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EFFECT OF HIGH-FAT DIET ON GENE EXPRESSION OF GLUT4 AND INSULIN RECEPTOR IN SOLEUS MUSCLE

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SUMMARY : Gene expression of GLUT4 and insulin receptor in soleus muscle of high-fat and high-carbohydrate diet fed rats was studied by measuring mRNA. High-fat diet feeding increased plasma glucose but decreased plasma insulin level. Glucose uptake in soleus muscle measured by 2-deoxy-D-glucose technique was lower in high-fat than high-carbohydrate diet fed rats. GLUT4 mRNA level in soleus muscle was significantly decreased but insulin receptor mRNA was similar in high-fat compared with high-carbohydrate diet fed rats. Insulin receptor exists in two forms generated by alternative splicing of a primary gene transcript. There was no difference in the relative expression of insulin receptor isoform mRNAs between high-fat and high-carbohydrate diet fed rats. These results suggest that high-fat diet impairs glucose metabolism in muscle by reducing transcription of GLUT4 without affecting gene expression of insulin receptor.

High-fat diet impairs glucose metabolism in skeletal muscle, which is the major site of insulin-stimulated glucose disposal (1-3). Although the molecular basis for the high-fat diet induced impairment in glucose metabolism in skeletal muscle remains unknown, it is conceivable that high-fat diet modulates insulin receptor and glucose transporter in skeletal muscle. High-fat diet feeding, which was so severe to stunt growth rate of rats, decreased mRNA of GLUT4 in skeletal muscle (4). It is not known if high-fat diet also affects gene expression of insulin receptor. Recent reports showed that insulin receptor exists in two isoforms generated by alternative splicing of a primary gene transcript. The two forms are possess distinct functional properties and are expressed in a tissue-specific fashion (5-7).

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The present studies were designed to examine the effect of high-fat diet on : 1) GLUT4 and insulin receptor gene expression and 2) alternative splicing of insulin receptor RNA in rats.

MATERIALS AND METHODS

Animal care. Forty male Spraque-Dawley rats (4 wk old) were obtained from CLEA Japan (Tokyo). Rats were isoenergetically meal-fed a high-fat diet (35% carbohydrate, 40% fat, 25% protein; caloric percentage) or a high-carbohydrate diet (70% carbohydrate, 5% fat, 25% protein) (8) at 0830-0930h and 2030-2130h for 10 wk . The animals were housed at 23 °C with light from 0700 to 1900h. On the final day of the experiment, rats were killed by decapitation at 2230h. The soleus muscle was rapidly removed, frozen in liquid nitrogen, and stored at -70°C until total RNA extraction.

Glucose uptake estimation. A nonmetabolizable glucose analogue 2-[^{3}H]deoxy-D-glucose (50μCi) (Amersham) was administered and plasma samples were obtained at 2, 5, 10, 15, 20, 30, and 45 min after the administration to determine glucose and tracer concentrations (9). An estimate of glucose uptake in soleus muscle was calculated from accumulation of phosphorylated 2-[^{3}H]deoxy-D-glucose in soleus muscle and plasma level of tracer (10).

RNA hybridization analysis. Total RNA was extracted from soleus muscle by guanidinium thiocynate method (11). For Northern blot analysis of GLUT4 and insulin receptor mRNA, RNA samples (40μg) were denatured, elctrophoresed in agarose gel containing formaldehyde, and transferred to Hybond-N$^+$ membranes (Amersham). For quantification of GLUT4 and insulin receptor mRNA, RNA samples (10μg) were spotted to Hybond-N$^+$ membranes by using a slot blot apparatus. The GLUT4 cDNA probe was kindly provided by Dr. G.Bell (12), and insulin receptor cDNA (3671-4419 bp) was amplified by PCR from a H4TG cDNA library (13) with primers corresponding to the coding regions of the published rat sequence (14). The membranes were hybridized with α-[^{32}P]dCTP (Amersham) -labeled probes using standard protocol (15) and exposed to a image plate (Fujix, Fuji Film, Japan). Image plate time was kept within a linear range. The amounts of mRNA in each samples were quantitated by a laser image analyzer (Fujix, BAS2000, Fuji Film) (16), and were normalized to that of ß-actin mRNA.

cDNA synthesis and PCR. First-stand cDNA synthesis was performed on 2μg total RNA using oligo(dT) primer as described by manufacturer's instructions (BRL SuperScript Kit, Life Technologies). Oligonucleotide primers spanning nucleotides 2474-2493 (sense primer) and 2724-2743 (antisense primer) of the rat insulin receptor were synthesized. The nucleotide numbering is according to Goldstein et al. (14). These primers are complementary to sequences that flank the region of the rat insulin receptor cDNA encoding exon 11 and yield fragments of 270 (Exon 11+) and 234 (Exon 11-) basepairs after PCR amplification. Before PCR amplification, the primers were labeled with γ-[^{32}P]ATP (Amersham) by using T4 polynucleotide kinase (Takara, Japan). PCR

reactions were carried out as described (17). Initial template denaturation was performed at 94 °C for 6 min followed by 25 cycles consisting of 1 min at 94 °C, 2 min at 58 °C, and 3 min at 72 °C. PCR products electrophoresed on 7.5% polyacrylamide gel and quantitated by a laser image analyzer.

Insulin, glucose, free fatty acid, triacylglycerol and total cholesterol determination. Plasma insulin concentration was determined by enzyme immunoassay (Sanko-Junyaku, Japan) and glucose, free fatty acid, triacylglycerol and total cholesterol concentration were analyzed enzymatically (Wako Pure Chemical Industries, Japan).

Statistical analysis. Data are expressed as mean ± SE. Statistical analysis was performed using Student's t test .

RESULTS

Basic profiles of rats. Characteristics of the rats from the two diet groups at 14 weeks of age are given in Table 1. After the 10 weeks of experimental period, there was no significant difference in body weight gain between high-carbohydrate and high-fat diet fed rats. When measured 1 hr after the meal, plasma glucose concentration was higher ($P<0.001$), whereas insulin concentration was lower ($P<0.05$) in high-fat than the high-carbohydrate diet fed rats (Table 1). Plasma free fatty acid, triacyglycerol and total cholesterol concentrations were significantly higher ($P<0.001$) in high-fat than high-carbohydrate diet fed rats. There was no significant difference in the weight of soleus muscle and its RNA content between high-carbohydrate and high-fat diet fed rats.

Table 1

Basic profiles of high-carbohydrate and high-fat diet fed rats for 10 wk

	High-carbohydrate (n=10)	High-fat (n=10)
Weight gain (g)	332 ± 5	334 ± 4
Plasma glucose (mg/dL)	122 ± 3	142 ± 4§
Plasma insulin (μU/mL)	57 ± 6	35 ± 6*
Plasma free fatty acid (μmol/L)	144 ± 17	624 ± 60§
Plasma triacylglycerol (mg/dL)	116 ± 5	141 ± 4§
Plasma total cholesterol (mg/dL)	59 ± 3	80 ± 2§

Data are means ± SE.

n: Number of rats.

*$P<0.05$, §$P<0.001$ vs high-carbohydrate diet fed rats.

Table 2
Soleus muscle characteristics of high-carbohydrate or high-fat diet fed rats for 10 wk

	High-carbohydrate (n=10)	High-fat (n=10)
Soleus muscle weight (mg)	309 ± 13	324 ± 10
Glucose uptake (μmole/100g/min)	27 ± 5	12 ± 2†
RNA (mg/g)	1.46 ± 0.07	1.56 ± 0.02

Data are means ± SE.
n: Number of rats.
†P<0.01 vs high-carbohydrate diet fed rats.

Glucose uptake of soleus muscle assessed by 2-deoxy-D-glucose method was decreased (P<0.01) in high-fat compared with high-carbohydrate diet fed rats (Table 2).

GLUT4 and insulin receptor mRNA levels in soleus muscle. GLUT4 and insulin receptor mRNA levels were measured using Northern blot and slot blot analysis in the soleus muscles of the high-carbohydrate and high-fat diet fed rats. GLUT4 mRNA was detected in a single band of 2.8 kb and insulin receptor mRNA was observed as two bands of 9.6 kb and 7.4 kb (Figure 1), as previously reported (18, 19). The level of GLUT4 mRNA was decreased (P<0.05) in high-fat compared with high-carbohydrate diet fed rats. High-fat diet feeding did not affect insulin receptor mRNA level (Table 3).

Relative expression of insulin receptor mRNA isoforms in soleus muscle. Two specific PCR products (270 and 234 bp) were present, showing the two insulin

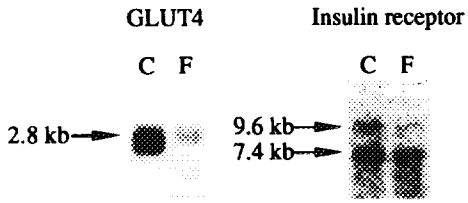

Figure 1. Northern blot analysis of GLUT4 and insulin receptor mRNA levels in soleus muscle from high-carbohydrate (C) and high-fat (F) diet fed rats. Forty μg of total RNA in soleus muscle was pooled and analyzed by Northern blotting.

Table 3
GLUT4 and insulin receptor mRNA in soleus muscle

	High-carbohydrate (n=10)	High-fat (n=10)
GLUT4 mRNA (arbitrary units)	33.5 ± 3.9	16.9 ± 0.3§
Insulin receptor (arbitrary units)	42.5 ± 0.9	42.3 ± 1.3
Relative expression of insulin receptor mRNA isoforms		
Exon 11- (%)	94.6 ± 0.4	93.5 ± 1.9
Exon 11+ (%)	5.4 ± 0.4	6.5 ± 1.9

Data are means ± SE.
n: Number of rats.
§P<0.001 vs high-carbohydrate diet fed rats.

receptor mRNA isoforms. The amounts of both PCR products increased exponentially until 26th PCR cycles (Figure 2). Therefore, insulin receptor mRNA isoforms were measured in PCR products after 25 cycles. There was no difference in the relative expression of the insulin receptor mRNA isoforms between high-carbohydrate and high-fat diet fed rats (Table 3).

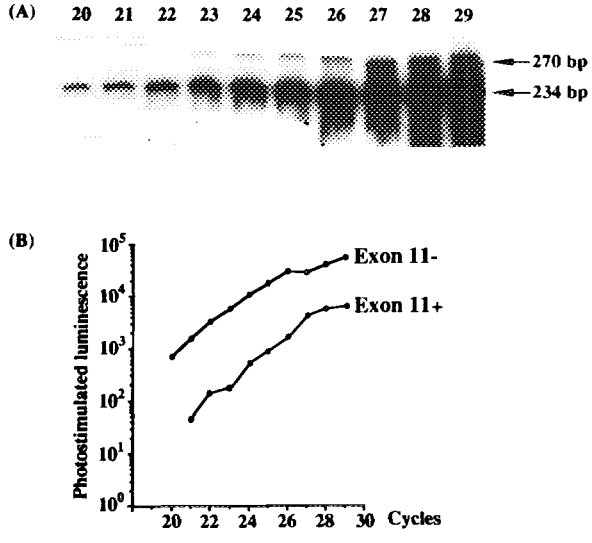

Figure 2. Optimization of PCR conditions. **A.** PCR products amplified with increasing number of PCR cycles. At the top, the numbers of amplification cycles are indicated, and the length of PCR products specific for Exon 11+ and Exon 11- are indicated to the right. **B.** The intensity of photostimulated luminescence (PSL) of each band in A was measured by a laser image analyzer and plotted on a logarithmic scale against the cycle number.

DISCUSSION

A high level of dietary fat intake impairs glucose metabolism in skeletal muscle (1-3). To elucidate the molecular mechanism of the effect of high-fat diet feeding on glucose metabolism, we studied gene expression of GLUT4 and insulin receptor in soleus muscle.

GLUT4 mRNA was decreased in high-fat compared with high-carbohydrate diet fed rats. Consistent with our findings obtained by using a modest level of high-fat diet (40% of energy), Kahn and Pedersen (4) reported that GLUT4 mRNA was reduced by very high-fat diet (80% of energy). Similarly Leturque et al. (20) observed a decreased GLUT4 mRNA level in skeletal muscle of weaning rats fed very high-fat diet (70% of energy). Effect of high-fat diet to decrease GLUT4 mRNA was associated with an increase in plasma glucose level in our experiment but not in others (4, 20). On the other hand, high-fat diet decreased plasma insulin level in our as well as the other reports, suggesting that high-fat diet decreases GLUT4 mRNA level in skeletal muscle via suppression of plasma insulin level.

Insulin receptor number in skeletal muscle is decreased by high-fat diet feeding without change in receptor affinity (21, 22). High-fat diet feeding did not affect insulin receptor mRNA level in soleus muscle, suggesting control at post-transcriptional level. The two spliced isoforms of insulin receptor, with exon 11 (Exon 11+) and without exon 11 (Exon 11-), are generated by alternative splicing of the 36 base-pair exon 11 (5-7). The expression of insulin receptor isoforms in rat skeletal muscle is not consistent with that of previous observations in human skeletal muscle (23, 24). The mechanism responsible for this species difference in the relative expression of insulin receptor isoforms remains to be identified. Recent studies suggest genetic (25) and hormonal (26) regulation of relative expression of the insulin receptor isoforms. We therefore examined the effect of nutritional status on insulin receptor isoforms. The relative expression of the insulin receptor mRNA isoforms was not affected by high-fat diet feeding. Thus, the level and splicing of insulin receptor RNA were not affected by high-fat diet feeding.

In conclusion, high-fat diet impairs glucose metabolism in muscle without affecting gene expression of insulin receptor but related to reduction in transcription of GLUT4.

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