

PROJECT REVIEW

“Manipulation of Muscle Mass Via the Growth hormone/Insulin like Growth Factor Axis”

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With the increasing availability of recombinant human growth hormone (rhGH) and insulin-like growth factor I (IGF-I) these two peptides are becoming increasingly used to improve performance in strength and power events in track and field athletics and other sports. Both can now be purchased over the internet and are relatively inexpensive. GH is hyper- and IGF-I hypo-glycaemic and instructions are provided on how these substances should be taken so that glucose homeostasis is not unduly disturbed, yet a marked growth effect may be produced. As both are human peptides that are naturally upregulated during athletic training it is difficult to detect exogenous from endogenous, GH and IGF-I peptides. Methods have been developed that enable us to distinguish between the different isoforms and determine the ratios of the different IGF-I splice variants so that it is now possible to distinguish between the introduced and the endogenous peptides. At the present time the abuse includes injection as well as the ingestion of the peptides. The latter is a grey area as far as banned substances is concerned as colostrum which can be purchased from health stores is also known to have high levels of IGF-I that survive pasteurization and digestion in the gastrointestinal (GI) system. It can do this because it is stabilized by binding to specific binding proteins. In the newborn, which derives many of its growth factors in this way, there is an uptake mechanism in the GI system and it is believed that may persist to some extent into adulthood. This would explain the apparent “beneficial” effects of ingesting colostrum or freeze dried IGF-I. Certainly it is known that the age-related loss of muscle mass is associated with a marked decrease in GH and IGF-I levels which decline by about 60% between the teenage years and old age (Rudman et al., 1981).

An anticipated problem in future years is gene doping using the cDNA of different forms of IGF-I. Several laboratories in the world including Professor Goldspink’s lab are developing gene therapy methods for maintaining muscle mass in medical conditions such as muscular dystrophy, prolonged space flight and in ageing. The experiments on animals have been very successful (Skarli et al, 1998; Goldspink et al, 1999; MacCoil et al, 1998; MacCoil et al, 2000; Barton-Davis et al, 1998)) and once the engineered gene becomes more widely available it is predicted that there will be an increasing misuse. It is anticipated that this will happen within 5 to 10 years. Further work is required to characterise these growth factors in relation to the development and maintenance of muscle mass and in relation to the misuse of these potent growth factors.

Previous work on the GH/IGF-I system

The growth hormone /insulin growth factor 1 (GH/IGF-I) axis is the main regulator of tissue mass during early life and IGF 1 is one of the main growth

factors that stimulates protein synthesis in muscle tissue. In adult muscles, increasing evidence suggests that IGF-I can act in an autocrine/paracrine fashion. As described below (Yang et al, 1997; Goldspink, 2000) showed that exercised muscle produces an autocrine splice variant of IGF-I that appears to be an important link between mechanical activity and the local cellular effects that result in muscle hypertrophy. Although the liver is usually thought of as the source of circulating IGFs, it has been shown that during exercise skeletal muscle not only produces much of the circulating IGF-I but the active musculature also utilises most of the IGF-I produced (Brahm et al, 1997).

It has long been appreciated that there is local as well as systemic regulation of muscle growth. Using Differential Display (RT-PCR) Professor Goldspink's group have identified and cloned the cDNA of two growth factors which are expressed by muscle when it is subjected to activity which are derived from the IGF-I gene by alternative splicing (Yang et al 1997). One (L.IGF-I) is very similar to the liver endocrine type of IGF-I. The other is a new growth factor that can only be detected in exercised/overloaded muscle and has been

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Results and Conclusions

The group at the Royal Free and UCL medical have presented results in relation to hGH doping that show:

1. Two weeks after hGH administration of young untrained and trained adult male subjects results in markedly increased serum levels of IGF-I
2. Two weeks after hGH administration PIIIP levels remain elevated longer after cessation hGH administration longer than IGF-I.
3. A single bout of exercise intensive raining does not affect IGF-I expression in thigh muscles. Hence it cannot be claimed that the elevated levels in 1 and 2 are due to exercise.
4. In addition to validating the use of IGF-I and PIIIR as markers, two further approaches to detecting hGh and IGF-I abuse have been pursued.

A. These include a rapid screening method using "cell biosensors". Serum from the athlete suspected of cheating following rapid screening is placed on muscle cells maintained in culture. Non physiological levels containing exogenous hGH result in the expression of MGF (human IGF-IEc) RNA which is a involved in the muscle hypertrophy process. This is a normal physiological process but in excess and therefore should stand up in a Court of Law.

B. In addition a new approach using computerised mass spectrometry method involving neural net work analyses has been studied as an extension of the present project. Human biopsy samples have been studied to determine if those receiving hGH had a different pattern of molecules in the serum. This work is being carried out with the HFL in NewMarket that carries out much of the equine doping tests and Nottingham Trent University who have experts in mass spectrometry analysis. Good repeatability is being obtained with murine as well as human serum samples following hGH administration and this approach would be very suitable for routine screening of athletes as only very small serum samples are required and hundreds of these can be analyses within a few days as the process is robotically controlled. The work is refined to identifying the marker molecules that differ in hGh doping so that it might also act as a confirmatory test.