AINO JOURNAL, Vol. 8, 2009, pp. 11 – 14

Original

The association between the thrifty genotype and energy expenditure during aerobic exercise in healthy males and females

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Abstract

The number of patients with diabetes is notably increasing in Japan. The thrifty genotype characteristics provide an explanation for this very high prevalence of diabetes. Especially, the Trp64Arg polymorphism in the β3-adrenergic receptor (β3-AR) gene is relatively common in Japanese people. Aerobic exercise is one of the major strategies for prevention and treatment of type 2 diabetes. The present study investigated whether the Trp64Arg polymorphism in the β3-AR gene is associated with a reduction in energy expenditure in aerobic exercise. Eighty-six non-obese healthy young people were recruited. Energy expenditure was measured using indirect calorimetry. The subjects performed an aerobic exercise program at 60% of their maximal heart rate for 30 minutes. The level of fat oxidation during aerobic exercise by male subjects with the Trp/Arg of the β3-AR gene was significantly lower than that by those with the Trp/Trp genotype. There was no difference observed in female subjects. It was demonstrated that the Trp64Arg polymorphism in the β3-AR gene is associated with the reduction of fat oxidation during aerobic exercise in healthy young males.

Key words: energy expenditure, aerobic exercise, β3-adrenergic receptor, Trp64Arg polymorphism

INTRODUCTION

The number of patients with diabetes is notably increasing in Japan. In 2008, the Japanese Ministry of Health, Labour, and Welfare announced that in the Japanese population, 8.2 million people were strongly suspected as being diabetic, and 10.5 million people were candidates for diabetes. The reasons why the prevalence of diabetes in Japan is increasing so explosively should be discussed considering that insulin resistance is enhanced by changes in life style and the thrifty genotype characteristics of many Japanese. Among these thrifty genes, the Trp 64Arg polymorphism in the β3-adrenergic receptor (β3-AR) gene has been reported to be associated with the prevalence of obesity and type 2 diabetes (Kogure et al., 1998). The prevalence of Japanese with Trp to Arg substitution at codon 64 of the β3-AR gene was documented to be relatively high, and associated with early onset of type 2 diabetes (Nagase et al., 1997). This polymorphism was also reported to be associated with abdominal obesity, BMI and insulin resistance (Valve et al., 1998; Walston et al., 2003). In addition, this polymorphism acts synergistically to lower the basal metabolic rate (Clément et al., 1996; Sivenius et al., 2000).

Meanwhile, exercise has positive benefits for people with diabetes. Especially, aerobic exercise is one of the major strategies for prevention and treatment of obesity and type 2 diabetes (Gordon et al., 2008). It is well known that aerobic exercise increases not only energy expenditure, but also glucose uptake of the muscle cells by translocating the glucose-transporter from the cytoplasm to the cell surface, leading to lowering plasma glucose levels and attenuating insulin resistance (Winder et al., 1999).

The effects of aerobic exercise on weight reduction vary among individuals. These are influenced by a variety of factors such as environmental factors, exercise intensity, muscle mass, level of circulating hormones, age, gender, and genotype. Genotypes of the genes were demonstrated to be associated with expenditure at rest, but it remains unclear whether the effects of aerobic exercise should be modified by the genotypes. The purpose of this study is to investigate whether energy expenditure and fat oxidation during aerobic exercise are affected by the Trp64Arg polymorphism of the β3-AR gene.

Materials and Methods

Subjects

Eighty-six healthy volunteer students (male: 45, female: 41, age: 22.2±3.6) were recruited to participate in this study. All subjects were free of metabolic, neuromuscular, cardiovascular disorders and recent illness. This study was approved by the Aino University ethics committees and conformed to the principles outlined in the Helsinki Declaration. Informed consent was obtained from each participant before participation in this study.

Determination of Trp64Arg in β3-AR gene

Genomic DNA was extracted from peripheral blood cells using a GFX Genomic Blood DNA Purification Kit (GE Healthcare, UK). The Trp64Arg polymorphism in the β3-AR gene was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), according to previously reported methods (Sakane et al., 1997). Briefly, the PCR primers were 5'- CCAAT ACCGCCA-ACACCAGT-3' (upstream) and 5'-AGGAGT-CCCATCACCAGGTC-3' (downstream), which flank the whole of exon 1 of the β3-AR gene. Genomic DNA (100ng) in a total volume of 20 μl was used for PCR. PCR was performed with initial denaturation at 94℃ for 5 min, 30 cycles at 94℃ for 30s, 67℃ for 30s, and 72℃ for 30s, and a final extension at 72℃ for 10 min. We incubated 5μl of the PCR product for 4 hr with 10U of MvaI at 37℃ in a final volume of 10μl. The samples were run on a 3.0% agarose gel, stained with ethidium bromide, and analyzed under ultraviolet light.

Energy expenditure, Fat oxidation, and Carbohydrate oxidation

Temperature and humidity of the measurement room were maintained at 23.0±1.0℃ and 50.0±7.0% throughout the experiments, respectively. All measurements were obtained within 2 hours after intake of 400kcal carbohydrate as lunch. Participants were requested not to smoke and not to expose themselves to high physical activity before exercise. Body weight, height and lean body mass were measured using a body fat analyzer (TANITA, BC-522, Japan), while body mass index (BMI) was calculated as weight (kg) divided by squared height (m^2). Using the Karvonen method, the maximal heart rate of the each participant was evaluated and the target heart rate during exercise was determined. Energy expenditure was measured in the sitting position for 30 minutes both at rest and during exercise. The subjects performed an aerobic exercise program with a bicycle ergometer (75XL2ME, COMBI WELLNESS, Japan) at the target heart rate (60% of their maximal heart rate) for 30 min, and the pedaling frequency was set at 60 rpm. Energy expenditure, fat oxidation, and carbohydrate oxidation at rest and during exercise were measured using a whole-body indirect human calorimeter (AE 300 S, MINATO Medical Science, Japan). All data were expressed as kilocalories per 30 minutes.

Statistical Analysis

Statistical analysis was performed using the Statcel97 PC software (OMS, Japan). All data were presented as the mean±SD. Significance of the differences between genotypes was evaluated using student's t-test. Significance was set at $p<0.05$.

RESULTS

Genotyping of the Trp64Arg polymorphism in the β3-AR gene demonstrated that the distribution of the Trp/Trp, Trp/Arg, and Arg/Arg genotypes was 52 (60.5%), 33 (38.4%), and 1 (1.1%), respectively. The allele frequency of the Arg64 was 20.3%. The subjects were separated into two groups, Trp/Trp and Trp/Arg + Arg/Arg, to analyze the association of the genotype with energy expenditure, because the number of subjects with the Arg/Arg was too small for statistical analysis.

Table 1 Physical characteristics and energy expenditure in subjects with the β3-AR Trp64Arg polymorphism.
Data are expressed as means ± SD. *P<0.05

	Male			Female		
	Trp/Trp	Trp/Arg Arg/Arg	P	Trp/Trp	Trp/Arg Arg/Arg	P
Number of subjects	29 (64.4%)	16 (35.6%)		23 (56.0%)	18 (44.0%)	
Height (cm)	172.7±5.1	171.5±6.8	0.55	158.3±5.1	159.8±6.5	0.39
Weight (kg)	68.7±9.3	68.3±15.2	0.90	56.8±7.3	53.7±7.5	0.18
BMI (kg/m^2)	23.0±2.7	23.2±5.0	0.87	22.7±3.2	21.0±2.5	0.06
Lean body mass (kg)	52.3±4.6	51.1±6.5	0.47	37.4±5.3	35.6±2.7	0.21
In the resting state (kcal/30 min)						
Energy expenditure	49.09±10.32	46.70±8.41	0.44	38.63±6.35	37.31±7.36	0.54
Fat oxidation	26.30±12.97	14.69±10.24	*0.01	21.10±9.97	16.99±7.86	0.15
Carbohydrate oxidation	23.79±11.57	32.01±11.12	*0.01	7.53±10.89	21.32±9.29	0.38
During the aerobic exercise (kcal/30 min)						
Energy expenditure	210.60±24.13	207.30±28.13	0.71	126.12±15.40	124.33±15.06	0.71
Fat oxidation	67.74±25.20	52.44±18.27	*0.04	41.98±16.14	40.35±17.98	0.76
Carbohydrate oxidation	143.86±25.33	155.86±26.75	0.15	85.14±22.35	84.98±21.70	0.98

Table 1 shows the physical characteristics and energy expenditure of subjects with Trp/Trp, and with Trp/Arg+Arg/Arg. In male subjects, there was no significant difference in the resting energy expenditure between the two groups, whereas it was demonstrated that the subjects with the Arg allele showed a higher level of resting carbohydrate oxidation and a lower level of resting fat oxidation. Energy expenditure during exercise in subjects with the Arg allele was comparable to that of those without the Arg allele. The level of fat oxidation in subjects without the Arg allele was significantly higher than that in those with the Arg allele, though there was no difference in the level of carbohydrate oxidation. Analysis of female subjects also demonstrated that there were no significant differences in energy expenditure, fat oxidation and the carbohydrate oxidation either at rest and during exercise between female subjects with and without the Arg64 allele.

DISCUSSION

This study demonstrated that the Trp64Arg polymorphism of the β3-AR gene is associated with reduction of fat oxidation not only at rest, but also during aerobic exercise in healthy young male subjects. As the polymorphism did not affect the level of energy expenditure, the present data would imply that prolonged exercise is necessary to utilize the energy stored as lipids in males with the Arg64 allele of the β3-AR gene, compared with that in individuals without the Arg64 allele.

Aerobic exercise in healthy subjects is reported to increase lipid oxidization during exercise by elevating the circulating catecholamine level, which facilitates lipolysis in the white adipose tissue through β3-AR signaling (Lönnqvist et al., 1993). The increased lipolysis raises the free fatty acid (FFA) level and delivers energy to skeletal muscles for fat oxidation (Mittendorfer et al., 2002). In subjects with Trp to Arg substitution at codon 64 of β3-AR gene, intracellular cAMP level after stimulation with catecholamine has been documented to be lower than that in those without the substitution, resulting in attenuated hormone-sensitive lipoprotein lipase activity, lipolysis and thermogenesis (Umekawa et al., 1999).

Differences in the lipolytic activity during aerobic exercise have been observed between males and females with the Arg64 allele. This might be explained by different distributions of visceral and subcutaneous fat in the two sexes. Males accumulate fat in the abdominal region through the action of testosterone, whereas females accumulate fat in the gluteal-femoral regions through the action of estrogen (Wahrenberg et al., 1989). It is well known that lipids stored in the visceral adipose tissue are consumed rapidly during aerobic exercise, compared with those stored in the subcutaneous adipose tissue (Ross et al., 1996). As it is shown that the visceral adipocytes express the β3-AR gene four times more abundantly than the subcutaneous fat (Hellmér et al., 1992), the fat oxidation level in males is thought to be highly influenced by the presence of Trp to Arg substitution at

codon 64 of β3-AR gene. The fat oxidation level during aerobic exercise in female subjects may also be influenced by this substitution, though only slightly, because of the smaller volume of visceral fat.

Our study suggests the necessity of an individualized menu of aerobic exercise for type 2 diabetics in order to achieve an efficient outcome according to their β3-AR genotypes. This finding has the potential to provide benefits for developing tailor-made regimens to treat diabetes in the finding. However, as our study involved short-term aerobic training on young healthy subjects, it is unclear whether the β3-AR Trp64Arg polymorphism influences fat oxidation after long-term aerobic training. It also remains unclear whether the effect of physical training in patients with type 2 diabetes is modified by the β3-AR Trp64Arg polymorphism similarly to that in the healthy subjects. Further investigations are needed to fully elucidate the effect of the β3-AR Trp64Arg polymorphism on fat oxidation during aerobic exercise in patients with type 2 diabetes.

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