CLINICAL STUDY

Interstitial IGF-I in exercising skeletal muscle in women

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Abstract

Objective: To study interstitial IGF-I concentrations in resting and exercising skeletal muscle in relation to the circulating components of the IGF–IGF binding protein (IGFBP) system.

Design and methods: Seven women performed endurance exercise with 1 leg (Ex-leg) for 1 h. The resting leg (Rest-leg) served as a control. IGF-I was determined in microdialysate (MD) and was compared with veno-arterial (v-a) concentrations of circulating IGF–IGFBP components.

Results: Median (range) basal MD-IGF-I was 0.87 (0.4–1.5) µg/l or 0.4 (0.2)% of total-IGF-I (t-IGF-I) determined in arterial serum and in the same concentration range as free dissociable IGF-I (f-IGF-I). Rest-leg MD-IGF-I decreased, reaching significance after exercise. Ex-leg MD-IGF-I was unchanged during exercise and declined after exercise at the level of significance ($P=0.05$). There was a release of f-IGF-I from the Ex-leg into the circulation at the end of and shortly after exercise. A small but significant increase in circulating IGFBP-1 was detected at the end of exercise and IGFBP-1 increased further after exercise. Although interleukin-6 (IL-6) has been associated with IGFBP-3 proteolysis, the circulating molecular forms of IGFBP-3 remained unchanged in spite of an IL-6 release from the muscle compartment.

Conclusions: Circulating IGFBP-1 is related to interstitial IGF-I in resting muscle although the temporal relationship may not be simple. Further studies should explore the role of local release of IGF-I and its impact on IGF-I activity during contraction.

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Introduction

Insulin-like growth factor-I (IGF-I) is expressed in a large range of tissues. It exerts anabolic, differentiating, and metabolic effects. The major source of circulating IGF-I is the liver as confirmed by studies of liver-specific IGF-I knockout mice which have 20% of wild-type circulating total IGF-I (t-IGF-I) concentrations. Despite skeletal muscle insulin resistance and the development of diabetes in the liver-specific IGF-I knock-out (KO) mice, tissue anabolism (body weight and length) appears to be less affected by the deficiency of endocrine IGF-I, suggesting that locally produced IGF-I is important for anabolism (1). In fact, IGF-I is expressed in human skeletal muscle and involved in processes such as differentiation, hypertrophy, and angiogenesis. These remodeling effects of IGF-I and its metabolic effects are mediated by the IGF type 1 receptor (IGF1R). Functional inactivation of the IGF1R in skeletal muscle in mice causes muscle hypoplasia during the early postnatal stages. In contrast to the effect in wild-type mice, exercise fails to increase muscle fiber diameter and satellite cell proliferation in these mice (2). IGF-I and insulin are structurally homologous and they equipotently stimulate glucose uptake into skeletal muscle preparations *ex vivo* (3)

and human muscle cell cultures *in vitro* (4). Transgenic mice lacking insulin receptors in skeletal muscle develop compensatory mechanisms to clear glucose (5), while mice with functional inactivation of both IGF1R and insulin receptors in skeletal muscle develop type 2 diabetes (6).

Altogether, the available data establish major biological effects of IGF-I and its importance for muscle tissue structural and functional adaptation to environmental demands. However, the reports on exercise-induced changes in skeletal muscle IGF-I mRNA and/or protein expression are rather sparse and, to some extent, contradictory (7–9). Moreover, the fact that the bioavailability of IGF-I is regulated by numerous factors other than changes in gene expression is somewhat overlooked.

IGF binding proteins (IGFBP-1–IGFBP-6) modulate the bioavailability of IGF-I in the circulation and tissues. In the circulation, most IGF-I is bound to IGFBP-3 and an acid-labile subunit in a large 150 kDa ternary complex that reduces IGF-I bioavailability. IGF-I in the ternary complex is restricted to the circulation, while unbound IGF-I and possibly IGF-I in binary complexes have access to the extracellular fluid. Supraphysiological levels of IGFBP-1 inhibit the hypoglycemic effects of IGF-I *in vivo* (10). IGFBP-1 has previously been reported to increase in

prolonged exercise (11). IGFBP-1 has been suggested to restrict IGF-I access to the tissues and prevent hypoglycemia, although the impact of IGFBP-1 on tissue concentrations of IGF-I has not previously been explored. Furthermore, increased serum proteolytic fragmentation of IGFBP-3 may increase IGF-I access to the tissues (12). Endocrine or locally produced IGF-I binds to IGFbps in the extracellular fluid or to IGFbps associated with the extracellular matrix or the cell surface (13). At the tissue level, IGFBP-1 inhibits IGF-induced glucose uptake in human skeletal muscle (14). Tissue-activated IGFBP proteases may further modulate IGF-I actions (15). Interleukin-6 (IL-6) increases the activity of proteases that degrade IGFBP-3 including matrix metalloproteinases (16, 17). Increased proteolytic degradation of IGFBP-3 has been detected in the circulation in human conditions of elevated IL-6 and in transgenic mice overexpressing IL-6 (18). This suggests that IL-6 released from exercising skeletal muscle (19, 20) may locally increase IGFBP-3 proteolysis and IGF-I bioavailability.

The aim of the present study was to explore unbound IGF-I concentrations by microdialysis in skeletal muscle interstitial fluid. We used a one-legged knee extension exercise model (the contralateral leg served as a control) to induce profound acute changes in the local skeletal muscle tissue milieu. Interstitial IGF-I concentrations in the muscle and the circulating IGF-IGFBP system, including free dissociable IGF-I (f-IGF-I), were determined from arteriovenous (v-a) differences over the exercising leg (Ex-leg). For the first time the (a-v) differences of IL-6 were determined in women.

Subjects and methods

Subjects

Seven healthy women were studied. Their mean (range) age, height, weight, and BMI were 28 years (23–39), 171 cm (165–177), 63 kg (54–72), and 21 kg/m² (20–23) respectively. They were not on any medication

and they had regular menstrual cycles. The experiment was performed during the follicular phase of the cycle, confirmed by measurements of luteinizing hormone, follicle-stimulating hormone, estradiol, and progesterone on the day of the experiment (data not shown). Pregnancy was excluded by urine human chorionic gonadotropin (hCG) determination (TestPack Plus, Abbott). The study conformed to the standards set by the Declaration of Helsinki and was approved by the Karolinska Institute Ethics Committee (Number 546/03). Written informed consent was obtained from all subjects.

Experimental protocol

On two occasions before the experiment, each subject was familiarized with the experimental apparatus, a modified cycle ergometer built for one-legged knee extension exercise (at 60 r.p.m.) (21). On the second occasion (at least one week before the experiment), the maximal one-legged performance capacity was determined in a test where the workload was increased in 5 W increments every minute to fatigue. The mean maximal workload during one-legged exercise was 36 (4) W or 0.6 (0.1) W/kg. Subjects were asked to avoid physical activity of moderate intensity or higher for 72 h before the day of the experiment. On that day subjects had a standardized breakfast at 0630 h. The study was conducted between 0800 and 1500 h (Fig. 1). Microdialysis catheters were inserted in both vastus lateralis muscles 2.5 h before the onset of exercise, as described in detail below. Teflon catheters were inserted into the femoral artery of the later Ex-leg and both femoral veins. Patency of the catheters was maintained by intermittent flushing with a saline solution. The subjects rested in the supine position for 2 h. Thereafter, they performed a one-legged knee extension exercise for 1 h in the sitting position with a workload corresponding to 60 (6)% of the previously assessed maximal one-legged workload at 60 r.p.m. The absolute workload was 22 (3) W. The resting leg (Rest-leg) was allowed to move freely in order to avoid a workload

Figure 1 Experimental protocol. Seven women performed one-legged knee extension exercise for 1 h (0–60 min). Microdialysate from the exercising (Ex-leg) and resting (Rest-leg) leg was collected during 60-min periods. Blood sampling was performed in the femoral artery and vein of Ex-leg and the femoral vein of Rest-leg at eight time points (0 through P120). Blood flow (BF) determinations in Ex-leg at five time points (0 through P30) were completed in four subjects.

associated with counteracting or balancing contractions while the Ex-leg was kicking. This resulted in simultaneous passive movements in the knee joint of the Rest-leg with an amplitude of approximately half that of the Ex-leg. The mean rate of perceived exertion, (RPE 6–20) as described in (22), was 15 (range 14–16). Finally, the subjects rested for 2 h in the supine position. Blood samples for hormone determinations were drawn from the femoral artery and vein of the Ex-leg before exercise (basal; time = 0), during exercise at 10 (Ex10), 30 (Ex30), and 60 (Ex60) min and at 10 (P10), 30 (P30), 60 (P60), and 120 (P120) min after exercise. Blood samples were kept on ice for 1 h to allow clotting before obtaining serum by centrifugation and storing at -70°C . Blood for a B-glucose determination was collected in heparin-fluoride plasma vials and kept on ice until centrifugation and analysis. Blood samples from the femoral veins of both legs were obtained to assess blood flow using Cardiogreen infusion as described below.

Hormones and biochemical markers

IGF-I t-IGF-I was determined after acid/ethanol extraction of serum samples to exclude interference of IGFBPs (23) or directly in microdialysate (MD) by an IGF-I DELFIA originally described by Frystyk *et al.* (24) with a few modifications (25). The detection limit was $0.007\text{ }\mu\text{g/l}$ and the intra-assay CV for serum samples, including the extraction step, was 6.4%. No interference of increasing concentrations of human albumin or ^{14}C inulin could be detected. Free IGF-I in serum was determined with a commercially available ELISA kit (DSL Inc., Webster, TX, USA).

IGFBP-I The total IGFBP-I serum levels were determined by a RIA described in (25) and modified from (26). The detection limit was $6\text{ }\mu\text{g/l}$ and the intra- and interassay CV was 5.6 and 11.8% respectively.

IGFBP-3 Immunoreactive total IGFBP-3 from the microdialysis *in vitro* and *in vivo* experiments was determined by ELISA, which is known to detect intact as well as fragmented IGFBP-3 (DSL Inc.; detection limit $0.73\text{ }\mu\text{g/l}$). IGFBP-3 fragmentation was determined in serum by western immunoblotting (WIB) using the enhanced chemiluminescence detection technique (27). The density of the bands was analyzed using the Image J computer program (National Institutes of Health, Bethesda, MD, USA). The serum IGFBP-3 *in vivo* fragmentation was expressed as a percentage of the major IGFBP-3 fragment (30 kDa) relative to the total IGFBP-3 intensity (40–42 kDa + 30 kDa).

IL-6 IL-6 was determined with a high sensitivity ELISA kit (QuantiGlo, R&D Systems, Abingdon, UK).

pH and Hb pH and Hb in venous blood from the Ex-leg were determined using an automatic spectrophotometric technique (ABL 520, Copenhagen, Denmark).

Glucose Plasma was kept on ice and analyzed on the day of the experiment. A hexokinase method was used to determine glucose concentrations (Gluco-quant, Roche Diagnostics GmbH) in the artery to and the vein from the Ex-leg. The detection limit was 0.11 mM (0.02 g/l). The intra- and inter-assay CV was 1 and 3% respectively.

^{14}C inulin in MD ^{14}C inulin reverse recovery (I-RR) was calculated from measurements of radioactivity in $10\text{ }\mu\text{l}$ aliquots of collected MD compared with the perfusion fluid. After the addition of 5 ml scintillation solution (Ready Safe, Beckman Coulter Inc., Fullerton, CA, USA), radioactivity was determined in a β -counter (Beckman LS 5000CE, Beckman Instruments, Fullerton, CA, USA).

Microdialysis

Probes, perfusion pumps, and perfusion fluid were all purchased from CMA Microdialysis (CMA, Solna, Sweden). Prior to the experiment, the microdialysis methodology was optimized and validated *in vitro*, as described later. The polyamide probe (CMA 60, 0.5–30 mm, cutoff 20 kDa) was perfused with a nonbuffered water solution containing $\text{Na}^{+} 147\text{ mM}$, $\text{K}^{+} 4\text{ mM}$, $\text{Ca}^{2+} 2.3\text{ mM}$, and $\text{Cl}^{-} 156\text{ mM}$, osmolality 290 mosm/kg and pH 6.0, provided by the manufacturer with an addition of 0.05% human serum albumin (HSA; Pharmacia). For assessment of changes in probe recovery of IGF-I (7.5 kDa) related to muscle contractions and blood flow (28–32), ^{14}C inulin ($0.05\text{ }\mu\text{Ci/ml}$; 5 kDa; Amersham Biosciences) was chosen as an internal reference substance. The internal reference method is based on the concept that the ratio of the *in vitro* recovery of any two compounds is equal to the ratio of the recovery of the same two compounds *in vivo*. Inulin (5 kDa) has previously been used as a marker molecule for insulin (5.8 kDa) due to similarity in molecular weight, lack of tissue binding, and metabolism of inulin and availability of a safe and stably labeled molecule (33–35). The perfusion flow rate was $2\text{ }\mu\text{l/min}$ (perfusion pump CMA 107). After inducing local anesthesia down to the muscle fascia (Mepivacainhydrochloride; Carbocain 10 mg/ml, Astra Zeneca), two microdialysis catheters were inserted into the vastus lateralis muscle of each leg by insertion in a cranial direction, 45° relative to the sagittal plane of the muscle. The distal end of the outlet tubing was cut off and inserted into a TreffLab polypropylene tube (Treff AG, Degersheim, Switzerland). To minimize the possible impact of skeletal muscle tissue response to the insertion trauma, microdialysis probes were inserted 2.5 h before onset of exercise and 1.5 h of equilibration was allowed before the collection of MD for IGF-I determinations. This approach has been taken in previous microdialysis studies (28, 36).

MD was collected from the exercising (Ex-leg) and resting (Rest-leg) leg in the basal state (−60 to 0 min), during exercise (Ex0–Ex60) as well as after exercise (Ex60–P60) and (P60–P120) for the determination of IGF-I and ^{14}C inulin concentrations. The collecting tubes were weighed before and after collection and stored at -70°C until analysis.

In a series of *in vitro* experiments, microdialysis probes were submerged in a polyethylene tube containing an experimental 'interstitial fluid' consisting of a modified Krebs Henseleit solution with 0.05% HSA (Pharmacia AB) and 0.19 (0.05) $\mu\text{g/l}$ (0.0248 (0.006) nM) of human recombinant IGF-I (kindly provided by Genentech Inc., South San Francisco, CA, USA) with or without IGFBP-3 (Upstate, NY, USA supplied by Chemicon Europe, Hampshire, UK). The composition of the perfusion fluid was the same as that *in vivo* described above (perfusion speed 2 $\mu\text{l/min}$). Microdialysis of the 'interstitial fluid' was performed for up to 24 h at 37°C under gentle shaking. MD was collected at 1-h intervals for 4 h for IGF-I determination by DELFIA ($n=4$ CMA 60 catheters). After the first 4 h, a 10 molar excess of IGFBP-3 was added to the 'interstitial fluid' in a final concentration of 10.6 $\mu\text{g/l}$ (0.264 nM).

Addition of a 10 molar excess of IGFBP-3 decreased IGF-I in the MD by 48 (7)% in accordance with the expected 50% calculated reduction in 'free IGF-I' using a K_D of 0.24 nM (12). Microdialysis of 'in vitro interstitial fluid' IGFBP-3 (GroPep, Adelaide, Australia) at 500 $\mu\text{g/l}$, was not detectable in MD by SDS-PAGE and WB after 16 h of collection (recovery of IGFBP-3 was <1.6%). We have previously reported (37) that larger IGFBP-3 fragments or IGF-IGFBP complexes (30–50 kDa) do not pass the microdialysis membrane.

Calculations The MD-IGF-I_{absolute} was corrected for IGF-I recovery *in vivo* according to the internal reference technique (33, 34). The reverse recovery of ^{14}C inulin ($\text{I-RR}_{in vitro}$) was calculated as the relative loss of ^{14}C inulin from the perfusion fluid = $(^{14}\text{C inulin}_{perfusion fluid} - ^{14}\text{C inulin}_{dialysate}) / ^{14}\text{C inulin}_{perfusion fluid}$. The mean ($\text{I-RR}_{in vitro}$) of ^{14}C inulin was 55 (4)% in the four catheters. The following formula was used for calculation of the IGF-I recovery *in vivo*: $(\text{IGF-I recovery}_{in vitro} \times \text{I-RR}_{in vitro} / \text{I-RR}_{in vitro})$. The mean relative recovery of IGF-I_{in vitro} = $(\text{IGF-I}_{dialysate} / \text{IGF-I}_{experimental 'interstitial fluid'})$ was 16 (6)%. The mean intracatheter CV over time was 10%. The actual measured interstitial IGF-I values (IGF-I_{absolute}) were corrected for IGF-I recovery *in vivo* by the following formula: $\text{MD-IGF-I} = (\text{IGF-I}_{absolute}) \times (\text{IGF-I recovery}_{in vivo})$.

Leg blood flow and plasma flow

Leg blood flow measurements before exercise (0), during exercise (Ex10, Ex30, and Ex60), and after exercise (P10 and P30) in Ex-leg were completed in four

subjects. Leg blood flow was estimated according to the indicator-dilution technique (38). Leg plasma flow was calculated according to the following formula: $\text{leg blood flow} \times (100 - \text{EVF}) / 100$. The EVF was determined exactly at the same time points as the leg blood flow determinations. The flux of substances over the leg was calculated according to the following formula: $\text{leg plasma flow} \times (\text{venous plasma concentrations} - \text{arterial plasma concentrations})$.

Statistical analysis

Results are given as the mean (s.d.) if normally distributed; otherwise, as the median (range) unless otherwise stated. Variables with a normal distribution were compared by repeated measures ANOVA with time and artery/vein as dependent factors. Planned comparisons were used to locate differences corresponding to the significant interaction in the ANOVA model. The f-IGF-I was log-transformed to obtain normality prior to parametric statistical analysis (ANOVA) and then transformed back to the original scale. A nonparametric test (Friedman's ANOVA) was used if normality did not apply. Wilcoxon's matched pair test was performed to define where in time the difference was significant. Correlations were investigated with Spearman's nonparametric correlation test. Only individuals with complete data sets (data on every time point) were included in the statistical analyses. $P < 0.05$ was considered significant.

Results

IGF-I in MD-IGF-I

IGF-I concentrations in (MD-IGF-I_{absolute}) were detectable in all subjects at all times. The MD-IGF-I_{absolute} as well as IGF-I concentrations corrected for calculated IGF-I recovery (MD-IGF-I) are given in Table 1. Basal MD-IGF-I was 0.87 (0.4–1.5) $\mu\text{g/l}$ equal to 0.4 (0.2)% of t-IGF-I determined in arterial serum and in the same concentration range as f-IGF-I. Basal MD-IGF-I was higher in younger individuals ($R = -0.77$, $P = 0.04$).

Basal *in vivo* I-RR was 30 (7)% in Ex-leg and 39 (3)% in Rest-leg, markedly lower than 55 (4)% *in vitro*. This difference resulted in a basal mean IGF-I recovery of 8 (1)% in Ex-leg significantly lower than 11 (1)% in Rest-leg. During exercise mean IGF-I recovery increased to 14 (2)% in the Ex-leg ($P < 0.001$ compared with basal) and returned back to basal levels during the first hour after exercise. Calculated IGF-I recovery did not change significantly over time in the Rest-leg ($P = 0.08$).

Rest-leg MD-IGF-I had already started to decline during exercise with a significant 58% decrease during the first hour after exercise (0.54 (0.4–0.8) $\mu\text{g/l}$, $P = 0.02$) as shown in Fig. 2. Ex-leg MD-IGF-I was unchanged during exercise and declined during the first hour after exercise at the limit of significance

Table 1 Changes in microdialysate (MD) insulin-like growth factor-I (IGF-I) from skeletal muscle of seven healthy women performing one-legged aerobic exercise during 1 h (exercising (Ex) 0–Ex60).

Collection period	MD-IGF-I ($\mu\text{g/l}$) absolute values		MD-IGF-I ($\mu\text{g/l}$) values corrected for IGF-I recovery	
	Ex-leg	Rest-leg	Ex-leg	Rest-leg
(–60–0)	0.07 (0.02)	0.09 (0.03) ^e	0.87 (0.4–1.5)	0.77 (0.4–1.2)
(Ex0–Ex60)	0.12 (0.04) ^{c, e}	0.07 (0.03)	0.92 (0.4–1.2)	0.55 (0.2–0.8)
(Ex60–P60)	0.07 (0.04)	0.06 (0.02)	0.58 (0.4–1.0) ^a ($n=6$)	0.54 (0.4–0.8) ^b ($n=5$)
(P60–P120)	0.06 (0.01)	0.06 (0.02) ^c	0.50 (0.3–0.6) ($n=4$)	0.50 (0.2–0.7) ^b ($n=6$)

The mean (s.d.) MD-IGF-I_{absolute} (absolute values) and median (range) MD-IGF-I (corrected for individual probe changes in IGF-I recovery), before, during, and after 60-min one-legged exercise. ^a $P=0.05$, ^b $P<0.05$, ^c $P<0.01$, ^d $P<0.001$ versus basal (–60–0) values in the same microdialysis probe and leg. ^e $P<0.05$ Ex-leg versus Rest-leg. $n=7$ unless otherwise stated.

($P=0.05$). There was no correlation between the changes in I-RR and MD-IGF-I_{absolute} from basal levels to exercise in the individuals probes.

t-IGF-I in serum

Basal t-IGF-I was significantly lower in the vein than in the artery (Table 2). The basal uptake of t-IGF-I in the four individuals in whom blood flow was determined is shown in Table 2. During or after exercise no significant veno-arterial (v-a) difference in t-IGF-I were observed. Venous t-IGF-I was increased by 11 (9)% at Ex10 and returned to basal levels at Ex60. Arterial t-IGF-I did not change significantly during exercise but it was significantly decreased at P60.

f-IGF-I in serum

There was no significant (v-a) difference in f-IGF-I in the basal state (Table 2). No significant changes in f-IGF-I were detected in the artery during or after exercise. Basal f-IGF-I in the vein was 0.38 (0.18–0.72)% of venous t-IGF-I. At Ex10, there was a minor but significant decrease in venous f-IGF-I. At Ex60, venous f-IGF-I had returned to basal values. At Ex60 and P10, f-IGF-I was higher in the vein than in the artery in all the subjects. Table 2 shows (v-a) differences and the calculated individual net release of f-IGF-I. Among subjects with a calculated net f-IGF-I release over the Ex-leg at Ex60, the highest release was found in those with the highest MD-IGF-I (Ex-leg) during exercise.

IGFBP-1 in serum

The arterial IGFBP-1 (Fig. 3) was increased at Ex60 ($P=0.04$), increased further at P60, and remained significantly elevated at P120 ($P=0.007$). The changes in IGFBP-1 and MD-IGF-I in the Ex-leg and/or Rest-leg did not correlate. However, the relative change in the Rest-leg MD-IGF-I_{absolute} during exercise was inversely correlated with the relative change in arterial IGFBP-1 ($R=-0.8$; $P=0.02$).

IGFBP-3 in serum

Basal total IGFBP-3 was 4625 (474) $\mu\text{g/l}$. There was no significant change in total IGFBP-3 fragmentation with time in the artery or the vein (data not shown). IGFBP-3 WIB detected intact glycosylated IGFBP-3 (doublet at 40–43 kDa) and a major IGFBP-3 proteolytic fragment (at 30 kDa) in both the artery and the vein. The size of the major IGFBP-3 fragment was similar to that found in pregnancy serum.

IL-6 in serum

IL-6 increased during exercise (Table 2), reaching significance in the artery at Ex60 ($P=0.008$), and in the vein at P10 ($P=0.008$). Basal IL-6 was significantly higher in the vein than in the artery ($P=0.007$). The (v-a) difference was significantly increased from basal values at P10 ($P=0.02$) and remained elevated at P60 ($P=0.004$). The net release of IL-6 was calculated in the four subjects where blood flow could be determined (Table 2).

Figure 2 The MD-IGF-I concentrations in the Ex-leg (hatched bars) and Rest-leg (grey bars) before (–60–0), during (0–Ex60), and after exercise (Ex60–P60) and (P60–P120). Boxes represent median with the 5th and 95th percentiles. * = $P<0.05$ versus basal values (–60–0).

Table 2 Total IGF-I (t-IGF-I), free dissociable IGF-I (f-IGF-I) and interleukin-6 (IL-6) in the artery (a) and the vein (v) of the exercising (Ex) leg in seven women performing one-legged aerobic exercise.

Time point		t-IGF-I ($\mu\text{g/l}$)	f-IGF-I ($\mu\text{g/l}$)	IL-6 (ng/l)
0	a, v	230 (42), 202 (38) ^e	0.68 (0.39, 1.19), 0.74 (0.42, 1.27)	4.0 (3.0–6.1), 6.1 (3.6–9.1) ^e
	v-a	–28.0 (19)	0.05 (–0.13, 0.23)	1.0 (0.5–3.1)
	Release/uptake ¹	–18/0.7/–10/–6.0	0.14/0.0/0.0/0.0	0.2/0.2/0.2/0.6
Ex10	a, v	227 (40), 227 (50) ^a	0.58 (0.30, 1.13), 0.66 (0.41, 1.06) ^a	N.D., N.D.
	v-a	–0.4 (11)	–0.06 (–0.47, 0.34)	N.D.
	Release/uptake ¹	10/–10/21/–16	0.37/– ² /0.05/–0.01	N.D.
Ex30	a, v	231 (56), 239 (49) ^c	0.60 (0.33, 1.09), 0.68 (0.40, 1.14)	6.1 (4.1–27.8), 7.9 (4.8–29.3)
	v-a	8.1 (14)	0.05 (–0.10, 0.20)	0.3 (0.0–2.6)
	Release/uptake ¹	N.D.	N.D.	N.D.
Ex60	a, v	228 (51), 215 (43)	0.69 (0.40, 1.22), 0.78 (0.48, 1.27) ^e	6.2 (4.1–27.7) ^b , 8.0 (4.8–29.3) ^f
	v-a	–12.4 (22)	0.12 (0.03, 0.21)	1.6 (0.7–2.3)
	Release/uptake ¹	5.5/–8.1/–117/–20	0.07/– ² /0.25/0.15	1.4, 1.1, 3.3, 2.4
P10	a, v	224 (60), 221 (55)	0.66 (0.41, 1.05), 0.74 (0.44, 1.23) ^f	6.9 (4.1–27.1) ^d , 14.2 (7.9–44.5) ^{c, f}
	v-a	–2.7 (16)	0.10 (0.01, 0.19)	5.2 (3.8–17.3) ^g
	Release/uptake ¹	–16/1.0/4.8/0.5	0.15/–0.03/0.03/0.03	3.4/1.1/0.7/3.9
P60	a, v	197 (55) ^c , 203 (48)	0.54 (0.32, 0.92), 0.51 (0.29, 0.93) ^a	5.7 (3.6–16.4) ^b , 11.0 (8.5–35.7) ^{b, f}
	v-a	5.6 (12)	0.01 (–0.16, 0.13)	5.8 (2.5–19.3) ^h
	Release/uptake ¹	N.D.	N.D.	N.D.

The t-IGF-I is given as the mean (s.d.) and the log-transformed f-IGF-I is transformed back and given as the mean (mean – s.d. and mean + s.d.). IL-6 is given as median (range); n.d., not determined. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$, ^d $P < 0.001$ versus basal (time = 0) values in the same catheter (artery/vein). ^e $P < 0.05$, ^f $P < 0.001$ vein vs. artery. ^g $P < 0.05$, ^h $P < 0.01$ versus basal (v-a) difference.

¹Blood flow was calculated in four subjects. Therefore, fluxes of t-IGF-I ($\mu\text{g/min}$), f-IGF-I ($\mu\text{g/min}$), and IL-6 (ng/min) are given as four individual values. Release is indicated by (+) and uptake as (–). ²Missing value

Glucose in plasma (P-glucose)

Arterial P-glucose decreased from a basal value of 5.4 (0.4) to 4.9 (0.5) mmol/l at Ex30 ($P = 0.0001$) and remained decreased at P120 ($P = 0.0001$). Basal glucose uptake was 0.05 (0.01–0.20) mmol/min ($n = 4$) and increased to 0.18 (0.08–0.31) mmol/min at Ex60 ($P = 0.001$; $n = 4$). The peak mean glucose uptake (P10) was 0.21 (0.17–0.51) mmol/min ($P = 0.09$; $n = 4$). No correlation between glucose uptake and MD-IGF-I in the Ex-leg could be detected at any time point.

Blood flow and pH

The Ex-leg venous pH was decreased from a basal value of 7.36 (7.32–7.37) to 7.28 (7.24–7.29) at Ex10 ($P = 0.001$) and 7.31 (7.29–7.32) at Ex30 ($P = 0.01$), and then returned to basal values at Ex60. Basal Hb was 133 (130–141) g/l and did not change significantly during exercise. At P30, Hb was decreased by 6 (3)% ($P = 0.03$). The mean increase in blood flow in the Ex-leg in the four subjects for whom measurements were complete was almost fourfold (0.45 (0.39–0.58) l/min versus 2.08 (1.7–2.7) l/min at Ex10, $P = 0.007$) and remained significantly elevated during exercise. Blood flow returned to basal values at P10.

Discussion

For the first time, we have assessed interstitial IGF-I concentrations by determining IGF-I concentrations in MD with correction for probe recovery in resting and

exercising human skeletal muscle in women. At rest, interstitial IGF-I in muscle was estimated to be in the same concentration range as f-IGF-I in serum. Rest-leg MD-IGF-I decreased after exercise concomitantly with an increase in circulating IGFBP-1, offering the first direct evidence that IGFBP-1 may alter peripheral tissue levels although the temporal relationship may not be simple. After correction for probe recovery, the increase in Ex-leg MD-IGF-I during exercise was attenuated and did not reach significance. Thereafter, Ex-leg MD-IGF-I decreased with an apparent delay compared with Rest-leg. This may suggest that in Ex-leg, exercise-induced mobilization of

Figure 3 Circulating arterial IGFBP-1 ($\mu\text{g/l}$) before, during, and after 60 min of one-legged exercise ($n = 7$). Values are given as median (25th–75th percentiles). * = $P < 0.05$, ** = $P < 0.01$, **** = $P < 0.0001$ versus basal (time = 0).

local tissue-bound IGF-I stores compensates for the impact of increased circulating IGFBP-1 on local IGF-I.

In this study, basal skeletal muscle MD-IGF-I levels, calculated by the internal reference technique for *in vivo* probe recovery, were in the same range as f-IGF-I in serum. Two other approaches have been reported to assess interstitial IGF-I in resting muscle. When skeletal muscle extracellular fluid was collected with a 3000 kDa probe (39, 40), t-IGF-I was reported to be tenfold higher than MD-IGF-I in the present study. The impact of IGFBPs collected along with IGF-I was not assessed by these authors. In the other approach, MD from a 60 kDa probe, also likely to contain IGFBPs was analyzed using a free IGF-I kit (41). MD-free IGF-I was tenfold higher than f-IGF-I in serum, suggesting the presence of a steep gradient of unbound IGF-I from the muscle to the circulation. Such a gradient would imply that endocrine IGF-I does not contribute to local tissue levels. This conclusion is not in accordance with the marked effects of endocrine IGF-I on glucose metabolism in mice (1) and humans (42). Our findings of MD-IGF-I in the same range as f-IGF-I in serum, support that endocrine IGF-I may have impact on resting skeletal muscle and whole body glucose homeostasis as previously reported (1, 42, 43). We further observed a significant uptake of t-IGF-I over skeletal muscle in the basal state, suggesting that IGF-I is cleared in the resting muscle possibly following stimulation of the IGF1R.

Rest-leg MD-IGF-I decreased, reaching significance after exercise. Circulating IGFBP-1 was already significantly increased at the end of exercise but increased further after exercise. If there is a temporal relationship between circulating IGFBP-1 and local IGF-I bioavailability, it may not be as simple as first assumed. Changes in IGFBP-1 in serum are to a large part determined by hepatic production but changes in IGFBP-1 transport to the tissues may contribute. Consequently, tissue changes in IGFBP-1 may not be completely reflected by the changes in circulating IGFBP-1. The exact mechanism by which circulating IGFBP-1 affects tissue IGF-I levels should be further explored in a less-complex study model.

In Ex-leg, the decrease in MD-IGF-I appeared to be delayed as a result of an increase in MD-IGF-I_{absolute} during exercise in accordance with our previous findings in men (44). In that study, the microdialysis methodology was not optimized and most of the basal levels were below the detection limit. The MD-IGF-I in Ex-leg was elevated during and after exercise. However, MD-IGF-I was not corrected for probe recovery and Rest-leg MD-IGF-I concentrations were not determined. It is known that muscle contraction induces changes in probe recovery (45). In the present study, we attempted to control for probe recovery by calculating IGF-I recovery from the reverse recovery of ¹⁴C inulin using the internal reference method (33, 34). In previous exercise studies, reference molecules with molecular weights similar to (29, 36) or different from (31, 32, 40) the substance of interest have been used.

Although the higher molecular weight of IGF-I compared with inulin resulted in a lower recovery than we expected, this result is not in conflict with the principle of the internal reference method (34). We found a significant difference between basal IGF-I recovery in the resting and Ex-leg. It is possible that this may reflect variability in recovery between probes within the same batch. Considering the limited number of subjects, this may alternatively represent a type 1 error. Further evaluation of the validity of the I-RR is required to resolve this issue.

The lack of correlation between the changes in I-RR and MD-IGF-I_{absolute} from rest to exercise in this study suggests that the increase in probe recovery does not fully account for the increase in MD-IGF-I_{absolute}. When MD-IGF-I_{absolute} was corrected for recovery, the rise in Ex-leg MD-IGF-I was attenuated and did not reach significance. It is likely that I-RR is influenced by the increase in Ex-leg blood flow. If so, corrected MD-IGF-I values may underestimate local bioavailable IGF-I in the muscle resulting from the increased IGF-I flux to the muscle. In addition, the difference in MD-IGF-I response to exercise in Ex-leg versus Rest-leg may be explained by local exercise-induced mobilization of tissue-bound IGF-I stores. This view is supported by the net release of Ex-leg f-IGF-I at the end of exercise, suggesting that the source of IGF-I is the muscle itself. The difference was observed already during the first hour after exercise which favors release of local stores of IGF-I rather than *de novo* synthesis. A local release would also explain that the impact of increased circulating IGFBP-1 on local IGF-I appears to be less pronounced in Ex-leg during the first hour after exercise. A release of IGF-I from local stores may be induced by proteolytic cleavage of IGFBP-3 (46, 47). IGFBP-3 proteolysis is associated with increased IL-6 (16, 17). For the first time, we demonstrate that the net release of IL-6 from skeletal muscle is increased during and after exercise in women and confirms previous reports in men (48). The absence of an increased IGFBP-3 proteolysis in serum does not exclude the possibility that IGFBP proteases are activated locally. The decrease in pH observed in the blood from Ex-leg reflects a decrease in pH in skeletal muscle tissue that theoretically may decrease IGF-I binding to the IGFBPs. Furthermore, an IGF-I protease with a pH optimum around 6.0 is known to cleave IGF-I to des - (1-3) IGF-I resulting in a reduced binding to IGFBPs (49). Further investigation of IGFBP protease activity in muscle interstitial fluid is warranted, but this will require probes with a larger pore size.

In the circulation, changes in IGF-I bioavailability associated with IGFBP-1 has previously been assessed by changes in f-IGF-I (50). The present study is the first to report (v-a) differences of f-IGF-I in exercising human skeletal muscle. In the vein, f-IGF-I was higher at the end of exercise and shortly thereafter, indicating a release of unbound IGF-I into the circulation. This finding supports the hypothesis that local exercise-induced mobilization of

tissue-bound IGF-I stores affects local levels of IGF-I in the exercising muscle. In previous exercise studies, circulating f-IGF-I has been determined in an antecubital vein and has been reported to be increased (51) or unchanged (52). In the present study, the quantity of f-IGF-I released was not sufficient to be detectable in the large pool of t-IGF-I in the circulation.

This study of interstitial IGF-I protein concentrations during and shortly after a single bout of aerobic exercise of moderate duration indicates rapid changes in IGF-I concentrations. Since IGF-I protein synthesis and release induced by changes in IGF-I mRNA are likely to take hours to peak (53), non-transcriptional regulation such as local changes in IGF-I bioavailability is a likely mechanism. The role of muscle interstitial IGF-I during and after aerobic exercise needs to be further explored. Single aerobic exercise bouts and/or training result in increased vascularization, skeletal tissue remodeling (such as fiber type transformation), and a sustained increase in glucose uptake after exercise (54). Changes in interstitial muscle IGF-I may potentially be important for these processes.

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