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INCREASED GLUCOSE TRANSPORTER (GLUT4) PROTEIN EXPRESSION IN HYPERTHYROIDISM

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SUMMARY: We have studied skeletal muscle glucose uptake by perfused hindquarter preparations from rats treated with thyroxine. Basal glucose uptake (in the absence of insulin) was approximately 2 fold higher in muscle of hyperthyroid rats compared to controls. Insulin (10^{-7}M) stimulated glucose uptake 4.0 and 6.8 fold in the 10 day and 30 day controls rats, respectively. Maximal glucose uptake (10^{-7}M insulin) was not different in control and hyperthyroid rats and thus insulin responsiveness in the hyperthyroid animals was reduced to 2.5 fold stimulation. The abundance of the insulin-sensitive glucose transporter protein (muscle/fat, GLUT-4), measured by Western blot analysis using polyclonal antisera, was higher in skeletal muscle from both groups of hyperthyroid rats. These studies indicate that thyroid hormones increase basal glucose uptake in skeletal muscle and this is due, at least in part, to an increment of GLUT-4 isoform. Increased expression of muscle glucose transporter proteins may be responsible for the increased peripheral glucose utilization seen in hyperthyroidism.

Tissue metabolic activity is enhanced by thyroid hormones (1) and high requirements of glucose have been observed both in human and in experimental hyperthyroidism (2-5). An increment of glucose uptake has been reported in cultured cells treated with thyroid hormones (6-8), in human-induced hyperthyroidism and spontaneous hyperthyroidism using the forearm glucose uptake (9,10) and in hindlimb from hyperthyroid rats (11). Glucose transport is mediated by a family of glucose transporter isoforms with distinct structure, function and tissue distribution (12-14). Since skeletal muscle is the major site of peripheral

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insulin stimulated glucose disposal, the aim of the present study was to investigate muscle glucose uptake and the abundance of the insulin sensitive glucose transporter (muscle/fat, GLUT-4) in muscle. In addition, the time-related effect of thyroid hormones on skeletal muscle glucose uptake and GLUT-4 content was investigated.

MATERIALS AND METHODS

Experimental animals: Male wistar rats (200-230 g body weight) were treated by intraperitoneal injection of L-thyroxine (375μg/kg BW/day), for 10 (HT10) or 30 days (HT30). Control rats were injected with saline during the same periods (C10 and C30).

Hindquarter perfusion: Hindquarter preparations were perfused as previously described (15). The hemicorpora of treated and control rats were perfused with Krebs-Henseleit solution containing 5.5 mM glucose, 0.15 mM pyruvate, 4% (w/v) bovine serum albumin and 30% washed bovine erythrocytes. The hindquarters were perfused for a total of 100 min: 40 min without insulin, 30 min with 10^{-9}M insulin and 30 min with 10^{-7}M insulin. When 10^{-7}M insulin was added, glucose was also added to the perfusion medium to get the initial concentration of 5.5 mM. Samples were taken every 5 min for glucose assay and calculation of the glucose uptake. After the perfusion, the gastrocnemius muscles were quickly removed and frozen in liquid nitrogen.

Analysis of glucose transporter protein: Frozen muscles were pulverized and homogenized (1/6, w/v) using a Polytron homogenizer at setting of 6 for 15 sec in a buffer containing 25 mM HEPES (pH 7.4), 4 mM EDTA, 25 mM benzamidine, 1% Triton X-100 and 1 μM each of leupeptin, pepstatin and aprotinin. The homogenate was incubated for 90 min at 4°C with occasional mixing, and then centrifuged for 2 min in a microfuge. For Western analysis, an aliquot of the supernatant (50μg protein) was mixed with 50 μl Laemmli's sample buffer containing 5% dithiothreitol, brought to a total volume of 100 μl with 25 mM Tris/0.19 M glycine (pH 8.3) containing 1% SDS, and left overnight at 4°C. Proteins were separated by SDS polyacrylamide gel electrophoresis on 8% resolving gel using Laemmli's method (16), and transferred to Immobilon membrane by electrotransfer. The membrane was blocked for 2 hr with 5% Carnation Low-fat Instant Milk in Tris buffered saline (TBS), followed by incubation with the antibody [polyclonal antisera ECU-4 raised in rabbits against the carboxyterminal peptide of GLUT4 as described by James et al (17)]. After 16 hr incubation the membrane was washed alternatively in TBS and TBS-0.05% Tween and probed overnight with ^{125}I-goat anti-rabbit IgG. Autoradiography was carried out for 48 hr at -70°C, and the resulting autoradiograph of the band corresponding to the GLUT-4 (45 kd) analyzed by densitometry. The results are expressed relative to an standard control (rat heart membranes) run on each gel.

For dot blot analysis of glucose transport protein, samples of supernate containing 20μg protein were applied to Immobilon

membrane in a dot blot template. The membrane was blocked and incubated in primary and secondary antibodies as described for the Western blots. The absorbance of the autoradiograph was determined on a Titerteck Multiskan MCC/340 plate reader at 550 nm.

Statistical analysis: Student's T-test was performed to compare control and hyperthyroid rats, and significance was accepted at $p<0.05$. All values are expressed as mean±SEM.

RESULTS

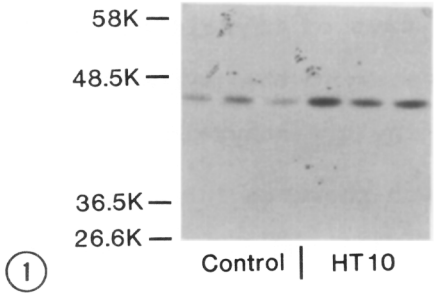
In Table 1 are shown the data on glucose uptake by the perfused hindquarters from control and hyperthyroid rats. Basal glucose uptake was approximately 2 fold higher in both HT10 and HT30 than in the corresponding controls. Although glucose uptake tended to be higher in HT10 compared to C10 at 10^{-9}M insulin, the difference was not statistically significant. There were no differences in maximal insulin stimulated glucose uptake between control and hyperthyroid rats. Consequently the fold stimulation by insulin was only about half as high in hyperthyroid as in controls (Table 1).

To study the effect of thyroid hormones on the insulin sensitive glucose transporter (GLUT4) Western blot analyses were performed using a polyclonal antisera to the carboxyterminus of GLUT4. Fig. 1 shows the 45 kd band corresponding to GLUT4,

Table I. Glucose uptake by perfused hindquarters from control and hyperthyroid rats

	Insulin Concentrations			
	0	10^{-9}M	10^{-7}M	Fold Stimulation
C10 (n=10)	2.3±0.5	4.4±0.8	9.3±1.0	4.0
HT10 (n=10)	3.6±0.3*	6.0±1.0	9.5±0.6	2.6
C30 (n=10)	1.5±0.4	7.4±1.0	10.3±0.8	6.8
HT30 (n=10)	3.6±0.4*	7.4±0.7	8.9±0.5	2.5

Values are expressed as μmol glucose/gm/hr. (Mean±SEM).
* $P<0.05$ vs control.



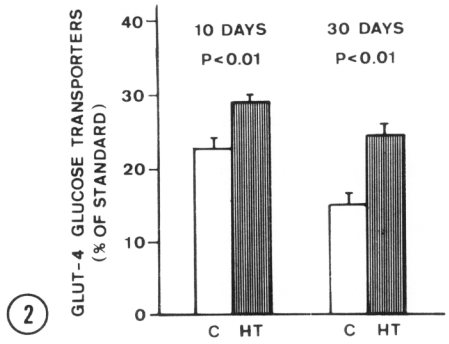


Figure 1. Representative Western blot of glucose transporter (GLUT4) protein from skeletal muscle. 50 μg protein from muscle of control rats and hyperthyroid rats were electrophoresed using SDS-PAGE. Proteins were transferred to Immobilon membrane and immuno-blotted with polyclonal antiserum ECU-4 as described in Materials and Methods.

Figure 2. Quantitation of GLUT4 in skeletal muscle of control and hyperthyroid rats (10 days treated: C10 and HT10, and 30 days treated: C30 and HT30). GLUT4 protein was quantitated using polyclonal antibody ECU-4 by dot blot analysis as described in the Methods.

obtained by autoradiography, from the gastrocnemius muscle of 3 control and 3 hyperthyroid rats. From the Western blot it was clear that hyperthyroidism increased the level of glucose transporter and densitometry of the autoradiographs confirmed this observation (C10, 114±15; HT10, 161±19; C30, 109±8; HT30, 175±52 expressed as a percent of rat heart membrane standards for 3 observation/group). Since the Western blot gave only one band, GLUT4 protein was quantitated on 10 samples per group using a dot blot assay. The absorbance of autoradiographs of the dot blots was linear with the amount of protein applied. The data in Fig. 2 confirm the observation made by Western analysis and the amount of GLUT4 protein was statistically higher ($p<0.05$) in muscle of hyperthyroid rats at both 10 and 30 days of treatment.

DISCUSSION

Our study demonstrated that thyroxine increases basal glucose uptake in skeletal muscle in both 10 and 30 days treated rats. After 10 days of treatment there appeared to be a small

increase in glucose uptake at submaximal insulin but this response was completely lost after 30 days of thyroid administration. In fact, after 30 days of treatment the maximal rate of glucose uptake tended to be lower in the hyperthyroid group.

Thyroid hormone treatment has been shown to increase glucose uptake and utilization in humans (3,9-11) and in cultured cells (6-8). The observation that basal glucose uptake was increased in muscle of hyperthyroid rats seems to be consistent with the effects seen in humans (3,9-11). The insulin resistance that we observed may relate to the degree of hyperthyroidism and in milder forms there may be enhanced submaximal insulin stimulation to account for the greater glucose utilization observed in humans.

Skeletal muscle expresses 2 isoforms of the glucose transporter (GLUT1 and GLUT4) with GLUT4 being quantitatively the predominant form. Because of the importance of glucose transport in the regulation of glucose metabolism in muscle, the factors which regulate the expression of GLUT4 have been of great interest (18). To our knowledge, this is the first report demonstrating that thyroid hormone may regulate the expression of GLUT4 in muscle. Recently, Weinstein et al (8) reported that T_3 increased abundance of GLUT1 protein and mRNA levels in a liver derived cell line (ARL 15). It would be interesting to know whether GLUT1 is also induced by thyroid hormones in skeletal muscle but our methods are not yet sensitive enough to confidently measure GLUT1 protein.

One question raised by these results is whether thyroid hormones play a role in regulation of GLUT4 levels under normal physiological conditions. Charron and Kahn (19) found that expression of both GLUT1 and GLUT4 in muscle increased with fasting. However, it has been shown that T_3 levels fall in

starvation (20) suggesting that thyroid hormones do not regulate GLUT4 expression during fasting.

In summary, our data indicated that thyroid hormones increase basal glucose uptake in skeletal muscle and this is due at least in part, to an increase in GLUT4 isoform. An increment of muscle glucose transporter proteins may be responsible for the high peripheral glucose utilization seen in hyperthyroidism.

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