Progressive Defect of Insulin Action on Glycogen Synthase in Obesity and Diabetes

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The purpose of the present work was to have a closer view on the changes in the regulation of glycogen synthase (GS) activity by insulin in relationship with the impairment of nonoxidative glucose disposal in human obesity. Obese patients with normal glucose tolerance (12), impaired glucose tolerance (11), diabetes (10), and lean control subjects (15) participated to the study. A euglycemic, hyperinsulinemic clamp was performed and associated with indirect calorimetry. Muscle needle biopsies were taken before and at the end of the 2-hour clamp for measurements of glycogen synthase fractional velocity and total activity. Total GS activity was significantly decreased ($P < .05$), while its percent activation by insulin was still normal in the obese glucose-tolerant group, and nonoxidative glucose disposal was decreased by 56% ($P < .001$) and glucose oxidation still normal. Total GS activity was decreased by about 50% ($P < .01$) and GS was unresponsive to insulin in the glucose-intolerant and diabetic groups. In conclusion, our data show that insulin-stimulated nonoxidative glucose disposal and total glycogen synthase are very early defects observed in obese patients.

THE ORIGIN OF glucose intolerance and insulin resistance in obesity and type 2 diabetes has been the subject of numerous investigations.[1] Defects in skeletal muscle are generally recognized to be at the origin of insulin resistance, as this tissue represents, together with the liver, the major site for insulin-stimulated glucose disposal.[2-4] The question has arisen whether the limitation of glucose storage as glycogen would be situated proximately at the entry of glucose into the muscle cell through inhibition of glucose transport or distally at the level of glycogen synthesis through inhibition of glycogen synthase (GS) activity.

Recent works have favored the first hypothesis of inhibition situated at the proximal level of glucose uptake.[1,5-8] Studies in obese patients and in normal subjects after an increase of plasma free fatty acid (FFA) concentration by means of a triglyceride emulsion combined with heparin have shown that the decrease in glucose synthesis measured by nuclear magnetic resonance was associated with a decrease in the increment of intramuscular glucose 6-phosphate (G6P), implying diminished glucose transport and/or phosphorylation.[9-10] These observations have given evidence for a defect at a proximal site causing lowering of G6P rather than at a distal site.

Studies at the distal site have shown a significant lack of activation of GS in response to insulin in obese subjects.[11-14] While glycogen may inhibit its own formation, insulin by dephosphorylating GS and G6P, as an allosteric activator of glycogen synthase b, both stimulate the activity of the enzyme. It is therefore evident that both proximal and distal sites of the pathway leading glucose from its entry into the cell to its deposition as glycogen might participate in the regulation of glucose tolerance. The relative contribution of these 2 processes in the decreased nonoxidative glucose disposal in type 2 diabetic patients is still being debated.[15]

The purpose of the present work was to have a closer view on the changes in GS fractional velocity in response to insulin in relationship with the impairment of glucose disposal observed in human obesity. For that purpose, obese patients were divided into 3 categories according to the presence or absence of glucose intolerance and diabetes. GS activity, total, and nonoxidative glucose disposal were studied after a hyperinsulinemic, euglycemic clamp.

SUBJECTS AND METHODS

A total of 48 subjects participated in the study: 12 obese patients with normal glucose tolerance according to the criteria of the National Diabetes Data Group[16] after a 75-g oral glucose tolerance test, 11 obese patients presenting impaired glucose tolerance, 10 obese diabetic patients, and 15 lean subjects served as controls. All the obese nondiabetic subjects have been diagnosed with a 75-g oral glucose tolerance test performed during the weeks preceding the study. None of the diabetic patients were insulin-treated or receiving any antidiabetic oral agent. The subjects were consuming a weight-maintaining diet containing at least 250 g carbohydrate as starchy food per day for the 2 days preceding the test. None of the obese patients, including those who were diabetic, were taking any drug for at least 7 days before the study. Body fat content was assessed by bioelectrical impedance[17] to calculate lean body mass. None of the participants were engaged in any intense physical exercise during a week prior to the test. The nature, purpose, and risk of the study were explained to all subjects, and their written agreement was obtained. The experimental protocol was approved by the Human Investigation Committee of the Department of Internal Medicine at the University Hospital of Geneva, Switzerland.

Experimental Protocol

All studies were performed with subjects in the recumbent position after a 10- to 12-hour overnight fast. An intravenous catheter (Venflon; Viggo-Spectramid, Helsinborg, Sweden) was inserted into an antecubital vein for infusing glucose and insulin. A second catheter was placed into a controlateral vein for blood withdrawal and kept patent with an infusion of isotonic saline. The glucose clamp was performed by the method of DeFronzo et al,[18] and all the patients were clamped

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Table 1. Physical Characteristics

	Control Subjects (N = 15)	Obese Subjects Glucose Tolerant (N = 12)	Obese Subjects Glucose Intolerant (N = 11)	Obese Diabetic Subjects (N = 10)
Gender (M + F)	6 M + 9 F	7 M + 5 F	9 M + 2 F	6 M + 4 F
Age (yr)	44.0 ± 2.6	47.4 ± 3.5	49.4 ± 3.3	55.3 ± 2.9*
Weight (kg)	67.8 ± 2.9	92.0 ± 3.9†	100.6 ± 4.8†	115.7 ± 8.2†
BMI (kg/m^2)	23.3 ± 0.6	30.9 ± 1.0†	35.3 ± 2.0†	40.8 ± 2.2†
Body surface (m^2)	1.71 ± 0.05	2.05 ± 0.06†	2.1 ± 0.05†	2.22 ± 0.09†
Lean body mass (kg)	50.0 ± 3.0	61.4 ± 3.9‡	66.0 ± 3.5*	70.7 ± 5.3*

Abbreviation: BMI, body mass index.
*$P \leq .01$; †$P \leq .001$; ‡$P \leq .05$ *v* control subjects.

at the same plasma glucose levels of 5 mmol/L. Insulin was infused at a continuous rate of 6 nmol/kg · min after a priming dose given at a decreasing rate over a period of 10 minutes.

Indirect Calorimetry

The experiments were performed in combination with continuous indirect calorimetry using a Deltatrac cart (Datex Instrumentorium Corp, Helsinki, Finland). The measurements started with a 30- to 45-minute baseline and continued during the 2-hour clamp study. Basal glucose and lipid oxidation were calculated by averaging the values obtained during the 30 minutes preceding the euglycemic, hyperinsulinemic clamp. Glucose and lipid oxidation were calculated by averaging the values for the last 60 minutes of the glucose clamp. Nonoxidative glucose disposal, which largely corresponds to glucose storage, was calculated by subtracting glucose oxidation and urinary loss from the total amount of glucose infused during the clamp.

Muscle Biopsies, Glycogen, and GS Activity

The first needle-biopsy was performed during the 15 minutes before the start of the clamp study and the second at the end of the clamp in the vastus lateralis muscle under local anesthesia with a Bergström muscle-biopsy needle (Depuy, Phoenix, AZ). Samples were immediately frozen in liquid nitrogen and stored at −70°C. They were subsequently assayed for GS activity, as previously reported.[19] Total GS activity was assessed by the activity in the presence of 10.8 mmol/L G6P. GS activity was also measured at 0, 0.1, 0.25, and 1.0 mmol/L G6P, and fractional velocity was calculated as the ratio of GS activity at 0.1 mmol/L G6P divided by the activity measured at 10.8 mmol/L G6P and expressed as percent.[20] Glycogen content was assayed as previously reported.[19]

Analytical Methods

Plasma glucose concentrations were determined by the glucose-oxidase method with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Urinary nitrogen concentration was measured by the method of Kjeldahl.[21] Plasma insulin was measured according to Herbert et al.[22] FFA were determined by commercial kits (NEFA-C; Wako Chemicals GmbH, Neuss, Germany).

Statistical Methods

Data are presented as a group means ± SEM. Intersituational comparisons were performed using 2-way analysis of variance (ANOVA) and unpaired *t* tests. Simple correlations are Pearson Product-Moment correlations.

RESULTS

Subjects

The anthropometric characteristics of the subjects are given in Table 1. The age of diabetic patients was significantly higher than that of the lean control group, but was not statistically different from that of the other obese groups. The body weight and the lean body mass in diabetic patients were significantly different from values in the lean control group but, again, not statistically different from values in the other groups.

Basal Plasma Values, Glucose Clamp, and Indirect Calorimetry

Basal plasma glucose was significantly higher in the 2 groups of obese glucose-intolerant and diabetic patients compared with the lean control subjects ($P < .001$, $P < .001$) (Table 2). During the 75-g oral glucose tolerance test, glycemia was 5.5 ± 0.2, 8.0 ± 0.4, 8.7 ± 0.5, 6.2 ± 0.5, and 4.9 ± 0.3 mmol/L after 0, 30, 60, 120, and 180 minutes in the glucose-tolerant group and 6.4 ± 0.2, 9.7 ± 0.5, 11.5 ± 0.6, 10.6 ± 0.9, and 7.6 ± 1.1 in the glucose-intolerant group. Basal plasma insulin was higher in the obese diabetic group ($P < .05$). Basal plasma FFA was statistically higher in the obese group with impaired glucose tolerance ($P < .05$) and in the diabetic obese group ($P < .01$). In the obese groups, plasma FFA was significantly higher at the end of the clamp than in the control group ($P < .01$, $P < .001$, and $P < .001$). During the clamp, the rate of lipid oxidation was significantly elevated in the obese groups with glucose intolerance and diabetes ($P < .001$) (Fig 1). Glucose oxidation was decreased in these same groups ($P < .001$). Nonoxidative glucose disposal, which essentially measures glucose storage as glycogen in the conditions of the glucose-insulin clamp, in normal lean subjects was significantly lowered in the 3 obese groups ($P < .001$), as was glucose uptake ($P < .001$ in the 3 groups). Muscle glycogen level was similar in all groups studied (Table 3) and not significantly changed after the euglycemic hyperinsulinemic clamp.

GS Activity

GS fractional velocity is currently used as an index of the state of activation (ie, dephosphorylation) of the enzyme. Table 3 shows that, in the basal state, this value is not different in the 4 groups studied. GS fractional velocity increased 2.1-fold ($P \leq .001$) after hyperinsulinemic clamp in the group of control

Table 2. Biological and Metabolical Characteristics

	Control Subjects (N = 15)	Obese Subjects Glucose Tolerant (N = 12)	Obese Subjects Glucose Intolerant (N = 11)	Obese Diabetic Subjects (N = 10)
Basal glycemia (mmol/L)	4.9 ± 0.1	5.1 ± 0.2	5.8 ± 0.1†	8.4 ± 0.6†
Clamp glycemia (mmol/L)	4.9 ± 0.2	5.0 ± 0.1	5.0 ± 0.1	5.6 ± 0.5
Basal insulin (pmol/L)	61 ± 26	64 ± 11	93 ± 26	110 ± 29‡
Clamp insulin (pmol/L)	414 ± 35	531 ± 72	480 ± 35	597 ± 77‡
Basal FFA (μmol/L)	630 ± 45	732 ± 62	825 ± 67‡	940 ± 78*
Clamp FFA (μmol/L)	71 ± 8	138 ± 25*	194 ± 19†	299 ± 78†
Basal lipid oxidation (mg/kg lbm · min)	1.21 ± 0.09	1.32 ± 0.15	1.69 ± 0.15*	1.46 ± 0.14
Λ lipid oxidation (clamp-basal)	0.60 ± 0.08	0.61 ± 0.1	0.64 ± 0.09	0.31 ± 0.1*
Suppression during clamp (%)	49	46	38	21
Basal glucose oxidation (mg/kg lbm · min)	1.52 ± 0.24	0.93 ± 0.22	0.58 ± 0.29‡	1.10 ± 0.25
Λ glucose oxidation (clamp-basal)	2.04 ± 0.21	1.99 ± 0.27	1.48 ± 0.22	0.95 ± 0.25*
Stimulation during clamp (%)	152	214	255	86

*$P \leq .01$; †$P \leq .001$; ‡$P \leq .05$ *v* control subjects.

lean subjects. A similar 1.7-fold ($P \leq .01$) increase in GS fractional activity is observed in the obese glucose-tolerant group. In this group, glucose uptake was decreased by 40% ($P \leq .001$) and nonoxidative glucose disposal by 56% ($P \leq .001$) compared with control subjects (Fig 1). In the groups of glucose-intolerant and diabetic subjects, insulin did not significantly change GS fractional velocity (Table 3).

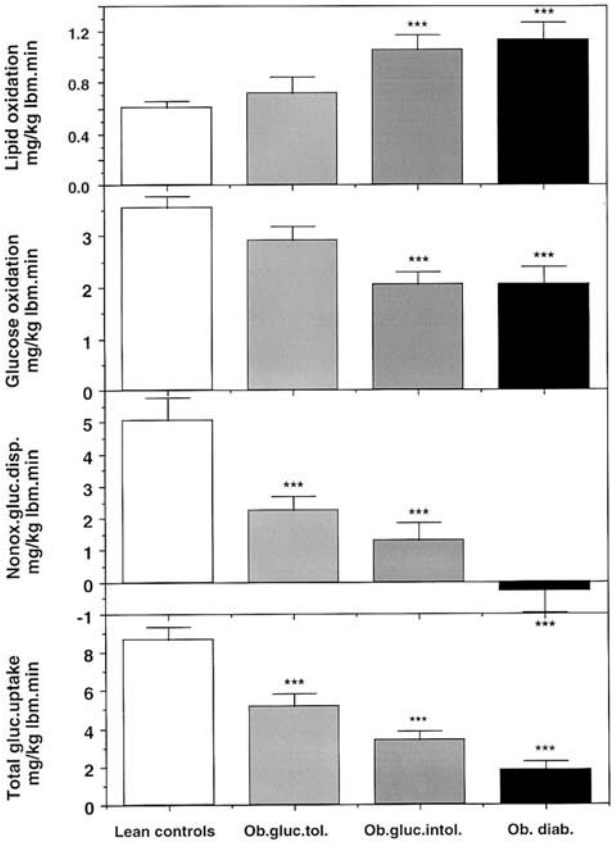

Fig 1. Lipid oxidation, glucose oxidation, nonoxidative glucose disposal, and total glucose uptake during the euglycemic, hyperinsulinemic clamp in lean controls (□), obese glucose-tolerant (ob. gluc. tol; ▨), obese glucose-intolerant (ob. gluc. intol.; ▩), and obese diabetic (ob. diab.; ■) subjects (* $P \leq .001$ *v* lean controls).**

These 2 groups also showed, respectively, a 75% and a total suppression of nonoxidative glucose disposal ($P \leq .001$ *v* control subjects), while glucose oxidation was only decreased by 42% ($P \leq .001$ *v* control subjects, Fig 1). GS activity measured at a maximal concentration of G6P is considered an indicator of total enzyme protein. Total GS activity was unchanged by insulin infusion in all groups studied (Table 3). Total GS activity decreased gradually from the glucose-tolerant group (not significant) to the glucose-intolerant and diabetic group ($P \leq .05$) (Table 3), suggesting a decrease in the total enzyme protein.

DISCUSSION

The 4 groups examined in the present study represent progressive changes from normal lean individuals to obese glucose-tolerant, glucose-intolerant, and diabetic individuals. This progression is accompanied by gradual increases in glycemia and insulinemia (Table 2), as well as decreased response of GS to insulin (Table 3), of overal glucose uptake, glucose oxidation, and nonoxidative glucose disposal (Fig 1). Among these parameters, nonoxidative glucose disposal was markedly decreased in the obese glucose-tolerant group and close to zero in the obese diabetic group, while total glucose uptake and oxidation decreased to the same extent.

Total GS activity decreases progressively from obese to diabetic patients. This decrease may be due to a proportional decrease in total GS protein rather than to a defect or impairment in the activity of the enzyme, as total GS activity is generally considered an indicator of GS enzyme protein. A reduced GS activity has been demonstrated in non–insulin-dependent diabetes mellitus (NIDDM) and persists in skeletal muscle culture.[23] Our study shows that this defect preceeds the development of diabetes. In the present study, although the total GS activity decreased as one progresses from the obese to the obese diabetic group (Table 3, Fig 2), the fractional velocity of GS is similar in all the groups. In the obese glucose-tolerant group, the effect of insulin on GS fractional velocity seems normal, but taking into account the lower total GS values, the

Table 3. Glycogen Synthase

	Control Subjects (N = 15)	Obese Subjects Glucose Tolerant (N = 12)	Obese Subjects Glucose Intolerant (N = 11)	Obese Diabetic Subjects (N = 10)
Basal glycogen synthase (total activity, nmol/min · mg protein)	8.1 ± 1.1	6.2 ± 0.6	4.8 ± 0.6*	4.5 ± 0.7*
Clamp glycogen synthase (total activity, nmol/min.mg protein)	8.6 ± 0.9	6.0 ± 0.7*	4.6 ± 0.5†	4.2 ± 0.6†
Basal glycogen synthase (fractional velocity) (% total activity, 0.1 mmol/L G6P/10.8 mmol/L G6P)	13.0 ± 2.2	17.6 ± 1.8	18.8 ± 2.7	14.2 ± 2.0
Clamp glycogen synthase (fractional velocity) (% total activity, 0.1 mmol/L G6P/10.8 mmol/L G6P)	27.7 ± 2.9‡	30.0 ± 4.3§	26.0 ± 2.5	21.4 ± 3.2
Basal muscle glycogen (μg glucose/mg wet muscle)	7.2 ± 1.3	6.3 ± 0.9	5.1 ± 0.7	6.7 ± 1.2
Clamp muscle glycogen (μg glucose/mg wet muscle)	7.2 ± 1.1	4.9 ± 0.8	5.4 ± 1.0	5.0 ± 0.5

*$P \leq .05$ *v* control subjects.
†$P \leq .01$ *v* control subjects.
‡$P < .001$ *v* basal GS.
§$P < .01$ *v* basal GS.

absolute value of the insulin effect is lower (Fig 2). This may explain, at least partially, the 50% reduction in the nonoxidative glucose disposal. In the obese glucose-intolerant and diabetic groups, insulin failed to significantly increase GS fractional activity and glycogen synthesis measured as nonoxidative glucose disposal.

The lack of insulin stimulation of GS in the glucose-intolerant and diabetic group could be the consequence of dysregulation of insulin signaling to glucose transport and glycogen synthesis. It should be mentioned that these 2 metabolic effects share the same signaling pathway at least up to PKB/Akt.[24] The lack of stimulation of GS could also result from the prevaling hyperinsulinemia. Indeed, recent studies have demonstrated that in normal subjects, physiologic hyperinsulinemia for 3 to 5 days leads to the development of insulin resistance of GS fractional velocity and nonoxidative disposal without changes in insulin's effect on glucose oxidation and glycolysis.[25]

Whether glycogen synthesis is entirely controlled at the level of glucose transport and phosphorylation (the "push" hypothesis) or at the level of glycogen synthase (the "pull" hypothesis)

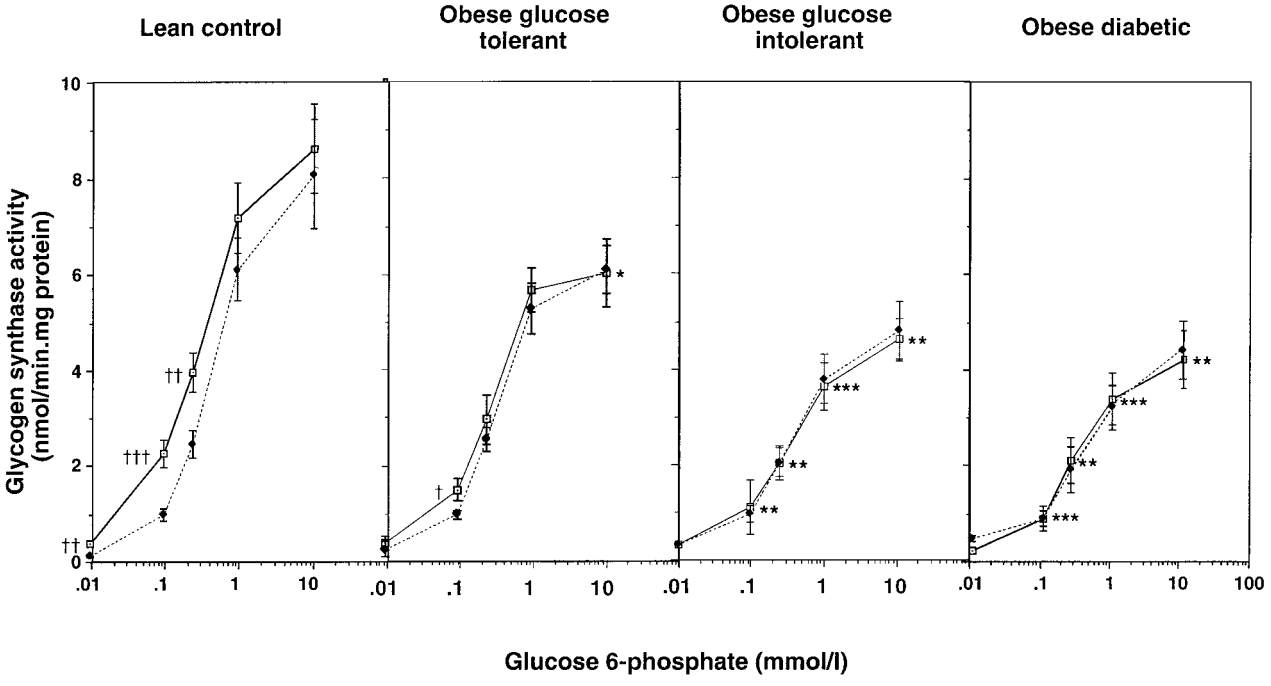

Fig 2. GS activity (nmol/min · mg protein) in the muscle biopsies taken before and after euglycemic hyperinsulinemic clamp. The activity was measured as a function of the concentrations of glucose 6-phosphate in the assay. Lean control subjects and obese glucose-tolerant, glucose-intolerant, and diabetic groups (*$P \leq .05$; **$P \leq .01$; ***$P \leq .001$ *v* lean control) (†$P \leq .05$; ††$P \leq .01$; †††$P \leq .001$ *v* basal); (●·····●) basal, before the clamp and (□—□) after the clamp.

is still matter of debate (reviewed in Lawrence and Roch[15]), and each hypothesis is supported by experiments using transgenic animals overexpressing glucose transporters or GS. Other approaches have measured the relative contribution of glucose transport/phosphorylation and of GS on glycogen synthesis by metabolic control analysis. The measurements suggest that the majority of the flux control of muscle glycogen synthesis is at the transport/phosphorylation step.[26]

Our results do not allow us to favor one or the other hypothesis, as glucose transport was not measured. However, Fig 1 clearly shows that in the glucose-tolerant group, the nonoxidative glucose disposal is decreased by 50%, while glucose oxidation is unchanged compared with lean controls. This suggests that GS could be responsible for an early defect in nonoxidative glucose disposal. The impairment of insulin resistance at the distal site of the glucose-glycogen pathway caused by chronic hyperinsulinemia, chronic hyperglycemia was shown to have its target on the glucose transport system.[27] Glucose toxicity might be a possible cause for insulin resistance in obese patients presenting glucose intolerance or diabetes. This would, however, not be the case in the obese glucose-tolerant patients who present only limited excursions of glycemia.

In conclusion, our data show that insulin stimulated nonoxidative glucose disposal and total GS are very early defects observed in obese patients when lipid and glucose oxidation are not yet significantly altered.

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