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Decreased Glucose Transporter Protein (GLUT4) in Skeletal Muscle of Hypertriglyceridaemic Insulin-Resistant Rat

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Summary

Protein levels (Western blot) of the major glucose transporter isoform (GLUT4) were measured in skeletal muscles (quadriceps femoris) of an animal model of human metabolic syndrome X, i.e. the hereditary hypertriglyceridaemic (HTG) insulin-resistant rats fed various diets. The results were compared with the data obtained in normal Wistar rats which underwent the identical protocol. In HTG rats fed the basal diet (B) or high-sucrose diet (HS) (known to induce hypertriglyceridaemia and to impair insulin action), a decrease of GLUT4 protein levels (B: Control 100±3 vs HTG 46±5 %, $p<0.005$; HS: Control 80±9 vs HTG 49±3 %, $p<0.005$) was observed. Furthermore, marine fish oil (FO) rich in n-3 polyunsaturated fatty acids (PUFA), added to the basal diet (30 wt % of n-3 PUFA) reduced the GLUT4 protein levels (B: 100±3 vs B+FO: 42±4 %, $p<0.005$) in control rats to values similar to those found in HTG rats (B: 46±4 %). However, dietary FO did not have any effect in HTG rats (49±3 %). Feeding the high-sucrose diet supplemented with FO to both the control and HTG rats was followed by a further decrement of GLUT4 protein (Control 15±5 vs HTG 14±4 %). In conclusions, a) the hereditary HTG rats had by about 50 % lower GLUT4 protein levels in the quadriceps femoris muscle in comparison to normal Wistar rats; b) high-sucrose diet or raised dietary intake of n-3 PUFA did not further alter the number of glucose carriers in quadriceps femoris muscle in HTG rats and c) feeding the high-sucrose diet with higher proportion of n-3 PUFA was associated with an additional reduction of the GLUT4 protein level in this muscle.

Key words

GLUT4 protein levels – Skeletal muscle – Hereditary hypertriglyceridaemic rat – Sucrose diet – Fish oil

Introduction

The strain of Wistar rats with hereditary hypertriglyceridaemia, that has recently been developed in Vrána's laboratory in Prague, is characterized by a hyperproductive type of fasting hypertriglyceridaemia (Vrána and Kazdová 1990), mild impairment of glucose tolerance, slightly elevated insulinaemia (Klimeš *et al.* 1989), normal insulin signalling in the liver (Ficková *et al.* 1991), insulin resistance in white adipose tissue and skeletal muscles (Štolba *et al.* 1993) and elevated blood pressure (Štolba *et al.* 1992). Therefore, it may be considered as an interesting animal model of the known human metabolic X syndrome (Klimeš *et al.* 1994). Feeding these animals with a high-sucrose diet results in an exaggeration of hypertriglyceridaemia, and severe augmentation the other metabolic abnormalities (Klimeš *et al.* 1989). Nevertheless, some of them are to a certain degree correctable by raised dietary intake of polyunsaturated fatty acids (PUFA) of the omega-3 (n-3) series (Klimeš *et al.* 1993).

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It has been almost generally accepted that the predominant role for the pathogenesis of insulin resistance is played by the skeletal muscle, involving the glucose transporter system (Klip and Paquet 1990). In the present study, we therefore measured protein levels of the major glucose transporter isoform (i.e. GLUT4) in the quadriceps femoris muscle obtained from hereditary hypertriglyceridaemic rats fed either basal or high-sucrose diets with or without omega-3 (n-3) PUFA supplementation.

Materials and Methods

Animals and diets

Adult male hypertriglyceridaemic (HTG) Wistar rats with initial weight of 250–280 g were obtained from VELAZ (Prague, Czech Republic) and housed by two in wire-mesh cages at a temperature of 22±2 °C and under a light regimen (12 h light:dark cycle; lights off at 18.00 h) and fed *ad libitum* for 21 days various diets prepared in our laboratory. One of the following fat mixtures was added to 900 g of basal diet or to 900 g of high-sucrose diet: 100 g beef tallow (Palma, Bratislava, Slovakia) or 100 g fish oil (Activepa 30 TG, Martens, Norway). A semipurified basal diet is commercially available (Velaz Prague, Czech Republic) (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 %). A 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 %. Further details about the composition of the diets were published recently (Šeböková *et al.* 1992). Using the above approach, the rats were randomly divided into 4 groups (8 rats per group), each of them being fed a different diet: basal (B), basal+fish oil (B+FO), high-sucrose (HS) and high-sucrose+ fish oil (HS+FO). In addition, control (NTG) (Velaz Prague, Czech Republic) Wistar rats underwent the same dietary intervention as did the HTG animals.

Biochemical analyses

Serum obtained from blood after decapitation of animals in the fed state was used for the assays of insulin (RIA kit, Opidi, Poland), glucose (glucose-oxidase kit, Lachema Brno, Czech Republic) and triglycerides (TG) (Lachema Brno, Czech Republic). Skeletal muscle (m. quadriceps femoris) was immediately removed, frozen and subsequently used for analysis of GLUT4 protein levels.

Analysis of GLUT4 protein

Total membrane preparation. For the preparation of total membrane (TM) fraction from muscle quadriceps femoris a modification of Kahn's method (Kahn *et al.* 1991) was used. Muscle homogenate in buffer A (250 mM sucrose, 20 mM HEPES, 1 mM EDTA, 1 mM phenylmethylsulphonyl fluoride, and 1 mM leupeptin, pH 7.4) was centrifuged at 1200 g_{max} and 9000 g_{max} for 10 min, followed by 200000 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 (Simpson and Sonne 1982) using bovine serum albumin (Sigma, St. Louis, MO) as standard. The final pellet (TM) was resuspended in water to approximate a concentration of 5 mg protein/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.* (1992) was used. The proteins (20 μg/lane) from the total membrane fraction in sodium dodecyl sulfate (SDS) sample buffer (containing 6 M urea and 100 mM dithiothreitol, 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 h) 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 % non-fat dried milk powder (Elk^R, Campina B.V., The Netherlands) in TBST buffer for 1 h. After three additional washes with TBST the filters were incubated with anti-GLUT4 (2 μg/ml of TBST with 1 % Elk^R) for 2 h. 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 (Davies *et al.* 1990). After washing with TBST buffer the filters were incubated with ^{125}I-labelled donkey anti-rabbit antibody (Cat. Nr. IM 134, Amersham, England; specific activity 740 kBq/μg), applied at a dilution of 1:500 (74 kBq/10 ml TBST with 1 % Elk^R). 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 the

autoradiograms analyzed by scanning densitometry using an LKB ultrascan densitometer. Relative values for glucose transporter concentrations were derived from the peak heights.

Statistical analyses

The effect of dietary fat supplements was examined by the analysis of variance procedure. Duncan's test (Duncan 1955) was used to evaluate the significance of differences ($p<0.05$) between the means.

Results

Body weight of hereditary HTG rats was slightly lower than that of age-matched NTG control animals (Table 1). Fed state serum insulin and glucose levels did not differ between the strains and dietary treatments except the serum glucose of HS+FO fed HTG rats (Table 1). However, trends in predictable directions (i.e. higher serum insulin in HS fed rats and lower serum insulin levels in fish oil supplemented groups) can be noted in serum insulin. On the other hand, serum TG were clearly higher in HTG rats in both basal and stimulated state, i.e. in animals fed the basal or high-sucrose diet (Table 1).

GLUT4 protein levels (Fig. 1) in quadriceps femoris muscle of HTG rats fed the basal diet were reduced by about 50 % compared with the control group fed identical chow. These low GLUT4 protein levels were further influenced by feeding the HTG rats neither with high-sucrose nor the basal diet supplemented with fish oil. It is, however, notable that fish oil supplementation of the basal diet also led to a decrease (about 60 %) of GLUT4 protein levels in the muscles of control rats. Moreover, the consumption of a high-sucrose diet supplemented with fish oil resulted in about 85 % decrease of GLUT4 protein concentration in muscles of both strains when compared to control rats fed the basal diet.

Table 1
Comparison between body weight, serum glucose, insulin and triglyceride concentrations in hereditary hypertriglyceridaemic (HTG) and control (NTG) Wistar rats

Strain Diet	Body weight [g]	Glucose [mmol/l]	Insulin [μU/ml]	Triglycerides [mmol/l]
NTG rats				
B	380 ± 9.8^a	8.47 ± 0.18^a	67.8 ± 6.9^a	1.85 ± 0.26^a
B+FO	368 ± 6.8^a	8.37 ± 0.38^a	51.8 ± 7.7^a	0.88 ± 0.12^b
HS	359 ± 6.4^a	8.58 ± 0.30^a	79.0 ± 10.0^a	2.74 ± 0.19^c
HS+FO	355 ± 6.3^a	8.30 ± 0.19^a	65.9 ± 13.1^a	1.01 ± 0.09^b
HTG rats				
B	317 ± 9.4^b	9.50 ± 0.54^a	53.8 ± 8.6^a	3.24 ± 0.23^c
B+FO	311 ± 6.2^b	8.80 ± 0.23^a	39.5 ± 6.7^a	1.04 ± 0.06^b
HS	306 ± 6.7^b	9.29 ± 0.35^a	65.8 ± 9.7^a	4.62 ± 0.53^d
HS+FO	301 ± 5.7^b	9.06 ± 0.30^a	41.5 ± 7.5^a	1.08 ± 0.09^b

Data are expressed as means ± S.E.M., n=8. Values without a common superscript $^{(a,b,c,d)}$ within the column are significantly different ($p<0.05$). B = basal diet; B+FO = basal diet supplemented with fish oil; HS = high-sucrose diet; HS+FO = high-sucrose diet supplemented with fish oil

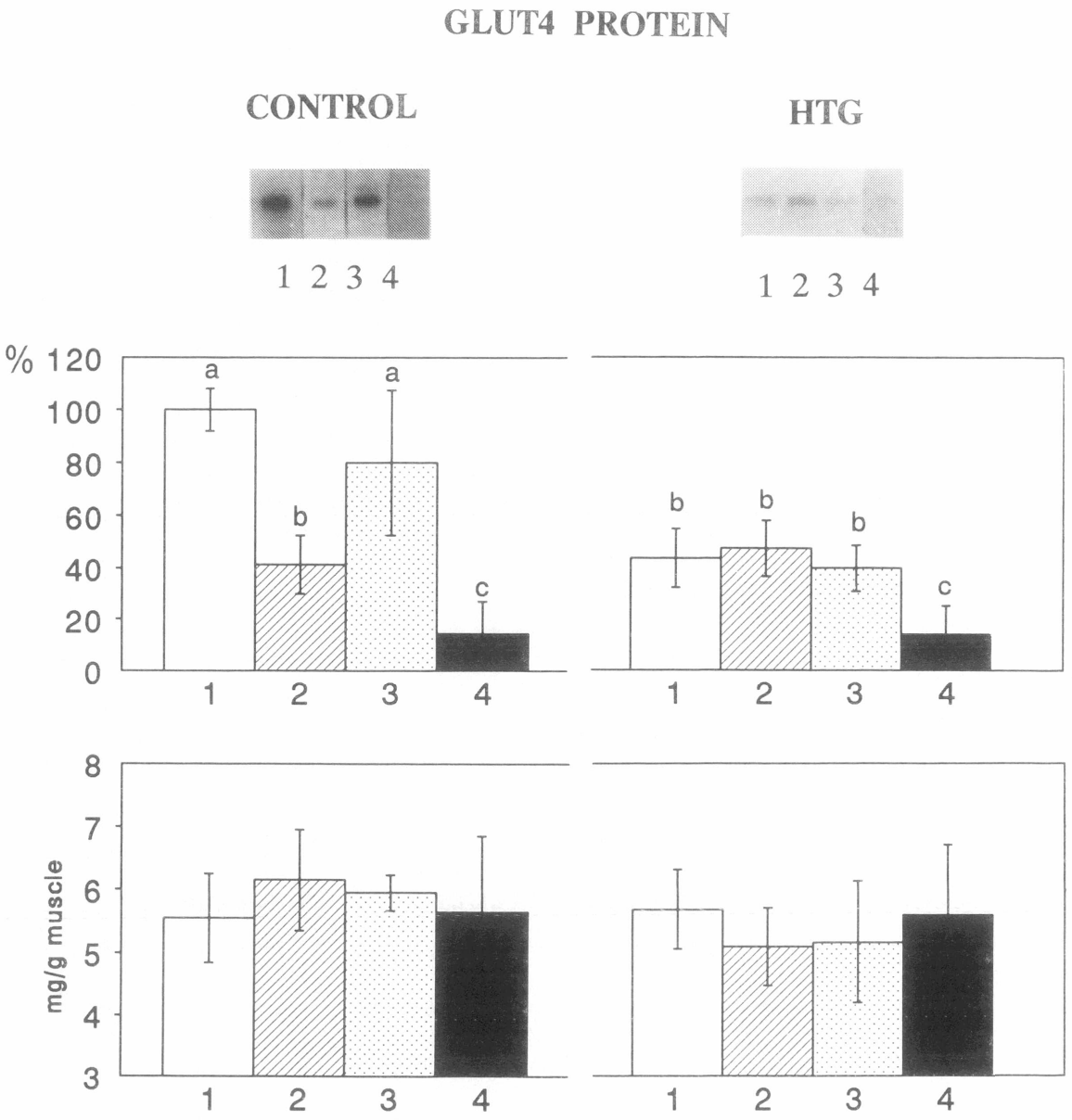

Fig. 1
Representative autoradiograms of Western blotting of GLUT4 proteins (first panels from the top) in quadriceps femoris muscle of control and hereditary hypertriglyceridaemic (HTG), insulin-resistant rats fed various diets: 1 = basal diet; 2 = basal diet supplemented with fish oil; 3 = high-sucrose diet and 4 = high-sucrose diet supplemented with fish oil. Muscle total membrane proteins were prepared and gel electrophoresis, Western blotting, and densitometric scanning were carried out as described in Materials and Methods. Each lane contains 20 μg membrane protein. Second panels from the top give values obtained by scanning of the individual autoradiograms (mean ± S.E.M., n=8 expressed as percentage of values obtained in control rats (group 1). Values without a common superscript $^{(a,b,c)}$ are significantly different ($p<0.05$). Third panels from the top show total membrane proteins recovered per gram of muscle tissue (mean ± S.E.M., n=8).

Discussion

The present results show that skeletal muscle (quadriceps femoris) of hereditary HTG insulin-resistant and moderately hypertensive rats, which were derived from the Wistar strain, contains only about half the amount of glucose transporters compared with control Wistar rats.

Previously, we found a similar decrease of muscle GLUT4 transporter levels in rats with the neonatally streptozotocin-induced Type 2 diabetes (Šeböková *et al.* 1993) which is accompanied by insulin

resistance. Moreover, our recently published data (Klimeš *et al.* 1994) indicate a tendency towards a decrease of GLUT4 protein levels in skeletal muscles of normal rats fed a high-sucrose diet which is also known to induce insulin resistance (Klimeš *et al.* 1989). On the contrary, Bader *et al.* (1992) who measured GLUT4 levels in the hindlimb muscles of spontaneously hypertensive rats (SHR), found no differences in carrier number in comparison with normal Wistar rats. There are few differences in the animal characteristics (lower blood pressure and elevated serum TG in the HTG than in SHR rats) and biochemical methods used (different polyclonal antibody against GLUT4 and different muscle membrane preparations) which might be responsible for this discrepancy. On the other hand, we may agree with Bader *et al.* (1992) that the interpretation of our data (and their as well) is limited to each particular specific animal model of insulin resistance and hypertension, respectively. Moreover, there are no doubts that the major regulation of GLUT4 (and hence of muscle glucose uptake) by insulin involves the translocation of the protein from intracellular vesicles to the plasma membrane together with alteration of intrinsic activity of the glucose transporter (Klip and Paquet 1990, Kahn *et al.* 1991) rather than the effect on total GLUT4 protein. Therefore, in future studies it might be very useful to investigate the subcellular distribution of muscle glucose transporters under basal and insulin-stimulated conditions.

Another important issue is highlighted in this study by varying the type of fat while holding the total fat content of the diet unchanged. Substitution of the basal diet with marine fish oil, rich in eicosapentaenoic and docosahexaenoic n-3 polyunsaturated fatty acids (PUFA), also reduced the GLUT4 protein level in muscles of control rats as we have already shown earlier (Klimeš *et al.* 1994). In addition, replacement of saturated fat in high-sucrose diet by fish oil led to a further striking reduction of GLUT4 levels in the muscle of both hereditary HTG and control rats. These findings may indicate that the prolonged intake of dietary fish oil might be, in a sense of current knowledge, paradoxically associated with impaired insulin action at the tissue level. Indeed, Ezaki *et al.* (1992) found that by high fish oil diet-induced increase of glucose uptake and GLUT4 protein levels in fat cells peaks at the end of the first week, and then vanishes (2–4 weeks). It was concluded that prolonged fish oil intake may cause insulin resistance.

We are aware of the different regulation of GLUT4 in muscle and adipose tissue (Charon 1990), but we have repeatedly observed the suppressive effect of dietary fish oil on GLUT4 protein levels in skeletal muscle (Šeböková *et al.* 1993, Klimeš *et al.* 1994). Therefore, either the change of total glucose transporter number is not directly related to the actual amounts of glucose being transported, or the intrinsic activity (i.e. moles of glucose transported by one transporter per time unit – Harrison *et al.* 1991) is able to compensate for the GLUT4 protein decrease (as dietary fish oil has been observed to improve the *in vivo* insulin action in the hereditary HTG rats –unpublished results).

It is also to be stressed that the regulation of glucose transport and GLUT4 protein production depends on the muscle fibre type. There is more GLUT4 protein in red, oxidative than in white, glycolytic fibres (Goodyear *et al.* 1991, Megeney *et al.* 1993). The GLUT4 protein level was analyzed in the present study only in the quadriceps femoris muscle, and in the total membrane preparation containing both fibre types. Thus, in the absence of information on other muscles (and their prevailing fibre types), our conclusions cannot be generalized.

In summary, 1. the hereditary HTG rat has lower GLUT4 protein levels in the quadriceps femoris muscle; 2. a high-sucrose diet or raised dietary intake of n-3 PUFA do not further alter the number of glucose carriers in this muscle of the hereditary HTG rat; 3. an unexpected decrease of GLUT4 protein levels in the quadriceps femoris muscle appears in response to feeding a high-sucrose diet supplemented with n-3 PUFA.

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