Stimulation of glucose uptake by insulin-like growth factor II in human muscle is not mediated by the insulin-like growth factor II/mannose 6-phosphate receptor

Bartolomé BURGUERA,* Charles W. ELTON,† Edward B. TAPSCOTT,† Walter J. PORIES,‡ Richard DIMARCHI,§ Katsu-ichi SAKANO|| and G. Lynis DOHM†¶

Departments of *Medicine, †Biochemistry and ‡Surgery, School of Medicine, East Carolina University, Greenville, NC 27858I, U.S.A., §Lilly Research Laboratories, Indianapolis, IN 46283, U.S.A., and ||Molecular Biology Research Laboratory, Daiichi Pharmaceutical Co. Ltd., 16–13 Kitakasai 1-chome, Edogawa-ku, Tokyo 134, Japan

Although the growth-promoting effects of insulin-like growth factor II (IGF-II) have been intensively studied, the acute actions of this hormone on glucose metabolism have been less well evaluated, especially in skeletal muscle of humans. We and other groups have shown that IGFs reduce glycaemic levels in humans and stimulate glucose uptake in rat muscle. The purpose of the present study was to evaluate the effect of IGF-II on glucose transport in muscle of normal and obese patients with and without non-insulin-dependent diabetes mellitus (NIDDM), as well as to identify the receptor responsible for this action. 2-Deoxyglucose transport was determined *in vitro* using a muscle-fibre strip preparation. IGF-II binding and stimulation of glucose transport by IGF-II were investigated in biopsy material of rectus abdominus muscle taken from lean and obese patients and obese patients with NIDDM at the time of surgery. In the lean group, IGF-II (100 nM) stimulated glucose transport 2.1-fold, which was slightly less than stimulation by insulin (2.8-fold) at the same concentration. Binding of IGF-II was approx. 25% of that of insulin at 1 nM concentrations of both hormones. Obesity with or without NIDDM significantly reduced IGF-II-stimulated glucose uptake compared with the lean group. In order to explore which receptor mediated the IGF-II effect, we compared glucose uptake induced by IGF-II and two IGF-II analogues: [Leu^{27}]IGF-II, with high affinity for the IGF-II/Man 6-P receptor but markedly reduced affinity for the IGF-I and insulin receptors, and [Arg^{54},Arg^{55}]IGF-II, with high affinity for the IGF-I and insulin receptors but no affinity for the IGF-II/Man 6-P receptor. The potency of [Arg^{54},Arg^{55}]IGF-II was similar to that of IGF-II, whereas [Leu^{27}]IGF-II had a very diminished effect. Results show that IGF-II is capable of stimulating muscle glucose uptake in lean but not in obese subjects and this effect seems not to be mediated via an IGF-II/Man 6-P receptor.

INTRODUCTION

Insulin-like growth factor II (IGF-II) is a single-chain polypeptide, which shows high sequence homology and similarities in tertiary structure to IGF-I and proinsulin [1]. In addition to growth promotion, IGFs also exert acute metabolic effects that are very similar to those produced by insulin. IGFs circulate in the blood bound to specific IGF-binding proteins which probably protect them from degradation and regulate the availability of both growth factors to the tissues [2–4]. IGFs are mainly produced in the liver, but they are also synthesized locally in several tissues, including skeletal muscle [5]. IGF-I, a mediator of the growth-promoting effect of growth hormone, also mimics many effects of insulin [6,7]. It has been shown to reduce glycaemic levels in normal [8–10] and diabetic humans [11,12]. Incubation of human or rat skeletal muscle with IGF-I increases the rates of glucose phosphorylation [6,13,14], glycolysis and glycogen synthesis [6,15,16].

IGF-II is present in plasma at three to four times the concentration of IGF-I and seems not to be regulated by growth hormone [17]. Several studies have shown that high levels of IGF-II in human patients with extrapancreatic tumours [17–21] appear to be responsible for the development of hypoglycaemia, as well as stimulation of synthesis of tissue glycogen and lipid in rats [16]. Furthermore, IGF-II increases glucose uptake in rat and human adipocytes [22–24], actions considered to be mediated via the insulin receptor. However, other authors have suggested that some biological effects of IGF-II are mediated by the IGF-II/Man 6-P receptor [25–28]. Several *in vitro* studies [27] have demonstrated the ability of IGF-II to stimulate glucose transport in rat, as well as in human, skeletal muscle [29]. Shimizu et al. [30] showed that, in myotubes, the effect of IGF-I and IGF-II appeared to be mediated via the IGF-I receptor. It seems likely that, in skeletal muscle, the metabolic effects of IGFs are mainly exerted via an IGF-I receptor [6,31,32]. The IGF-I receptor has an α-subunit with binding capacity and a β-subunit with tyrosine kinase activity [33,34]. The IGF-I receptor binds both IGF-I and IGF-II, although the affinity for IGF-I is somewhat higher. In contrast with the IGF-I and insulin receptors, the IGF-II/Man 6-P receptor is a single transmembrane protein which does not have tyrosine kinase activity [35–38]. IGF-II is capable of reacting with three different receptors: those for IGF-II/Man 6-P and IGF-I as well as the insulin receptor, although with a lower affinity. The existence of at least three different receptors with overlapping specific binding complicates the assignment of a particular response to a particular receptor and obscures the identity of the receptor via which the biological actions of IGF-II are mediated.

In the present study, we have investigated the IGF-II stimulation of glucose transport into human muscle from lean and obese subjects and obese patients suffering from non-insulin-dependent diabetes mellitus (NIDDM). Furthermore, we have

Abbreviations used: IGF-I, IGF-II, insulin-like growth factor I, II; NIDDM, non-insulin-dependent diabetes mellitus.

¶ To whom correspondence should be addressed.

looked at which receptor mediates the IGF-II effect, by comparing glucose uptake induced by IGF-II with that induced by two IGF-II analogues [39,40], [Leu^{27}]IGF-II and [Arg^{54},Arg^{55}] IGF-II.

MATERIALS AND METHODS

Materials

^{125}I-insulin, ^{125}I-IGF-I, ^{125}I-IGF-II, IGF-I and IGF-II were gifts from Eli Lilly, Indianapolis, IN, U.S.A. IGF-II was also purchased from Gro-pep. [U-^{14}C]Sorbitol (150–250 mCi/mmol) and 2-[1,2-^{3}H(n)]-deoxy-D-glucose (30.3 Ci/mmol) were obtained from Du Pont–NEN (Boston, MA, U.S.A.). [Arg^{54},Arg^{55}]IGF-II and [Leu^{27}]IGF-II (mutant recombinant human IGF-II analogues) were obtained from Daiichi Pharmaceutical Co. (Tokyo, Japan). Unless otherwise stated, all other chemicals were obtained from Sigma (St. Louis, MO, U.S.A.).

Human subjects

Abdominal-muscle samples were obtained from five morbidly obese patients with NIDDM and five morbidly obese and ten lean patients with normal glucose-tolerance tests. All the patients not known to have NIDDM underwent an oral glucose-tolerance test, as recommended by the National Diabetes Data Group [41], to establish the diagnosis of normal or NIDDM. The morbidly obese patients had been admitted to the hospital to undergo gastric bypass for the treatment of morbid obesity and the lean patients had been admitted for elective hysterectomy. Other than obesity and NIDDM, none of the subjects had any diseases or had taken any medications known to alter carbohydrate or lipid metabolism. NIDDM patients who were taking sulphonylureas and/or insulin discontinued the medication 3 weeks and 1 week respectively before surgery. Detailed clinical information on this patient population is presented in Table 1. Written consent was obtained from all patients after they had been informed about the nature and potential risks of the study. The patients underwent surgery after an overnight fast. General anaesthesia was induced with a short-acting barbiturate and maintained by fentanyl and a nitrous oxide/oxygen mixture. Only saline was given intravenously before the biopsy. After exposure of the rectus abdominus muscle, a piece 3 cm × 2 cm × 2 cm was removed.

The project was approved by the East Carolina University Policy and Review Committee on Human Research.

Table 1 Clinical and biochemical characteristics of the three groups of patients

Values are means or means ± S.E.M.

	Lean control ($n = 10$)	Obese control ($n = 5$)	Obese NIDDM ($n = 5$)
Age (years)	41.4 ± 3	35.8 ± 3	38.8 ± 4
Weight (kg)	63	164	120
Height (cm)	160	164	159
Body mass index (kg/m^2)	24.6 ± 2	46.1 ± 6	48 ± 4
Fasting glucose concentration (mM)	4.5 ± 0.2	5.7 ± 1	10 ± 2.5

Binding assays for insulin, IGF-I and IGF-II

Muscle samples for receptor-binding studies were frozen between aluminium tongs, cooled in solid CO_2 as quickly as possible after excision and stored at −70 °C until analysed. Partial purification of the insulin and IGF receptors were accomplished by wheat-germ-agglutinin chromatography. Binding of ^{125}I-insulin, ^{125}I-IGF-I and ^{125}I-IGF-II to the partially purified receptors was measured at 4 °C for 16 h as previously described [42]. The data were expressed as specific hormone binding after subtraction of non-specific binding from total binding. Non-specific hormone binding is the radioactivity associated with the receptor in the presence of 1 μM labelled hormone, and in this study was less than 20% of the total binding. Cross-linking of solubilized receptors with ^{125}I-IGF-II was accomplished with disuccinimidyl suberate [43,44], and PAGE was performed in 7.5% gel under reduced conditions.

Transport of 2-deoxyglucose by muscle-fibre strips

A specially constructed clamp (3 cm wide) was placed on muscle tissue before excision, and then the clamp and muscle biopsy material were quickly transported to the laboratory in oxygenated Krebs–Henseleit buffer. Muscle-fibre strips weighing 50–80 mg were teased from the mounted muscle, and a smaller clamp was placed on each muscle strip before it was cut free [45]. The muscle-fibre strip in the clamp was then incubated *in vitro* in Krebs–Henseleit buffer with BSA and pyruvate as previously described [45] or by a modification (muscle in 1 cm small clamps incubated at 29 °C with 0.5 mM Ca^{2+}). 2-[^{3}H]Deoxyglucose transport was assayed as previously described [45]. These changes in the incubation conditions reduced basal glucose uptake. The maximum hormone-stimulated glucose uptake was similar under both conditions.

Statistical analysis

Stimulation of glucose transport induced by the different hormones were analysed using one-way analysis of variance and expressed as a mean ± S.E.M. Differences between groups were detected using the Student–Newman–Keuls test with significance set at the $P < 0.05$ level.

RESULTS

IGF-II binding

To demonstrate the specific binding of IGF-II to its own receptor, displacement of ^{125}I-insulin, ^{125}I-IGF-I and ^{125}I-IGF-II was investigated (Figure 1). Approx. 100 times more IGF-II than insulin was required to displace half of the bound insulin (Figure 1a), showing that IGF-II has a relatively low affinity for the insulin receptor. IGF-II, however, did have a reasonably high affinity for the IGF-I receptor as shown by the fact that only about 10 times more IGF-II was required to displace half of the ^{125}I-IGF-I than unlabelled IGF-I (Figure 1b). On the other hand, IGF-I has a very low affinity for the IGF-II/Man 6-P receptor (Figure 1c).

To demonstrate further the presence of IGF-II/Man 6-P receptor, ^{125}I-IGF-II was cross-linked to wheat-germ-agglutinin-purified receptors and then separated by electrophoresis. Figure 2 shows a 230 kDa band which is displaced by unlabelled IGF-II. Specific binding of IGF-II to the α-subunit (130 kDa) of the IGF-I receptor can be seen. A 70 kDa band was displaced by both IGF-I and IGF-II, at a 1 μM concentration of each of the growth factors. It is our belief that this protein could be a

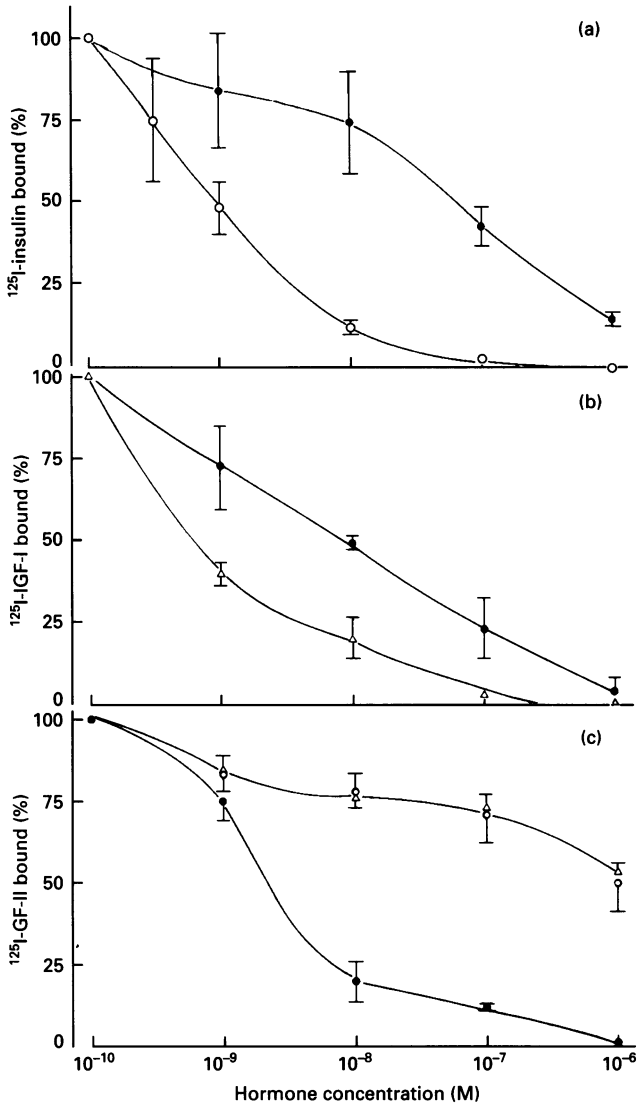

Figure 1 Displacement of ^{125}I-insulin (a), ^{125}I-IGF-I (b), ^{125}I-IGF-II (c) by unlabelled insulin, IGF-I and IGF-II from wheat-germ-agglutinin-purified receptors

Each data point is the mean ± S.E.M. for four observations. ○, Displacement by insulin; △, displacement by IGF-I; ●, displacement by IGF-II.

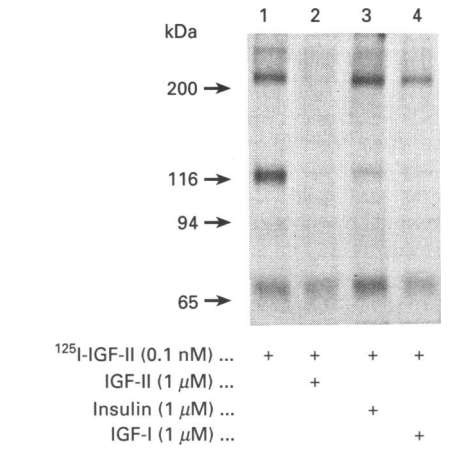

Figure 2 Electrophoretic separation of wheat-germ-agglutinin-purified receptors affinity-cross-linked to ^{125}I-IGF-II

Displacement of ^{125}I-IGF-II from the IGF-II/Man 6-P receptor (230 kDa) and the α-subunit of the IGF-I receptor (130 kDa) is demonstrated in lane 2. Insulin and IGF-I displace ^{125}I-IGF-II from the α-subunit of the IGF-I receptor but not the IGF-II/Man 6-P receptor (lanes 3 and 4).

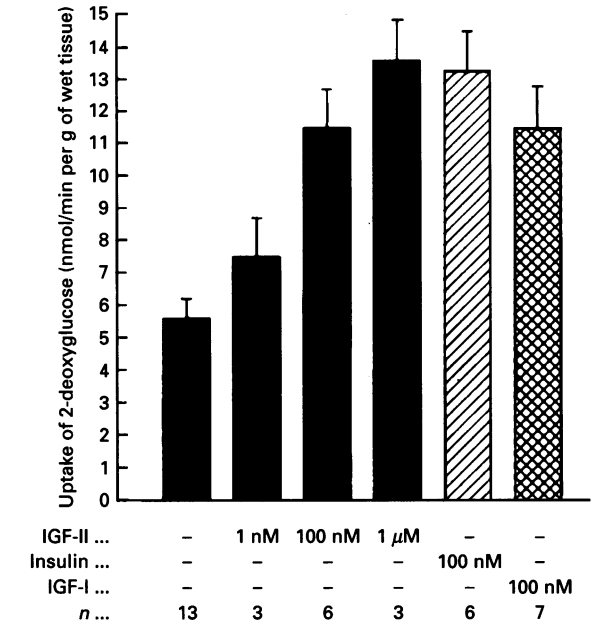

Figure 3 Stimulation of 2-deoxyglucose transport into muscle-fibre strips by IGF-II, insulin and IGF-I

Values are means ± S.E.M. for the number of observations (*n*) indicated.

soluble fraction of the IGF-II/Man 6-P receptor or an as yet undescribed IGF-binding protein.

Binding of ^{125}I-insulin, ^{125}I-IGF-I and ^{125}I-IGF-II was measured at 0.1 nM hormone to determine the relative binding capacities of these hormones to a wheat-germ-agglutinin-purified preparation of receptors from human skeletal muscle in the presence and absence of a large excess of unlabelled hormone (1 μM). As we have shown elsewhere [42], there was approx. equal binding for IGF-I and IGF-II, and IGF-II binding was approx. 25% of that for insulin.

Stimulation of glucose transport

To determine whether IGF-II can increase glucose transport in human muscle, 2-deoxyglucose transport was measured in muscle-fibre strips from lean patients at several hormone concentrations. The data in Figure 3 demonstrate that IGF-II at a concentration of 100 nM maximally stimulates glucose transport approx. 2.1-fold. Increasing the concentration of IGF-II from 1 nM to 1 μM induced a dose–response increase in the 2-deoxyglucose transport. At concentrations as low as 1 nM, IGF-II stimulated 2-deoxyglucose transport to 36% above basal.

For comparison, stimulation of 2-deoxyglucose transport by insulin and IGF-I is also shown in Figure 3. At 100 nM concentration, insulin stimulated 2-deoxyglucose transport

Table 2 Stimulation of 2-deoxyglucose transport (nmol/min per g of wet tissue) into muscle-fibre strips from lean, obese and obese–NIDDM patients by IGF-II (100 nM), [Arg^{54},Arg^{55}]IGF-II (100 nM) and [Leu^{27}]IGF-II (100 nM)

Values (means ± S.E.M.) are expressed as increase above basal. Basal glucose-transport rates for lean, obese and obese–NIDDM patients were 2.6 ± 0.15, 2.3 ± 1 and 2.4 ± 0.4 nmol/min per g of wet tissue respectively. *$P < 0.05$ compared with lean patients.

	Lean ($n = 4$)	Obese ($n = 5$)	Obese–NIDDM ($n = 5$)
IGF-II	2.58 ± 0.15	0.42 ± 1*	1.26 ± 0.4*
[Leu^{27}]IGF-II	1.05 ± 0.4	0.32 ± 0.2	0.75 ± 0.41
[Arg^{54},Arg^{55}]IGF-II	2.67 ± 0.55	0.5 ± 0.2*	1.56 ± 0.6*

slightly more than IGF-II or IGF-I, but the differences were not statistically significant. Insulin (100 nM) stimulated glucose transport 2.5-fold or 2.8-fold depending on the conditions (see the Materials and methods section).

In order to evaluate further which receptor was responsible for this increase in 2-deoxyglucose transport induced by the administration of IGF-II, we used two IGF-II analogues with different affinities for the IGF-II/Man 6-P receptor: [Leu^{27}]IGF-II, with high affinity for the IGF-II/Man 6-P receptor but markedly reduced affinity for the IGF-I and insulin receptors, and [Arg^{54},Arg^{55}]IGF-II, with high affinity for the IGF-I and insulin receptors but no affinity for the IGF-II/Man 6-P receptor [39]. Uptake of 2-deoxyglucose increased 2.1-fold when stimulated with [Arg^{54},Arg^{55}]IGF-II at a concentration of 100 nM. This effect was very similar to that induced by IGF-II at the same concentration. In contrast, [Leu^{27}]IGF-II caused a very much reduced stimulation of glucose uptake (1.4-fold) (Table 2, lean patients). To determine whether IGF-II (or any of its analogues) can increase glucose utilization in obesity or NIDDM, 2-deoxyglucose transport was measured in muscle-fibre strips from obese and obese–NIDDM patients at a concentration of 100 nM. In contrast with the lean group, IGF-II did not significantly stimulate the uptake of 2-deoxyglucose transport in either of the obese groups (Table 2). [Arg^{54},Arg^{55}]IGF-II at a concentration of 100 nM induced a larger stimulation of glucose uptake than [Leu^{27}]IGF-II in all groups. Stimulation of glucose transport by [Arg^{54},Arg^{55}]IGF-II or [Leu^{27}]IGF-II was not statistically significant compared with the basal 2-deoxyglucose transport in the obese or obese–NIDDM groups. In Table 2, values are expressed as increase above basal.

DISCUSSION

In a previous study [12] we demonstrated the effectiveness of IGF-I as an alternative treatment to insulin in circumstances of severe insulin-resistance. Furthermore we postulated that IGF-I administered in very high doses could, in part, mediate its effects through the IGF-II/Man 6-P receptor. It is apparent that there is an overlapping of the physiological functions of these two ligands [46].

To our knowledge, there are no data in the literature evaluating the effect *in vivo* of IGF-II on glucose metabolism in humans. However, an indirect evaluation of the effect that high levels of IGF-II can produce in humans comes from studies performed in patients with an extrapancreatic tumour that produces IGF-II or larger forms of IGF-II (big IGF-II) [17–21]. Even though several investigators have shown that high levels of IGF-II may lead to sustained hypoglycaemia, there is uncertainty about which receptor mediates this effect.

Yu and Czech [7] using multiplication stimulating activity (IGF-II) showed stimulation of glucose transport in rat soleus muscle apparently via an IGF-I receptor. Likewise, it has been shown that IGF-II stimulates glucose transport in L6 myoblasts probably mediated by an IGF-I receptor [47,48]. The insulin receptor has also been suggested to be responsible for the acute metabolic actions of IGF-II on carbohydrate metabolism observed *in vivo*. Compared with insulin, about 50 times more IGF-II is required to produce similar responses [49]. In contrast with these studies, the recent data of Bevan et al. [27] suggest that IGF-II acts through its own receptor to increase insulin activity in rat soleus muscle.

Another approach to the determination of which receptor mediates IGF-II action is the use of specific antibodies to block the IGF-I receptor or affinity IGF-II cross-linking, thereafter correlating binding to the IGF-II/Man 6-P receptor with actions exerted via this receptor [27,50,51]. However, receptor antibodies may only partially block the binding of ligands and some may not be fully specific. Therefore IGF-II analogues would appear to be more useful than receptor antibodies in detecting which receptor mediates the IGF-II effects [40,52]. We used two IGF-II analogues with different affinities for the IGF-II/Man 6-P receptor in order to evaluate which receptor is responsible for the IGF-II stimulation of glucose transport in human muscle [40,52].

IGF-II at a concentration of 1 nM stimulated basal transport by 36%, whereas insulin at the same concentration stimulated glucose transport by 59%. The observation that submaximal stimulation of glucose transport occurred at approximately the same concentration for insulin and IGF-II was an unexpected finding. As there are fewer IGF-I and IGF-II receptors than insulin receptors in muscle, we had expected that a higher concentration of IGF-II would be required to produce stimulation of glucose transport. This result may suggest that a stronger signal is generated by IGF receptors and that a smaller percentage occupancy is required to produce a maximal response. However, IGF-II maximally stimulated the uptake of 2-deoxyglucose transport at a concentration of 1 μM, making it 10-fold less potent than insulin. However, in a previous report [29], a maximal glucose-transport effect was found in human quadriceps femoris muscle at an IGF-II concentration of 13 nM (20 times less potent than insulin). The discrepancy in concentration between these studies could in part be explained by methodological differences, especially the type of muscle and glucose tracer used. Furthermore the dose–response curve of Zierath et al. [29] does not show saturation with higher concentrations of IGF-II.

Our data show that the stimulation of glucose uptake induced by [Leu^{27}]IGF-II in the human muscle strips was significantly lower than that exerted by IGF-II. However, [Arg^{54},Arg^{55}]IGF-II stimulated 2-deoxyglucose transport in a similar fashion to insulin and IGF-I. These two facts indicate that IGF-II mainly stimulates glucose transport in human muscle via the IGF-I receptor, and the IGF-II/Man 6-P receptor seems to play a smaller role in this effect. Stimulation via the insulin receptor is unlikely because of the low affinity of IGF-II for it.

Our previous data [13] showed a decrease in stimulation by insulin and IGF-I of glucose transport in muscle of morbidly obese patients with or without NIDDM. One of the objectives of the present study was to investigate further the mechanism of insulin resistance by evaluating whether the muscle of morbidly obese patients is also resistant to IGF-II. The present report clearly demonstrates that IGF-II does not stimulate glucose transport in muscle-fibre strips from obese patients. It seems

unlikely that the beneficial effect of IGF-I administration in severe cases of insulin resistance would be mediated via an IGF-II/Man 6-P receptor, at least in human muscle. However it is our belief that, in circumstances of severe insulin resistance, IGF-I (and probably IGF-II) seems to increase insulin sensitivity. Usala et al. [12] showed that lower concentrations of insulin were required to maintain euglycaemia for several days after IGF-I administration in a severe insulin-resistant diabetic patient.

The present report provides a unique evaluation of the role of the IGF-II/Man 6-P and IGF-I receptors in the mediation of IGF-II effects on glucose uptake in muscle. The question of whether IGF-II stimulation of glucose transport in skeletal muscle has physiological significance remains unanswered.

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