Volume 307, number 3, 379–382 FEBS 11377 August 1992

Insulin-like growth factor II stimulates glucose transport in human skeletal muscle

Juleen R. Zierath[a], Peter Bang[b], Dana Galuska[a], Kerstin Hall[b] and Harriet Wallberg-Henriksson[a]

Departments of [a]Clinical Physiology and [b]Endocrinology, Karolinska Hospital, Karolinska Institute, Stockholm, Sweden

Received 12 June 1992

We investigated the effect of insulin-like growth factor II (IGF-II) and insulin-like growth factor binding protein-1 (IGFBP-1) on 3-*O*-methylglucose transport in incubated human skeletal muscle strips. Increasing physiological concentrations of IGF-II stimulated glucose transport in a dose-dependent manner. Glucose transport was maximally stimulated in the presence of 100 ng/ml (13.4 nM) of IGF-II, which corresponded to the effect obtained by 100 μU/ml (0.6 nM) of insulin. Exposure of muscle strips to IGFBP-1 (500 ng/ml) inhibited the maximal effect of IGF-II on glucose transport by 40%. Thus, it is conceivable that IGF-II and IGFBP-1 are physiological regulators of the glucose transport process in human skeletal muscle.

3-*O*-Methylglucose transport; Insulin-like growth factor II; Insulin-like growth factor binding protein-1; Insulin

1. INTRODUCTION

The IGF-I and IGF-II polypeptides are insulin-like growth factors which have structural homology with insulin [1]. Although the major effects of the IGF's are on cell replication and differentiation [1,2], the IGF's demonstrate effects similar to insulin on glucose metabolism [3,4]. IGF-I exerts a stimulatory effect on glucose transport in rat [4] and human skeletal muscle [5]. Furthermore, in rats, glucose uptake is increased in insulin target tissue, including adipose tissue, heart muscle and striated muscle [6–8] in response to IGF-II exposure.

One striking difference between the IGF's and insulin is the existence of a family of six specific serum insulin-like growth factor binding proteins (IGFBP's) [9]. In the circulation, IGF's are mainly associated with a 150 kDa binding complex which contains the IGF binding subunit, IGFBP-3 [10], or are coupled to the IGFBP-1 in smaller quantities [11]. Circulatory IGF's originate in the liver, and in their free form, can be transported to the target tissues [12]. When the IGF's are bound to the IGFBP's, their distribution to the target tissues appears to be modulated [13]. Furthermore, at the target tissue, the IGFBP's modulate the action of the IGF's by interacting with the receptor binding processes [2,14]. In human target tissue, the interaction between the IGFBP's and IGF-II has not been defined.

IGF-I and II operate as hypoglycemic peptides which may play a functional role in the maintenance of glycemia [3,15]. In response to physical activity, a temporary increase in the circulatory level of IGF-II has been observed, and may contribute to the in vivo increase in glucose transport activity associated with exercise [16]. Here we investigate the response of the human skeletal muscle glucose transport process to IGF-II. Since the biological activity of the IGF's may be altered by their binding to the IGFBP peptides, we also investigated the interaction between IGFBP-1 and IGF-II on skeletal muscle glucose transport. For these purposes, we employed an open muscle biopsy technique to obtain human muscle specimens [17] suitable for in vitro incubations [18].

Correspondence address: J. Zierath, Department of Clinical Physiology, Karolinska Hospital, Box 60500, S-104 01 Stockholm, Sweden. Fax: (46) (8) 32 90 22.

2. MATERIALS AND METHODS

2.1. *Subjects*

Fifteen healthy male volunteers (age, 28.7 ± 1.5 year; weight, 75.2 ± 1.9 kg; height, 179.6 ± 3.9 cm; body mass index, 23.5 ± 0.6 kg/m^2) participated in the study. None of the subjects were taking medications, or had a family history of metabolic disorders. The subjects were moderately engaged in physical activity, 2–3 times per week, and all were non-smokers. The study protocol was reviewed and approved by the institutional ethical committeee of the Karolinska Institute. The nature, purpose, and possible risks of the study were carefully explained to each subject before giving their consent to participate.

2.2. *Open muscle biopsy procedure*

The subjects reported to the laboratory following an overnight fast. Local anaesthesia (Mepivakain chloride 5 mg/ml) was administered and a 4-cm incision was made 15 cm above the proximal border of the patella. Thereafter, the skin and fascia were reflected to expose the intact muscle fibers of the vastus lateralis portion of the quadriceps femoris muscle [17]. Two muscle specimens (~250 mg) were clamped at resting length in vivo, excised, and immediately transferred to a beaker which contained oxygenated Krebs–Henselit (KHB) bicarbonate buffer, 5 mM HEPES (*N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), 0.1% bovine serum albumin (RIA Grade, Fraction V), 38 mM mannitol and 2 mM pyruvate. The two samples were

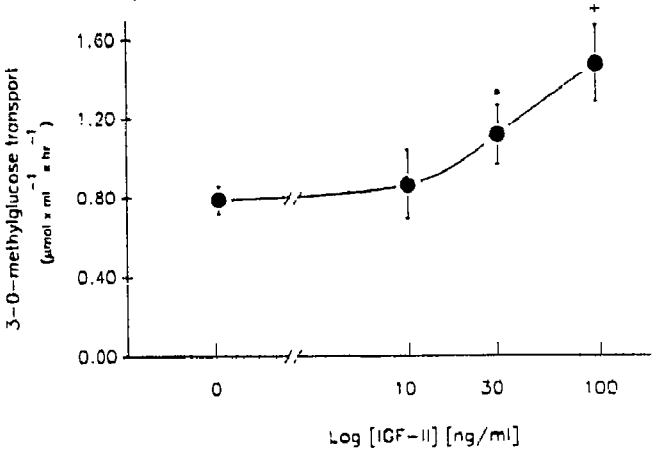

Fig. 1. IGF-II stimulated 3-*O*-methylglucose transport in human skeletal muscle strips. Muscle strips from 7 subjects were incubated in vitro, in increasing concentrations of IGF-II and the rate of 3-*O*-methylglucose transport was determined as described in section 2. Results represent a complete dose–response curve for each subject. Values are means ± S.E.M. *$P < 0.05$ vs. 0 ng/ml of IGF-II, †$P < 0.01$ vs. 0 ng/ml of IGF-II.

transported to the laboratory (<2 min), whereafter 5–8 smaller muscle strips (~20 mg) were dissected free from each sample, mounted on plexi-glass clamps (0.9 cm wide) and removed from the tissue specimen as described earlier [17,18].

2.3. *In vitro muscle strip incubation*

Immediately following tissue preparation, the muscle strips were placed in individual covered flasks, in a shaking incubator (110 times/min) maintained at 35°C. All media were prepared daily from a KHB stock (pH 7.4), which was gassed with 95% O_2/5% CO_2. Following dissection, the muscle strips were allowed to recover for 10 min in KHB containing 2 mM pyruvate and 38 mM mannitol. Thereafter, the muscle strips were transferred to pre-incubation media which contained KHB, 5 mM glucose, and 35 mM mannitol. The muscle strips were exposed to IGF-II, insulin, or IGFBP-1 for 60 min as described in Table I and Fig. 1. Media concentrations of IGF-II, insulin, or IGFBP-1 were maintained throughout the entire incubation protocol. Glucose was rinsed from the extracellular space of the muscle strips by means of a 10 min exposure to a KHB solution which contained 40 mM mannitol. Thereafter, 3-*O*-methylglucose transport activity was assessed in the muscle strips during a 20 min incubation in KHB medium whereby 5 mM 3-*O*[^{3}H]methylglucose (437 μCi/mmol) and 35 mM [^{14}C]mannitol (8 μCi/mmol) were substituted for unlabeled glucose and mannitol, respectively. Following incubation, the muscles were processed by the method described by Wallberg-Henriksson and Holloszy [19].

2.4. *IGF-II and IGFBP-1 release*

Basal pre-incubation media (0 ng/ml IGF-II) was collected from six muscle strips following the 60-min pre-incubation treatment. The media was pooled (total 12 ml) and dialyzed in a 1000 MW cutoff dialysis tube against 0.01 M acetic acid. The dialysate was lyophilized and re-suspended in 1 M acetic acid. The solution was then chromatographed on a Sephadex G 50 column (0.9 × 30 cm) (FPLC equipment, Pharmacia, Uppsala, Sweden) and eluted with 1 M acetic acid, and 0.02 M NaCl (flow rate 0.5 ml/min at 4°C) to separate the IGF-II and the IGFBP's fractions. The IGF-II immunoreactivity in the IGF fraction (K_d 0.32–0.85) was determined by radioimmunoassay [20]. The IGFBP fraction (K_d 0.0–0.32) was analyzed by Western ligand blotting as originally described by Hossenlopp et al. [21] with minor modifications. Briefly, approximately 1/5 the original volume was processed by SDS-PAGE (~2.4 ml), and trans-blotted to a nitrocellulose filter paper. After blocking the filter paper with albumin, the binding proteins were probed with ^{125}I-labeled IGF-II and visualized on X-ray film. The detection limit for the Western ligand blotting is in the order of 10 ng of IGFBP-1.

2.5. *Analytic techniques and chemicals*

Plasma glucose was analyzed with a Beckman Glucose Analyzer (Beckman Instrument Inc., Fullerton, CA, USA). Serum insulin concentration was determined by standard radioimmunoassay (Phadeseph Insulin RIA method, Pharmacia, Uppsala, Sweden). The lower limit of sensitivity of this method is 3.0 μU/ml of insulin.

All chemicals, unless stated otherwise, were of the highest purity and obtained from Sigma Chemical Company (St. Louis, MO, USA). Recombinant human IGF-II and IGFBP-1 were generous gifts provided by Kabigen, Kabi Pharmacia (Stockholm, Sweden). The insulin (Actrapid) was a product of Novo Industry A/S, Copenhagen, Denmark. All radioactive products were purchased from New England Nuclear (Boston, MA, USA).

2.6. *Statistical analysis*

The statistical significance of the differences between the effect of IGF-II on glucose transport in human skeletal muscle strips incubated in the presence or absence of IGFBP-1 was determined by Student's paired *t*-test. A one-way analysis of variance (ANOVA) was employed to evaluate the differences between various concentrations of IGF-II. When the ANOVA resulted in a significant *F*-value, the difference between the means was identified by the Newman–Keuls test. A significance level of $P < 0.05$ was employed. Results are expressed as means ± S.E.M.

3. RESULTS

3.1. *Subject characteristics*

The participants exhibited normal fasting plasma glucose and serum insulin values of 5.01 ± 0.10 mM and 6.29 ± 0.57 μU/ml, respectively.

3.2. *Release of IGF-II and IGFBP-1 to media*

Pooled basal (0 ng/ml of IGF-II) pre-incubation media, collected from six muscle strips following 60 min incubation, contained 0.6 ng/ml of IGF-II. The observance of IGF-II in the basal media indicates that each muscle strip released approximately 1.2 ng of IGF-II into the media during the basal incubation period. This release corresponded to ~6% of the lowest concentration of IGF-II tested (10 ng/ml). No IGFBP's could be detected in the pooled media when assessed by Western ligand blot analysis. Thus, the concentration of IGFBP-1 in the pooled pre-incubation media was less than 4 ng/ml.

3.3. *Effect of IGF-II on glucose transport*

The basal rate of 3-*O*-methylglucose transport in the incubated skeletal muscle strips increased from 0.95 ± 0.06 ($n = 7$) to 1.74 ± 0.17 ($n = 7$) μmol·ml^{-1}·h^{-1} ($P < 0.01$) when IGF-II at 100 ng/ml (13.4 nM) was present in the media. No further increase in the IGF-II stimulated glucose transport process was observed in the presence of 1000 ng/ml (134 nM) of IGF-II when compared to the effect elicited by 100 ng/ml of IGF-II (1.87 ± 0.24 μmol·ml^{-1}·h^{-1} for 1,000 ng/ml of IGF-II; $n = 7$). Thus, a maximal effect of IGF-II on the rate of glucose transport was obtained at a concentration of 100 ng/

A dose dependent relationship for physiological concentrations of IGF-II was established by exposing muscle strips to increasing concentrations of IGF-II (0, 10, 30 and 100 ng/ml). The rate of 3-*O*-methylglucose transport in the skeletal muscle strips increased in a dose-dependent manner (Fig. 1). Basal glucose transport increased 1.4-fold ($P < 0.05$) when stimulated with IGF-II at a concentration of 30 ng/ml (4 nM), whereas 10 ng/ml (1.3 nM) of IGF-II failed to elicit an effect. IGF-II, at a concentration of 100 ng/ml (13.4 nM), stimulated the glucose transport system (1.47 ± 0.19 μmol·ml^{-1}·h^{-1}, $P < 0.01$) to the same extent as observed in the presence of 100 μU/ml (0.6 nM) of insulin (1.51 ± 0.18 μmol·ml^{-1}·h^{-1}, $P < 0.01$).

3.4. *Effect of IGFBP-1 on glucose transport*

Exposure of muscle strips to IGFBP-1 did not alter the basal rate of glucose transport in human skeletal muscle strips (Table I). In the presence of IGF-II (100 ng/ml), an equimolar concentration of IGFBP-1 (500 ng/ml) (20 nM) inhibited the increase in IGF-II stimulated glucose transport by 40% ($P < 0.05$) (Table I).

4. DISCUSSION

In this report, we provide evidence which indicates that IGF-II is a regulator of the glucose transport process in human skeletal muscle. During in vitro incubation, increasing concentrations of free IGF-II stimulated skeletal muscle glucose transport in a dose-dependent manner. The maximal effect of IGF-II observed at 100 ng/ml (13.4 nM), corresponded with the stimulatory effect of insulin (100 μU/ml, 0.6 nM) on skeletal muscle glucose transport. Thus, as a stimulator of the skeletal muscle glucose transport process, IGF-II is approximately 20 times less potent than insulin.

In adult serum, the molar concentrations of IGF-I and IGF-II are 10- to 100-fold higher than required for their effect in vitro [22]. Of the total serum IGF-I's, the free form of IGF-I has been estimated to be in the order of 1–5% [15]. Since the total serum concentration of IGF-II is approximately 800 ng/ml, the estimated free form of IGF-II, based on the reported percentage of free IGF-I, would correspond to 8–40 ng. We have observed a stimulatory effect of IGF-II on skeletal muscle glucose transport in the presence of 30 ng/ml. Thus, our data indicates that physiological concentrations of IGF-II activate the human muscular glucose transport process.

The liver is the main source of the circulatory IGF's, however, the IGF's are also produced locally in many tissues including skeletal muscle [23,24]. Although the extracellular concentrations of the IGF's in the target tissues are not known, the possibility remains that the IGF's may accumulate in the extracellular space of the musculature, thus resulting in a high concentration of IGF's at the site of the muscle.

Elevated or depressed levels of the IGFBP's will potentially influence the metabolic effects of the IGF's. When the IGF's are coupled to the binding proteins, the binding of the hormone to the insulin or IGF receptor is inhibited [25] and the stimulatory effects of the IGF's on glucose metabolism are altered [26]. Evidence for an inhibitory effect of IGFBP-1 on the IGF-II stimulated increase in skeletal muscle glucose transport is presented in this report (Table I). Factors which alter the regulation of the IGFBP's must be considered when investigating the effects of the IGF's. Insulin is reported to play a regulatory role in the concentration of IGFBP-1 [27]. Consequently, fluctuations in the concentration of binding proteins as a result of alterations in the serum insulin level may modify the physiological effect of IGF's.

Of the two insulin-like growth factors, the biological function of IGF-1 is more clearly defined [1,26]. The biological action of IGF-I on human skeletal muscle glucose transport has been reported to elicit a response similar to that observed by insulin [5]. In human skeletal muscle there is cross-reactivity between the structurally homologous insulin and IGF-I receptors, whereby the insulin-like effects of IGF-I have been demonstrated to be mediated [5,8,28,29]. Results from hormone displacement experiments reveal that IGF-I stimulates glucose transport through its specific receptor and not simply in association with the insulin receptor [5]. Although IGF-II receptors are present in various tissues, the physiological role of IGF-II in adult humans has not been clarified. In the present report, physiological concentrations of IGF-II appear to regulate the skeletal muscle glucose transport system, nevertheless, the cellular mechanism by which this polypeptide operates remains to be elucidated. Almost all of the reported in vitro and in vivo effects of IGF's have been described to be mediated over the IGF-I receptor [26]. Thus, it is likely that the effects of IGF-II on the glucose transport are mediated in part by the IGF-I receptors.

IGF's have been described as hypoglycemic peptides

Table I

Effect of IGFBP-1 on the rate of basal and IGF-II stimulated glucose transport in human skeletal muscle

Media	Glucose transport (μmol·ml^{-1}·h^{-1})	
	No IGFBP-1	IGFBP-1 (500 ng/ml)
Basal	0.88 ± 0.14	0.93 ± 0.11
IGF-II (100 ng/ml)	1.78 ± 0.28	1.44 ± 0.19*

Values are means ± S.E.M. for $n = 6$ subjects. One observation was obtained for all experimental conditions for each of the 6 subjects. Skeletal muscle strips were prepared and incubated in the presence or absence of insulin-like growth factor II (IGF-II) with the addition or omission of insulin-like growth factor binding protein 1 (IGFBP-1) as outlined in section 2. *$P < 0.05$ vs. No IGFBP-1.

[3,15,26] which as reported here, and by others [5], have profound effects on glucose metabolism in skeletal muscle from healthy subjects. Although insulin is the primary stimulator of skeletal muscle glucose transport, the IGF's may be notable contributors to the regulation of glycemia in vivo. Patients with non-insulin-dependent diabetes mellitus and obesity demonstrate decreased insulin [18,30] and IGF-I [5] stimulated skeletal muscle glucose transport. The effects of IGF-II on skeletal muscle glucose metabolism in patients with peripheral insulin resistance remains to be established.

In conclusion, the present study reveals that physiological concentrations of IGF-II stimulate the glucose transport process in healthy, human skeletal muscle. Furthermore, IGFBP-1 appears to play a counter-regulatory role in the IGF-II stimulated glucose transport.

Acknowledgements: This study was supported by grants from the Swedish Medical Research Council (5117 and 4224), the Nordisk Insulin Fund, the Swedish Diabetes Association, Gustav V's research fund, the Thurings Fund for Medical Research, the Nordisk Grant for the Study of Growth, and from Novo-Nordisk A/B.

REFERENCES

[1] Froesch, E.R. and Zapf, J. (1985) Diabetologia 28, 485–493.
[2] Blum, W.F., Jenne, E.W., Reppin, F., Kietzmann, K., Ranke, M.B. and Bierich, J.R. (1989) Endocrinology 125, 766–772.
[3] Guler, H.P., Zapf, J. and Froesch, E.R. (1987) N. Engl. J. Med. 317, 137–140.
[4] Poggi, C., Le Marchand-Brustel, Y., Zapf, J., Froesch, E.R. and Freychet, P. (1979) Endocrinology 105, 723–730.
[5] Dohm, L.G., Elton, C.W., Raju, M.S., Mooney, M.D., DiMarchi, R., Pories, W.J., Flickinger, E.G., Atkinson, S.M. and Caro, J.F. (1990) Diabetes 39, 1028–1032.
[6] Meuli, C. and Froesch, E.R. (1977) Biochem. Biophys. Res. Commun. 75, 689–695.
[7] Meuli, C. and Froesch, E.R. (1976) Arch. Biochem. Biophys. 177, 31–38.
[8] Zapf, J., Schoenle, E. and Froesch, E.R. (1978) Eur. J. Biochem. 87, 285–296.
[9] Lamson, G., Giudice, L.C. and Rosenfeld, R.G. (1991) Growth Factors 5, 19–28.
[10] Zapf, J., Waldvogel, M. and Froesch, E.R. (1975) Arch. Biochem. Biophys. 168, 638–645.
[11] Luthman, H., Söderling-Barros, J., Persson, B., Engberg, C., Stern, I., Lake, M., Franzén, S.-Å., Israelsson, M., Rådén, B., Lindgren, B., Hjelmqvist, L., Enerbäck, S., Carlsson, P., Bjursell, G., Póvoa, G., Hall, K. and Jörnvall, H. (1989) Eur. J. Biochem. 180, 259–265.
[12] Cascieri, M.A., Saperstein, R., Hayes, N.S., Green, B.G., Chicchi, G.G., Applebaum, J. and Bayne, M.L. (1989) Endocrinology 123, 373–381.
[13] Bar, R.S., Boes, M., Clemmons, D.R., Busby, W.H., Sandra, A., Dake, B.L. and Booth, B.A. (1990) Endocrinology 127, 497–499.
[14] Clemmons, D.R., Han, V.K.M., Elgin, R.G. and D'Ercole, A.J. (1987) Mol. Endocrinol. 1, 339–347.
[15] Lewitt, M.S., Denyer, G.S., Cooney, G.J. and Baxter, R.C. (1991) Endocrinology 129, 2254–2256.
[16] Bang, P., Brandt, J., Degerblad, M., Enberg, G., Kaijser, L., Thorén, M. and Hall, K. (1990) Eur. J. Clin. Invest. 20, 285–292.
[17] Zierath, J.R., Galuska, D., Engström, Å., Johnson, K.H., Betsholtz, C., Westermark, P. and Wallberg-Henriksson, H. (1992) Diabetologia 35, 26–31.
[18] Dohm, G.L., Tapscott, E.B., Pories, W.J., Dabbs, D.J., Flickinger, E.G., Meelheim, D., Fushiki, T., Atkinson, S.M., Elton, C.W. and Caro, J.F. (1988) J. Clin. Invest. 82, 486–494.
[19] Wallberg-Henriksson, H. and Holloszy, J.O. (1985) Am. J. Physiol. 249, C233–C237.
[20] Bang, P., Eriksson, U., Sara, V., Wivall, I.-L. and Hall, K. (1991) Acta Endocrinol. (Copenh.) 124, 620–629.
[21] Hossenlopp, P., Seurin, D., Segovia-Quinson, B., Hardouin, S. and Binoux, M. (1986) Anal. Biochem. 154, 138–143.
[22] Sara, V.R. and Hall, K. (1990) Physiol. Rev. 70, 591–614.
[23] Florini, J.R., Magri, K.A., Ewton, D.Z., James, P.L., Grindstaff, K. and Rotwein, P.S. (1991) J. Biol. Chem. 266, 15917–15923.
[24] Hall, K. and Bozovic, M. (1969) Horm. Metab. Res. 1, 235–240.
[25] Zapf, J., Schoenle, E., Waldvogel, M., Sand, I. and Froesch, E.R. (1981) Eur. J. Biochem. 113, 605–609.
[26] Froesch, E.R., Guler, H.P., Schmid, C., Ernst, M. and Zapf, J., in: Diabetes Mellitus (4th Edn) (H. Rifkin and D. Porte, Eds.) Elsevier, New York, 1990, pp. 154–169.
[27] Brismar, K., Gutniak, M., Povoa, G., Werner, S. and Hall, K. (1988) J. Endocrinol. Invest. 11, 599–602.
[28] Livingston, N., Pollare, T., Lithell, H. and Arner, P. (1988) Diabetologia 31, 871–877.
[29] Zorzano, A., James, D.E., Ruderman, N.B. and Pilch, P.F. (1988) FEBS Lett. 234, 257–262.
[30] Andréasson, K., Galuska, D., Thörne, A., Sonnenfeld, T. and Wallberg-Henriksson, H. (1991) Acta Physiol. Scand. 142, 255–260.