ANZAC Research Institute

ANZAC Research Institute
Sydney NSW 2139
Australia
Telephone +61 2 9767 9100
Facsimile +61 2 9767 9101
Email: djh@anzac.edu.au
Web: www.anzac.edu.au

David J Handelsman *MB BS FRACP PhD*
Professor of Reproductive Endocrinology and Andrology
Director

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Report on Doping Status of Certain Peptides

This report is an expert statement on background physiology and pharmacology including the basis for prohibition under Prohibited List of the following areas and substances

- Regulation of growth hormone (GH) secretion
- Growth hormone releasing-peptide (GHRP-6)
- Hexarelin
- CJC-1295
- Thymosin beta-4
- SARM S22
- IGF-2
- Follistatin

This report will make preliminary comments to set the context for the subsequent detailed summaries on physiology, pharmacology and potential performance enhancing effects for each of these substances.

My relevant professional expertise is summarised in an appendix.

I confirm that this report is based on my professional expertise. I acknowledge my overriding duty to assist the Tribunal on matters within my expertise in an impartial manner

Preliminary Comments:

1. **Nature of evidence for sports performance effects.**
   In human medicine or physiology, the pinnacle in the hierarchy of evidence for therapeutic effects is data from well-controlled, prospective therapeutic trials featuring randomization, placebo controls and the specific therapeutic endpoint in question.
   For the sports performance enhancing effects of any drug, there is not, nor can there ever be, such evidentiary certainty because it is ethically and logistically impossible to conduct the necessary controlled experiments using banned drugs during elite competitive sporting events. In anti-doping science, it is therefore necessary to make the most plausible inference from the best available surrogate evidence on sports performance enhancement.

2. **Use of salient surrogate variables for establishing performance enhancement**
   2.1. Despite the limitation in obtaining direct evidence of sports performance enhancement of drugs, classical studies using suitable, closely related surrogate variables provide compelling evidence. For example, a strong biological basis for androgen doping was provided by Bhasin et al in studies showing a tight linear relationship between testosterone dose and muscle mass or strength, extending from below to well above physiological testosterone levels, displaying additive effects with physical exercise, but without any plateau even at 6 times regular testosterone replacement dosage[1, 2].

2.2. Similarly, the strong linear relationship between acute changes in circulating hemoglobin and maximal oxygen consumption [3] explains the effectiveness of doping by increasing hemoglobin level by blood transfusion or other means (eg administration of erythropoiesis-stimulating agents (ESA)).

2.3. These findings form a sound basis for banning administration of both exogenous androgens and increasing hemoglobin (ie via blood transfusion) which directly enhance sports performance.

2.4. Furthermore, they also provide a rationale for banning of indirect doping methods. That refers to where substances or methods, which are not themselves intrinsically performance enhancing, are used to increase or supplement endogenous hormones (testosterone, erythropoietin) which do or can (depending on dose and drug combinations) enhance sports performance.

2.5. Such indirect androgen doping methods include use of human chorionic gonadotrophin (hCG), luteinizing hormone (LH), anti-estrogens (including estrogen blockers or aromatase inhibitor drugs) all of which are banned on the basis that androgens are banned as a class of doping drugs [4].

2.6. Similarly, indirect hemoglobin doping methods include ESAs such as erythropoietin and its analogs, hypoxic-mimetics and artificial oxygen carriers are banned on the basis that they are likely to increase circulating hemoglobin [5].

2.7. It is neither necessary nor feasible to evaluate explicitly the performance enhancing effects of each of the growing list of such putative doping substances. It is sufficient to show that for any substance in question, the key surrogate variable which can induce performance enhancement (endogenous testosterone for indirect androgen doping; hemoglobin for indirect blood doping) is increased. This demonstrates that the substance that produces such increases in endogenous hormones or hemoglobin is potentially performance enhancing and warrants being included on the Prohibited List. In effect, this is operationalised by the "catch-all" provisions under S1 (1a) and S2 categories of the Prohibited List.

2.8. It is germane to this consideration that the doses of approved drugs that can be used safely and acceptably in demonstrative therapeutic trials under ethical supervision are likely to be lower than the doses used illicitly (and in combinations with other ergogenic drugs) by athletes for doping purposes.

3. Lessons from anti-doping history.

In considering the limitations of surrogate evidence available for novel forms of doping, it is paramount to remember the lessons of history. Until the mid 1990's when it was directly refuted by Bhasin et al[1], it was widely held that healthy eugonadal male athletes could not benefit from exogenous androgens as their androgen receptors were already fully saturated and down-regulated by exposure to natural endogenous testosterone. This was largely due to inadequate studies which, specifically, used only low doses of androgens that did not match the doping practices involving much higher doses[6]. Tenacious adherence to this fallacy has been costly in credibility among athletes who were either androgen abusers themselves (and their support staff) or suffered disadvantage against those who were. The legacy of this misadventure is the experience of doctors that in obtaining the crucial detailed and accurate medical history, discussing doping practices now often features omission and deception [7]. It is crucial that wherever convincing evidence from supraphysiological and/or multi-drug doping regimens is not available, but where some effects are demonstrated at lower doses, it is prudent not to rule out ergogenic effects unless and until the testing can replicate characteristic doping regimens, especially as regards high doses and drug combinations.

4. WADA definition of the S0 "Non-Approved Substances"

The definition of S0 in the Prohibited List refers to ".... any pharmacological substance which ... (has) ... no current approval by any governmental regulatory health authority for human therapeutic use" (underline emphasis added) is banned at all times. Operationally, in Australia this is equivalent to whether that

substance is contained on the Australian Register of Therapeutic Goods (ARTG). None of these peptides are listed on the ARTG or other major national regulatory agencies (see also #17.6). This means they are covered by the WADA S0 category.

4.1. The WADA S0 definition makes an important distinction between diagnostic and therapeutic use as widely accepted by major drug regulatory agencies. Diagnostic use involves a single dose, usually in a medically equipped testing facility where blood samples are obtained to measure a biological response to the administered stimulus. This is quite different from therapeutic use which involves prolonged or repeated administration for the purpose of producing a therapeutic response to ameliorate a medical condition. In particular, the safety profile of a single use diagnostic drug is very different from approval for therapeutic use[1], which allows for not only the approved use but also tacitly, potentially open-ended off-label usage.

4.2. Therapeutic use may be either according to an approved medical indication or "off-label" usage. "Off-label" use is the administration of an approved drug for an indication (a justified medical reason for use), age, dose or using a formulation of it outside the terms of its approved registration for therapeutic use. "Off-label" usage also assumes the treatment is based on a valid prescription written by an approved person - a fully registered and suitably qualified doctor legally authorised to write a prescription for pharmaceutical drugs. Typically, off-label usage is for a non-approved indication or for an approved indication but significantly beyond the original approval (e.g. use in children when approved for adults, use of different dosage or form of the drug).

4.3. The focus on any governmental regulatory health authority for human therapeutic use does not stipulate which regulatory agencies are those of record. Globally, pharmaceutical drug marketing is subject to registration and approval from national drug regulatory agencies. Among these national drug regulatory agencies the most expert and experienced are those of the most economically developed countries, notably USA (FDA), Canada (Health Canada), UK (MHRA), Germany (BfARM), Sweden (MPA), Netherlands (MEB) and Australia (TGA). It is a strategy adopted by some pharmaceutical companies to seek drug registration from national regulatory agencies of less developed and developing countries whose national regulatory agencies have limited local expertise. In fact these less experienced regulatory agencies are often reliant on decisions of the more major regulatory agencies and in many cases they defer to such approvals. WADA's reliance on approval by <u>any</u> national regulatory agency leaves a loophole to circumvent the otherwise important S0 category.

5. Use of non-approved peptides in humans

5.1. The manufacture of peptide products for therapeutic use by reputable pharmaceutical companies requires strict compliance to Good Manufacturing Practice (GMP) standards which are subject to licensing and regular critical review by independent regulators. These are designed to (a) ensure the authenticity and expected biological activity of the product as labelled on the vial or packaging and (b) eliminate the possibility of adulteration of drugs with chemicals used in drug manufacture as well as *inter alia* sterility, non-pyrogenicity and shelf-life stability testing. Naturally this compliance has a major impact on increasing the costs of production.

5.2. Good manufacturing practice (GMP) is an internationally harmonised set of standards endorsed and enforced by major pharmaceutical drug regulatory agencies that control marketing authorisation/licensing in various countries or regions that aim to ensure drug products are safe and effective for therapeutic use. GMP standards were originally developed by the FDA in 1963 (following the USA's avoidance of the thalidomide tragedy because the FDA had declined to register thalidomide for therapeutic use in the USA). Consistent GMP regulations are now promulgated by the WHO, EU (European Medicines Agency) and International Conference on Harmonisation (ICH), the latter involving most economically developed countries. Within ICH signatory countries (including Australia

[1] "Therapeutic use" and "clinical use" are largely just alternative terminologies for the use of drugs in medicine aiming to prevent, treat or cure disease, based on sound knowledge of the drug's safety and efficacy for treatment in that setting.

and China), GMP regulations and licensing are implemented by their peak pharmaceutical regulatory agency – in Australia by the Therapeutic Goods Administration (TGA) and in China by the China Food and Drug Administration (CFDA). GMP requires, among many other things, thorough documentation of the source, process, quality controls and finished product specifications. This aims to ensure that end users can be confident of product manufacture to very high quality standards, subject to ongoing quality control monitoring including accurate labelling and freedom from contamination by infectious or toxic adulterants. Accurate and detailed record keeping covering all stages of manufacturing are mandatory, onerous and subject to regular inspection to maintain licensing.

5.3. Non-approved therapeutic drugs may be used under certain circumstances. For life-saving circumstances, the Special Access Scheme (SAS) allows for compassionate use of specific drugs for individual patients with the approach documentation and expert specialist supervision. For less urgent use of non-approved therapeutic or diagnostic drugs (including therapeutic research), the Clinical Trials Notification (CTN) scheme allows a competent institutional human research ethics committee (HREC) to take responsibility together with its specialist doctors for the risk-benefit evaluation and supervision of safe conduct of therapeutic trials. Where no HREC is available or the relevant institutional HREC is unwilling or unable to judge risk-benefit and safety, the Clinical Trials Exemption (CTX) scheme allows for the TGA to make such evaluation.

5.4. In these circumstances of non-approved substances, the availability of pharmaceutical GMP grade products, provide reasonable assurance of safety, with regard to authenticity, purity and sterility. However, although non-approved drugs without a pharmaceutical company sponsor may also be considered, these are usually non-sterile oral or topical products rather than injectable drugs where the adulteration, sterility and pyrogenicity are additional major risks.

5.5. None of these safety assurances are available when peptides or other chemicals are obtained from any of the numerous low-cost peptide synthesis facilities around the world, either directly from the plant or via the internet. Among confiscated drugs intended for doping counterfeit [8] and fake packaging or labelling [9, 10] are well known. The extent of clean-up from toxic adulterants used in manufacture is unknown and/or unverifiable. In order to forestall any legal action, the vials are usually clearly marked "for research use" or a similar designation which is affixed to indicate they are not sold as fit for human use. It is perplexing by what scientific process such raw material substrate, purchased as not fit for human use, is then rendered fit for human use by a compounding chemist for use without fully informed consent for a non-approved substance, as well as the additional safety assurance and independent supervision by a competent human ethics committee.

5.6. It has been my personal experience, that a competent HREC would not approve use of such non-marketed products in human volunteers even for single dose experiments as the safety of the product could not be reasonably verified.

5.7. An important feature of the therapeutic use of non-approved substances like peptides is the necessity to obtain fully informed, written consent to the administration. In achieving this, modern standards require provision of a written Information Sheet which gives the name of the drug, its source, the commercial sponsor of the study or other agency taking responsibility for the drug administration, the medical reason (indication) for the administration, the likely expected effects and side-effects, warning about teratogenic risk and the extent of clinical experience in using that product. Use of such substances without fully informed consent would be a major dereliction of duty by any doctor and a matter of even more grave concern if undertaken without medical supervision and/or by an unqualified person.

6. Safety

6.1. For any drug, proof of safety is essentially the proof of a negative - that is no significant or serious adverse effects. Hence any judgement on safety has to be carefully circumscribed by the conditions of the safety testing undertaken. These considerations include especially the size of the population studied, the intensity of the surveillance for harm and duration of follow-up, all of which combine to

determine the likelihood of detecting uncommon, subtle or indirect but potentially serious adverse effects. Specifically, serious adverse effects can be missed in small samples with minimal surveillance and limited follow-up. Over recent decades these issues have repeatedly led to FDA withdrawal from the market of drugs which were approved under the usual rigorous therapeutic development program involving phase I-III clinical trials but were subsequently found to have serious but infrequent adverse effects. Hence reliance on simple survival and/or spontaneous complaints of harm experienced by the drug exposed person are inadequate to meet modern safety standards of detecting and predicting long-term toxic organ damage following drug exposure.

6.2. An important and often under-estimated risk is that associated with dose finding for non-approved drugs. The catastrophic effects of a 2006 phase I therapeutic trial in the UK have been well reported (see recent BBC update report (http://www.bbc.co.uk/news/health-22556736) and Annex to this report). These events highlight that even with the most diligent pre-clinical evaluation of drug effects in the laboratory and in animal models, disastrous miscalculations with devastating effects can occur. This misadventure has had a galvanizing effect on conduct and approval of therapeutic trials world-wide. Any competent person intending to administer new, injectable non-approved substances should be acutely unaware of the risk and accompanying responsibilities.

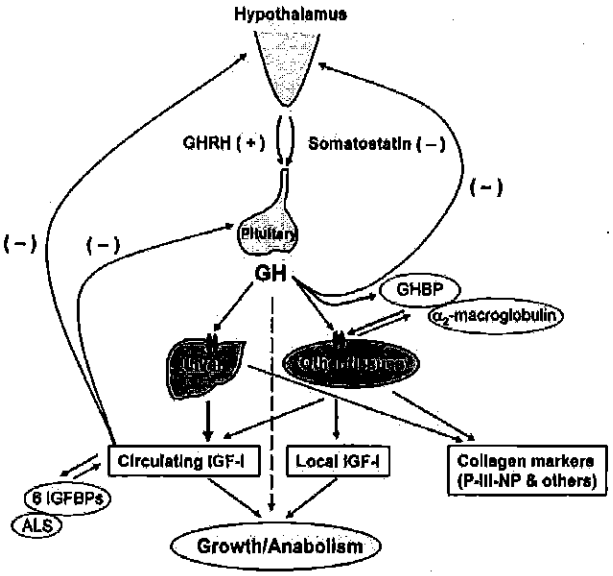
6.3. Similarly, concern about teratogenicity would preclude the use of non-approved substances in pregnancy (or in fertile women where pregnancy is not ruled out), unless there was a major and serious medical disorder justifying treatment. Otherwise, the teratogenic risk makes administration of non-approved substances to potentially fertile women without valid medical indication a reckless and highly irresponsible activity.

6.4. A further safety concern is that drugs such as tissue growth factors listed under S2 which promote cellular proliferation may enhance healing from injury; however, they are also likely to enhance proliferation of latent or metastatic cancer cells so that careful and ongoing safety evaluation is essential for people exposed to such treatment.

Specific Comments

7. Physiological regulation and pharmacological stimulation of growth hormone (GH) secretion

The physiological regulation of GH secretion is complex. GH is secreted exclusively by the somatotroph cells of the anterior pituitary gland. Endogenous GH secretion is primarily under dual regulation by stimulatory effects of GH releasing hormone (GHRH) and inhibitory effects of somatostatin, both short peptides secreted by the hypothalamus. GH is secreted in a markedly pulsatile fashion with bursts of highly variable magnitude at 2-3 hour intervals. Only minimal GH secretion occurs between these intermittent bursts. This intermittent pattern of GH secretion is entrained by a hypothalamic pulse generator, which coordinate the two hypothalamic peptides that govern GH secretion from the pituitary somatotrophs. Hence, circulating GH concentrations are mostly at very low or undetectable levels with only brief episodes of high circulating levels. The largest and most active pulses of GH secretion occur during sleep (stage IV, slow wave) sleep. Overall, net GH secretion is gradually reduced with age from the 3rd decade onwards as well as by obesity whereas undernutrition, acute stress and exercise increase GH secretion.



A third potent regulatory influence on GH secretion is negative feedback by GH itself (via hypothalamus)

as well as by circulating IGF-I (produced mainly by the liver) at the pituitary level. The effects of many factors such as ageing, gender, estrogen/androgen effects, stress, trauma, sleep, exercise, fasting/nutrition and some GH-sensitive metabolic factors (fatty acids, glucose) as well as pharmacological stimulators of GH secretion (arginine, lysine, L-dopa) are all exerted by means of these more final common pathway drivers (or inhibitors) of GH secretion although the detailed mechanisms of action are not always fully characterized.

Ghrelin, a gastric peptide with both a bioactive (3-octanoyl) and inactive forms has only a subordinate minor role [11] in physiological regulation of GH secretion with a greater role in appetite (satiety feedback) regulation. However, Ghrelin analogs which act upon the Ghrelin receptor do have potent pharmacological effects on short-term GH secretion.

The most potent pharmacological drugs that stimulate endogenous GH release (indirect GH doping) are either synthetic GHRH or various Ghrelin agonists which are short peptides pharmaceutically engineered to have more potent and long-lasting duration of action leading to sustained GH secretion. To achieve effective, sustained pharmacological stimulation of GH secretion, a secretagogue must overcome several obstacles. It must have (a) a prolonged depot-like duration of release, (b) it must be protected against the usually rapid metabolism of short peptide in the circulation, and (c) it must also overcome the negative feedback and inhibitory somatostatin effects. Virtually none of the GH secretagogues developed based on GHRH or Ghrelin structures have been approved for marketing, mainly because despite provoking GH release on initial dosing, they proved unable to sustain increased endogenous GH release. To the best of my knowledge, the sole exception is GHRP-2 (pralmorelin) which was approved for marketing by Kaken Pharmaceuticals only in Japan for diagnostic use (ie as a single dose test for GH deficiency) and not for therapeutic use (ie repeated administration to induce sustained GH secretion).

Although such indirect GH doping may not detected in either of the two current GH doping tests (isoform or biomarker), there is evidence that Ghrelin analog administration may have a masking effect on GH doping tests [12].

8. **Direct performance enhancing effects of GH**
The two best, well-designed studies of the direct performance enhancing effects of GH show only marginal effects at the relatively low doses used (reviewed in [11, 13, 14]).

8.1. In one study, 30 healthy participants (15 men, 15 women) were randomized to treatment with one of two doses of hGH (0.038 or 0.067 mg/kg/day, equivalent to ~2.3 or ~4.6 mg/day) or placebo for 28 days. Neither GH dosage produced any significant increase power output or maximal oxygen consumption [15].

8.2. A larger and more definitive study examined 96 recreational athletes (63 men, 33 women) who were randomized to treatment with GH (2 mg/day) or placebo for 8 weeks; in addition, the men were randomized to additional testosterone treatment (injection of 250 mg testosterone esters weekly) or placebo for the last 5 weeks [16]. One performance measure (anaerobic sprint capacity, Wingate test) was significantly increased (by 5.5%) in men, but not women, and the effects in men were further increased when combined with testosterone (+8.3%). There were no other effects of GH on 3 other performance measures (maximal oxygen consumption, dead lift or jump height). GH had effects on body composition (increased lean and decreased fat mass) in both men and women.

8.3. Two additional placebo-controlled studies of GH effects on performance were less convincing. One reported significant improvement in maximal oxygen consumption but only studied very short-term, low dose GH treatment in abstinent former androgen abusers using an incompletely masked study design [17]. The other did not report any recognised exercise performance variables [18].

8.4. Caveats arising from both the well designed and conducted studies are that higher doses of GH, of testosterone and their interaction were not studied. These higher doses and combination regimens

more closely replicate the reported doping practices.

8.5. It is therefore concluded at this time that GH is likely to enhance sports performance especially in combination with androgens, but the demonstrated magnitude of effect is less than that shown for the major ergogenic agents (androgens, ESA). It is likely, however, that greater effects may be detected at higher GH doses than have been tested and especially in combination with higher androgen doses. This supports the rationale of the banning GH under S2.

9. Indirect performance enhancing effects of GH via tissue repair and/or injury healing

9.1. The other potential benefit of hGH relevant to sports performance enhancement is the claim that GH improves tissue repair and/or healing recovery from injury. If true, this would expedite recovery from sporting injuries and/or from intensive training allowing faster return to competition from injury and/or the ability to tolerate more intensive training regimens. This claim is difficult to evaluate for the diversity of the claims and the mechanisms involved, with a corresponding lack of widely accepted surrogate measures. Nevertheless, effects of GH on healing in burns, fractures and skin wounds have been studied as the nearest available surrogates to injury healing.

9.2. GH effects on recovery from burns injury are the most investigated and also the subject of a recent Cochrane review [19]. This meta-analysis notes a small but (statistically) significant benefit in skin healing with large burns and reduced hospital stay but no benefit in mortality or scarring together with an increase in adverse effects (hyperglycemia). The increased mortality due to high dose GH treatment in critical illness reported in another influential study [20] has overshadowed these findings, although this study was not included in the Cochrane meta-analysis as it did not focus solely on burns injury. As a result, GH treatment for burns injury has not been adopted as having a sufficient benefit-risk-cost profile for therapeutic management. The warnings about the increased mortality of high dose GH in critical illness [20] together with concern that long-term GH treatment may increase risk of subsequent cancers [21, 22], are relevant to the safety criterion in the WADA Code for placing substances on the Prohibited List.

9.3. The effect of GH treatment on fracture healing was examined in one well-designed study where patients with tibial fractures (n=408) were randomized to GH treatment (1, 2 or 4 mg per day) or placebo for up to 16 weeks [23]. No benefit was observed in healing although a post-hoc trend to a benefit for high dose GH in patients with closed fractures was reported.

9.4. The effect of GH on wound healing (excluding bone) has not been investigated by well designed clinical studies. There are numerous pre-clinical studies using animal models or *in vitro* showing a wide range of effects of GH from beneficial to neutral or deleterious. The findings on wound healing are therefore inconclusive.

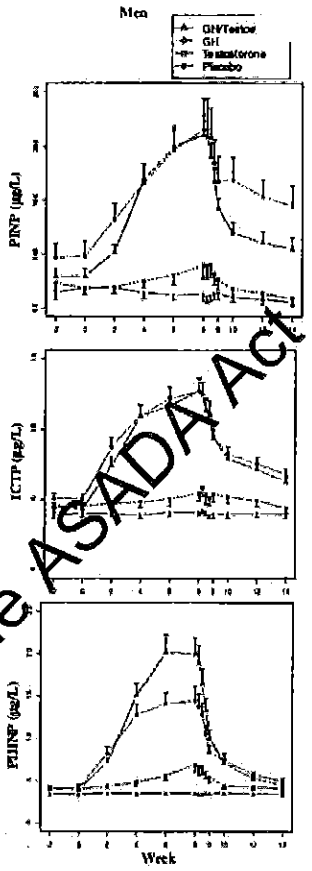
9.5. In summary, the effect of GH for improved injury healing shows a minimal to modest benefit. However, the available surrogate evidence cannot rule out effects from higher GH doses with or without combination with other drugs, notably androgens but also possibly tissue growth or angiogenic factors, for the milder sports related injuries including effects of vigorous training. Consequently this forms an additional basis for the banning of GH secretagogues under the "catch-all" provisions of S2.

10. Cumulative effects of long-term or repeated GH administration or exposure

A key issue is what would be the effects of prolonged or repeated cycles of administration of these peptides. On this issue, noting the lack of definitive clinical studies, some insight is available from experience of cumulative effects of administering GH and other hormones.

10.1. The effects GH on tissues are best exemplified by onset and offset of GH effects in the longer, well-controlled study (see #4.2) [24]. During GH treatment, serum IGF-I and related biomarkers

(IGFBP-3, ALS) usually peaked at the 1st time-point measured during treatment (2 weeks) and were largely reversed by a week after cessation of GH administration.
By striking contrast, the tissue effects of GH on 3 collagen peptides[2] were much slower to peak and persisted much longer (see figure).



The peak GH effect on collagen peptides was apparent only at the end of the 8 week treatment period and did not reach any plateau, which means that even higher effects may be evident with prolongation of GH treatment.
Furthermore, the collagen peptide responses were only slowly and partly reversed after cessation of GH treatment, with most effects persisting without having returned to baseline at the end of the 6 week post-treatment follow-up period. Hence this study provides only minimal estimates of the likely impact of GH treatment on tissues if treatment was prolonged beyond 8 weeks or even if repeated cycles of treatment were instituted before the effects of the previous treatment had fully worn-off. Furthermore, as the GH effects did not reverse by 6 weeks after cessation of treatment, it is likely that full reversal of GH effects may take several months. Thus if repeated cycles of GH treatment were to be re-started before full reversibility of GH tissue effects (assuming these effects are eventually fully reversible), then a "stair-case" pattern of a rising between-treatment plateau would be created. Presumably this re-creates the pattern of major tissue changes of acromegaly, a hypothalamic-pituitary disease which features persistent and prolonged excess of endogenous GH secretion leading to characteristic pathological tissue over-growth effects on bone, muscle, cartilage and joints.

10.2. The reversibility of hormone effects following cessation of exposure varies widely from full to partial reversal or to completely irreversible. Short-term biochemical responses are more often reversible whereas tissue effects, notably those of growth and pubertal maturation, are more often largely or fully irreversible. For example, virilisation effects of testosterone at male puberty or the growth effects of GH prior to and during puberty are largely irreversible, or at most, only partly and slowly reversed, even if hormone exposure is subsequently reduced or ceases.

10.3. One practical example of these effects is the eligibility of transgender people for sport in their transitioned gender. Whereas female-to-male (F2M) athletes are acceptable in male sports and male-to-female (M2F) are acceptable in female sports if the cross-gender transition and hormonal treatments commence prior to puberty, M2F transitioning after puberty is not generally considered reasonable as the gender-disproportionate bone and muscle growth during normal male puberty is largely irreversible, even if ongoing endogenous testosterone exposure is removed (see IOC consensus statement on transgender athletes, 2003).

10.4. Another therapeutic example of the partial reversibility of hormonal effects from a course of treatment enabling greater responses to repeated treatments is from the hormonal induction of testis development leading to spermatogenesis and fertility in gonadotrophin-deficient infertile men. In these gonadotrophin deficient infertile men, second and subsequent cycles of gonadotrophin replacement therapy are faster to reach the therapeutic endpoints (sperm output, fertility) than the first cycle [25]. This effect is because the testis growth produced by the first cycle of treatment is only partially reversed when hormone administration ceases. As a result, second and subsequent cycles start from a larger testis size baseline resulting in faster re-initiation of spermatogenesis.

10.5. Consequently it is likely that GH effects may last for up to several months even after only moderate doses with or without co-administration of androgens. The reversibility, and possibility of

[2] N-terminal propeptide of type I procollagen (PINP), C-terminal telopeptide of type I collagen (ICTP), N-terminal propeptide of type III procollagen (PIIINP)

additive, stair-case effects, depends on the dose and duration of GH treatment. While comparable details are not available for the GHS peptides, similar additive effects with other hormones as well as non-GH mediated effects may be produced by prolonged and/or repeated courses of drug exposure and which vary in their degree and tempo of reversibility after multiple cycles of hormone administration.

10.6. A further aspect of prolonged or repeated GHS peptide treatment is desensitisation of endogenous GH responses to stimulation. This is due to both down-regulation and desensitisation of the GHS receptor [26-29] as well as via effects mediated negative feedback inhibition of GH on its own secretion[11], a common feature of pituitary-dependent hormones that are characteristically regulated by negative feedback mechanisms.
As a result, prolonged or repeated doses of GH or GHS (via its effects on stimulating GH secretion), causes suppression of endogenous GH secretion, the magnitude and duration of which is not well defined. However the resulting GH deficiency state may persist well beyond the time when GH or GHS treatment ceases. Although the functional GH deficiency may be ultimately reversible, prolonged periods of post-treatment GH deficiency may have deleterious effects on health and sports performance.

10.7. This is analogous to the effects of exogenous androgens which inhibit endogenous testosterone production. That inhibition of endogenous testosterone production can last for many months to over a year beyond cessation of treatment. The recovery time depends on the dose and duration of exogenous androgen abuse. For example, heavy androgen abusers (eg bodybuilders who have used high doses for prolonged periods (years) without a break), have a characteristic suppression of their own reproductive system (subnormal serum testosterone, impaired spermatogenesis and infertility) which may take 12 months or more to recover full functionality. This is also analogous to the post-pill amenorrhea, a feature of the first generation of high estrogen dose oral contraceptives.

10.8. In practice, this might mean that athletes using GHS for prolonged periods or in repeated doses or cycles may experience functional GH deficiency as a withdrawal effect with deleterious effects on performance. How long this lasts until endogenous GH secretion recovers is not known but could be for many months or up to a year.
The prolonged tissue effects of GH or GHS including recovery may therefore extend for many months until the normal GH axis functionality returns.

11. Definition of GH releasing factors

11.1. The term releasing factor is a generic "term of art" in endocrinology referring to any substance which causes physiological or pharmacological release of another chemical, usually a hormone, which (by definition) then in turn enters the circulation to act on a distant cell or tissue. It is not a specific appellation of any particular chemical or hormones but rather it refers to a class or grouping of chemical substances which may have no chemical similarity but share biological effects. This term is also congruent with the concept of indirect doping, which is the use of a substance or method to cause increased release of a potentially ergogenic endogenous hormone (e.g. testosterone, GH) or substance (e.g. hemoglobin).

11.2. This definition is consistent with the term "releasing factors" in the section 2 (notably 2.4) of the Prohibited List in that releasing factors refers to any chemical which causes release of endogenous GH. This clearly includes GHRH and Ghrelin, together with their analogs.

11.3. It is less clear whether or not this extends to chemicals that have been used pharmacologically in single (high) dose, short-term (<2 hours) provocative tests of GH release to diagnose GH deficiency by stimulating endogenous GH secretion. These include insulin, arginine, lysine, clonidine, l-dopa, and glucagon. Their precise mechanism of action in stimulating acute GH release remains incompletely defined though the best evidence is that they involve modulation of the hypothalamic dual release and negative feedback mechanism that regulates endogenous GH release, rather than any novel

receptor-mediated mechanisms [30-35]. On that basis as well as the fact that there is no evidence that these chemicals produce sustained endogenous GH release, the minimal medical or pharmacological significance means the mechanism is unlikely to be elucidated in the near future.

12. Rationale for banning GH releasing peptides.

12.1. The banning of GH releasing factors, also known as GH secretagogues, is dependent on their effects in stimulating endogenous GH secretion. As GH itself is banned under S2, the administration of substances that stimulate endogenous GH would constitute indirect GH doping and therefore warrant banning.

12.2. Moreover, it is likely that there are additional performance enhancement effects of GH secretagogues via more speculative but plausible claims of non-GH mediated effects as well as masking effects [12]. These claims include improved tissue healing and therefore recovery from injury and/or supporting higher intensity training with use of Ghrelin [36-41] or GHRH [38, 42] analogs. These would constitute an additional basis for banning GHRH analogs or GH secretagogues under the "catch-all" provision of S2 for various growth factors with similar chemical or biological effects.

13. Specific GH releasing peptides

Most of the peptides under review are Ghrelin analogs acting via the GHS receptor with the common features being they are short peptides, making them easily and cheaply synthesized by widely available commercial peptide production facilities. They all contain artificial amino acids which extend the duration of action of the peptide by inhibiting the otherwise very rapid metabolism by endogenous peptidases, which creates a very brief duration of action. The artificial amino acids are also valuable xenobiotic signatures that permit more facile detection of these peptides.

13.1. **The structure of the peptides are listed in the table following:**

Table 1. Growth Hormone Releasing Peptides, Metabolite for GHRP-2, and the Used ISTDs with Their Amino Acid Sequence, Elemental Composition, Monoisotopic Masses, and Dominant Charge State[a]

name	amino acid sequence	monoisotopic mass [Da]	elemental composition	dominant charge state (ESI)
GHRP-2	(D-Ala)-(*D*-β-Nal)-Ala-Trp-(*D*-Phe)-Lys-NH_2	817.427	$C_{45}H_{55}N_9O_6$	2+
GHRP-1	Ala-His-(*D*-β-Nal)-Ala-Trp-(*D*-Phe)-Lys-NH_2	954.486	$C_{51}H_{62}N_{12}O_7$	2+
GHRP-6	His-(*D*-Trp)-Ala-Trp-(*D*-Phe)-Lys-NH_2	872.444	$C_{46}H_{56}N_{12}O_6$	2+
GHRP-5	Tyr-(D-Trp)-Ala-Trp-(D-Phe)-NH_2	770.354	$C_{43}H_{46}N_8O_6$	1+
GHRP-4	(D-Trp)-Ala-Trp-(D-Phe)-NH_2	607.292	$C_{34}H_{37}N_7O_4$	1+
alexamorelin	Ala-His-(*D*-Mrp)-Ala-Trp-(*D*-Phe)-Lys-NH_2	957.497	$C_{50}H_{63}O_7N_{13}$	2+
hexarelin	His-(*D*-Mrp)-Ala-Trp-(*D*-Phe)-Lys-NH_2	886.460	$C_{47}H_{58}N_{12}O_6$	2+
ipamorelin	Aib-His-(*D*-β-Nal)-(*D*-Phe)-Lys-NH_2	711.385	$C_{38}H_{49}N_9O_5$	1+/2+
GHRP-2 metabolite	(D-Ala)-(*D*-β-Nal)-Ala	357.168	$C_{19}H_{23}N_3O_4$	1+
ISTD1	(D-$^{[2]}H_3$-Ala)-(*D*-β-Nal)-Ala	360.187	$C_{19}H_{20}{}^{[2]}H_3N_3O_4$	1+
ISTD2	(D-Trp)-$^{[2]}H_4$-Ala-Trp-(D-Phe)-NH_2	611.315	$C_{34}H_{33}{}^{[2]}H_4N_7O_4$	1+

[a] Nonstandard abbreviations: Nal = naphthylalanine, Mrp = 2-methyltryptophan, Aib = aminoisobutyric acid.

Table from: Thomas et al, Anal Chem 84: 10252-10259, 2012

14. ***GHRP-6***

14.1. **WADA Status:** S0 & S2
S2: GHRP-6 is a releasing factor of endogenous GH.
S0: GHRP-6 has never been approved for therapeutic use by any regulatory agency

14.2. **Chemical structure:** See table. GHRH-6 was the first synthetic Ghrelin agonist to be purpose-developed. It arose from the surprising discovery of potent GH releasing activity of the pentapeptide

enkephalins[43, 44]. GHRP-6 is a synthetic hexapeptide modelled on the enkephalin structure but modified to feature a terminal amide and two synthetic D amino acids to inhibit proteolytic degradation and prolong bioactivity.

14.3. **Physiology & Pharmacology:** GHRH-6 is a synthetic Ghrelin agonist which stimulates GH secretion via the GHS (Ghrelin) receptor. Additional effects of GHRP-6 on other pituitary hormones (prolactin, ACTH) and on tissue protective effects (antioxidant, re-perfusion recovery) are also reported. The duration of effect GHRP-6 effects is brief with blood levels of GHRP-6 remaining detectable for <12 hours after a single dose [45] while the GH secretion response lasts no more than 2-3 hours [46, 47]. Although substantive data are lacking, it is a reasonable speculation that any tissue effect of additional GH exposure stimulated by GHRP-6 administration would last no more than a few weeks beyond the last GHRP-6 dose.

14.4. Thus, as a drug designed to and which does cause release of endogenous hGH, GHRP-6 is considered as a doping agent under S2.

14.5. **Safety:** Most therapeutic use of GHRP-6 reported has been proof of concept studies using single doses for comparison of GH releasing and other effects compared with GHRH and/or Ghrelin agonists. The longest duration study of GHRP-6 administration in humans was in 7 older women who were administered 300 µg/kg twice daily for 4 days without reported adverse effects [47]. This minimal extent of safety exposure is inadequate to support use of GHRP-6 outside carefully monitored and designed therapeutic trials under supervision of a competent HREC.

15. ***Hexarelin***

15.1. **WADA Status:** S0, S2

S2: Hexarelin is a releasing factor of endogenous hGH

S0: Hexarelin has never been approved by any regulatory agency for human therapeutic use

15.2. **Chemical structure:** See table. Hexarelin was developed as a more potent Ghrelin agonist than the first Ghrelin agonist, GHRP-6. It differs from GHRP-6 only in one amino acid (D-methyl tryptophan replacing D-Trp at position 2).

15.3. **Physiology & Pharmacology:** Hexarelin has very similar pharmacology to GHRP-6 and stimulates GH secretion in a similar pattern to other GHRH or Ghrelin analogs. A single dose of hexarelin increases serum GH levels for up to 3 hours (and that of other hormones like ACTH, cortisol and prolactin for up to 1 hour). Continued dosing leads to desensitization and no consistent increase in serum IGF-I (an integrated measure of GH effects) [48-53]. Although there is no data, it is most likely that the consequential GH effects might persist for a longer period, perhaps a week at most.

15.4. Thus, as a drug designed to and does cause release of endogenous hGH, hexarelin is considered as a doping agent under S2.

15.5. **Safety:** Hexarelin has been used in over 50 clinical research studies each involving a median of 12 (range 6-54) participants using intravenous or subcutaneous injections in doses ranging from 1-2 µg/kg. Nearly all were single dose experimental studies so that knowledge of the safety profile for multi-dose usage is minimal. The longest studies investigating repeated administration of hexarelin have used (a) twice daily subcutaneous injection of 1.5 µg/kg for 16 weeks in 12 elderly volunteers [52, 53], (b) 18 mg/kg for 8 days or 300 µg/kg for 15 days in 7 elderly volunteers [50] or (c) thrice daily intranasal spray of 60 µg/kg in 7 children for up 10 months [48, 49]. The studies showed no consistent increase in serum IGF-I levels and a consistent partial desensitization (reduction of hexarelin stimulation of GH secretion) during prolonged hexarelin administration. No serious adverse effects were reported in any of these studies. The lack of any more than single dose studies of hexarelin reported since 2000 indicates the status of hexarelin is as a clinical research diagnostic tool, without prospect of therapeutic use primarily due to its weak and ill-sustained efficacy (judged by sustained

elevation of serum IGF-I effects). This is sufficient safety exposure for therapeutic research studies under HREC supervision but not for wider, unsupervised therapeutic use especially without a valid medical indication.

16. ***CJC-1295***

16.1. **WADA Status:** S0, S2
S2: CJC-1295 is a releasing factor of endogenous hGH
S0: CJC-1295 has never been approved by any regulatory agency for human therapeutic use.

16.2. **Chemical structure:** CJC-1295 is a 30 amino acid analog of 1-29 hGHRH stabilised by 4 amino acid substitutions plus an additional 3-maleimidopropionic acid (MPA) unit added to extra lysine at the C terminus.

16.3. **Physiology & Pharmacology:** CJC-1295 retains full GH releasing bioactivity of hGHRH but has a prolonged circulating half-life (and therefore duration of action) because the C terminal MPA unit forms an *in vivo* bioconjugate with circulating serum albumin through its free thiol group on Cys34[54]. A proof of principle study in GH deficient mice showed that daily, but not 2nd or 3rd daily, injections of CJC-1295 fully rectified growth to achieve normal weight, length and body composition as well as increased serum IGF-1. Injections every 2nd or 3rd day normalised body composition but not growth or serum IGF-I levels.

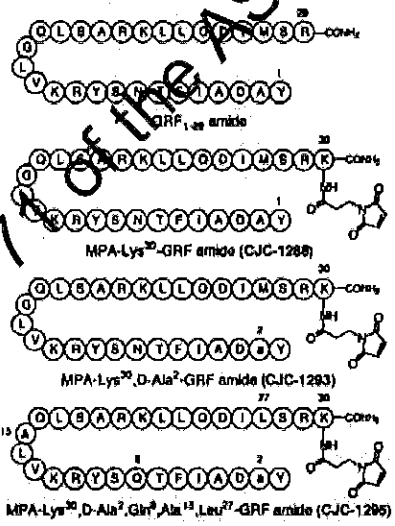


FIG. 1. Molecular structures of $hGRF_{1-29}$ amide and the three maleimido derivatives CJC-1288, CJC-1293, and CJC-1295, prepared by solid-phase synthesis. CJC-1288 is $hGRF_{1-29}$ with an extra lysine (Lys) at the 30-position to accommodate a MPA; CJC-1293 is equivalent to CJC-1288 but with a D-alanine (D-Ala) at the 2-position; and CJC-1295 is a tetrasubstituted analog of CJC-1288 but with a D-Ala at the 2-position, a glutamine (Gln) at the 8-position, an Ala at the 15-position, and a leucine (Leu) at the 27-position.
Jette et al Endocrinology 146: 3052-8, 2005

CJC-1295 has been reported in only two therapeutic trials conducted by single principal investigator (Dr L Frohman, Chicago). The first studied pulsatile GH secretion following a single subcutaneous injection of CJC-1295 (either 60 or 90 µg/kg) in 12 healthy men [55]. The second study involved a dose-finding study comprising 42 single doses (ranging from 30 to 250 µg/kg) and then 24 participants of whom 12 received two doses at 2 week intervals (30 or 60 µg/kg) and another 12 who received three doses at weekly intervals (30 or 20 µg/kg)[56]. Plasma CJC-1295 levels were detectable for up to 14 days after a single injection at the highest doses (125 & 250 µg/kg) with and serum IGF-I was elevated for ~10 days at all doses and 14 days at the highest dose (250 µg/kg)[56].

16.4. Thus, as a drug designed to and does cause release of endogenous hGH, CJC-1295 is considered as a doping agent under S2

16.5. **Safety:** The safety experience of CJC-1295 pooling both reported studies consisting of 114 injections in 66 individuals (assuming none participated more than once). Both studies reported injection site reactions which were dose dependent with induration lasting up to 5 days at higher dose but all resolved spontaneously. In the single dose study, tachycardia and injection site irritation were reported in some men but no serious adverse effects. In the multi-dosing study, flushing, dizziness, hypotension, headache, diarrhea, incoordination with leg muscle contractions were all reported but resolved spontaneously without lasting sequelae. There were no abnormalities detected in routine safety lab tests (biochemistry, hematology). No other serious adverse effects were noted. This safety experience is neither alarming nor reassuring and could be considered sufficient to support carefully monitored therapeutic research study under supervision of a HREC but not for wider unsupervised therapeutic use.

17. ***Thymus Extract Peptides, Thymomodulin and Thymosins***

17.1. Thymomodulin is a term that refers to a crude extract of calf thymus produced in Europe during the early to mid-20th century. It has sometimes been referred to as "thymic" or "thymus" hormones. At that time, prior to the modern detailed understanding of immunology, the thymus was known to be present at a young age and to virtually disappear by adulthood but its precise function was not yet known. By a process of little more than wishful thinking it was considered as a potential means of rejuvenation of youthful vigour and healing capacity. The calf thymus extract was described as a cell-free acid lysate so that, like any biological extract, it is a mixture of probably hundreds or thousands of active and inactive proteins – including ones that have opposing effects - making it subject to batch-to-batch variation in composition and effects. This makes it difficult, if not impossible, to standardise dosage or to evaluate therapeutic safety by modern standards.

17.2. Such crude extracts were used to important effect in the 19th and early 20th century laboratory research to identify and purify hormonal effects and ultimately to fully characterize the hormones we now know. Such crude extracts including thymomodulin were also popularly promoted by quack rejuvenation clinics, which proliferated in mid-20th century Europe. Till the middle of the 20th century crude biological extracts (eg dessicated thyroid extract, posterior pituitary snuff, equine estrogens) were still used therapeutically in medicine but have been supplanted by purified hormones as they became properly identified in the latter part of the 20th century. Crude extracts are an important first step along the discovery pathway of identifying important biological proteins, but they are definitely outmoded and unacceptable as therapeutic substances by the standards of medicine in the 21st century.

17.3. Thymomodulin was partially purified into subfractions called thymosins [57]. Some forms of thymomodulin continued to be marketed and used into the late 20th century in Europe [58, 59]. Thymosin fraction 5 (TF5) was used in some small therapeutic trials [60] but it appears never to have been formally marketed. TF5 was a family of at least 40 (and probably many more) mostly small acidic polypeptides with molecular weights 1,000 to 15,000 [61]. Subsequently, further purifications of TF5 by isoelectric focussing divided TF5 into 3 broad subsets, based on their pH, comprising highly acidic (α), acidic (β) and basic (γ) fractions. Each of these pH fractions comprised many distinct proteins which were then given numerical subscripts (α_1, α_2, α_3, β_1, β_2, β_3 etc) according to their appearance as bands on the purifying gels. However, even these gel fractions are not necessarily single proteins but can also be mixtures. Further work has clarified the precise molecular structure of many of these thymosins.

17.4. Thymosin α_1 and β_4 have been fully characterized structurally according to their precise amino acid sequence and developed for therapeutic trials.

17.5. **Thymosin Beta-4**

17.5.1. **WADA Status:** S0, S2

S2: Thymosin β4 is a growth factor affecting muscle, tendon or ligament, vascularisation and regenerative capacity.
S0: Thymosin β4 has never been approved by any regulatory agency for human therapeutic use

```
thymosin-β4   Ac-SDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETIEQEKQAGES
thymosin-β10  Ac-ADKPDMGEIASFDKAKLKKTETQEKNTLPTKETIEQEKRSEIS
thymosin-β15  Ac-SDKPDLSEVETFDKSKLKKTNTEEKNTLPSKETIQQEKEYNQRS
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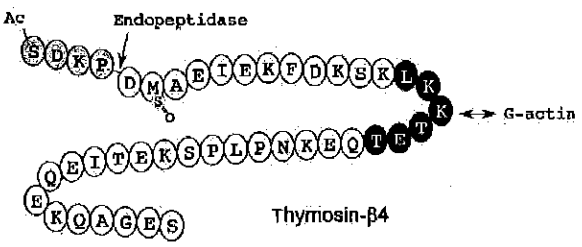


Thymosin β-4 structure. From Hara Vitam Horm 2011

17.5.2. **Chemical structure:**

Thymosin β4 is a 43 amino acid peptide. The molecular structure of Thymosin β4 is shown in the diagram opposite where each letter indicates one of the 20 different amino acids

17.5.3. **Physiology & Pharmacology:**

Thymosin β4 is a member of the family of thymosins, a highly conserved family of 40-60 small peptides

originally purified from calf thymus. They now are divided into 3 groups (α, β, γ) according to their isoelectric points. The thymosin β family have a neutral pH (5.0-7.0) and includes ubiquitin (thymosin β-1) but with thymosin β-4 together with several others being highly homologous and having overlapping tissue regeneration and recovery functions [62].

17.5.4. **TB-500, an analog of thymosin β4**

TB-500, a short peptide analog of thymosin beta 4 has been identified in horse doping [63, 64] and the prospects of thymosin ß-4 as a doping agent has been outlined [65]. As TB-500 was invented as an analog of thymosin β4, it is presumed by design to have the same properties as thymosin β4. These include acting as a growth factor which affects muscle, tendon or ligament vascularisation and regenerative capacity hence banned under WADA category S2. TB-500 has not been marketed for human therapeutic use anywhere. Hence, TB-500 is banned under the WADA Prohibited List categories S0 and S2.

17.5.5. Thymosin β-4 has both intracellular and extracellular functions [66]. The intracellular function is primarily as a G-actin monomers binding protein which acts to sequester the actin in the form of monofilaments in dynamic balance with F-actin polymers. These stabilise cellular shape and mobility including muscle contractility. Such intracellular functions are likely to be impervious to administration of exogenous thymosin β-4.

17.5.6. Thymosin β-4 has both intracellular and extracellular functions [66]. The intracellular function is primarily as a G-actin monomers binding protein which acts to sequester the actin in the form of monofilaments in dynamic balance with F-actin polymers. These stabilise cellular shape and mobility including muscle contractility. Such intracellular functions are likely to be impervious to administration of exogenous thymosin β-4.

17.5.7. The extracellular functions of thymosin β-4 include angiogenesis [67-78], wound healing [79-88] and chemotaxis of cells involved in inflammation [89, 90] and tissue regeneration including skeletal and cardiac muscle [90-94]. The angiogenic effects vascularisation) involve interactions with hypoxia-inducing factor [70-72, 75] and Notch signalling [78, 95, 96], a pathway involving on hypoxia-inducing factor.

17.5.8. These functions of thymosin β-4 may not be entirely beneficial as noted in cautions from experimental studies suggesting that thymosin β-4, via enhancing cell migration and angiogenesis, may promote the metastatic potential of certain cancers [97, 98].

17.5.9. Thus, as a growth factor affecting muscle, tendon or ligament, vascularisation and regenerative capacity as well as having interaction with hypoxia-inducing factor, thymosin beta 4 is considered a doping agent under section 2 of the Prohibited List.

17.5.10. **Safety:** Thymosin Beta-4 has been administered in one phase I, one small therapeutic trial and an uncontrolled case series. The single phase I study investigated the effects of single and multiple doses of thymosin β-4 in healthy volunteers who underwent intravenous administration of a sterile pharmaceutical company manufactured product in doses ranging from 42 mg up to 1260 mg[99]. After a single dose to 40 participants, the multi-dose phase of the study was conducted involving 20 volunteers from the first single dose group plus another 20 volunteers who all underwent daily injections for 14 days. A wide range of mostly mild and reversible adverse effects (as judged by a drug in development for therapy of patients with serious illness), more frequently in those receiving thymosin β-4 compared with placebo, were recorded but no serious adverse effects, dose-limiting toxicity or deaths were reported. Follow-up for risk of cancer promotion was limited to 6 months.

A placebo-controlled therapeutic study involved 72 patients with venous stasis ulcers who were randomised to one of 3 doses (concentrations) of topical application of a dermal gel containing thymosin β-4 or placebo for 12 weeks. Despite a study design that was favourable to the trial product by excluding common underlying diseases that delay wound healing (eg arterial disease, diabetes), the study found no significant overall benefit of any dose of thymosin β-4 on wound healing. The failure of

thymosin β4 to effectively heal venous ulcers in a single study has many possible explanations which remains consistent with thymosin β4 still being an effective drug. These reasons include suboptimal study design for some or all of the following reasons: wrong patient population, inadequate dosage regimen, too small a sample or too short treatment. For a first-in-human therapeutic trial, safety precautions always dictate the use of the minimum dosage regimen likely to be effective. This standard precaution may tend to underestimate the drug's optimal efficacy. Hence inadequate efficacy in the first human therapeutic trial is not surprising and does not mean the drug is necessarily ineffective. It is well understood that even if a drug does ultimately prove ineffective or unsafe for human therapeutic use, it may still be abused by elite athletes with doping intent.

A small and uncontrolled case series based on compassionate use approval claimed benefits of thymosin β-4 ophthalmic solution for improving epithelial regrowth of chronic non-healing corneal ulcers [100].

17.5.11. The use of thymosin β4 in pre-registration human therapeutic trials is not the same as the drug having been approved or registered for marketing. Early, pre-registration therapeutic trials for a new, unapproved drug are always conducted under the ethical jurisdiction of, and monitoring by, a human research ethics committee (HREC). Among many other conditions, this requires the patient to provide written informed consent to the unproven treatment. Registration of a drug for therapeutic use requires a sequence of large and complex clinical therapeutic trials which must be completed satisfactorily before the drug dossier is submitted for registration. If it is successful, the drug is approved for general marketing as a proven treatment of a specific medical disease or condition. After registration the therapeutic use of the drug no longer requires ethical approval and informed consent for treatment and may be prescribed by a duly qualified and registered medical practitioner for that indication.

17.5.12. No form of thymosin β_4 is yet approved for human therapeutic use anywhere in the world. In concert, these findings would only support the safety of thymosin β-4 for therapeutic use using a pharmaceutical grade product under the ethical approval and supervision of a HREC for a valid medical indication. No usage outside carefully monitored and ethically approved therapeutic trials is acceptable medical practice in 21st century Australia.

17.6. Thymosin α1

17.6.1. WADA Status: Not banned

Thymosin α1 is registered for human therapeutic use in several countries so is not S0. The countries that registered thymosin α1 for therapeutic use are less developed and developing countries with national drug regulatory affairs bureaus having limited within-agency expertise and uncertain transparency. Thymosin α1 is not registered by any major national or regional regulatory agency. Although immune modifying effects can be considered as a growth factor for lymphocytes, thymosin α1 does not have any of the specific physiological or pharmacological growth factor properties outlined under S2.

17.6.2. Chemical structure:

Thymosin α1 is 28 amino acid peptide depicted in the adjacent figure using standard three letter codes for the different amino acids. The peptide is not glycosylated and the N terminus is acetyated.

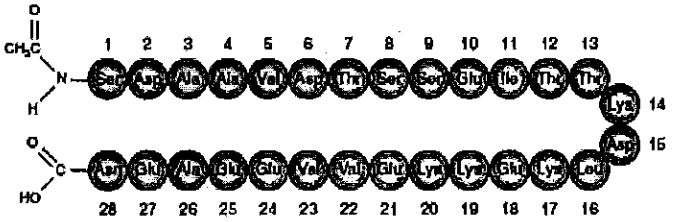


Figure 1. Structural formula of thymosin α_1.

17.6.3. Physiological and pharmacological effects

Thymosin α1 has a wide variety of physiological and pharmacological effects based on experimental studies in animals, cells and cell-free systems. The major physiological and pharmacological effects of thymosin α1 are immunomodulatory or immunostimulant effects that include induction of immune

competence for maturing lymphocytes within the thymus, enhancing immune responses to infective agents or anti-cancer activity via stimulation of immune function of lymphocyte subpopulations.

17.6.4. **Clinical therapeutic trials and registration**

Thymosin α_1 has been marketed in a variety of countries for treatment of hepatitis B and C and "immune stimulant and adjuvant" effects involving co-ordinate activation of the innate and adaptive immune systems [101]. Other potential therapeutic benefits, none having sufficient proof to achieve marketing status, include adjuvant boosting of vaccine effectiveness, anti-cancer efficacy, enhancing recovery from infectious illness, immunodeficiency and cancer chemotherapy-induced myelosuppression [102-107]. It is notable that these approvals were solely in less developed and developing countries whose national regulatory agencies have limited in-agency drug regulatory expertise. They are often reliant on decisions of the major regulatory agencies in developed countries such as USA (FDA), Canada (Health Canada), UK (MHRA), Germany (BfARM), Sweden (MPA), Netherlands (MEB) and Australia (TGA). Notably thymosin α_1 is not approved by any of the major national regulatory agencies.

18. *Human Therapeutic Trials*

18.1. A valid indication for medical treatment is a reason that makes it advisable to administer a specific drug or treatment to prevent, treat or cure a medical disease or condition. An indication must be well justified by correct diagnosis of an established medical disease, sound understanding of the disease pathophysiology and adequate clinical evidence of therapeutic benefit with acceptable safety. On the contrary, there is no medical indication to treat a healthy person without any known disease with a prescription medication.

18.2. Conduct of human therapeutic trials in Australia is a highly regulated activity. In Australia, the administration to any person of a new, unapproved drug for therapeutic purposes can only occur with prior approval from a Human Research Ethics Committee (HREC). No such therapeutic trial can commence without full and final prior HREC approval. This approval requires the trial sponsor (the person, institution or agency who takes legal responsibility for the proposed therapeutic trial) and the responsible doctor to submit a detailed clinical trial protocol for review to the HREC. Typically, this protocol must include details of a reasonable rationale for the study balancing risks against benefits. It must also provide an acceptable justification for the proposed treatment (dose, duration, drug formulation) based on the drug's known physiology, pharmacology, pre-clinical toxicology and the available experience from previous human therapeutic trials. In evaluating the safety of any new non-marketed drug, production in a GMP-licensed facility would be expected especially for a drug intended to be administered to the whole body by injection, implantation or transdermal application.

18.3. A mandatory component of any therapeutic trial is the requirement for written informed consent for the participants. This is achieved by providing the potential participants with an approved patient information statement and consent forms (PIS/CF) which must explain, to the satisfaction of the HREC, in clear, non-technical terms the reason for the study, the requirement for participation in the study, the risks and benefit of participating in the trial, what are the alternatives to participation and what remedies are available in the event of adverse effects. The explanation in the PIS/CF must be sufficient to make clear to potential participant all the study requirements as well as likely risks and benefits so that their signature can be deemed to constitute informed consent for participation in the study. Meeting these requirements, including responding to question from the HREC usually requires multiple submission over a couple of months.

18.4. In addition, for a therapeutic trial of any new, non-approved drug or even approved drugs when used "off-label" in an experimental setting, the Therapeutic Goods Administration (TGA) must give its approval for the study to proceed. This can be through either the Clinical Trial Notification (CTN) or Clinical Trial Exemption (CTX) schemes. Only after all approvals are completed can the study commence. During the study the HREC continues to monitor the study's safety by requiring timely

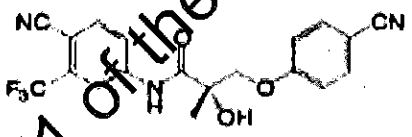
reports of any adverse effects with an evaluation of their severity and likelihood of being due to the drug. In addition, study lead investigators must complete an annual report to the HREC on the study's progress which summarises all adverse effects observed.

19. ***SARM S22***

19.1. **WADA Status:** S0, S1.1, S1.2
S1.2: S22 is a SARM
S1.1: S22 is an exogenous androgen, a substance with similar chemical structure and biological effects as other synthetic androgens
S0: S22 has never been approved by any regulatory agency for human therapeutic use

19.2. **Chemical structure:** S22 [108] is an aryl propionamide derivative, one of the early lead compounds in the class of non-steroidal androgens. Its chemical structure is S-3-(4-nitrophenoxy) and S-3-(4-cyanophenoxy) 2-hydroxy-2-methyl-N-(4-cyano-3-trifluromethylphenyl) propionamide [109].

S22



19.3. **Physiology & Pharmacology:** S22 is one of the early generation of non-steroidal androgens collectively referred to as Selective Androgen Receptor Modulators (SARM) [110].

19.4. This novel class of non-steroidal androgens was developed since the 1990s with the aim to develop more selective androgens which would have certain desirable properties, mainly stimulation of muscle growth and strength, without perceived adverse effects on the prostate. Historically this development program is a revival of the failed enterprise to develop a pure anabolic steroid, which is an androgen-based steroid that had the desirable muscle stimulating (anabolic) properties of testosterone without its adverse properties (undesirable virilisation) that render testosterone unsuitable for use in children and women. The remarkable Golden Age of steroid pharmacology - the post-war decades up to the 1970's - developed oral contraception and synthetic glucocorticoids both remaining major components of modern clinical pharmacology and therapeutics. However, during that Golden Age one quest was unsuccessful, the search for a pure anabolic steroid failed comprehensively and was abandoned by the pharmaceutical industry by the 1970's. Subsequent molecular biology explained that this failure was due to the existence of only a single identical androgen receptor in all tissues, rather than different mechanisms of action for testosterone muscle and other androgen target tissues.

19.5. Nevertheless, the wishful impulse for a more selective androgen persisted to be revived in recent decades. The modern revival of this quest for a selective androgen followed developments in the estrogens field where serendipitous discoveries showed that, for still largely unexplained reasons, some anti-estrogens could have beneficial estrogenic effects in certain tissues (e.g. bone, brain) but have equally advantageous effects as anti-estrogens (ie blocking estrogen effects) in other estrogen target tissues (e.g. breast, uterus). These chemicals (based on non-steroidal anti-estrogens) featuring mixed partial agonist/antagonist properties were then termed as being members of a novel class of "selective estrogen receptor modulator" (SERM), although this is actually a marketing term rather than precise pharmacological classification. By wishful analogy, hope triumphing over experience, the existence of SARMs was postulated and has been pursued in a modified framework – this time selectivity is framed as still desirable anabolic effects on muscle but the adverse effects are now stimulatory effects on the prostate (which might promote prostate diseases like prostate cancer).

19.6. S22 is a simple non-steroidal chemical developed in the second generation of orally active, aryl

propionamide SARMs with favourable metabolic effects and prolonged duration of action [108]. As a non-steroidal compound, it would have direct androgenic effects via its interaction with androgen receptors. However, it would lack other testosterone effects such as those mediated via aromatisation (testosterone's conversion to estradiol by the enzyme aromatase) or via androgen amplification (testosterone activation to a more potent androgen, dihydrotestosterone by 5α-reductase enzymes). It is therefore almost certain that S22 would have ergogenic effects due to increasing muscle mass and strength in humans, although this remains to be confirmed for this specific SARM. It is highly likely that this is a correct assumption as the first therapeutic studies of a closely related SARM, enobosarm (also known as osterine, GTx-024 & MK2866) show significant increases in muscle mass, strength and performance [111, 112].

19.7. Although S22 was synthesized and reported as part of a pharmaceutical company pre-clinical development program, it is among the vast majority of compounds that end up as discarded by-products of the search for a promising lead drug that warrants the large-scale investment required to enter a formal therapeutic development program. There is no evidence, nor any likelihood in the foreseeable future, that S22 will ever be developed for therapeutic registration and marketing. On the other hand as a relatively simple chemical it is readily adaptable to large scale industrial manufacture and is readily available from Chinese research chemical websites.

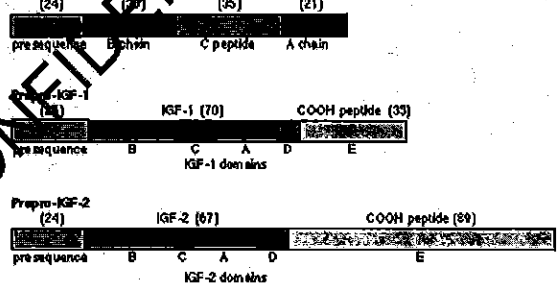
19.8. Thus, as a drug designed to act as an androgen, S22 is considered a doping agent under section 1.2 of the Prohibited List.

19.9. **Safety:** As a non-marketed androgen, there is no human safety data. The use of this compound in Australia would require formal approval of a therapeutic trial by a competent, registered human ethics committee and a CTN or CTX approval for use of a non-marketed drug from the TGA. The pre-clinical data on the use of S22 is too limited to provide any reliable guidance let alone conclusions on its human safety.

20. *Insulin-like Growth Factor 2 (IGF2)*

20.1. **WADA Status:** S2.5 (2011, 2012), S2.4 (2013, 2014), S0
S2.4 or 5: IGF2 has similar chemical and biological effects to insulin and IGF1
S0: IGF2 has never been approved by any regulatory agency for human therapeutic use

20.2. **Chemical structure:** IGF2 is a single chain polypeptide of 67 amino acids as a member of the insulin and insulin-like growth factor family of peptides with underlying structural and functional homology. It is initially secreted in a precursor form of 180 amino acids which is trimmed to the mature peptide in a sequence of processing steps within the secreting cell.



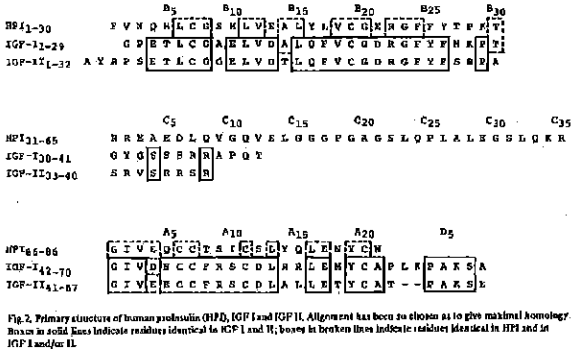
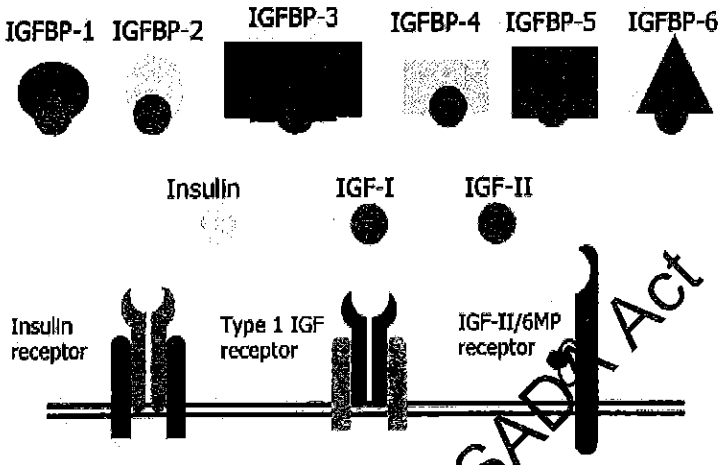


Fig.2. Primary structure of human proinsulin (HPI), IGF I and IGF II. Alignment has been so chosen as to give maximal homology. Boxes in solid lines indicate residues identical to IGF I and II; boxes in broken lines indicate residues identical in HPI and at IGF I and/or II.

20.3. **Physiology & Pharmacology:** In mammals including humans IGF2 is predominantly a fetal growth factor which is preferentially expressed in early embryonic and fetal development in a wide variety of somatic tissues[113, 114]. In fetal life IGF2 has a major role in the regulation of cell proliferation differentiation, growth, migration and cell survival including the musculoskeletal system whereas in adults its role is unclear but may have local tissue effects supporting cellular maintenance. In fetal and adult humans, IGF2 circulates largely bound to insulin-like growth factor binding proteins (IBFBP) 2 and 3. In adults IGF2 in the bloodstream is principally secreted by the liver but IGF2 is also produced locally within many other mature tissues. IGF2 action is primarily exerted via the IGF1 receptor and the mitogenic isoform type A insulin receptor while binding to the IGFBPs inhibits its effects and binding to the IGF2/mannose-6-phosphate receptor, a non-signalling "sink" receptor, contributes to clearance of IGF2 from the circulation.



Although IGF2 has predominantly prenatal roles, its high circulating levels in adult life together with its actions via the insulin family of receptors, suggests IGF2 has important ongoing physiological roles in postnatal and adult life. IGF2 has growth promoting activity in a wide variety of mature tissues including placenta, blood vessels, immune, bone and bone marrow cells. In the musculoskeletal system, IGF2 has a prominent role in stimulating muscle development, growth and maturation in the fetus. Whether IGF2 has a similar role in mature muscle and especially muscle healing and recovery from muscular injury (including severe training) remains speculative.

20.4. Thus, as a drug designed to act as an insulin-like growth factor with similar biological effects to insulin and IGF1, IGF2 is considered a doping agent under section 2 of the Prohibited List.

20.5. **Safety:** There are no therapeutic trials using IGF2 reported so its drug safety at any dose in humans has not been assessed. Excessive secretion of IGF2 by certain bulky human tumors causes a distinctive syndrome of tumor-related hypoglycaemia (dangerously low blood glucose). Based on its known physiology, pharmacological doses of IGF2 would be expected to risk mitogenic effects (such as promotion of cell proliferation in cancers) and/or causing hypoglycaemia.

21. ***Follistatin***

21.1. **WADA Status:** S0, S4.4
S4.4: Follistatin is an agent modifying myostatin function, a myostatin inhibitor
S0: Follistatin has never been approved by any regulatory agency for human therapeutic use

21.2. **Chemical structure:** Follistatin is a single chain polypeptide with complex substructural features reflecting its binding properties.

21.3. **Physiology & Pharmacology:** Follistatin is a member of the inhibin-activin-follistatin family of proteins which interact with the transforming growth factor (TGF) ß superfamily of proteins. Follistatin was originally identified as an activin-binding protein and subsequently wider interactions with the TGFß superfamily, notably with myostatin for the purposes of this report, have been defined.

21.4. Myostatin (also known as GDF8) is a muscle-specific member of the TGFß superfamily of proteins. Its characteristic physiological property is to limit muscle growth during pre-natal development by limiting the numbers of muscle fibres grown. There is strong evidence that inactivation of myostatin by genetic knockout in mice [115-120] and a variety of others species (cattle, sheep, dogs)[116] including humans [121], leads to excessive muscle growth although the quality of muscular function may be compromised [122]. There is also evidence that myostatin has a postnatal role in limiting growth of the existing stock of muscle fibres. This latter role has led to speculation that myostatin inhibition – by antibodies, dominant negative regulatory proteins or decoy receptor mechanisms - may have beneficial effects on muscle regrowth or turnover in adult life, such as for genetic muscular diseases [123, 124] and after injury [125]. Thus, based on these speculations, inhibition of myostatin has been considered as a mechanism to increase muscle mass and therefore strength and performance in power sports.

Structure of Myostatin-Follistatin-like 3

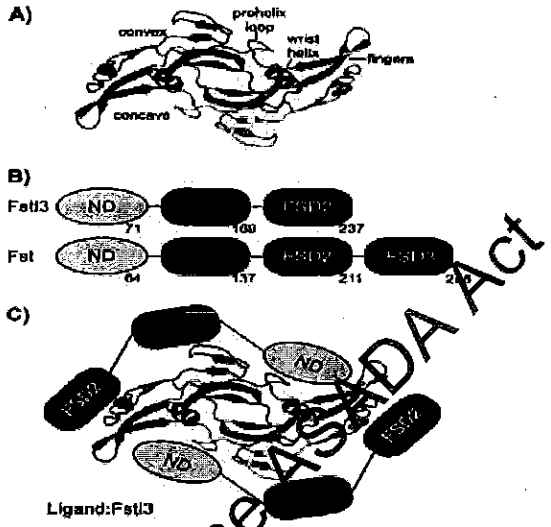


FIGURE 1. TGF-β and Fst-type protein family architecture and interaction. *A*, TGF-β family ligands form disulfide-bonded dimers with distinct architectural features, as labeled. Myostatin is shown as a representative. *B*, Fst and Fstl3 domain layout with the last residue of each domain shown. *C*, schematic of Fstl3 binding to ligand. The ND blocks the concave type I receptor-binding site, whereas FSD1 and FSD2 bind the convex type II receptor-binding site.

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21.5. Among various means to inhibit myostatin, follistatin has been considered a likely candidate. Follistatin binds to myostatin and inhibits its myogenic activity [115, 126]. Hence administration of follistatin may be considered as a potential doping agent with non-androgenic effects to increase muscle mass and strength. Whether this is effective or not in humans remains to be assessed.

21.6. **Safety:** There are no reported therapeutic trials with any form of follistatin so that human safety of this protein has not been assessed.

Conclusion

All these peptides and chemicals are covered by the WADA category of S0 and all by at least one other category.

None has been approved for any human therapeutic use rendering them all covered by S0.

In Australia, as non-approved drugs, these chemicals may only be used under supervision of a competent HREC for a valid medical indication or justifiable therapeutic research trial. This requires an approved, fully informed consent procedure and with TGA approval under the CTN or CTX scheme. In the absence of these clinical governance features, administration by injection or other means of these non-approved peptides to healthy humans is unacceptably risky and constitutes reckless and irresponsible behaviour.

Sourcing of peptides for injection into healthy humans using material manufactured outside a properly certified GMP production facility is unacceptable for safety reasons. GMP documentation is required to prove the authenticity of the product, purity from adulteration, sterility and non-pyrogenicity.

In considering the risks of administration, in addition to the authentic pharmacological effects of the peptides themselves and the uncertainty of safe dosing, the additional risks of non-approved products include toxic effects of unknown adulterants, of infections from non-sterile formulations, teratogenicity and, with repeated

use, carcinogenicity. As a result it is advisable that athletes exposed to repeated or prolonged use of peptides with GH stimulating effects be considered for long-term surveillance for the common cancers of the young adult age (testis, lymphomas).

The administration of such non-approved peptides by injection or other means, or even sanctioning their use by unqualified persons, by a doctor could be considered professional misconduct by the Medical Board of Australia. Administration by any medically unqualified person is risky, reckless and such behaviour should be considered as practising medicine without a license.

DJ Handelsman
November 2014

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Appendix - Relevant expertise:

Current appointment:

- Inaugural Professor/Director, ANZAC Research Institute (1998-present)
- Inaugural Head, Andrology Department, Concord Hospital (1999-present)
- Professor of Reproductive Endocrinology & Andrology (1996, Personal Chair, Univ of Sydney)

Professional training:

- MB BS (1974, Univ of Melbourne)
- Medical specialist qualification in Endocrinology (1980, FRACP)

Research training:

- PhD (1984, Univ of Sydney)
- NHMRC Neil Hamilton Fairley Postdoctoral Fellow, Harbor-UCLA (1984-6)
- Wellcome Senior Research (Postdoctoral) Fellow, Univ of Sydney (1987-9)

Service to research, professional and health policy advisory bodies:

- WHO Human Reproduction Programme (1988-1994)
- Australian Drug Evaluation Committee (1994-1998)
- President, Endocrine Society of Australia (1992-4)
- Secretary, International Society of Andrology (1997-2001)
- Chair, Endocrine Society of Australia's writing group (2000) to create the 1st national testosterone prescribing guidelines; adopted and remain the PBS prescribing criteria
- NHMRC Grants (Reproduction, Endocrinology) & Fellowship Panels for >25 years
- Inaugural member, Board of Andrology Australia (1999-present)
- Inaugural Chair, Scientific Advisory Board, Freemasons Foundation Centre for Men's Health, University of Adelaide (2007-present)
- Crown expert witness, Full Bench, Federal Court of Australia, highest court hearing testimony from non-legal experts
- Invited submission, House of Representatives Standing Committee on Health and Ageing's review of impotence medications.

Anti-doping research and expertise:

- Expert advisory panel, Australian Sports Drug Medical Advisory Committee (1999-present)
- Anti-Doping Research Panel (2002-14)
- World Anti-Doping Agency's Health, Medicine and Research Committee (2011-6)
- ASADA Advisory Group (2011-present)

Research track-record (since 1980):

- 340 peer-reviewed papers; 132 book chapters, reviews & reports; 439 scientific abstracts.
- Papers cited >12,000 times, average 23 citation/paper, h factor 58 (ISI Web of Science).
- Most actively cited author world-wide on "testosterone" (GOPUBMED database)
- Invited chapters in major textbooks of Endocrinology (De Groot's *Endocrinology*; Wass & Shalet's *Oxford Textbook of Endocrinology and Diabetes*) and Reproductive Biology (Knobil & Neill, *Physiology of Reproduction*).
- Served 14 editorial boards of peer-reviewed journals including currently Associate Editor, Male Reproduction, *JCEM* (2010-14) & Deputy Editor, *Asian Journal of Andrology* (2007-present).
- Invited ad hoc reviewer for 127 different peer-review journals
- Continuous research grant and contract funding since 1980 from peer-reviewed international and national funding agencies as well as the pharmaceutical industry
- Awards: Royal Australasian College of Physician's Susman Prize (1994); inaugural AMA Men's Health Award (2003); Honorary Life Member, Endocrine Society of Australia (2008).
- Supervised or co-supervised 22 PhD and 11 other graduate students.

BBC NEWS

HEALTH

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Northwick Park drug trial disaster - could it happen again?

By Philippa Roxby
Health reporter, BBC News

Before any new medicine can be given to patients, detailed information about how it works and how safe it is must be collected.

Clinical trials are the key to getting that data - and without volunteers to take part in the trials, there would be no new treatments for serious diseases such as cancer, multiple sclerosis and arthritis.

But one disastrous drug trial at a London hospital in 2006 threatened to derail that system.

In what became known as the Elephant Man trial, **six healthy young men were treated for organ failure** after experiencing a serious reaction within hours of taking the drug TGN1412 in a clinical trial.

After they were all admitted to intensive care, two became critically ill, the worst affected lost his fingers and toes, and all the men were subsequently told they would be likely to develop cancers or auto-immune diseases as a result of their exposure to the drug.

In follow-up interviews, **the men described feeling like their brains were "on fire" and their "eyeballs were going to pop out"**.

Experts queued up to say the outcome of the trial had been unprecedented and exceptional, but could it happen again?

Prof David Webb, professor of therapeutics and clinical pharmacology at the University of Edinburgh and vice president of the British Pharmacological Society, says it is "much less likely to happen again".

He says things have changed for the better since 2006, following a number of recommendations made in the Duff Report, written in response to the trial.

"The MHRA [Medicines and Health products Regulatory Agency] now ensures committees look at pre-clinical data, to decide whether the first dose given to humans is the right dose and has rules for stopping if things don't go as expected."

This is particularly important when trials involve drugs that affect the immune system, he says.

But is it possible to eliminate the risks entirely?

"You can mitigate against the risks, but nothing is 100% certain. We can never be sure," Prof Webb says.

The trial, which was privately run at a research facility at Northwick Park Hospital in north London, involved the first testing of a new drug on humans. This is the initial phase in assessing the safety of a drug before moving onto larger-scales studies in patients themselves.

The report said **Parexel**, the company managing the trial, had been unclear about a safe dose to start testing on humans and it should have tested the drug on one person at a time.

The MHRA, which regulates clinical trials and medicines in the UK, and which was criticised at the time for giving the green light to

the TGN1412 trial, says the conduct on these phase-one trials "has moved on significantly".

"Additional provisions and guidance has been put in place for certain novel products to provide as much assurance on safety as possible," the agency says.

It adds that it has simplified and streamlined the regulation of clinical trials and collaborated with other bodies and experts to collect as much information as possible on risk factors before a trial is authorised.

Phase-one trials, when drugs are tested on humans for the first time, only happen after extensive testing on tissue samples and animals in the lab.

Getting this stage right before moving onto research in humans is crucial.

Dr Catherine Elliott, director of clinical research interests at the Medical Research Council, which funds clinical trials in the UK and globally, says there is a move to refine the models used at the pre-clinical stage.

"Animal models are the mainstay, but we are trying to develop other models too to have more tailored disease models."

She says researchers are making use of brain imaging to understand the mechanisms of illness in humans and using IT to predict the effects of new drugs.

Testing on animals, which has its own controversies, can get scientists so far - but someone always has to be the first person to test a new medicine.

The volunteers for phase-one clinical tests always have to be healthy young men because of the risk to a woman's eggs or foetus.

Prof Webb says we are indebted to the 50 to 100 people in the UK each year who step forward to begin the testing of every new drug.

"There are so many effective medicines for cancer, heart disease et cetera - and they all come from volunteers who have taken part in small, early studies."

He believes that everyone who wants to should be able to register themselves available for clinical research through their GP.

"I would argue that everyone should be a volunteer. We'd get the payback eventually because by the time we're in our 60s and 70s most of us will end up on medicines."

Although volunteers are compensated for their time and inconvenience during the trial, they are not paid for taking part - and Dr Elliott says this is the correct approach.

"There shouldn't be an incentive to do something they wouldn't otherwise do. It shouldn't be related to risk. People have to be able to give free consent."

Despite all this, there appears to have been no reduction in interest in participating in early-stage trials since Northwick Park.

The MHRA says the number of UK clinical trial authorisation applications has been fairly stable at 900-1,000 per year since May 2004.

Prof Webb says he has always found it relatively easy to find volunteers for the "first in man" trials he oversees at his approved research centre in Edinburgh.

The MHRA is in no doubt about the safety of drug trials, seven years on from Northwick Park.

A representative said: "Clinical trials in the UK have an excellent safety record and they play a vital role in the development of new medicines, providing evidence so that clinicians can make informed prescribing decisions.

"Safety problems associated with clinical trials are rare and the risk of a repeat of the incident in 2006 concerning the TGN1412 drug is extremely low."

More Health stories

Drug trials process

Before a drug is tested on humans, it goes through laboratory and animal testing. Medicines are also tested for toxicity before being given to people.

Then there are four stages of drug testing in humans.

Phase I - studies, on a small number of healthy volunteers, to understand what effects a new medicine has on human subjects - what happens to the compound in the body from the time it is swallowed or injected until it is excreted. Study participants are monitored for side effects.

Phase II - designed to evaluate the safety and efficacy of a drug in patients who are at the same stage of a specific disease or condition. They are given various doses of a compound and closely monitored.

Phase III - used to confirm a new drug's safety and efficacy, while working out the best dosage regimen. Studies are carried out in large numbers of patients with a specific disease or condition. Safety and efficacy is compared to the currently accepted standard treatment.

Phase IV - these studies take place after the drug has been approved for marketing. They evaluate the long-term effects of the drug in larger numbers of patients, sub-populations of patients. Less common adverse events may be detected.