Decreased Activation of Skeletal Muscle Glycogen Synthase by Mixed-Meal Ingestion in NIDDM

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Glycogen synthase (GS) catalyzes the formation of glycogen in human skeletal muscle, the tissue responsible for disposal of a significant portion of an oral carbohydrate load. Non-insulin-dependent diabetes mellitus (NIDDM) is characterized by fasting and postprandial hyperglycemia in conjunction with reduced rates of insulin-stimulated glucose disposal and storage in peripheral tissues, including muscle. Our objectives in this study were to determine whether ingestion of a mixed meal activates GS in control nondiabetic subjects and whether meal-related GS activation is reduced in NIDDM. To accomplish this, mixed formula meals were administered to 11 NIDDM and 9 age- and weight-matched nondiabetic control subjects. Plasma glucose and insulin values were measured before and for 90 min after meal ingestion. Skeletal muscle biopsies were performed just before and 90 min after meal ingestion for measurement of GS activity. Compared with control subjects, NIDDM subjects had significantly higher postprandial hyperglycemia and reduced postprandial hyperinsulinemia. GS was activated by meal ingestion in control subjects to a significantly greater extent than in NIDDM subjects. In NIDDM subjects, activation of GS was inversely correlated with fasting plasma glucose ($r = .69$, $P < .05$). Therefore, NIDDM is characterized by reduced activation of a key step in the process of muscle glycogen repletion after a meal. Reduced activation of GS by a mixed meal in NIDDM may contribute to the reduced glucose disposal after a meal, thus contributing to the hyperglycemia observed in these subjects. *Diabetes* 37:436–40, 1988

The mechanisms responsible for hyperglycemia in non-insulin-dependent diabetes mellitus (NIDDM) remain controversial; however, both hepatic and peripheral insulin resistance are thought to be important contributing factors (1–3). Bogardus et al. (4), using steady-state infusions of insulin and glucose in conjunction with isotopically determined glucose disposal rates and indirect calorimetry, showed that in peripheral NIDDM tissues there was decreased glucose storage. In their studies, *glucose storage* was defined as the difference between the overall rate of glucose disposal and the rate of glucose oxidation. Thus, glucose storage represented not only glycogen formation but also lactate production from glucose. Nevertheless, glucose storage in vivo was found to be correlated with the activity of skeletal muscle glycogen synthase (GS), a key regulatory enzyme in the pathway of glycogen formation. Because skeletal muscle is the tissue responsible for most glucose disposal during insulin infusion (5), during hyperinsulinemia, glucose storage probably largely reflects insulin-stimulated glycogen formation in muscle.

However useful the technique of combined steady-state insulin and glucose infusion has been in defining the insulin resistance of NIDDM, these conditions are not physiological. Increased basal rates of hepatic glucose output (1) and excess postmeal glycemia (6) contribute from day to day to hyperglycemia in NIDDM. After meal ingestion, there is both defective suppression of hepatic glucose output and decreased disposal of glucose by peripheral tissues (6). In addition, Boden et al. (7) found that after an oral glucose load, glucose storage estimated with indirect calorimetry in NIDDM subjects was reduced to ~60% of the storage of control subjects. Although the relative contributions of the liver and skeletal muscle in disposal of an oral glucose load is controversial, skeletal muscle probably accounts for one- to two-thirds of this process (6,8–15).

Because activation of GS is an important step in activation of glucose disposal and storage during steady-state infusion of glucose and insulin, activation of this enzyme may also help to regulate glucose storage in skeletal muscle after meal ingestion. Therefore, our goals were to determine whether

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Received for publication 10 June 1987 and accepted in revised form 28 September 1987.

activation of GS by meal ingestion is reduced in NIDDM and, if so, whether this defect is correlated with the postprandial metabolic abnormalities of NIDDM.

MATERIALS AND METHODS

Subjects. All NIDDM subjects (n = 11) were withdrawn from antidiabetic therapy at least 2 wk before the study. Control subjects (n = 9) were age and weight matched with the diabetic subjects and had normal oral glucose tolerance tests (16). All subjects gave written informed consent before participating. These studies were approved by the Human Studies Committee of the University of California, San Diego.

Basic study design. After an overnight fast, a needle muscle (vastus lateralis) biopsy was performed by the method of Bergstrom (17) under local anesthesia. Muscle specimens (50–100 mg) were frozen in liquid nitrogen in <15 s and stored in liquid nitrogen until assay. A liquid formula meal (7 kcal/kg; 45% carbohydrate, 15% protein, 40% fat) was then ingested for 5 min. Ninety minutes after the start of meal ingestion, a second muscle biopsy was performed in the same leg ~1–2 cm distal to the first biopsy. (Ninety minutes approximately coincides with maximum glucose disposal after meal ingestion; 6.) Serum glucose and insulin were measured periodically before and for 90 min after the meal.

Analytical methods. Serum glucose was measured by a glucose oxidase method with a YSI glucose analyzer (Yellow Springs, OH). Insulin was assayed by double-antibody radioimmunoassay.

In vitro methods. Muscle samples were weighed while still frozen and were homogenized (50 mg tissue/ml buffer) at 4°C with a Polytron homogenizer (Brinkmann, Westbury, NY) in a buffer consisting of 2 mM EDTA, 2 mM 1,4-dithiothreitol, 20 mM sodium fluoride, and 50 mM potassium phosphate, pH 7.4. The crude extract was then centrifuged at 20,000 × g for 20 min. The supernatant, which contained the GS activity, was retained, and the pellet, which contained no GS activity, was discarded. GS was assayed by a modification of the method of Thomas et al. (18). The supernatant was diluted 1:5 in a buffer of 20 mM EDTA, 25 mM sodium fluoride, and 50 mM Tris-HCl, pH 7.8. Reactions were started by addition of 30-μl aliquots of cell extracts to 60 μl of a reaction mixture composed of the same buffer used to dilute the supernatant, plus 1% glycogen, 0.7 μCi [U-^{14}C]uridine diphosphate glucose (UDPG), 0.3 mM UDPG, and 0–10 mM glucose 6-phosphate (G6P). Maximal GS activity was determined at saturating concentrations of G6P (10 mM) and UDPG (5 mM). Reactions were allowed to proceed for 15 min at 30°C and were terminated by precipitating 75-μl aliquots of the reaction mixture on 2 × 2-cm squares of filter paper immediately dropped into cold 66% ethanol. After 30 min of washing, the filter papers were washed twice for 20 min each in cold 66% ethanol and once for 5 min in acetone, then dried and placed in organic counting scintillant (Amersham, Arlington Heights, IL) for determination of radioactivity. Enzyme activity was expressed as nanomoles of UDPG incorporated into glycogen per minute per milligram of extract protein. Protein was determined by the method of Lowry et al. (19).

We performed the assay with a range of G6P concentrations to allow determination of the Hill coefficient and an $A_{0.5}$ for G6P (the concentration of G6P that half-maximally activated GS). The activity of GS assayed at 0.1 mM G6P divided by the activity of GS assayed at 10 mM G6P is termed the *fractional velocity* (FV) at 0.1 mM G6P and has been found to be a sensitive measurement of changes in GS activity in experimental diabetes, fasting, and exercise (20,21).

Statistics. Comparisons among means of pre- and postmeal values between groups were made by two-way repeated-measures analysis of variance (ANOVA), except where noted otherwise.

RESULTS

Clinical characteristics. The groups were well matched for age (Table 1). NIDDM subjects had a mean fasting plasma glucose of 240 ± 25 mg/dl. The range of severity of diabetes was wide, with fasting plasma glucose 114–391 mg/dl. On average, both groups were obese [body mass index (BMI) 29 ± 1 kg/m² for control subjects vs. 33 ± 2 kg/m² for NIDDM subjects, NS], although each group was heterogeneous, consisting of both obese and lean individuals. This heterogeneity in BMI was consistent with heterogeneity in fasting serum insulin levels, with the more obese subjects having the higher serum insulin concentrations.

Effect of meal ingestion on glucose metabolism. NIDDM subjects had a significantly greater integrated response of plasma glucose to the meal, over basal values (5894 ± 1114 vs. 3447 ± 880 mg · min^{-1} · dl^{-1}, $P < .05$; Fig. 1). Compared with control subjects, NIDDM subjects exhibited a delayed insulin response to the meal. Insulin concentration peaked at 45 min in control subjects and appeared to reach a plateau between 60 and 90 min in NIDDM subjects. Incremental

TABLE 1
Clinical characteristics

Subjects	Age (yr)	Body mass index (kg/m²)	Fasting plasma glucose (mg/dl)	Fasting serum insulin (μU/ml)
NIDDM				
1	57	33	176	13
2	59	45	333	20
3	53	33	243	12
4	39	35	302	39
5	52	34	391	17
6	55	28	169	14
7	59	19	114	12
8	44	46	151	53
9	64	26	282	6
10	49	32	262	19
11	49	32	216	25
Mean ± SE	52 ± 2	33 ± 2	240 ± 25*	21 ± 4
Control				
1	64	25	99	11
2	45	31	90	9
3	38	28	92	12
4	44	33	93	29
5	65	25	89	12
6	60	26	106	12
7	25	28	76	6
8	66	34	99	29
9	30	33	94	18
Mean ± SE	49 ± 5	29 ± 1	93 ± 3	15 ± 3

*$P < .001$ for fasting plasma glucose greater than that of control rats.

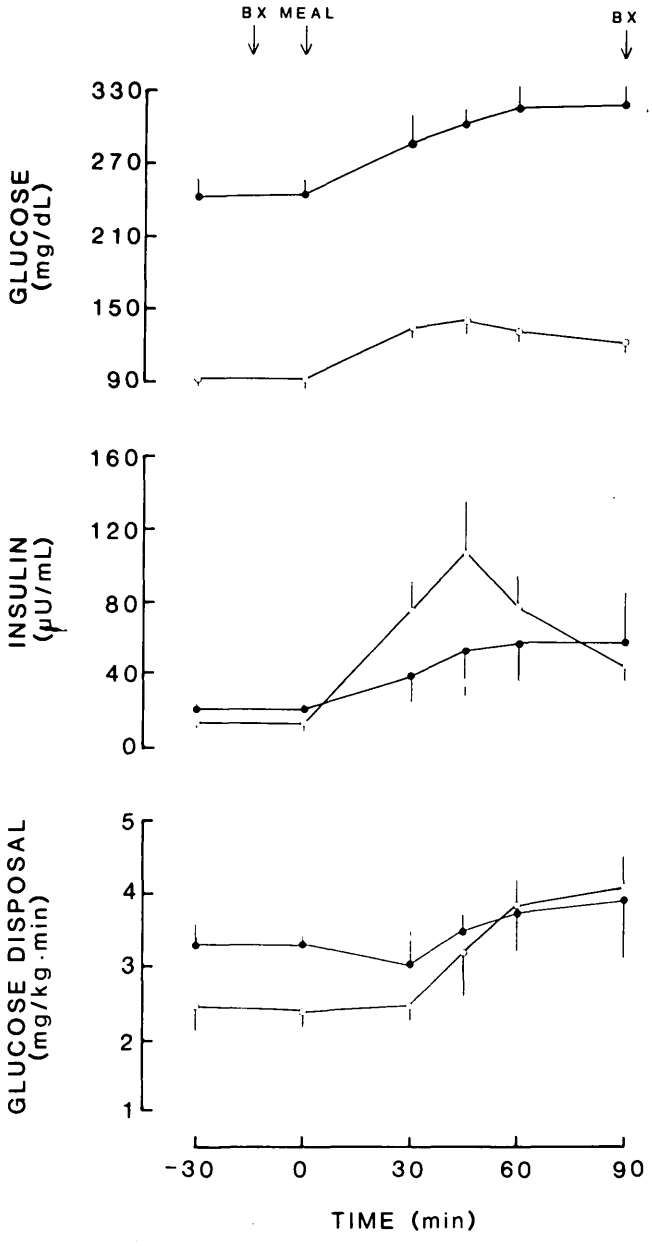

FIG. 1. Effect of meal ingestion on plasma glucose concentration (*top*), serum insulin concentration (*middle*), and glucose disposal rates (*bottom*). ○, Control subjects; ●, NIDDM subjects. For glucose and insulin concentrations, data are means ± SE of 11 NIDDM and 9 control subjects; for glucose disposal rates, data are means ± SE of 7 NIDDM and 6 control subjects, as noted in Table 1. BX, muscle biopsy performed.

insulin area was significantly greater in control than in NIDDM subjects, although there was wide variability (4138 ± 1084 vs. 2362 ± 1123 μU · min^{-1} · ml^{-1}, $P < .05$).

Effect of meal ingestion on GS activity. In control subjects, meal ingestion significantly activated the GS assayed at 0.1 mM G6P (2.88 ± 0.78 nmol · min^{-1} · mg^{-1} in the basal state vs. 4.23 ± 1.17 nmol · min^{-1} · mg^{-1} after the meal, $P < .001$, repeated-measures ANOVA; Table 2). Meal ingestion also activated GS in muscle from NIDDM subjects (2.12 ± 0.44 vs. 2.66 ± 0.38 nmol · min^{-1} · mg^{-1}, $P < .01$, repeated-measures ANOVA), although this activation was not as great as in the control subjects. Premeal GS activity did not differ between groups.

The activity of GS assayed at 0.1 mM G6P expressed as a proportion of maximal GS activity (assayed at 10 mM G6P), termed $FV_{0.1}$ (20,21), is a sensitive indicator of changes in the activation state of the enzyme (21). Expression of GS activity in this manner (Table 2) reduced the interindividual variability among control subjects but not among NIDDM subjects. In the basal state, the $FV_{0.1}$ for NIDDM subjects (0.166 ± 0.034) did not differ from control subjects (0.182 ± 0.036). However, meal ingestion increased $FV_{0.1}$ for control subjects to a significantly greater value than for NIDDM subjects (0.211 ± 0.037 vs. 0.282 ± 0.041, $P < .001$, repeated-measures ANOVA).

Effects of G6P on GS activity. The $A_{0.5}$, or the concentration of G6P required to half-maximally activate the enzyme above its activity in the absence of G6P, was 0.79 ± 0.23 and 0.91 ± 0.26 mM (NS) for control and NIDDM subjects, respectively, before the meal (Table 3). These values were decreased after the meal to 0.47 ± 0.15 and 0.65 ± 0.14 mM, respectively ($P < .01$). Although both pre- and postmeal values for the $A_{0.5}$ for control subjects were slightly lower compared with the values for the NIDDM subjects, the differences were not statistically significant. Maximal activation of the enzyme in the presence of 10 mM G6P was the same for control and NIDDM subjects, and no effect of the meal on this parameter was observed.

Because it has been reported that skeletal muscle GS kinetics exhibit positive cooperativity with respect to interactions with G6P (20,21), Hill coefficients were also estimated from the data. Premeal Hill coefficients were 1.12 ± 0.06 and 1.12 ± 0.05 for control and NIDDM subjects, respectively ($P < .05$). After the meal, the Hill coefficients decreased to 1.03 ± 0.04 ($P < .05$, one-tailed *t* test) and 1.04 ± 0.03 ($P < .01$, one-tailed *t* test) in control and NIDDM subjects, respectively.

Relationships between the metabolic effects of the meal and GS activity. To examine the relationships between metabolic changes and activation of muscle GS activity after meal ingestion, the fold increases in $FV_{0.1}$ values induced by the meal were correlated with both fasting plasma glucose concentrations and integrated responses to the meal of plasma glucose and insulin concentrations. In NIDDM, the fold increase in $FV_{0.1}$ after the meal was inversely correlated with fasting plasma glucose concentration ($r = -.69$, $P < .05$). There was no correlation between fasting plasma glucose and fold increase in $FV_{0.1}$ in controls. Increases in $FV_{0.1}$ were not correlated with integrated glucose ($r = .42$ for NIDDM subjects and .56 for control subjects, NS) or insulin ($r = -.10$ for NIDDM subjects and $-.01$ for control subjects, NS) responses to the meal.

DISCUSSION

Our objective was to determine whether activation of GS by meal ingestion is reduced in NIDDM and, if so, whether this defect is correlated with the postprandial metabolic abnormalities of the disease.

In control subjects, meal ingestion activated GS fractional velocities ($FV_{0.1}$) at 0.1 mM G6P. This may be physiologically significant, because G6P concentrations in human muscle have been calculated in the range of 0.1–0.5 mM. In subjects

TABLE 2
Response of glycogen synthase activity to meal ingestion

Subjects	Glycogen synthase activity (nmol · min^{-1} · mg^{-1})		Fractional velocity (at 0.1 mM glucose 6-phosphate)	
	Premeal	Postmeal	Premeal	Postmeal
NIDDM				
1	0.773	1.59	0.041	0.096
2	4.28	2.74	0.252	0.151
3	3.13	3.31	0.171	0.230
4	4.61	4.72	0.334	0.332
5	2.49	3.00	0.135	0.147
6	0.399	1.07	0.034	0.078
7	0.266	1.25	0.014	0.061
8	1.64	1.64	0.179	0.244
9	1.85	1.91	0.147	0.228
10	1.60	4.03	0.180	0.270
11	2.33	3.97	0.340	0.478
Mean ± SE	2.12 ± 0.44	2.66 ± 0.38*	0.166 ± 0.034	0.211 ± 0.037†‡
Control				
1	3.71	6.20	0.233	0.341
2	6.47	11.20	0.246	0.388
3	6.42	5.40	0.340	0.370
4	0.522	1.42	0.031	0.076
5	0.230	0.86	0.021	0.082
6	3.17	4.76	0.217	0.384
7	2.66	5.21	0.200	0.336
8	1.55	0.86	0.246	0.311
9	1.16	2.20	0.102	0.250
Mean ± SE	2.88 ± 0.78	4.23 ± 1.17	0.182 ± 0.036	0.282 ± 0.041†

*$P < .01$, †$P < .0001$, values greater than those before meal.
‡$P < .001$, value less than those of control rats.

with NIDDM, meal ingestion also activated the $FV_{0.1}$ for GS, although to a significantly lesser extent than in control subjects. Absolute activities of GS were activated in control subjects when assayed with 0.1 mM G6P, where the extent of activation was about 50%. At 0.1 mM G6P, presumably a physiologic concentration, GS activity was minimally activated by meal ingestion in NIDDM.

Although absorption and disposal of a mixed meal is probably not complete 90 min after its ingestion, the diabetic subjects had significantly greater postprandial hyperglycemia than the control subjects during the period studied. This hyperglycemia was accompanied by a reduced, delayed insulin response in the diabetic subjects. In this period, the insulin response to the meal was absolutely lower in subjects with NIDDM than in control subjects.

To determine whether the extent of activation of GS was associated with postprandial hyperglycemia or insulin action, the fold stimulation of $FV_{0.1}$ after the meal was correlated with integrated meal responses of plasma glucose and insulin. Activation of GS was not correlated with postprandial hyperglycemia or insulin response in either control or NIDDM subjects.

Because muscle is an important site of glucose disposal after a meal (6,8–11), disposal of glucose via glycogen storage could be a quantitatively important step regulated in part by activation of GS. Decreased meal activation of GS in NIDDM may then be proposed to be the cause of decreased nonoxidative glucose metabolism after a meal (7). However, the proportion of glucose absorbed by muscle that is stored as glycogen after a meal is controversial, so decreased activation of skeletal muscle GS in NIDDM by a meal cannot completely explain decreased meal-related glucose disposal rates in this disease.

In subjects with NIDDM, the extent of activation of GS after a meal was inversely correlated with the fasting plasma glucose concentration. Therefore, the more severely diabetic subjects had little meal-related activation of the enzyme. In subjects with fasting plasma glucose >300 mg/dl, the fold stimulation of $FV_{0.1}$ by the meal was 0.93 ± 0.12, indicating that the meal had no effect on GS activity. This may be merely

TABLE 3
Effects of meal ingestion on the allosteric activation of glycogen synthase by glucose 6-phosphate

Subjects	*n*	$A_{0.5}$ (mM)		Maximal activity (nmol · min^{-1} · mg^{-1})		Hill coefficient	
		Premeal	Postmeal	Premeal	Postmeal	Premeal	Postmeal
Control	9	0.79 ± 0.23	0.47 ± 0.15*	14.9 ± 1.9	14.3 ± 2.4	1.12 ± 0.06	1.03 ± 0.04†
NIDDM	11	0.91 ± 0.26	0.65 ± 0.14*	14.1 ± 1.4	14.2 ± 1.4	1.12 ± 0.05	1.04 ± 0.03*

*$P < .01$, †$P < .05$ vs. premeal values.

a reflection of the more severe insulin resistance present in these subjects (1), or it may indicate that severe sustained hyperglycemia may be partly due to a failure to activate GS after meals. However, the variability in $FV_{0.1}$ was wide and considerably overlapping in control and NIDDM subjects, so the significance of this correlation in NIDDM is not clear. It is also possible that the decreased activation of GS observed in subjects with NIDDM was related to their decreased response of insulin after the meal. Although no correlation was found between insulin response and enzyme activation, the wide variability in insulin response may have obscured a relationship if one existed.

Premeal GS activities in NIDDM subjects were not significantly different from control values. Thus, despite hyperglycemia and presumably increased glucose flux, GS activities were not increased. All else being equal, this would imply that hyperglycemia per se did not increase GS activity. However, it is conceivable that without hyperglycemia, GS activities in the NIDDM subjects would be lower. In this manner, hyperglycemia may compensate for insulin resistance as has been suggested previously (22).

Reduced GS activation after meal ingestion by NIDDM subjects suggests that these individuals may have a reduced capacity to replete muscle glycogen by meal ingestion, although increased glucose flux due to hyperglycemia may somewhat compensate for this. Because intracellular G6P concentrations in NIDDM are not known, it is difficult to state with precision what the magnitude of this defect may be. The contribution of this defect to basal and postprandial hyperglycemia requires further investigation.

ACKNOWLEDGMENTS

The excellent technical assistance of J. Bell, R. Thorne, P. Elliott, and T. Morrison is gratefully acknowledged. Thanks are also due to C. Knowles and J. Lockwood for editorial assistance.

These studies were supported by grants from the American Diabetes Association, General Clinical Research Center Grant RR-0827, the Danish Research Council, and the NATO Research Council.

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