

Normalization of Plasma Glucose Concentration by Insulin Therapy Improves Insulin-Stimulated Glycogen Synthesis in Type 2 Diabetes

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Considerable evidence suggests that skeletal muscle insulin resistance is an inherent feature of type 2 diabetes and contributes to the pathogenesis of the disease. In patients with poorly controlled diabetes, hyperglycemia is thought to produce additional insulin resistance in muscle. The magnitude and nature of hyperglycemia-induced insulin resistance is not known. The purpose of the present study was to determine the biochemical mechanisms responsible for increased insulin-stimulated glucose disposal after the achievement of tight glycemic control with a mixed-split regimen. We performed hyperinsulinemic-euglycemic clamps with indirect calorimetry and vastus lateralis muscle biopsies in eight type 2 diabetic patients who had poor glycemic control (HbA_{1c} 10.1%) and again after 3 months of intensive insulin therapy designed to produce near-normoglycemia (HbA_{1c} 6.6%). Improved glycemic control increased insulin-stimulated glucose disposal (5.16 ± 0.32 vs. 3.69 ± 0.33 mg · kg⁻¹ · min⁻¹; $P < 0.01$); nonoxidative glucose disposal, which primarily reflects glycogen synthesis (2.11 ± 0.26 vs. 0.90 ± 0.16 mg · kg⁻¹ · min⁻¹; $P < 0.01$); and glycogen synthase fractional velocity (0.094 ± 0.017 vs. 0.045 ± 0.007 ; $P < 0.05$). There was no improvement in insulin-stimulated glucose oxidation (3.05 ± 0.25 vs. 2.79 ± 0.20 mg · kg⁻¹ · min⁻¹), hexokinase II mRNA expression (increase over basal values), or hexokinase II enzymatic activity (0.51 ± 0.16 vs. 0.42 ± 0.18 pmol · min⁻¹ · μg⁻¹ protein). All of the increase in insulin-stimulated glucose disposal could be accounted for by increased glycogen synthesis, which is likely attributable to increased activation of glycogen synthase by insulin. *Diabetes* 51: 462–468, 2002

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FFA, free fatty acid; FFM, fat-free mass; G6P, glucose 6-phosphate; GS_{0.1}, glycogen synthase determined using 0.1 mmol/l G6P; GS₁₀, glycogen synthase determined using 10 mmol/l G6P; GS_n, glycogen synthase fractional velocity; HK, hexokinase.

Insulin resistance in skeletal muscle is a characteristic feature of type 2 diabetes (1). The insulin resistance is thought to derive from two components. One component is primary or hereditary in nature, and is present before the development of diabetes and its attendant metabolic abnormalities. Abundant evidence shows that normal glucose-tolerant individuals with a strong family history of type 2 diabetes are insulin resistant (2–7), thereby suggesting a hereditary component to the defect in insulin action. The second component of the muscle insulin resistance is acquired secondary to alterations in the metabolic milieu, including elevated plasma glucose (8–11), free fatty acid (FFA) (12–14), and insulin (15–17) levels. Experimental hyperinsulinemia has been shown to cause insulin resistance both in vitro (10,18) and in vivo (15–17,19). Similarly, a physiological elevation in plasma FFA concentrations has been shown to induce muscle insulin resistance (20–22). Finally, chronic hyperglycemia itself is known to impair insulin action (8,9), and reversal of hyperglycemia in rats with phlorizin (23–25) improves insulin sensitivity. In type 1 diabetic patients, intensive insulin therapy also has been shown to augment insulin action (26–29). Some (30–36), but not all (37), studies have shown that insulin therapy can improve insulin resistance in type 2 diabetic patients as well. However, the improvement in insulin resistance is less uniformly observed in type 2 than in type 1 diabetic subjects and, when observed, is of lesser magnitude. No previous study has examined the biochemical basis for the improvement in insulin resistance observed after improved glycemic control with insulin in type 1 or type 2 diabetic individuals.

Results from several recent studies in rodents have suggested that increased hexosamine biosynthesis leads to skeletal muscle insulin resistance in vivo and in vitro (38–40). In other studies, glucose-induced activation of protein kinase C isoforms has been shown to interfere with insulin receptor signaling and produce insulin resistance (41–44). Kurowski et al. (45), using rat extensor digitorum longus muscle, provided evidence that glucose-induced insulin resistance affects 2-deoxy-glucose uptake and glycogen synthesis and suggested that the defects in glucose transport and phosphorylation might be independent of the defect in glycogen synthesis.

In the present study, we used a mixed-split insulin treatment regimen to produce tight glucose control in type 2 diabetic subjects in an attempt to determine the biochemical mechanisms responsible for impaired insulin-stimulated glucose disposal in skeletal muscle. Hyperinsulinemic-euglycemic clamps with indirect calorimetry and vastus lateralis muscle biopsies were performed while diabetic subjects were in poor glycemic control and again after they had sustained 3 months of near-normoglycemia through insulin therapy. Insulin-stimulated whole-body glucose uptake, glucose oxidation, and nonoxidative glucose disposal (primarily representing glycogen synthesis) were quantified and compared with insulin-stimulated hexokinase (HK) II activity and mRNA levels and glycogen synthase activity in muscle biopsies. The results demonstrated that improved glycemic control enhances insulin-stimulated glucose disposal, nonoxidative glucose disposal (glycogen synthesis), and glycogen synthase activity, without improving glucose oxidation, hexokinase enzymatic activity, or hexokinase mRNA expression.

RESEARCH DESIGN AND METHODS

Subjects. Eight subjects with poorly controlled type 2 diabetes (five men and three women, aged 51 ± 3 years, BMI 34.7 ± 2.1 kg/m²) participated in this study. In all subjects, the diagnosis of type 2 diabetes was established using American Diabetes Association criteria. All subjects had long-standing diabetes (mean duration 6 ± 2 years), had a history of poor glycemic control (HbA_{1c} >8%), and were being treated with diet plus sulfonylureas. None of the patients had previously taken metformin or a thiazolidinedione. Other than having type 2 diabetes, all subjects were in good general health as determined by medical history, physical examination, routine blood tests, urinalysis, and electrocardiogram. No subject was taking any medication known to adversely affect glucose metabolism. In all subjects, body weight was stable for at least 4 months before the study. No subject participated in exercise on a regular basis. On the day of the subjects' initial visit and on the day of the hyperinsulinemic-euglycemic clamp, the HbA_{1c} was determined to provide a measure of glycemic control. Subjects were instructed to maintain their usual diet and not to engage in any vigorous exercise for at least 3 days before the study. The purpose, nature, and potential risks of the study were explained to all subjects, and their written consent was obtained before their participation. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, TX.

Study design. All patients received a hyperinsulinemic-euglycemic clamp study with biopsies of the vastus lateralis muscle before and after 12 weeks of tight glycemic control, which was achieved with a mixed-split insulin treatment regimen (see below). The hyperinsulinemic-euglycemic clamps were performed at the General Clinical Research Center of the University of Texas Health Science Center at San Antonio, TX, as previously described (46). Studies began at 0700 h after a 10-h overnight fast. An antecubital vein was cannulated for infusion of [3-³H]glucose, 20% dextrose, and insulin (Humulin; Eli Lilly, Indianapolis, IN). A hand vein was cannulated in retrograde fashion, and the hand was placed in a heated box (60°C) for sampling of arterialized blood. A primed ($25 \times$ fasting plasma glucose/90 μ Ci)-continuous (0.25 μ Ci/min) infusion of [3-³H]glucose was begun 3 h before the start of insulin infusion to ensure isotopic equilibration. After 120 min of bed rest, a percutaneous muscle biopsy was obtained with a Bergstrom cannula from the vastus lateralis muscle under local anesthesia (47). Muscle biopsy specimens (75–200 mg) were immediately blotted free of blood, frozen, and stored in liquid nitrogen until used. Blood was drawn every 5–10 min during the last 30 min of the isotopic equilibration period for measurement of tritiated glucose radioactivity and plasma glucose, insulin, and FFA concentrations. Continuous indirect calorimetry was performed with a ventilated hood system (DeltaTrac, Sensor Medics, Anaheim, CA) during the last 40 min of the tracer equilibration and insulin clamp periods for the measurement of carbohydrate and lipid oxidation rates. After completion of the 3-h isotopic equilibration period, an infusion of insulin was started at the rate of $40 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 240 min. Plasma glucose was measured every 5 min throughout the study with a glucose oxidase analyzer (Beckman Instruments, Fullerton, CA). After the start of the insulin infusion, the plasma glucose concentration was allowed to decline to 100 mg/dl, at which level it was maintained using a variable infusion rate of 20% glucose. At the end of the study, a second percutaneous

muscle biopsy was obtained from the opposite vastus lateralis muscle and then the insulin infusion was stopped.

After completion of the hyperinsulinemic-euglycemic clamp, all subjects met with a diabetes nurse educator and were instructed in home blood glucose monitoring. Then patients stopped their oral hypoglycemic medication and were started on a multiple insulin injection regimen, which consisted of bedtime and morning NPH insulin with two injections of regular insulin 20–30 min before breakfast and dinner. On average, the diabetic subjects took 32 ± 5 units of NPH insulin and 9 ± 1 units of regular insulin in the morning, 14 ± 2 units of regular insulin with dinner, and 24 ± 2 units of NPH insulin at bedtime. Home self-monitoring of blood glucose concentrations was performed four times per day. Subjects spoke with the investigators two to three times per week and returned to the clinic every 2 weeks for a review of blood glucose self-monitoring and insulin dosage adjustment. HbA_{1c} was measured every month and twice during the last week of the study. After 12 weeks of intensified glycemic control, the hyperinsulinemic-euglycemic clamp study with indirect calorimetry and muscle biopsies was repeated in all subjects, as described above. Fat-free mass (FFM) was measured before and after intensified glycemic control by a bioelectrical impedance method (RJL Systems, Clinton, MI).

Enzyme activity assays. Glycogen synthase, HKI, and HKII activities were assayed as previously described, with some minor modifications (48). A portion of the muscle biopsy specimen was homogenized using a Polytron Homogenizer (Brinkman Instruments) for 20 s at high speed in a buffer consisting of 50 mmol/l potassium phosphate (pH 7.4), 2 mmol/l dithiothreitol, 2 mmol/l EDTA, 20 mmol/l sodium fluoride, 10 μ g/ml leupeptin, 10 μ g/ml soybean trypsin inhibitor, 20 μ g/ml *p*-aminobenzamidine, 70 μ g/ml *N*_α-*P*-tosyl-L-lysine chloromethyl ketone, and 170 μ g/ml phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 13,000*g*, and the supernatant (soluble fraction) was removed and saved for assay of glycogen synthase activity. Glycogen synthase activity was assayed in the soluble fraction using 0.1 and 10 mmol/l glucose 6-phosphate (G6P). Glycogen synthase fractional velocity (GS_{fv}) was calculated as the ratio of the activity determined using 0.1 mmol/l G6P (GS_{0.1}) to that determined using 10 mmol/l G6P (GS₁₀), as previously described (49). A separate portion of the whole homogenate was solubilized in the above homogenization buffer containing 1% Triton X-100 and used for assaying HKI and HKII activity, as previously described (48).

HKI and HKII mRNAs. Hexokinase mRNA content was determined in total RNA isolated from a portion of each muscle biopsy using an RNase protection assay (Ambion, Austin, TX), as previously described (48). Muscle was extracted using a guanidinium isothiocyanate method (Tel-Test, Friendswood, TX). The content of HKI and HKII mRNA was determined on 4- μ g aliquots of total RNA. Riboprobes were generated that would yield protected products of 396 nt for HKI and 231 nt for HKII (48,50). HKI and HKII RNAs were quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and were normalized to a 28S rRNA internal control signal (Ambion, Austin, TX). Thus, within each sample, 28S rRNA, HKI mRNA, and HKII mRNA were quantified simultaneously. All signals ((PhosphorImage density units) were normalized to the 28S rRNA value within that sample. Insulin stimulation of HK mRNA was then assessed by setting the basal value for each subject to 1.0 and expressing the insulin-stimulated value relative to that subject's basal value. Therefore, values for HKI and HKII mRNA are expressed as dimensionless ratios.

Laboratory analyses. Plasma insulin concentration was determined by radioimmunoassay (Diagnostic Product, Los Angeles, CA), and plasma FFA concentration was determined by an enzymatic method (Wako Pure Chemical Industry, Osaka, Japan). Plasma-tritiated glucose specific activity was determined on barium hydroxide/zinc sulfate-precipitated plasma samples.

Calculations. During the postabsorptive state, the rate of glucose appearance equals the rate of glucose disappearance and is calculated by dividing the tritiated glucose infusion rate (disintegrations per minute \times minute⁻¹) by the plasma tritiated glucose specific activity (disintegrations per minute \times milligram⁻¹). During the insulin clamp, non-steady-state conditions exist and the rate of whole glucose disappearance was calculated using Steele's non-steady-state equation (51), with a distribution volume of 250 ml and a pool correction factor of 0.65, as previously described (52). The rate of glucose oxidation was calculated from the rates of respiratory gas exchange, as previously described (53). The rate of nonoxidative glucose disposal, which primarily represents muscle glycogen synthesis (54), was calculated by subtracting the rate of glucose oxidation from the rate of total body glucose disposal during the hyperinsulinemic-euglycemic clamp.

Statistics. Differences in glucose metabolic rates, enzyme activities, and mRNA levels before and after intensified glycemic control were compared for statistical significance by ANOVA or paired *t* test, where appropriate.

TABLE 1
Subject characteristics

Body weight (kg)		Lean body mass (%)		Fasting plasma glucose (mg/dl)		HbA _{1c} (%)	
Before	After	Before	After	Before	After	Before	After
97.3 ± 7.7	103.6 ± 8.7	64 ± 4	63 ± 4	236 ± 16	119 ± 8*	10.1 ± 0.5	6.6 ± 0.2

Data are means ± SE. Characteristics of diabetic subjects ($n = 8$) are before and after 3 months of intensive insulin therapy designed to achieve near-normal plasma glucose concentrations. * $P < 0.001$ vs. before treatment.

RESULTS

Effects of insulin therapy on metabolic control and body weight. After 3 months of insulin therapy, fasting blood glucose concentrations were significantly improved (236 ± 16 to 119 ± 8 mg/dl; $P < 0.001$), as were HbA_{1c} levels (10.1 ± 0.5 to $6.6 \pm 0.2\%$; $P < 0.01$), despite an increase in body weight from 97.3 ± 7.7 to 103.6 ± 8.7 kg ($P = 0.06$) (Table 1). Fasting plasma FFA concentrations were 819 ± 65 and 570 ± 37 μmol before and after insulin therapy, respectively ($P < 0.01$). Similarly, during the last 30 min of the hyperinsulinemic-euglycemic clamp, plasma FFA concentrations were more suppressed by insulin infusion after insulin therapy (232 ± 30 vs. 131 ± 7 μmol ; $P < 0.01$).

Glucose metabolism. When the diabetic subjects had poor glucose control, the basal rate of endogenous (hepatic) glucose production was 3.89 ± 0.21 $\text{mg} \cdot \text{kg}^{-1}$ FFM $\cdot \text{min}^{-1}$ and was suppressed to 0.79 ± 0.14 $\text{mg} \cdot \text{kg}^{-1}$ FFM $\cdot \text{min}^{-1}$ during the insulin clamp. After 12 weeks of intensified insulin therapy, basal hepatic glucose production decreased to 3.13 ± 0.20 $\text{mg} \cdot \text{kg}^{-1}$ FFM $\cdot \text{min}^{-1}$ ($P < 0.05$ vs. before insulin therapy), and the rate of glucose production during the insulin clamp was suppressed to 0.06 ± 0.06 $\text{mg} \cdot \text{kg}^{-1}$ FFM $\cdot \text{min}^{-1}$ ($P < 0.01$ vs. insulin clamp before insulin therapy). The basal rate of hepatic glucose production was strongly correlated with the fasting plasma glucose concentration ($r = 0.722$, $P < 0.01$). Insulin-stimulated glucose disposal increased by $47 \pm 12\%$, from 3.69 ± 0.33 to 5.16 ± 0.32 $\text{mg} \cdot \text{kg}^{-1}$ FFM $\cdot \text{min}^{-1}$ ($P < 0.01$) after 12 weeks of intensified glycemic control with insulin (Fig. 1). During the baseline insulin clamp study, glucose oxidation increased from 1.57 ± 0.31 (basal) to 2.79 ± 0.76 $\text{mg} \cdot \text{kg}^{-1}$ FFM $\cdot \text{min}^{-1}$ ($P < 0.05$ vs. basal). After 12 weeks of insulin therapy, the increase in glucose oxidation during the insulin clamp (from 1.48 ± 0.24 to 3.05 ± 0.34 $\text{mg} \cdot \text{kg}^{-1}$ FFM $\cdot \text{min}^{-1}$) was similar to that during the baseline insulin clamp study. Insulin-stimulated nonoxidative glucose metabolism, which primarily represents skeletal muscle glycogen synthesis, was significantly increased by insulin therapy from 0.90 ± 0.22 to 2.11 ± 0.26 $\text{mg} \cdot \text{kg}^{-1}$ FFM $\cdot \text{min}^{-1}$ ($P < 0.001$) (Fig. 1). The increase in whole-body glucose disposal after 12 weeks of insulin therapy was primarily attributable to the increase in nonoxidative glucose disposal.

Glycogen synthase activity. Glycogen synthase activities are summarized in Table 2, and the insulin-stimulated GS_{FV}, a sensitive indicator of insulin action in skeletal muscle (48), is shown in Fig. 2. When the subjects were in poor glycemic control, insulin failed to increase glycogen synthase activity assayed in the presence of physiological concentrations of G6P (GS_{0.1}) (Table 1) and only marginally stimulated GS_{FV} (Fig. 2). After 12 weeks of good

glycemic control, insulin significantly enhanced GS_{FV} ($P < 0.01$) and increased GS_{0.1} ($P = 0.05$). The insulin-stimulated increase in GS_{FV} was significantly increased ($P < 0.05$) in subjects after good glycemic control had been achieved.

HK mRNA and activity. The effect of the 4-h insulin infusion on HKI and HKII mRNA is shown in Fig. 3. Relative to a 28S ribosomal RNA internal control signal, the 4-h insulin infusion failed to increase HKI or HKII mRNA when the diabetic subjects had poor glycemic control. After subjects had achieved 3 months of good glycemic control, insulin still had no effect on HKI or HKII mRNA. Consistent with this, insulin infusion did not increase HKI or HKII activity in muscle biopsies, whether patients had poor or good glycemic control (Fig. 4).

DISCUSSION

Type 2 diabetes is characterized by skeletal muscle insulin resistance (1). A proportion of this insulin resistance is considered to be present before the development of hyperglycemia and is genetic in origin (2–7,55). However, hyperglycemia, once manifest, also contributes to the worsening of insulin resistance (8,9). The magnitude and nature of insulin resistance that is primary, and presumably genetic in origin (55), versus that which is acquired secondarily to metabolic disturbances, such as hyperglycemia, is unclear. In vitro, high media glucose concentra-

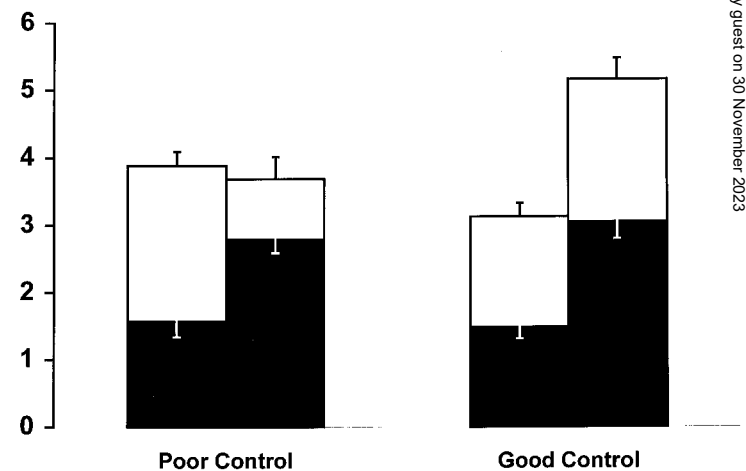


FIG. 1. Insulin-stimulated whole-body glucose metabolism in eight patients with type 2 diabetes before (Poor Control) and after (Good Control) 12 weeks of insulin therapy. Subjects had a hyperinsulinemic-euglycemic ($40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) clamp with measurement of total body glucose disposal (total height of the bar) using tritiated glucose and glucose oxidation (■) using indirect calorimetry. Nonoxidative glucose disposal, or glycogen synthesis (□), represents the difference between total glucose disposal and glucose oxidation. Data are presented as means ± SE

TABLE 2
Glycogen synthase activities

	Before glycemic control			After glycemic control		
	GS _{0.1}	GS ₁₀	GS _{fv}	GS _{0.1}	GS ₁₀	GS _{fv}
Basal	0.252 ± 0.071	9.269 ± 2.969	0.034 ± 0.005	0.229 ± 0.028	6.547 ± 1.638	0.044 ± 0.008
Insulin	0.279 ± 0.028	7.097 ± 2.033	0.045 ± 0.007	0.466 ± 0.163*†	5.216 ± 1.321	0.094 ± 0.017*†

Data are means ± SE. Glycogen synthase activities in muscle biopsies taken from diabetic subjects ($n = 8$) basally and after 240 min of insulin infusion before and after 3 months of intensive insulin therapy designed to achieve near-normal plasma glucose concentrations. * $P < 0.05$ vs. before treatment; † $P < 0.05$ insulin vs. basal.

tions induce insulin resistance in glycogen synthase in primary cultures of human skeletal muscle cells (10). However, it is not known whether the insulin resistance that develops secondary to hyperglycemia differentially affects specific pathways of glucose metabolism. In the present study, we examined these questions by assessing the impact of reversal of hyperglycemia with an insulin therapy regimen on skeletal muscle glucose metabolism in type 2 diabetic patients.

The results of the present study showed that 12 weeks of a mixed-split insulin regimen with near-normalization of the mean day-long plasma glucose concentration increased insulin-stimulated glucose disposal rates by ~50%. The average rate of insulin-stimulated whole-body glucose disposal ($3.26 \pm 0.32 \text{ mg} \cdot \text{kg}^{-1} \text{ body wt} \cdot \text{min}^{-1}$) after achievement of near-normoglycemia is similar to that observed in normal glucose-tolerant obese subjects who were previously studied in our laboratory with a $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ hyperinsulinemic-euglycemic clamp ($3.30 \pm 0.3 \text{ mg} \cdot \text{kg}^{-1} \text{ body wt} \cdot \text{min}^{-1}$) (56). In that study, the obese subjects had no family history of type 2 diabetes and were of comparable obesity to the diabetic subjects in the present study (56). These results suggest that, after achieving good metabolic control, obese patients with type 2 diabetes are inherently no more insulin resistant than obese nondiabetic subjects, and that the additional insulin resistance of the diabetic subjects when they had poor glycemic control was caused by hyperglycemia or other alterations in the metabolic milieu. This result is consis-

tent with previous observations from our laboratory (57) and others (58) that have demonstrated that the addition of type 2 diabetes to obesity confers little or no further reduction in insulin sensitivity.

The present results also showed that the achievement of good metabolic control through an intensified insulin regimen preferentially improved insulin-stimulated non-oxidative glucose disposal (glycogen synthesis) without any effect on glucose oxidation. Because glucose oxidation accounts for >90% of the flux through glycolysis (56), it can be inferred that intensified glycemic control has little or no effect on insulin-stimulated glycolytic flux, and that essentially all of the improvement in whole-body glucose utilization was attributable to increased nonoxidative glucose disposal, which primarily reflects skeletal muscle glycogen synthesis (54). The ability of insulin to stimulate glycogen synthase activity also was markedly improved after insulin therapy. The results of the glycogen synthase assays strongly support the conclusion that achievement of near-normoglycemia by intensified insulin therapy improves insulin-stimulated skeletal muscle glycogen synthesis. Insulin activation of glycogen synthase activity is strongly correlated with nonoxidative glucose disposal or glycogen synthesis during a euglycemic insulin clamp (59). It is therefore likely that at least one mechanism through which insulin therapy brings about improved glucose disposal is by increasing the ability of insulin to activate glycogen synthase. These results are consistent with the observations of Henry et al. (10), who reported that human skeletal muscle cells cultured in media containing a high glucose concentration developed a marked impairment in insulin-stimulated glycogen synthase activity. On the other hand, it should be noted that skeletal muscle cells cultured from patients with type 2 diabetes

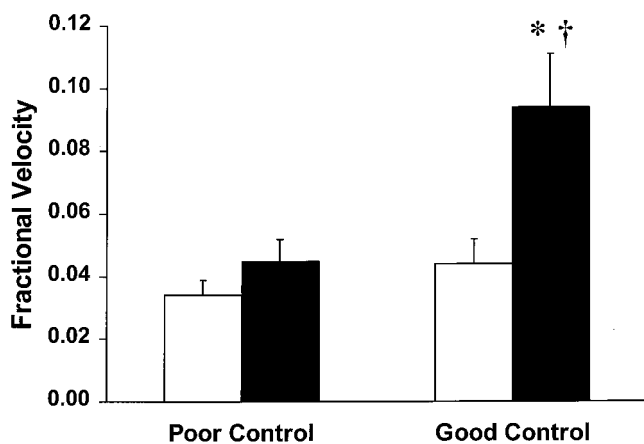


FIG. 2. Insulin-stimulated GS_{fv} in eight patients with type 2 diabetes before (Poor Control) and after (Good Control) 12 weeks of insulin therapy. Subjects had a hyperinsulinemic-euglycemic clamp ($40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) with biopsies of the vastus lateralis muscle basally and at the end of the 240-min euglycemic insulin clamp. GS_{fv} was calculated as the ratio of GS_{0.1} to GS₁₀. □, basal values; ■, insulin-stimulated values. Data are means ± SE. * $P < 0.05$ vs. basal values; † $P < 0.05$ vs. Poor Control values.

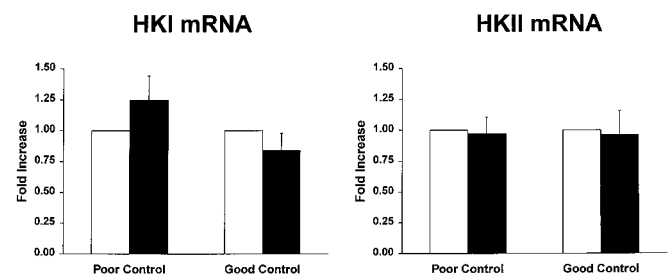


FIG. 3. Lack of effect of insulin on HKI or HKII mRNA in eight patients with type 2 diabetes before (Poor Control) and after (Good Control) 12 weeks of insulin therapy. Subjects had a hyperinsulinemic-euglycemic clamp ($40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) with biopsies of the vastus lateralis muscle basally and at the end of the 240-min euglycemic insulin clamp. HKI and HKII mRNA expression levels were determined in the muscle biopsies using an RNase protection assay, as described in RESEARCH DESIGN AND METHODS. □, basal values; ■, insulin-stimulated values. Data are means ± SE.

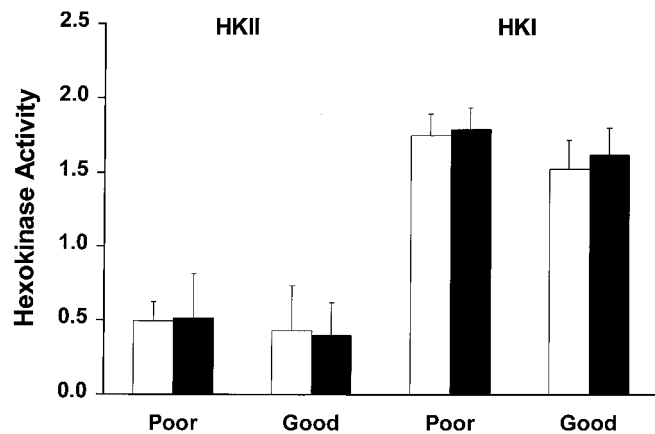


FIG. 4. Lack of effect of insulin infusion on HKI or HKII activity in eight patients with type 2 diabetes before (Poor Control) and after (Good Control) 12 weeks of insulin therapy. Subjects had a hyperinsulinemic-euglycemic clamp ($40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) with biopsies of the vastus lateralis muscle basally and at the end of the 240-min euglycemic insulin clamp. HKI and HKII activities were determined in the muscle biopsies, as described in RESEARCH DESIGN AND METHODS □, basal values; ■, insulin-stimulated values. Data are means \pm SE.

retain an abnormality in glycogen synthase activation by insulin, suggesting that the defect in glycogen synthase may not be completely reversible (60).

In contrast to nonoxidative glucose disposal, tight glucose control produced by insulin therapy did not increase insulin-stimulated whole-body glucose oxidation in the present diabetic subjects. This implies that the abnormality in glucose oxidation could be either primary (inherited) or secondary (acquired), but not reversible by achieving near-normoglycemia. In support of the former, Gulli et al. (4) and Niskanen et al. (61) have reported decreased insulin-stimulated glucose oxidation in normal glucose-tolerant subjects with a family history of type 2 diabetes. In contrast, several investigators have reported that insulin-stimulated glucose oxidation is normal in subjects with a strong family history of type 2 diabetes. Differences in ethnicity or subject selection could explain this discrepancy in findings. Alternatively, the persistent defect in glucose oxidation after improved glycemic control could be related to the persistent or worsened obesity in the present study. It is well established that insulin-stimulated glucose oxidation is decreased in obese nondiabetic subjects (62–65) and that weight loss ameliorates the defect in insulin-stimulated glucose oxidation (66,67). Thus, the irreversible defect in glucose oxidation in the present group of patients could be secondary to obesity. Because insulin therapy does not reduce obesity (rather, in the present study, subjects gained body mass), under this scenario one would not expect to see a reversal of a defect in glucose oxidation.

Because essentially all of the increase in glucose disposal was accounted for by nonoxidative glucose disposal (glycogen synthesis), this implies that glucose flux through glycolysis was not increased. Consistent with this observation, insulin therapy had no effect on HKI or HKII activity. We have previously shown that HK activity is decreased in muscle from type 2 diabetic subjects (68), and that in vivo insulin-stimulated flux through HK in muscle of type 2 diabetic subjects is severely impaired (69). The present results suggest that the defect in hexoki-

nase activity is not secondary to hyperglycemia, given that achievement of good glycemic control did not increase HK activity. Alternatively, if the defect in HK is acquired secondary to chronic hyperglycemia, one must postulate that after a certain duration of poor glycemic control, the defect becomes fixed and cannot be reversed by near-normalization of the plasma glucose concentration with insulin therapy. We (48) and others (70,71) have also shown that a 4-h insulin infusion, although maintaining euglycemia, increases HKII mRNA in skeletal muscle from healthy nondiabetic subjects. The response of HKII mRNA and activity to insulin is severely impaired in type 2 diabetic individuals. In the present study, improved glycemic control failed to ameliorate these defects in the diabetic subjects, suggesting that the defects in insulin-stimulated HKII mRNA and HKII activity are not secondary to hyperglycemia. Moreover, it is likely that the continued non-response of HKII mRNA to insulin is related to, if not responsible for, the defect in hexokinase activity. These findings suggest that the defect in insulin stimulation of HKII mRNA expression in type 2 diabetes may be present before hyperglycemia develops and represents an important defect responsible for the pathogenesis of insulin resistance in obese type 2 diabetic individuals. In support of this, obese nondiabetic subjects also have a profound defect in insulin stimulation of HKII mRNA expression. We reported in a previous study that an insulin infusion of $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ (the same as that used in the present study) increased HKII mRNA expression by 50% in lean control volunteers but had no effect on HKII mRNA in obese nondiabetic control subjects. Similarly, HKII activity was decreased in obese nondiabetic subjects compared to lean healthy control subjects (68). Because there is no information concerning HKII expression or activity in normal glucose-tolerant, nonobese subjects with a family history of type 2 diabetes, it is still unclear whether the defect in HKII is hereditary or secondary to obesity. Thus, it appears that the abnormality in insulin stimulation of HKII expression in patients with type 2 diabetes is not related to hyperglycemia or poor metabolic control. However, it is possible that the defect in HK expression may be secondary to obesity and not related to a hereditary abnormality in type 2 diabetes.

Insulin therapy improved not only fasting plasma glucose concentrations and HbA_{1c} levels, but also fasting and insulin-suppressed plasma FFA concentrations. It is possible, therefore, that some of the improvement in whole-body glucose disposal, nonoxidative glucose disposal, and glycogen synthase activity was attributable to amelioration of the adverse effects of high plasma FFA concentrations. Infusion of a triglyceride emulsion to increase plasma FFAs induces insulin resistance in glucose disposal and the glycogen synthetic pathway (20–22). Therefore, it is reasonable to expect that a decrease in elevated plasma FFA levels might improve insulin sensitivity in the nonoxidative/glycogen synthase pathway. However, despite the reduction in plasma FFAs with insulin therapy, there was no improvement in glucose oxidation or HKII activity.

In conclusion, the achievement of near-normoglycemia by 3 months of insulin therapy in type 2 diabetic patients improved insulin-stimulated muscle glucose uptake by

increasing insulin-stimulated glucose storage and glycogen synthase activity. In contrast, insulin stimulation of glucose oxidation and HKII mRNA expression and HK activity remained impaired after insulin therapy. The irreversibility of these defects would be consistent with an underlying hereditary cause, an acquired defect that is not reversible by achieving near-normoglycemia and does not have an etiology secondary to obesity.

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