IGF-I–Stimulated Glucose Transport in Human Skeletal Muscle and IGF-I Resistance in Obesity and NIDDM

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Based on the observation that insulinlike growth factor I (IGF-I) can stimulate glucose utilization in nondiabetic subjects and that the action of the IGF-I receptor is normal in the skeletal muscle of patients with non-insulin-dependent diabetes mellitus (NIDDM), it seems possible that IGF-I might provide an effective acute treatment for the hyperglycemia of NIDDM. Using our recently developed in vitro human muscle preparation, we investigated the hypothesis that IGF-I might be an effective alternative to insulin in stimulating glucose transport in diabetic muscle. Abdominal muscle samples from nonobese nondiabetic, obese nondiabetic, and obese NIDDM patients were obtained during elective abdominal surgery. Plasma levels of IGF-I in diabetic patients were lower than those in either of the nondiabetic groups. Binding studies with wheat-germ–agglutinin–chromatography–purified receptors demonstrated the presence of IGF-I receptors in human muscle, with IGF-I binding being ~24% that of insulin. There was no change in IGF-I binding in muscle from obese or diabetic subjects, and the structural characteristics of the IGF-I receptor were not altered, as determined by electrophoretic mobility. IGF-I stimulated glucose transport approximately twofold in incubated muscle from control subjects, but there was no IGF-I stimulation of transport in muscle from obese subjects with or without NIDDM. These results confirm a previous report that human muscle contains receptors for IGF-I and demonstrate for the first time that IGF-I can stimulate glucose transport in human muscle. However, muscle from obese subjects with or without NIDDM is resistant to the action of IGF-I. *Diabetes* 39:1028–32, 1990

Insulinlike growth factor I (IGF-I) has several short-term metabolic effects that mimic those of insulin, including stimulation of glucose metabolism and inhibition of lipolysis in adipose tissue (1) and stimulation of glucose and amino acid transport into muscle (2–5). In adipose tissue, the insulinlike effects of IGF-I may be mediated through the insulin receptor (1,6,7), but in muscle, there are significant numbers of IGF-I receptors (8,9), and the insulinlike effects are probably mediated through the IGF-I receptors.

Guler et al. (10) demonstrated that IGF-I can have a hypoglycemic effect when administered to nondiabetic human subjects. On a molar basis, IGF-I was only ~6% as potent as insulin in producing hypoglycemia, but an equivalent lowering of plasma glucose could be achieved with either insulin or IGF-I. This finding suggests that IGF-I might be a useful tool to lower blood glucose in patients with non-insulin-dependent diabetes mellitus (NIDDM). This possibility seems more attractive after the report by Livingston et al. (8) that, in NIDDM, the number and tyrosine kinase activity of the IGF-I receptors in muscle were normal. If the insulin-receptor defect observed in muscle of NIDDM patients is a primary cause of the insulin resistance (8,11,12), circumventing the defect by going through the IGF-I receptor seems an attractive possibility.

Using a newly developed in vitro human muscle preparation, we demonstrated insulin resistance of glucose transport in muscle of morbidly obese patients with or without NIDDM (13). With this preparation, it was possible to investigate the hypothesis that IGF-I may be able to overcome the insulin resistance of muscle from obese patients with or without NIDDM. Besides the obvious importance of this hypothesis for the possible treatment of NIDDM, this question has relevance to the causes of insulin resistance in muscle. Resistance to insulin but not IGF-I suggests that the primary defect is in the insulin receptor, because the IGF-I receptor has been shown to be normal in obese diabetic patients (8).

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TABLE 1
Characteristics of human subjects

	Nonobese nondiabetic	Obese nondiabetic	Obese NIDDM
n	13 F	5 F/1 M	5 F/1 M
Age (yr)	39 ± 1	33 ± 2*	41 ± 2
Body mass index (kg/m²)	25 ± 1*	49 ± 4*	60 ± 5*
Fasting plasma glucose (mM)	4.7 ± 0.2	5.2 ± 0.6	10.5 ± 2.1*
Fasting plasma insulin (pM)	60 ± 12*	120 ± 6*	222 ± 36*
Fasting plasma IGF-I (nM)	22.9 ± 1.8	19.6 ± 1.6	13.3 ± 0.9*

Values are means ± SE. NIDDM, non-insulin-dependent diabetes mellitus; IGF-I, insulinlike growth factor I. IGF-I concentrations were taken from a larger group of patients from the same general population. There were 21 observations for the nonobese nondiabetic, 20 for obese nondiabetic, and 26 for obese NIDDM patients.
*$P < 0.05$ vs. other 2 groups.

RESEARCH DESIGN AND METHODS

^{125}I-labeled insulin and ^{125}I-labeled IGF-I were gifts from Lilly (Indianapolis, IN). [U-^{14}C]sorbitol (150–250 mCi/mmol) and 2-[1,2-^{3}H(N)]deoxy-D-glucose (30.2 Ci/mmol) were obtained from Du Pont-NEN (Boston, MA). Unless otherwise stated, all other chemicals were obtained from Sigma (St. Louis, MO).

Abdominal muscle samples were obtained from 13 nonobese nondiabetic women and 6 morbidly obese nondiabetic (5 women, 1 man) and 6 morbidly obese diabetic (5 women, 1 man) patients during elective abdominal surgery. Nonobese patients were admitted for elective hysterectomies, whereas the morbidly obese patients had gastric bypass surgery for the treatment of their obesity.

None of the subjects had any disease other than diabetes or had taken any drugs known to alter carbohydrate metabolism for at least 2 wk before surgery. The experimental protocol was explained to all subjects, and informed consent was obtained. The project was approved by the East Carolina University Policy and Review Committee on Human Research. All subjects maintained constant body weight during the month before surgery. Because of the effect that calorie intake and its distribution might have on this study, the morbidly obese subjects received a weight-maintaining diet providing 50% of the calories as carbohydrates, 30% as fat (polyunsaturated–saturated fat ratio 0.4, cholesterol content 600 mg), and 20% as protein for 4 days before surgery.

Morbidly obese nondiabetic patients had a 75-g oral glucose tolerance test. The criteria of the National Diabetes Data Group were used to classify patients as nondiabetic (14). Oral glucose tolerance tests were not performed in the morbidly obese patients who had previously been diagnosed as having diabetes.

The subjects underwent surgery after an overnight fast. General anesthesia was induced with a short-acting barbiturate and maintained by fentanyl and N_2O-O_2 mixture. Only saline was given intravenously before the biopsy. After exposing the rectus abdominis muscle, a 3 × 2 × 2-cm biopsy was obtained.

Muscle samples for receptor-binding studies were frozen between aluminum tongs cooled in dry ice as quickly as possible after excision and stored at −70°C until analyzed. Partial purification of the insulin and IGF-I receptors was accomplished by wheat-germ–agglutinin chromatography as previously described (11). ^{125}I-insulin and ^{125}I–IGF-I binding to the partially purified receptors was measured at 4°C for 16 h as previously described (11). Cross-linkage of solubilized receptors with ^{125}I-insulin and ^{125}I–IGF-I was accomplished with disuccinimidyl suberate by the method of Pilch and Czech (15), and polyacrylamide gel electrophoresis was performed according to the method of Laemmli (16) in 7.5% gel under reduced conditions.

2-Deoxyglucose transport by muscle fiber strips was measured as follows. A specially constructed clamp (3 cm wide) was placed on muscle tissue before it was excised, and the

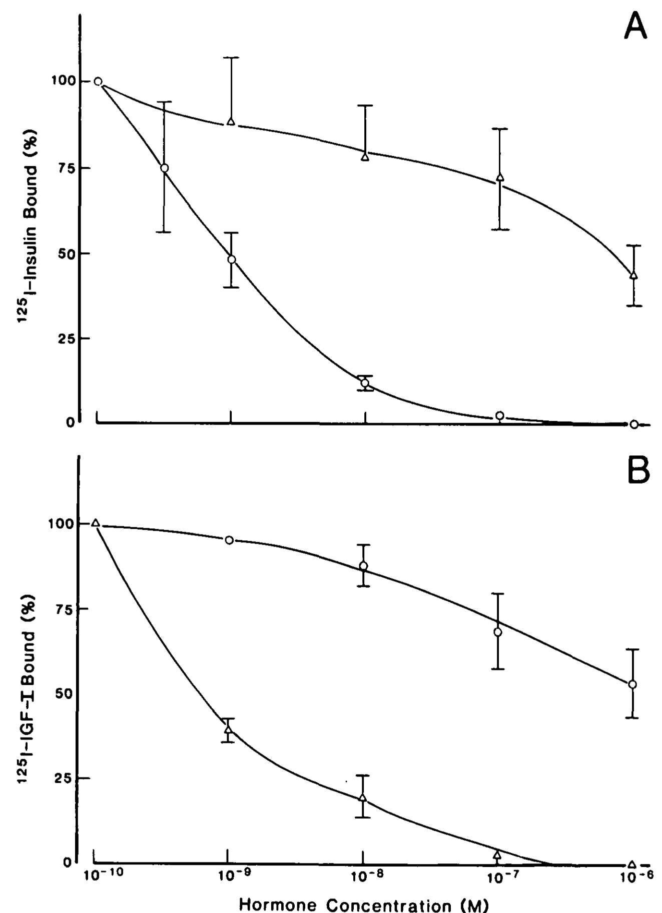

FIG. 1. Displacement of ^{125}I-labeled insulin (*A*) and ^{125}I-labeled insulinlike growth factor I (IGF-I; *B*) by insulin and IGF-I from wheat-germ–agglutinin chromatography–purified receptors from nonobese subjects. Each data point represents mean of 4 observations. ○, displacement by insulin; △, displacement by IGF-I.

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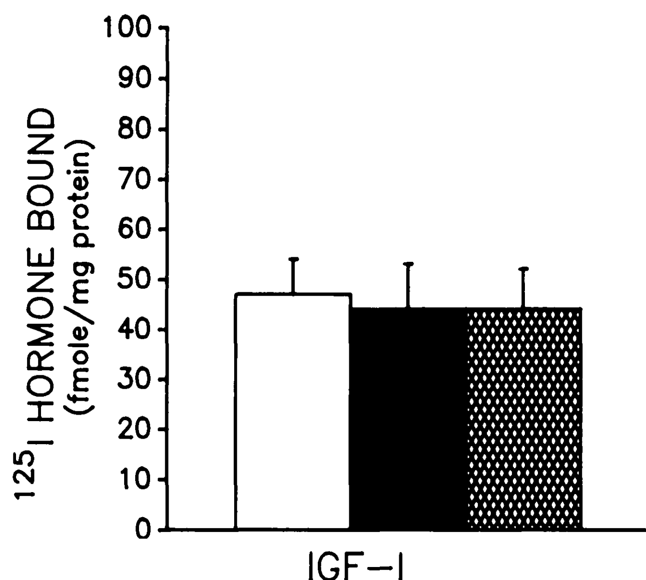

FIG. 2. Binding of insulinlike growth factor (IGF-I) by wheat-germ–agglutinin chromatography–purified receptors from nonobese nondiabetic (*open bar*), obese nondiabetic (*solid bar*), and obese diabetic (*crosshatched bar*) patients. Values are means + SE for 4 observations/group. Hormone concentration for binding was 0.1 nM.

clamp and muscle biopsy were quickly transported to the laboratory in oxygenated Krebs-Henseleit buffer. Muscle fiber strips weighing 50–80 mg were teased from the mounted muscle, and a smaller clamp (2 cm) was placed on the muscle fiber strip before it was cut free (13). The muscle fiber strip in the clamp was then incubated in vitro in 4 ml of Krebs-Henseleit buffer with 1% bovine serum albumin and 1 mM pyruvate. [^{3}H]-2-deoxyglucose transport was assayed as previously described (13).

RESULTS

The characteristics of the three groups of patients are shown in Table 1. Generally, the parameters reported for these patients are similar to those in previous studies (11,13). The nondiabetic morbidly obese subjects had fasting plasma glucose concentrations in the normal range but elevated plasma insulin, indicating insulin resistance.

In a separate group of patients from the same patient pool, we analyzed the fasting plasma concentration of IGF-I. Note that the assay measured total plasma level of the hormone and did not differentiate between free and bound forms (17). The data in Table 1 demonstrate that the IGF-I concentration is significantly lower in the diabetic group than either the obese nondiabetic or the nonobese nondiabetic control groups. There was a trend toward lower levels in the obese nondiabetic group compared with the nonobese nondiabetic group, but the difference did not reach statistical significance.

Insulin and IGF-I binding confirmed that there was specific binding of these hormones. At 0.1 nM hormone concentration, binding of IGF-I was 24% that of insulin. (Insulin binding was 0.29 ± 0.12 pmol/mg protein; IGF-I binding was 0.07 ± 0.01 pmol/mg protein.) Hormone-displacement experiments were performed to demonstrate that IGF-I binds to specific receptors and does not simply associate with the insulin receptor. Unlabeled insulin displaced 50% of the tracer ^{125}I-insulin at ~1 nM, whereas 50% displacement by IGF-I occurred at 100- to 1000-fold higher concentrations (Fig. 1). Half-displacement of tracer ^{125}I–IGF-I by unlabeled IGF-I occurred at ~1 nM, whereas insulin appears to have a very low affinity for the IGF-I receptor (Fig. 1).

We previously demonstrated that insulin binding was depressed in muscle of morbidly obese subjects with or without NIDDM (11). In this study, we measured IGF-I binding in the three groups of patients to see if the same were true of this hormone. The data in Fig. 2 show that there were no differences in binding in obese nondiabetic or obese diabetic

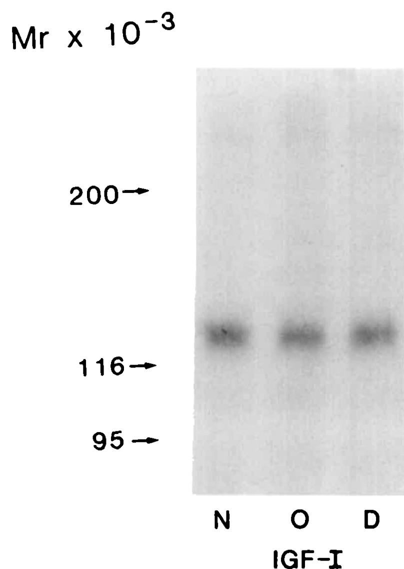

FIG. 3. Electrophoretic separation of wheat-germ–agglutinin chromatography–purified receptors affinity cross-linked to ^{125}I-labeled insulinlike growth factor I (IGF-I). Mr, molecular weight. Representative sample from nonobese nondiabetic (N), obese nondiabetic (O), and obese diabetic (D) patients is shown for each hormone.

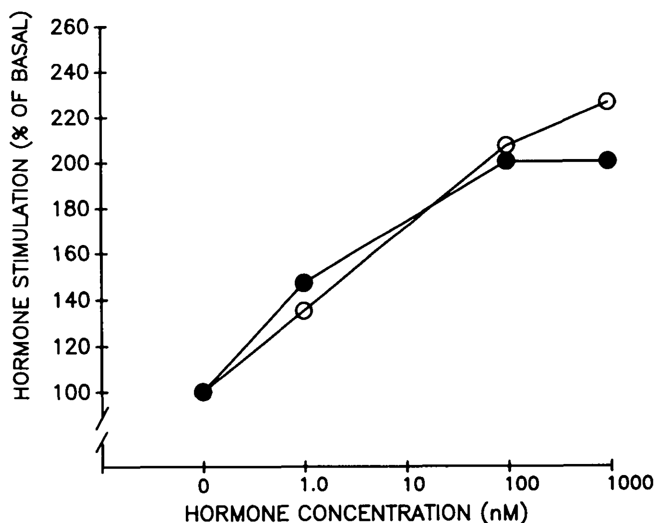

FIG. 4. Stimulation by insulin (○) and insulinlike growth factor I (IGF-I; ●) of 2-deoxyglucose transport into muscle fiber strips incubated in vitro. Each data point is mean of 3–8 observations from muscle of nonobese patients. Six to 12 muscle fiber strips were prepared from each muscle biopsy, and triplicate determinations of 2-deoxyglucose transport rate were done at each insulin concentration. Thus, complete dose-response curve could not be obtained for all muscles. Glucose transport rates were as follows (in nmol · min^{-1} · g^{-1}): 23 ± 2 (n = 7), 30 ± 3 (n = 4), 45 ± 8 (n = 7), 48 ± 9 (n = 3) for 0, 1, 100, and 1000 nM IGF-I, respectively, and 26 ± 3 (n = 8), 35 ± 3 (n = 4), 54 ± 5 (n = 6), and 59 ± 12 (n = 3) for 0, 1, 100, and 1000 nM insulin, respectively.

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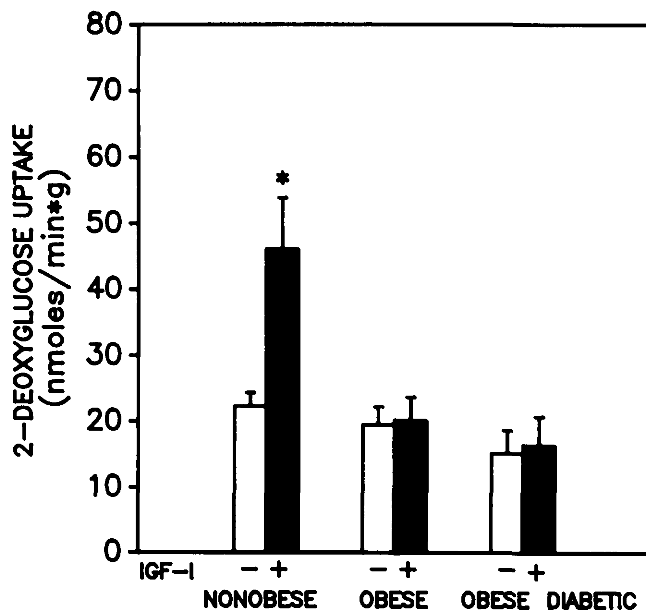

FIG. 5. Insulinlike growth factor I (IGF-I; 100 nM) stimulation of 2-deoxyglucose uptake (nmol · min^{-1} · g^{-1}) in muscle fiber strips incubated in vitro from nonobese nondiabeteic, obese nondiabetic, and obese diabetic patients. Values are means + SE for 6–13 observations. *$P < .05$ vs. IGF-I^{-} nonobese and IGF-I^{+} obese diabetic.

patients compared with nonobese nondiabetic control subjects. Likewise, cross-linking and electrophoresis demonstrated that neither obesity nor diabetes caused a change in the IGF-I–receptor structure as assessed by electrophoretic mobility (Fig. 3).

To determine whether IGF-I can increase glucose utilization in human muscle, 2-deoxyglucose transport was measured in muscle fiber strips from nonobese nondiabetic patients at several hormone concentrations. Insulin and IGF-I stimulated glucose transport ~2.0- to 2.5-fold, and the dose-response curves for the two hormones were very similar (Fig. 4). Although it is not possible to determine a precise ED_{50} from these limited data, glucose transport appears to be half-maximally stimulated at ~3 nM concentration of both hormones.

Stimulation of 2-deoxyglucose transport by IGF-I in nonobese nondiabetic, obese nondiabetic, and obese diabetic muscle is shown in Fig. 5. Basal 2-deoxyglucose transport (in the absence of hormone) tended to show a graded decrease from nonobese nondiabetic to obese nondiabetic to obese diabetic, but the differences were not statistically significant. In muscle from nonobese nondiabetic patients, IGF-I stimulated 2-deoxyglucose transport 2.0- to 2.5-fold. In contrast to the nonobese nondiabetic group, there was no significant stimulation of 2-deoxyglucose transport in either of the obese groups by IGF-I.

DISCUSSION

In agreement with previous investigators, we found specific receptors for IGF-I in muscle (8,9). Like Livingston et al. (8), we found no change in IGF binding in muscle from morbidly obese patients with or without NIDDM.

IGF-I stimulated glucose transport into muscle fiber strips incubated in vitro, and the degree of stimulation was approximately the same as that of insulin. The hormone concentration required to half-maximally stimulate glucose transport was approximately the same for insulin and IGF-I. This was somewhat surprising, because there are appreciably more insulin receptors than IGF-I receptors. However, IGF-I probably acts through its own receptor, because the affinity of the insulin receptor for IGF-I was 100–1000 times less than for insulin. These results are in contrast to IGF-I stimulation of glucose transport in human adipocytes, because Sinha et al. (6,7) found that IGF-I acts through the insulin receptor.

Our previous data demonstrated a decrease in insulin stimulation of glucose transport in muscle of morbidly obese patients with or without NIDDM (13). One of the purposes of this study was to explore the mechanism of insulin resistance by investigating whether the muscle of morbidly obese patients is also resistant to IGF-I. The data clearly demonstrate that IGF-I does not stimulate glucose transport in muscle fiber strips from obese patients. Resistance to both insulin and IGF-I may be a common feature in muscle; Poggi et al. (3) and Cascieri et al. (18) found resistance to both insulin and IGF-I in soleus muscle of obese mice, and Sowell et al. (2) observed resistance to both insulin and IGF-I in denervated muscle.

We believe that the resistance of muscle from obese subjects with or without NIDDM to insulin and IGF-I may suggest that the primary defect causing insulin resistance is distal to the tyrosine kinase activity of the insulin receptor. We (11) and others (8) have observed a defect in the muscle insulin-receptor kinase activity of morbidly obese patients, but if this were the primary cause of resistance, then IGF-I should have stimulated glucose transport normally, because the tyrosine kinase activity of the IGF-I receptor has been shown to be normal in obese diabetic subjects (8). Instead, it seems more plausible that the primary defect may be a depletion of glucose transporters, as has been shown in adipose tissue by Garvey et al. (19). Alternatively, there could be a defect in the ability to translocate glucose transporters to the plasma membrane or in the activation of the intrinsic activity of glucose transporters.

Because our results were obtained with morbidly obese subjects, we do not know whether these conclusions would also hold true for moderately obese individuals and/or lean NIDDM patients. Nevertheless, we feel that these morbidly obese patients represent a good model of obesity and NIDDM from which we can formulate working hypotheses that can be tested in a larger population of patients.

In summary, our study demonstrates that, in vitro, human skeletal muscle from obese patients with or without NIDDM is as resistant to acute exposure of IGF-I as it is to insulin. Thus, it is unlikely that the acute hypoglycemic effect of IGF-I administration observed by Guler et al. (10) in nondiabetic subjects would also be observed in NIDDM patients. Our study, however, does not exclude the possibility that prolonged administration of IGF-I might have a beneficial effect in NIDDM—particularly because Guler et al. (20), in a separate study, administered IGF-I to nondiabetic subjects for 6 days to increase IGF-I serum levels over sixfold. A transient hypoglycemia was observed on day 1, followed by euglycemia in the presence of decreased insulin secretion and degradation and normal serum insulin levels. However, unless the IGF-I molecule is modified to separate its metabolic effects from those on growth, chronic exposure to high con-

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centrations of IGF-I may not have a salutary effect. Nevertheless, because most studies have demonstrated low IGF-I serum levels in patients with diabetes (21), replenishment therapy to normalize IGF-I serum concentrations might result in reduction of growth hormone and insulin concentrations and in a resultant improvement of insulin resistance.

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