Irreversibility of the Defect in Glycogen Synthase Activity in Skeletal Muscle From Obese Patients With NIDDM Treated With Diet and Metformin

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OBJECTIVE — To assess the reversibility of the defect in glycogen synthase (GS) activity in skeletal muscle from obese patients with NIDDM treated with a hypocaloric diet and metformin.

RESEARCH DESIGN AND METHODS — Eighteen obese patients newly diagnosed with NIDDM were included in a randomized placebo-controlled double-blind parallel group trial and followed for 3 months. Euglycemic-hyperinsulinemic clamp including indirect calorimetry and biopsy of *m. vastus lateralis* was performed before and after treatment with a hypocaloric diet plus metformin or placebo. The patients were studied at basal, low, and high insulin concentrations.

RESULTS — The impaired GS activity in muscle biopsies was not reversed either by acute normalization of glycemia (for 8 h) or by chronic reduction of hyperglycemia by diet plus metformin. In both treatment groups, comparable effects on glycemic control and weight loss were found together with marked insulin suppression of nonesterified fatty acids and increased glucose oxidation. Total glucose disposal at euglycemic-hyperinsulinemic clamp increased significantly in the metformin group by 25% at high insulin level (259 ± 31 vs. 207 ± 21 mg · m^{-2} · min^{-1}, $P < 0.05$). An insignificant increase by 13% was found in the placebo group. There were no significant changes in nonoxidative glucose metabolism. GS activity and glucose utilization showed no significant differences between the two treatment groups when regression coefficients, expressed as incremental changes by increments of insulin, were compared.

CONCLUSIONS — Defective GS activity in obese NIDDM patients is not secondary to hyperglycemia. Metformin and diet had no significant influence on GS activity. The added effect of metformin to that of a hypocaloric diet in improving insulin-stimulated glucose utilization is marginal when blood glucose reduction is obtained by weight loss.

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Glycogen synthase (GS) is the key enzyme in glycogen synthesis. Its activity is reduced in NIDDM (1–3) and in nondiabetic subjects with insulin resistance (1,4,5). Correspondingly, it has been found that nonoxidative glucose metabolism is impaired in NIDDM (1,3,6), in Pima Indians with impaired glucose tolerance (7), and in people at increased risk for NIDDM (8). Direct evidence of defects in muscle glycogen synthesis in NIDDM has also been presented (9).

In a previous investigation (1), we found reduced GS activity in obese subjects with and without NIDDM. This defect could not be reversed despite normal plasma glucose for 8 h prior to the investigation. This indicates that the enzyme defect is not secondary to hyperglycemia itself. The findings of impaired GS activity in subjects without hyperglycemia (4,5) also point to other factors in insulin resistance affecting the enzyme. A quantitatively important nongenetic component appears to be involved in the defective GS activity in NIDDM (3). Long-term reduction of hyperglycemia in NIDDM by diet (10) as well as by sulfonylurea (11,12) improves the ability of insulin to stimulate GS. This might be related to amelioration of the insulin resistance caused by glucotoxicity, even if a direct drug effect could not be excluded in one of the studies with gliclazide (11). The other study (12) showed unchanged gene expression of muscle GS after drug-induced improvement of glycemic control. Therefore, an effect of gliclazide on enzyme synthesis seems unlikely.

Metformin ameliorates insulin resistance both in NIDDM and in nondiabetic conditions (13). The biochemical mechanism for this effect is not completely understood. Metformin inhibits hepatic gluconeogenesis (14,15) and increases muscular glucose uptake (16,17) and glucose oxidation (15,18). An effect on glycogen synthesis has been demonstrated in animal experiments (19,20) and in human erythrocytes (21). It is supported by the increased nonoxidative glucose metabolism after metformin observed in NIDDM patients (22,23) and their relatives (24). The effect of metformin on GS in humans has been assessed only in a single study (22) showing no improved insulin activation of the enzyme in muscle samples from obese newly diagnosed NIDDM patients.

The aim of the present investigation was to study the reversibility of GS activity in skeletal muscle biopsies from obese patients with newly diagnosed NIDDM

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Abbreviations: FBG, fasting blood glucose; G-6-P, glucose-6-phosphate; G_{ox}, glucose oxidation; GS, glycogen synthase; NEFA, nonesterified fatty acid; N_{ox}, nonoxidative glucose metabolism; R_d, total glucose disposal; UDPG, uridine diphosphate glucose.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Patient characteristics at baseline and before and after treatment*

	Placebo (n = 9)		Metformin (n = 9)	
	Before	After	Before	After
Age in years (range)	53 (40–63)		51 (40–60)	
Sex (M/F)	6/3		7/2	
Body weight (kg)	93.6 ± 2.8	87.5 ± 3.2	101.2 ± 4.0	94.7 ± 3.5
BMI (kg/m^2)	31.9 ± 1.5	29.7 ± 1.5	31.6 ± 1.2	29.6 ± 1.1
FBG (mmol/l)	10.9 ± 0.9	8.0 ± 0.8	12.7 ± 1.2	8.1 ± 1.0
HbA_{1c} (%)	9.9 ± 1.3	6.6 ± 0.5	9.5 ± 0.6	6.7 ± 0.3

Data are means ± SEM.

after acute normalization of glycemia and chronic treatment with metformin or diet alone. Glucose metabolism was assessed using the euglycemic-hyperinsulinemic clamp technique and indirect calorimetry.

RESEARCH DESIGN AND METHODS

Subjects

Eighteen obese patients with newly diagnosed NIDDM referred from general practitioners to the Steno Diabetes Center within weeks after diagnosis with the aim of initiating antidiabetic treatment were included in the study. The patients had received no antidiabetic drugs and were not treated with other drugs influencing glucose homeostasis. They had no clinical or biochemical evidence of impaired renal, hepatic, or cardiac function and no significant concurrent disease or diabetic late complications. Obesity was defined as a BMI >25 kg/m^2. A fasting C-peptide value of >0.4 pmol/l was required to exclude IDDM. Patient characteristics are shown in Table 1 for the two treatment groups (placebo and metformin). Before participation, the purpose and risks of the study were carefully explained to the patients, and oral informed consent was obtained. The study was conducted in accordance with the Helsinki Declaration, and the protocol was approved by the local ethics committee.

Study design

The study was designed as a randomized, double-blind trial of parallel groups. The patients were treated with either placebo or metformin for 3 months and were also seen at interim visits after 1 and 2 months. Metformin was supplied as Glucophage tablets of 0.5 g together with matching placebo tablets (Lipha Pharmaceuticals, West Drayton, Middlesex, U.K.). The initial dose of metformin was 1.0 g daily, followed by increments of 0.5 g every week to a maximum of 3 g daily or the maximum tolerated dose aiming at a fasting blood glucose (FBG) concentration of <7.8 mmol/l. Intake of placebo tablets was increased similarly. Tablets were taken at meals twice daily. A hypocaloric diet (6,000 kJ) was instituted at inclusion and was continued unchanged during the study. Before inclusion (randomization), the patients had only received general dietary instructions from their general practitioner. Concurrent medication at baseline remained unchanged. Compliance was checked by tablet counting, and adverse events were reported by patients at each of the follow-up visits. Glycemic control (FBG and HbA_{1c}) and body weight were recorded before and after 1, 2, and 3 months of treatment. The euglycemic-hyperinsulinemic clamp combined with indirect calorimetry and muscle biopsy was performed before and after 3 months' treatment, as described below.

Euglycemic-hyperinsulinemic clamp

The procedure was carried out as described previously (1). In short, the patients were admitted to the hospital in the evening before the day of the study and connected to a glucose-controlled insulin infusion system (Biostator; Ames, Germany). A catheter was inserted into a dorsal wrist vein for continuous blood sampling and blood glucose analysis, with the hand placed in a heated box. Another catheter was inserted into a contralateral antecubital vein for insulin infusion and labeled glucose. Blood glucose was normalized at 5.0 ± 0.5 mmol/l within 2 h by insulin infusion. Thereafter, insulin was continued overnight to maintain normoglycemia for 8 h. In the morning, a bolus of 25 μCi [^{3}H]glucose followed by a continuous infusion (0.25 μCi/min) was given to assess total glucose disposal (25). The patients were studied during 30-min periods at three different insulin levels: basal, low, and high. The studies included blood sampling (glucose, insulin, lactate, and nonesterified fatty acids), indirect calorimetry, and muscle biopsy. Step 1 (basal) was initiated 90 min after the bolus injection of labeled glucose. Step 2 (low insulin) was performed after a steady-state level around 40 μU/ml was reached. This was obtained by continuous insulin infusion (20 mU · m^{-2} · min^{-1}) over a period of 120 min. The infusion was continued during the next 30 min, when the above studies were performed. Blood was sampled every 10 min during this period. Step 3 (high insulin) was performed as in step 2 but after a new steady-state level of ~160 μU/ml was reached. This was obtained by continuous insulin infusion (80 mU · m^{-2} · min^{-1}) over another 120-min period. The insulin levels were chosen to approach the physiological insulin concentrations found in obese NIDDM patients (1). The insulin infusion procedure at the above three insulin levels (0, 40, and 160 μU/ml) is a modification of the technique of DeFronzo et al. (26). Euglycemia was maintained by infusion of 20% glucose by the Biostator and external infusion pumps.

Blood samples and analytical methods

The sampling of blood at the clamp procedure was performed as described previously (1). Plasma glucose was analyzed by a routine glucose oxidase method and HbA_{1c} by thin-layer isoelectric focusing (27). Standard radioimmunologic methods were used for the determination of plasma concentrations of insulin (28) and C-peptide (29), the latter only before inclusion. Plasma nonesterified fatty acids (NEFAs) and lactate concentrations were determined with commercial kits using an automated analyzer (Cobas Mira; Roche, Basel, Switzerland). For the determination of lactate, blood was deproteinized by precipitation with perchloric acid. Arterialized venous blood was collected from a hand vein while the hand was in a heated box. All blood samples were immediately centrifuged at 5°C, and the collected plasma was stored at −70°C until assayed.

Glucose disposal and calculations

Total glucose disposal (R_d) was calculated from plasma concentrations of tritiated glucose and plasma glucose using Steele's nonsteady situations equation (30). At the elevated insulin levels, when hepatic glucose production was totally suppressed, the glucose infusion rate corresponded to R_d. Glucose oxidation was calculated from oxygen and carbon dioxide concentrations in

inspired and expired air during indirect calorimetry, which was performed by a computerized flow-through canopy gas analyzer system (Deltatrac Metabolic Monitor; Datec Industrial, Helsinki, Finland), as described previously (1). Basal metabolic rate was also calculated by this method. Nonoxidative glucose disposal was calculated as the difference between R_d and oxidized glucose. All variables of glucose were expressed as milligrams per meter squared per minute.

Muscle biopsies

Muscle biopsies were obtained from *m. vastus lateralis*, as described previously (1). Biopsy was taken at the end of each 30-min period at different steady-state insulin levels, as described above. Before enzyme analysis, the biopsies were freeze-dried and microdissected free from nonmuscle constituents (blood, fat, and connective tissue). GS activity was estimated by incubation of homogenized muscle fibers with ^{14}C-UDPG (uridine diphosphate glucose) at glucose-6-phosphate (G-6-P) concentrations of 0, 0.1, and 10 mmol/l. The enzyme activity was expressed as millimole of radioactive UDPG incorporated in glycogen per minute per milligram protein. Fractional velocity was calculated as percentage of the maximal activity (at 10 mmol/l G-6-P).

Statistical analysis

Intragroup comparisons were made at the three different insulin levels and before and after treatment in each group (placebo and metformin) by nonparametric statistics (Wilcoxon's test for paired data). A significance level of 0.05 was chosen. All values are presented as means ± SEM. Intergroup comparisons were made of regression coefficients, expressed as incremental changes by increments of insulin, using Fisher's permutation test.

RESULTS

Glycemic control and body weight

Glycemic control, FBG, and HbA_{1c}, improved considerably in both treatment groups (Table 1). The improvement was already evident after 1 month during the titration phase, with a further decrease after 2 months, whereupon glycemia remained stable. All patients lost weight, and BMI decreased in both groups (Table 1), with a weight loss of about 6 kg. No serious side effects were recorded, and there were no dropouts.

Table 2—*Values before and after treatment for plasma concentrations of NEFA, lactate, and insulin in the basal and insulin-stimulated state during euglycemic-hyperinsulinemic clamp*

	Placebo		Metformin	
	Before	After	Before	After
NEFA (mmol/l)				
Basal	0.59 ± 0.13	0.37 ± 0.08*	0.53 ± 0.11	0.41 ± 0.10
Low	0.41 ± 0.10	0.19 ± 0.04*	0.41 ± 0.07	0.25 ± 0.06*
High	0.09 ± 0.02†	0.06 ± 0.03‡	0.21 ± 0.05*	0.11 ± 0.05*‡
Lactate (mmol/l)				
Basal	0.67 ± 0.09	0.63 ± 0.07	0.80 ± 0.08	0.86 ± 0.07
Low	0.49 ± 0.03	0.54 ± 0.04	0.63 ± 0.03	0.88 ± 0.07
High	0.61 ± 0.04	0.76 ± 0.04	0.69 ± 0.06	0.97 ± 0.07
Insulin (nmol/l)				
Basal	0.21 ± 0.03	0.14 ± 0.02	0.23 ± 0.03	0.16 ± 0.02
Low	0.28 ± 0.05	0.24 ± 0.02	0.27 ± 0.01	0.26 ± 0.02
High	0.95 ± 0.09	0.85 ± 0.07	0.96 ± 0.06	0.91 ± 0.06

Data are means ± SEM. Insulin infusion: 20 mU · m^{-2} · min^{-1} (low) and 80 mU · m^{-2} · min^{-1} (high). *P < 0.05 vs. before; †P < 0.01 vs. basal; ‡P < 0.02 vs. basal.

Insulin, NEFA, and lactate

Compared with lean control subjects in the previous study (1), the patients in the present investigation were hyperinsulinemic. The mean fasting plasma insulin concentration (± SEM) was 16.7 ± 1.7 μU/ml in the placebo group, i.e., slightly lower than the mean value of 23.2 ± 3.3 μU/ml in the metformin group (NS). The values achieved during the clamp can be found in Table 2. The basal insulin level is determined by the insulin infusion rate necessary to keep the patients normoglycemic at the clamped blood glucose. Basal insulin levels were lower after 3 months' treatment in both groups.

NEFA and lactate concentrations during the clamp are also shown in Table 2. The values at baseline represent the mean of two samples (before the infusion of tritiated glucose and after 120 min). The values at low and high insulin levels each represent the mean of four samples at steady state. NEFA was significantly suppressed at high insulin levels independent of treatment. In the placebo group, values at basal and low insulin levels were lower after treatment; and in the metformin group, the values at low and high insulin levels were lower after treatment. Lactate concentrations were unchanged during the clamp and were not affected by treatment.

Glucose disposal

R_d, glucose oxidation (G_{ox}), and nonoxidative glucose metabolism (N_{ox}) increased from the basal state to the high insulin level at clamp, but not from the basal to the low insulin level (Table 3). R_d increased significantly ($P < 0.05$) after metformin treatment at high insulin level, whereas no significant changes were recorded at basal and low level and at all levels in the placebo group. G_{ox} increased significantly in both groups at low ($P < 0.01$) and high ($P < 0.01$ for metformin and $P < 0.05$ for placebo) insulin levels. No significant changes were seen for N_{ox}. Basal metabolic rate was unchanged during the clamp and after treatment (data not shown).

GS activity

The fractional velocity of GS assayed when no G-6-P was added to the homogenate and in the presence of physiological G-6-P concentrations (0.1 mmol/l) is shown in Table 4 for the three insulin levels during the clamp. There were no significant differences in GS activity at the various insulin levels before and after treatment in each group. A comparison of regression coefficients, expressed as incremental GS changes by increments of insulin, confirmed the lack of treatment effect and showed no significant differences between placebo and metformin. Small increases in GS activity from basal to high insulin were seen after treatment in both groups, reaching statistical significance in the metformin group.

CONCLUSIONS — The present investigation confirmed previous findings (1,3) of a defect in GS activity in skeletal muscle from obese NIDDM patients. The basal values were not different from those found in lean control subjects (1), but insulin did

Table 3—*Values before and after treatment for R_d, G_{ox}, and N_{ox} in the basal and insulin-stimulated state during euglycemic-hyperinsulinemic clamp*

	Placebo		Metformin	
	Before	After	Before	After
R_d				
Basal	86 ± 4	104 ± 8	91 ± 4	120 ± 20
Low	96 ± 10	111 ± 14	85 ± 6	137 ± 27
High	239 ± 23	271 ± 27	207 ± 21	259 ± 31*
G_{ox}				
Basal	52 ± 10	80 ± 11	56 ± 7	88 ± 12
Low	58 ± 6	87 ± 7†	60 ± 6	89 ± 8†
High	103 ± 7	143 ± 13*	95 ± 8	128 ± 8†
N_{ox}				
Basal	36 ± 7	26 ± 10	35 ± 8	35 ± 15
Low	38 ± 9	28 ± 10	25 ± 4	49 ± 25
High	137 ± 25	128 ± 16	112 ± 20	132 ± 25

Data are means ± SEM and are given in milligrams per meter squared per minute. Insulin infusion: 20 mU · m^{-2} · min^{-1} (low) and 80 mU · m^{-2} · min^{-1} (high). *$P < 0.05$ vs. before; †$P < 0.01$ vs. before.

not stimulate GS activity to the same extent (Table 4). In lean control subjects, the fractional velocity of GS (at G-6-P = 0) increased from (mean ± SEM) 4.6 ± 1.2% at basal level to 15.6 ± 3.8% at high insulin (1). The defect was not secondary to hyperglycemia, as physiological insulin concentrations did not stimulate the enzyme after 8 h of acutely induced normoglycemia. Chronic reduction of hyperglycemia by diet treatment had no significant effect on the enzyme activity. Metformin added to the same hypocaloric diet appeared to improve the defective insulin stimulation of GS activity, both at physiological G-6-P concentration and without addition of this enzyme activator (Table 4), but this could not be confirmed by further analysis, which showed no significant difference in incremental GS changes by increments of insulin when compared between groups and before versus after treatment in each group. Insulin-stimulated peripheral glucose utilization (measured as R_d at euglycemic-hyperinsulinemic clamp) improved significantly after metformin treatment (Table 3), but as discussed below, these findings can be interpreted as indicative of only a marginal effect of metformin beyond that of the hypocaloric diet.

First, there was no difference between groups in the glycemic level achieved (Table 1). Hyperglycemia was considerably reduced with HbA_{1c} values (6.6 and 6.7%) close to the normal range (4.1–6.2%). The improved glycemic control after placebo can be explained by the considerable weight loss, which averaged 6.1 kg over 3 months with a mean BMI reduction of 2.2 kg/m². It is well known that weight reduction can improve insulin sensitivity (31), and this is a possible explanation for the above finding. In the metformin group, an average weight loss of the same magnitude (6.5 kg with a mean BMI reduction of 2.0 kg/m²) was observed. Second, insulin-stimulated R_d was only marginally improved in the metformin group, which might be due to a small basal effect of the drug (Table 3). The improvement was mainly due to an improvement in glucose oxidation. Third, both treatments had identical effects on G_{ox} (Table 3), and nonoxidative glucose disposal did not increase significantly. Fourth, the effectiveness of insulin to suppress NEFA was the same with and without metformin (Table 2). Finally, it should be emphasized that no differences between treatment groups were found when regression coefficients of incremental changes in relation to increments of insulin were compared. Therefore, it appears that the effect of weight loss overshadowed any effect of metformin on glucose utilization.

The lowered basal clamp insulin levels after treatment in both groups (Table 2) indicated an improved insulin sensitivity leading to reduced insulin requirements. The increase of insulin-stimulated R_d in the metformin group averaged 25% at high insulin level (versus 13% in the placebo group) (Table 3), which is in accordance with previous findings (13). In the study by Johnson et al. (22), insulin-stimulated glucose disposal increased by 23% while G_{ox} was unchanged. This study used a two-way, 3-month crossover design, and the same methodology was used for assessing glucose metabolism (clamp, indirect calorimetry). GS activity was measured in muscle biopsies taken from the same muscle, but there might have been a slight difference of the tissue obtained. Johnson et al. did not mention any pretreatment in order to be free from nonmuscle material. Although the study showed an increase in N_{ox} after metformin, there was no measurable effect on insulin activation of GS. Johnson et al. did not measure the enzyme activity at physiological G-6-P concentrations. We found a very dubious effect of metformin on GS and no effect on N_{ox}. The NIDDM patients in both studies were obese and newly diagnosed, but there was no significant weight loss in the study by Johnson et al., as opposed to the considerable weight loss in our study, which might explain the improved G_{ox}.

Table 4—*Values before and after treatment for skeletal muscle GS activity fractional velocity (%), when no G-6-P was added to the suspension and at physiological G-6-P (0.1 mmol/l) in the basal and insulin-stimulated state during euglycemic-hyperinsulinemic clamp*

	Placebo		Metformin	
	Before	After	Before	After
G-6-P = 0				
Basal	6.4 ± 1.9	3.3 ± 0.7	4.2 ± 1.2	3.3 ± 0.4
Low	6.3 ± 2.1	3.8 ± 0.8	5.2 ± 1.5	6.0 ± 1.4
High	8.3 ± 1.5	8.2 ± 2.2	6.2 ± 1.3	10.1 ± 3.0*
G-6-P = 0.1				
Basal	21.7 ± 4.4	14.8 ± 3.7	20.5 ± 3.9	18.4 ± 1.8
Low	19.6 ± 5.1	15.0 ± 3.9	17.8 ± 3.1	18.6 ± 2.8
High	26.9 ± 3.7	25.8 ± 6.2	26.3 ± 4.1	30.9 ± 3.5†

Data are means ± SEM. Insulin infusion: 20 mU · m^{-2} · min^{-1} (low) and 80 mU · m^{-2} · min^{-1} (high).*$P < 0.01$ vs. basal; †$P < 0.02$ vs. basal.

Changes in lipid metabolism and effects on G_{ox} via the glucose fatty acid cycle play a major role in the hyperglycemia of NIDDM (3,32). The increased suppression of NEFA after therapy (Table 2) was in accordance with improved insulin action and increased G_{ox}. Marked suppression of NEFA levels during hyperinsulinemic clamp has been demonstrated in a previous study after combined metformin and sulfonylurea treatment in nonobese NIDDM patients with sulfonylurea failure (23). In the study by Johnson et al. (22), NEFA levels, glycerol, and 3-hydroxybutyrate were suppressed to comparable extent under euglycemic clamp conditions by placebo and metformin. Inhibition of fatty acid oxidation has been suggested in the past as an explanation of biguanide action (33) independent of any effect on insulin action. Recently, several studies have examined NEFA levels in response to acute administration (15) and chronic therapy with metformin (34–36). An insulin-independent effect mediated via decreased NEFA oxidation could influence glucose metabolism and thereby explain the antihyperglycemic action of the drug. The increased G_{ox} seen with metformin in our study and other investigations (15,18,36) and the decreased gluconeogenesis in acute (15) and chronic (14) studies are in accordance with this concept. On the other hand, improvement of lipid metabolism by weight loss might well explain the increased G_{ox} and blood glucose reduction in our study as demonstrated in both treatment groups, with the added effect of metformin being only marginal.

A possible action of metformin on GS cannot be ruled out totally from our results (Table 4). However, neither was it possible to demonstrate significant differences after treatment compared with baseline, nor between metformin and placebo. The reason for this could be the high variability in individual values and the relatively small sample size. The significant difference between basal and high insulin level after metformin could have arisen by chance.

Finally, the lactate results (Table 2) support previous findings (13,19,22) that blood lactate concentrations remain within the normal range during metformin treatment. Metformin does not inhibit oxidative processes (13), and as in the present study, G_{ox} can be increased.

In conclusion, the study showed that the defect in skeletal muscle GS activity in obese NIDDM patients is not secondary to hyperglycemia. Metformin and diet had no significant influence on GS activity. The added effect of metformin to that of a hypocaloric diet in improving insulin-stimulated glucose utilization is marginal when blood glucose reduction is obtained by weight loss.

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