Regulation by Insulin of Gene Expression in Human Skeletal Muscle and Adipose Tissue

Evidence for Specific Defects in Type 2 Diabetes

Pierre-Henri Ducluzeau,[1] Noël Perretti,[1] Martine Laville,[1,2,3] Fabrizio Andreelli,[1,3] Nathalie Vega,[1] Jean-Paul Riou,[1,2,3] and Hubert Vidal[1,2]

Defective regulation of gene expression may be involved in the pathogenesis of type 2 diabetes. We have characterized the concerted regulation by insulin (3-h hyperinsulinemic clamp) of the expression of 10 genes related to insulin action in skeletal muscle and in subcutaneous adipose tissue, and we have verified whether a defective regulation of some of them could be specifically encountered in tissues of type 2 diabetic patients. Basal mRNA levels (determined by reverse transcriptase–competitive polymerase chain reaction) of insulin receptor, insulin receptor substrate-1, p85α phosphatidylinositol 3-kinase (PI3K), p110αPI3K, p110βPI3K, GLUT4, glycogen synthase, and sterol regulatory-element-binding protein-1c (SREBP-1c) were similar in muscle of control (n = 17), type 2 diabetic (n = 9), type 1 diabetic (n = 9), and nondiabetic obese (n = 9) subjects. In muscle, the expression of hexokinase II was decreased in type 2 diabetic patients (P < 0.01). In adipose tissue, SREBP-1c (P < 0.01) mRNA expression was reduced in obese (nondiabetic and type 2 diabetic) subjects and was negatively correlated with the BMI of the subjects (r = −0.63, P = 0.02). Insulin (±1,000 pmol/l) induced a two- to threefold increase (P < 0.05) in hexokinase II, p85αPI3K, and SREBP-1c mRNA levels in muscle and in adipose tissue in control subjects, in insulin-resistant nondiabetic obese patients, and in hyperglycemic type 1 diabetic subjects. Upregulation of these genes was completely blunted in type 2 diabetic patients. This study thus provides evidence for a specific defect in the regulation of a group of important genes in response to insulin in peripheral tissues of type 2 diabetic patients. *Diabetes* 50: 1134–1142, 2001

From the [1]Institut National de la Santé et de la Recherche Médicale (INSERM) U.449 and [2]Human Nutrition Research Center of Lyon, Faculty of Medicine R. Laennec; and the [3]Department of Endocrinology, Diabetology, and Nutrition, E. Herriot Hospital, Lyon, France.

Address correspondence and reprint requests to Hubert Vidal, PhD, INSERM U.449, Faculté de Médecine R. Laennec, Rue G. Paradin, F-69372 Lyon Cédex 08, France. E-mail: vidal@laennec.univ-lyon1.fr.

Received for publication 31 July 2000 and accepted in revised form 5 February 2001.

P.-H.D. and N.P. contributed equally to this work.

IRS, insulin receptor substrate; PCR, polymerase chain reaction; PI3K, phosphatidylinositol 3-kinase; p85αPI3K, p85α regulatory subunit of phosphatidylinositol 3-kinase; p110αPI3K, p110α catalytic subunit of phosphatidylinositol 3-kinase; p110βPI3K, p110β catalytic subunit of phosphatidylinositol 3-kinase; Rad, *Ras* protein associated with diabetes; RT-cPCR, reverse transcriptase–competitive polymerase chain reaction; SREBP-1c, sterol regulatory-element-binding protein-lc.

Insulin resistance is the main metabolic feature of type 2 diabetes (1,2), and several studies indicate that it generally precedes the onset of the disease (2,3). In vivo, skeletal muscle is the major site for insulin-dependent glucose disposal, and type 2 diabetic patients are characterized by a marked decrease in insulin-stimulated glucose utilization in muscle mainly due to reduced glucose uptake and storage (1,2). Insulin stimulates glucose uptake by increasing the translocation of GLUT4-containing vesicles to the plasma membrane and by modifying the activity of enzymes involved in glucose metabolism (4). Insulin action is initiated by binding of the hormone to cell membranes and activation of the insulin receptor tyrosine kinase that results in the stimulation of intracellular signaling cascades (4). Among these cascades, the phosphatidylinositol 3-kinase (PI3K) pathway is thought to play a crucial role in the effects of insulin on glucose metabolism (5). Several defects in the insulin signaling pathways have been identified in skeletal muscle of type 2 diabetic patients. Impaired phosphorylation of insulin receptor and insulin receptor substrate (IRS)-1 in response to insulin has been reported (6–8), and the induction of PI3K and Akt kinase activities have been found to be reduced (8–10). The stimulation of glycogen synthase activity is also decreased in skeletal muscle of patients with type 2 diabetes (11). All of these alterations take place at the level of the acute posttranslational regulation of key enzyme activity.

In addition to this level of regulation, insulin also controls the transcription of important genes in its target cells (12). This action is crucial for insulin to sustain its metabolic effects and also to adapt the organism to environmental changes. It is well known that environmental factors play a major role in type 2 diabetes, in addition to genetic predisposition. Alterations in the transcriptional mechanisms involved in the adaptation of the cells to environmental changes may thus participate in the pathogenesis of the disease (13,14). In keeping with this hypothesis, the expression of some important genes involved in insulin action and glucose metabolism has been found to be altered in peripheral tissues of type 2 diabetic patients. For example, the basal expression levels of hexokinase II (15,16) and glycogen synthase (17) in skeletal muscle and of GLUT4 (18) and IRS-1 (19) in adipose tissue are significantly reduced. Moreover, it has been reported that

TABLE 1
Characteristics of the subjects

	Control 50 9	Obese 9	Type 2 diabetes 9	Control 25 8	Type 1 diabetes 9
Men/women	3/6	3/6	6/3	1/7	4/5
Age (years)	51 ± 2	43 ± 4	54 ± 3*	25 ± 1	33 ± 3†
BMI (kg/m^2)	23 ± 1	35 ± 1‡	30 ± 1‡*	22 ± 1	24 ± 1
Basal					
Glucose (mmol/l)	5.0 ± 0.2	5.0 ± 0.2	10.5 ± 0.6*‡	4.4 ± 0.1	12.6 ± 0.8‡
Insulin (pmol/l)	37 ± 4	99 ± 17‡	66 ± 10‡	36 ± 4	–
Nonesterified fatty acid (μmol/l)	399 ± 48	629 ± 49†	621 ± 67†	492 ± 48	519 ± 97
Triglycerides (mmol/l)	0.7 ± 0.1	1.2 ± 0.1†	1.4 ± 0.1‡	0.6 ± 0.1	0.4 ± 0.02
Glucose disposal rate (mg · kg^{-1} · min^{-1})	2.2 ± 0.1	1.6 ± 0.1‡	2.4 ± 0.2*	nd	nd
Glucose oxidation rate (mg · kg^{-1} · min^{-1})	1.2 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.2	1.5 ± 0.2
Clamp study					
Glucose (mmol/l)	4.3 ± 0.2	4.5 ± 0.1	4.9 ± 0.1	4.5 ± 0.1	5.6 ± 0.2‡
Insulin (pmol/l)	919 ± 82	1376 ± 168	1179 ± 65	875 ± 48	949 ± 115
Glucose disposal rate (mg · kg^{-1} · min^{-1})	9.6 ± 0.9	4.5 ± 0.5‡	4.3 ± 0.6‡	10.4 ± 0.6	8.6 ± 0.8
Glucose oxidation rate (mg · kg^{-1} · min^{-1})	3.2 ± 0.2	2.1 ± 0.1‡	1.9 ± 0.1‡	3.5 ± 0.2	3.4 ± 0.2
Nonoxidative glucose disposal rate (mg · kg^{-1} · min^{-1})	6.3 ± 0.8	2.4 ± 0.4‡	2.5 ± 0.6‡	6.9 ± 0.7	5.3 ± 0.8

†$P < 0.05$ and ‡$P < 0.01$ vs. the respective control subjects; *$P < 0.05$ between type 2 diabetic and obese subjects; nd, not determined.

the induction of GLUT4 (20,21) and hexokinase II (22) expression in response to hyperinsulinemia is impaired in skeletal muscle of type 2 diabetic patients. Recently, we have demonstrated that the regulation by insulin of the p85α regulatory subunit of PI3K (p85αPI3K) is also altered in muscle and adipose tissue of patients with type 2 diabetes (23). Taken together, these data suggest that the regulation of a cluster of genes involved in insulin action and glucose metabolism may be affected in type 2 diabetes. It could thus be hypothesized that a common mechanism, involved in the regulation of these genes and probably others that have yet to be identified, is altered in the peripheral tissues of type 2 diabetic patients.

However, this hypothesis is based on results from unrelated studies that have been performed using different hyperinsulinemic conditions and different procedures to estimate gene expression levels. Moreover, in most of the studies, the regulation of one individual gene was investigated, although we have reported the regulation of three mRNAs (insulin receptor, IRS-1, and p85αPI3K) in parallel (23). It is thus difficult to conclude whether the same mechanism is involved in the defective regulation of GLUT4, hexokinase II, and p85αPI3K gene expression. In addition, it is still unclear whether the observed defects in the regulation of gene expression result from a specific alteration linked to type 2 diabetes or are secondary to the metabolic state of the patients, such as insulin resistance or chronic hyperglycemia.

The present study was performed with the aim of verifying these points. To this end, we investigated the concerted regulation by insulin of the expression of 10 candidate genes, measured by similar reverse transcriptase–competitive polymerase chain reaction (RT-cPCR) assays, in skeletal muscle and in adipose tissue of type 2 diabetic patients. In addition to the regulation of genes that have already been studied individually (20–24), we also investigated, for the first time, the regulation of the p110α and p110β catalytic subunits of PI3K (p110αPI3K and p110βPI3K, respectively) and the regulation of the transcription factor sterol regulatory-element-binding protein-1c (SREBP-1c).

To verify whether an impaired regulation of the expression of some of these genes is specific or secondary to the metabolic state of type 2 diabetes, age-matched control subjects, type 2 diabetic patients, and insulin-resistant nondiabetic obese subjects were investigated in parallel. Moreover, the contribution of a deleterious role of chronic hyperglycemia was verified with a group of type 1 diabetic subjects with HbA_{1c} similar to that in the type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

Subjects. The characteristics of the 44 subjects involved in the study are presented in Table 1. The 17 healthy lean volunteers were divided into two groups on the basis of their age. None of these subjects had impaired glucose tolerance or a familial or personal history of diabetes, obesity, dyslipidemia, or hypertension. One group of control subjects (six women and three men, age 51 ± 2 years, BMI 23 ± 1 kg/m^2) was age-matched with nine patients with type 2 diabetes (three women and six men, age 54 ± 3 years, BMI 30 ± 1 kg/m^2, duration of diabetes 7 ± 1 years) and nine nondiabetic obese subjects (six women and three men, age 43 ± 4 years, BMI 35 ± 1 kg/m^2). The type 2 diabetic patients interrupted, under medical control, their usual treatment with oral antidiabetic agents at least 1 week before the investigation. None of the obese subjects had impaired glucose tolerance as assessed by classic oral glucose tolerance test. The metabolic data of some of these subjects (all of the control and type 2 diabetic subjects and six of the nine obese patients) have been presented in a previous study (23). An unrelated group of healthy subjects (seven women and one man, age 25 ± 1 years, BMI 22 ± 1 kg/m^2) served as control subjects for a group of type 1 diabetic patients (five women and four men, age 33 ± 3 years, BMI 23 ± 1 kg/m^2, duration of diabetes 16 ± 3 years, C-peptide <0.05 ng/ml) with HbA_{1c} (9.2 ± 0.3%) similar to that in the type 2 diabetic subjects (10.9 ± 0.3%, $P = 0.252$). Type 1 diabetic patients had no familial antecedent of type 2 diabetes, none had complications, and they were all treated with daily injections of insulin (45 ± 5 IU/day). The last dose of insulin was administered the day before beginning the clamp study. On the morning of the experiment, type 1 diabetic patients showed marked hyperglycemia (12.6 ± 0.8 mmol/l) that was not significantly different from the glycemia of the type 2 diabetic subjects (10.5 ± 0.6 mmol/l, $P = 0.401$). To avoid further increase in plasma glucose concentrations during the 3 h of the basal period that preceded the hyperinsulinemic clamp, an intravenous low-dose insulin infusion (0.5 IU/h) was administrated to the type 1 diabetic subjects. This infusion was maintained during the 3 h of the basal period so that both type 1 and type 2 diabetic patients had similar levels of hyperglycemia at the beginning of the insulin clamp period.

All participants gave their written consent after being informed of the nature, purpose, and possible risks of the study. The experimental protocol was approved by the Ethical Committees of the Hospices Civils de Lyon and performed according to the French legislation (Huriet law).

Study design. All studies were performed after an overnight fast. So that insulin action on glucose metabolism and on target gene expression could be investigated, the subjects were submitted to a 3-h euglycemic-hyperinsulinemic clamp (24).

Euglycemic-hyperinsulinemic clamp. Before the hyperinsulinemic period, basal glucose turnover rate was determined during the last 30 min of a 3-h basal period by tracer dilution methodology using a primed [6,6-^{2}H2]glucose (Eurisotop, St. Aubain, France) infusion (0.02 mg · kg^{-1} · min^{-1}). Then, a 3-h euglycemic-hyperinsulinemic clamp was started by the infusion of insulin (Actrapid Novo, Copenhagen, Denmark) at a rate of 450 pmol · m^{-2} · min^{-1}. Primed [6,6-^{2}H2]glucose was infused (0.1 mg · kg^{-1} · min^{-1}) during the clamp to determine glucose turnover rate, while any decrease in blood glucose was prevented by adapted infusion of 20% glucose solution (Aguettant, Lyon, France). For the determination of metabolites, hormones, and [6,6-^{2}H2]glucose isotopic enrichment, blood samples were drawn every 10 min during the last 30 min of the basal and hyperinsulinemic periods. Metabolite and hormone concentrations were measured using enzymatic methods and radioimmunoassays. Plasma isotopic enrichment of [6,6-^{2}H2]glucose was determined by gas chromatography–mass spectrometry (5,971 MSD; Hewlett-Packard, Palo Alto, CA), and glucose turnover rates were calculated using steady-state equations as previously described (25). For the diabetic patients, glucosuria was subtracted from glucose turnover rates to calculate glucose utilization. For the young control and the type 1 diabetic subjects, [6,6-^{2}H2]glucose was not used and thus basal glucose disposal was not determined. For these subjects, the rates of glucose infusion during the clamp were used to estimate whole-body glucose disposal rates, since it has already been established that endogenous glucose production is suppressed with the level of hyperinsulinemia reached during the clamp (26). To estimate glucose and lipid oxidation rates, respiratory exchange measurements were performed during the final 30 min of both the basal and the hyperinsulinemic periods, using a flow-through canopy gas-analyzer system (Deltatrac Metabolic Monitor; Datex, Helsinki, Finland) (25).

Muscle and adipose tissue biopsies. Muscle and adipose tissue biopsies were taken under local anesthesia (2% lidocaine), at the beginning of the basal period and at the end of the hyperinsulinemic clamp period, as described previously (24). Briefly, skeletal muscle samples were obtained by percutaneous biopsies of the vastus lateralis muscle using Weil-Blakesley pliers. The size of the biopsies averaged 60 mg, with no difference between samples from control, obese, and diabetic subjects or before and after clamp. Abdominal subcutaneous adipose tissue was aspirated from the periumbilical area through a 15-gauge needle (27). About 250 mg wet wt of tissue was used for total RNA preparation, with no significant difference in average size between groups or before and after clamp. Tissue samples were immediately frozen in liquid nitrogen and stored at −80°C for further analysis.

Total RNA preparation. Tissue samples were pulverized in liquid nitrogen and total RNA was prepared according to a procedure based on the method of Chomczynski and Sacchi (28) for muscle samples and using the RNeasy total RNA kit from Qiagen (Courtaboeuf, France) for adipose tissue (27). Average yields of total RNA were 26 ± 2 μg/100 mg of muscle (wet weight) and 1.2 ± 0.2 μg/100 mg of adipose tissue (wet weight), and were not significantly different in tissues from control, obese, and diabetic subjects, before or after the clamp. Total RNA solutions were stored at −80°C until quantification of the target mRNAs.

Quantification of messenger RNAs. The concentrations of the mRNAs corresponding to the genes of interest were measured by RT-cPCR. The method relies on the addition of known amounts of a competitor DNA molecule in the polymerase chain reaction (PCR) to standardize the amplification process (29). The construction of the competitors, the sequences of the primers, the validation of the assays, and the conditions of the RT-cPCR assays have been described in detail previously (16,24,29). For each mRNA, the specific first-strand cDNA was synthesized from 0.1 μg of total RNA in experimental conditions that warranted optimal efficiency of the reaction (29). During the competitive PCR, Cy-5 5′-end-labeled sense primers were used to generate fluorescent PCR products that were analyzed with an automated laser fluorescence DNA sequencer (ALFexpress; Pharmacia, Uppsala, Sweden) in 4% denaturing polyacrylamide gels. The initial concentration of target mRNA was determined at the competition equivalence point as previously described (29).

New competitors were constructed for the quantitation of the mRNAs encoding the p110α and p110β catalytic subunits of PI3K and the transcription factor SREBP-1c. For p110αPI3K, a 517-nucleotide-long cDNA fragment was obtained by RT-PCR on human skeletal muscle total RNA using 5′-$_{79}$CCAAATGGAATGATAGTGAC$_{98}$-3′ as sense primer and 5′-$_{593}$ACTATTACCCAAATCACCAC$_{574}$-3′ as antisense primer. The competitor was obtained by deleting 61 bp using a two-step PCR overlap extension method. For p110βPI3K, a 457-nucleotide-long cDNA fragment was generated by RT-PCR using 5′-$_{2,712}$CTGTGCTGGCTACTGTGTA$_{2,730}$-3′ as sense primer and 5′-$_{3,166}$TAGTAGTCCAG CTTTCCCTG$_{3,147}$-3′ as antisense primer. The competitor was obtained by deleting 49 bp. For SREBP-1c, a 311-nucleotide-long cDNA fragment was synthesized by RT-PCR from human adipose tissue total RNA using 5′-$_{8}$GCGGAGCCATGGATTGCAC$_{11}$-3′ as sense primer (specific of the exon 1c of the SREBP-1 gene) and 5′-$_{311}$CTCTTCCTTGATACCAGGCCC$_{291}$-3′ as antisense primer. The competitor was obtained by adding 20 bp in the SREBP-1c cDNA fragment. The checked sequences of the cDNAs and the competitors of p110αPI3K, p110βPI3K, and SREBP-1c perfectly matched the published ones. For the assays by RT-cPCR, the antisense primers were used for specific reverse transcriptase (RT) reactions, and CY-5 5′-end-labeled sense primers were used during the PCR. Both reactions were performed under the conditions indicated above and previously described (29). The new RT-cPCR mRNA assays were validated using a large range of in vitro synthesized RNA (0.25–50 amol in the reaction) as recommended (29).

To accurately determine the effect of insulin, total RNA of the two muscle (or adipose tissue) biopsies from the same individual (before and after clamp) were prepared simultaneously and the assays of the target mRNAs were always made in the same run of PCR, with the same working solutions of competitor. The data presented in this work generally corresponded to the single determination of a target mRNA in a given biopsy. The coefficient of variation of the RT-cPCR method was <15% (24,29). Because the method allowed the real amount of target mRNAs to be determined (29), the results were presented as absolute concentrations, in attomoles per microgram of total RNA. Control experiments were routinely carried out to verify the absence of PCR contamination and/or genomic DNA amplification.

Statistical analysis. All data in text and figures are presented as means ± SE. Statistical significance of the results was determined using Kruskal-Wallis analysis followed by nonparametric Mann-Whitney's test when comparing groups of subjects. A nonparametric Wilcoxon's test for paired values was used when comparing mRNA levels before and after clamp in the same group of subjects. Correlations were analyzed using Spearman's rank correlation test. The threshold for significance was set at $P < 0.05$.

RESULTS

Characteristics of the subjects and effects of insulin on glucose metabolism (Table 1). After the subjects fasted overnight, plasma concentrations of insulin, nonesterified fatty acids, and triglycerides were higher in obese and type 2 diabetic patients than in lean control subjects. Type 1 and type 2 diabetic patients had higher fasting glycemia. Basal glucose disposal rate was slightly reduced in obese patients compared with age-matched control subjects and tended to be higher in type 2 diabetic patients, although the difference was not significant ($P = 0.09$). During the hyperinsulinemic clamp, the stimulation by insulin of glucose utilization rate was profoundly reduced by >50% in obese and in type 2 diabetic patients. Both insulin-stimulated nonoxidative glucose disposal and glucose oxidation rates were decreased when compared with healthy lean subjects. In addition, nonesterified fatty acid concentrations remained higher in obese (56 ± 5 μmol/l, $P = 0.003$) and type 2 diabetic patients (74 ± 10 μmol/l, $P = 0.002$) than in age-matched control subjects (28 ± 5 μmol/l) during the clamp. Type 1 diabetic patients displayed a slight reduction (17%) in glucose disposal rate during the clamp, which was very close to being significant ($P = 0.053$) (Table 1). This result thus indicates that the type 1 diabetic patients involved in this study were not characterized by a marked insulin resistance, in contrast to the obese and type 2 diabetic subjects.

Basal mRNA expression pattern in skeletal muscle. We have first investigated the regulation of the expression of nine genes that encode major proteins and enzymes related to insulin action on glucose metabolism. They include insulin receptor, IRS-1, p85αPI3K, p110αPI3K, p110βPI3K, *Ras* protein associated with diabetes (Rad), GLUT4, hexokinase II, and glycogen synthase. The basal concentrations of the nine transcripts in vastus lateralis muscle, determined by RT-cPCR, are presented in Fig. 1.

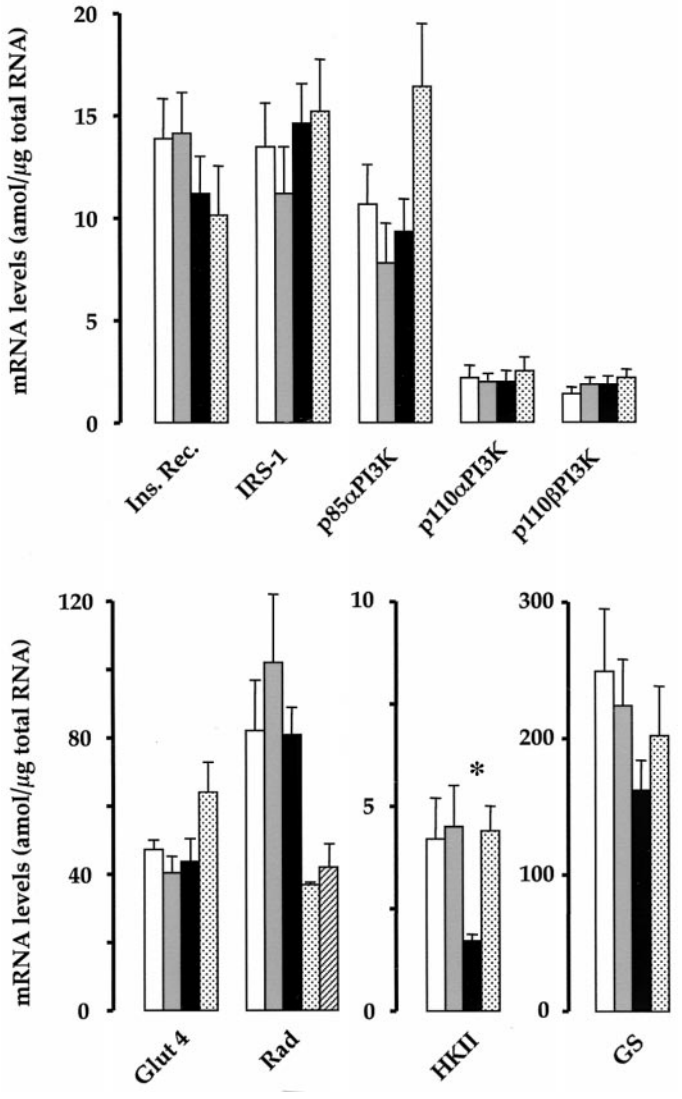

FIG. 1. Comparison of the mRNA levels of the nine target genes in skeletal muscle in the different groups of subjects. Specific mRNA levels were quantified by RT-cPCR in total RNA preparations from vastus lateralis skeletal muscle biopsies in healthy control (□), nondiabetic obese (■), type 2 diabetic (■), and type 1 diabetic (▨) subjects. For clarity, the data of the young control subjects (▨) are shown only for Rad mRNA. For the other target mRNAs, there was no significant difference between the two groups of control subjects. GS, glycogen synthase; HKII, hexokinase II; Ins. Rec., insulin receptor. *$P < 0.01$ vs. age-matched control subjects.

For clarity, the results obtained in young control subjects are not shown, except for Rad mRNA. When the data from the five groups were compared (Kruskal-Wallis analysis), there was no statistical difference between groups in the mRNA levels of insulin receptor, IRS-1, p85αPI3K, p110αPI3K, p110βPI3K, GLUT4, and glycogen synthase. Significant differences were found for Rad ($P = 0.003$) and hexokinase II ($P = 0.001$) mRNA levels. When the same analysis was performed taking into account the data of the age-matched control, nondiabetic obese, and type 2 diabetic subjects, the differences remained significant for hexokinase II ($P < 0.001$) only. Using the nonparametric Mann-Whitney's test, we found that the mRNA levels of hexokinase II were significantly reduced in the skeletal muscle of type 2 diabetic patients with respect to both the control ($P = 0.007$) and the nondiabetic obese ($P = 0.003$) subjects. Regarding Rad, Fig. 1 shows that there was a twofold reduction in Rad mRNA levels in the groups of young subjects (control and type 1 diabetic subjects) when compared with the groups of older subjects ($P < 0.001$), suggesting an age-related difference in the expression of this gene. When all subjects were considered, there was a significant positive correlation ($r = 0.44$, $P = 0.008$) between Rad mRNA and the age of the subjects. Importantly, there was no difference in the expression of Rad between type 2 diabetic patients and age-matched healthy control subjects ($P = 0.954$) or age-matched nondiabetic obese subjects ($P = 0.391$). Glycogen synthase mRNA levels tended to be reduced in the muscle of type 2 diabetic patients when compared with their age-matched control subjects, and the difference was very close to being significant ($P = 0.064$). On the other hand, type 1 diabetic patients showed a tendency for an increased expression of p85αPI3K and GLUT4 mRNAs, but the difference was not significant when tested using the Kruskal-Wallis analysis ($P = 0.222$ and 0.144 for GLUT4 and p85αPI3K mRNA, respectively). However, when the type 1 diabetic patients were directly compared with the young control subjects (Mann-Whitney's test), the diabetic patients had a slightly higher GLUT4 mRNA levels (64 ± 9 vs. 44 ± 3 amol/μg total RNA, $P = 0.043$).

Effects of 3-h insulin infusion on mRNA expression. Table 2 shows the insulin-induced changes in mRNA levels of the nine target genes in the skeletal muscle of the five groups of subjects. The data are presented as percentage changes over the basal (preclamp) values. In the two groups of healthy control subjects, the mRNA levels of p85αPI3K, hexokinase II, and Rad were markedly increased by insulin. The effect on GLUT4 expression was less pronounced (1.5-fold increase), but it was highly significant ($P = 0.011$). In contrast to these four genes, the expression of IRS-1 was significantly reduced after the hyperinsulinemic clamp. Finally, the mRNA levels of insulin receptor, the p110α and p110β catalytic subunits of PI3K, and glycogen synthase were not significantly modified by insulin infusion in control subjects. The effects of insulin in type 1 diabetic patients were similar to what was observed in the control subjects, with significant increases in the mRNA levels of p85αPI3K, hexokinase II, GLUT4, and Rad and a decrease in the expression of IRS-1 (Table 2).

In contrast, in insulin-resistant patients we identified several defects in the regulation of gene expression by insulin. The insulin-induced rise in p85αPI3K, hexokinase II, and GLUT4 mRNA levels was completely blunted in type 2 diabetic patients. Rad mRNA levels were still significantly increased by insulin in type 2 diabetic patients, but the effect of insulin appeared attenuated (~60% increase in diabetic vs. 200% increase in control subjects, $P = 0.064$). As in control subjects, insulin receptor and glycogen synthase mRNA expression did not change in type 2 diabetic subjects. In addition, insulin induced a significant decrease in p110α and p110β PI3K mRNA concentrations in type 2 diabetic muscle that was not observed in any of the other groups (Table 2). Finally, in insulin-resistant nondiabetic obese subjects, an impaired regulation by insulin was observed only for GLUT4 mRNA. All other transcripts responded to insulin in the same way

TABLE 2
Relative effects of insulin infusion on the expression of the target genes in skeletal muscle

	Control 50	Obese	Type 2 diabetes	Control 25	Type 1 diabetes
Insulin receptor	36 ± 20	−8 ± 12	12 ± 20	20 ± 12	32 ± 18
IRS-1	−47 ± 6*	−31 ± 6*	−27 ± 9*	−34 ± 4*	−31 ± 9*
p85αPI3K	99 ± 20*	78 ± 18*	−1 ± 12†	128 ± 46*	67 ± 20*
p110αPI3K	−19 ± 20	18 ± 18	−48 ± 9*†	−7 ± 15	−5 ± 15
p110βPI3K	−23 ± 12	31 ± 17	−42 ± 9*†	12 ± 19	47 ± 32
GLUT4	45 ± 10*	21 ± 9†	17 ± 23†	48 ± 10*	66 ± 31*
Hexokinase II	93 ± 20*	127 ± 35*	9 ± 18†	194 ± 37*	88 ± 16*
Rad	223 ± 68*	112 ± 34*	60 ± 22*	190 ± 37*	339 ± 88*
Glycogen synthase	32 ± 29	−2 ± 21	19 ± 13	15 ± 9	56 ± 45

The results are presented as percentage changes of specific mRNA levels between the values measured at end of the 3-h hyperinsulinemic clamp and the basal values. *Significant change ($P < 0.05$ with Wicoxon's nonparametric test for paired values) when comparing the values before and after insulin infusion. †Significant difference in the effect of insulin in a group of patients when compared with the age-matched control group.

and with similar magnitude in nondiabetic obese and in healthy lean subjects (Table 2).

Figure 2 shows the individual data regarding the regulation by insulin of p85αPI3K and hexokinase II mRNA levels in skeletal muscle, clearly demonstrating that there was a specific defect in the skeletal muscle of type 2 diabetic subjects that was not encountered in insulin-resistant obese subjects and in hyperglycemic type 1 diabetic patients.

Regulation of gene expression in adipose tissue. We further investigated whether the impaired regulation of gene expression observed in skeletal muscle also existed in adipose tissue of type 2 diabetic patients. Due to low yield in total RNA recovery in adipose tissue (~1 μg/100 mg tissue), we did not measure the mRNA levels of all target genes and we first studied insulin receptor, p85αPI3K, hexokinase II, and GLUT4 mRNA expression. Insulin receptor, p85αPI3K, and particularly hexokinase II mRNAs were expressed at higher levels in abdominal subcutaneous fat tissue than in skeletal muscle (Fig. 3). There was no significant difference regarding the mRNA levels of these three genes between the investigated groups (age-matched control, obese, and type 2 diabetic subjects). In contrast, basal GLUT4 mRNA expression was significantly reduced in adipose tissue in obese (17.4 ± 2.7 amol/μg total RNA, $P = 0.006$) and in type 2 diabetic (13.3 ± 1.5 amol/μg total RNA, $P = 0.001$) patients when compared with control subjects (46 ± 7.6 amol/μg total RNA). There was a significant negative correlation between GLUT4 mRNA levels and the BMI of the subjects ($r = -0.68$, $P = 0.041$). As in skeletal muscle, insulin markedly increased p85αPI3K (79 ± 27%, $P = 0.018$), hexokinase II (130 ± 27%, $P = 0.018$), and GLUT4 (114 ± 29%, $P = 0.018$) mRNA expression in adipose tissue of control subjects (Fig. 3). Similar positive effects of insulin were observed in nondiabetic obese patients (44 ± 15, 102 ± 32, and 64 ± 18% for p85αPI3K, hexokinase II, and GLUT4, respectively; $P = 0.018$). In contrast, Fig. 3 clearly shows that the effect of insulin on the mRNA expression of these three genes was, as in muscle, completely impaired in the adipose tissue of type 2 diabetic patients (18 ± 25%, $P = 0.575$, 1 ± 15%, $P = 0.888$ and 4 ± 11%, $P = 0.401$ for p85αPI3K, hexokinase II, and GLUT4, respectively).

Expression of SREBP-1c in skeletal muscle and adipose tissue. Recent evidence supports a crucial role of the transcription factor SREBP-1c in the effect of insulin on the transcription of several genes that encode enzymes of glucose and lipid metabolism (30–32). Therefore, SREBP-1c could potentially be involved in the impaired regulation of gene expression observed in tissues of type 2 diabetic patients. We set up a new RT-cPCR assay for SREBP-1c mRNA and studied its expression and regulation by insulin in skeletal muscle and adipose tissue from age-matched control, nondiabetic obese, and type 2 diabetic subjects ($n = 8$ per group). Figure 4 shows that SREBP-1c mRNA was more abundant in adipose tissue that in skeletal muscle in humans. In adipose tissue, the mRNA expression of SREBP-1c was significantly reduced both in the nondiabetic obese subjects ($P = 0.005$) and the type 2 diabetic patients ($P = 0.009$). Moreover, a significant negative correlation was found between SREBP-1c mRNA levels in adipose tissue and the BMI of the subjects ($r = -0.633$, $P = 0.022$). In skeletal muscle, the expression of SREBP-1c mRNA was not significantly different between groups, although it tended to be lower in type 2 diabetic patients than in control subjects ($P = 0.083$). Three hours of hyperinsulinemia produced a two- to threefold increase in SREBP-1c mRNA expression in skeletal muscle ($P = 0.025$) and in subcutaneous adipose tissue ($P = 0.012$) in control subjects. Similar upregulation was observed in tissues from nondiabetic obese subjects ($P = 0.028$ in muscle and $P = 0.042$ in adipose tissue). In contrast, the effect of insulin was completely impaired in tissues of type 2 diabetic patients ($P = 0.124$ in muscle and $P = 0.123$ in adipose tissue for the difference in SREBP-1c mRNA level after versus before clamp).

DISCUSSION

One of the main objectives of the present work was to verify whether the regulation of genes other than those encoding GLUT4, hexokinase II, and p85αPI3K (20–23) could be altered, in a concerted manner, in tissues of type 2 diabetic patients. This was important to strengthen the hypothesis that, under the same experimental conditions, the regulation of a cluster of genes may be impaired during type 2 diabetes. To this end, we measured, in parallel in the same samples, the regulation by insulin of the expression of 10 candidate genes using validated RT-cPCR assays.

The second objective of the work was to define whether the observed defects in the regulation of gene expression resulted from a specific alteration in type 2 diabetes or were

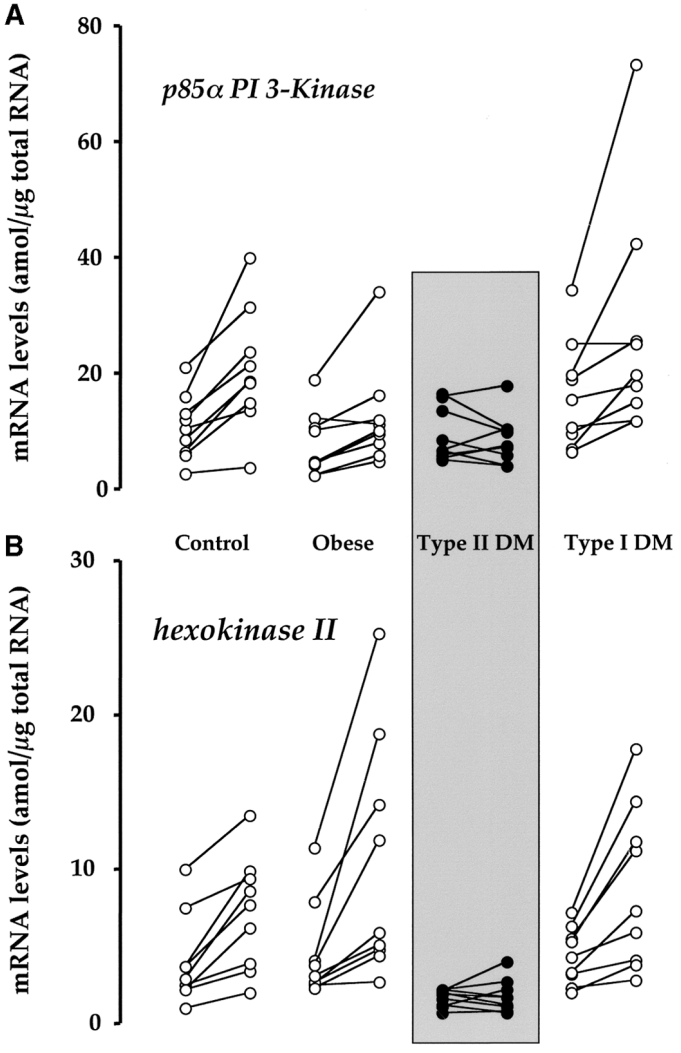

FIG. 2. Effect of insulin infusion on the mRNA levels of p85αPI3K (*A*) and hexokinase II (*B*) in skeletal muscle. The mRNA levels of the target genes were measured before and at the end of a 3-h euglycemic-hyperinsulinemic clamp. The individual data obtained in 50-year-old control (Control), nondiabetic obese (Obese), type 2 diabetic (Type II DM), and type 1 diabetic (Type I DM) subjects are presented.

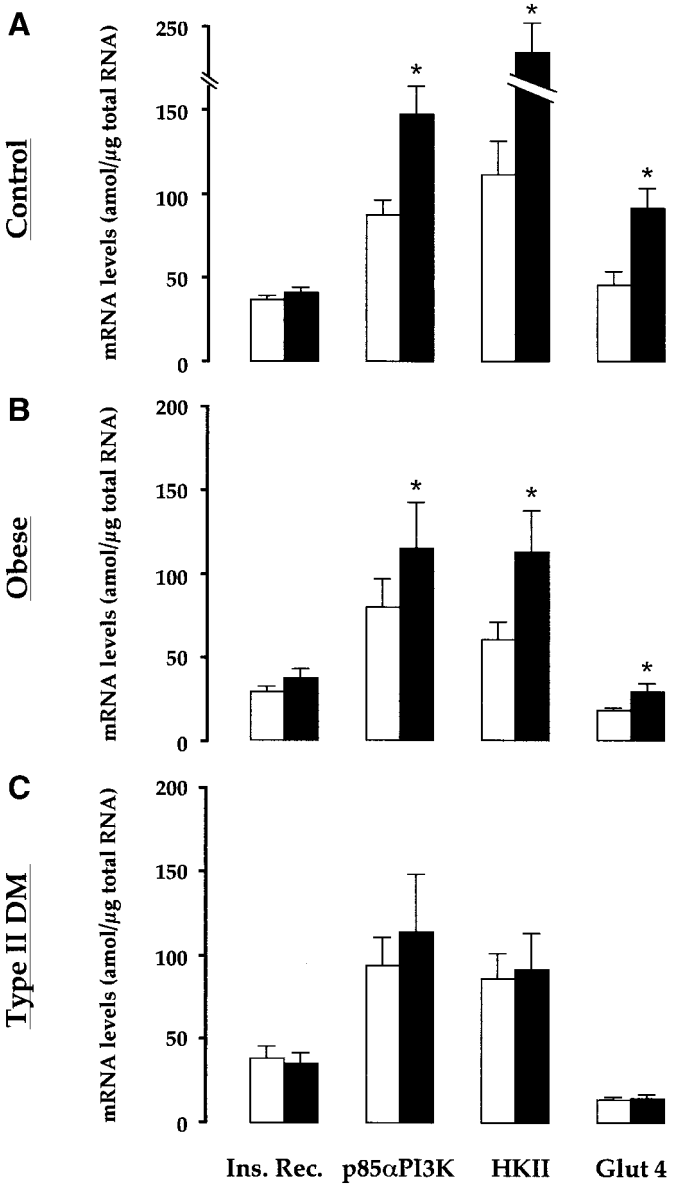

FIG. 3. Effects of insulin infusion on the mRNA levels of insulin receptor, p85αPI3K, hexokinase II, and GLUT4 in adipose tissue. The mRNA levels of the target genes were measured in subcutaneous abdominal adipose tissue biopsies taken before (□) and at the end (■) of a 3-h hyperinsulinemic clamp. The experiment was performed in age-matched control (*A*), nondiabetic obese (*B*), and type 2 diabetic (*C*) subjects. **P* < 0.05 after vs. before 3 h of insulin infusion.

secondary to the metabolic state of the patients. Therefore, the regulation of the candidate genes was studied in tissues of healthy control subjects, type 2 diabetic patients, nondiabetic obese subjects, and type 1 diabetic patients, in parallel. These groups of subjects were selected to verify the contribution of either obesity-related insulin resistance (nondiabetic obese subjects) or chronic hyperglycemia (type 1 diabetic patients) on the defective regulation of gene expression observed in type 2 diabetic patients.

The expression of GLUT4, hexokinase II, glycogen synthase, insulin receptor, and IRS-1 mRNAs in muscle or adipose tissue of type 2 diabetic patients has been previously reported (15–23). The between-group differences found in the present study were globally in agreement with what was previously observed for these genes (15–23). For example, we confirmed the marked reduction in the mRNA level of hexokinase II in the muscle of type 2 diabetic patients (15,22). Interestingly, we showed that this reduction was not observed in subcutaneous adipose tissue, indicating thus a tissue-specific alteration. In addition, our work provided several new pieces of information. We demonstrated that the basal mRNA expression of the p110α and p110β catalytic subunits of PI3K was not altered in the muscle of insulin-resistant subjects. There is thus no defect in the basal expression of the main actors of insulin signaling (insulin receptor, IRS-1, and the regulatory and catalytic subunits of PI3K) in the skeletal muscle of type 2 diabetic patients. We also found that Rad mRNA concentration was similar in muscle of age-matched lean, obese, and type 2 diabetic subjects, confirming previous studies (33). Nevertheless, we observed a significant positive correlation between Rad mRNA and the age of the subjects, thus suggesting that Rad expression increases with age in skeletal muscle, independently of obesity and diabetes. Finally, we demonstrated that SREBP-1c mRNA expression is profoundly decreased in subcutaneous adi-

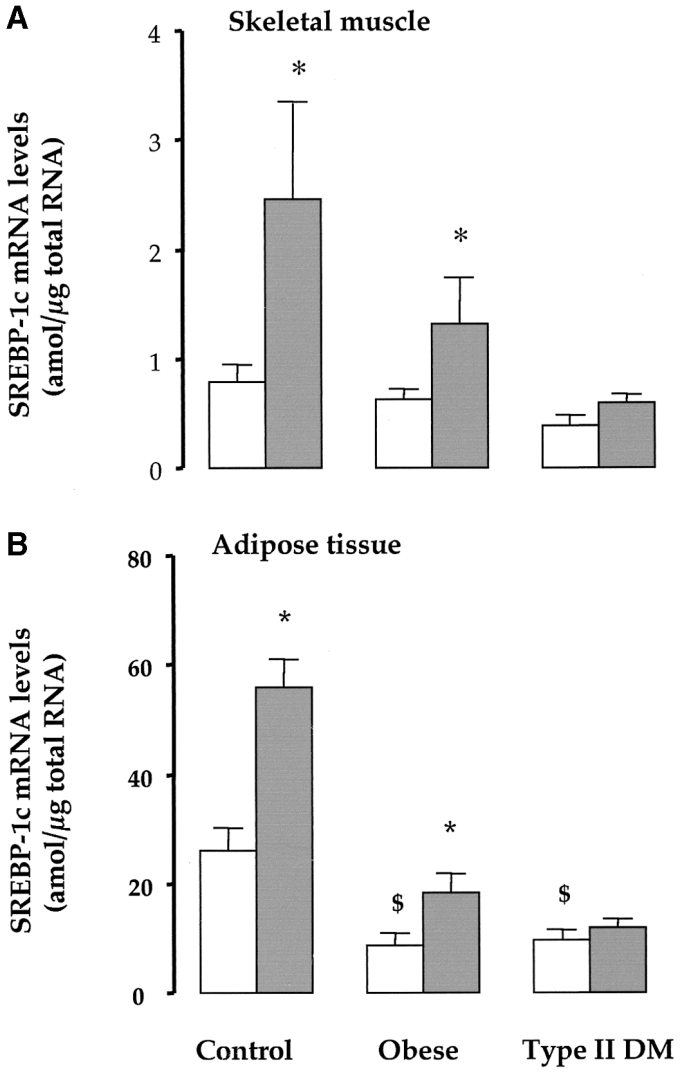

FIG. 4. Effects of insulin infusion on the mRNA levels of SREBP-1c in skeletal muscle and in subcutaneous abdominal adipose tissue. The mRNA levels of SREBP-1c were measured in muscle (*A*) and in adipose tissue (*B*) biopsies taken before (□) and at the end (■) of a 3-h hyperinsulinemic clamp. The experiment was performed in age-matched control, nondiabetic obese, and type 2 diabetic subjects (n = 8 per group). *P < 0.05 after vs. before 3 h of insulin infusion and \$$P$ < 0.05 obese or diabetic patients vs. control subjects.

pose tissue of nondiabetic and type 2 diabetic obese subjects, while there was no significant difference between groups in skeletal muscle. The reduction in SREBP-1c mRNA in adipose tissue correlated with the BMI of the subjects, indicating an association between obesity and the downregulation of this transcription factor. This observation, made in human fat tissue, was in keeping with recent results obtained in mice, using DNA microarray technology, that showed that the expression of SREBP-1c is two- to threefold decreased in white adipose tissue of obese and diabetic animals (34,35).

Hyperinsulinemia produced several changes in the mRNA levels of the investigated genes. Under our experimental conditions (insulin concentration of ~1,000 pmol/l for 3 h), these genes could be classified into the following four categories: *1*) those that were not regulated by insulin in any of the groups of subjects; *2*) those that are regulated (upregulated or downregulated) by insulin in a similar way in all groups; *3*) those with an impaired regulation by insulin in insulin resistance (nondiabetic obese and type 2 diabetic patients); and *4*) those with an altered regulation by insulin in type 2 diabetic patients specifically.

The mRNA expression of insulin receptor and glycogen synthase was not modified in skeletal muscle after 3 h of insulin infusion, in any of the groups. This was in agreement with most of the preceding studies (23,24,36).

IRS-1 mRNA levels were significantly reduced and Rad expression markedly increased in skeletal muscle in all groups, although the magnitude of the insulin effect on Rad mRNA seemed lower in type 2 diabetic patients. These results indicated thus that the regulation of IRS-1 and Rad mRNA expression are not significantly affected by obesity, insulin resistance, or diabetes in skeletal muscle.

The regulation by insulin of GLUT4 mRNA expression in muscle was altered both in nondiabetic obese and type 2 diabetic patients—the two groups of frankly insulin-resistant subjects. These data thus suggest that the impaired regulation of GLUT4 expression by insulin may result from the reduced insulin sensitivity of these patients. Interestingly, we found a normal induction of GLUT4 mRNA in response to insulin infusion in skeletal muscle of type 1 diabetic patients, whereas other investigators have previously reported an impaired regulation of GLUT4 expression in these subjects (26). Type 1 diabetic patients are classically considered as mildly insulin resistant (26,37,38). The difference between our results and those of Yki-Järvinen et al. (26) are likely to be due to the higher levels of hyperinsulinemia maintained during the clamp in our study (±1,000 vs. 700 pmol/l). When moderate concentrations of insulin were used during the clamp, a significant reduction in insulin-induced glucose disposal rate has been observed in type 1 diabetic patients (26,37,38). Here, with a higher level of hyperinsulinemia, we found only a slight reduction in insulin-induced glucose utilization when compared with control subjects. This finding clearly indicated that insulin resistance in type 1 diabetic subjects was compensated when the concentration of insulin was increased. Under such conditions, the regulation of gene expression by insulin was found to be similar in control subjects and in type 1 diabetic patients. Taken together, these results strongly support the assumption that the regulation of GLUT4 gene by insulin is firmly associated with the responsiveness of the tissue to insulin.

Insulin induced a significant reduction in the mRNA levels of the two p110 catalytic subunits of PI3K in the muscle of type 2 diabetic patients. This result was not observed in the other groups of subjects. The consequences of this downregulation of p110αPI3K and p110βPI3K mRNAs are yet unknown but will require further studies to verify, at the protein and kinase activity levels, whether this regulatory mechanism may play a role in the transduction of the insulin signal.

The insulin-induced regulation of p85αPI3K, hexokinase II, and SREBP-1c mRNA expression was impaired only in type 2 diabetic patients, both in skeletal muscle and subcutaneous adipose tissue. Because the regulation of these three genes was normal in insulin-resistant nondiabetic obese subjects and in type 1 diabetic patients, one might thus suggest that the observed defects in type 2 diabetes were not secondary to insulin resistance, obesity,

or chronic hyperglycemia. It has been recently reported, however, that the regulation by insulin of hexokinase II expression in skeletal muscle was impaired not only in type 2 diabetic but also in nondiabetic obese subjects when low levels of insulin were maintained during the clamp (400–500 pmol/l) (22). Moreover, the effect of insulin was restored, in both groups, in the presence of very high concentrations (4,000 pmol/l) of insulin (22). With intermediate levels of hyperinsulinemia (1,000 pmol/l), we found that the defective regulation of hexokinase II gene expression was observed in the tissues of the type 2 diabetic patients specifically. Taken together, these results suggest that the concentration of insulin required to compensate for insulin resistance is an important parameter in the regulation of gene expression. However, under our experimental conditions, both the nondiabetic obese subjects and the type 2 diabetic patients had a similar level of insulin resistance as assessed by measurement of glucose disposal rate during the hyperinsulinemic clamp. This finding thus suggests that insulin resistance may not be the only cause of the defective regulation of gene expression in type 2 diabetes.

In addition to insulin resistance, a defect in the transcriptional machinery could contribute to the observed alterations. This attractive hypothesis is supported by the recent identification of mutated transcription factors in subtypes of maturity-onset diabetes of the young (39,40) and in other particular forms of type 2 diabetes (13,14). If this also occurs in the common form of type 2 diabetes, one can predict that the impaired regulation of gene expression may play a primary role in the pathogenesis of the disease. Recently, the transcription factor SREBP-1c has been involved in the effect of insulin on the transcription of several genes that encode enzymes of glucose and lipid metabolism (30–32). Moreover, overexpression of SREBP-1c in adipose tissue in mice is associated with insulin resistance, diabetes, and lipodystrophy (41). The promoter regions of hexokinase II and p85 αPI3K genes (E. Lefai and H. Vidal, unpublished observations) contain several SRE and E-box sequences that could be putative response elements for SREBP-1c. In addition, there is evidence that SREBP-1c could control the expression of its own gene (34). Therefore, SREBP-1c is a potential candidate to participate in the defective regulation of gene expression observed in type 2 diabetes. We have found that SREBP-1c mRNA expression is decreased in subcutaneous adipose tissue of type 2 diabetic obese subjects. However, this observed reduction is not likely to play a predominant role in the defective regulation of gene expression in type 2 diabetes. There was indeed no major alteration in the basal mRNA levels of SREBP-1c in skeletal muscle, while the defective regulation of gene expression in response to insulin was observed in both adipose tissue and skeletal muscle. In addition, the reduction in SREBP-1c in adipose tissue appeared to be mainly associated with obesity, and we have found that the regulation of gene expression by insulin was not altered in the tissues of nondiabetic obese subjects. However, it has been recently shown that, in addition to upregulating SREBP-1c gene expression (42,43), insulin also activates SREBP-1c transcriptional activity (43). Therefore, involvement of this transcription factor in the regulation of gene expression in human tissues and its putative role in the defective regulation observed in type 2 diabetes require further investigation.

The regulation of hexokinase II, p85αPI3K, and SREBP-1c gene expression is altered in skeletal muscle and adipose tissue of type 2 diabetic patients. These three genes could thus belong to a cluster of genes with impaired regulation by insulin in type 2 diabetes. In keeping with such a hypothesis, a common mechanism involved in their regulation, and probably of other yet unidentified genes, should be altered in the peripheral tissues of type 2 diabetic patients. Importantly in this context, it has been reported that insulin requires the PI3K pathway to control the expression of hexokinase II and p85αPI3K genes at the transcriptional level in cultured muscle cells (44,45). In hepatocyte, the same pathway is also involved in the effects of insulin on SREBP-1c expression and activation (43). Moreover, it has been clearly demonstrated that the activation by insulin of the PI3K pathway is altered in muscle of type 2 diabetic patients (8,9). Therefore, altered transmission of the insulin signal through the PI3K pathway could be involved in the impaired regulation of gene expression. However, the same pathway is also required in the actions of insulin on glucose metabolism (4,5), and we have shown that the response of the three genes to insulin was normal in tissues of insulin-resistant nondiabetic obese subjects. This finding suggests that the pathways involved in the regulation of gene expression and in the control of glucose metabolism may diverge after the activation of the PI3K and that type 2 diabetes may have additional defects in the pathway leading to the transcriptional regulation in the nuclei. Further works are clearly needed to decipher the mechanism of action of insulin from its receptor to the promoters of its target genes and to identify a common element that may be involved in the altered regulation of a cluster of genes.

In summary, insulin modulates in a coordinate fashion the mRNA levels of several genes involved in insulin action and glucose metabolism in skeletal muscle and in adipose tissue. We have found that the regulation of p85αPI3K, hexokinase II, and SREBP-1c gene expression by insulin is impaired in the tissues of type 2 diabetic patients. This defect appears to be independent from obesity-related insulin resistance and chronic hyperglycemia. These results suggest that type 2 diabetes may be associated with a specific alteration in the signaling to the nucleus or in the transcriptional machinery.

ACKNOWLEDGMENTS

This work was supported in part by a grant from INSERM (PROGRES no. 4P020D).

The authors acknowledge C. Urbain, J. Peyrat, and M. Odeon for excellent technical assistance.

REFERENCES

1. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15:318–368, 1992
2. Yki-Järvinen H: Role of insulin resistance in the pathogenesis of NIDDM. *Diabetologia* 38:1378–1388, 1995
3. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn RC: Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet* 340:925–929, 1992
4. Kahn RC: Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes* 43:1066–1084, 1994

5. Shepherd PR, Navé BT, O'Rahilly SO: The role of phosphoinositide 3-kinase in insulin signalling. *J Mol Endocrinol* 17:175–184, 1996
6. 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. *Diabetologia* 30:437–440, 1987
7. Björnholm M, Kawano Y, Lehtihet M, Zierath JR: Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after in vivo insulin stimulation. *Diabetes* 46:524–527, 1997
8. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ: Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest* 105:311–320, 2000
9. Krook A, Björnholm M, Galuska D, Jiang XJ, Fahlman R, Myers MG, Wallberg-Henriksson H, Zierath JR: Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. *Diabetes* 49:284–292, 2000
10. Krook A, Roth RA, Jiang XJ, Zierath JR, Wallberg-Henriksson H: Insulin-stimulated Akt kinase activity is reduced in skeletal muscle from non-insulin-dependent diabetic subjects. *Diabetes* 47:1281–1286, 1998
11. Damsbo P, Vaag A, Hother-Nielsen O, Beck-Nielsen H: Reduced glycogen synthase activity in skeletal muscle from obese patients with and without type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 34:239–245, 1991
12. O'Brien RM, Granner DK: Regulation of gene expression by insulin. *Physiol Rev* 76:1109–1161, 1996
13. Brunetti A, Brunetti L, Foti D, Accili D, Goldfine ID: Human diabetes associated with defects in nuclear regulatory proteins for the insulin receptor gene. *J Clin Invest* 97:258–262, 1997
14. Barroso I, Gurnell M, Crowley VE, Agostini M, Schwabe JW, Soos MA, Maslen GL, Williams TD, Lewis H, Schafer AJ, Chatterjee VK, O'Rahilly S: Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature* 402:880–883, 1999
15. Vestergaard H, Bjorbaek C, Hansen T, Larsen FS, Granner DK, Pedersen O: Impaired activity and gene expression of hexokinase II in muscle from non-insulin-dependent diabetes mellitus patients. *J Clin Invest* 96:2639–2645, 1995
16. Andreelli F, Laville M, Vega N, Riou J-P, Vidal H: Regulation of gene expression during severe caloric restriction: lack of induction of p85α phosphatidylinositol 3-kinase mRNA in skeletal muscle of patients with type II (non-insulin-dependent) diabetes mellitus. *Diabetologia* 43:356–363, 2000
17. Vestergaard H, Lund S, Larsen FS, Bjerrum OJ, Pedersen O: Glycogen synthase and phosphofructokinase protein and mRNA levels in skeletal muscle from insulin-resistant patients with non-insulin-dependent diabetes mellitus. *J Clin Invest* 91:2342–2350, 1993
18. Garvey WT, Maianu L, Huecksteadt TP, Birnbaum MJ, Molina JM, Ciaraldi TP: Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with non-insulin-dependent diabetes mellitus and obesity. *J Clin Invest* 87:1072–1081, 1991
19. Rondinone CM, Wang L-M, Lonnroth P, Wesslau C, Pierce JH, Smith U: Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus. *Proc Natl Acad Sci U S A* 94:4171–4175, 1997
20. Andersen PH, Lund S, Vestergaard H, Junker S, Kahn BB, Pedersen O: Expression of the major insulin regulatable glucose transporter (Glut 4) in skeletal muscle of non insulin dependent diabetic patients and healthy subjects before and after insulin infusion. *J Clin Endocrinol Metab* 77:27–32, 1993
21. Schalin-Jänti C, Yki-Järvinen H, Koranyi L, Bourey R, Lindstrom J, Nikulas-Ijas O, Franssila-Kallunki A, Groop LC: Effect of insulin on Glut 4 mRNA and protein concentrations in skeletal muscle of patients with NIDDM and their first-degree relatives. *Diabetologia* 37:401–407, 1994
22. Pendergrass M, Koval J, Vogt C, Yki-Järvinen H, Iozzo P, Pipek R, Ardehali H, Printz R, Granner D, DeFronzo RA, Mandarino LJ: Insulin-induced hexokinase II expression is reduced in obesity and NIDDM. *Diabetes* 47:387–394, 1998
23. Andreelli F, Laville M, Ducluzeau P-H, Vega N, Vallier P, Khalfallah Y, Riou J-P, Vidal H: Defective regulation of phosphatidylinositol 3-kinase gene expression in skeletal muscle and adipose tissue of non-insulin-dependent diabetes mellitus patients. *Diabetologia* 42:358–364, 1999
24. Laville M, Auboeuf D, Khalfallah Y, Vega N, Riou JP, Vidal H: Acute regulation by insulin of phosphatidylinositol-3-kinase, Rad, Glut 4 and lipoprotein lipase mRNA levels in human muscle. *J Clin Invest* 98:43–49, 1996
25. Laville M, Rigalleau V, Riou J-P, Beylot M: Respective role of plasma non esterified fatty acid oxidation and total lipid oxidation in lipid-induced insulin resistance. *Metabolism* 44:639–644, 1995
26. Yki-Järvinen H, Vuorinen-Markkola H, Korany L, Bourey R, Tordjman K, Mueckler M, Permutt AM, Koivisto VA: Defect in insulin action on expression of the muscle/adipose tissue glucose transporter gene in skeletal muscle of type 1 diabetic patients. *J Clin Endocrinol Metab* 75:795–799, 1992
27. Vidal H, Auboeuf D, De Vos P, Staels B, Riou J-P, Auwerx J, Laville M: The expression of *ob* gene is not acutely regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. *J Clin Invest* 98:251–255, 1996
28. Chomczynski P, Sacchi N: Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
29. Auboeuf D, Vidal H: The use of the reverse transcription–competitive polymerase chain reaction to investigate the in vivo regulation of gene expression in small tissue samples. *Anal Biochem* 245:141–148, 1997
30. Kim JB, Sarraf P, Wright M, Yao KM, Mueller E, Solanes G, Lowell BB, Spiegelman B: Nutritional and insulin regulation of fatty acid synthase and leptin gene expression through ADD1/SREBP-1. *J Clin Invest* 101:1–9, 1998
31. Foretz M, Pacot C, Dugail I, Lemarchand P, Guichard CC, Le Liepvre X, Berthelier-Lubrano C, Spiegelman B, Kim JB, Ferré P, Foufelle F: ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Mol Cell Biol* 19:3760–3768, 1999
32. Foretz M, Guichard C, Ferré P, Foufelle F: Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc Natl Acad Sci U S A* 96:12737–12742, 1999
33. Garvey WT, Maianu L, Kennedy A, Wallace P, Ganaway E, Hamacher LL, Yarnall DP, Lenhard JM, Burns DK: Muscle Rad expression and human metabolism: potential role of the novel Ras-related GTPase in energy expenditure and body composition. *Diabetes* 46:444–450, 1997
34. Soukas A, Cohen P, Socci ND, Friedman JM: Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev* 14:963–980, 2000
35. Nadler ST, Stoehr JP, Schueler KL, Tanimoto G, Yandell BS, Attie AD: The expression of adipogenic genes is decreased in obesity and diabetes mellitus. *Proc Natl Acad Sci U S A* 97:11371–11376, 2000
36. Mandarino LJ, Printz RL, Cusi KA, Kinchington P, O'Doherty RM, Osawa H, Sewell C, Consoli A, Granner DK, DeFronzo RA: Regulation of hexokinase II and glycogen synthase mRNA, protein activity in human muscle. *Am J Physiol* 269: E701–E708, 1995
37. DeFronzo RA, Hendler R, Simonson D: Insulin resistance is a prominent feature of insulin-dependent diabetes. *Diabetes* 31:795–801, 1982
38. Hother-Nielsen O, Schmitz O, Bak J, Beck-Nielsen H: Enhanced hepatic insulin sensitivity, but peripheral insulin resistance in patients with type 1 (insulin-dependent) diabetes. *Diabetologia* 30:834–840, 1987
39. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Turner RC, Velho G, Chèvre JC, Froguel P, Bell GI: Mutations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young (MODY3). *Nature* 384:455–458, 1996
40. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4α gene in maturity-onset diabetes of the young (MODY1). *Nature* 384:458–460, 1996
41. Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, Brown MS: Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. *Genes Dev* 12:3182–3194, 1998
42. Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL: Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc Natl Acad Sci U S A* 96:13656–13661, 1999
43. Azzout-Marniche D, Bécard D, Guicharrd CC, Foretz M, Ferré P, Foufelle F: Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes. *Biochem J* 350:389–393, 2000
44. Osawa H, Sutherland C, Robey RB, Printz RL, Granner DK: Analysis of the signaling pathway involved in the regulation of hexokinase II gene transcription by insulin. *J Biol Chem* 271:16690–16694, 1996
45. Roques M, Vidal H: A phosphatidylinositol 3-kinase/p70 ribosomal S6 protein kinase pathway is required for the regulation by insulin of the p85α regulatory subunit of phosphatidylinositol 3-kinase gene expression in human muscle cells. *J Biol Chem* 274:34005–34010, 1999