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Brief Genetics Report

Uncoupling Protein 3 Content Is Decreased in Skeletal Muscle of Patients With Type 2 Diabetes

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Recently, a role for uncoupling protein-3 (UCP3) in carbohydrate metabolism and in type 2 diabetes has been suggested. Mice overexpressing UCP3 in skeletal muscle showed reduced fasting plasma glucose levels, improved glucose tolerance after an oral glucose load, and reduced fasting plasma insulin levels. However, data regarding the expression of UCP3 in patients with type 2 diabetes is inconsistent, and so far, there have been no reports of UCP3 protein content. Here we compared, for the first time, the protein levels of UCP3 in vastus lateralis muscle in 14 male type 2 diabetic patients (age 49.8 ± 2.1 years; BMI 27.2 ± 1.2 kg/m^2; mean ± SE) with 16 male control subjects (age 48.0 ± 1.9 years; BMI 23.4 ± 0.6 kg/m^2). We found that UCP3 protein levels were twice as low in patients with type 2 diabetes compared with control subjects (117 ± 16 vs. 58 ± 12 AU; P = 0.007). There was no correlation between UCP3 content and BMI. In conclusion, UCP3 content is lower in type 2 diabetic patients compared with healthy control subjects. These results are consistent with a role for UCP3 in glucose homeostasis and suggest a role for UCP3 in type 2 diabetes. *Diabetes* 50: 2870–2873, 2001

The human uncoupling protein UCP3 uncouples oxidative phosphorylation from ATP production and is therefore thought to play a role in human energy metabolism and obesity (1). Recently, a role for UCP3 in carbohydrate metabolism and in type 2 diabetes has been suggested (2). Previous reports have shown that treatment of muscle and fat cells with chemical uncouplers of ATP production leads to increased glucose uptake, most probably linked to synthesis and translocation of GLUT4 (3). Furthermore, high levels of UCP3 mRNA have been observed in skeletal muscle of mice overexpressing GLUT4, which have high levels of glucose uptake (4). Similarly, exposure of rats to cold (4°C), which is known to increase glucose utilization, leads to a concerted upregulation of UCP3 and GLUT4 mRNA after 6–24 h and a concerted downregulation of both genes after 6 days of cold exposure (5). In addition, after endurance exercise, UCP3 and GLUT4 mRNA increased in parallel between 0 and 3 h after the cessation of exercise, when glucose uptake was high, and returned in parallel to control levels 20–30 h after exercise, when glucose uptake had returned to normal values (6). These results indicate that changes in glucose metabolism are accompanied by changes in UCP3 expression, although the results do not provide evidence for a functional role of UCP3 in controlling glucose metabolism. However, evidence that UCP3 (over)expression improves glucose metabolism comes from the generation of mice overexpressing UCP3. These mice showed reduced fasting plasma glucose levels, improved glucose tolerance after an oral glucose load, and reduced fasting plasma insulin levels (2). Furthermore, overexpression of UCP1 in skeletal muscle (to a level of only 1% of the UCP1 expression naturally occurring in brown adipose tissue) increases skeletal muscle glucose transport and protects against the insulin resistance and hyperglycemia induced by high-fat feeding (7). These results, obtained in cell lines and rodents, indicate that high levels of uncoupling proteins in skeletal muscle could improve glucose homeostasis. Because type 2 diabetes is characterized by impairments in glucose homeostasis, it is tempting to speculate that type 2 diabetic patients have impaired regulation of UCP gene expression. However, whether patients with type 2 diabetes indeed have altered levels of UCP3 is still unclear. Krook et al. (8) reported lower levels of UCP3 mRNA in type 2 diabetic patients, which would be consistent with a functional role for UCP3 in type 2 diabetes. In contrast, Bao et al. (9) showed 85–170% increased levels of UCP3 mRNA in skeletal muscle of type 2 diabetic patients. Similarly, Vidal et al. (10) showed three- to fourfold higher levels of UCP3 mRNA in obese type 2 diabetic patients compared with obese control subjects. However, mRNA levels do not necessarily reflect protein content, and none of the studies performed so far determined UCP3 protein content. Therefore, the aim of the present study was to investigate UCP3 content in type 2 diabetic subjects and compare them with healthy control subjects, using a recently validated UCP3 antibody (11,12).

Skeletal muscle biopsies were taken after an overnight fast in 16 male healthy control and 14 male type 2 diabetic subjects. Subject characteristics are shown in Table 1. BMI

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Received for publication 6 April 2001 and accepted in revised form 12 September 2001.

UCP, uncoupling protein.

TABLE 1
Subject characteristics

	Type 2 diabetic subjects	Control subjects	*P*
n	14	16	
Age (years)	49.8 ± 2.1	48.0 ± 1.9	NS
Height (m)	1.76 ± 0.02	1.75 ± 0.02	NS
Weight (kg)	84.6 ± 4.9	71.6 ± 1.8	<0.05
BMI (kg/m^2)	27.2 ± 1.2	23.4 ± 0.6	<0.05
Insulin (μU/ml)	8.9 ± 1.2	5.3 ± 0.7	<0.05
Glucose (mmol/l)	9.2 ± 0.9	5.3 ± 0.1	<0.005
HbA_{1c} (%)	7.1 ± 0.3	—	—

and body weight was significantly higher in type 2 diabetic patients compared with healthy control subjects ($P < 0.05$). Height and age were not significantly different between type 2 diabetic patients and healthy control subjects (Table 1). As expected, fasting glucose and insulin levels were significantly higher in the type 2 diabetic patients ($P < 0.001$). The main finding, however, was a significantly lower level of UCP3 protein in patients with type 2 diabetes compared with healthy control subjects ($P = 0.007$). Figure 1 shows a representative Western blot showing the results from five diabetic and five control subjects. On average, UCP3 content was 50% lower in type 2 diabetic patients compared with healthy control subjects (58 ± 12 vs. 117 ± 16 AU) (Fig. 2). This result was not caused by an effect of body weight or BMI on UCP3 content, because *1*) there was no correlation between UCP3 content and body weight ($r = 0.15$; $P = 0.43$) or BMI ($r = 0.18$; $P = 0.35$), and *2*) the difference in UCP3 content between type 2 diabetic patients and control subjects remained significant after the exclusion of three subjects with BMI >30 kg/m^2 (BMI 23.4 ± 0.6 vs. 25.3 ± 0.9 kg/m^2, NS; UCP3 content: 59 ± 14 vs. 117 ± 16 AU, $P = 0.016$). There were significant negative correlations between UCP3 content and plasma glucose concentration ($r = -0.43$; $P = 0.017$) (Fig. 3*A*) and plasma insulin concentration ($r = -0.42$; $P = 0.028$) (Fig. 3*B*). Also, age was negatively correlated with UCP3 content ($r = -0.40$; $P = 0.015$). No correlations between UCP3 content and height or HbA_{1c} were found.

Suggestions have been made that patients with type 2 diabetes have altered levels of UCP3 (8–10). However, so far only data on the mRNA levels of UCP3 have been reported in humans, showing either increased or decreased levels of UCP3 mRNA in diabetic subjects (8–10). In the present study, we have measured, for the first time,

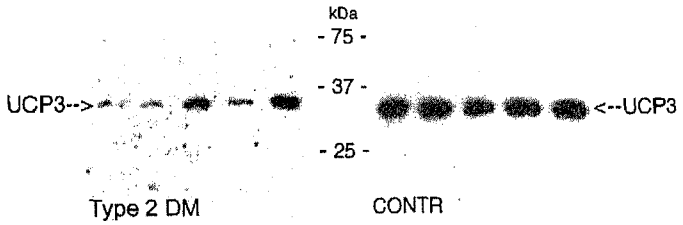

FIG. 1. Representative sample of UCP3 using Western blot. Equal amounts of protein were loaded in every lane. Protein was normalized on the band, which was identified as actin. Blotting and antibody incubation were performed simultaneously for control and diabetic samples, and all samples were exposed to the same film.

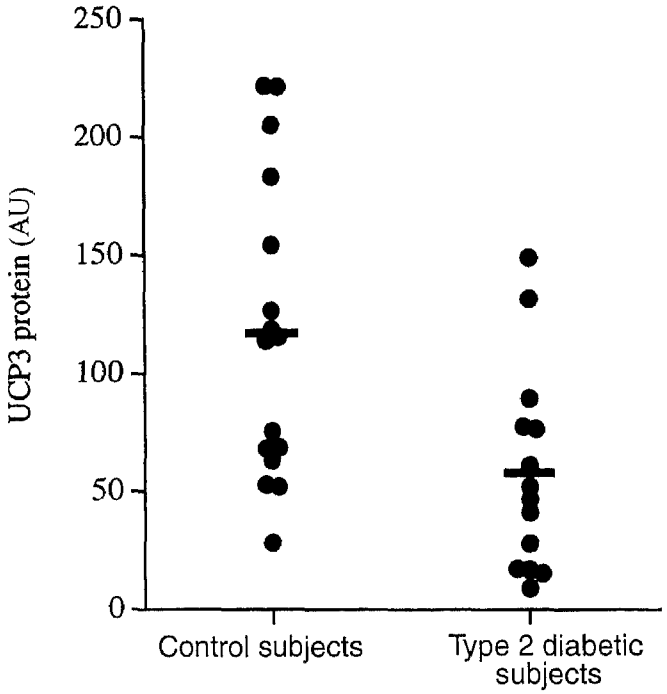

FIG. 2. Skeletal muscle UCP3 protein content in type 2 diabetic (n = 14) and healthy control (n = 16) subjects.

UCP3 content in type 2 diabetic subjects. Patients with type 2 diabetes had 50% lower UCP3 content compared with control subjects who were matched for age. This effect could not be attributed to differences in BMI, and similar results were found when we compared a subgroup of the type 2 diabetic patients and healthy control subjects matched for BMI, age, and body weight.

A decreased level of UCP3 content in type 2 diabetic patients is consistent with recent findings, suggesting involvement of UCP3 in type 2 diabetes in humans. We have recently discovered a novel polymorphism in the proximal promoter region of UCP3, consisting of a C to T substitution, which was associated with increased skeletal muscle UCP3 mRNA expression. Thus, skeletal muscle UCP3 mRNA expression was higher in the C/T and T/T group compared with the C/C homozygotes (13). Interestingly, Meirhaeghe et al. (14) recently found that the variant T allele of this polymorphism was associated with a decreased risk of developing type 2 diabetes. Combining these results, it is tempting to suggest that high levels of UCP3 can protect against the development of type 2 diabetes. This hypothesis is in accordance with the observation that mice overexpressing UCP3 are resistant to the development of diet-induced diabetes (7).

How UCP3 could be involved in type 2 diabetes is currently unknown. Treatment of L6 myotubes with the strong chemical uncoupler dinitrophenol stimulated glucose uptake (3). Recently, Huppertz et al. (15) showed that overexpression of UCP3 in L6 myotubes increases glucose uptake through increased recruitment of GLUT4 to the cell surface. Overexpression of UCP3 in transgenic mice resulted in increased glucose tolerance and reduced fasting plasma glucose levels (2). Overexpression of UCP1 in skeletal muscle of mice had similar results and also resulted in increased expression of GLUT4 (7). Together,

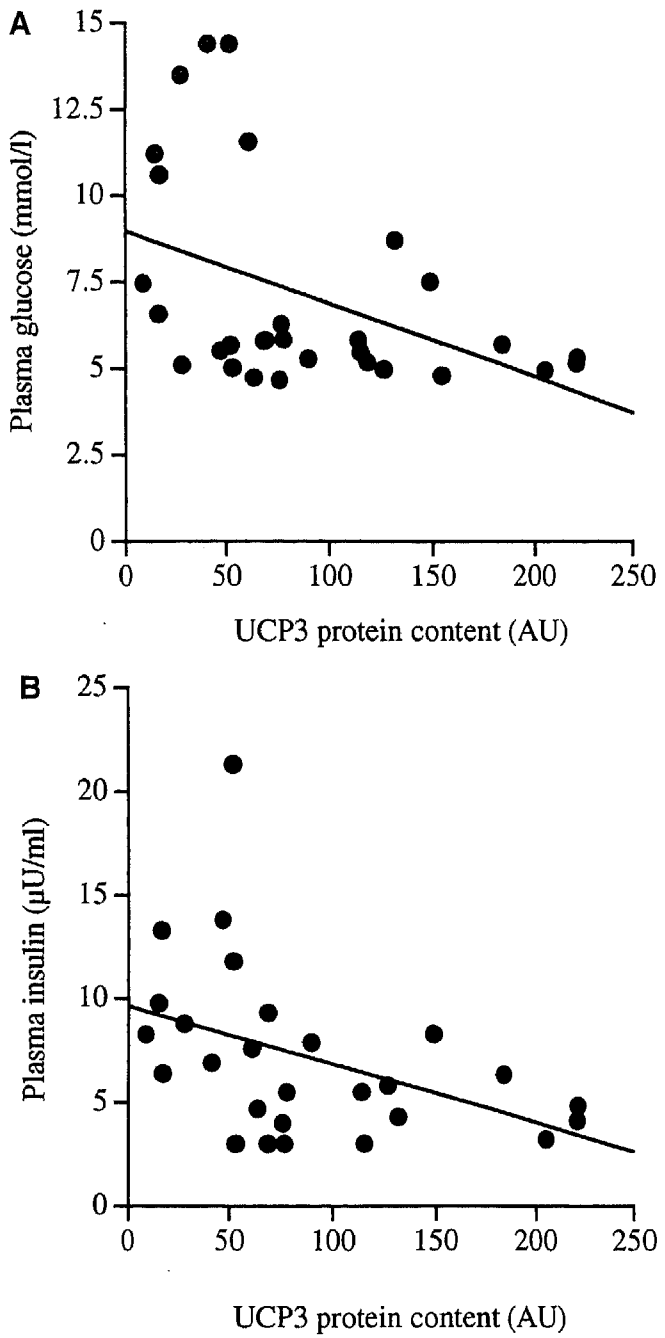

FIG. 3. Relation between plasma glucose concentration (*A*) ($r = -0.43$; $P = 0.017$) and plasma insulin concentration (*B*) ($r = -0.42$; $P = 0.028$) and skeletal muscle UCP3 content.

these results suggest that the uncoupling of oxidative phosphorylation leads to increased glucose uptake, probably via GLUT4 translocation, and improved glucose tolerance. Because type 2 diabetic subjects have impaired glucose tolerance and insulin-mediated glucose uptake, our finding of decreased UCP3 content in type 2 diabetic patients is consistent with an association between UCP3 and glucose homeostasis. This is further illustrated by our finding that UCP3 content was negatively correlated with plasma glucose and insulin levels, stressing the relationship between UCP3 and glucose metabolism.

Using immunofluorescence, we have recently shown that UCP3 is expressed more abundantly in type 2b muscle fibers compared with type 1 muscle fibers (11), both in healthy control and type 2 diabetic subjects. This expression pattern is consistent with a role for UCP3 in glucose homeostasis because type 2b muscle fibers are characterized by a high glycolytic capacity, whereas type 1 muscle fibers primarily use lipids as substrates. Because type 2 diabetic patients are characterized by an increased amount of type 2b fibers compared with control subjects (16), we previously hypothesized that type 2 diabetic subjects might have increased levels of UCP3. However, our present study shows that the opposite is true: type 2 diabetes is characterized by 50% lower levels of UCP3 protein content. It is therefore not likely that the reduction in UCP3 content in type 2 diabetes is attributed to differences in muscle fiber type composition.

A possible role for UCP3 in glucose homeostasis makes UCP3 a potential pharmacological target for upregulation. Thiazolidinediones are a novel class of blood glucose–lowering drugs, improving glycemic control and insulin sensitivity (17). Interestingly, thiazolidinediones have been shown to upregulate skeletal muscle UCP3 mRNA in rodents (18,19). Whether this upregulation of UCP3 by thiazolidinediones is responsible for the improved glucose tolerance is currently unknown. Future studies should reveal the effect of thiazolidinediones on UCP3 content and glucose metabolism in humans.

In conclusion, we found for the first time that patients with type 2 diabetes have decreased levels of UCP3 protein content. This data, together with results found in mice overexpressing UCP3, suggests a role for UCP3 in type 2 diabetes. Therefore, UCP3 is a potential pharmacological target for upregulation by thiazolidinediones. However, further studies regarding the exact function of UCP3 are necessary to reveal the role of UCP3 in type 2 diabetes.

RESEARCH DESIGN AND METHODS

A total of 14 male type 2 diabetic patients and 16 male control subjects participated in the study. All subjects gave their written informed consent, and the study was approved by the medical ethical committee of Maastricht University. Type 2 diabetic patients were treated by first- or second-generation sulfonylurea derivatives and diet ($n = 11$) or diet alone ($n = 3$). Medication was withheld for 2 days before the experiment. At the time they were enrolled in the study, the subjects had been diagnosed as having type 2 diabetes for 1–5 years.

Muscle biopsy, blood sampling, and UCP3 content determination. After an overnight fast, muscle biopsies were taken from the mid-thigh region from musculus vastus lateralis according to the technique of Bergström et al. (20). The subjects were required to abstain from blood glucose–lowering medication and training or vigorous exercise for 48 h before the biopsy. Before subjecting the samples to homogenization, special care was taken to remove visible blood, connective tissue, and fat. Muscle biopsies were homogenized in ice-cold Tris-EDTA buffer at pH 7.4, and then the homogenates were sonicated for 4 × 15 s. Subsequently, two volumes of each skeletal muscle homogenate and one volume of SDS-sample buffer were boiled for 4 min. Next, 13% polyacrylamide gels containing 0.1% SDS were loaded with equal amounts of protein from each sample, and electrophoresis was performed using a Mini-Protean 3 Electrophoresis Cell (Bio-Rad Laboratories, Hercules, CA). After gel electrophoreses, this gel was scanned, and the optical density of the 43-kDa band, previously immuno-identified to represent actin, was assessed. Then, a second gel was prepared and loaded with the sample volume (which had been recalculated based on the optical density of the actin band), after which Western blotting was performed using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories) as described previously (11). We used a rabbit polyclonal UCP3 antibody (code 1331; kindly provided by LJ Slieker, Eli Lilly) prepared against a 20–amino acid (aa) peptide (human sequence aa 147–166), which recognizes both the long and the short form of UCP3 and was previously shown to not recognize UCP2. The antibody was affinity-purified on a Sulfolink column (Pierce, Omnilabo International, Breda,

the Netherlands) containing the peptide coupled through a COOH-terminal cysteine. Cross-reaction of the antibody with other proteins was checked by examining the entire 5- to 94-kDa range for additional bands. Other specificity checks are presented in the study by Hesselink et al. (11).

Blood was sampled after an overnight fast. Glucose concentrations were determined using the hexokinase method (Roche, Basel, Switzerland), and insulin concentrations were measured using radioimmunoassay (Linco Research, St. Charles, MO). HbA_{1c} was measured with high-performance liquid chromatography (Recipe, Munich, Germany; and Bio-Rad Diamat, Munich, Germany).

Statistical analysis. Statistical differences were determined using Student's unpaired *t* test. Pearson correlation coefficients were calculated to determine the relationships between selected variables. Data are represented as the means ± SE, and $P < 0.05$ was considered as statistically significant.

ACKNOWLEDGMENTS

P.S. was supported by a grant from the Netherlands Organization for Scientific Research (NWO). L.B.B. was supported by a grant from the Dutch Diabetes Research Foundation. E.E.B was supported by grants from the Netherlands Organization for Scientific Research (NWO) and the Dutch Diabetes Research Foundation.

We thank Lawrence J. Slieker from Eli Lilly for providing us with the UCP3 antibody.

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