Insulin and Insulin-Like Growth Factor-1 Action on Human Skeletal Muscle: Preferential Effects of Insulin-Like Growth Factor-1 in Type 2 Diabetic Subjects

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The metabolic actions of insulin and insulin-like growth factor-1 (IGF-1) were compared in cultured skeletal muscle cells from nondiabetic (ND) and type 2 diabetic subjects. Insulin stimulated glucose uptake with comparable sensitivity in ND (EC_{50} = 2.0 ± 0.7 nmol/L) and diabetic (1.3 ± 0.4) cells. IGF-1 sensitivity for uptake stimulation was similar in ND (EC_{50} = 0.30 ± 0.06 nmol/L) and type 2 cells (0.37 ± 0.01). In ND cells, insulin and IGF-1 were equally potent for stimulation of glucose uptake and glycogen synthase (GS) activity. However, in diabetic cells, maximal insulin stimulation of both responses was only half of the increases due to IGF-1. Final absolute activities after IGF-1 stimulation were still lower in diabetic cells compared with cells from ND subjects. Hormonal stimulation of Akt phosphorylation exhibited the same behavior as metabolic responses; comparable for insulin and IGF-1 in ND muscle, while IGF-1 was significantly more effective in diabetic cells. Both insulin receptor (IR) binding and receptor protein expression were similar in ND and diabetic cells. IGF-1 binding and receptor protein expression were not significantly different in diabetic compared with ND cells. The expression of IGF-binding proteins (IGFBP) 3, 5, and 6 were similar in ND and diabetic cells; IGFBP-4 was slightly, but significantly higher, in diabetic cells. While insulin and IGF-1 are equally effective on metabolic responses in ND muscle, diabetic muscle cells are markedly more resistant to insulin than IGF-1. The greater metabolic activity of IGF-1 in type 2 diabetic muscle may provide new insights into the mechanisms of insulin resistance in skeletal muscle.

SKELETAL MUSCLE IS the major tissue involved in glucose disposal in response to insulin and the primary site of insulin resistance. In type 2 diabetes, this is expressed as defects in the ability of skeletal muscle to respond to insulin with increases in glucose uptake and utilization.[1] Skeletal muscle also responds to insulin-like growth factor-1 (IGF-1) in ways both similar to and different from insulin.[2] Insulin and IGF-1 effects are mediated by hormone binding to specific receptors, and receptors for both IGF-1 and insulin have been identified in skeletal muscle.[3-6]

The insulin receptors (IR) and IGF-1 receptors (IGF-1R) are structurally similar, consisting of 2 extracellular α subunits, which bind the ligand, and 2 membrane spanning β subunits with an intrinsic tyrosine kinase activity within the cytoplasmic domain.[7] Cellular responses to insulin and IGF-1 occur when hormone binds to its receptor and stimulates tyrosine kinase activity and receptor autophosphorylation.[8] While many subsequent signaling events appear to be common to insulin and IGF-1R activation,[8] the final responses in muscle do differ somewhat. Insulin primarily influences metabolic processes, such as as glycogen synthesis and glucose transport, with some effect on cell growth,[9] while IGF-1 regulates mitogenesis and myogenesis,[10,11] as well as metabolism.[2,3,6,12] Delineating the elements in insulin and IGF-1 signaling that lead to this divergence of responses is the focus of a considerable research effort.[13-15] An additional consideration is that both insulin and IGF-1 are capable of binding to and activating the heterologous receptor ("cross talk"), although with a considerably reduced affinity.[8] Further complicating the issue is the presence of insulin/IGF-1R hybrids, formed from IR$\alpha\beta$ and IGF-IR$\alpha\beta$ heterodimers.[16]

While insulin and IGF-1 display many similarities at the receptor level, when infused in vivo, IGF-1 expresses a severely reduced potency compared with insulin (3% to 5%) to influence glucose disposal in both animals and humans,[17,18] leading to the hypothesis that IGF-1 might be acting mainly through the IR. Despite this lower efficacy, IGF-1 has been tested as a potential therapy in insulin-resistant subjects with variable results.[12,19,20] IGF-1 treatment of type 2 diabetic subjects results in a lowering of blood glucose levels[19,21] and appears to improve insulin action, seen as a reduction of insulin levels or requirements.[21] Understanding the relationship between insulin and IGF-1 action in vivo is complicated by several factors, including the influence of insulin on IGF-1 production by the liver[22] and the presence of IGF-binding proteins (IGFBPs). The majority of IGF-1 in the circulation is complexed to IGFBPs[23]; the expression of many of these binding proteins can be regulated by insulin in a tissue-specific manner.[24,25] In addition, IGFBPs can either augment[26] or inhibit[26-28] IGF-1 action. Studies in an isolated cell system, where many of these variables can be controlled independently, provide unique information about the actions of insulin and IGF-1 on muscle metabolism. This is particularly important when such a system serves as a model of insulin resistance in type 2 diabetes.

Studies from our laboratory have shown that human skeletal muscle cells can be grown in culture from needle biopsy specimens and induced to fuse and differentiate into mature

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Table 1. Clinical Characteristics

	Age (yr)	BMI (kg/m^2)	HbA_{1c} (%)	Fasting Glucose (mmol/L)	Fasting Insulin (pmol/L)	GDR (mg/kg/min)
ND (n = 30)	42 ± 1	28.6 ± 1.0	5.2 ± 0.1	5.0 ± 0.1	42 ± 6	10.2 ± 0.5
Type 2 diabetic (n = 22)	52 ± 2*	34.5 ± 1.8*	8.1 ± 0.5*	8.0 ± 0.5*	138 ± 24*	6.0 ± 0.5*

NOTE. Results are average ± SEM.

Abbreviations: BMI, body mass index; HbA_{1c}, glycosylated hemoglobin; GDR, glucose disposal rate.

*$P < .05$ *v* ND.

myotubes displaying many of the insulin-regulated properties of intact skeletal muscle.[29] Myotubes from type 2 diabetic subjects also retain impairments in glucose uptake and glycogen synthase (GS) reflective of those observed in vivo.[30,31] This culture system allows for direct comparison of the effects of IGF-1 and insulin on skeletal muscle from nondiabetic (ND) and type 2 diabetic subjects.

SUBJECTS AND METHODS

Human Subjects

Thirty healthy ND subjects and 22 subjects with type 2 diabetes mellitus participated in these studies. Glucose tolerance was determined from a 75-g oral glucose tolerance test.[32] Insulin action was determined by a 3-hour hyperinsulinemic (300 mU/m^2/min) euglycemic (5.0 to 5.5 mmol/L) clamp; the glucose disposal rate (GDR) was measured during the last 30 minutes of the clamp.[33] Subject characteristics are summarized in Table 1. Twenty-seven of the ND subjects were men, 3 were women. Gender had no effect on the results. All 22 type 2 subjects were men. Five of the type 2 patients were treated with oral sulfonylureas, 3 with insulin, and 4 with diet therapy only. Type 2 subjects had their medication withheld on the morning of the biopsy. Both groups were obese, the diabetic group significantly more so, as well as older (Table 1). Impairments of the maximally insulin-stimulated GDR confirmed the insulin resistance of the diabetic group (Table 1). The experimental protocol was approved by the Committee on Human Investigation of the University of California, San Diego. Informed written consent was obtained from all subjects after explanation of the protocol.

Materials

Human biosynthetic insulin and IGF-1 were purchased from Calbiochem (La Jolla, CA). Long R3-IGF-1 was obtained from Sigma (St Louis, MO). Cell culture materials were purchased from Irvine Scientific (Irvine, CA) except for skeletal muscle basal medium (SkGM), which was obtained from Clonetics (San Diego, CA). Fetal bovine serum (FBS) was purchased from Gemini (Calabasas, CA). Bovine serum albumin (BSA, Cohn fraction V) was supplied by Boerhinger Mannheim (Indianapolis, IN). ^{125}I-Insulin and ^{125}I-IGF-1, 2-[1,2-^{3}H]-deoxy-D-glucose, L-[1-^{14}C]-glucose and uridine diphosphate (UDP)-[^{14}C] glucose were purchased from New England Nuclear (Boston, MA). Reagents for electrophoresis were obtained from Bio-Rad (Richmond, CA). A monoclonal antibody against the IGF-1R was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), as were polyclonal antisera against IGFBPs-4, 5, and 6. Polyclonal antisera against the IR β subunit and IGFBP-3 were purchased from Upstate Biotechnology (Lake Placid, NY). A polyclonal antibody against pSer^{473}-Akt was purchased from New England Biolabs (Beverly, MA). Antimouse and antirabbit IgGs complexed to horseradish peroxidase (HRP) were obtained from Amersham (Arlington Heights, IL); antigoat IgG-HRP was from Santa Cruz Biotechnology. SuperSignal chemiluminescence substrate was from Pierce Chemical (Rockford, IL).

Human Muscle Cell Culture

Biopsy of the vastus lateralis muscle was performed according to published procedures.[33] Human skeletal muscle cells were isolated and grown in culture as described in detail previously.[29] When myoblasts atttained 80% to 90% confluency, the growth media was changed to α-minimum essential medium (α-MEM) supplemented with 2% FBS and antibiotics to induce fusion/differentiation to multinucleated myotubes. The media was changed every other day during cell fusion. Differentiation was complete within 4 days. All studies were performed on cells after 1 passage. Due to the limited number of cells available from a biopsy, not all studies were performed in each subject.

Glucose Uptake Assay

Glucose uptake measurements were performed as described previously.[30] Media was added to the cells together with insulin (0 to 33 nmol/L) or IGF-1 (0 to 24 nmol/L) and the cells incubated for 60 to 90 minutes in a 5% CO_2 incubator before washing and transport assay. An aliquot of the suspension was removed for protein analysis using the Bradford method.[34] The uptake of L-glucose was used to correct each sample for the contribution of diffusion.

GS Assay

GS enzyme activity was determined according to methods described previously.[29,35] Briefly, fully differentiated myotubes were incubated in serum-free α-MEM containing 5.5 mmol/L D-glucose for 1 hour at 37°C in a 5% CO_2 incubator, followed by a 1-hour treatment with or without insulin or IGF-1. GS activity was assayed at a physiologic concentration of substrate (0.3 mmol/L UDP-[^{14}C] glucose) in parallel incubations with 0.1 and 10 mmol/L glucose-6-phosphate (G-6-P).[35] GS activity is expressed as nanomole of UDP-glucose incorporated into glycogen · min^{-1} · mg of total protein or as fractional velocity (FV), the ratio of activity at 0.1 mmol/L G-6-P/activity at 10 mmol/L G-6-P.

Insulin and IGF-1 Binding Assays

Hormone binding assays were performed by a modification of a method described previously.[29] Fully differentiated muscle cells were washed 4 times with reaction buffer, then incubated with reaction buffer and [^{125}I-Tyr A14] insulin (final concentration = 67 pmol/L) or [^{125}I-Tyr A14] IGF-1 (final concentration = 39 pmol/L) for 4 hours at 12°C in the absence or presence of varying concentrations of unlabeled hormones. Cell-associated hormone was determined as described earlier.[29]

Cell Solubilization

Cells were washed free of media and then treated with varying concentrations of insulin or IGF-1 for 15 minutes at 37°C. Reactions were terminated by washing with 4°C phosphate-buffered saline (PBS) and extraction buffer (20 mmol/L Tris HCl, 145 mmol/L NaCl, 10% glycerol, 5 mmol/L EDTA, 1% Triton X-100, 0.5% NP-40, 200 μmol/L sodium vanadate, 200 μmol/L phenylmethyl sulfonyl fluoride (PMSF), 1 μmol/L leupeptin, 1 μmol/L peptstatin, 10 μg/mL aprotinin,

100 mmol/L NaF, 40 mmol/L sodium pyrophosphate, pH 7.5) added. Cells were scrapped into tubes, solubulized by incubating for 30 minutes on ice, and centrifuged at 14,000 × *g* for 10 minutes at 4°C. The supernatant was analyzed for protein content using the Bradford method.

Western Blotting

The method for Western blot analysis was as described previously.[29,36] Total cell lysate preparations were diluted 3:1 in 4× Laemmli's buffer and boiled for 5 minutes at 100°C. Proteins were separated on 7.5 or 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and then transferred to a nitrocellulose membrane.[37] Immune complexes were detected using an enhanced chemiluminescence kit (SuperSubstrate) according to the manufacturer's instructions, followed by autoradiography. Quantification was performed with a scanning laser densitometer (Scan Analysis, Biosoft, Cambridge, UK). All gels included a sample extract of CHO-IR cells[38] as an internal control. Results were normalized to this value to correct for gel-to-gel variation.

Statistical Analysis

Data calculations and statistical analysis were preformed using the GraphPad Prism program (Intuitive Software for Science, San Diego, CA). Hormone sensitivities (EC_{50} values) were obtained from log-logit transformations of dose-response curves. All data are expressed as mean ± SEM. EC_{50} values presented in the text are the average of the values determined from the individual dose-response curves and may differ from those apparent in the figures, which are the curves from the averaged data. Statistical significance was tested with 2-tailed Student's *t* test, using paired analysis when appropriate. Results presented as percentage or fold-change were obtained from paired comparisons with the appropriate control for each set of cells.

RESULTS

Insulin and IGF-1 Effects on Metabolic Responses

The basal (no added hormone) rate of glucose uptake in muscle cells of ND subjects was 16.0 ± 1.8 pmol/mg/min (n = 16). Basal uptake in type 2 cultures (11.0 ± 2.1, n = 10) was reduced compared with NDs ($P < .05$), in confirmation of previous results.[29] In ND cells, both insulin and IGF-1 stimulated uptake in a dose-dependent manner (Fig 1). Cells were more sensitive to IGF-1 (EC_{50} = 0.30 ± 0.06 nmol/L) than insulin (2.0 ± 0.7 nmol/L). The maximal glucose uptake response was similar for insulin (70% ± 14% increase over basal) and IGF-1 (84% ± 16%, P = not significant [NS]) (Fig 2A).

Glucose uptake in diabetic muscle cells also responded to the hormones in a dose-dependent manner (Fig 1B). Sensitivity to IGF-1 (EC_{50} = 0.37 ± 0.01 nmol/L) did not differ from that seen in ND cells. Unlike in vivo, insulin sensitivity (EC_{50} = 1.3 ± 0.4 nmol/L) was also maintained in diabetic muscle cells. The relative response to IGF-1 (70% ± 14% increase) was similar to that in ND cells (Fig 2A). However, as basal uptake was lower in type 2 diabetes, absolute IGF-1-stimulated uptake remained below the stimulated values in ND muscle cells ($P <$ .05). Unlike the situation in ND cells, the maximal insulin response (35% ± 8% increase) was lower than that to IGF-1 (Fig 2A). In diabetic muscle cells, the relative response to IGF-1 was 177% ± 16% ($P < .002$ *v* insulin) of the insulin response in cells from the same subject. Thus, insulin resistance for glucose uptake stimulation in diabetic muscle cells was more profound than IGF-1 resistance.

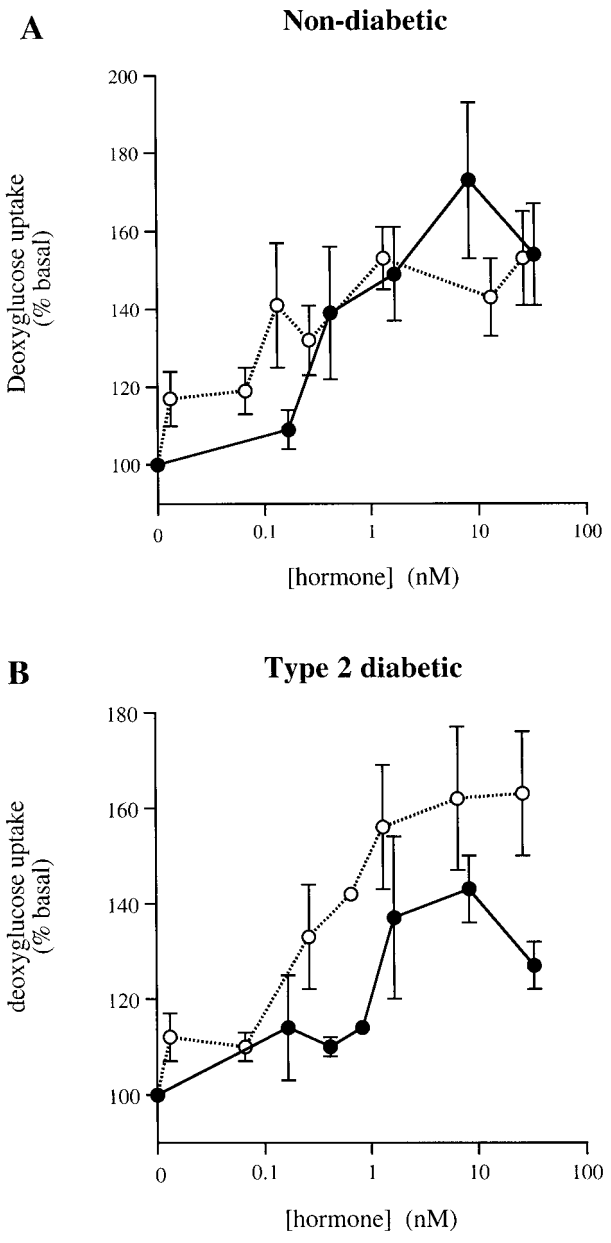
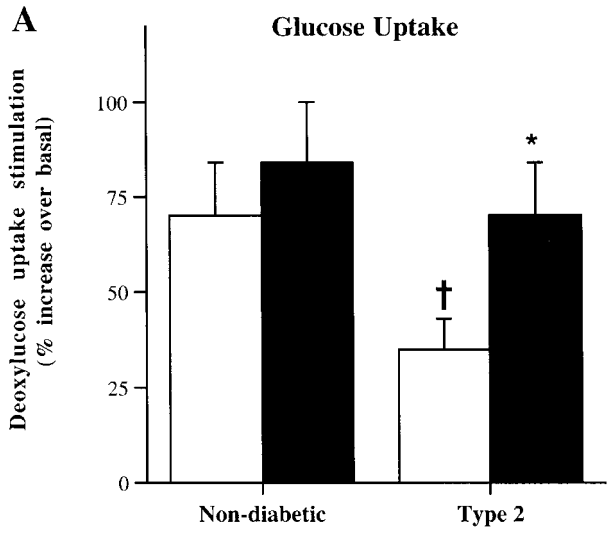


Fig 1. Concentration dependence of insulin and IGF-1 stimulation of deoxyglucose uptake in cultured muscle cells. Cells were treated for 90 minutes at 37°C with the indicated concentration of hormone before assay of initial rates of deoxyglucose uptake. Dose response curves calculated as a function of the basal (no added insulin) activity in each individual set of cells. (A) Insulin (○, n = 7 to 15) and IGF-1 (●, n = 7 to 15) dose-response curves for cells from ND subjects. (B) Insulin (n = 5 to 12) and IGF-1 (n = 5 to 12) dose-response curves for muscle cells from type 2 diabetic subjects. Results are average ± SEM.

Cultured muscle cells from type 2 diabetic subjects also display defects in GS activity.[31] The basal FV of GS in diabetic



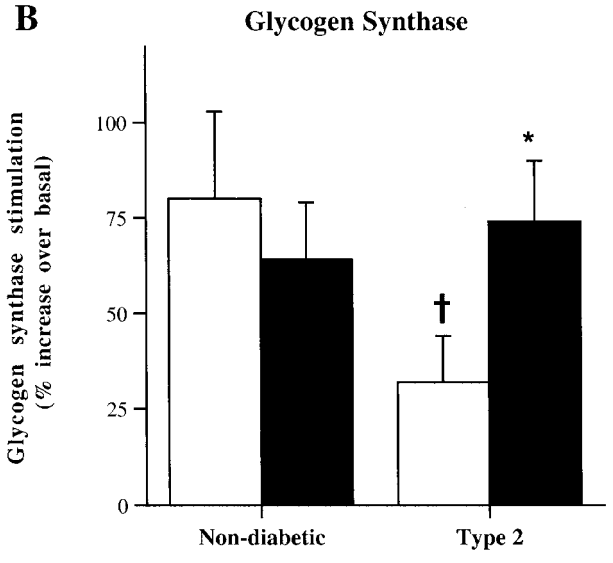


Fig 2. Insulin and IGF-1 responsiveness of muscle cells from ND and type 2 diabetic subjects. Cells were treated with insulin (□) or IGF-1 (■) before assay of glucose uptake or GS as described in the Methods. Results are presented as the percentage increase above basal activity for each set of cells. (A) Glucose uptake stimulation, n = 15 for ND, n = 13 for type 2. (B) GS stimulation, n = 7 for ND, n = 9 for type 2. Results are average + SEM. *$P < .05$ *v* insulin response in same subjects (paired comparison); †$P < .05$ *v* response in ND subjects.

muscle cells (0.073 ± 0.008) was lower than in cells from ND subjects (0.117 ± 0.015, $P < .025$). In ND cells, the maximal responses to insulin (80% ± 23% increase) and IGF-1 (64% ± 15%) were similar (P = NS, Fig 2B). However, in type 2 diabetic cells, the maximal response to insulin (38% ± 12% increase) was considerably lower than that to IGF-1 (74% ± 16%). The relative IGF-1 response was 183% ± 30% of the insulin response in the same cells ($P < .005$), another instance of insulin resistance exceeding IGF-1 resistance.

Signal Transduction

The serine threonine kinase Akt has been identified as an important downstream element in acute regulation of both glucose transport and GS.[39] Phosphorylation of Akt on Ser-473 is a crucial step in hormonal activation of the kinase.[40] Both insulin and IGF-1 stimulated S473 phosphorylation of Akt (Fig 3A) in a dose-dependent manner (data not shown). Sensitivities for this response were similar to those for stimulation of glucose uptake (half maximal stimulation in ND cells at 0.2 nmol/L and 0.3 nmol/L for insulin and IGF-1, respectively). In ND muscle cells, maximal responsiveness was not different for insulin and IGF-1 (Fig 3B). As was the case for glucose uptake and GS, in diabetic muscle cells, final responsiveness

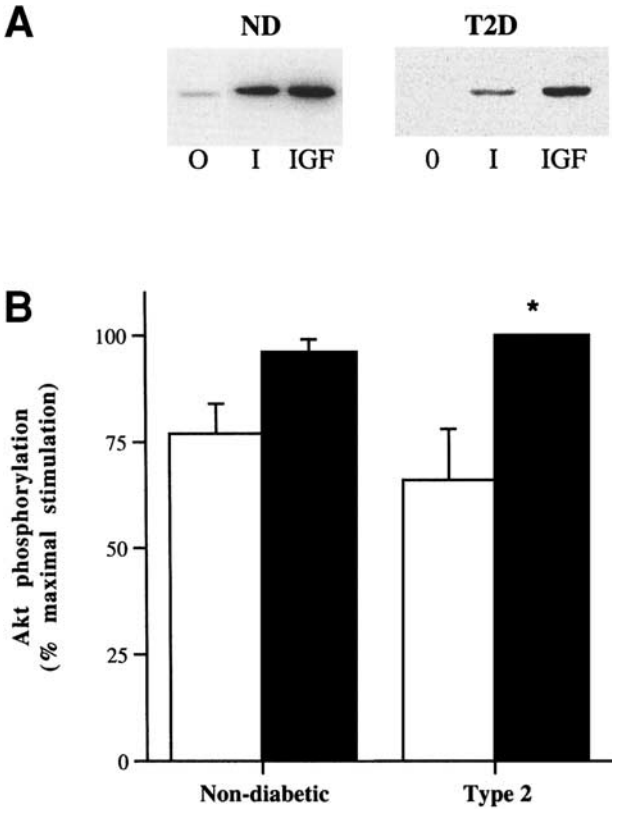


Fig 3. Effects of insulin and IGF-1 on Akt serine phosphorylation in human skeletal muscle cells. Cells were treated with insulin or IGF-1 (0 to 30 nmol/L) for 15 minutes at 37°C before cell extraction. Proteins detected by Western blotting with a phosphospecific antibody. (A) Representative autoradiogram in cells from ND and diabetic subjects. (B) Maximal stimulation of Akt phosphorylation in muscle cells. Results are normalized against the maximal response attained for each individual subject, average + SEM, n = 5 each for ND and diabetic subjects. *$P < .05$ *v* insulin.

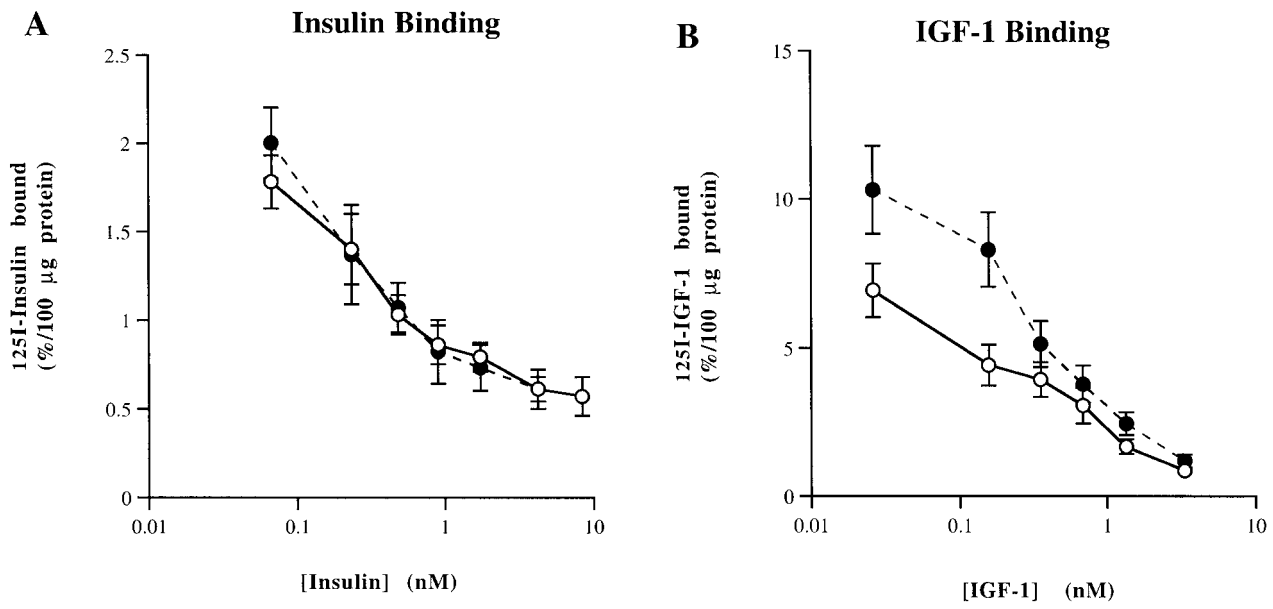


Fig 4. Insulin and IGF-1 binding to cultured human muscle cells. (A) Comparison of IR binding to muscle cells from ND (○, n = 18) and diabetic (●, n = 13) subjects. Binding was measured for 4 hours at 12°C with ^{125}I-insulin (67 pmol/L) in the presence of increasing concentrations of unlabeled insulin. (B) IGF-1 binding to muscle cells from ND (n = 13) and diabetic (n = 10) subjects. Binding was measured with ^{125}I-IGF-1 (13 pmol/L) in the presence of increasing concentrations of unlabeled IGF-1. Results presented as displacement of specific hormone bound, normalized to protein. Results are average ± SEM; each subject studied in triplicate.

to IGF-1 was significantly ($P < .05$) greater than that to insulin (Fig 3B).

Insulin and IGF-1 Binding

Possible causes for the differences in insulin and IGF-1 action in diabetic muscle were investigated at the level of receptor binding. Conditions for the receptor binding assays (4 hours at 12°C) were selected so that only cell surface binding was measured. Under these conditions, internalization of ligand is minimal.[41] Exocytosis/release of intracellular proteins, such as IGFBPs, should be greatly reduced. Specific binding of a tracer concentration of insulin was similar in cultured muscle cells from ND (1.78% ± 0.15%/100 μg protein) and type 2 diabetic subjects (2.00 ± 0.20, P = NS) (Fig 4A). The concentration of unlabeled insulin required to displace 50% of the bound hormone (EB_{50}), an indicator of receptor affinity, did not differ between ND (1.01 ± 0.17 nmol/L) and diabetic (0.78 ± 0.18, P = NS) muscle cells.

Tracer binding of IGF-1 was higher than that of insulin (Fig 4B). The ratio of tracer specific IGF-1 to insulin binding in the same subject's cells was 5.1-fold ± 0.7-fold in ND cells and 4.5-fold ± 0.9-fold in type 2 muscle cells. IGF-1 binding in ND cells (6.94% ± 0.90%/100 μg) was lower than that in diabetic cells (10.30 ± 1.48), although this difference did not attain statistical significance (P = .055). The affinity for IGF-1 binding was identical in both groups of cells (EB_{50} = 0.39 ± 0.03 nmol/L for both). Long R3- IGF-1, an analog that binds to IGFBPs with greatly reduced affinity,[23] displaced 70% of specifically bound IGF-1 (not shown). Thus, of the IGF-1 binding to muscle cells measured under these conditions, approximately 30% may be to IGFBPs.

The potential for interaction between insulin and IGF-1Rs was tested by measuring the ability of each hormone to displace binding of the heterologous hormone. IGF-1 was far less effective than insulin in competing for IR binding. Because displacement did not consistently exceed 50% of initial binding over the concentration range tested, it was not possible to calculate a binding affinity. However, by comparing displacement at points where the insulin and IGF-1 curves were parallel, it could be estimated that IGF-1 competed for insulin binding to the IR with 1% to 2% of the affinity of insulin (not shown). This extent of interaction was the same in ND and diabetic muscle cells.

Insulin was even less effective in competing for IGF-1 binding; displacement rarely exceeded 30%. The estimated relative sensitivity of insulin for IGF-1 binding is approximately 0.25% that of IGF-1 and was similar in ND and diabetic muscle.

Insulin and IGF-1R and Binding Protein Expression

In agreement with the ligand binding results, total cell IR protein expression was similar in ND (7.5 ± 1.8 arbitrary units/10 μg protein) and diabetic (6.6 ± 1.4) muscle cells (Fig 5A). Total cell IGF-1R protein expression was similar in diabetic cells compared with ND cells (Fig 5A), generally mirroring the ligand binding results.

Muscle cells can produce IGFBPs that are either retained by the cell or released into the circulation.[23,27] As muscle cells were washed extensively before extraction, the same procedure as used for hormone binding, only cell-associated IGFBPs should be detected. IGFBPs 3, 4, 5, and 6 are all present in human myotubes (Fig 5B). Due to differing affinities of the

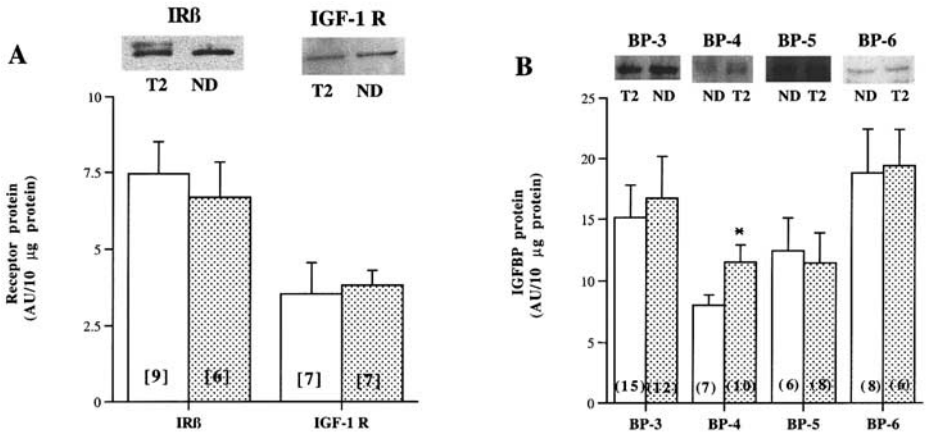


Fig 5. Insulin and IGF-1R and IGFBP expression in muscle cells of ND (□) and type 2 diabetic (▨) subjects. (A) Top, representative autoradiographs of Western blots for the IR and IGF-1R. Bottom, quantitation of Western blots. (B) Top, representative autoradiographs of Western blots for IGFBP-3, -4, -5, and -6. Bottom, quantitation of Western blots. All results are average ± SEM; number of individuals studied indicated in brackets in each bar.

antibodies used, the relative content of the different IGFBPs cannot be established by this analysis. The most important comparison is between subject groups for each IGFBP. There was considerable variability in IGFBP expression, but only for the case of IGFBP-4 was there a small, but statistically significant, increase in type 2 diabetic muscle cells (Fig 5B).

DISCUSSION

Skeletal muscle is a major target tissue for both insulin and IGF-1. The influence of type 2 diabetes on insulin and IGF-1 actions was investigated in cultured human muscle cells. The similar potencies of insulin and IGF-1 to stimulate multiple aspects of glucose metabolism in ND human muscle cells (Figs 1 and 2) contrasts with the situation in vivo[42,43] or in rat muscle strips[3] where IGF-1 is far less potent. Yet, muscle cells display greater sensitivity to IGF-1 than to insulin, probably reflecting the greater number and/or affinity of IGF-1Rs. These results indicate that IGF-1 is operating through its own receptor, and possibly a portion of hybrid receptors, to stimulate glucose metabolism and not by cross talk through IR, and that skeletal muscle is highly sensitive to IGF-1. The similar final responsiveness, even in the presence of IGF-1 binding that exceeds insulin binding, suggests that, at least in ND muscle cells, it is factors downstream of the receptors that determine the extent of the final response. There may be several reasons for the lower IGF-1 effectiveness observed in vivo including; clearance of IGF-1 by nonmuscle tissues,[44] contribution to whole body glucose disposal by tissues with poor sensitivity to IGF-1, such as adipose tissue,[45] and association with IGFBPs.[23] All of these factors could be reduced in the culture system, allowing the high intrinsic sensitivity of muscle to IGF-1 to be revealed.

Hormone action is defined both by hormone sensitivity, measured by EC_{50}, and responsiveness, the final maximal effect. Diabetic muscle cells displayed normal sensitivities to insulin and IGF-1, unlike in vivo, in which insulin sensitivity is reduced. One reason for this discrepancy may be the maintenance of normal levels of receptor binding in culture, possibly due to removal from the hyperinsulinemic environment present in vivo. One of the more interesting results in the current work is the observation that for the metabolic events glucose uptake and GS, diabetic muscle cells are more responsive to IGF-1 than to insulin, while the hormones are equivalent in ND cells. The relative response to IGF-1 is the same in ND and diabetic cells, while the relative insulin effect is halved in diabetic cells. It is important to recognize that, due to the lower basal uptake and synthase activities in diabetic cells, the absolute increases in these activities in diabetic cells are still lower than normal, but the impairment due to diabetes is far less for IGF-1 than for insulin. Thus, IGF-1 is nearly twice as effective as insulin in trying to normalize glucose uptake and GS in diabetic muscle cells. The current result contrasts with a report in which IGF-1 had no effect on glucose transport in muscle strips from obese ND and obese type 2 diabetic subjects.[6] Explanations for this discrepancy may include differences between the features of the muscle strip and muscle cell culture systems. In agreement with the current results, there are other reports of insulin resistant states in which IGF-1 responses for glucose metabolism are either intact or at least greater than those to insulin. These conditions, all established in rats, include: liver cirrhosis,[46] chronic renal failure,[47] tumor necrosis factor (TNF)α treatment,[48] and gold thioglucose-induced obesity.[49] In addition, cultured human muscle cells from a myotonic dystrophy fetus were resistant to insulin, but responsive to IGF-1.[50] Thus, despite the similarities in insulin and IGF-1 signaling and action, they also involve unique events that can be regulated independently of each other.

Differentiated human myotubes contain both insulin and IGF-1Rs (Figs 4 and 5). IGF-1 binding is present at 4-fold to 5-fold greater levels than insulin binding in muscle cells. Relative higher IGF-1 binding (3-fold to 4-fold) has also been observed in muscle biopsies.[4,5] Tracer binding, as measured in this report, is a product of receptor number and affinity, and the higher IGF-1 binding is partially due to the higher affinity. The most important comparison is that between subject groups, in which the affinities did not differ. Thus, IGF-1R number is most likely also similar between groups, as also indicated by Western blotting for the IGF-1R protein. The affinity of IGF-1 for its homologous receptor and binding proteins is greater than that of insulin for its own receptor, a common finding in many tissue and cell types.[7] In human muscle cells, IGF-1 is somewhat more effective than insulin in displacing the other ligand at high concentrations of hormone. Other than this small dif-

ference, insulin and IGF-1Rs in human muscle cells appear to behave much like those in other cell types and tissues, including skeletal muscle.

In type 2 diabetes, IR binding is decreased in both adipose tissue and muscle,[51] while IGF-1 binding is either elevated[5] or unaltered.[6] A factor in these differences may be the hyperinsulinemia and reduced IGF-1 levels characteristic of diabetes.[44] In the muscle culture system, cells from ND and diabetic subjects are maintained in the same low levels of insulin and IGF-1. The fact that insulin and IGF-1 binding do not differ, on average, between ND and diabetic muscle cells suggests that the differences seen in biopsies and freshly isolated cells might reflect the hormonal environment of the tissue.

The IR can exist as 2 alternatively spliced isoforms, either including (+11) or omitting (-11) exon 11, in a tissue-specific manner.[52] Skeletal muscle contains primarily the -11 form.[52] Several investigators have reported that the proportion of the +11 isoform is increased in type 2 diabetes,[53,54] although this is not a universal finding.[55,56] The current work measured only IR binding and did not distinguish between receptor isoforms. However, the +11 isoform has a lower affinity for insulin,[57] and it would be expected that if there were a greater proportion of +11 receptors in diabetic muscle cells, then the average binding affinity would be lower than in ND cells. That was not the case, as affinities were comparable in both groups of cells.

One consequence of the similarity of the structures for insulin and IGF-1Rs is the ability to form hybrid receptors consisting of IR$\alpha\beta$ and IGF-1R$\alpha\beta$ heterodimers.[16] The expression of such hybrid receptors has been reported to be elevated in muscle[5] and adipose tissue[58] from type 2 diabetic subjects. Because insulin/IGF-1 hybrid receptors typically behave like IGF-1Rs, with a low affinity for insulin,[16] a predicted consequence of increased hybrid receptor formation would be a reduction in insulin binding affinity and sensitivity, contributing to insulin resistance. While we did not directly determine the level of hybrid receptors in cultured muscle cells, the fact that insulin binding affinities were similar in ND and diabetic muscle cells again argues against the increased presence of lower affinity forms of the IR in diabetic muscle cells, be they hybrid receptors or the +exon 11 isoform. Augmented formation of hybrid receptors may be an acquired property of diabetic tissues, as the proportion of hybrid receptors is positively correlated with insulin levels[5] and is also elevated in muscle from subjects with insulinomas.[59] The contribution of hyperinsulinemia to hybrid receptor formation would be absent in the muscle cultured in media containing normal insulin concentrations ($\approx$20 pmol/L).

While insulin binding measured under the conditions used in this report is a measure of insulin association with specific cell surface receptors, the situation is not as simple for IGF-1. The washing and reaction conditions were selected to reduce the release of intracellular IGFBPs, but could not eliminate IGF-1 binding to membrane-localized IGFBPs. Thus, the IGF-1 binding measured here represents association with both the IGF-1R and IGFBPs, the latter representing approximately 30% of specific binding. As IGFBPs can modulate bioavability of IGF-1[22] and even directly influence IGF action,[26-28] a change in the balance of receptors and IGFBPs could alter final action. Indeed, IGFBPs have also been shown to inhibit insulin action.[60] Production of IGFBPs has been demonstrated in cultured muscle cell lines[61-63]; the current findings extend this behavior to human muscle skeletal cells. Several lines of evidence suggest that changes in IGFBPs are not responsible for the results presented here. First, expression of IGF-1R protein is similar in ND and diabetic cells, in general agreement with the IGF-1 binding results. In addition, the expression of IGFBP-3, IGFBP-5, and IGFBP-6, measured by Western blotting, did not differ between ND and diabetic cells, while IGFBP-4 was only slightly elevated in diabetic cells. Little is known about the impact of type 2 diabetes on IGFBP production, especially in muscle. The current results suggest that, when removed from the hyperinsulinemic/hyperglycemic diabetic environment, diabetes has only limited impact on the production by muscle of a number of IGFBPs. While the role of IGFBPs in insulin and IGF-1 action, especially in insulin-resistant states, is an important topic for future investigation, IGFBPs do not appear to account for the differences in insulin and IGF-1 action that are seen in diabetic muscle cells.

The comparable responsiveness of ND cells to insulin and IGF-1 indicate that some common elements(s) determine the magnitude of the final response. However, in diabetic muscle cells, there must be some process available to IGF-1 and not insulin that is responsible for the augmented final response. One obvious quantitative difference between insulin and IGF-1 is the receptor binding. In ND cells, postreceptor events determine the final responses to insulin and IGF-1, suggesting that there may be "spare" receptors. Diabetic muscle may not have these reserves, and the final level of occupied receptors would set the response, thus reflecting the greater IGF-1R occupancy. There might also be qualitative differences, such as differential substrate phosphorylation in response to IGF-1, which would activate signaling pathways not influenced by insulin. Another possible explanation would be the presence in diabetic muscle of a specific inhibitor of insulin action. Support for this possibility is included in a recent report showing that the membrane glycoprotein PC-1, whose expression is elevated in type 2 diabetic tissues,[64] inhibits IR kinase activity, while not influencing the IGF-1R kinase.[65] This latter observation would be consistent with the diabetes-related difference in insulin and IGF-1 action arising between receptor binding and phosphorylation of Akt, as this step mirrors the differences seen in final responses. In addition, the ultimately lower final responses in diabetic muscle, regardless of the hormone, would be reflective of defects in the effector systems that are characteristic of diabetes.

Several general conclusions can be drawn from the current work. One is that skeletal muscle intrinsically has a high sensitivity to IGF-1 in both diabetic and ND states. Second, results in the culture system confirm that human skeletal muscle can produce IGFBPs. Removed from the metabolic environment characteristic of the diabetic state, diabetic muscle has, in most instances, a generally normal content of IGFBPs. Most importantly, type 2 diabetes might have differential impacts on insulin and IGF-1 signaling. This last finding suggests that differences in insulin and IGF-1 signaling could be exploited to identify sites of insulin resistance in muscle tissue.

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