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Rapid communications

Expression of insulin regulatable glucose transporters in skeletal muscle from Type 2 (non-insulin-dependent) diabetic patients

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Summary. A prominent feature of Type 2 (non-insulin-dependent) diabetes mellitus is the inability of insulin to appropiately increase the transport of glucose into target tissue. In adipocytes from individuals with Type 2 diabetes, insulin resistance has been shown to be associated with a depletion of glucose transporters. Similarly, streptozotocin induced diabetes causes a diminished expression of the insulin regulatable glucose transporter in rat adipocytes. The expression of this glucose transporter isoform has not yet been investigated in muscle tissue from patients with Type 2 diabetes. We have measured the content of the insulin regulatable glucose transporter in a vesicular fraction isolated from muscle biopsies from fasting individuals with Type 2 diabetes and control subjects, and we found that the number of the insulin regulatable glucose transporters expressed in skeletal muscle was unaffected by Type 2 diabetes (0.208 vs 0.205, arbitrary units, $p > 0.5$, control subjects and diabetic patients). Thus, the decreased glucose disposal in Type 2 diabetes is not associated with a diminished number of insulin regulatable glucose transporters.

Key words: Type 2 (non-insulin-dependent) diabetes mellitus, skeletal muscle, glucose transporters, insulin regulatable glucose transporters.

In patients with Type 2 (non-insulin-dependent) diabetes peripheral glucose disposal is reduced, and a direct measurement of glucose uptake in isolated skeletal muscle fibers has demonstrated decreased insulin responsiveness [1]. It has also been reported that adipocytes from these patients have a decreased insulin stimulated glucose transport and a decreased number of cytochalasin B binding sites [2]. In rats with streptozotocin-induced (STZ) diabetes it was recently found that the translation and expression of the insulin regulatable glucose transporter (IRGT) is decreased in fat and muscle [3, 4]. The IRGT's of rat adipocytes have been shown to constiute about 90% of the total number of glucose inhibitable cytochalasin B binding sites [5], whereas in skeletal muscle the relative number of IRGT's expressed is unknown. However, the report of a decreased translation of IRGT in skeletal muscle from STZ-rats [3] have led to the theory that the peripheral insulin resistance in Type 2 diabetes may be caused by a decreased expression of IRGT in skeletal muscle and adipose tissue [3]. Our aim was therefore to investigate if the number of IRGT's in skeletal muscle from Type 2 diabetic patients is reduced.

Subjects and methods

Materials

Synthetic peptide CT-13 and reagents for immunoblotting were made by Kem-En-Tec (Copenhagen,DK), reagents and protocol for SDS-PAGE and protein determination were from Biorad (Munich, FRG) whereas Sigma (St. Louis, MO, USA) provided the chemicals for membrane preparation.

Design

Eleven obese patients with Type 2 diabetes and 12 healthy control subjects, matched for age (52.1 vs 52.5 years) and sex, participated in the study. Mean body mass index was significantly higher in the diabetic group (34.7 vs 25.1 kg/m^2, $p < 0.001$), as were fasting plasma glucose (9.5 vs 5.3 mmol/l, $p < 0.001$) and insulin (0.19 vs 0.08 nmol/l, $p < 0.01$) as well as glycated haemoglobin (7.4 vs 5.6%, $p < 0.01$) according to the nature of their disease. Informed consent was obtained from all participants prior to the study which was conducted according to the Helsinki declaration. After an overnight fast, skin and fascia overlying the vastus lateralis muscle were anaesthesized, muscle biopsies taken using a Bergstrøm needle and immediately frozen in liquid nitrogen.

Preparation of antibodies towards IRGT

Antibodies towards the IRGT were produced by immunization of rabbits with a synthetic peptide (CT-13) coupled to haemocyanin. CT-13 is identical to the 13 C-terminal aminoacids of the IRGT [7], which are specific for this isoform of glucose transporters. The antiserum was affinity purified on CT-13 coupled to agarose.

Crude membrane preparation

Fifty mg of frozen muscle was thawed in 50 mmol/l Hepes pH 7.4, containing 0.25 mol/l sucrose, 20 mmol/l disodiummolybdate, 1.5 mmol/l phenyl-methyl-sulfonyl-flouride (PMSF), 10 mmol/l EDTA, 2 µmol/l pepstatin A, 2 µmol/l leupeptin and 400,000 Kallikrein inhibitor units/l aprotinin. Preparation of crude membranes was performed by a centrifugation procedure, essentially as in [7]. The final pellet was resuspended in the Hepes/Sucrose buffer with 10 mmol/l EDTA, and the protein content was determined using the Bradford method (BIORAD). The present protein recovery (2.8 ± 0.2 mg/g frozen muscle, mean ± SEM) was the same for the two groups ($p > 0.1$) and was comparable to that reported in rat muscle (1.6 mg/g fresh rat muscle, [7]). Membrane proteins were separated by SDS-PAGE. A sample from a "reference" pool of rat adipocyte microsomal membranes with a known concentration of glucose inhibitable cytochalasin B-binding sites was applied in three dilutions on each gel.

Western blotting and quantification

Western blotting was performed as described in [8] using the antipeptide antibody towards the IRGT as primary antibody. The stained immunoblots were analysed by densitometry and the results were expressed relatively to the protein content and the reference preparation.

Statistical analysis

Comparison of groups was performed with the two-sample rank sum test, and the Spearman test was used for analysis of co-variation between groups.

Results

When the presently used preparatory procedure is applied to the human muscle biopsies, contamination of the resulting vesicular fraction with erythrocyte membranes is probably unavoidable. Figure 1, lanes a–c represents immunoblots of human erythrocyte membranes. The lack of labelling with the CT-13 antibody and the intense labelling with HepG2/erythroid antibody demonstrate that our antipeptide antibody towards IRGT is specific in the sense that it does not crossreact with even high concentrations of the HepG2/erythroid glucose transporter. Furthermore, our antibody could be used to monitor insulin induced translocation of IRGT from low density microsomes to plasma membranes in rat adipocytes in immunoblots (results not shown).

A representative immunoblot of the present vesicular muscle fraction from a patient is shown in three dilutions in Figure 1, lanes d–f. There are two bands, a broader one at M_r of 46,000 and a more distinct band around 53,000. When blocking the primary antibody by addition of a high concentration of the (CT-13) peptide or when omitting the primary antibody both stained bands disappeared (results not shown). These results indicate that both bands represent the IRGT even though crossreactivity with a different protein could explain the results as well. However, only the 46,000 band was present in our reference micro-

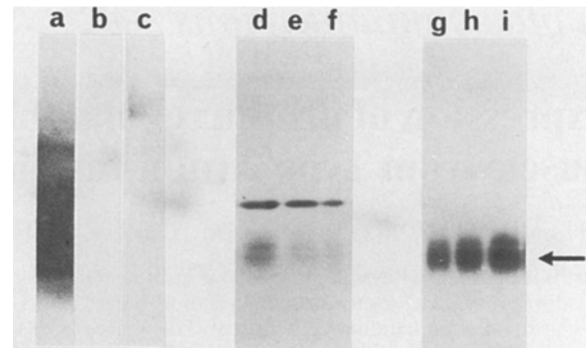

Fig. 1a–i. Immunoblots of the crude membrane fraction from human skeletal muscle and of human erythrocyte membranes. **Lanes a–c.** Crossreactivity between the IRGT anti-peptide antibody and the HepG2 transporter was tested using human erythrocyte membranes. After separation of membrane proteins by 10% SDS-PAGE followed by electrotransfer to nitrocellulose paper, the membranes were incubated with antibody towards the HepG2 transporter (F18) **(lane a),** buffer **(lane b)** or CT-13 antipeptide antibody **(lane c).** Lanes d–f show a representative immunoblot of human muscle membranes in twofold dilutions, using the CT-13 antibody as primary antibody. **Lanes g–i** represent immunoblots of rat microsomal membranes in twofold dilutions from our reference pool using the CT-13 antibody. The arrow indicates the position of the 45,000 Mw marker

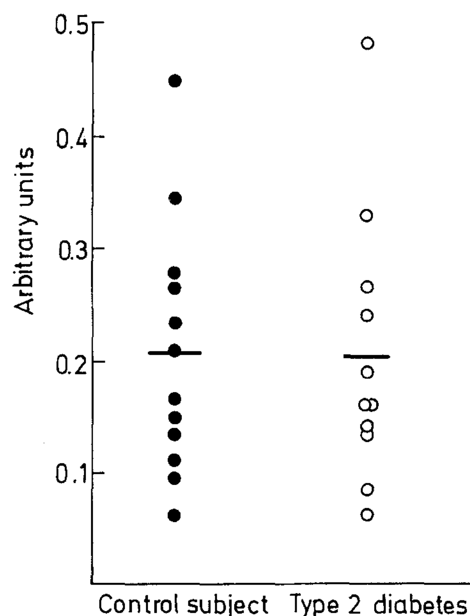

Fig. 2. Individual measurements of the immunoreactivity of IRGT in muscle membrane from Type 2 (non-insulin-dependent) diabetic patients and control subjects expressed in arbitrary units. Immunoblots were scanned and the absorptions expressed relatively to protein content and to the absorption of the reference preparation of rat microsomal membranes. Mean values in each group are indicated by horizontal bars

some preparation from rat adipocytes (Fig. 1, lanes g–i), therefore the sharp band with M_r 53,000 was ignored.

Quantification of the immunoblots was done by densitometry and the results were normalized to our reference preparation as well as to protein content (Fig. 2). The Type 2 diabetic patients had an immunoreactivity of IRGT of 0.208 ± 0.031 and the control subjects 0.205 ± 0.035 (arbitrary units, mean ± SEM, $p > 0.5$). Our results showed no difference in the content of IRGT in skeletal muscle from patients with Type 2 diabetes compared to control subjects, and IRGT content was not significantly correlated to any of the other parameters measured ($p > 0.1$).

Discussion

A distinctive feature of Type 2 diabetes is a decreased insulin sensitivity of peripheral glucose disposal, and the quantitatively most important tissue for this process is skeletal muscle. In support of the notion that glucose permeability of the sarcolemmal membrane is an important determinant of glucose utilization in muscle also in this condition, a decreased insulin responsiveness of glucose transport in isolated strips of skeletal muscle from Type 2 diabetic patients has recently been demonstrated [1]. In addition, it has been shown that Type 2 diabetes is associated with a reduction of insulin stimulated glucose transport along with a depletion of glucose transporters in adipocytes [2], another major insulin target tissue.

That the IRGT isoform is likely to be of importance for glucose permeability of the plasma membrane is indicated by the finding that IRGT constitutes 90% of the glucose transporters in rat adipocytes [5] and it has been shown to be quantitatively dominating among the known glucose transporter isoforms in rat skeletal muscle [6].

In STZ-rats insulin resistance is present but the metabolic state differs from Type 2 diabetes in several respects, notably by being catabolic. Insulin stimulated glucose clearance was reduced to 30–50% of its normal value and the glucose inhibitable cytochalasin B binding to muscle vesicles enriched in plasma membranes was decreased correspondingly [9]. Whether this reduced binding was due to an impaired efficiency of insulin stimulated translocation of transporters to the plasma membrane and/or to a decreased total cellular content of transporters could, however, not be determined [9]. The present results indicate that total IRGT content in biopsies of non-insulin stimulated muscle is unaffected by Type 2 diabetes.

The reported insulin induced translocation of glucose transporters to plasma membrane enriched fractions from muscle are far smaller than the insulin responsiveness of glucose transport in this tissue (about 2- vs 20-fold, [7]). This suggests an insulin effect on the intrinsic activity of glucose transporters in the plasma membrane, as also claimed in several papers on the mechanism of insulin action in adipocytes [2, 5].

The present demonstration of an unchanged content of IRGT in diabetic muscle could thus be taken to indicate that the insulin effect on translocation or on intrinsic activity of transporters is impaired in Type 2 diabetes. Using rather sophisticated indirect techniques it has recently been demonstrated that in non-obese Type 2 diabetic patients intracellular metabolic pathways in muscle are also affected [10]. The importance of these changes for the reduction in overall muscular glucose uptake remains to be determined.

At variance with findings in adipocytes, our data indicate that reduced glucose disposal in Type 2 diabetes is not due to a decreased content of IRGT in skeletal muscle. However, insulin stimulated translocation of IRGT and intrinsic activity of IRGT remains to be studied in skeletal muscle from patients with this disease.

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