GROWTH HORMONE AND EXERCISE

Exercise modulation of growth hormone isoforms: current knowledge and future directions for the exercise endocrinologist

B C Nindl

Importance of growth hormone molecular heterogeneity as a partial mechanism for somatogenic adaptations to physical activity

Growth hormone (GH) exhibits a great deal of molecular heterogeneity (there are over 100 isoforms in circulation) and we are only beginning to understand how exercise influences concentrations of GH isoforms.

As the field of exercise endocrinology moves forward in the 21st century, greater recognition will be given to molecular heterogeneity that exists for many protein hormones within the paradigm of exercise and physical activity. Specifically, it is well established, but not well recognised, that GH exhibits a great deal of molecular heterogeneity, as over 100 molecular isoforms are thought to exist in the circulation. The importance of gaining a greater understanding of the exercise-mediated influences on GH molecular heterogeneity resides in the fact that the GH isoforms have diverse downstream metabolic and anabolic actions in target tissues.

GH MOLECULAR HETEROGENEITY

The human GH-N gene expresses the main pituitary molecular mass variant in the GH family, which is the 22 kDa form. Monomeric 22 kDa GH is a 191 amino acid sequence and represents ~21% of all circulating plasma GH. The next most prevalent form is the monomeric 20 kDa molecule, representing ~6% of all circulating plasma GH, formed through alternative mRNA splicing during which amino acid residues 32–46 are cleaved out. GH can also undergo post-translational modification and peripheral tissue proteolytic cleavage at its site of action to form variants and aggregates (ie, dimers, trimers, pentamers, oligomers) and fragments that exist in the circulation. The existence of high-affinity and low-affinity GH-binding proteins, which are released from the pituitary and/or cleaved from the extracellular domain of the GH receptor, adds further complexity to the nature and spatial arrangement of circulating GH moieties. GH should no longer be regarded as a single hormone, but rather as a family of related polypeptides that are all derived from one gene (table 1). This molecular heterogeneity seems to have physiological significance, as the different forms have been shown to possess different biological activities (eg, relative potency in bioassays), different effects on lipid, carbohydrate and protein metabolism, as well as different immunodetectabilities.[1 3 6] For example, of the main monomer GH variants (ie, 22 and 20 kDa isoforms) that comprise approximately 56% of circulating GH, the 22 kDa form has been shown to have a greater effect on inhibiting lipoprotein lipase activity and lipolysis, and a weaker effect on antidiuretic and diabetogenic effects than the 20 kDa form. Table 1 lists the relative proportions of circulating GH at rest.[1]

It is critical to consider the molecular heterogeneity when interpreting the meaning of GH concentrations and to understand that the proportions of GH molecules can potentially be influenced by exercise and physical activity. To date, few people have attempted to characterise GH concentrations using different assays before and after exercise. Exercise alterations in GH isoforms may represent an important regulatory step in the adaptational process by which downstream target tissues are somatogenically modulated by physical activity patterns.

MEASUREMENT AND EXERCISE MODULATION OF GH ISOFORMS

Strasburger *et al*[11] have developed a novel, unique ELISA on the basis of the molecular interaction between the hormone and its receptor necessary for receptor dimerisation

Table 1 Estimated proportions of growth hormone forms in plasma 15 min after secretion[1]

GH form	Proportion of total GH (%)
Monomeric	56
22 kDa total	43
22 kDa free	21
22 kDa in high-affinity complex	20
22 kDa in low-affinity complex	2
20 kDa total	8
20 kDa free	5.5
20 kDa bound in high-affinity complex	0.5
20 kDa bound in low-affinity complex	2
Acidic GH (desamido-GH and acyl-GH) total	5
Acidic GH-bound fractions	Unknown
Dimeric	29
22 kDa non-covalent dimer total	14
22 kDa disulphide dimer total	6
22 kDa dimer-bound fraction	Unknown
20 kDa non-covalent dimer total	5
20 kDa disulphide dimer total	2
20 kDa dimer-bound fraction	Unknown
Acidic GH non-covalent dimer total	1.5
Acidic GH disulphide dimer total	0.5
Acidic dimer-bound fraction	Unknown
Trimeric to pentameric	13.5
22 kDa non-covalent oligomer total	7
22 kDa disulphide oligomer total	3
22 kDa oligomer-bound fraction	Unknown
20 kDa non-covalent oligomer total	1
20 kDa disulphide oligomer total	0.5
20 kDa oligomer-bound fraction	Unknown
Acidic GH oligomer (non-covalent and S–S) total	1
Acidic GH oligomer-bound fraction	Unknown
Non-S–S-linked covalent oligomer total	1
Fragments	
16, 12, and 30 kDa immunoreactive fragments	Variable

GH, growth hormone.
Monomeric, dimeric and trimeric to pentameric fragments comprise approximately 98.5% of all circulating GH, whereas presumably lower molecular mass fragments comprise approximately 1.5%.

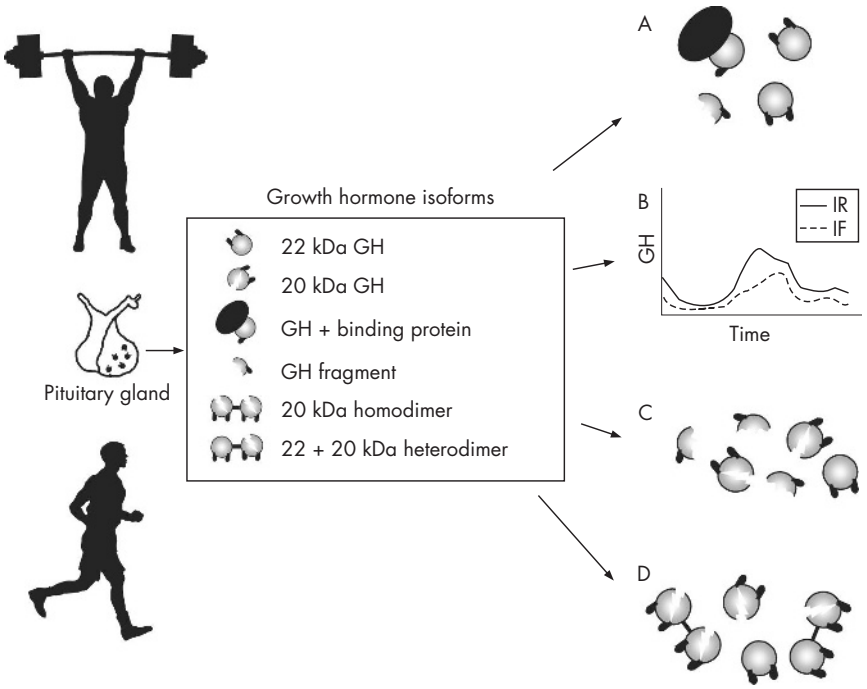

Figure 1 Schematic summarising the current available findings concerning GH molecular heterogeneity and exercise. (A) One half of the immunoreactive (IR) GH released after resistance exercise in men and women is able to dimerise the GH receptor and is measured as immunofunctional (IF). The other half of IR GH exists as either GH fragments or GH bound to the GH binding protein.[5] (B) Overnight IR and IF GH released after resistance exercise exhibit similar qualitative pulsatility profiles, but differ quantitatively.[4 8] (C) Aerobic exercise increases the proportion of non-22 kDa GH isoforms after exercise, and these isoforms have a slower disappearance rate. The biological significance of this may be an enhanced diabetogenic effect preventing post-exercise hypoglycaemia.[9] (D) GH dimers are preferentially released after resistance exercise and may serve to prolong the biological activity of GH.[2 7]

and subsequent induction of signal transduction. This assay only detects those GH isoforms actually capable of having a physiological effect. Strasburger termed this assay "immunofunctional" and reported that GH measured in this assay had a higher correlation than a conventional polyclonal assay when compared with the in vitro NB2 bioassay, thus lending credence to the notion that GH as measured by this assay detection system may be more biologically relevant than other conventional assays. The assay is now termed "bioactive" GH and is commercially available from Diagnostic Systems Laboratories (DSL-10-11100, Webster, Texas, USA). Other novel immunoassays for the measurement of GH mainly involve antibodies directed at specific epitopes on the GH isoforms.

Our laboratory conducted the first two studies that evaluated immunofunctional GH responses after exercise.[4–6 8] Using resistance exercise, the first study was a gender comparison reporting that post-exercise immunofunctional GH was significantly lower (~50%) than immunoreactive GH and that men and women demonstrated similar ratios of immunofunctional vs immunoreactive GH.[5] The second study considered the pulsatile, episodic release of GH and measured overnight immunofunctional and immunoreactive GH every 10 min for 12 h after a bout of heavy resistance exercise. This study also reported that immunofunctional GH was lower (~50%) than immunoreactive GH and that, while deconvolution analyses revealed qualitatively similar pulsatile profiles, the estimated half-life of immunofunctional GH when compared with immunoreactive GH suggests that GH molecules that are not capable of initiating signal transduction are released, both in exercised and non-exercised conditions.

Further, Wallace *et al*[9] measured GH with seven different immunoassays with antibodies specific for the 22 kDa, non-22 kDa, 20 kDa and immunofunctional isoforms during aerobic exercise. This study reported that the proportion of GH isoforms changed across exercise and into recovery. Specifically, non-22 kDa GH increased during the post-exercise recovery period. The biological significance of this may be an enhanced diabetogenic effect preventing post-exercise hypoglycaemia as well as a GH isoform profile favouring lipolysis in order to meet fuel needs. The authors also postulated that likely factors explaining the increase in non-22 kDa forms after exercise are those inherent to the molecular heterogeneity of the GH molecule, such as the influence of GH-binding proteins, GH fragments that may interfere with detection in the immunofunctional GH assay as well as inhibiting GH biological action, and GH isoforms of higher molecular mass.

In an effort to glean more information on the nature of GH isoforms in the post-exercise milieu, two studies have used glutathione (GSH) as a reducing agent to break the disulphide bonds linking GH aggregates. Hymer *et al*[3] fractionated plasma into three molecular mass size classes and added GSH to pre-resistance and post-resistance exercise samples. GSH-treated samples showed greater increases in GH concentrations post-exercise, but not pre-exercise, for the molecular isoform sizes of 30–60 kDa (ie, dimers) and >60 kDa (ie, trimers). Rubin *et al*[8] confirmed and extended these findings in aerobic exercise by demonstrating that GSH reduction also resulted in increased GH concentrations during exercise and into post-exercise recovery. These data suggest that exercise results in the preferential release of disulphide-linked dimeric isoforms of GH. The physiological significance of these findings would be that exercise may prolong the half-life of GH isoforms, thereby sustaining biological activity.

SUMMARY AND FUTURE DIRECTIONS

From the few studies that have examined the effects of exercise on GH molecular heterogeneity, the concept is emerging that acute exercise, both resistance and aerobic, alters the relative proportions of circulating GH molecular isoforms (specifically non-22 kDa and dimeric isoforms), suggesting that acute exercise may potentiate the bioactivity of GH by inducing the release of molecular isoforms with extended half-lives and thereby sustaining biological action (fig 1). As exercise introduces an acute stressor in terms of meeting energy demands, the potential enhanced lipolytic and diabetogenic effect of these non-22 kDa GH isoforms would seem beneficial in terms of maintaining metabolic homoeostasis.

With the blending of biochemical techniques (fractionation, chemical reduction

and so on) and novel assays procedures (both in vitro and in vivo) within the paradigm of exercise protocols, the field of exercise endocrinology will continue to provide definitive information with regard to the relative importance of GH molecular heterogeneity as a partial mechanism for somatogenic adaptations to physical activity. Future studies aimed at further separating different GH isoforms during longitudinal physical training and exploring the mechanisms and biological consequences of such responses would seem prudent.

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Correspondence to: Dr B C Nindl, Military Performance Division, US Army Research Institute of Environmental Medicine, Natick, MA 1760, USA; bradley.nindl@na.amedd.army.mil

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COMMENTARY

Since the growth hormone response to exercise is such a critical aspect of exercise physiology, it is important to understand that not all circulating forms of the hormone are identical. Previous research has typically not appreciated that there are different forms of the hormone, and that each variant has potentially very different physiological effects. These data are very helpful when interpreting the growth hormone responses for future studies.

Andrew Fry
Exercise and Sport Science Laboratories, University of Memphis, Memphis, Tennessee, USA; afry@memphis.edu

EDITORIAL BOARD MEMBER

Jill Cook

Jill Cook is a physiotherapist who after a clinical career of 20 years has become primarily a musculoskeletal researcher. Her main interests are in connective tissue function, injury and healing. Her particular interests are risk factors for tendon injury and the tendon in growth and development. She is currently working in the Musculoskeletal Research Centre at La Trobe University and at the Centre for Physical Activity and Nutrition Research at Deakin University.

Figure 1 Jill Cook.