Exercise and Circulating Insulin-Like Growth Factor I

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Key Words
Exercise · Insulin-like growth factor I · Muscle · Insulin-like growth factor binding protein · Monitoring

Abstract
Determinations of serum concentrations of total insulin-like growth factor I (tIGF-I) are important in the diagnosis, monitoring of treatment and safety evaluation of patients with growth disorders and/or metabolic disease. It is well established that tIGF-I status varies over time. Changes in tIGF-I levels in relation to an acute bout of exercise or repeated bouts, known as training, are likely to contribute to this variation. Serum tIGF-I has also been found to be of predictive value in growth prediction models employed before the start of growth hormone (GH) treatment. Furthermore, IGF-I generation tests have been suggested to be of value in the assessment of the growth response to GH administration in patients suspected of GH deficiency with or without some degree of GH insensitivity. This is discussed elsewhere in this issue. Recent progress in our understanding of growth hormone-dependent and -independent expression of the IGF1 gene in skeletal muscle and the role of sufficient energy intake during training for muscle and liver generation of IGF-I raises important questions regarding their relative contribution to the circulating pool of IGF-I. The present review is focused on circulating levels of tIGF-I in relation to a single bout of exercise or to a period of training. In addition, the expression of IGF-I locally in muscle in response to these stimuli will be discussed.
The rapid increase of tIGF-I in response to exercise suggests that there is a release of IGF-I from tissue stores rather than induction of peptide synthesis. Furthermore, the short half-life suggests that IGF-I is unbound or in a readily available form, i.e. bound in binary complexes with one of the IGF binding proteins (IGFBP-1 through -6). An alternative explanation was suggested by the study of Schwartz et al., demonstrating that serum IGFBP-3 proteolysis was induced concomitantly with the increase in tIGF-I [3]. If IGFBP proteolysis is involved, IGF-I may be released from tissue stores in skeletal muscle or from IGFBP-3-bound stores associated with the endothelium [20]. Previous studies in the rat have demonstrated that large IGF-I stores are readily available to associate with IGFBP-3 administered intravenously [21]. The intensity of exercise and the number of muscle groups engaged may be of importance for the activation of IGFBP-3 proteases [3] (Berg et al., unpublished data). Similarly to the IGF-I response, induction of IGFBP-3 proteolysis occurs within 5–10 min and may [17] or may not [9] be detectable after the exercise bout. We have recently found marked degradation of circulating IGFBP-3 in the absence of serum IGFBP-3 proteolytic activity after repeated extremely strenuous 30-second exercise bouts, suggesting that tissue-associated and not circulating proteases may be involved (Berg et al., unpublished data). Studies demonstrating that IGF-II is increased in response to exercise, to the same relative extent as IGF-I, would also argue for a mechanism by which IGFs are released from tissue stores [1]. In contrast to this view, Wallace et al. demonstrated similar relative changes in serum levels of tIGF-I, IGFBP-3 and acid-labile subunit (ALS) during a 30-min bout of moderate exercise [5]. However, increased IGF-I concomitantly with unchanged IGFBP-3 has also been reported [22].

Redistribution of body water during the initial phase of moderate to strenuous exercise may contribute to the rapid changes in tIGF-I. This haemoconcentration is dependent on the duration, the intensity and the modality of exercise and may result in changes in plasma volume as large as 20–25%. For example, in the study of Schwarz et al., low-intensity exercise below the lactate or anaerobic threshold (LT) resulted in a significant increase in tIGF-I (~8%) in the absence of a significant change in haematocrit, while the more pronounced tIGF-I (~13%) increase during above-LT exercise was accompanied by a significant increase in haematocrit (~14%) [3]. Thus, tIGF-I levels determined at various time-points during exercise and corrected for changes in haematocrit display only modest or no increase in tIGF-I [23, 24]. The possible impact of this haemoconcentration on IGF-I bioavailability in the circulation and, more importantly, in the extracellular space surrounding IGF-I receptors (IGF-IR) in muscle cannot easily be predicted.

It is well established that in the presence of excess high-affinity binding proteins such as the IGFBPs (Kd in the nanomolar to picomolar range), the effects of several-fold dilution/concentration does not have any significant impact on the concentration of unbound IGF-I (for this discussion see Bang et al. [25]). With decreasing affinity of the binding proteins, haemoconcentration and extracellular fluid dilution may have a more significant impact on the equilibrium between bound and unbound IGF-I.

Consequently, in the case of IGFBP proteolysis, resulting in low-affinity IGFBP fragments, redistribution of body water may be of importance. Further complicating the picture, the presence of extracellular matrix and cell surface-bound IGF-I stores may be affected by redistribution of body water. We believe that if the focus is the possible changes in tissue actions of IGF-I during exercise it is not meaningful to correct changes in circulating tIGF-I for changes in haematocrit. In the case that a blood sample is obtained for clinical assessment of growth/metabolism in an individual that has been engaged in physical activity just prior to sampling, the tIGF-I value may be largely affected and, obviously, correction for haematocrit will not be considered.

The intensity of exercise and whether one or more muscle groups are engaged appear to play a role in the tIGF-I response. Only a few studies have addressed this issue in a strictly experimental way. Schwartz et al. demonstrated that high-intensity cycle ergometry resulted in a more pronounced increase in tIGF-I compared with low-intensity exercise [3]. Further studies are required to resolve this question.

Although GH is rapidly increased in the majority of healthy individuals in response to exercise, we reported that tIGF-I (~38%) and tIGF-II (~48%) are increased in GH-deficient individuals in response to exercise [1]. Although several animal studies have demonstrated that IGF-I mRNA expression in skeletal muscle in response to exercise/training is GH-independent, this finding is likely to be related to the release process rather than new synthesis of IGF-I.
Fig. 1. Determinations of serum tIGF-I levels in blood samples collected from the femoral artery supplying, and the femoral vein draining, an exercising leg give information about the net fluxes of IGF-I with time. Although circulating stores of IGF-I are large, it has been possible to demonstrate a rapid net release of IGF-I during short bouts of high-intensity exercise as shown here in four healthy young individuals (Berg and Saltin, unpublished data). We have also reported that extended moderate intensity exercise for 2 h results in a net release of IGF-I, but not until the end of the exercise period [24]. The modality and duration of exercise are some of the factors that must be considered in order to make any general statement about tIGF-I responses to exercise.

Cappon et al. studied the tIGF-I response to a bout of exercise and how it was affected by one of two isocaloric, isovolaemic liquid meals high in either fat or glucose or an isovolaemic, noncaloric placebo provided just prior to exercise [2]. After each of the three different meals a 10% increase in tIGF-I was observed. In contrast, the effect of repeated bouts of exercise (training) on tIGF is likely to be dependent on the composition of the diet as well as on the energy content, as we will discuss later.

**IGF-I Release from Skeletal Muscle during Exercise**

Exercise results in a markedly increased perfusion of the contracting skeletal muscle. As we have discussed, the major source of IGF-I leading to increased serum tIGF-I levels may be the skeletal muscle itself. This is not unlikely, considering that IGF-I is largely expressed in skeletal muscle and that IGF-I stores on IGFBPs in the muscle may be available for release. In fact, skeletal muscle was one source of IGF-I purification in the early 1970s, when the IGF molecules were isolated and characterized for the first time by different laboratories [26].

To further explore this hypothesis, a few studies have been conducted to assess if there is a net release of IGF-I from human skeletal muscle during exercise (fig. 1). By catheterization of both the femoral vein and the femoral artery, we demonstrated that after 2 h of moderate two-legged cycle ergometry in nine healthy young men, tIGF-I was elevated in the vein and there was a significant net release of IGF-I (range of venous concentration minus arterial concentration [VA difference]: 10–28 µg/l) after cessation of exercise [24]. In contrast, no net release was seen during muscle contraction.

Brahm et al., on the other hand, studied 12 men and women during and after 30 min of one-legged knee extension on a modified cycle ergometer with graded increases in workloads [27]. During the last 5 min of maximal work, mean tIGF-I was 198 µg/l, not significantly different from the resting value of 181 µg/l. During that time, the net muscle IGF-I release indicated by a mean VA difference of 16 µg/l was significantly different from that recorded before exercise (~10 µg/l). The leg blood flow was 0.36 l/min at rest and increased to more than 5 l/min during the last 5 min of maximal work. Thus, IGF-I was released at a rate of 60 µg/min (although it is not clear if blood or plasma flow was used for this calculation). If we assume that this release rate is maintained during 5 min
of maximal work, a total of 300 μg IGF-I is released to the bloodstream. In perspective, this is more than 50% of the total circulating pool of IGF-I in a 65-kg person (estimated plasma volume 3 litres; mean serum tIGF-I concentration 181 μg/l). Therefore, it is of interest that the arterial serum tIGF-I concentration did not increase significantly. This may at least partly be explained by an increased bioavailability of circulating IGF-I during the bout of exercise, suggesting that systemic IGF-I effects such as improved brain function associated with recruitment of hippocampal neuroprogenitor cells and improved neuroglia function [28, 29] or improved insulin sensitivity associated with exercise/training may be accounted for.

Again, activation of cell-associated IGFBP-3 proteases such as the urokinase plasminogen activator (UPA)/tissue plasminogen activator (tPA)-plasminogen/plasmin cascade [30] or matrix metalloproteinases (MMPs) [31] would explain both muscle IGF-I release and an increased clearance rate of IGF-I resulting from IGFBP-3 fragmentation. Activation of IGFBP-3 proteases such as cathepsin D [32] may be associated with the marked drop in pH that occurs locally in skeletal muscle, particularly during anaerobic exercise. In addition, the affinity of IGF-I for IGFBPs is markedly decreased at low pH and may by itself increase tIGF, as suggested by the study of Kraemer et al. [33]. Other potential activators of IGFBP proteases may include interleukin-6 (IL-6) [34] and tumour necrosis factor-alpha [35], both of which are released from contracting skeletal muscle. Interestingly, IGF-I itself may be converted to des(1-3)IGF-I by protease activity at low pH, as suggested by Murphy et al. [36]. As IGFBP-3 affinity for des(1-3)IGF-I is 10-fold decreased, such proteolytic cleavage of IGF-I would also result in increased clearance.

The above studies should also be viewed in light of the study by Fernqvist-Forbes et al., in which catheterization of the hepatic vein failed to show any significant IGF-I release from the liver, the major source of circulating IGF-I [37]. VA sampling has been advantageous in studies of substrates largely taken up or metabolites mainly secreted by contracting skeletal muscle. However, due to the large circulating pool of IGF-I, a relatively large quantity of IGF-I has to be released in order to detect a significant VA difference given that most IGF-I assays display a typical intra-assay coefficient of variation of 5–8%. These conditions may partly explain the large individual variations in the VA differences in the study of Brahm et al. [27] and in a study that we have recently completed (Berg et al., unpublished data). In addition, an alternative methodological approach is required to disclose the actual changes in local muscle tissue concentrations of IGF-I during exercise.

A New Approach in the Assessment of Interstitial IGF-I Concentrations in Skeletal Muscle during Exercise

We believe that exercise-induced changes in circulating tIGF-I are of limited help in understanding the physiological role of responses mediated by IGF-I and IGF-IR in resting or contracting skeletal muscle. IGF-I stimulates glucose transport in skeletal muscle as potently as insulin and is involved in skeletal muscle remodelling. In addition, IGF-I deficiency results in impaired insulin actions specifically in skeletal muscle [38]. Studies of arteriovenous differences over an exercising leg suggest that a net release of IGF-I takes place during or immediately after exercise. As we have mentioned, release of IGF-I from tissue stores on IGFBPs in the extracellular fluid or associated with extracellular matrix or cell surfaces may significantly contribute to the fast release of IGF-I (fig. 2). This implies that the local tissue changes in IGF-I bioavailability may far exceed changes in tIGF-I detected in the venous outflow from the muscle. Accordingly, determinations of serum free dissociable IGF-I will hardly be of further help in the assessment of tissue IGF-I bioavailability. What would then be an appropriate methodological approach to assess IGF-I bioavailability in skeletal muscle tissues in vivo?

Microdialysis has been used to assess skeletal muscle concentrations of insulin, IL-6 and other peptide hormones and we are currently applying this technique to assess changes in interstitial IGF-I concentrations during exercise. In a preliminary study, we have found that IGF-I concentrations in skeletal muscle microdialysis perfusate collected during 45 min of moderate work on a cycle ergometer are increased 5-fold compared with resting levels (Berg, unpublished data). After optimizing this technique, we are currently performing further studies to assess the physiological role of IGF-I in human muscle in relation to physical activity.

Effects of Exercise and Training on Skeletal Muscle IGF-I Production

In 1996, Yang et al. [39] reported that, in rabbits, the IGF-IEb mRNA splice variant first described by Chew et al. [40], encoding parts of exon 5 on the rat IGF1 gene, was expressed in a stretch model inducing rapid muscle hypertrophy. In addition to the common liver mRNA splice variant, IGF-IEa, expressed in the resting muscle, the IGF-IEb splice variant was exclusively induced by
Fig. 2. Contraction in exercising skeletal muscle will result in a rapid release of stores of local IGF-I bound to IGFBPs in the extracellular fluid or associated with cell surfaces or extracellular matrix (ECM). Lowering of pH as a result of anaerobic metabolism lowers the binding affinity of IGF-I for IGFBPs and may – in addition to other tissue activators – induce IGFBP proteases. Stores of IGF-I on IGFBPs associated with the endothelium may also be affected. In the circulation total IGF-I increases, with peak levels 5–10 min after the start of moderate to strenuous exercise. IGF-I is rapidly cleared from the blood, suggesting that IGF-I is present in bioavailable binary complexes or in ternary complexes with fragmented IGFBP-3/IGFBP-5. Exercise-induced IGF-I mRNA expression may preferentially lead to a specific mRNA species, IGF-IE or mechano growth factor (MGF). In contrast, GH-dependent expression of IGF-I mRNA in skeletal muscle leads to IGF-I Ea production. Skeletal muscle-derived IGF-I contributes to total circulating IGF-I levels, although liver-derived IGF-I, which is energy- and protein-dependent, is the major determinant of the changes in serum tIGF-I in response to training.

stretch. The IGF-IEb mRNA species was called mechano growth factor (MGF), which is unfortunate, as it is predicted to result in two peptides, IGF-I and a predicted E-peptide. Due to the introduction of an additional 52-bp insert originating from exon 5, a reading frame shift predicts a unique amino acid sequence for this E-peptide.

In humans, the equivalent to IGF-IEb in the rat is the splice variant IGF-IEc, which differs from the common human liver IGF-I Ea variant by a 49-bp insert, similarly resulting in a reading frame shift and a predicted unique E-peptide sequence. The human counterpart induced by exercise was detected by rtPCR in the study of Yang et al. [39]. So far, the predicted E-peptides have not been identified in rat or human tissues.

Rat and human myotubes are incapable of undergoing mitosis. However, adjacent mesenchymal progenitor cells may in response to IGF-I undergo mitosis and subsequently differentiate into myotubes. Interestingly, a 24-amino acid peptide representing a small part of the predicted human IGF-IEc-derived E-peptide was synthesized and demonstrated to induce proliferation in the murine C2C12 primary myoblast-like cell line [41]. In contrast to mature IGF-I that also stimulated proliferation, this peptide did not stimulate differentiation into myotubes.

In humans, Hameed et al. recently demonstrated that IGF-IEc but not IGF-I Ea mRNA levels were increased in response to 10 sets of repetitive knee extensor exercise [42]. Although the responses showed large individual variations, it is interesting that a significant increase in IGF-IEc was observed in the group of eight young men (~30 years) but not in seven elderly men (~75 years). In support of this age-related response, Owino et al. demonstrated that in a rat muscle overload model, up-regulation of IGF-IEb mRNA expression was age-related [43].

The initiation of transcription at promoter 2 of the IGF1 gene is GH-dependent and is thought to result predominantly in the IGF-I Ea splice variant. In contrast,
promoter I appears to be less responsive to GH and, at least hypothetically, may be initiated by exercise, resulting in the IGF-IEc transcript. However, Hameed et al. found that, in the resting condition, the IGF-IIEa mRNA species was expressed in 1,000-fold higher quantities in muscle compared with IGF-IIEc [42]. Thus, the change in total IGF-I mRNA in that study, comprising no change in IGF-IIEa and a limited increase in the 1,000-fold lower level of IGF-IIEc, appears insignificant.

This result is in contrast to other human studies in which IGF-I mRNA is increased by exercise. For example, Bamman et al. demonstrated that a bout of repetitive eccentric exercise resulted in a more pronounced and significant increase in IGF-I mRNA (most likely IGF-IIEa) of 64%, compared with concentric exercise [44]. On the other hand, in a recent study of young men performing four sets of 6–12 repetitions on a leg press and knee extensor machine, the investigators found that IGF-IIEa was down-regulated by 44% after 1 and 6 h of recovery while IGF-IIEc mRNA levels were unchanged [45]. This study did not allow comparisons between the relative quantities of IGF-IIEa and IGF-IIEc transcripts. Increased IGF-I peptide in human muscle in response to exercise has been reported [46].

Previous studies of IGF-I expression in rodent skeletal muscle in response to exercise have consistently demonstrated several-fold increases in IGF-I mRNA. In rats, functional overload models in which, for example, hypertrophy is induced in the soleus and plantaris by surgical excision of the synergistic gastrocnemius muscle have demonstrated two- to ten-fold increases in IGF-I mRNA with apparently larger responses after prolonged overload [47, 48]. In these studies, hypophysectomized rats display relatively more pronounced responses, demonstrating that the exercise-induced IGF-I expression is GH independent (see also Frost and Lang [49]). In the study of Adams and Haddad, concomitant increases in muscle extractable IGF-I peptide were seen [48]. In the study of Czerwinski et al., IGF-I mRNA increases in response to stretch in rats were not blocked by fasting, which is known to uncouple GH induction of hepatic IGF-I [50].

At this point, the physiological role of IGF-IIEc expression in response to exercise in humans and its relative contribution to total skeletal muscle IGF-I production and circulating tIGF-I appears to be limited. On the other hand, future isolation and characterization of the predicted E-peptide resulting from translation of the IGF-IIEc splice variant and determination of its mitogenic potency may provide insights into its physiological significance in skeletal muscle regeneration and growth.

Effects of Training on tIGF-I Concentrations

Despite the prevailing evidence that a single bout of exercise as well as training increases muscle IGF-I production, studies investigating tIGF-I concentrations in relation to fitness or change in tIGF-I concentrations after a period of training are apparently less consistent. Obviously, the fact that circulating levels of IGF-I are primarily liver derived may offer an important explanation for that. Liver IGF-I expression is largely dependent on nutritional factors and GH status, which is influenced by age, pubertal stage, body composition, body stature, concurrent disease, etc. Insulin status is important for hepatic GH receptor function, hence factors such as dietary energy intake, dietary composition, and body composition influence IGF-I production at least partly by modulating hepatic GH receptor function. In contrast, we have discussed that IGF-I expression in muscle appears to be less dependent on most of these factors, with the possible exception of age. Therefore, exercise may induce IGF-I production in muscle, but if the increased energy and protein demands associated with training are not met, exercise may be expected to decrease liver IGF-I production [51].

In accordance with our early study demonstrating exercise-induced increases in tIGF-I concentrations [1], a positive correlation between tIGF-I and fitness measured as VO$_{2\text{max}}$ in young and elderly men and women [52–54] or quadriceps maximal muscle power (Wmax) in elderly men and women [55] has been reported. A negative correlation between tIGF-I and fitness was later suggested in a study where physically active elderly were compared with sedentary elderly, but fitness and energy expenditure were not assessed [56]. More recently, Eliakim et al. have demonstrated a positive correlation between tIGF-I and VO$_{2\text{max}}$ in pre-pubertal girls [57], while this apparent relationship did not reach significance in adolescent girls [58]. In adolescent boys, Eliakim et al. failed to find any such relationship [59]. All of these studies are cross-sectional in nature and the power of such studies is compromised by the large intra-individual variation of tIGF concentrations, particularly in adolescence. Consequently, longitudinal studies investigating changes in tIGF-I after a period of training should be more informative.

Endurance training has generally been found to result in decreased tIGF-I levels [58, 60–63], although unchanged [57, 64, 65] or slightly increased levels [66] have been reported. Whether these changes can be explained by an energy/protein deficiency associated with endurance training has not been elucidated. Poehlman et al. reported
a 19% increase in men and unchanged tIGF-I levels in women after an 8-week endurance training programme
[67]. Eliakim et al. demonstrated that adolescent girls had decreased tIGF-I (~14%) after endurance training [58]
while pre-pubertal girls had unchanged tIGF-I levels [57]. Whether a higher physical activity level in the pre-puber-
tal girls prior to the training period, which may be expected, explains this difference has not been addressed.
Also, Scheett et al. found a decrease in tIGF-I levels (~13%) in pre-pubertal or early pubertal boys after 1.5 h of aerobic training daily for 5 weeks [60]. Recently, Nemet et al. were able to demonstrate that young men performing 3 h of aerobic high- and low-intensity training per day for 7 days demonstrated decreased tIGF-I levels if fed a low-energy diet, while this decrease could be prevented by a high-energy diet resulting in a ~15% increase in tIGF-I levels [68]. In accordance, elite swimmers, who are most likely supervised to maintain positive energy-
balance, increased their tIGF-I levels as much as ~70% by intensified training [69]. Extremely energy-demanding training programmes, such as intense mixed military training for 2–4 h daily for 12 weeks resulted in decreased tIGF-I in young men, and this was more pronounced in less fit individuals [63].

Resistance training, on the other hand, may generally require less increase in energy intake and thus be more prone to result in increases in tIGF-I levels. Borst found that resistance training 3 days a week for 25 weeks in middle-aged men and women resulted in increases in tIGF-I levels (~20%) [70]. In the study by Parkhouse et al., resistance training in elderly women with low bone mineral density resulted in a ~70% increase in tIGF-I [71], while no change in tIGF-I was found by Bermon et al. after 8 weeks of resistance training in elderly participants [72]. This is interesting, given that exercise-induced increases in skeletal muscle IGF-I mRNA have been found to be attenuated with age, as we have previously discussed.

In the future, more strict measurements of the changes in energy demands, energy intake, nitrogen balance etc.
should enable us to determine how training under energy-neutral conditions affects tIGF-I. Moreover, it would be of importance to design studies to determine whether skeletal muscle is an important source of circulating IGF-I.

**Conclusions**

IGF-I is produced by skeletal muscle and has an important physiological role in the fetal and post-natal growth and development of this tissue [73]. IGF-I stimu-
lates the division of mesenchymal stem cells, so-called satellite cells, in skeletal muscle, and also stimulates differ-
entiation of these cells into myoblasts that subsequently fuse and form myotubes. Skeletal muscle production of
IGF-I is partly GH-dependent in the resting state. In con-
trast, contraction-induced increases in muscle IGF-I mRNA are GH-independent and so is the acute release of
tIGF-I in response to exercise. Circulating IGF-I as well
as locally produced IGF-I has important metabolic ef-
fects. IGF-I stimulates glucose uptake via the IGF-IR and
IGF-I/insulin hybrid receptors and is at least as potent as
insulin at the receptor level. In addition, IGF-I supports
insulin receptor function in skeletal muscle partly by feed-
back inhibition of GH (which negatively affects insulin
receptor function) and, most probably, partly by direct
IGF-IR-mediated events. Consequently, IGF-I has an im-
portant impact on glucose uptake in resting andcontracting
muscle, although exercise initiates glucose uptake via
intracellular signalling molecules that at least initially differ from those activated by insulin and IGF-I. The present
review has focused on exercise- and training-induced
changes in circulating tIGF-I that could influence the
interpretation of tIGF-I measurements used in the diag-
osis and monitoring of growth disorders. Although skele-
tal muscle production of IGF-I is significant, further stud-
ies are needed to determine its relative contribution to the
circulating pool of IGF, and its relation to gender, age,
pubertal stage, etc. A bout of exercise can cause rapid and
marked increases in tIGF-I, independently of source. In
addition, training may result in increased tIGF-I if the
higher energy demand is sufficiently met, or result in
decreased tIGF-I if the negative energy balance results in uncoupling of GH-induced hepatic IGF-I production.
Therefore, participation in acute exercise and the current training state should be considered when tIGF-I measure-
ments are to be interpreted.
References


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