GLUT4 gene expression and GLUT4 protein levels in muscle of high sucrose-fed rats: effect of dietary fish oil

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High sucrose feeding is known to induce the symptoms of so-called metabolic X syndrome (e.g., hypertriglyceridemia, hyperinsulinemia, insulin resistance, and hypertension) in rats. To elucidate whether the latter may also involve an alteration of the glucose transport in skeletal muscle, we determined GLUT4 gene expression and GLUT4 protein levels in muscle quadriceps femoris of Wistar rats fed ad libitum a basal or high (63 cal%) sucrose diet for 21 days. To test a possible beneficial effect of dietary omega-3 polyunsaturated fatty acids on the above parameters, additional groups of rats were fed the diets supplemented with marine fish oil (30 wt% omega-3 polyunsaturated fatty acids). A twofold increase in GLUT4 gene expression (dot blot analysis) was observed in the muscle of rats fed the high sucrose diet. Dietary fish oil reduced the GLUT4 gene expression in high sucrose-fed animals by about 50% of the values obtained in rats fed the basal diet plus fish oil. Western blot analysis of total muscle membrane fractions using specific antibodies against GLUT4 revealed no changes in the transporter levels in high sucrose-fed rats, and even a decrease in both, with fish oil-supplemented groups of animals. These data suggest that feeding high sucrose diet to rats is associated with an uncoupling between muscle GLUT4 mRNA and protein levels. On the other hand, it is notable that the decrease of GLUT4 protein found in rats fed the fish oil-supplemented diets was more pronounced in the group of animals fed the high sucrose diet. (J. Nutr. Biochem. 5:389–396, 1994.)

Keywords: GLUT4 gene expression; GLUT4 protein level; skeletal muscle; sucrose diet; dietary fish oil; rat

Introduction

Deleterious effects of raised sucrose (and/or fructose) intake on carbohydrate and lipid metabolism and insulin action have been known for more than two decades.[1–3] In particular, a high sucrose diet is known to be associated with significant hypertriglyceridemia, hyperinsulinemia (at euglycemia), increased blood pressure, and insulin resistance, i.e., a complex of symptoms recently called the metabolic X syndrome.[4]

Insulin resistance might be caused by abnormalities of insulin signal transduction at any level of the signal transmitting chain in the target cell of the hormone[5] or directly at the level of major effector systems such as the glycogen synthase or the glucose transport system. In human Type 2 diabetes, insulin resistance controversial results were obtained on the glucose transporter system in the skeletal muscle.[6–9] However, in streptozotocin-induced diabetes, the defects at the level of glucose transporter in skeletal muscle were observed.[10–12]

The high fructose-diet-induced insulin resistance includes a decrease of 2-deoxyglucose transport into rat diaphragm muscle.[13] Similarly, a high sucrose diet is accompanied by significant insulin resistance at the level of the liver,[14,15] with additional impairment of insulin action in skeletal muscles.[15] The data obtained in other animal models of insulin resistance[10,11,16,17] show a predominant participation of skeletal muscle in that phenomenon.

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Thus, the aim of our study was to ascertain whether skeletal muscle of high sucrose-fed rats shows an alteration at the level of the glucose transporter, and therefore, the GLUT4 mRNA and the GLUT4 protein levels were determined. Raised dietary intake of omega-3 (n-3) polyunsaturated fatty acids (PUFA) has been repeatedly demonstrated[14,18–20] to have beneficial effects on several of the consequences of high sucrose feeding. Therefore, the skeletal muscle glucose transporter system was also analyzed in two additional groups of rats, i.e., fed either basal or high sucrose diet, and both supplemented with marine fish oil rich in n-3 PUFA.

Methods and materials

Animals and diets

All experiments reported were approved by the Institute of Experimental Endocrinology Animal House Ethics Committee. Adult male Wistar rats weighing 275 to 285 g, obtained from Velaz (Prague, Czech Republic), were housed by two wire-mesh cages in a temperature-controlled room (22 ± 2° C) on a 12 hr-light-dark cycle (lights off at 6:00 p.m.). Eight rats per group were fed ad libitum for 21 days various diets, which were prepared as indicated in *Table 1*. One of the following fat mixtures was added to 900 g of the basal diet or to 900 g of the high sucrose diet: 100 g beef tallow (Palma, Bratislava, Slovakia) or 100 g fish oil (Activepa 30 TG, Martens, Norway). Semipurified basal diet is commercially available (Velaz) (protein:lipid:carbohydrates = 26:13:61 cal%, originating from wheat bran 50 wt%, oats bran 17 wt%, casein 15 wt%, dry milk 13 wt%, vitamins and biofactors 5 wt%). High sucrose diet (protein:lipid:carbohydrates = 25:12:63 cal%) was prepared from casein 12.5 wt%, dry milk 9 wt%, lucerne 3 wt%, dry yeast powder 9 wt%, sucrose 63 wt%, mineral and vitamin mix 3.5 wt%. The fatty acid composition of the diets used is shown in *Table 2*. Lipids were extracted from the diet by the method of Folch et al.[21] The methyl esters of fatty acids were prepared (after alkaline hydrolysis of lipids) by esterification with diazomethane and analyzed by gas-liquid chromatography using an HRGC 4160 apparatus (Carlo Erba, Italy) equipped with a flame detector. A glass capillary column with stationary phase SP 2340 was used. The details of the gas chromatographic analysis were reported elsewhere.[22] The results were quantified with an SP 4000 integrator (Spectra Physics, Germany).

The diets were prepared fresh every 3 to 4 days and stored at 4° C under a nitrogen atmosphere. The aliquot daily amount of the diets was offered in small glass dishes shortly before the beginning of the dark period. Body weight was recorded at the beginning of the study and after 21 days of the dietary treatment. Using the above approach, the rats were randomly divided into four groups that were fed four different diets: basal (B), basal + fish oil (B + FO), high sucrose (HS), and high sucrose + fish oil (HS + FO).

Table 1 Composition of experimental diets

Diet	B	B + FO	HS	HS + FO
Commerical basal diet	900*	900	—	—
Semipurified sucrose diet	—	—	900	900
Beef tallow	100	—	100	—
Fish oil	—	100	—	100

*g/kg diet. Energy content of 100 g diet is about 350 calories.

Table 2 Fatty acid composition of experimental diets (wt %)

Diet	B	B + FO	HS	HS + FO
14:0	3.6	7.0	4.0	8.1
16:0	24.5	19.5	25.5	19.9
16:1	2.1	8.6	3.5	8.9
18:0	15.6	6.0	16.9	6.4
18:1 (9)	29.2	14.0	32.0	14.6
18:1 (7)	1.6	2.9	1.7	3.1
18:2 (6)	18.1	9.3	7.6	2.3
18:3 (3)	2.1	0.9	0.9	0.8
20:4 (6)	0.1	0.6	0.1	0.6
20:5 (3)	0.2	16.9	0.2	16.9
22:5 (3)	—	1.7	—	1.9
22:6 (3)	—	11.1	—	11.3
Others	2.9	1.5	7.6	5.2
Total				
SFA	43.7	32.5	46.4	34.4
MUFA	32.9	25.5	37.2	26.6
Omega-6 PUFA	18.2	9.9	7.7	2.9
Omega-3 PUFA	2.3	30.6	1.1	30.9

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Routine biochemical analyses

Blood was obtained after cervical dislocation of rats fasted overnight (16 hr). The aliquots of obtained serum were stored for assays of insulin (RIA kit from Opidi, Poland), glucose (glucose-oxidase kit, Lachema, Brno, Czech Republic), triglycerides (Lachema). Skeletal muscle (muscle quadriceps femoris) was immediately removed, frozen, and subsequently used for determination of tissue triglyceride content, analysis of GLUT4 gene expression, and GLUT4 protein levels. Skeletal muscle triglycerides were determined using an enzymatic method (Lachema) after tissue homogenization in 10 mmol l^{-1} TRIS-HCl, pH 7.4, containing 0.9% sodium chloride. Tissue proteins were determined according to the modification of the Bio-Rad Laboratories method[23] using bovine serum albumin (BSA) (Sigma, St. Louis, MO USA) as standard.

Analysis of GLUT4 gene expression

GLUT4 gene expression was estimated using the Northern dot blot technique. Total RNA from muscle quadriceps femoris was extracted by the guanidium isothiocyanate using the phenol/chloroform method.[24] The quantity and purity of RNA was determined by absorbance at 260 and 280 nm. After isolation, 20 μg of RNA was transferred to hybond N^+ membrane using a dot blot apparatus (the quantitative linearity of this method was present in our conditions between 10 and 80 μg of mRNA per dot). The blots were subsequently hybridized with digoxigenin-labeled dUTP probes for GLUT4 and Human Elongation Factor 1 (HEF-1). The cDNA probe for GLUT4 was a 2.95 kB plasmid p-bluescript/Eco RI fragment corresponding to a region of the gene encoding the adipose/muscle glucose transporter obtained from Dr. D.E. James[25] (Washington University, St. Louis MO USA). The cDNA probe for HEF-1 was a plasmid pBR 322/Eco RI fragment having the sequence for HEF-1 given to us by Dr. T. Maassen[26] (Sylvius Laboratories, Leiden, The Netherlands). Labeling of the cDNA probes, hybridization of labeled cDNA to homologous RNA, and detection of hybrids by enzyme immunoassay with an antidigoxigenin-antibody conjugate was performed using a nonradioactive DNA labeling and detection kit from Boehringer Mannheim (Germany, Cat. No. 1093 657). Prior to prehybridization and hybridization with a new cDNA probe, the previously bound probe was

removed by immersing the filters in boiling water for 1 min. The blots obtained were then quantitatively evaluated by scanning densitometry using an LKB densitometer (Sweden). Peak heights were used as relative values for mRNA concentrations.

Analysis of the GLUT4 protein

Total membrane preparation. For the preparation of total membrane (TM) fraction from muscle quadriceps femoris, a modification of Kahn's[27] method was used. Muscle homogenate in buffer A (250 mM sucrose, 20 mM HEPES, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 mM leupeptin, pH 7.4) was centrifuged at 1,200 g_{max} and 9,000 g_{max} for 10 min, followed by 200,000 g_{max} centrifugation for 80 min. Protein was determined on the final total membrane preparation according to a modification of the Bio-Rad Laboratories method[23] using BSA (Sigma) as standard. The final pellet (TM) was resuspended in water to approximate a concentration of 5 mg/mL and was used for the determination of glucose transporter concentration by Western blotting.

Western blotting. In this case a slightly modified procedure of Sugden et al.[28] was used. The proteins (20 μg/lane) from total membrane fraction in sodium dodecyl sulfate (SDS) sample buffer (containing 6 M urea and 100 mM dithiotreitol, 2% SDS Pierce) were resolved by SDS-polyacrylamide (12%) gel electrophoresis under denaturating conditions (200 V for 45 min) in TRIS/glycine/SDS buffer (25 mM/192 mM/0.5%; pH 8.3) using the Bio-Rad Mini Protean II system (Bio-Rad Laboratories, Richmond, CA USA). After electrophoresis, proteins were transferred in blotting buffer (25 mM TRIS, 192 mM glycine, 20% vol/vol methanol, pH 8.3; at 100 V for 1 hour) to nitrocellulose filters (Schleicher & Schuell, 0.45 μm pore size). The equivalence of blotting was confirmed by the absence of staining on the gel by Coomassie Brilliant Blue and identical staining of proteins on the nitrocellulose using Ponceau S. The filters were washed in TBST (250 mM NaCl, 20 mM TRIS, 0.2% Tween-20, pH 7.5) and blocked by using 2.5% nonfat dried milk powder (Elk, Campina B.V., The Netherlands) in TBST buffer for 1 hr. After three additional washes with TBST, the filters were incubated with anti-GLUT4 (2 μg/mL of TBST with 1% Elk) for 2 hr. The polyclonal antibody was raised in rabbits against synthetic peptide corresponding to residue 494–509 of rat GLUT4 and affinity purified by chromatography on columns of immobilized peptides.[29] After washing with TBST buffer, the filters were incubated with ^{125}I-labeled donkey anti-rabbit antibody (catalogue N° IM 134, Amersham, England; specific activity 740 kBq/μg) applied at a dilution of 1:500 (74 kBq/10 mL TBST with 1% Elk). The filters were then intensively washed; first with TBST containing 0.5% Elk, followed by three additional washes with TBST. The filters were radiographed (Fuji RX-80 film), and autoradiographies analyzed by scanning densitometry using an LKB ultrascan densitometer. Relative values for glucose transporter concentrations were derived from peak heights.

Statistical analysis

All data were expressed as mean values ± SEM. The effect of dietary supplements was evaluated by the analysis of variance (ANOVA) procedure, followed by Duncan's multiple range test,[30] which was used to test the significance of differences ($P < 0.05$) between the means.

Results

Body weight changes

Mean growth rate throughout the study did not differ significantly among the groups of rats subjected to different dietary regimens *(Table 3)*. However, a higher scatter in the body weight increment data was observed in the group of rats that had been fed the fish oil-supplemented sucrose diet.

Table 3 Effects of fish oil on body weight increment and daily food consumption in rats fed a high sucrose diet

Diet	Body weight increment [g]	Food consumption [g]
B	69 ± 15	20.9 ± 0.3
B + FO	62 ± 10	20.1 ± 0.9
HS	76 ± 17	21.1 ± 1.4
HS + FO	58 ± 27	20.5 ± 2.0

Given values represent the mean ± SEM.
B: basal diet-fed controls; B + FO: basal diet supplemented with fish oil; HS: high sucrose diet; HS + FO: high sucrose diet supplemented with fish oil.

Serum glucose, insulin and triglycerides

Table 4 summarizes the effects of dietary interventions used on serum glucose, insulin, and triglyceride levels. In the high sucrose group the fasting levels of triglycerides and insulin were significantly elevated, but glucose levels did not differ from the other groups. The addition of fish oil to

Table 4 Fasting serum glucose, insulin, and triglycerides and muscle (m. quadriceps) triglyceride content in rats fed basal or high sucrose diet supplemented with fish oil for 21 days

Diet	B	B + FO	HS	HS + FO
Glucose (mmol/L)	5.6 ± 0.1	5.5 ± 0.1	5.8 ± 0.3	5.4 ± 0.2
Insulin (μU/mL)	36.9 ± 2.2[a]	36.6 ± 1.9[a]	55.9 ± 3.8[b]	36.6 ± 1.9[a]
TG (serum) (mmol/L)	1.52 ± 0.13[a]	0.83 ± 0.06[b]	2.23 ± 0.42[c]	1.1 ± 0.05[b]
TG (muscle) (nmol/mg protein)	31.3 ± 2.3[a]	29.7 ± 2.4[a]	49.5 ± 2.7[b]	35.7 ± 3.0[a]

Values are mean ± SEM.
B: basal diet-fed controls; B + FO: basal diet supplemented with fish oil; HS: high sucrose diet; HS + FO: high sucrose diet supplemented with fish oil.
Values without a common superscript [a,b,c] are significantly different ($P < 0.05$).

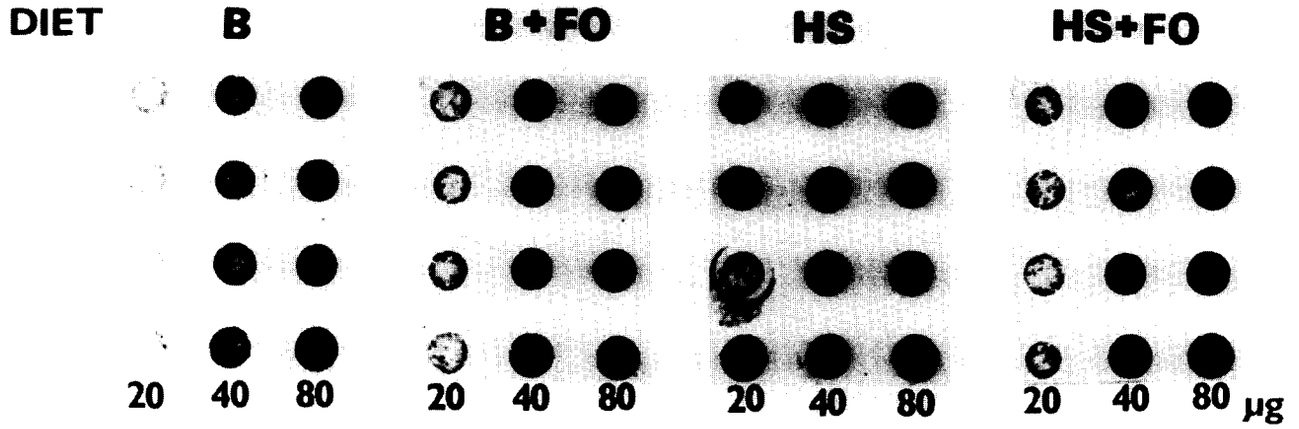

Figure 1 Dot blot analysis of GLUT4 gene expression of m. quadriceps femoris of rats fed various diets. GLUT4 mRNA was detected after hybridization with a cDNA probe[25] (details in *Methods and materials*). This is an example of a typical experiment. Relative values for GLUT4 mRNA levels (see *Figure 2*) were obtained as peak areas (mean ± SEM) from scanning densitometry. B: basal diet; B + FO: basal diet supplemented with fish oil; HS: high sucrose diet and HS + FO: high sucrose diet supplemented with fish oil.

the high sucrose diet prevented the increase of both the serum insulin and triglyceride levels, which did not differ from control values.

Tissue triglycerides

High sucrose diets resulted in a significant increase of triglyceride content in muscle quadriceps femoris (plus ≈63%) *(Table 4)*. Though the supplementation of basal diet with fish oil did not alter the muscle triglycerides, the addition of fish oil to high sucrose diets completely prevented the accumulation of triglycerides in the muscles.

GLUT4 gene expression

In the muscle of high sucrose fed rats, a twofold increase in the expression of the GLUT4 gene (i.e., GLUT4 mRNA levels per µg of total RNA) was observed *(Table 5)*. However, the addition of fish oil to the basal diet led also to a 1.5-fold increase of GLUT4 mRNA levels *(Figures 1 and 2)*. It is also notable that feeding the high sucrose diet supplemented with fish oil was accompanied by an approximately equal GLUT4 mRNA level, as seen in muscle of rats fed the fish oil-supplemented basal diet *(Figures 1 and 2)*.

To determine the specificity of muscle GLUT4 mRNA changes under various dietary regimens, the HEF-1 mRNA levels in the muscle were also investigated. However, they

Table 5 Individual data for GLUT4 gene expression in m. quadriceps femoris of the rats fed a basal or high sucrose diet without or with fish oil supplementation

Diet	B	B + FO	HS	HS + FO
	111.0	130.6	210.1	160.4
	101.4	144.0	197.3	140.0
	94.0	159.5	207.5	168.9
	94.0	150.0	194.0	201.3
Mean	100.0[a]	146.0[b]	202.2[c]	167.7[b]
± SEM	4.0	6.1	3.9	12.7

The results are expressed as percent of control (rats fed basal diet).
Values given are mean ± SEM of four rats.
Values without a common superscript [a,b,c] are significantly different ($P < 0.05$).

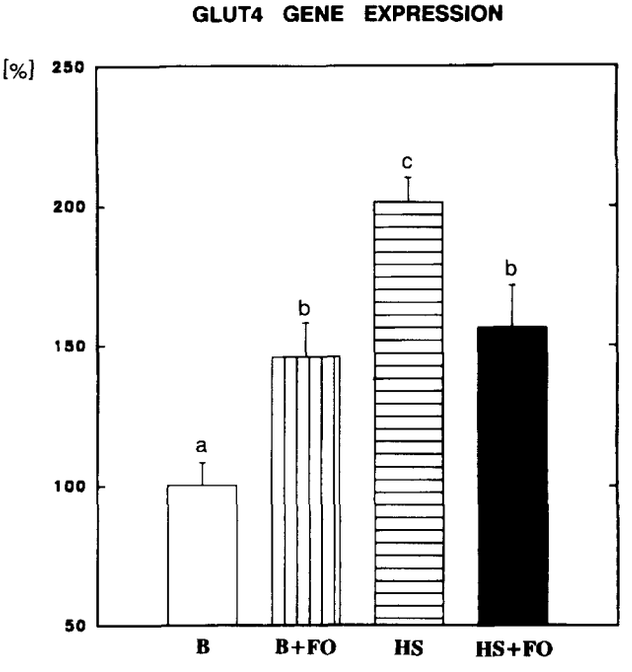

Figure 2 GLUT4 gene expression in m. quadriceps femoris of the rats fed basal or high sucrose diet without or with fish oil supplementation. The results are expressed as percent of control (rats fed basal diet). Bars represent mean ± SEM of four rats. [a,b,c] Values without a common superscript are significantly different ($P < 0.05$).

did not differ significantly among the groups of rats fed various diets *(Table 6)*.

GLUT4 mRNA levels correlated positively with muscle triglyceride content *(Figure 3)*. Moreover, muscle triglyceride content also correlated positively with fasting serum insulin levels (data not shown).

GLUT4 protein levels

In membranes from high sucrose-fed rats no change in density of the radioactive spot was detected *(Figure 4)*. GLUT4 protein levels were, however, decreased in the muscles of both groups of rats fed the fish oil-supplemented diets, i.e., either the basal (− 58%) or the high sucrose (− 85%) group *(Figure 5)*.

Table 6 Biochemical characteristics of m. quadriceps of rats fed basal or high sucrose diet supplemented with fish oil for 21 days

Diet	B	B + FO	HS	HS + FO
Membrane protein/g muscle [μg] n = 8	5588 ± 267	6175 ± 290	6013 ± 101	5688 ± 441
Total mRNA/g muscle [μg] n = 4	436 ± 33	511 ± 25	502 ± 36	473 ± 45
HEF mRNA [% of control] n = 4	100 ± 4	92 ± 11	105 ± 9	85 ± 10

Values are mean ± SEM.
B: basal diet-fed controls; B + FO: basal diet supplemented with fish oil; HS: high sucrose diet; HS + FO: high sucrose diet supplemented with fish oil.

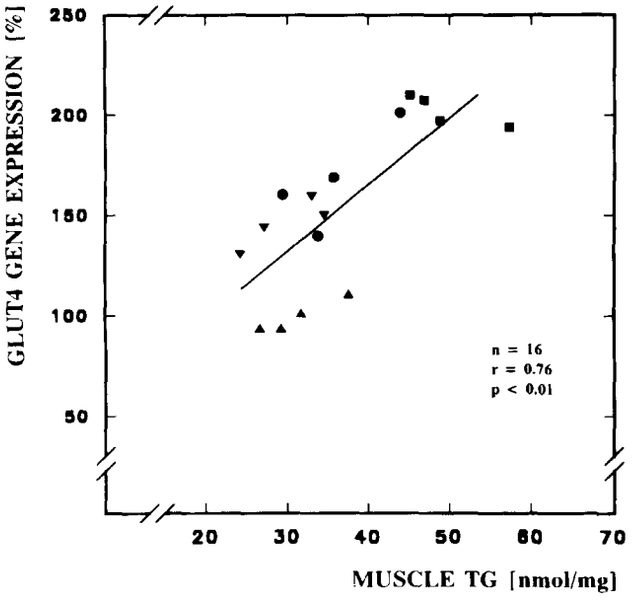

Figure 3 Correlation between the triglyceride content and GLUT4 gene expression in skeletal muscle of rats fed different fat supplemented diets. ▲: basal diet; ▼: basal diet supplemented with fish oil; ■: high sucrose diet; and ●: high sucrose diet supplemented with fish oil.

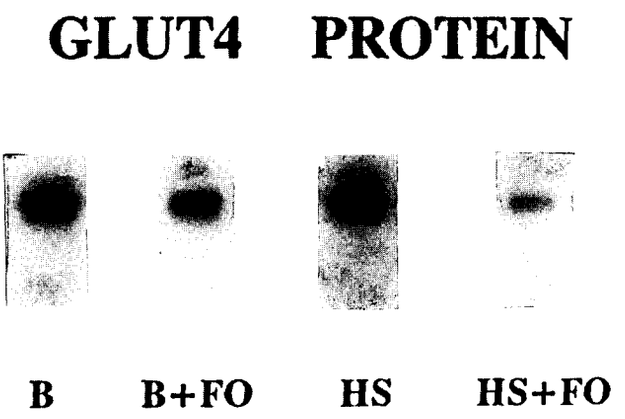

Figure 4 Western blot analysis of GLUT4 protein in total membrane fractions of m. quadriceps femoris of rats fed different diets. Total membrane proteins were subjected to SDS PAGE, transferred to nitrocellulose, and immunoblotted with a polyclonal antibody to GLUT4 protein[29] as described in Methods and materials. This is an example of a typical experiment. Relative values for GLUT4 protein levels (see *Figure 4*) were obtained as means (n = 8 per group) of peak heights from densitometric evaluation of radiographies. B: basal diet; B + FO: basal diet supplemented with fish oil; HS: high sucrose diet; and HS + FO: high sucrose diet supplemented with fish oil.

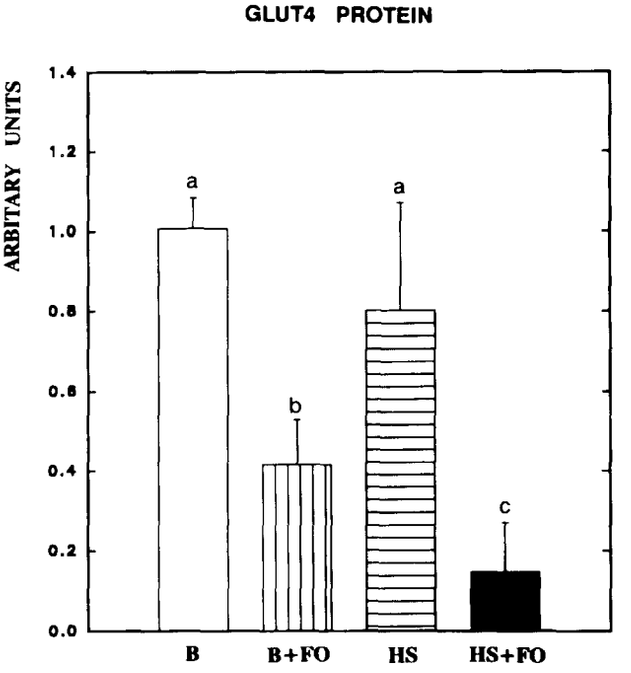

Figure 5 Effect of high sucrose diet and n-3 PUFA supplementation on GLUT4 protein levels in rat quadriceps femoris muscle. Bars represent mean ± SEM of eight rats. [a,b,c] Values without a common superscript are significantly different (P < 0.05).

Discussion

These results indicate that feeding rats the high sucrose diet results in a dissociation between transcriptional and translational events of the GLUT4 pathways of rat skeletal muscle (muscle quadriceps femoris).

High sucrose feeding is always accompanied by fasting hyperinsulinemia[2,18] at euglycemia. Because insulin deficiency results in a reduction of GLUT4 mRNA in the muscle, while insulin treatment reverses this defect,[31–33] the increase in GLUT4 mRNA in high sucrose diet fed rats could be, at least in part, the result of hyperinsulinemia. This idea is further supported by the fact that GLUT4 mRNA levels in the muscle of rats fed the sucrose diet with fish oil supplement decreased by approximately 50%. Moreover, we previously reported evidence, using the in vitro system of isolated islets of Langerhans, suggesting that dietary n-3 PUFA inhibit the insulin release from B cells.[34]

Nevertheless, the role of circulating plasma insulin levels should not be overemphasized. Leturque et al.[35] found low GLUT4 mRNA and protein levels in skeletal muscles of suckling rats while a twofold to threefold increase was observed after weaning when the rats were fed a high carbohydrate diet and were also hyperinsulinemic. However, if such rats were fed a high fat diet, the level of plasma insulin was 50% higher than that in suckling rats, but there was no increase in GLUT4 protein and mRNA in skeletal muscles. On the contrary, the infusion of insulin into normal rats continuously for 4 days via a minipump was accompanied by decreased in vivo insulin-induced glucose utilization in skeletal muscles and decreased GLUT4 mRNA in skeletal muscle.[36]

Besides the liver insulin resistance, the decrease of in vivo insulin action in the rat induced by sucrose feeding also involves insulin resistance in skeletal muscles.[15] Marine fish oil, mixed into the sucrose diet, improves insulin sensitivity in muscle tissues[19] at normal fasting insulinemia. It could be suggested, therefore, that the increase in muscle GLUT4 mRNA level in the sucrose diet-fed rats may not have resulted from impaired insulin action.

In any case, all of these results suggest that hyperinsulinemia per se may participate in divergent modifications of the response of skeletal muscle to insulin, which probably depend on the different hormonal/metabolic milieus specific for each physiological and/or pathological situation studied.

The increased GLUT4 mRNA levels in muscles should be directly related to GLUT4 protein levels.[37] In our recent studies, however, we did not find any change in GLUT4 protein levels in the muscle total membrane fraction of high sucrose-fed rats, which indicates a dissociation between transcriptional and translational events of the GLUT4 pathways in the experimental setting used.

Indeed, this interpretation should be qualified in view of other data suggesting that mRNA levels may not be always directly related to the levels of glucose transporter protein.[38–40] Thus, in normal rats infused for 72 hr with glucose, Hager et al.[39] found that GLUT4 mRNA abundance in muscles of hyperglycemic rats was 2.5-fold greater when compared with saline-infused controls, and yet the muscle GLUT4 protein levels did not change. It was concluded that the increase in GLUT4 mRNA could result from increased transcription or from increased stability of GLUT4 mRNA along with a partial block in GLUT4 mRNA translation. Alternatively, GLUT4 protein degradation might have been increased with increased transcription and translation. This would result in an increase in GLUT4 mRNA with no net change in GLUT4 protein abundance. In quadriceps muscle of spontaneously obese mice, Koranyi et al.[40] also did not find any changes of GLUT4 protein levels with reduced muscle GLUT4 gene expression and suggested that the transcription of GLUT4 gene may not be proportional to the steady-state level of protein in different tissues and/or different metabolic states.[25,40]

There are no doubts that the major regulation of GLUT4 (and hence muscle glucose uptake) by insulin involves the translocation of the protein from intracellular vesicles to the plasma membrane with the alteration of intrinsic activity of the glucose transporter,[37,41] rather than as an effect on total GLUT4 protein. In light of the above suggestion, it might be also obvious that the increased mRNA levels in muscle of rats fed a high sucrose diet could be a consequence of a less active protein, and thus a result rather than a cause of this model of insulin resistance.

There are also several important issues highlighted in this study by varying the type of fat while holding the total fat content of the diet unchanged. First, the substitution of beef tallow into a high sucrose diet with marine fish oil, rich in eicosapentaenoic and docosahexaenoic n-3 PUFA, seems to support a certain role of raised circulating insulin plasma levels for elevated GLUT4 mRNA levels in skeletal muscle of rats fed the sucrose diet (as n-3 PUFA inhibit insulin release[34]).

Second, it suggests that the increase of skeletal muscle GLUT4 mRNA in the high sucrose diet-fed rats may not result from insulin resistance as: (a) the levels of circulating plasma insulin and triglycerides and (b) the muscle triglyceride content returned to normal values by fish oil supplement, (c) the muscle GLUT4 mRNA levels remained increased by about 50% compared with basal diet-fed control rats, and (d) insulin action in fish oil-supplemented rats was improved.[19,42] Very similar conclusions were made in high fat diet-induced insulin resistance, where an extreme improvement of insulin action, as achieved by the substitution of fish oil in high fat diet, was not accompanied by any significant differences in GLUT4 mRNA levels assessed in several skeletal muscles.[43]

Third, the long-chain PUFA of the n-3 family seem to have their own pathway for regulating the GLUT4 transporter system in rat skeletal muscle. Replacing the beef tallow of the basal diet with an equivalent amount of marine fish oil led to an almost identical (approximately 1.5-fold) increase of GLUT4 mRNA levels in the quadriceps muscle as was obtained in the fish oil-substituted high sucrose diet group. Moreover, GLUT4 protein levels were decreased in muscles of both groups of rats fed the fish oil-supplemented diets, i.e., either in the basal (-58%) or the high sucrose (-85%) group. The marked decrease in GLUT4 protein seen with fish oil supplementation, which normalizes serum insulin levels and hence presumably rectifies the sucrose diet-induced insulin resistance, seems to indicate that the GLUT4 protein level may not be the most important parameter for insulin action in muscle. Nevertheless, further careful studies are required, as the decrease of muscle GLUT4 protein levels in both groups with raised dietary fish oil intake could be also understood as a negative consequence of such dietary manipulations as recently suggested (although under different conditions) by Ezaki et al.[44]

The present data show that the well-established model

of high sucrose diet-induced hypertriglyceridemia, hyperinsulinemia, and insulin resistance is accompanied by a divergence between GLUT4 mRNA and protein abundance in skeletal muscle of the rat. The results reported here also suggest that the fatty acid composition of food is one of the important regulators of the glucose transport system in rat skeletal muscle and may also have differential effects on the expression and functional activity of the GLUT4 in vivo.

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