Evidence Against Altered Expression of GLUT1 or GLUT4 in Skeletal Muscle of Patients With Obesity or NIDDM

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Studies of experimental diabetes in rodents induced by the β-cell toxin streptozocin have shown that the insulin-resistant glucose transport of peripheral tissues (muscle and adipose) in these animals can be ascribed in part to a pretranslational reduction of the major insulin-sensitive glucose transporter (GLUT4) in these tissues. Because a central feature of non-insulin-dependent diabetes mellitus (NIDDM) is an impaired ability of insulin to enhance glucose disposal in skeletal muscle, we examined the hypothesis that reduced expression of GLUT4 is a characteristic finding in the skeletal muscle of subjects with NIDDM. Biopsies of skeletal muscles were obtained from 17 patients with NIDDM and 10 lean and 9 obese nondiabetic subjects. Among the diabetic subjects, 7 were newly diagnosed and untreated. Compared with age-matched and body-weight-matched healthy control subjects, there was no significant alteration in the level of GLUT4 mRNA demonstrated by Northern blot and slot blot or GLUT4 protein determined by immunoblotting muscle membranes. Neither GLUT4 mRNA nor protein concentration correlated with the degree of glycemic control, fasting plasma insulin or glucose, diabetes duration, body mass index, sex, or age. GLUT1 mRNA and protein levels were also not significantly different between diabetic and matched control subjects. Thus, unlike streptozocin-induced diabetes in rodents, there is no evidence that impaired expression of the major insulin-responsive glucose transporter is responsible for insulin-resistant glucose transport in the skeletal muscle of these lean and moderately obese NIDDM patients. Diabetes 39:865–70, 1990

Although patients with non-insulin-dependent diabetes mellitus (NIDDM) are heterogeneous with respect to clinical and pathophysiological manifestations, they share two major abnormalities in the pathogenesis of hyperglycemia: a relative insulin deficiency and tissue resistance to the action of insulin (1). In the postprandial state, most insulin-regulated glucose disposal takes place in skeletal muscle, and in NIDDM patients, insulin resistance in muscle makes a large contribution to the observed postprandial hyperglycemia (1). Studies of the molecular mechanisms that may be involved in the insulin resistance of muscle from NIDDM patients are limited. Decreased binding and kinase activity of solubilized muscle insulin receptors of NIDDM patients have been demonstrated (2–4). Moreover, in muscle-strip preparations from morbidly obese diabetic and nondiabetic subjects, diminished insulin-stimulated glucose transport has been reported (5). Insight into the mechanisms for this defect could come from studies of adipose tissue, another peripheral insulin target tissue, where cellular glucose transport has been well characterized (6). The reduced insulin-stimulated glucose transport in fat cells from patients with NIDDM is due in part to an inability of insulin to elicit a translocation of adequate numbers of glucose transporters from an intracellular pool to the plasma membrane (7). It is clear that insulin-sensitive peripheral tissues such as adipose and muscle tissue contain at least two different species (GLUT1 and GLUT4) of glucose-transporter proteins (for review, see ref. 8). Previous experiments with rats, in which acute diabetes was induced by streptozocin, have shown that the insulin-resistant glucose transport in adipose cells from these animals is associated with a significant reduction of the major insulin-responsive glucose transporter (GLUT4; 9–12). GLUT4 mRNA levels have also been shown to be reduced in muscle from diabetic rats (10). Consequently, we tested the hypothesis that the molecular mechanisms responsible for the insulin resistance of patients with NIDDM involve reduced expression of GLUT4 in skeletal muscle.
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RESEARCH DESIGN AND METHODS

The study protocol was approved by the ethical committee of the county of Aarhus, Denmark. Informed consent was obtained from all subjects in accordance with Helsinki Declaration II. Lean and obese control subjects had normal HbA1c and fasting plasma glucose and had no known family history of diabetes. They were all sedentary, and they were asked to continue their usual diet until the examination. A body mass index (BMI) of 25.0 kg/m\(^2\) was considered the upper limit for a lean subject. Every obese control subject exhibited normal glucose tolerance with a standard 75-g oral glucose tolerance test. Subjects with NIDDM as defined by the National Diabetes Data Group (13) were selected from the outpatient clinic of the Diabetes Unit of Aarhus Amtssygehus, Denmark, according to the following criteria: Patients with a plasma C-peptide level of >0.6 nM 6 min after an intravenous bolus injection of 1 mg glucagon were included in the protocol.

In our study, we did not measure in vivo insulin action; however, in a group of NIDDM patients with the same ethnic background and similar metabolic control and BMI, we reported severe insulin resistance of glucose disposal in peripheral (muscle) tissue measured by euglycemic clamp (14). Only patients in good health, apart from diabetes; without evidence of infectious disease; and with normal serum electrolytes and liver, renal, cardiac, and thyroid functions on routine laboratory examination were selected for study. All diabetic subjects were sedentary. Seven of the diabetic patients (4 lean and 3 obese) were newly diagnosed diabetic patients (4 lean and 3 obese) were newly diagnosed and received no diet or drug treatment before examination. Of the 10 previously diagnosed patients, 7 were treated with insulin and diet, and 1 with diet only. For the last week before the study, the diabetic patients were asked to eat weight-maintaining diets that typically contained 48% carbohydrates, 40% fat, and 12% protein. Patients with diabetes were classified according to metabolic control: Patients with HbA1c ≤7.5% were considered in regular acceptable control, whereas patients with HbA1c >7.5% were considered in poor glycemic control.

All examinations were made between 0800 and 0900 after an overnight fast (no food or medication for 10 h). A venous blood sample was taken. Plasma glucose, insulin, C-peptide, and HbA1c were measured by conventional methods. A percutaneous needle biopsy of vastus lateralis muscle (~500 mg) was taken with local anesthesia 20 cm above the patella on the lateral part of the thigh.

The muscle tissue was rinsed and dissected free of erythrocytes and connective tissue and frozen at −70°C. RNA was extracted by the guanidinium isothiocyanate–CsCl method (15). Quantity and purity of RNA was determined by absorbance at 260 and 280 nm. DNA was measured spectrophotometrically (16). Total muscle membranes (plasma membranes and microsomes) were prepared from ~100 mg of the biopsy that was polytronized in a buffer containing 10 mM NaHCO\(_3\), 0.25 M sucrose, and 5 mM NaN\(_2\), pH 7.4, 4°C, according to a modification of the total membrane preparation of Klip et al. (17). Homogenates were centrifuged at 1200 × g for 10 min at 4°C. The supernatant was saved, and the pellet was resuspended, homogenized, and centrifuged at 1200 × g for 10 min at 4°C. The first and second supernatants were combined and centrifuged at 9000 × g for 10 min at 4°C. The supernatant from this spin was centrifuged at 227,000 × g for 75 min at 4°C, and the pellet was resuspended. Protein was determined by the Biorad protein determination method. To ensure that significant amounts of GLUT4 protein were not discarded, we immuno-blotted the pellets from the 1200 × g centrifugation that contained fibrous material, the pellet from the 9000 × g spin that contained mitochondria and nuclei, and the supernatant from the final ultracentrifugation. Insignificant amounts of GLUT4 were detected by anti-GLUT4 antisera in each of these three components. Similarly, additional GLUT4 proteins were not released from the fibrous pellet after DNase treatment in the presence of protease inhibitors.

Muscle membranes prepared as described in the previous paragraph were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by immunoblotting (100 μg/lane) with monoclonal antibody (MoAb) 1F8, specific for the COOH-terminal of GLUT4 (18), and polyclonal antisera raised against COOH-terminal peptides for GLUT1 and GLUT4. Immunolabeled bands were visualized with a 35S-labeled sheep antimouse R(ab')\(_2\) fragment (Amersham, Arlington Heights, IL) after the MoAb 1F8 immunoblotting and 35S-protein A (DuPont-NEN, Boston, MA) after the antipeptide antisera (9). Bands were quantitated by scanning densitometry.

Total muscle RNA, extracted as described before, was subjected to Northern-blot analysis (10 μg/lane) on 1.2% formaldehyde-agarose gels, blotted and fixed onto nylon membranes, and probed under stringent conditions with 32P-labeled GLUT1, GLUT4, and β-actin probes (9). Equivalent loading of RNA in each lane was assessed by ultraviolet shadowing. The GLUT1 cRNA probe and the actin cDNA probe were labeled, and washes were performed as previously described (9). The GLUT4 cDNA probe was labeled by random priming and washed as described for other glucose-transporter cDNA probes (9). The polymerase chain reaction (PCR) was used to isolate the human muscle GLUT4 cDNA as follows. Two oligonucleotide primers, (A) 5'-CGAAGCTTTCATCTTCGCCGCCCCT-3' and (B) 5'-CCGAAATTCGCTTCTCATCCTTTACAG-3', which are complementary to the published human GLUT4 cDNA sequence, were synthesized and used to amplify an 852-base pair (bp) GLUT4 cDNA fragment (19). Primer B and Moloney murine leukemia virus reverse transcriptase (Bethesda Research, Gaithersburg, MD) were used to generate first-strand cDNA from 10 μg of total RNA from human skeletal muscle as previously described (20,21). The cDNA mixture was amplified by PCR for 40 cycles with primers A and B and Taq DNA polymerase (Perkin-Elmer/Cetus, Norwalk, CT), as previously described (20,21). The 852-bp PCR product was purified by agarose gel electrophoresis and subsequent treatment with Gene Clean (Bio 101, San Diego, CA). Approximately 100–200 ng of the purified PCR-product cDNA was directly sequenced with the deoxy-chain-termination method (22). The results of direct sequencing confirmed that the PCR-product cDNA sequence was identical to the published human GLUT4 cDNA in this (proximal coding) region (19). Blots were exposed to Kodak XAR-5 film with an intensifying screen. The abundance of GLUT1, GLUT4, and
actin mRNA was determined by scanning densitometry (Hoeffer, San Francisco, CA). Human muscle GLUT4 mRNA was also analyzed using the slot-blot technique. Total muscle RNA was applied in triplicate (0.5 μg/slot) to nitrocellulose filters with a Hybri-slot manifold (Bethesda Research). Rat liver RNA was applied to slot blots as a negative control. Filters were hybridized with a GLUT4 cDNA probe, as described above, and mRNA levels were quantitated by scanning densitometry.

Results are given as means ± SE. Student's unpaired t test was used to compare mean values. The Beth Israel Hospital analyzer system was employed in correlation studies and analysis of variance.

RESULTS
Clinical characteristics of the participants of the study are outlined in Table 1. Obese subjects with and without NIDDM had fasting hyperinsulinemia indicating insulin resistance. To assess whether the glucose transport system was altered at the level of gene expression, we isolated total RNA from biopsies of the vastus lateralis muscle of the thigh. The RNA of 33 subjects was analyzed by the Northern-blot technique under stringent hybridization conditions with 32P-labeled GLUT4 cDNA. The cDNA recognized a single 3.0- to 3.5-kilobase mRNA, the size of which was unaltered by diabetes or obesity (Fig. 1). When Northern blots were loaded with equivalent amounts of total RNA/lane, the abundance of the specific glucose-transporter mRNA showed a 1.5- to 3-fold variation in each group of individuals, with no significant difference in the mean between the groups (obese nondiabetic, 154 ± 24% of lean control; diabetic, 98 ± 11% of lean control). When expressed in relation to actin mRNA abundance, the GLUT4 mRNA levels were still minimally affected by obesity or diabetes (Fig. 1C). The GLUT4/actin mRNA values (ratio of optical density units) for the Northern blots were obese nondiabetic, 84 ± 6% of lean control; obese diabetic, 95 ± 10% of obese control; lean diabetic, 95 ± 12% of lean control; and all diabetic, 93 ± 7% of all control.

To better quantitate the GLUT4 transcripts, we also performed slot-blot analysis in triplicate on RNA from all 36 individuals, using the same cDNA probe. Quantitative densitometric scanning of slot blots also failed to detect any significant differences between the groups at the level of GLUT4 mRNA, despite demonstrating an ability to distinguish small known differences with a standard curve (Fig. 2).

The normalization of glucose-transporter mRNA relative to total RNA is justified only if diabetes or obesity is not accompanied by perturbations in the amount of total RNA per unit of muscle tissue. Therefore, it might be physiologically more relevant to normalize the results per gram of muscle or per DNA, assuming that equal concentrations of DNA reflect the same number of multinucleated muscle cells. RNA/g of muscle was unaltered with obesity or diabetes.

TABLE 1
Characteristics of study participants

|                  | Lean (M/F) | Obese (M/F) | NIDDM (M/F) |
|------------------|------------|-------------|-------------|
| n (M/F)          | 5/5        | 0/9         | 4/3         | 8/2         |
| Age (yr)         | 53 ± 4     | 52 ± 5      | 51 ± 3      | 55 ± 3      |
| Body mass index (kg/m²) | 23.6 ± 0.4 | 34.5 ± 2.0* | 22.7 ± 0.7  | 30.2 ± 1.3* |
| Plasma glucose (mM) | 4.6 ± 0.2  | 4.8 ± 0.1   | 11.9 ± 1.8* | 10.9 ± 0.9* |
| HbA1c (%)        | 4.7 ± 0.2  | 4.8 ± 0.2   | 7.7 ± 0.6*  | 8.0 ± 0.5*  |
| Plasma insulin (mM) | 4.7 ± 0.7  | 11.2 ± 2.2* | 3.5 ± 0.5   | 18.7 ± 3.3* |

Values are means ± SE. NIDDM, non-insulin-dependent diabetes mellitus. Plasma concentrations of glucose and insulin were measured in fasting state in morning.

*P < 0.05 vs. lean control subjects.
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3.0-1
2.5-1
2.0-1
1.5-1
1.0-1
0.5-1
0.0
0.0 1.0 2.0
TOTAL RNA (µg)

FIG. 2. Slot-blot analysis of human muscle GLUT4 mRNA levels in all 36 subjects characterized in Table 1 was performed in triplicate (0.5 µg/slot) as described in RESEARCH DESIGN AND METHODS. Diabetic subjects were divided according to ± therapy and glycemic regulation (HbA1c >7.5%). Horizontal bars, mean values. Inset, standard curve with 6 different concentrations (each in triplicate) of single RNA sample was performed to verify linearity of densitometric comparisons.

TABLE 2
Characteristics of tissue from vastus lateralis muscle of study participants

|                  | Lean | Obese | Lean | Obese | NIDDM | Lean | Obese |
|------------------|------|-------|------|-------|-------|------|-------|
| RNA/g of tissue (µg/g) | 72 ± 4 | 64 ± 3 | 58 ± 11 | 56 ± 4 |
| DNA/g of tissue (µg/g) | 623 ± 72 | 676 ± 63 | 789 ± 100 | 705 ± 67 |
| RNA/DNA (µg/µg) | 0.128 ± 0.014 | 0.094 ± 0.008 | 0.077 ± 0.015* | 0.084 ± 0.010* |
| Protein/g of tissue (µg/g) | 4096 ± 498 | 3895 ± 503 | 3309 ± 613 | 4271 ± 600 |
| Protein/DNA (µg/µg) | 6.98 ± 1.01 | 5.97 ± 0.91 | 5.51 ± 1.95 | 6.79 ± 1.43 |

Values are means ± SE. NIDDM, non-insulin-dependent diabetes mellitus. RNA, DNA, and protein were analyzed as described in RESEARCH DESIGN AND METHODS.  *P < 0.05 vs. lean control subjects.

In muscle biopsies from obese nondiabetic subjects, the amount of RNA/DNA was not statistically different from that of lean control subjects (Table 2). In lean diabetic individuals, the ratio was decreased by 40%, and in obese diabetic individuals, the ratio was decreased by 35% (P < 0.05 vs. lean control individuals; Table 2). Applying this denominator, the abundance of GLUT4 mRNA was slightly decreased in diabetic subjects; however, this decrease was not seen at the GLUT4 protein level.

We also studied the expression of the specific mRNA for GLUT1 in skeletal muscle, with Northern-blot analysis (Fig. 1A). The transcript size was similar to that of GLUT4. Again, overlapping values for mRNA between diabetic subjects and matched control subjects was demonstrated, although the variability for GLUT1 mRNA was smaller than for GLUT4 mRNA.

The amount of GLUT4 protein in a crude membrane preparation of human skeletal muscle was quantitated by Western blotting with two antiserums specific for GLUT4 (18). In all groups, a dominant band of ~47,000–49,000 M, was identified (Fig. 3). Because pre-stained molecular-weight standards were used, the precise molecular weight of this protein cannot be determined; however, it comigrates with the GLUT4 protein in rat adipose cell low-density microsomes, suggesting that it may be 43,000–45,000 Mr (18; Fig. 3B). Because of the fainter lower-molecular-weight bands detected with antisemum 1F8 (Fig. 3A), immunoblots were repeated with an anti-GLUT4 COOH-terminal peptide antiserum (Fig. 3B). This revealed only a single band of the same molecular weight as the one detected by antisemur 1F8.

Densitometric scanning of autoradiograms showed no difference in the relative abundance of GLUT4 protein between groups when results were normalized for equal amounts of membrane protein (obese, 83 ± 15% of lean control; diabetic, 103 ± 19% of lean control). When diabetic subjects were separated into lean and obese groups, GLUT4 protein levels were still unchanged (lean diabetic, 96 ± 29% of lean control; obese diabetic, 90 ± 21% of obese nondiabetic). Normalization of the glucose-transporter protein/DNA did not change the interpretation of results, because the amounts of total protein recovered in the crude membrane preparations were similar in all groups (Table 2). Immunoblotting of muscle membranes with an anti-GLUT1 antisemur revealed a broad and intense signal, which probably reflected the highly glycosylated glucose transporter in erythrocytes present in muscle (data not shown).

We also analyzed the specific mRNA and protein data after the diabetic subjects were divided according to therapy (untreated newly diagnosed vs. diet ± drug therapy) and degree of glycemic control (HbA1c ≥7.5%); however, no significant differences in levels of GLUT4 mRNA (Fig. 3) or protein (data not shown) could be demonstrated between...
the groups. To identify factors that might play a role in the regulation of GLUT4, we calculated correlation coefficients for GLUT4 mRNA and protein levels and the following variables: age, sex, BMI, diabetes duration, fasting plasma insulin and glucose concentrations, and HbA1c. No significant relationships were demonstrable in the complete group or its subgroups.

**DISCUSSION**

Recently, cDNAs encoding several genetically distinct glucose transporters have been cloned (for a review, see ref. 8). These include a glucose transporter isolated from human HepG2 hepatoma cells and rat brain (GLUT1) and expressed in a wide variety of tissues; a glucose transporter isolated from liver and pancreatic islets; and liver, kidney, intestine, and β-cells (GLUT2). One was isolated from a fetal skeletal muscle cDNA library (GLUT3), which is also widely expressed. The most recently characterized member of this family of glucose transporters is the so-called “major insulin-responsive” glucose transporter (GLUT4), cloned from skeletal muscle, heart and adipose cells and expressed in significant levels exclusively in classic insulin-responsive tissues.

Given the existence of multiple transporter species with overlapping tissue distribution, it is essential to determine the function of these species and the extent to which altered expression or function of individual transporters may play a role in the pathogenesis of insulin resistance in diabetic or altered nutritional states. Several lines of evidence have led to the belief that GLUT4, not GLUT1, may be the dominant species responsible for insulin-sensitive glucose transport in those tissues that express it and that reduced expression of GLUT4 at the pretranslational level may be a central feature of the pathogenesis of insulin-resistant glucose transport. First, although GLUT1 is expressed in adipose and skeletal muscle tissue, the concentration of this transporter protein increases only ~2-fold in the plasma membrane of rat adipocytes in response to insulin, whereas the concentration of GLUT4 protein increases ~10-fold in adipocyte plasma membranes after insulin stimulation (18). Second, GLUT1 is much less abundant in rat adipose cells than GLUT4 (23). Finally, several groups of investigators have reported that, in adipocytes from rats made acutely diabetic by streptozocin injection, there is a substantial decrease in both GLUT4 mRNA and protein, which is reversed by insulin treatment (9–12), whereas GLUT1 is unaltered by diabetes (9).

Investigations of experimental diabetes also included studies in rat skeletal muscle. Changes in GLUT4 mRNA (10) and glucose transporters assessed by cytochalasin-B binding (24), which detects both GLUT1 and GLUT4 (23), show the same tendency as changes in adipocytes, although the changes are less pronounced. We found a 1.5- to 3-fold variability of GLUT1 and GLUT4 mRNA and GLUT4 protein within each group of subjects. This degree of biological variability is not unexpected and is in accordance with the range of values for related phenomena. That is, even within groups of lean healthy subjects, 3-fold variations have been demonstrated in basal and insulin-stimulated glucose transport in isolated adipose cells (25) and in basal and insulin-mediated glucose disposal to peripheral tissues as assessed by euglycemic clamp (14). In this study of lean and obese nondiabetic subjects, newly diagnosed untreated patients with NIDDM, and long-term treated patients with NIDDM, we did not find impaired expression of GLUT1 or GLUT4 in skeletal muscle. Hence, the pathophysiology of insulin-resistant glucose transport appears to differ between streptozocin-induced diabetes in rats and human NIDDM. In this regard, it is notable that, in the fasting rat, another metabolic model associated with insulin-resistant glucose uptake in vivo (26), GLUT4 expression is actually increased in muscle while GLUT4 mRNA and protein are markedly reduced in adipose cells (27). Thus, in animal models, GLUT4 expression in muscle does not necessarily correlate with in vivo insulin responsiveness. In conclusion, our findings argue against the hypothesis that impaired expression of GLUT1 or GLUT4 in muscle is responsible for the pathogenesis of insulin-resistant glucose disposal to peripheral tissues in patients with moderate obesity or mild NIDDM.
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REFERENCES
1. DeFronzo RA: The triumvirate: β-cell, muscle, liver: a collusion responsible for NIDDM. Diabetes 37:667–87, 1988
2. Caro JF, Sinha MS, Raju SM, Ittoo O, Pories WJ, Flickinger EG, Meelheim D, Dohm GL: Insulin receptor kinase in human skeletal muscle from obese subjects with and without non-insulin dependent diabetes. J Clin Invest 79:1330–37, 1987
3. Arner P, Pollare T, Lithell H, Livingston JN: Defective insulin receptor tyrosine kinase in human skeletal muscle in obesity and type 2 (non-insulin dependent) diabetes mellitus. Diabetesologia 30:437–40, 1987
4. Obermaier-Kusser B, White MF, Pongratz DE, Su Z, Ermel B, Multibacher C, Häring HU: Defective intramolecular autoactivation cascade may cause the activity of the skeletal muscle insulin receptor from patients with non-insulin dependent diabetes mellitus. J Biol Chem 264:9497–504, 1989
5. Dohm GL, Tapscott EB, Pories WJ, Dabbs DJ, Flickinger EG, Meelheim D, Fushiki T, Atkinson SM, Elton CW, Caro JF: An in vitro human muscle preparation suitable for metabolic studies: decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. J Clin Invest 82:486–94, 1988
6. Kahn BB, Cushman SW: Subcellular translocation of glucose transporters: role in insulin action and its perturbation in altered metabolic states. Diabetes Metab Rev 1:203–27, 1985
7. Garvey WT, Huecksteadt TP, Matthaei S, Olefsky JM: Role of glucose transporters in the cellular insulin resistance of type II non-insulin dependent diabetes mellitus. J Clin Invest 81:1528–36, 1988
8. Mueckler M: Family of glucose-transporter genes: implications for glucose homeostasis and diabetes. Diabetes 39:6–11, 1990
9. Kahn BB, Charron MJ, Lodish HF, Cushman SW, Flier JS: Differential regulation of two glucose transporters in adipose cells from diabetic and insulin-treated diabetic rats. J Clin Invest 84:404–11, 1989
10. Garvey WT, Huecksteadt TP, Birnbaum MJ: Pretranslational suppression of an insulin-responsive glucose transporter in rats with diabetes mellitus. Science 245:60–63, 1989
11. Shivitz W, DeSanctis SL, Kayano T, Bell GI, Pessin JE: Regulation of glucose transporter messenger RNA in insulin deficient states. Nature (Lond) 340:72–74, 1989
12. Berger J, Biswas C, Vicario PP, Strout HV, Saperstein R, Pilch PF: Decreased expression of the insulin responsive glucose transporter in diabetes and fasting. Nature (Lond) 340:70–72, 1989
13. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039–57, 1979
14. Hoffer Nielsen O, Schmitz O, Andersen PH, Pedersen O, Beck-Nielsen H: In vivo action of glibenclamide in obese subjects with mild type 2 (non-insulin dependent) diabetes. Diabetes Res 6:63–70, 1988
15. Chirgwin JM, Przylasa AJ, MacDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–98, 1979
16. Labarca C, Paigen K: A simple rapid and sensitive DNA assay procedure. Anal Biochem Exp Med (Calcutta) 102:344–51, 1980
17. Klip A, Ramial T, Young DA, Holloszy JO: Insulin induced translocation of glucose transporters in rat hindlimb muscles. FEBS Lett 224:22–28, 1987
18. James DE, Brown R, Navarro J, Pilch PF: Insulin-regulatable tissues express a unique insulin sensitive glucose transport protein. Nature (Lond) 333:183–85, 1988
19. Fukumoto H, Kayano T, Buse JB, Edwards Y, Pilch PF, Bell GI, Seino S: Cloning and characterization of the major insulin-responsive glucose transporter expressed in human skeletal muscle and other insulin-responsive tissues. J Biol Chem 264:7776–79, 1989
20. Moller DE, Flier JS: Detection of an alternation in the insulin receptor gene in a patient with insulin resistance, acanthosis nigricans, and the polycystic ovary syndrome (type A insulin resistance). N Engl J Med 319:1526–29, 1988
21. Moller DE, Yokota A, Caro JF, Flier JS: Tissue-specific expression of two alternatively spliced insulin receptor mRNAs in man. Mol Endocrinol 3:1263–69, 1989
22. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–67, 1977
23. Zorzano A, Wilkinson W, Kotter N, Thoidis G, Wadzinki BE, Rucho AE, Zorzano A, Wilkinson W: Detection of two alternatively spliced insulin receptor mRNAs in human tissues. Mol Endocrinol 3:1263–69, 1989
24. Ramlal T, Rastogi S, Vranic M, Klip A: Decrease in glucose transporter number in skeletal muscle of mildly diabetic (streptozotocin-treated) rats. Endocrinology 224:5258–63, 1989
25. Ramial T, Rastogi S, Niiranen K, Klip A: Decrease in glucose transporter number in skeletal muscle of mildly diabetic (streptozotocin-treated) rats. Endocrinology 125:850–97, 1989
26. Hjollund E, Pedersen O, Richelsen B, Beck-Nielsen H: In vivo action of glibenclamide in obese subjects with mild type 2 (non-insulin dependent) diabetes mellitus. Diabetes 39:63–70, 1988
27. Hjollund E, Pedersen O, Richelsen B, Beck-Nielsen H, Sorensen NS: Increased insulin binding to adipocytes and monocytes and increased insulin sensitivity of glucose transport and metabolism in adipocytes from non-insulin-dependent diabetics after a low-fat, high starch and high fiber diet. Metabolism 32:1067–75, 1983
28. Pericau L, Kinda J, LeMagney J, Girard JR: Insulin action during fasting and refeeding in rat determined by euglycemic clamp. Am J Physiol 249:ES14–19, 1985
29. Charron MJ, Kahn BB: Divergent molecular mechanisms for insulin resistant glucose transport in muscle and adipose cells in vivo. J Biol Chem 265:7994–8000, 1990