meal (14.5% ± 0.5% vs 14.0% ± 0.3%). The characteristics of obese boys with long and short durations of obesity are shown in Table 5. The boys with a long duration of obesity had greater body mass index compared with the boys with a short duration of obesity, but percentage of body fat was not significantly different between the 2 groups.

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

Explanatory variable	Preprandial coefficient (95% CI)	*P*
HC meal		
TEF (% intake)	R = 0.49	
UCP1 (A→G variant)	−0.3 (−2.1-1.0)	.659
β_3-AR (Trp^{64}Arg variant)	0.7 (−1.0-2.2)	.390
Obesity	−1.5 (−3.0-0.0)	.046
Fat oxidation (% intake)	R = 0.28	
UCP1 (A→G variant)	18.8 (−15.7-53.4)	.268
β_3-AR (Trp^{64}Arg variant)	8.3 (−28.6-45.3)	.642
Obesity	3.5 (−31.1-38.0)	.835
Carbohydrate oxidation (% intake)	R = 0.28	
UCP1 (A→G variant)	−3.9 (−12.0-4.3)	.333
β_3-AR (Trp^{64}Arg variant)	−2.9 (−9.1-7.2)	.498
Obesity	1.0 (−11.6-5.8)	.808
HF meal		
TEF (% intake)	R = 0.46	
UCP1 (A→G variant)	−1.4 (−3.0-0.0)	.040
β_3-AR (Trp^{64}Arg variant)	0.1 (−1.5-1.7)	1.702
Obesity	−0.5 (−2.0-1.0)	1.020
Fat oxidation (% intake)	R = 0.58	
UCP1 (A→G variant)	6.2 (−1.1-13.4)	.090
β_3-AR (Trp^{64}Arg variant)	7.6 (−0.2-15.3)	.054
Obesity	4.4 (−2.9-11.6)	.223
Fat oxidation (% intake)	R = 0.53	
UCP1 (A→G variant)	−19.6 (−43.4-4.0)	.099
β_3-AR (Trp^{64}Arg variant)	−18.8 (−44.2-6.6)	.137
Obesity	−13.7 (− 37.4-10.0)	.240

CI indicates coefficient interval.

3.1.4. Carbohydrate oxidation

Postprandial carbohydrate oxidation, expressed as absolute and relative values after each test meal, is presented in Table 1. Obese boys consumed a significantly higher amount (P < .01) of carbohydrates; however, carbohydrate oxidation values (% intake) after both HC and HF meals appeared to be lower in the obese group than in the lean group but were not statistically significant (Table 1). Moreover, obesity was not a significant variable contributing to the variances in carbohydrate oxidation induced by each test meal (Table 4). Regarding the duration of obesity, however, carbohydrate oxidation value (% intake) obtained from the boys with a short duration of obesity was significantly lower than the boys with a long duration of obesity and the lean boys (42.7% ± 8.8% [short] vs 85.5% ± 9.4% [long], P < .05, and vs 80.0% ± 7.8% [lean], P < .05)

Table 5
Characteristics of obese children with long (≥3 years) and short (<3 years) durations of obesity

	Obese ≥3 years (n = 5)	Obese <3 years (n = 5)
Age (y)	10.0 (0.6)	8.4 (0.2)
Body mass index (kg/m^2)	25.3 (1.9)	24.0 (2.1)*
Body fat (%)	31.0 (2.6)	28.4 (2.1)

n = 10. Data are expressed mean (±SE).
* P < .001 vs ≥3 years, by unpaired t test.

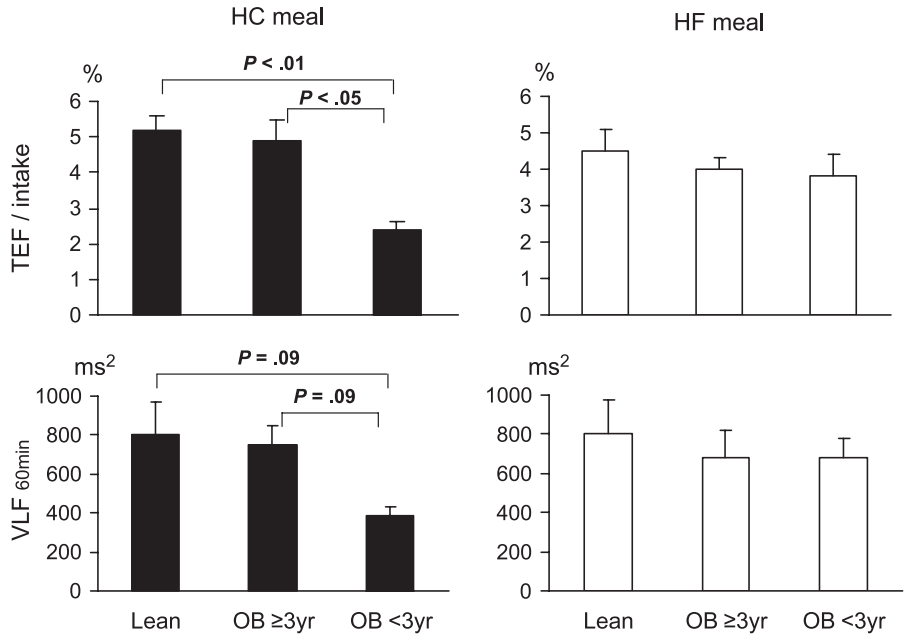

Fig. 3. Thermic effect of food (percentage of energy intake, top) and VLF component of heart rate variability (absolute values at 60 minutes after feeding, bottom) after the HC meal (left) and the HF meal (right) in lean (n = 13) and obese groups with obesity of 3 years or more (n = 5) and obesity of less than 3 years (n =5).

after the HF meal. These significant differences however disappeared after the HC meal (28.1% ± 4.0% [short] vs 33.2% ± 4.0% [long], and vs 32.1% ± 2.6% [lean], $P > .05$).

3.2. Sympathetic nervous system response

Fig. 3 shows the TEF, expressed as percentage of energy intake, and the VLF values (60 minutes after meals) after the HC and HF meals in the boys with leanness, long duration of obesity, and short duration of obesity, respectively. After the HC meal, the boys with a short duration of obesity had a significantly lower TEF as well as a slightly diminished thermogenic SNS response compared with the remaining obese and lean boys, whereas there were no group differences in values of the TEF and the VLF after the HF meal.

4. Discussion

The present study provides information regarding a potential etiologic association between metabolic alterations and childhood obesity. The main findings were that obese boys possessed blunted thermogenic responses to the HC meal, whereas such an alteration was not found after the HF meal. In addition, much lower TEF as well as reduced thermogenic SNS activity in response to the HC meal was observed in the boys with a recent onset of obesity.

Regarding characteristics of lipid use in obese children, Maffeis et al [2] demonstrated that obese and nonobese children had comparable TEF values after HF feeding, indicating that obese children may possess a normal metabolic response to the HF meal. Our data are consistent with this result [2] because the percentage of fat of the meal we used was considerably higher (70% vs 40%) than that of the previous study [2], which can confirm the major role of specific macronutrient (fat) composition on postprandial lipid use. Some studies indicate that enhanced fat oxidation is observed in obese children during postabsorptive [9,27,28] and postprandial periods [9]. Moreover, the study [9] using isotope tracers verified that both postabsorptive fat oxidation and postprandial fat oxidation are related to the fat mass of children and that increased exogenous (meal intake) fat oxidation as well as decreased endogenous (adipose tissue lipolysis) fat oxidation are correlated with greater fat mass. Taken together, previous findings [2,9] and our present results indicate that obese children may possess an enhanced or at least normal response for lipid use when they consume a high proportion of dietary fat.

Considering the feature of carbohydrate use in obese children, LeStunff and Bougnères [28] investigated fat and glucose use during the postabsorptive period and provided the hypothesis that increased fat oxidation may induce a lower glucose oxidation, insulin resistance, and a higher fasting insulin secretion. In addition, when adjusted for LBM, glucose oxidation was progressively decreased with the duration of obesity. This result is partly inconsistent with the present study because we found a reduced thermic response to the HC meal among the boys in the early phase of obesity. The reason for the conflict is not clear but may be accounted for by a lack of homogeneity in the experimental population, that is, LeStunff and Bougnères [28] examined obese children with longer durations of obesity more severely than our subjects. Rueda-Maza et al [8] examined the origin of carbohydrate oxidation using an isotope tracer

in obese and normal-weight children who had physical characteristics (ie, age and degree of obesity) similar to those of our subjects and reported a finding that obese children manifested a smaller proportion of endogenous (glycogenolysis) carbohydrate oxidation due to decreased glycogen turnover. Taking the above findings into consideration, insulin resistance together with a reduced endogenous glycogen appearance in obese children might be a conceivable early sign, predicting that metabolic disorder will occur in the future.

In the present study, we also found that diminished thermic and sympathetic responses to the HC meal were observed in the boys with a recent onset of obesity. As shown in Fig. 3, the TEF (% intake) and the postprandial VLF (60 minutes after meals) were comparable after the HF meal in lean subjects as well as in those with long and short durations of obesity, supporting the assumption that obese children have a normal thermogenic and SNS response to the HF intake. After the HC meal however, lower TEF in parallel with decreased VLF, a parameter of thermoregulatory SNS function, in boys with a recent onset of obesity might provide indirect evidence of a role of the SNS in postprandial thermogenesis. Although the coordination of energy homeostasis relies largely not only on neural but also on hormonal regulation, we evaluated only ANS activities in the present study. Because thyroid hormones (THs) are potent modulators of adaptive thermogenesis, altered TH release is considered to be a potential factor for the development of obesity [29]. Therefore, further experiments, including assessment of TH release during resting as well as postprandial periods, are needed to confirm the differences of thermogenic effects of various meals.

Molnár et al [3] indicated that children with a recent onset of obesity had a much lower TEF compared with those with long-standing obesity. In addition, Tounian et al [7] reported that the obese children with a familial history of obesity also possessed much reduced TEF compared with obese children without such a familial history. These results suggest that obese children can be classified according to their own metabolic characteristics. In the present study, we examined genetic characteristics (ie, the variants of the β_3-AR and UCP1 genes) to verify the effect on the TEF in children with and without variants of these genes, but the differences were not statistically significant. Consequently, based on our previous study [12] and that of Molnár et al [3], we categorized the obese boys into groups with short (<3 years) and long (≥ 3 years) durations of obesity to determine whether the stage of obesity influences the TEF. Although genetic effect remains unclear, our results confirm those of the previous studies [4-6] and the assumption [3] that a blunted TEF appears during the initial phase of childhood obesity. Considering previous findings [7-9,27,28] as well as our results, we hypothesize that predominant fat metabolism as generally shown in obese children can attenuate carbohydrate use. Further long-term studies are needed to scrutinize whether such altered carbohydrate metabolism has a significant impact on the maintenance or development of childhood obesity.

In summary, the present study's obese boys possessed a normal metabolic and sympathetic response to the HF meal but showed a diminished thermogenic response to the HC meal, especially during the early phase of obesity. The present results suggest that diet composition, even within the usual range for normal daily energy requirements, can affect the regulation of energy balance in obese boys. We make a further implication that excessive carbohydrate intake such as that with soft drinks or snacks popular among children might be one of the causative factors of the development of obesity in children during the early phase of obesity.

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