Increments in insulin sensitivity during intensive treatment are closely correlated with decrements in glucocorticoid receptor mRNA in skeletal muscle from patients with Type II diabetes

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ABSTRACT

To test the hypothesis that changes in the expression of the glucocorticoid receptor (GCR) and the \(\beta_2\)-adrenoceptor (\(\beta_2\)-AR) contribute significantly to the abnormal glucose metabolism in skeletal muscle from patients with Type II diabetes, we have examined (1) the levels of total GCR (\(\alpha_{1}+\beta\) isoforms), the \(\alpha/\alpha_2\) isoform of GCR and \(\beta_2\)-AR mRNAs in skeletal muscle from insulin-resistant patients with Type II diabetes (\(n = 10\)) and healthy controls (\(n = 15\)), and (2) the effects of 8 weeks of intensive treatment on the whole-body glucose disposal rate and on total GCR, \(\alpha/\alpha_2\) GCR and \(\beta_2\)-AR mRNA levels in diabetic patients. The total glucose disposal rate was measured by the euglycaemic hyperinsulinaemic (2 m-units \(\text{[min}^{-1}\text{kg}^{-1}\)) clamp technique, and mRNA levels were assessed by reverse transcriptase–PCR and HPLC for separation of standard and unknown and quantification. Mean levels of total GCR and \(\alpha/\alpha_2\) GCR mRNAs were increased in patients with Type II diabetes when compared with control subjects (total GCR, 2.06 \(\pm\) 0.30 and 1.47 \(\pm\) 0.10 amol/\(\mu\)g of total RNA respectively (\(P = 0.09\)); \(\alpha/\alpha_2\) GCR mRNA, 1.69 \(\pm\) 0.31 and 0.92 \(\pm\) 0.09 amol/\(\mu\)g of total RNA respectively (\(P = 0.02\)), whereas mRNA levels of the \(\beta\) isoform of GCR (total GCR minus \(\alpha/\alpha_2\) GCR) were decreased (\(P = 0.006\)). \(\beta_2\)-AR mRNA levels were comparable in diabetic patients and control subjects (0.53 \(\pm\) 0.05 and 0.45 \(\pm\) 0.02 amol/\(\mu\)g of total RNA respectively; \(P = 0.2\)). Intensive treatment for 8 weeks was associated with improved glycaemic control (\(P = 0.019\)), and during the clamp a 75\% (\(P = 0.001\)) increase in the whole-body insulin-stimulated glucose disposal rate was demonstrated. Total GCR (\(P = 0.005\)), \(\alpha/\alpha_2\) GCR (\(P = 0.005\)) and \(\beta_2\)-AR (\(P = 0.03\)) mRNA levels all decreased significantly after intensive insulin treatment. A close correlation was found between increments in glucose uptake during intensive treatment and decrements in skeletal muscle total GCR mRNA (\(r = 0.95, P < 0.001\); multiple regression analysis), and between glucose uptake and \(\alpha/\alpha_2\) GCR mRNA levels (\(r = 0.88, P < 0.001\); simple correlation). In conclusion, the abnormal regulation of GCR mRNA is likely to play a significant role in the insulin resistance observed in obese patients with Type II diabetes.

INTRODUCTION

Decreased whole-body insulin sensitivity, or insulin resistance, defined as a lower than expected effect of insulin on glucose uptake, plays a significant role in numerous conditions, including pathological entities such as Type II (non-insulin-dependent) diabetes mellitus and obesity, and physiological states such as a high

Key words: clamp, glucocorticoid receptor mRNA, insulin resistance, obesity.
Abbreviations: AR, adrenoceptor; FFM, fat-free body mass; GCR, glucocorticoid receptor; HbA\(_{1c}\), glycated haemoglobin; RT, reverse transcriptase.
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intake of saturated fat and physical inactivity [1,2]. In most patients with Type II diabetes, both the peripheral tissues (muscle and adipose tissue) and the liver are insulin resistant, and the patients are furthermore characterized by an abnormal pancreatic β-cell response to glucose [3]. Under clamp conditions of euglycaemia and hyperinsulinaemia, glucose uptake in patients with Type II diabetes is characteristically decreased by 30–50% compared with that of controls [2]. Treatment with sulphonylurea drugs [4], diet [5] and insulin [6] partially reverses the insulin insensitivity of patients with Type II diabetes.

Upper-body obesity is an important risk factor for the development of Type II diabetes, and most patients with Type II diabetes are obese [7]. It has been shown that the insulin resistance associated with obesity is clearly more pronounced in subjects with centrally localized obesity [7]. The mechanism behind the development of upper-body obesity is unknown, but this condition may result from defects in the breakdown of triacylglycerols (triacylglycerides) by lipolysis in adipocytes. In humans, catecholamines are the most important lipolytic hormones [7]. Lipolysis in adipocytes is stimulated by catecholamines through β1-adrenoceptors (β1-ARs) and β2-ARs. However, cortisol also has an influence on lipid mobilization through a permissive effect on catecholamine-dependent lipid mobilization [7].

Little is known about ARs and glucocorticoid receptors (GCRs) in obesity. Previous studies of obese non-diabetic subjects has shown a significant decrease in adipocyte lipolytic β2-AR sensitivity and receptor number, whereas β1-AR mRNA levels were normal [8,9]. In recent studies it was found that GCR number was decreased in mononuclear leucocytes from obese non-diabetic subjects, and a positive correlation was found between receptor number and insulin sensitivity [10,11]. These studies indicate the existence of lipolytic resistance in obesity, which may be of importance for insulin resistance.

To gain further insight into the abnormal glucose metabolism in patients with Type II diabetes, we have (1) examined under basal conditions the gene expression of the GCR and the β2-AR in muscle from patients with Type II diabetes and healthy control subjects; and (2) examined in patients with Type II diabetes the effects of 8 weeks of intensive treatment with the sulphonylurea drug gliclazide, insulin or diet on the inter-relationships between the whole-body glucose disposal rate and the gene expression of the GCR and β2-AR in muscle, using the euglycaemic hyperinsulinaemic clamp technique.

METHODS

Subjects

Altogether, 10 patients with Type II diabetes and 15 healthy control subjects participated in the study. A subgroup of the control subjects has been studied previously [12]. All subjects were Caucasians, and had sedentary lifestyles. The control subjects had normal fasting plasma glucose, normal blood pressure and no family history of diabetes or other known insulin-resistant states. Only patients with Type II diabetes as defined by the National Diabetes Data Group [13], with a fasting serum C-peptide level of > 0.3 nmol/l, were included in the protocol. The patients had no clinical or biochemical signs of diabetic complications. None of the participants in the study suffered from liver or kidney disease, as evaluated by clinical and standard laboratory examinations, and no subject was taking any other medication that is known to influence glucose and lipid metabolism. All anti-hyperglycaemic medication was withdrawn 2 weeks prior to the study. Clinical data are presented in Table 2 (see below).

The protocol was approved by the local Ethics Committee of Copenhagen, and was in accordance with the Declaration of Helsinki. All participants gave informed consent.

Study protocol

All experiments were started at 08.00 hours after a 10 h overnight fast. A venous blood sample was drawn, and a single percutaneous muscle biopsy was obtained from each control subject. Insulin resistance was estimated using the HOMA model [14]. The muscle biopsies from patients with Type II diabetes were obtained on the day of the euglycaemic hyperinsulinaemic clamp. The diabetic patients were studied twice, before and after 8 weeks of intensive treatment with diet plus gliclazide (n = 4), diet plus insulin (n = 2) or diet alone (n = 4).

Each patient with Type II diabetes was prescribed an individualized diet by a dietician; the diet typically contained 45% carbohydrate, 40% fat and 15% protein (by wt.). The dietary treatment was continued throughout the period of the study. The participants were instructed to continue their usual physical activity. During the 8 weeks of intensive diabetes treatment, patients were seen regularly in the outpatient clinic in order to confirm that the prescribed diet was being adhered to and, at the same time, for blood glucose monitoring in order to evaluate whether the dose of sulphonylurea or insulin should be increased.

Euglycaemic hyperinsulinaemic clamp

All patients with Type II diabetes underwent a 6 h euglycaemic hyperinsulinaemic clamp after a 10 h overnight fast, both before and after 8 weeks of intensive treatment. No medications were given on the day of the clamp, and major physical activity was avoided for 24 h before examination. Details of the clamp technique have been described previously [12,15]. Total insulin-stimulated glucose uptake was calculated from the rate of
infusion of glucose during steady state. Steady state was
defined as the last 30 min of insulin infusion (240 min).

**Muscle biopsies**
Percutaneous muscle biopsies were obtained under local
anaesthesia (1% lidocaine without adrenaline) from the
vastus lateralis muscle, approx. 20 cm above the knee,
using a modified Bergström needle (Stille-Werner,
Copenhagen, Denmark). Muscle samples were blotted to
remove blood and were frozen within 30 s in liquid
nitrogen, and stored at −80 °C until assayed.

**Other analytical procedures**
Glucose in plasma and in urine was measured by a
hexokinase method [16]. Serum insulin and C-peptide
concentrations were analysed by a two-site sandwich
ELISA method and RIA respectively [17,18]. Glycated
haemoglobin (HbA1c) was measured by an HPLC
method (normal range 4.1–6.1%). Non-esterified fatty
acids in plasma were determined by the method of Itaya
and Michio [19]. Fat-free body mass (FFM) was measured
using an impedance technique [20].

**Isolation of RNA, and determination of total RNA and DNA**
RNA was isolated from approx. 10 mg of muscle tissue
by the following method: (1) homogenization in an RLT-
buffer (Qiagen) containing β-mercaptoethanol, followed
by (2) extraction using a mixture of phenol/chloro-
form/3-methylbutan-1-ol (50:49:1, by vol.) and (3)
isoamyl alcohol using a QIAamp kit (Qiagen). After
the lysis step, an aliquot of 20 μl was removed for the
determination of DNA concentration. Elution was with
200 μl of diethyl pyrocarbonate-treated water. Samples
of 70 μl were used for measuring the total RNA con-
centration (Pharmacia Gene-Quant II). The DNA
centration was measured by H33258 fluorescence
(DyNAQuant 200 apparatus; Hoefer Pharmacia Bio-
tech).

**Reverse transcription**
Before reverse transcription, the RNA was treated with
1 unit of RQ1 RNase-free DNase (Promega) for 15 min
at 37 °C. The DNase was subsequently heat-inactivated
by incubation at 60 °C for 5 min.

The reverse transcription mixture contained RNA from
typically 0.5 mg of muscle tissue, internal standard
RNA (see Table 1), 225 pmol of 3′-primer, 1 mM of each
dNTP, 60 units of MMLV (Moloney murine leukaemia
virus) reverse transcriptase (RT) (Promega) and 40 units
of RNA-Guard in 25 μl of Promega RT-buffer (50 mM
Tris/HCl, pH 8.3, 75 mM KCl, 3 mM MgCl2 and
10 mM dithiothreitol). Incubation was at 37 °C for
60 min, and the cDNA was stored frozen at −80 °C
or used immediately.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Amounts of internal standards added to PCR, and annealing temperatures</th>
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<tr>
<td></td>
<td>GCR (α + β)</td>
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<td>Internal standard (amol)</td>
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<tr>
<td>Annealing temp. (°C)</td>
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</table>

**Quantification of GCR and β2-AR mRNAs in muscle tissue**
mRNA in muscle biopsies was quantified by RT-
PCR/HPLC. The technique has been described in detail
elsewhere [21,22].

**Primers and construction of internal standards**
The oligonucleotide primers were synthesized at DNA
Technology (Aarhus, Denmark). For quantification of
total GCR mRNA we used the following primers, which
do not distinguish between differentially spliced α/α2
and β transcripts (U01351): 5′ primer, CAG CAG GCC
ACT ACA GGA GT (residues 1997–2016); 3′ primer,
CCC AGA GCA AAT GCC ATA AG (residues 2322–2303). We also used a set of primers specific for the
GCR α/α2 isoform (U01351): 5′ primer, GTA TTG
AAT TCC CCG AGA TG (residues 2733–2752); 3′
primer, ACA GAC TTT GGG CAC TGG (residues
3158–3141). For quantification of β2-AR mRNA we used
the following primers (M15169): 5′ primer, CGG TTC
CAT GTC CAC AGA TG (residues 2302–2321); 3′
primer, CTG TTC CAC GTG ATA TCC AC (residues
2697–2678).

Internal standard DNAs for the total GCR mRNA,
the GCR α/α2 isoform mRNA and the β2-AR
mRNA were constructed using the above-described sets
of primers and the PCR-MIMIC™ construction kit
(Clontech). The sizes of the internal standards were
designed to be 596 bp for the total GCR, 238 bp for the
α/α2 isoform and 240 bp for the β2-AR.

Internal standard RNAs for the total GCR mRNA, the
α/α2 GCR isoform mRNA and the β2-AR mRNA were
constructed as described by Faure et al. [23]. The resulting
internal standard RNAs were quantified by UV-
detection (Gene-Quant II; Pharmacia). The resulting
RT-PCR products were indistinguishable from the
internal standard DNAs.

**PCR reaction**
For the PCR reactions, 3 μl aliquots of cDNA were
combined with 40 pmol of each primer, 150 μM of each
dNTP and 0.2 unit of Taq polymerase (Pharmacia) in the
supplied PCR reaction buffer at 0 °C in a volume of
100 μl, and overlayed with mineral oil. The ampli-
fication took place in a Perkin Elmer Model 480
thermocycler. After initial denaturation at 95 °C for
2 min, 27 cycles were performed, each comprising 45 s of
denaturation at 94 °C, 45 s of annealing (Table 1) and 90 s of extension at 72 °C. After the last cycle, the incubation continued for 5 min, whereupon the temperature was lowered to 4 °C. The PCR products were either used for HPLC immediately or stored frozen at −80 °C.

**Quantification of PCR products by HPLC**

The HPLC system consisted of a TSK® DEAE-NPR column (4.6 mm internal diam. × 35 mm, with a short guard column), thermostatted at 30 °C. The mobile phase was a gradient, comprising buffer A (25 mM Tris/HCl, pH 9.0, and 1.0 M NaCl) and buffer B (25 mM Tris/HCl, pH 9.0). The gradient was from 25% to 54% (v/v) buffer A in 0.5 min, from 54% to 59% A in 6.5 min, from 59% to 70% A in 0.5 min, 70% A for 1.0 min, from 70% to 25% A in 0.5 min, and 25% A for at least 3.0 min, all with a flow rate of 1.0 ml/min. The pump was a Waters Model 616 gradient pump controlled by Millennium® 32 software, which was also applied for data acquisition and processing. Detection was by an Applied Biosystems Model 759A UV-detector at 254 nm.

An aliquot of 90 µl was injected by a manual injector. The PCR product was quantified relative to the internal standard using areas, and corrected for the size differences between the two products. mRNA concentrations were expressed relative to the total (QIAamp) RNA content of the samples, in units of amol of mRNA/μg of total RNA.

**Validation of the technique**

The amplification rate was exponential up to at least 27 cycles for both standard and unknown. The amplification was close to the theoretical rate of 2^n (where n = number of cycles). The standard curve was linear, provided that the cDNA/standard area ratio was between 0.5 and 4. When the calculated ratio exceeded this limit, the sample was re-analysed with a reduced amount of mRNA added to RT.

Controls with no RT and no cDNA were run frequently. No contamination of sample mRNA with genomic DNA was observed. The sequences of the PCR products were confirmed by dideoxy-sequencing using Perkin-Elmer dRhodamine or Big Dye Terminator Cycle Sequencing Chemistry and an ABI 310 Applied Systems apparatus for separation and fluorescence detection.

The sensitivity of the assay was approximately 0.004 amol of RNA, corresponding to 5000 μ-absorbance units/s. Reproducibility was assessed as follows: a large muscle biopsy was cut into six pieces, and mRNA was isolated from each part and measured by RT-PCR/HPLC. The coefficients of variation were 14.9% and 15.4% for GCR and β2-AR mRNAs respectively.

**Statistics**

Statistical analysis was performed using the SigmaStat program, version 1.02, and the SPSS package (SPSS Inc., Chicago, IL, U.S.A.). The following statistical tests were applied: Mann–Whitney test for unpaired data and Wilcoxon test for paired data, correlations among variables by least-squares linear regression, the Spearman test, linear regression analysis, multiple regression analysis, backward stepwise regression analysis, and power analysis of performed tests. A P value of < 0.05 was considered significant. Results are presented as means ± S.E.M.

**RESULTS**

**Characterization of subjects**

The patients with Type II diabetes were overweight (P = 0.06), with a significant higher values for body mass index (P = 0.002), HbA1c (P < 0.001), fasting plasma glucose (P = 0.002), serum insulin (P < 0.001) and plasma triacylglycerol levels (P = 0.002) (Table 2). On estimating insulin resistance by the HOMA model, patients with Type II diabetes were severely insulin resistant (Type II diabetics, 31.8 ± 4.1; controls, 9.7 ± 1.8; P < 0.001).

After 8 weeks of intensive treatment of patients with Type II diabetes, basal plasma glucose and HbA1c had improved significantly (P = 0.047 and P = 0.02 respectively) (Table 3). There was no change in the body weight of the patients. Plasma levels of non-esterified fatty acids in the basal state were similar before and after intensive treatment, whereas a decrease was seen in plasma triacylglycerols (P = 0.09) (Table 3).

**Euglycaemic clamp studies**

Comparable serum insulin levels were found in the basal state before and after intensive intervention for 8 weeks (Table 3). Likewise, during insulin infusion, similar steady-state serum insulin levels were obtained. After intensive treatment for 8 weeks, the total insulin-stimulated glucose disposal rate increased significantly (before treatment, 8.4 ± 1.0 mg · min⁻¹ · kg⁻¹ FFM; after treatment, 14.5 ± 2.0 mg · min⁻¹ · kg⁻¹ FFM; P = 0.005) (power of performed test with α = 0.05:0.99).

**Levels of GCR and β2-AR mRNAs**

Using the HPLC technique, the abundance of mRNAs for total GCR (α +β isoforms), the GCR α/α2 isoform and the β2-AR, expressed in amol/μg of total RNA, was assessed from muscle biopsies taken in the post-absorptive state in control subjects, and both before and after intensive treatment in patients with Type II diabetes. Total GCR and GCR α/α2 mRNA levels were higher in patients with Type II diabetes than in control subjects, whereas the level of GCR β isoform mRNA was significantly lower (total GCR, 2.06 ± 0.30 and 1.47 ± 0.10 amol/μg of RNA (P = 0.09); GCR α/α2 isoform, 1.69 ± 0.31 and 0.92 ± 0.09 amol/μg (P = 0.02); GCR β isoform, 0.36 ± 0.08 and 0.66 ± 0.06 amol/μg (P = 0.006),
Table 2  Clinical characteristics of patients with Type II diabetes and control subjects
NEFA, non-esterified fatty acids. Data are means ± S.E.M. (range). Significance of differences: *P = 0.06; **P = 0.002; ***P < 0.001.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Type II diabetic patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54 (41–65)</td>
<td>53 (40–65)</td>
</tr>
<tr>
<td>Sex (female:male)</td>
<td>7:3</td>
<td>4:11</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>92.3 ± 4.9</td>
<td>80.9 ± 3.8</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>51.1 ± 3.2</td>
<td>52.3 ± 2.3</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>32.1 ± 1.1</td>
<td>26.1 ± 1.0**</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.95 ± 0.03</td>
<td>0.94 ± 0.02</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>9.1 ± 0.6</td>
<td>5.4 ± 0.1***</td>
</tr>
<tr>
<td>Fasting levels</td>
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<tr>
<td>Plasma glucose (mM)</td>
<td>14.4 ± 1.7</td>
<td>5.2 ± 0.1**</td>
</tr>
<tr>
<td>Serum insulin (pM)</td>
<td>73 ± 11</td>
<td>41 ± 7***</td>
</tr>
<tr>
<td>Serum C-peptide (pM)</td>
<td>0.52 ± 0.05</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>Plasma NEFA (mM)</td>
<td>0.60 ± 0.10</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td>Plasma triacylglycerols (mM)</td>
<td>4.15 ± 2.32</td>
<td>1.15 ± 0.11**</td>
</tr>
</tbody>
</table>

Table 3  Clinical characteristics of patients with type II diabetes before and after 8 weeks of intensive treatment
NEFA, non-esterified fatty acids. Data are means ± S.E.M. of four samples in the basal (—30 to 0 min, where the first muscle biopsy was taken at 0 min) and the hyperinsulinaemic (210 to 240 min) periods. Significance of differences: *P = 0.02; **P = 0.047; ***P = 0.09; ****P = 0.005.

<table>
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<tr>
<th>Parameter</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>92.3 ± 4.9</td>
<td>92.6 ± 4.9</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>51.1 ± 3.2</td>
<td>51.3 ± 3.2</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>32.1 ± 1.1</td>
<td>32.1 ± 1.3</td>
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<tr>
<td>Waist/hip ratio</td>
<td>0.95 ± 0.03</td>
<td>0.93 ± 0.03</td>
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<tr>
<td>HbA1C (%)</td>
<td>9.1 ± 0.6</td>
<td>8.2 ± 0.6*</td>
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<tr>
<td>Basal period</td>
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<tr>
<td>Plasma glucose (mM)</td>
<td>13.8 ± 1.6</td>
<td>11.1 ± 0.8***</td>
</tr>
<tr>
<td>Serum insulin (pM)</td>
<td>66 ± 10</td>
<td>84 ± 13</td>
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<tr>
<td>Serum C-peptide (pM)</td>
<td>0.49 ± 0.05</td>
<td>0.52 ± 0.06</td>
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<tr>
<td>Plasma NEFA (mM)</td>
<td>0.82 ± 0.10</td>
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<tr>
<td>Plasma triacylglycerols (mM)</td>
<td>4.15 ± 2.32</td>
<td>2.58 ± 0.36***</td>
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<tr>
<td>Hyperinsulinaemic period</td>
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<tr>
<td>Plasma glucose (mM)</td>
<td>5.8 ± 0.3</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td>Serum insulin (pM)</td>
<td>1047 ± 73</td>
<td>1094 ± 81</td>
</tr>
<tr>
<td>Total glucose disposal rate (mg·min⁻¹·kg⁻¹ FFM)</td>
<td>8.4 ± 1.0</td>
<td>14.5 ± 2.0****</td>
</tr>
</tbody>
</table>

for patients with Type II diabetes and control subjects respectively (Figure 1) (power of performed test with α = 0.05:0.60 for total GCR mRNA and 0.80 for GCR α/α2 isoform mRNA). β2-AR mRNA levels were comparable in patients with Type II diabetes and control subjects (0.53 ± 0.05 and 0.45 ± 0.02 amol/µg of RNA respectively; P = 0.20) (power of performed test with α = 0.05:0.65).

β2-AR mRNA levels were positively correlated with GCR mRNA levels (r = 0.46, P = 0.001) in the whole group of participants. When analysing the groups separately, in patients with Type II diabetes the correlation was significant (r = 0.58, P = 0.01), whereas no correlation was found in the control group. In the control group there was a negative correlation between GCR mRNA level and fat mass (r = −0.6, P = 0.026).

In the patients with Type II diabetes, the levels of total GCR, α/α2 GCR isoform and β2-AR mRNAs were assessed from muscle biopsies taken in the basal period both before and after 8 weeks of intensive treatment.
Total GCR, α/α2 GCR and β2-AR mRNA levels had all decreased significantly after 8 weeks of intensive treatment (total GCR, 2.06 ± 0.30 and 1.37 ± 0.14 amol (P = 0.005); αGCR, 1.69 ± 0.31 and 0.97 ± 0.15 amol (P = 0.005); β2-AR, 0.53 ± 0.05 and 0.41 ± 0.02 amol (P = 0.03), for pre- and post-intensive treatment respectively) (Figure 1) (power of performed test with α = 0.05:0.90 for total GCR mRNA and 0.80 for β2-AR mRNA).

Before intensive treatment of the diabetic subjects, there was a positive correlation between total glucose uptake and β2-AR mRNA levels (P = 0.001) (power of performed test with α = 0.05:0.84) and a negative correlation between total glucose uptake and basal glucose levels (P = 0.006) (multiple linear regression; r = 0.95, P = 0.001).

When assessing the patients with Type II diabetes before and after 8 weeks of intensive treatment, close correlations were found between the increment in glucose uptake and the decrement in GCR mRNA (P < 0.001) (Figure 2), and in the decrement in basal glucose (P = 0.02) and the increment in basal C-peptide levels (P = 0.04) multiple linear regression; r = 0.95, P < 0.001). By simple linear regression, the increment in glucose uptake was also correlated with the decrement in GCR α/α2 isoform mRNA levels (r = 0.88; P < 0.001).

**Correlations between GCR α + β and α/α2 isoforms**

Both isoforms were measured in all men in the control group (n = 11), and in all the diabetic patients (n = 10) both before and after intensive treatment. There was a close correlation between the two isoforms in patients with diabetes both before (r = 0.93, P < 0.0001) and after (r = 0.91, P < 0.0001) intensive treatment, and in control men (r = 0.84, P = 0.001). Figure 3 shows the relationship between the two isoforms in the 10 diabetic subjects studied before and after intensive treatment.

**DISCUSSION**

Previous studies of obese non-diabetic patients have shown a decrease in GCR number in mononuclear leucocytes when compared with lean subjects, but a normal cortisol concentration [10,11]. Since glucocorticoids may also have a direct effect on the pancreatic β-cell, causing changes in insulin secretion [24], these associations are compatible with the existence of hyper-

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**Figure 1** Total GCR and GCR α/α2 and β isoform mRNA levels in patients with Type II diabetes and in control subjects

mRNA levels were assessed from muscle biopsies taken in the postprandial state (hatched bars) and after 4 h of hyperinsulinaemia (closed bars) in patients with Type II diabetes, and in the postabsorptive state in control subjects (open bars). In the postabsorptive state, total GCR (P = 0.09) and GCR α/α2 isoform (P = 0.02) mRNA levels were higher in patients with Type II diabetes than in controls, whereas that of the GCR β isoform (P = 0.006) was lower. In patients with Type II diabetes, total GCR (P = 0.005) and GCR α/α2 isoform (P = 0.005) mRNA levels had decreased after 4 h of hyperinsulinaemia; no differences were found when compared with the control group.

Total GCR, α/α2 GCR and β2-AR mRNA levels had all decreased significantly after 8 weeks of intensive treatment [total GCR, 2.06 ± 0.30 and 1.37 ± 0.14 amol (P = 0.005); αGCR, 1.69 ± 0.31 and 0.97 ± 0.15 amol (P = 0.005); β2-AR, 0.53 ± 0.05 and 0.41 ± 0.02 amol (P = 0.03), for pre- and post-intensive treatment respectively] (Figure 1) (power of performed test with α = 0.05:0.90 for total GCR mRNA and 0.80 for β2-AR mRNA).

**Figure 2** Relationship between increments in insulin sensitivity and decrements in GCR mRNA after 8 weeks of intensive treatment in patients with Type II diabetes (P < 0.001)

**Figure 3** Relationship between GCR α/α2 isoform and total GCR (α + β) mRNA levels

Results were obtained in 10 patients with Type II diabetes. Data are presented as amol/µg of total RNA.
sensitivity to cortisol in various target tissues in obesity, leading to increased fat accumulation, enhanced insulin secretion and insulin resistance.

In the present study we have used the RT-PCR/HPLC technique to assess the abundance of total GCR, GCR α/α2 isoform and β2-AR mRNAs in muscle biopsies taken in the postabsorptive state from patients with Type II diabetes and from control subjects. We found that levels of total GCR and GCR α/α2 isoform mRNAs were higher in obese patients with Type II diabetes than in control subjects, whereas the level of GCR β isoform mRNA was significantly lower. In the control subjects, the GCR mRNA level in muscle tissue decreased with increasing fat mass. In a previous study of non-diabetic subjects of normal body weight [22], we demonstrated that the GCR mRNA level in lymphocytes had a negative relationship with the serum leptin concentration. These results may suggest that, in non-adipose tissue, the rate of transcription of GCR mRNA decreases with increasing fat mass and serum leptin. This may be a compensatory response to increased insulin levels with increasing fat mass and increasing amounts of fatty acids available for oxidation. The decrease in GCR mRNA is unlikely to be due to increased plasma cortisol levels in obese subjects, since in our previous study [22] no relationship between plasma cortisol and GCR mRNA levels was found. Furthermore, no decrements in lymphocyte GCR mRNA were observed in patients with Cushing’s syndrome and high plasma cortisol values (N. J. Christiansen, unpublished work), or in GCR number and affinity in mononuclear leucocytes from patients with Cushing’s syndrome [10]. The findings in the patients with Type II diabetes also support the hypothesis that insulin availability is important for the decrease in GCR mRNA. During intensive treatment, GCR mRNA abundance (total and the α/α2 isoform) decreased markedly, concomitant with the increase in insulin sensitivity. A limitation of this part of the study is that we only measured the GCR mRNA and not the receptor protein concentration, which cannot be measured very precisely. It is likely, however, that the marked decrease in the rate of transcription of GCR is associated with a decreased cortisol response. As indicated above, we have found no evidence suggesting that elevated plasma cortisol levels lead to the decrease in the GCR mRNA level.

Although insulin sensitivity in patients with Type II diabetes increased during intensive treatment, the sensitivity was not normalized. Several factors are likely to contribute to the decreased insulin sensitivity observed in Type II diabetes. Our study suggests that a contributory factor may be a lack of suppression of the rate of transcription of GCR mRNA. Further studies are warranted in order to elucidate the mechanism behind the abnormal GCR response, and to quantify GCR protein levels and the activities of enzymes regulated by GCR.

Our results are compatible with a recent publication [25] which reported that: (1) glucocorticoid hormone action in skeletal muscle is determined principally by autoregulation of GCR α and β isoforms and of type I 11β-hydroxysteroid dehydrogenase expression by the ligand-binding GCR α form, and (2) insulin regulation of type I 11β-hydroxysteroid dehydrogenase may represent a novel mechanism that maintains insulin sensitivity in skeletal muscle tissue by diminishing the glucocorticoid antagonism of insulin action. Moreover, there were no effects of insulin or of increasing concentrations of glucose on GCR α mRNA expression in the myoblasts [25].

The correlation observed in the diabetic patients between the levels of β2-AR mRNA and GCR mRNA is not surprising, considering that the promotor region of the β2-AR gene contains a GCR-responsive element. Previous studies have indicated that, in animals with experimental diabetes, there is a defect in the β-AR signalling pathway. In these animals administration of large doses of catecholamines did not result in ‘catecholamine myocarditis’ as observed in control animals [26]. Our results indicate that the β2-AR may also function abnormally in muscle tissue in Type II diabetes.

In accordance with our previous study [22], we found a rather close correlation between the total GCR (α + β) mRNA level and that of the α/α2 isoform. The α isoform constituted approx. 60% of total GCR mRNA in both diabetic patients and control subjects.

In conclusion, we have shown that, in obese insulin-resistant patients with Type II diabetes, the level of GCR mRNA is increased when compared with control subjects. Furthermore, during intensive treatment of diabetics, the level of GCR mRNA decreased concomitant with the improvement in insulin sensitivity. The abnormal GCR mRNA response in Type II diabetes is likely to play a significant role in the abnormal regulation of insulin sensitivity observed in these patients.

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