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Effects of Acarbose on the Expression of Obese and Neuropeptide Y (NPY) Genes in Mice on High-Carbohydrate Diet

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Two components of the neuroendocrine-hormonal response to long-term treatment of acarbose, adipose tissue-derived leptin and central neuropeptide Y (NPY), were investigated in the ICR mice on a high-carbohydrate diet. Acarbose, administered 5 or 50 mg per 100 g diet for four weeks, dose dependently suppressed body weight gain. The body weight gain was reduced along with the amount of daily food intake in 50 mg acarbose-treated group at 7^{th} and 28^{th} day. 5 or 50 mg acarbose treatment administered for four weeks reduced leptin mRNA levels to 62% and 77% of the control group, demonstrating that the amount of leptin mRNA in adipocytes correlates with body weight. As dose of acarbose increased, leptin mRNA level also increased, suggesting that potent inhibition of α-glycosidase by a higher dose of acarbose furthers the enzyme activity and leptin gene consequently. On the other hand, central expression level of NPY gene was increased significantly compared with the control group at the same amount of acarbose administered, reflecting that leptin and NPY operate in a negative-feedback circuit to regulate body fat stores.

Key Words: Acarbose, α-glycosidase, Leptin, Neuropeptide Y

INTRODUCTION

Acarbose is a competitive inhibitor of intestinal sucrase, glucosidase, and maltase is efficient in reducing postprandial plasma glucose and insulin levels in diabetic subjects (Willms et al, 1980; Reaven et al, 1990) and in non-diabetic and diabetic rats fed with carbohydrate diets containing acarbose (Gray & Olefsky, 1982; Lee et al, 1983). Acarbose is also efficient treating obesity as it decreases plasma insulin levels since metabolic abnormalities can be partly ascribed to hyperinsulinemia (Jeanrenaud, 1990). In genetically obese animals (*fa/fa* rats or *db/db* mice), acarbose treatment prevented hyperglycemia and decreased insulin levels. Hyperinsulinemia plays a key role not only in the development of the obese syndrome, since it promotes adipose and liver lipogenesis, but also in the establishment and maintenance of insulin resistance (Jeanrenaud, 1979).

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Leptin, the product of the *ob* gene, is a regulator of food intake and energy expenditure (Zhang et al, 1994). Leptin is an afferent signal molecule that interacts with appetite and satiety centers in the brain to regulate body weight (Meier, 1995). NPY, a 36-amino acid neurotransmitter distributed throughout the nervous system, is an important feeding stimulant in the brain (Eva et al, 1998). The aim of the present study is to investigate the relationship among acarbose treatment, leptin, and neuropeptide Y (NPY) message in mice on a high-carbohydrate diet. In order to test the hypothesis that acarbose treatment to mouse on a high carbohydrate diet would change energy balance, we investigated the effects of acarbose on daily food intake, body weight, and leptin and NPY mRNA levels in epididymal adipose tissue and brain, respectively.

METHODS

Animals

Three-week-old male ICR mice (12~15 g), ob-

tained from DaeHan Experimental Animal Center (Eumsung, Korea), were maintained at 22±2°C in a room at a 12 h light-dark cycle. Water and diet were freely available throughout the experiment. Food intake, body weight, and general condition of the mice were checked daily. These mice were on a high-carbohydrate diet with or without acarbose. The composition of the high-carbohydrate diet was as follows (kcal %): carbohydrate 67.5 kcal %, lipid 20.8 kcal %, protein 11.7 kcal %.

One group (n=5), designated as L5, received an acarbose (5 mg/100 g food) for 4 weeks and second group (n=5), designated as L50, also received the acarbose (50 mg/100 g food) during the same period of time. Control mice for this experiment (n=5) were fed with high-carbohydrate diet only. Food was freely available to both the acarbose-treated and control group.

At the completion of the experiment, all animals were sacrificed by cervical dislocation, and epididymal adipose tissue and brain were removed immediately, snap frozen in liquid nitrogen, and stored at −70°C until analysis.

Cloning ob cDNA

486 base pairs of the coding region of the leptin gene were amplified by polymerase chain reaction (PCR) with total RNA from white adipose tissue as the template (upstream primer 5'-CTG TGT CGG TTC CTG TGG-3', reverse primer 5'-GCA TTC AGG GCT AAC ATC-3') (Igel et al, 1996). The reaction product was separated on agarose gel and subcloned into the SmaI site of pUC19 (Sureclone kit, Pharmacia). Plasmid DNA was prepared and digested with BamHI/EcoRI in order to isolate the leptin cDNA.

NPY cDNA was digested with EcoRI (NPY cDNA was kindly provided by Dr. Baik at Kyunghee University, School of Medicine.). A human b-actin probe was used to monitor the amount of total RNA in each sample.

RNA extraction and Northern blotting

Total RNAs were extracted from epididymal adipose tissue and brain, fractionated and blotted onto charge-modified nylon membranes (Amersham) as previously described (Chirguin et al, 1979). The leptin, NPY cDNA, and β-actin probes were labeled with [α-^{32}P]dCTP (Amersham) by random priming of cDNAs using DNA labeling kit according to the manufacturer's instructions (Boehringer Mannheim). Membranes were hybridized overnight at 42°C. Blots were washed twice in 2X SSC/0.1% SDS at 42°C for 15 min, and washed once in 0.1X SSC/0.5% SDS at room temperature for 10 min, and then exposed to Kodak XR film at −70°C. Autoradiographic images were analyzed using Bio-Rad Image Analysis Software (Bio-Rad). Final results were expressed as the ratio of integrated intensities of *ob* or NPY and β-actin mRNA.

Statistical analysis

Statistical significance was assessed by Student's t-test for unpaired comparisons (Snedector & Cochran, 1967). Results were presented as means SE, and differences were considered significant when $p < 0.05$.

RESULTS

Body weight and food intake profiles

Effects of acarbose treatment on body weight and daily food intake in mice fed with a high-carbohydrate diet were evaluated. By inhibiting absorption of glucose through intestine, acarbose suppressed body weight gain dose dependently (Table 1). Male ICR mice treated with 50 mg of acarbose (L50) did not gain weight since the first week of acarbose treatment, and its body weight reduced significantly during the experimental period compared with the control group. On the other hand, 5 mg of acarbose-treated group (L5) showed statistically lower body weight than the control group after the third week of administration. The body weight gain was reduced along with the amount of daily food intake (Table 2). At 7th and 28th day, the daily food intake of 50 mg acarbose treated group was significantly reduced than that of the control group.

The ob gene expression

Northern blot of leptin mRNA in epididymal adipose tissue from acarbose-treated and control mice was analyzed (Fig. 1). Compared with the control group, scanning densitometry showed the acarbose-

Table 1. Effect of acarbose on body weight

Treatment duration (days)	Body weight (g)			
	Normal	Control	Acarbose-treated	
			5 mg	50 mg
0	14.13±0.53	12.07±1.53	12.21±1.88	12.38±2.34
7	26.41±0.90	19.65±2.64	16.08±3.26	15.23±3.19*
14	28.56±0.71	28.51±3.39	23.24±3.96	17.16±3.82**
21	34.54±1.19	38.75±2.26	34.62±2.53*	27.05±2.62**
28	36.28±1.67	42.86±2.27	38.32±1.88*	30.96±2.78**

Body weight was measured daily. Initial weight is the value of 3rd week of birth, and final weight is the value of 7th week after birth. Values are means±SE (n=5). Statistically significant differences were shown as * and ** for $p<0.05$ and $p<0.01$, respectively. Normal and control designate groups fed with normal diet and high-carbohydrate diet, respectively.

Table 2. Effect of acarbose on daily food intake

Treatment duration (days)	Food intake (g/day)			
	Normal	Control	Acarbose-treated	
			5 mg	50 mg
7	23.28±1.33	23.60±4.16	22.00±5.19	33.78±9.03*
14	29.67±2.81	43.37±8.96	39.06±8.52	41.99±7.22
21	28.02±3.57	59.50±13.32	52.98±7.22	54.54±16.87
28	30.15±3.92	59.60±2.69	59.75±7.16	62.81±4.03*

Food intake was measured daily during 4 weeks of acarbose treatment. Values are means±SE (n=5). Statistically significant difference was shown as * for $p<0.05$ between high-carbohydrate diet control and acarbose-treated mice (50 mg/100 g diet). Normal and control designate groups fed with normal diet and high-carbohydrate diet, respectively.

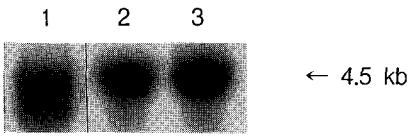
treated groups expressed significantly lower leptin mRNA levels (expressed as the ratio to human β-actin mRNA level). The *ob* (leptin) gene expression in L5 and L50 groups was 62% and 77% compared with the control group, respectively. This data clearly showed that the amount of leptin mRNA in adipocytes correlated with body weight.

The NPY gene expression

Northern blot for NPY mRNA in brain tissue from acarbose-treated and control mice was analyzed (Fig. 2). NPY mRNA levels were all significantly higher in L5 and L50 groups (158% and 220% of the control, respectively) than in the control group. This result correlated with the increase in daily food intake. NPY gene expression was increased following a long term administration of acarbose to high carbohydrate fed mice, an experimental model associated with moderate obese and hyperglycemia.

DISCUSSION

The results presented in this study showed that long term treatment of acarbose, especially L50, partially prevented the development of obesity and hyperglycemia (Table 3) in mice which received a diet



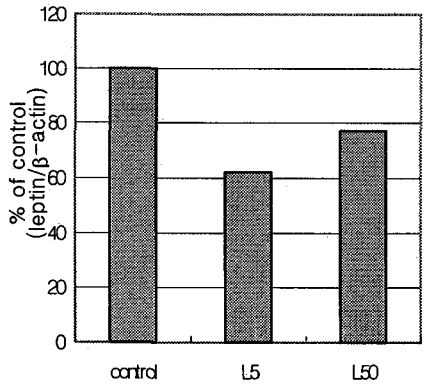
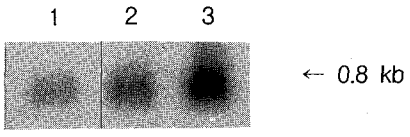


Fig. 1. Northern blot analysis of leptin gene expression in white adipose tissue. Total RNA extraction, gel electrophoresis, hybridization, and scanning densitometry were performed as outlined in methods.
Lane 1: fed high-carbohydrate diets only as a control; lane 2: 5 mg acarbose administered for 4 weeks; lane 3: 50 mg acarbose administered for 4 weeks



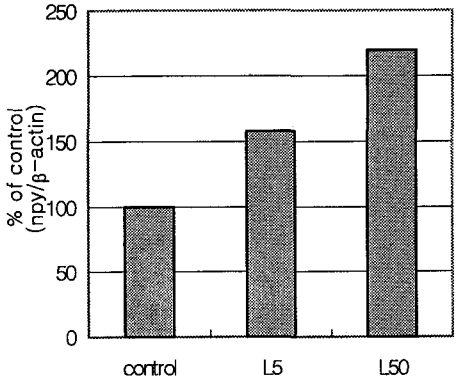


Fig. 2. Northern blot analysis of NPY mRNA expression in whole brain. Total RNA extraction, gel electrophoresis, hybridization, and scanning densitometry were performed as outlined in methods.
Lane 1: fed high-carbohydrate diets only as a control; lane 2: 5 mg acarbose administered for 4 weeks; lane 3: 50 mg acarbose administered for 4 weeks

Table 3. Effect of acarbose on fasting blood glucose

Treatment duration (days)	Fasting blood glucose (mg/dl)			
	Normal	Control	Acarbose-treated	
			5 mg	50 mg
0	65.10±13.17	70.90±40.41	65.52±16.91	87.30±19.76
7	101.38±32.23	115.68±12.36	99.40±35.35	108.94±22.72
14	127.58±18.38	119.98±14.86	87.02±6.00*	87.82±16.65*
21	100.00±24.03	189.98±58.03	94.26±28.94*	79.20±34.45*
28	97.90±25.21	187.04±33.05	176.90±21.64	160.28±20.77

Fasting blood glucose level was measured weekly during 4 weeks of acarbose treatment. Values are means±SE (n=5). Statistically significant difference was shown as * for $p<0.05$ between high-carbohydrate diet control and acarbose-treated mice. Normal and control designate groups fed with normal diet and high-carbohydrate diet, respectively.

enriched in carbohydrate. While daily food intake of the high-carbohydrate diet group was about twice as large as that of the normal diet group, food intake was not noticeably modified by acarbose except on the 7th and 28th day in L50 group (Table 2). These results are similar to the observation made in *db/db* mice (Reaven, 1988). The significantly reduction in body weight of acarbose-treated mice can be therefore ascribed only to the metabolic effects of acarbose. Following the α-glycosidase inhibition, the rate of in-

Table 4. Effect of acarbose on sucrase activity

Group	Sucrase activity (μmole of released glucose/mg protein)		
	Proximal	Middle	Distal
Normal	15.1	15.1	5.2
Control	15.4	22.8	13.4
Acarbose 5 mg	16.7	23.4	17.1
Acarbose 50 mg	16.2	28.3	18.2

At the completion of the experiment, animals were sacrificed. Immediately, the entire small intestine was removed and divided into three segments with equal length. Normal and control designate groups fed with normal diet and high-carbohydrate diet, respectively.

testinal carbohydrate absorption was reduced (William-Olsson, 1986), and consequently, blood insulin levels were significantly lowered, resulting in the decreased rates of liver and adipocyte lipogenesis compared with the control mice. The effect of acarbose in preventing the body weight gain became prominent since the second week of treatment.

Two components of the neuroendocrine-hormonal response to acarbose treatment, adipose tissue-derived leptin and central NPY, were investigated in ICR mice fed with a high-carbohydrate diet (Fig. 1 & 2). In the long term study, the leptin mRNA levels were reduced compared with the those of control. As the dose of acarbose increased, the leptin mRNA level also increased (62% *vs* 77% of control for L5 and L50). Nonetheless, leptin mRNA level in L50 group was still reduced by 23% compared with that of the control. The discrepancy between body weight and leptin mRNA expression level in L5 and L50 groups may result from the fact that potent α-glycosidase inhibition by a higher dose of acarbose induces significant changes in the small intestine disaccharidase activity and obese gene. The intestinal α-glycosidase activity was induced dose-dependently, especially at the distal portion of the intestine (Table 4). Leptin is an increasingly convincing candidate in the central regulation of energy homeostasis, and an inhibitory effect of leptin on hypothalamic NPY neurons has been confirmed by other studies (Stephens et al, 1995; Schwartz et al, 1996). A substantial body of evidence now suggests that leptin levels parallel with body fat mass. This relationship would be anticipated if leptin and the NPY neurons operate in a negative-feedback circuit to regulate body fat stores. In this study, leptin and NPY mRNA expression levels were reciprocal in L5 and L50 groups compared with the control group, because mRNA level of leptin influenced NPY's mRNA level negatively. Increased mRNA level of NPY reflected an increase in daily food intake at the first and fourth week of acarbose treatment in L50 group.

In conclusion, the present study demonstrates for the first time that *ob* and NPY gene expressions were modified in adipose and brain tissues in mice on a high-carbohydrate diet containing acarbose. This study may suggest that acarbose may be of use in reducing the development of obesity induced by a high-carbohydrate diet.

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