

Exercise and the Growth Hormone–Insulin-Like Growth Factor Axis

JAN FRYSTYK

The Medical Research Laboratories, Clinical Institute of Medicine, and Medical Department M (Diabetes and Endocrinology), Aarhus University Hospital, Aarhus, DENMARK

ABSTRACT

FRYSTYK, J. Exercise and the Growth Hormone–Insulin-Like Growth Factor Axis. *Med. Sci. Sports Exerc.*, Vol. 42, No. 1, pp. 58–66, 2010. Exercise is a robust physiological stimulator of the pituitary secretion of growth hormone (GH), and within approximately 15 min after the onset of exercise, plasma GH starts to increase. GH and its primary downstream mediator, insulin-like growth factor I (IGF-I), play a critical role in formation, maintenance, and regeneration of skeletal muscles. Consequently, it seems logical to link the exercise-induced stimulation of GH with the hypertrophy observed in exercising muscles. GH stimulates circulating (endocrine) as well as locally produced (peripheral) IGF-I, which acts through autocrine/paracrine mechanisms. However, it remains to be clarified whether skeletal muscle hypertrophy after exercise is stimulated primarily by endocrine or paracrine/autocrine IGF-I. Early cross-sectional studies have observed positive correlations between circulating IGF-I levels and GH secretion, respectively, and indices of fitness. However, longitudinal exercise studies have shown that it is possible to increase muscle strength, performance, and $\dot{V}O_{2\max}$ without concomitant and robust changes in circulating IGF-I, indicating that the effect of exercise on skeletal muscles is mediated via paracrine/autocrine IGF-I rather than endocrine IGF-I. So far, most exercise studies have investigated the concentration of immunoreactive IGF-I in serum or plasma, obtained after extraction of the IGF-binding proteins (i.e., total IGF-I). However, several of the newer exercise studies have included measurement of free IGF-I as well as bioactive IGF-I. The aim of this review was to discuss whether measurement of free and/or bioactive IGF-I have increased our knowledge on the processes that link exercise, muscle hypertrophy, and GH/IGF-I axis. Thus, the current review will discuss (i) the different IGF-I assay methodologies and (ii) the current literature on free, bioactive, and immunoreactive (total) IGF-I in exercising subjects. **Key Words:** FREE IGF-I, BIOACTIVE IGF-I, IGF-BINDING PROTEINS, ENDOCRINE IGF-I, PARACRINE/AUTOCRINE IGF-I

AN OVERVIEW OF THE GROWTH HORMONE–INSULIN-LIKE GROWTH FACTOR SYSTEM

Insulin-like growth factor I (IGF-I) possesses widespread anabolic and insulin-sensitizing effects, mediated through endocrine (i.e., circulating) as well as paracrine/autocrine (i.e., locally produced) mechanisms. IGF-I serves as ligand for the ubiquitously expressed tyrosine kinase IGF-I receptor (IGF-IR), which upon ligand occupancy by protein phosphorylation activates intracellular signaling cascades, which favor proliferation via the mitogen-activated protein kinase pathway and insulin-like effects via the phosphati-

dylinositol 3-kinase pathway. Furthermore, IGF-IR activation inhibits apoptosis, hereby linking the IGF-I with the development of neoplasias (13,42).

Growth hormone (GH) is the principal regulator of the hepatic synthesis of IGF-I, IGF-binding protein 3 (IGFBP-3), the major IGF carrier in plasma, and the acid labile subunit (ALS). ALS binds preformed complexes of IGF-I and IGFBP-3 and is responsible for the long turnover of circulating IGF-I (34). However, the hepatic responsiveness to GH is sensitive to nutritional changes (13). During fasting, the liver develops resistance to GH and its ability to synthesize IGF-I becomes markedly reduced (8,13), whereas in obesity, the GH-induced IGF-I generation is increased (44). Nutritionally regulated changes in the hepatic responsiveness to GH appear to be controlled by the portal supply of insulin, which increases GH receptor availability on the hepatocytes (43). The important role of portal insulin supplies is stressed by findings showing that patients with type 1 diabetes, who are C-peptide positive, have higher levels of free and total IGF-I than C-peptide-negative patients with a similar metabolic control (30).

Apart from controlling the hepatic responsiveness to GH, and in this way indirectly the synthesis of IGF-I, insulin may directly influence IGF-I bioactivity by controlling the hepatic synthesis of the insulin-suppressible and IGF-I

Address for correspondence: Jan Frystyk, M.D., Ph.D., D.M.Sc., The Medical Research Laboratories, Clinical Institute of Medicine, Aarhus University Hospital, Nørrebrogade 44, DK-8000 Aarhus C, Denmark; E-mail: jan@frystyk.dk.

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inhibitory binding protein, IGFBP-1. Insulin promptly inhibits the hepatic synthesis of IGFBP-1 at the transcriptional level, resulting in detectable reductions in circulating IGFBP-1 levels with 60 min after insulin exposure (6). Thus, IGFBP-1 levels are affected primarily by exercise of long duration and during the recovery after short-lasting exercise (see next section).

MEASUREMENTS OF IGF-I

Since the introduction of the first specific immunoassay for IGF-I more than 30 yr ago (25), quantification of immunoreactive IGF-I levels in plasma or serum has constituted the backbone of clinical IGF research. However, as the IGFBP influence the antibody recognition of IGF-I, all immunoassays require dissociation of IGFBP/IGF-I complexes followed by removal or “neutralization” of the IGFBPs before assay. As a result, immunoreactive IGF-I (~total IGF-I) represents an integrated sum of various IGF-I/IGFBP complexes, which differ with respect to plasma half-life, tissue accessibility, and possible bioactivity (21,22,51). The latter idea was supported by recent experimental studies suggesting that it is the composition of IGFBP/IGF-I complexes and their tissue accessibility rather than the absolute serum levels of IGF-I that determine the endogenous IGF-I bioactivity (74). Whether these experimental findings can be extrapolated to humans remains to be investigated, and it should be acknowledged that quantification of immunoreactive (total) IGF-I levels has yielded important and biologically meaningful information on the IGF system in many clinical conditions.

Assays for free IGF-I were introduced more than a decade ago (23,24,65). Two different approaches have been used. In our laboratory, we separate free from bound IGF-I (or IGF-II) using ultrafiltration by centrifugation, performed at 37°C, followed by immunoassay of ultrafiltered free IGF-I (24). The other methodology is based on a sandwich assay, which uses a solid phase antibody specific for free IGF-I, hereby allowing serum to be incubated directly without prior processing. However, although the capture antibody is specific for free IGF-I, it is able to extract IGFBP-complexed IGF-I during assay incubation, and consequently the sandwich technique is considered to measure truly free plus “readily dissociable” IGF-I. Accordingly, levels as determined by the sandwich assay are higher than those after ultrafiltration (21–23).

The ability of IGF-I to interact with its cell-membrane receptor depends on several aspects, including the free ligand levels, the ligand occupancy (or saturation) of the IGFBP, the ability of the IGFBP to dissociate IGF-I in the neighborhood of the IGF-IR (which depends on the proportion of IGFBP relative to the cell-surface density of the IGF-IR), and the presence of IGFBP proteases. Furthermore, IGF-II-mediated activation of the IGF-IR should be remembered. However, none of the immunoassays (whether it is total or free IGF-I) take the ligand competition between IGFBP and IGF-IR, the presence of IGFBP proteases, or the contribution of IGF-II into account. Therefore, we established a specific IGF-I bioassay based on the kinase receptor activation (KIRA) principle originally described by Sadick (59). In the KIRA assay, cells transfected with the human IGF-IR gene are stimulated with either serum or IGF-I standards for 15 min at 37°C. Then, the samples are aspirated, the transfected

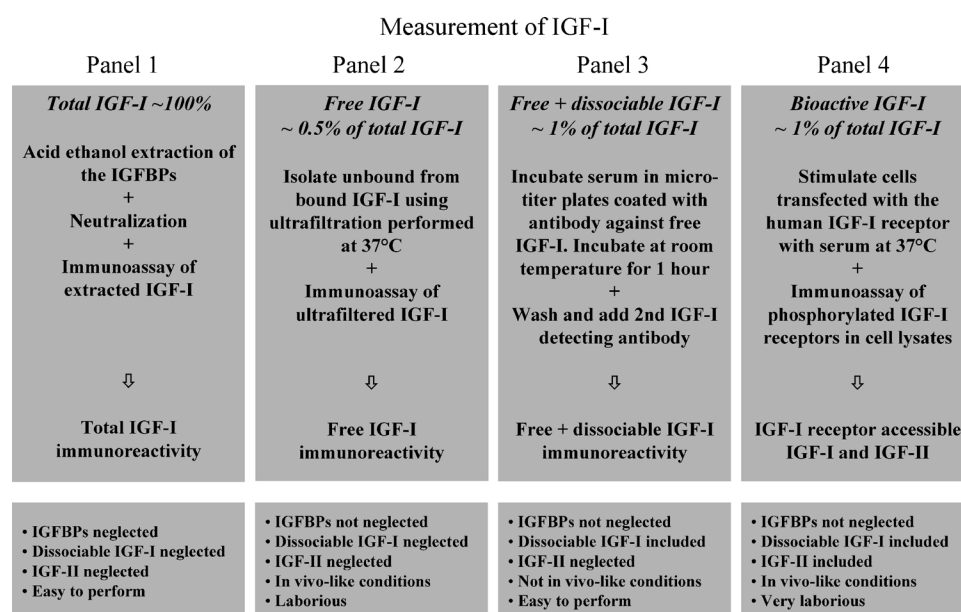


FIGURE 1—Four different methodologies used to estimate the endogenous bioactivity of IGF-I *in vivo*: total (extractable) IGF-I (panel 1); free IGF-I determined by ultrafiltration (panel 2); free IGF-I determined by enzyme-linked immunosorbent assay (ELISA; panel 3); and finally bioactive IGF-I determined by a cell-based bioassay (panel 4). Several studies have included a comparison of the four methods (see references [7,8,21,23]).

cells lysed, and the crude cell lysates transferred to a sandwich assay specific for the phosphorylated IGF-IR. Thus, the IGF-I KIRA assay enables determination of the ability of serum to phosphorylate (i.e., activate) the IGF-IR *in vitro* during conditions approaching those *in vivo* (~bioactive IGF-I) (9). The different IGF-I assay technologies are summarized in Figure 1. For further information on IGF-I assays and their pros and cons, refer to references (9,12,21–24).

It is currently debated whether the actions of IGF-I are maintained primarily by endocrine (i.e., liver-derived/circulating) or locally produced (autocrine/paracrine) IGF-I. Studies in liver IGF-I knockout mice and clinical case reports indicate that peripherally produced IGF-I is the main determinant of somatic growth, whereas the liver is responsible for circulating IGF-I levels, which by negative feedback controls pituitary GH secretion (18,21,41). On the basis of these considerations, there has been an increasing interest in measuring tissue-specific IGF-I levels, and microdialysis appears to provide a suitable methodology. However, the microdialysis technology needs to be refined; the recovery remains problematically low, and the perfusate only yields a limited volume for assay. At the time of writing, there are three microdialysis reports on tissue levels of IGF-I in skeletal muscle/connective tissue (3,17,50); data are summarized in Table 1. The author expects the microdialysis technology to be optimized and increasingly used in the years to come.

THE EFFECT OF EXERCISE ON GH

All types of exercise potently stimulate the secretion of GH, and within 10 to 20 min after the onset plasma GH levels are markedly increased. This physiological response was originally described in 1963 (58), and since then, hundreds of investigations have been published, describing the impact of different types of exercise (endurance and

resistance, sprint and marathon running, single and repetitive bouts of exercise, etc.) on the GH secretion in different study populations (young and old, trained and untrained, lean and obese, GH sufficient and GH deficient, etc.). Nevertheless, the precise mechanisms by which exercise elicits an increased secretion of GH remain to be clarified. The secretion of GH appears to be controlled by numerous hypothalamic hormones, neurotransmitters, and circulating factors, among others IGF-I. However, during exercise, circulating IGF-I is virtually unchanged (see next section), indicating that there are no alterations in the negative feedback regulation of the somatotrophs by circulating IGF-I. Consequently, the exercise-induced stimulus for GH appears to be primarily of central origin, probably involving GH-releasing hormone and somatostatin (27). It is beyond the scope of this review to cover the relationship between exercise and GH secretion; this topic has already been extensively reviewed by others (26,27,33,62,73). Instead, previous findings are briefly summarized in Table 2. As illustrated, the GH response to exercise is affected by numerous factors that are often physiologically linked, and consequently it may be difficult to segregate the contribution of the individual factors (for instance age, obesity, and $\dot{V}O_{2\max}$).

THE EFFECT OF EXERCISE ON CIRCULATING IGF-I AND IGFBP

IGF-I is the primary downstream mediator of GH actions, and circulating IGF-I plays an important role in the feedback regulation of GH secretion (21). Consequently, it was obvious to investigate the association between circulating IGF-I and GH during exercise. However, in contrast to the robust stimulation of GH, the acute IGF-I response to exercise appeared less predictable: quantification of immunoreactive (total) IGF-I levels in serum or plasma has yielded inconsistent results, with levels being reported to

TABLE 1. Extravascular concentrations of IGF-I obtained by microdialysis in relation to exercise.

Desvigne et al. (17)	Berg et al. (3)	Olesen et al. (50)
Study design and methods <ul style="list-style-type: none"> Resting vastus lateralis 60-kDa cutoff membrane Seven resting healthy males • Free IGF-I by ELISA <ul style="list-style-type: none"> Flow rate: $3\text{--}6\ \mu\text{L}\cdot\text{min}^{-1}$ Recovery: 1–7% Results <ul style="list-style-type: none"> Plasma-free IGF-I $\sim 0.4\ \mu\text{g}\cdot\text{L}^{-1}$ Interstitial IGF-I $\sim 6.8\ \mu\text{g}\cdot\text{L}^{-1}$ i.e., $17\times$ higher levels of free IGF-I in muscle 	Study design and methods <ul style="list-style-type: none"> Resting and working vastus lateralis 20-kDa cutoff membrane Seven healthy women investigated by comparing circulating and interstitial levels of free IGF-I in the exercising (1 h) versus resting leg • Free IGF-I by ELISA <ul style="list-style-type: none"> Flow rate: $2\ \mu\text{L}\cdot\text{min}^{-1}$ Recovery: <15% Results <ul style="list-style-type: none"> Plasma-free IGF-I $\sim 0.4\text{--}1.2\ \mu\text{g}\cdot\text{L}^{-1}$ Interstitial IGF-I $\sim 0.4\text{--}1.2\ \mu\text{g}\cdot\text{L}^{-1}$ i.e., similar levels of free IGF-I in muscle and plasma Interstitial IGF-I decreased in the resting leg but remained constant in the exercising leg, suggesting an exercise-induced mobilization of IGF-I 	Study design and methods <ul style="list-style-type: none"> Achilles peritendinous area 3000-kDa cutoff membrane Six male marathon runners before and after 36 km of treadmill running compared with six trained, resting males • Total IGF-I after acid-ethanol extraction <ul style="list-style-type: none"> Flow rate: $2\ \mu\text{L}\cdot\text{min}^{-1}$ Results <ul style="list-style-type: none"> Serum total IGF-I lower in marathon runners: 165 ± 11 vs $241 \pm 12\ \mu\text{g}\cdot\text{L}^{-1}$ Microdialysis IGF-I higher in marathon runners: 8.3 ± 1.0 vs $1.6 \pm 0.3\ \mu\text{g}\cdot\text{L}^{-1}$ No changes in IGF-I as a result of exercise Serum and interstitial IGFBP-1 increased approximately threefold immediately after running

The cutoff value is usually defined as the size of a neutral, globular protein which is retained by at least 90%. ELISA, a commercially available enzyme-linked immunosorbent assay from DSL, Webster, TX. This assay has been evaluated previously (23).

TABLE 2. Factors that influence the physiological response of GH to exercise in humans.

	Comments
<p>The acute GH response is stimulated by</p> <ul style="list-style-type: none"> • Increased core temperature 	<p>The increase in tympanic temperature and serum GH is almost parallel during exercise. The GH response to exercise is markedly blunted in cold rooms (11).</p>
<ul style="list-style-type: none"> • Fitness • Exercise intensity • Exercise duration 	<p>The GH response to exercise increases in a curvilinear fashion with increasing $\dot{V}O_{2\max}$ (31). There is an individual relationship between the release of GH during and after exercise and the exercise intensity (54). The GH response is positively correlated to the duration of exercise. However, irrespective of the duration (from <1 min and up to 120 min), plasma GH is normalized within 2 h after exercise (54,63,72).</p>
<ul style="list-style-type: none"> • Repetitive bouts of exercise 	<p>The GH response to exercise is augmented with repeated bouts of exercise (37). However, when performing two bouts of exercise, the GH response to the second bout of exercise appears to be dependent on recovery time. Shortening of the recovery time between two bouts of exercise may decrease the second GH response as compared with the first (37,62,63).</p>
<p>The acute GH response is inhibited by</p> <ul style="list-style-type: none"> • Age 	<p>The GH response declines with increasing age (28). This may be an effect of age (32) or of accumulation of adipose tissue with age (67).</p>
<ul style="list-style-type: none"> • Adiposity 	<p>Obesity <i>per se</i> inhibits spontaneous, stimulated, and exercise-induced GH secretion. Weight loss restores GH secretion (36,56).</p>
<p>The acute GH response is dependent on</p> <ul style="list-style-type: none"> • Gender 	<p>The relationship between GH secretion and exercise intensity is steeper in women than that in men (55). By contrast, the relationship between GH secretion and exercise duration is steeper in men than women (72).</p>
<p>The acute GH response in relation to</p> <ul style="list-style-type: none"> • Chronic exercise 	<p>After periods of long-term training, the GH response to acute exercise has been variously reported to decrease, increase, or remain unchanged. The variable findings are most likely explained by different training protocols and by different study groups (64,73).</p>
<p>Diurnal GH secretion may depend on</p> <ul style="list-style-type: none"> • Acute exercise 	<p>Single bouts as well as repetitive bouts of exercise may increase 24-h GH secretion when integrated GH secretion is estimated by frequent (10 min) sampling. The increase in GH secretion appears to be restricted to the daytime (37,71,73).</p>
<ul style="list-style-type: none"> • Chronic exercise 	<p>After 1 yr of endurance training (running), it was possible to increase 24-h GH secretion approximately twofold, provided that some of the training was above the lactate threshold (70). Other less intensive training programs have yielded no effect (73).</p>

Modified from Table 4 of reference (26). Due to the amount of literature on GH, it has been necessary to limit the number of references to one or two within each category. For more extensive information, several excellent reviews are available in references (26,27,33,62,73).

decline (39), to increase (2,16,60), or to remain unchanged (35,49,63) after the onset of exercise.

In addition, studies of the chronic effects of exercise on the circulating IGF-I have yielded inconsistent results. Early cross-sectional studies reported positive associations between $\dot{V}O_{2\max}$ and GH secretion (19,66) and immunoreactive IGF-I levels (19,52), respectively, suggesting that an improvement in fitness would result in a higher serum IGF-I level. However, this hypothesis has only been supported by some longitudinal studies (40,53); by contrast, other studies have observed reductions in immunoreactive IGF-I levels after several weeks of exercise despite an improved physical performance (muscle strength and/or $\dot{V}O_{2\max}$) (19,20,48).

The IGFBP are important modulators of IGF-I actions *in vivo* (34), and short-term as well as long-term exercise may alter the IGFBP. This is particularly true for IGFBP-1, which consistently increases severalfold during prolonged exercise (10,39) as well as in the recovery period after short-term exercise (69). It is well known that the IGFBP may alter plasma levels of free IGF-I without affecting total IGF-I (21), and accordingly many of the newer exercise studies have included measurements of IGFBP as well as free IGF-I. Proteolysis of IGFBP-3 may also affect levels of free IGF-I (61). However, exercise does not consistently lead to an increased IGFBP-3 proteolytic activity: both increased (46,60) and unchanged (15,35) IGFBP-3 proteolytic activities have been reported in association with exercise. Interestingly, one study found IGFBP-3 proteolysis to depend on the level of fitness, as it was increased in untrained but not in trained subjects (57).

At the time of writing, more than 10 studies have investigated the concomitant changes in “free IGF-I” (i.e., ul-

trafiltered free IGF-I, ELISA-free IGF-I, or bioactive IGF-I), total IGF-I, and IGFBP. These studies are summarized in Table 3A (acute studies) and Table 3B (long-term studies).

The acute studies ($n = 8$; Table 3A) cover exercise programs from 30 s to 2 h, and obviously the different training regimens make a direct comparison difficult. However, most studies find that “free IGF-I” (whether measured by ultrafiltration, direct ELISA, or bioassay) remains unchanged during exercise but may decrease during recovery. A notable exception is the study by Bermon et al. (4), who found significant increases in free IGF-I up to 6 h after exercise. As this study contained the oldest group of participants (67–80 yr), it can be speculated that the observed increment in free IGF-I is age specific and that it cannot be extrapolated to younger individuals.

In the acute studies, total IGF-I remains either unchanged or increases after exercise, and the same appears to be true for IGFBP-3. The author believes that the reported increases in total IGF-I observed in some studies are primarily explained by plasma volume changes induced by exercise, which is known to induce rapid changes in plasma volume related to exercise intensity (15). However, in the study by Wallace et al. (69), increases in total IGF-I, IGFBP-3, and ALS occurred in the absence of changes in hematocrit, and based on this observation, the authors suggested that ternary-complexed IGF-I was able to leave and to enter the circulation in relation to exercise. This “dynamic theory” is attractive but needs confirmation.

The long-term studies (Table 3B) cover exercise programs lasting from 3 wk to 6 months. In the four studies carried out in nonprofessionals, free IGF-I generally changed in parallel with total IGF-I, indicating that inclusion of “free IGF-I” does not add novel information. Furthermore,

TABLE 3. Summary of published exercise studies of free or bioactive IGF-I.

Reference	Study Cohort	Exercise Protocol	Changes in Free or Bioactive IGF-I	Changes in Immunoactive (Total) IGF-I	IGFBP Changes	Comments
A. Acute studies						
Study 1, Stokes et al. (63)	5 healthy males, studied after two sprints with either 60 or 240 min of recovery between	30-s sprint, IGF-I determined before and 5 min after sprint	→ ultrafiltered free IGF-I before vs after exercise	→ immunoactive IGF-I	No conclusive changes in IGFBP-1	Free IGF-I tended ($P = 0.06$) to be reduced after 60 and 240 min of recovery from a sprint with greater reductions after 60 min
Study 2, De Palo et al. (16)	20 professional cyclists	2 d of cycling: 1) 15 min exhaustion; 2) 15 min + 15 min at 70%–80% $\dot{V}O_{2max}$ and exhaustion, respectively	↓ free IGF-I by 16% study 1 → free IGF-I study 2	↑ by 6% study 1 ↑ by 11% study 2 ↓ free/total IGF-I ratio by ~15%–20%	None reported	The ratio free over total IGF-I was suggested to represent a new approach in exercise studies
Study 3, Wallace et al. (69)	8 very fit athletes	30 min cycling, samples collected before, after, and during 90 min of recovery	→ ultrafiltered free IGF-I comparing 0 vs 30 min	↑ at 30 min and returned to baseline during recovery Changes not explained by changes in plasma volume	ALS and IGFBP-3 changed in parallel with IGF-I, not explained by plasma volume shifts ↑ IGFBP-1 during recovery	Based on changes in IGF-I, IGFBP-3, and ALS, the ternary complex was suggested to enter and leave the circulation in response to exercise
Study 4, Dall et al. (15)	Acute study in 8 elite rowers	30 min of rowing, samples collected before, after, and during 120 min of recovery	↓ ultrafiltered free IGF-I by 25% 60 min after exercise, where after levels normalized	→ IGF-I and IGF-II (levels were albumin adjusted)	→ IGFBP-3 → IGFBP-3 proteolysis	The reduction in free IGF-I concurred with the peak in IGFBP-1
Study 5, Kanaley et al. (35)	8 GHD ± GH substitution and 8 controls	45 min of cycling at LT, followed by 75 min of recovery	→ bioactive IGF-I	→ IGF-I and IGF-II	↑ IGFBP-2 and -3, but only in GHD ↑ IGFBP-1 all groups → IGFBP-3 proteolysis	GH status does not affect the IGF-I response to exercise
Study 6, Berman et al. (4)	32 healthy 67–80 yr randomized to 8 wk of strength training or habitual activity	75 min of standardized strength test performed before and after 8 wk of strength training	↑ free IGF-I by 90% at 2 h and by 30% at 6 h in both groups	↑ acutely by 17% at 2 h and by 7% at 6 h in both groups	→ IGFBP-3	This study also contains data on the effect of 8 wk of training on baseline levels (see below)
Study 7, Nemet et al. (46)	>11 healthy adolescent boys aged ~16 yr	90 min of wrestling, containing aerobic and aerobic exercise. Samples collected immediately before and after the 90 min	→ free IGF-I	↓ by ~10% after correction for serum albumin	→ IGFBP-3 ↑ IGFBP-3 proteolysis ↑ IGFBP-1 by fourfold	Fitness was inversely correlated with the magnitude of the increase in IGFBP-1
Study 8, Nindl et al. (49)	10 healthy, fit men	2 h of heavy resistance exercise vs resting Samples collected for 13 h after exercise and during rest	→ free IGF-I both 1 h after exercise and O/N when compared with the resting situation	→ 1 h after exercise → O/N levels when compared with the resting situation	↑ IGFBP-3 by 8% 1 h after exercise but not O/N ↑ IGFBP-2 by 12% O/N but not after exercise	The authors suggested that exercise alters the molecular distribution of IGF-I rather than its levels
B. Long-term studies						
Study 9, Chicharro et al. (10)	Short-term study in 17 professional cyclists	3 wk competition study (Vuelta a Espana), baseline samples collected at start and at weeks 1 and 3	→ free IGF-I week 1 ↓ free IGF-I by 32% week 3	↑ 68% week 1 ↑ 78% week 3	IGFBP-1: ↑ 48% week 1 ↑ 63% week 3 → IGFBP-3	Free IGF-I and IGFBP-1 correlated inversely Cyclists lost 4 kg BW without changes in % body fat
Study 6, Berman et al. (4)	32 healthy 67–80 yr randomized to 8 wk of strength training or habitual activity	8 wk of strength training, 75 min standardized strength test performed before and after	→ baseline free IGF-I	→ baseline levels	→ IGFBP-3	No change in plasma volume was observed Training increased 1-RM significantly

Study 10, Rosendahl et al. (57)	UT (n = 7) and WT (n = 12) young male soldiers	11 wk of military-specific training, samples collected at baseline and after 4 and 11 wk	Ultrafiltered free IGF-I ↓ in UT week 4 ↓ in WT week 4 ↓ in UT week 11 → in WT week 11	Total IGF-I ↓ in UT week 11 ↓ in WT week 4 ↓ in UT week 4 → in WT week 11	IGFBP-3 transiently reduced at 4 wk in UT ↑ IGFBP-3 proteolysis in UT, but not WT at weeks 4 and 11	In UT, an increased IGFBP-3 proteolysis was unable to keep free IGF-I normalized during the study
Study 11, Nindl et al. (48)	10 ESRF patients on long-term dialysis	12 wk of resistance training (two times per week) using weight machines. Samples collected at -4, 0, 6, and 12 wk	→ free IGF-I	→ week 6 ↓ week 12 (~15%)	→ IGFBP-3 ↓ IGFBP-3 week 12 → IGFBP-2	Training significantly improved strength and performance
Study 12, Koziris et al. (40)	Long-term study in 14 university swimmers (9 females and 5 males) subdivided into three teams	6 months swimming: increased training more than 4 months, followed by 2 months taper phase. First samples collected after 2 months of training	↑ free IGF-I by two- to sevenfold at all training points but reductions in one group during tapering	↑ by ~1.5-fold at 4 months and until end of training	→ IGFBP-3 → IGF-/IGFBP-3 → IGFBP-1	Data based on small groups. However, training improved swimming speed in all three teams

Study 6 (2) has been included in panels A and B because there are acute as well as long-term data reported.

→, unchanged levels; ↑, a significant increase; ↓, a significant decrease; free IGF-I, ELISA-determined free IGF-I, otherwise as indicated by ultrafiltration (24) or by KIRA (9); LT, lactate threshold; GHD, growth hormone deficiency; O/N, overnight; ESRF, end-stage renal failure; BW, body weight; 1-RM, one repetition maximum; UT, untrained; WT, well trained.

as suggested in the study by Rosendahl et al. (57), untrained subjects respond differently to several weeks of exercise than trained subjects, with an increased IGFBP-3 proteolysis and a longer-lasting suppression of IGF-I levels. The 3-wk professional cycling contest is an exception because total and free IGF-I changed in opposite directions, most likely because it represents an extreme amount of exercise (10). In that study, all athletes completed the race with marked reductions in free IGF-I, which correlated inversely with increases in IGFBP-1. By contrast, total IGF-I increased by approximately 50%, whereas IGFBP-3 remained unchanged, hereby illustrating that the ratio between IGF-I and IGFBP-3 may be a poor indirect estimate of free IGF-I (21). On a molar basis, the increase in total IGF-I levels ($\sim 10 \text{ nmol} \cdot \text{L}^{-1}$) exceeded those of IGFBP-1 ($\sim 2 \text{ nmol} \cdot \text{L}^{-1}$), making it unlikely that IGFBP-1 can solely explain the reduction in free IGF-I. Accordingly, the authors speculated that after prolonged exercise, there was an increased transport of IGF-I from the circulation to the peripheral tissues, hereby explaining the reduction in free but not total IGF-I (10). Alternatively, because free IGF-I is more sensitive to catabolism (fasting) than total IGF-I (8), the reduction in free IGF-I may be caused by exercise-induced wasting. Supportive of this idea, the cyclists lost body weight during the last part of the race, concomitant with the reduction in free IGF-I (10). Two recent studies confirm that nutritional intake (in particular, calories rather than protein intake) affects the circulating IGF system during exercise. Alemany et al. (2) studied two groups of matched soldiers undergoing an 8-d high-energy expenditure military exercise. The two study groups received an isocaloric, low-energy diet with either low- or high-protein content, but neither of these two diets could abolish the reductions in free and total IGF-I (2). Conversely, overfed subjects (15% positive energy balance) undertaking 7 d of strenuous exercise were able to maintain levels of free and total IGF-I, whereas underfed subjects (33% negative energy balance) showed marked and parallel reductions in serum free and total IGF-I (47).

As illustrated in Table 3A and B, the inclusion of “free IGF-I” (irrespective of methodology) has not substantially altered the impression that the response of IGF-I to exercise is variable and most likely reflects differences in study protocol (exercise intensity and duration) and study populations (baseline level of fitness and age). Furthermore, it seems fair to conclude that the exercise-induced GH peak as a rule does not increase endocrine IGF-I levels. In comparison, subcutaneous injection with recombinant human GH, eliciting similar peak levels but longer-lasting elevations in serum GH than those observed after sprint exercise, was able to increase serum IGF-I levels of free and total IGF-I within 4 h after injection (63).

Numerous studies verify that IGF-I exerts anabolic effects on skeletal muscles (68). Therefore, the observation that it is possible to increase muscle strength, performance, and $\dot{V}O_{2\text{max}}$ without concomitant and robust changes in

circulating IGF-I indicates that the effect of exercise on muscle strength is mediated via locally produced (i.e., paracrine/autocrine) rather than endocrine IGF-I. Indeed, as excellently reviewed recently, there is solid (albeit primarily experimental) evidence that locally produced IGF-I is more important than endocrine-derived IGF-I (68). For instance, animal experiments have shown that exercise leading to muscle hypertrophy is accompanied by increases in IGF-I DNA, messenger RNA (mRNA), and peptide within the exercising muscle (1). Transgenic overexpression of IGF-I in skeletal muscles leads to significant hypertrophy without affecting circulating IGF-I levels (14), and mice lacking skeletal muscle IGF-IR have hypoplastic muscles, which in contrast to wild-type littermates are not stimulated by GH treatment (38). Interestingly, human studies have confirmed that exercise is accompanied by an increased IGF-I mRNA expression in exercising muscles without concomitant changes in circulating IGF-I levels (29).

A very recent experimental study in liver IGF-I-deficient (LID) mice stresses the importance of locally produced IGF-I (45). In the LID mouse, the hepatic generation of IGF-I has been genetically silenced, and these mice therefore show markedly reduced circulating levels of total IGF-I (approximately 20% of levels in wild-type littermates) and secondary GH hypersecretion. Matheny et al. (45) subjected adult LID mice to 16 wk of endurance training (ladder climbing) and observed changes, which generally resembled those found in wild-type littermates. Thus, training doubled the lifting capacity and increased hind leg muscle mass, IGF-I mRNA, and IGF-IR phosphorylation. On the basis of these studies, the authors concluded that normal muscle performance may be seen even in the setting of severe circulating IGF-I deficiency and that up-regulation of local IGF-I appears to be involved in compensatory growth of muscle in response to endurance exercise. More surprisingly, the same authors (45) found a

reduction in the intracellular signaling of GH, indicating that local increases in IGF-I are in fact GH independent. Although this finding appears controversial, earlier studies in hypophysectomized rats and in rats made GH deficient by treatment with neutralizing GH-releasing hormone antibodies support that muscular IGF-I expression may not be strictly GH dependent (75). However, in the author's view, this hypothesis needs further evaluation.

CONCLUSION AND PERSPECTIVES

On the basis of the current literature, it appears that the stimulatory impact of exercise on skeletal muscles is mediated by an augmented pituitary GH secretion, leading to an increased local IGF-I synthesis. This hypothesis may explain why training studies generally have failed to link an improved muscle performance with changes in circulating IGF-I levels. However, it should be acknowledged that a role of circulating IGF-I cannot completely be ruled out. For instance, patients with GH insensitivity (~Laron syndrome) do respond to subcutaneous IGF-I by an increased muscle mass, although the response was less pronounced than the response observed in GH-deficient subjects treated with GH (5). In the author's view, future research needs to compare circulating versus locally produced IGF-I and their impact on skeletal muscles to elucidate the link between exercise, GH, and muscle hypertrophy. Consequently, to gain more information, we need to optimize methodologies for the measurement of tissue IGF-I levels in humans.

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