

HORMONES AND SPORT

Insulin, growth hormone and sport

P H Sonksen

Guy's, King's and St Thomas' School of Medicine, St Thomas' Hospital, London SE1 7EH, UK; Email: peter.sonksen@kcl.ac.uk

Abstract

This review examines some interesting 'new' histories of insulin and reviews our current understanding of its physiological actions and synergy with GH in the regulation of metabolism and body composition. It reviews the history of GH abuse that antedates by many years the awareness of endocrinologists to its potent anabolic actions. Promising methods for detection of GH abuse have been developed but have yet to be sufficiently well validated to be ready for introduction into competitive sport. So far, there are two promising avenues for detecting GH abuse. The first uses immunoassays that can distinguish the isomers of pituitary-derived GH from the monomer of recombinant human GH. The second works through demonstrating circulating concentrations of one or more GH-sensitive substances that exceed the extremes of normal physiological variability. Both methods require

blood rather than urine samples. The first method has a window of opportunity lasting about 24 h after an injection and is most suitable for 'out of competition' testing. The second method has reasonable sensitivity for as long as 2 weeks after the last injection of GH and is uninfluenced by extreme exercise and suitable for post-competition samples. This method has a greater sensitivity in men than in women. The specificity of both methods seems acceptably high but lawyers need to decide what level of scientific probability is needed to obtain a conviction. Both methods need further validation before implementation. Research work carried out as part of the fight against doping in sport has opened up a new and exciting area of endocrinology.

Journal of Endocrinology (2001) **170**, 13–25

Introduction

Doping in sport has a very long history going as far back as the original Olympic Games and is mainly driven by the desire to win at all cost. In order to maintain an environment where cheats do not win, it is essential to develop methods of combating abuse of performance-enhancing drugs. To this end the International Olympic Committee (IOC) Medical Commission and Sub-Commission 'Doping and Biochemistry in Sport' publish annually a list of 'banned substances' and have developed a sophisticated system for detecting drug abuse. Recent evidence indicates that the protein hormones insulin and growth hormone (GH) have now become a significant threat to the level playing field essential in sport. The IOC was swift to ban GH and insulin but no tests are available to detect their abuse. As endogenous substances secreted in bursts they pose particular problems in developing satisfactory methods of detection. Our understanding of how insulin and GH work in the regulation of metabolism and body composition has evolved to demonstrate a remarkable degree of synergy, something the athletes may have discovered before the endocrinologists.

GH has been used as a drug of abuse in sport since the early 1980s – 10 years before endocrinologists recognised and understood its potency as an anabolic agent and as a hormone regulating body composition in adults. Insulin appears to have a shorter history as a 'doping agent' – it was at the Winter Olympic Games in Nagano in 1998 when a Russian medical officer enquired as to whether the use of insulin was restricted to insulin-dependent diabetes. This drew attention to its role as a potential performance-enhancing drug and the IOC were swift to act and immediately placed it on its list of banned substances. Subsequent evidence from a needle-exchange programme in the UK (R T Dawson, personal communication) has confirmed its widespread use in body building and other sports although it remains unclear exactly how it is used.

This review presents a brief but important (and not very well-known) history of the physiology of insulin that is essential for understanding the way in which it is used as a performance-enhancing agent. It points out how, somewhat paradoxically, the advances of 'modern science', through inappropriate extrapolation from *in vitro* to *in vivo*, confused the thinking and teaching to hide the truth behind insulin action right up to the present day!

It attempts to 'set the record straight' about our current understanding of how insulin and GH interact as anabolic agents, about why athletes use them and outlines a way forward in detecting their abuse. It says nothing about anabolic steroids that are still major drugs of abuse and may well be synergistic with the effects of insulin and GH.

Insulin physiology

Sir Edward Schafer was Professor of Physiology in Edinburgh when he published in 1916 a wonderful book called *The Endocrine Organs*. The book is based on a series of lectures he delivered at Stanford University in California in 1913 (Schafer 1916). As well as containing a wealth of interesting insights into the early days of endocrinology, this book is most notable for the fact that it was the first time that the then hypothetical hormone insulin was named (8 years before it was discovered). What is even more remarkable, he predicted the formation of insulin from 'pro-insulin' 54 years before it was actually discovered!

Schafer was a contemporary of Baylis and Starling – two eminent academic rivals from University College in London. Shortly before Schafer delivered his lectures to his American audiences, Baylis and Starling had isolated, characterised and published about Secretin, the first 'hormone' (a term coined by them to describe a substance produced in one part of the body, carried by the blood stream and acting elsewhere in the body) to be isolated. Schafer questioned the use of the word 'hormone' and proposed two alternative names:

Autacoids – excitatory substances
Chalones – inhibitory substances

He went on to describe how 'his' new hypothetical hormone 'insuline' exhibited properties that resembled both *autacoids* and *chalones* and that the *chalone* or 'inhibitory' actions were physiologically the most important. It was, he proposed, lack of this *chalone* (inhibitory) action of insulin that led to a failure to store glucose in the liver with the net result that the liver overproduced glucose and glucose accumulated in the circulation, and this led to the hyperglycaemia that is characteristic of diabetes. This was indeed advanced thinking.

The 'black ages' of endocrinology followed early *in vitro* experiments in the 1950s that showed insulin to be capable of stimulating glucose uptake into bits of rat muscle and fat. Before long the biochemists had extrapolated from these experiments to conclude (wrongly) that the hyperglycaemia of diabetes was due to a 'damming back' of glucose in the blood stream as a result of a failure of glucose to enter cells as a direct consequence of insulin deficiency. The concept of glucose uptake into muscle being 'insulin dependent' was born, and has been prevalent in textbooks and teaching up to the present day, even though it was shown to be rubbish 25 years ago.

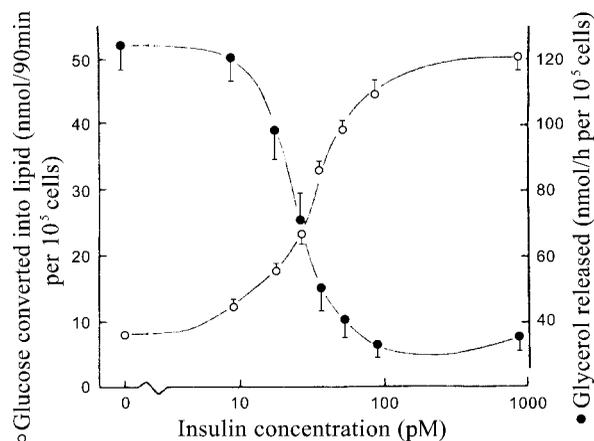


Figure 1 Insulin exhibits both inhibitory (*chalone*) and excitatory (*autacoid*) actions via the same receptor. In these experiments carried out on rat adipose tissue, *in vitro* insulin simultaneously inhibits lipolysis (the release of glycerol from stored triglyceride) and stimulates lipogenesis (formation of stored triglyceride from glucose). Thus its anabolic action is due to two mechanisms working synergistically.

The truth is that insulin acts exactly as Schafer had predicted – it acts as both an *autacoid* and a *chalone*. Through stimulating the translocation of 'Glut 4' glucose transporters from the cytoplasm of muscle and adipose tissue to the cell membrane it increases the rate of glucose uptake to values greater than the uptake that takes place in the basal state without insulin. This is most easily shown in isolated adipocytes from young rats and is illustrated in Fig. 1 (Thomas *et al.* 1979). What the 1950s biochemists failed to take notice of was the considerable uptake of glucose that takes place in all tissues even when insulin is absent. We now know that there is a sufficient population of glucose transporters in all cell membranes at all times to ensure enough glucose uptake to satisfy the cell's respiration, even in the absence of insulin. Insulin can and does increase the number of these transporters in some cells but glucose uptake is *never truly insulin dependent* – in fact, even in uncontrolled diabetic hyperglycaemia, whole body glucose uptake is inevitably *increased* (unless there is severe ketosis). Even under conditions of extreme ketoacidosis there is no significant membrane barrier to glucose uptake – the block occurs 'lower down' in the metabolic pathway where the excess of ketones competitively blocks the metabolites of glucose entering the Krebs cycle. Under these conditions, glucose is freely transported into the cell but the pathway of metabolism is effectively blocked at the entry point to the Krebs cycle by the excess of metabolites arising from fat and protein breakdown. As a result of this competitive block at the entry point to the Krebs cycle, intracellular glucose metabolites increase 'damming back' throughout the glycolytic pathway, leading to accumulation of free intracellular glucose and inhibiting initial glucose phosphorylation. As a result,

much of the 'free' intracellular glucose transported into the cell is transported back out of the cell into the extracellular fluid. Thus under conditions of ketoacidosis, glucose metabolism (but not glucose uptake) is impaired as a direct consequence of the metabolism of fat – the 'glucose–fatty acid' cycle (Randle *et al.* 1965).

In Fig. 1 it can be seen that simultaneously with insulin's *autacoid* effect in stimulating lipogenesis it also exhibits a *chalone* effect in inhibiting glycerol release. It is this inhibitory effect on lipolysis (and also glycolysis, gluconeogenesis, ketogenesis and proteolysis) that accounts for most of insulin's physiological effects *in vivo* in man. It is also this inhibitory effect that is mainly responsible for insulin's net anabolic actions.

It was the introduction of dynamic tracer studies that enabled us to resolve the misunderstanding about how insulin acts *in vivo* in man. Through infusing glucose (and other substrates) labelled with either radioactive or stable isotopes it is easily possible to measure accurately the *rates of glucose production* ('rate of appearance' – Ra) and *rates of glucose utilisation* ('rate of disappearance' – Rd) of glucose in the circulating blood. When these techniques were applied in people with uncontrolled diabetes it was found that the fasting hyperglycaemia was associated with rates of glucose appearance that were increased several fold above normal. Somewhat unexpectedly, it was also found that fasting glucose uptake was also increased. This is inevitable, since the fasting hyperglycaemia in diabetes is sustained and there is a 'dynamic steady state' where $Ra = Rd$. Thus both Ra and Rd are elevated. This finding clashed with the dogma obtained from extrapolating from *in vitro* experiments that had become embedded in the literature – namely that glucose uptake was 'insulin dependent' and was reduced in states of insulin deficiency. Despite a wealth of evidence confirming these early studies, this 'new concept' of insulin action remains unrecognised by the majority of teachers of physiology and biochemistry. A remarkable example of how difficult it sometimes is to reverse dogma even when it has been proven to be wrong (Sonksen & Sonksen 2000).

The facts are that in diabetes the fasting blood glucose is a very good measure of the severity of insulin deficiency. There is a linear correlation between the fasting blood glucose and the rate of hepatic glucose production (Ra) and thus with the rate of glucose disappearance (Rd). Since, in diabetes, the fasting blood glucose exceeds the renal threshold, not all glucose leaving the circulation is actually being metabolised. By collecting the urine and quantifying the urinary glucose losses it is easy to measure the true *rate of glucose utilisation* and the *rate of urinary glucose loss*. Glycosuria can account for as much as 30% of *glucose turnover* but even under these conditions, after correcting whole body glucose turnover for urinary glucose losses, tissue glucose utilisation is increased compared with normal. Thus insulin is NOT needed for glucose uptake and utilisation in man – glucose uptake is *NOT insulin dependent*.

When insulin is administered to people with diabetes who are fasting, blood glucose concentration falls. It is generally assumed that this is because insulin increases glucose uptake into tissues, particularly muscle. In fact this is NOT the case and is another error arising from extrapolating from *in vitro* rat data. It has been shown quite unequivocally that insulin at concentrations that are within the normal physiological range lowers blood glucose through inhibiting hepatic glucose production (Ra) without stimulating peripheral glucose uptake (Brown *et al.* 1978). As hepatic glucose output is 'switched off' by the *chalone* action of insulin, glucose concentration falls and glucose uptake actually decreases. Contrary to most textbooks and previous teaching, *glucose uptake is therefore actually increased in uncontrolled diabetes and decreased by insulin administration!* The explanation for this is that because, even in the face of insulin deficiency, there are plenty of glucose transporters in the cell membranes. The factor determining glucose uptake under these conditions is the concentration gradient across the cell membrane; this is highest in uncontrolled diabetes and falls as insulin lowers blood glucose concentration primarily (at physiological insulin concentrations) through reducing hepatic glucose production.

When insulin is given to patients with uncontrolled diabetes it switches off a number of metabolic processes (lipolysis, proteolysis, ketogenesis and gluconeogenesis) by a similar *chalone* action. The result is that free fatty acid (FFA) concentrations fall effectively to zero within minutes and ketogenesis inevitably stops through lack of substrate. It takes a while for the ketones to clear from the circulation, as the 'body load' is massive as they are water and fat soluble and distribute within body water and body fat. Since both ketones and FFA compete with glucose as energy substrate at the point of entry of substrates into the Krebs cycle, glucose metabolism increases inevitably as FFA and ketone levels fall (despite the concomitant fall in plasma glucose concentration). Thus insulin increases glucose metabolism more through reducing FFA and ketone levels than it does through recruiting more glucose transporters into the muscle cell membrane.

In fact, insulin does have a direct action to recruit more glucose transporters into muscle cell membranes and the effect of this is to facilitate glucose uptake – this is reflected as an increase in the metabolic clearance rate (MCR) of glucose. The MCR measured with tracer technology is a very important physiological measurement. It is defined as 'the amount of blood irreversibly cleared of glucose in unit time'. Expressed normally as ml/kg per min it is a non-linear function of blood glucose concentration (increasing as glucose concentration falls) and is highly sensitive to insulin (increasing with increasing insulin levels). It can readily be measured relatively non-invasively *in vivo* using non-radioactive tracers (or radioactive tracers but their use has been superseded by stable isotopes) and is of great importance in understanding substrate utilisation and its

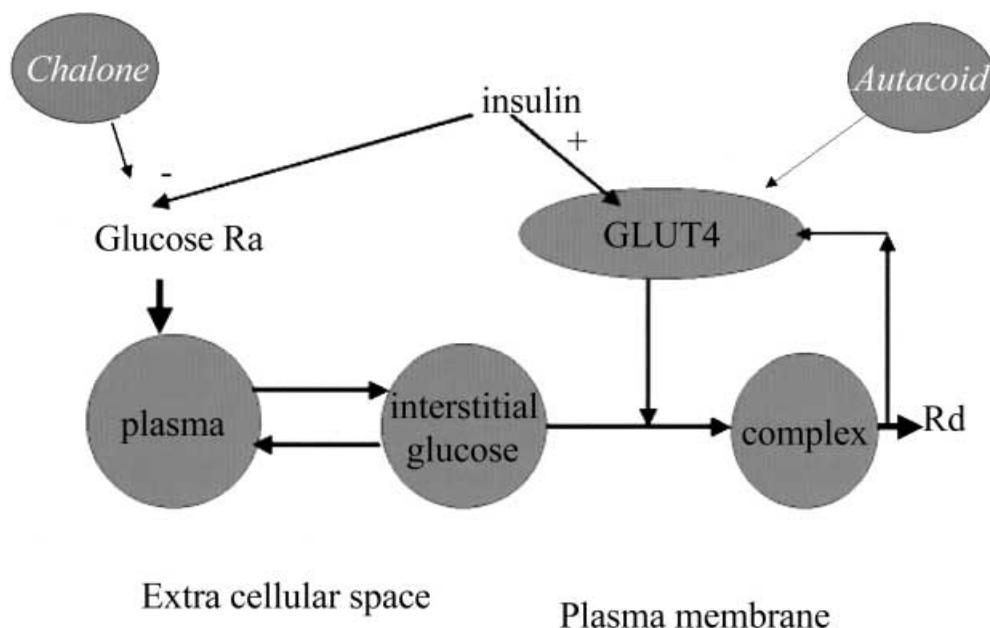


Figure 2 The entry of a water-soluble substrate such as glucose across an impermeable lipid bi-layer into a cell requires a specific transport mechanism. These protein carriers are known as 'transporters'. In the case of glucose there are at least six types and they tend to be tissue-specific. In the case of muscle the transporter is called 'Glut 4'. It is normally present in excess in the cell membrane even in the absence of insulin and is not rate limiting for glucose entry into the cell. Glucose transport into the cell is mainly determined by the concentration gradient between the extracellular fluid and the intracellular 'free' glucose. Normally, 'free' glucose is very low inside the cell as it is immediately phosphorylated. In uncontrolled diabetes, particularly where there is a high concentration of FFA and ketones, glycolysis is inhibited, phosphorylation of 'free' glucose stops and intracellular 'free' glucose rises. Insulin recruits more transporters into the cell membrane from an intracellular pool. This increases the rate of glucose entry for a given glucose concentration and this is reflected *in vivo* by an increase in the MCR of glucose. Thus MCR is an *in vivo* measure of substrate transporter activity.

control *in vivo*. Although glucose is used here for demonstration purposes, the process appears to be generic for all polar (water-soluble) substrates, as 'transporters' are the mechanism by which they are transported across the highly non-polar (lipid) cell membranes.

Figure 2 illustrates our model of the role of substrate transporters in facilitating water-soluble substrate entry into cells and it shows the ways in which we believe insulin exerts its normal control on glucose metabolism (Boroujerdi *et al.* 1995). There is a direct *chalone* action inhibiting glucose production by the liver while simultaneously insulin exerts an *autacoid* action stimulating transporter translocation into the cell membrane and, through that mechanism, increasing glucose uptake at any given blood glucose concentration.

Experiments in normal subjects using hyperglycaemic and hyperinsulinaemic 'clamps' have shown quantitatively the importance of both glucose and insulin concentrations in determining glucose uptake. Some studies illustrating these points are shown in Fig. 3. In these experiments, subjects were studied in the overnight-fasted state with fasting insulin averaging 18 mU/l and on two other

occasions when they were infused with insulin at rates that resulted in mean plasma insulin concentrations of 80 and 150 mU/l. They were also studied at the same insulin concentrations but with plasma glucose increased and maintained at a steady level by an exogenous glucose infusion. Four glucose concentrations ranging from 5 to 10 mmol/l were studied with insulin levels maintained at normal fasting values. During the insulin infusions, subjects were studied at three glucose concentrations spanning the same range. Using tracer methodology the authors were able to calculate Ra, Rd and MCR at each glucose and insulin concentration (Gottesman *et al.* 1982). The data from Gottesman *et al.* (1982) have been fitted to the model of glucose metabolism shown in Fig. 2. The important points of note are as follows.

1. Total glucose uptake (Rd) is a non-linear function of blood glucose concentration. Initially, uptake increases as blood glucose concentration rises but plateaus at higher glucose concentration. Although detectable within the range of glucose concentrations studied, it is made more obvious through extrapolation to higher

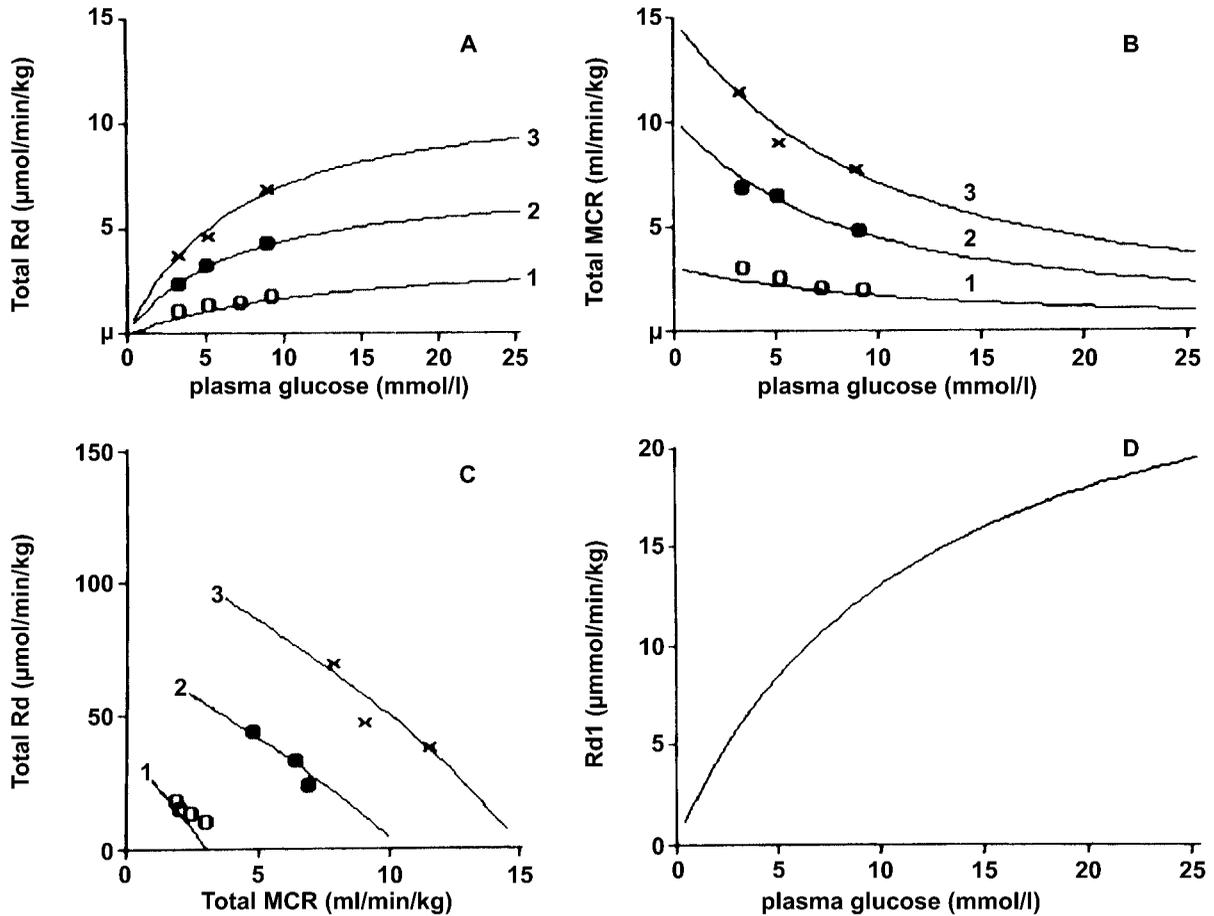


Figure 3 The data used in this illustration were obtained from healthy normal subjects using a series of euglycaemic and hyperglycaemic clamps at basal or increased insulin concentrations. Rd1: insulin independent glucose uptake. The data have been fitted to the generic model shown in Fig. 2 (see text for details).

glucose concentrations by use of the model. These high glucose values are unobtainable in normal subjects with existing technology. The shape of the curve suggests simple ‘saturation’ kinetics obeying Michaelis–Menten laws.

2. Glucose MCR falls with increasing plasma glucose concentration irrespective of the ambient plasma insulin concentration. This is in keeping with saturation of the glucose transporter system as plasma glucose rises.
3. MCR increases with increasing plasma insulin concentration, irrespective of the ambient plasma glucose concentration. This is in keeping with translocation of more glucose transporters into the cell membrane under the influence of increasing insulin concentrations.
4. The parallel nature of the plots shown in Fig. 3C (which is, in fact, a Scatchard plot of the data) indicates that increasing insulin concentrations are

associated with increasing number of ‘receptors’ – in this case, glucose transporters. There is no sign of a change in ‘affinity’ of the transporters under the influence of insulin, just the number present to facilitate glucose entry into cells.

Is insulin a performance-enhancing drug?

Thus from our understanding of insulin physiology we can see different ways in which insulin might be a performance-enhancing agent.

1. Through facilitating glucose entry into cells in amounts greater than needed for cellular respiration it will stimulate glycogen formation. Thus hyperinsulinaemic clamps will both increase muscle glycogen concentrations prior to events and in the recovery phase after events. Since performance in many events is known to be a function of muscle glycogen stores,

'bulking up' these stores will most probably enhance performance. There is no documentary proof that this technique is being used but informed 'street talk' indicates that it is not uncommon.

2. Through use of similar hyperinsulinaemic clamps post-event and during training, it is likely that recovery and stamina will be improved.
3. 'Street talk' indicates that insulin is also being used in a more haphazard way, particularly to increase muscle bulk in body builders, weight lifters and power lifters. This use is allegedly by regular injections of short-acting insulin together with high carbohydrate diets. Through this therapeutic regime it is almost certainly possible to increase muscle bulk and performance not only through increasing muscle glycogen stores on a 'chronic' basis but also by increasing muscle bulk through inhibition of muscle protein breakdown. Just as insulin has a *chalone* action in inhibiting glucose breakdown in muscle glycogen, it also has an equally important *chalone* action in inhibiting protein breakdown. Indeed, the evidence now indicates that insulin does NOT stimulate protein synthesis directly (this process is under the control of GH and insulin-like growth factor-I (IGF-I)). It has long been known that insulin-treated patients with diabetes have an increase in lean body mass when compared with matched controls (Sinha *et al.* 1996).

Taken together, all these points support the concern shown by the Russian medical officer in Nagano and the immediate response of the IOC to ban the use of insulin in those without diabetes.

GH and IGF-I

GH was written up as a potent performance-enhancing anabolic agent in *The Underground Steroid Handbook* first published in California in the early 1980s. The first scientific studies demonstrating a clear regulatory role for GH in adults was, however, only published in the peer-reviewed medical literature in 1989 (Jorgensen *et al.* 1989, Salomon *et al.* 1989). It had been alleged that many elite athletes had been abusing GH for many years and indeed several had confessed to having done so. The most eminent of these being Ben Johnson who, after losing his Gold Medal after testing positive for anabolic steroids at the Seoul Olympic games, admitted during subsequent investigation to using GH over many years (in combination with anabolic steroids). Although there are still no proper scientific studies proving GH to be performance enhancing in normal subjects (such studies would most likely be considered unethical and would be unlikely to receive research funding), few doubt this ability. GH has now been shown to have a very important role in regulating body composition in adult humans and also in other species. In cattle, GH is known as a 'partitioning

agent' – it specifically diverts calories in food towards protein synthesis and away from fat synthesis. Animals made transgenic for GH have greatly increased lean tissues and reduced fat. Similar changes in body composition are seen in humans with acromegaly. On the other hand, GH-deficient (GHD) adults have reduced lean body mass and increased fat mass, particularly central abdominal fat mass. Recombinant GH given in physiological 'replacement' doses to adults with GHD results in remarkable changes in body composition with, on average, a 5 kg increase in lean body mass within the first month (Salomon *et al.* 1989) and a comparable loss of 5 kg of fat. The fat loss is particularly from the intra-abdominal region where fat accumulates in the GHD state. In parallel with these changes in body composition, the subnormal exercise performance and strength of adults with GHD are returned to normal (Cuneo *et al.* 1991a,b).

Evidence about the abuse of GH in sport is largely anecdotal and circumstantial since, although banned by the IOC, there is as yet no recognised test for detecting its abuse. Perhaps the strongest circumstantial evidence for GH abuse comes from the losses admitted by the pharmaceutical industry of recombinant human GH (rhGH) from the production line, the distribution networks and the wholesale and retail outlets. There has also been a documented case of the re-sale to an athlete of a medicinal supply of rhGH by the mother of a GHD child.

Supplies of rhGH have been found in a team car during the Tour de France, in the personal possession of a Chinese swimmer in the World Championship in Perth, Australia and in the personal baggage of a national team trainer entering Australia for the Sydney 2000 Olympic Games. Six months before these recent Sydney Olympic Games there was a carefully targeted burglary on a wholesale pharmacy in Sydney. A massive supply of rhGH was stolen – nothing else was touched. It seems that none of this was ever recovered as described in the following report.

Olympic Jitters at Power Drug Theft

By Deborah Cameron (16 February 2000)

"The theft of a huge quantity of an undetectable bodybuilding drug from a Sydney importer has raised serious concerns among sports officials and doctors about whether Australia's elite athletes are 'clean'. With just six months to go until the Olympics, and as some sports prepare for selection trials, the timing of the theft of 1575 (*multidose*) vials of human GH (hGH) is seen as highly significant".

Perhaps of even greater concern is the knowledge that GH is readily available from gymnasia and other sporting establishments and that it is being used by school-children. Supplies of pituitary-derived GH are still in circulation indistinguishable from rhGH. There are several well-recognised sources of pituitary-derived GH appearing in the world market and it is highly likely that some of these

batches will be contaminated with Creutzfeld–Jacob prion. There were press reports recently of the arrest of a Russian who was found with a large jar with >1000 pickled human pituitary glands in his apartment in Moscow.

GH physiology

GH is a polypeptide hormone secreted by the pituitary gland. The predominant form has a molecular weight of 22 kDa and has a half-life in the plasma of between 15 and 20 min after secretion or intravenous injection. After subcutaneous or intramuscular injection, blood concentrations of GH reach a peak between 1 and 3 h after injection and fall to undetectable levels after 24 h. As it is a protein hormone it has to be administered by injection as it is completely digested to its constituent amino acids when administered by mouth. The circulating GH is cleared from the blood stream through receptor-mediated degradation, predominantly in the liver and kidney. The liver and kidney internalise the GH–receptor complex and completely degrade it to its basic amino acids. Only minute quantities of GH appear in the urine and the pattern of urinary excretion has been shown to be too low and variable to be considered of any value in developing a test of GH abuse.

GH receptors are present on all cells in the body. One GH molecule binds to two receptors and leads to dimerisation of the receptors. This dimerisation process is essential for initiation of intracellular signalling. Analogues of GH that prevent dimerisation are inhibitors of GH. The pituitary gland secretes GH in bursts and the major stimuli to GH secretion in man are sleep, exercise and stress. The sleep-related burst of GH secretion occurs most consistently during the phase of deep slow-wave sleep most commonly occurring during the early hours of sleep. In conditions where sleep pattern is disturbed, GH secretion is impaired; the introduction of effective therapy for the sleep disturbance (such as continuous positive airways pressure for obstructive sleep apnoea) restores GH secretion to normal. Hypnotics that reduce the period of slow-wave sleep impair GH secretion while drugs that enhance slow-wave sleep enhance GH secretion (Van Cauter & Copinschi 2000).

GH secretion reaches its maximum around late teenage life and falls progressively thereafter. The total amount of GH secreted over 24 h in normal adults over the age of 65 is, in the majority of cases, overlapping with people with organic GHD secondary to pituitary pathology or its treatment (Toogood *et al.* 1996). There is thus evidence of the development of functional GHD with increasing age – the so-called ‘Somatopause’. The majority of middle-aged and elderly normal subjects may be considered incompletely GHD.

GH stimulates many metabolic processes in all cells but one of its best-known actions is the generation of IGF-I

(and its binding proteins). GH stimulates IGF-I gene expression in all tissues. In most tissues, this IGF-I has local ‘autocrine’ and ‘paracrine’ actions but the liver actively secretes IGF-I (and its binding proteins) into the circulation. Until recently it was thought that this ‘hormonally’ secreted IGF-I produced by the liver was responsible for many of GH’s *in vivo* actions. Recent data from hepato-specific IGF-I knock-out mice have shed serious doubt on this, since their growth and metabolism appear to be quite normal despite very low circulating IGF-I levels. *Circulating IGF-I should now be considered more as a ‘marker’ of GH action on the liver than as the mechanism by which GH exerts its effects.* Hepatic IGF-I production is regulated by factors other than GH, most notably nutritional and thyroid status. Undernutrition, such as is seen in anorexia nervosa and poorly controlled Type 1 diabetes are both associated with low plasma IGF-I and high GH secretion. It appears that in these circumstances it is the portal insulin status that is one of the key factors regulating hepatic GH receptor expression and hence hepatic sensitivity to GH.

IGF-I has many actions that resemble GH and at one stage it was thought that most, if not all, of GH’s actions were mediated through IGF-I. Recent experience with the hepato-specific IGF-I knock-out mouse has shown this may be an oversimplification of a complex system and the exact roles of GH and IGF-I still have to be defined.

There are GH receptors on all cells in the body and it appears that GH exerts effects on most, if not all, of these cells. There are literally hundreds, if not thousands, of GH-dependent ‘markers’ produced under the influence of GH. IGF-I just happens to be the best known of these but since the majority of circulating IGF-I comes from the liver, we should now think of IGF-I more as a marker of GH action on the liver rather than the ‘second messenger’ of GH action.

GH administration leads to the production of a whole series of markers of its action that appear in the circulation and, as we shall see later, these can be used as a way of detecting GH abuse.

What are the metabolic effects of GH that make it attractive as a drug of abuse?

GH’s major action is to stimulate protein synthesis. It is at least as powerful as testosterone in this effect and, as they both operate through distinct pathways, their individual effects are additive or possibly even synergistic. In addition to stimulating protein synthesis, GH simultaneously mobilises fat by a direct lipolytic action. Together, these two effects are responsible for the ‘partitioning’ action of GH whereby it diverts nutritional calories to protein synthesis, possibly through using the energy derived from its lipolytic action. It most likely stimulates protein synthesis through mobilisation of amino acid transporters in a manner analogous to insulin and glucose transporters

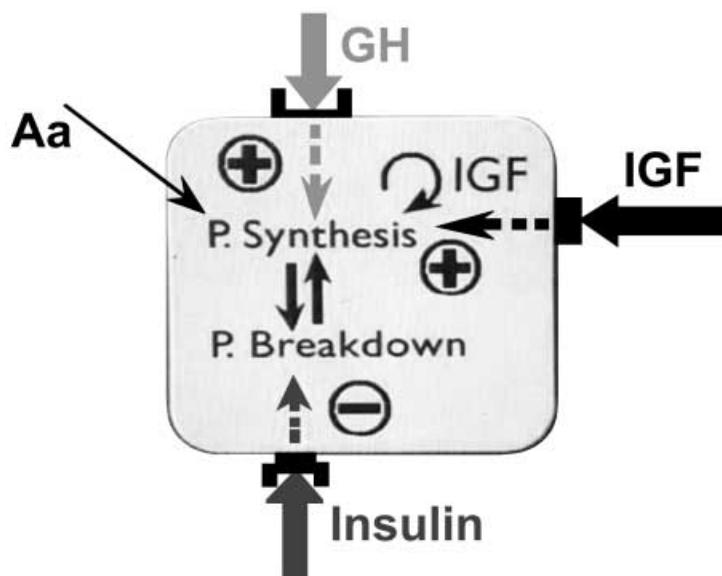


Figure 4 This diagram illustrates our current understanding about the synergistic action between insulin, IGF-I and GH in regulating protein (P) synthesis. Without insulin, GH loses much (if not all) of its anabolic action. GH and IGF-I stimulate protein synthesis directly, while insulin is anabolic through inhibiting protein breakdown. The anabolic action of both GH and IGF-I appears to be mediated through induction of amino acid (Aa) transporters in the cell membrane. This is reflected *in vivo* by an increase in amino acid MCR. It is not yet clear how much of IGF-I's action is through locally generated IGF-I ('autocrine' and 'paracrine') or through circulating IGF-I that is largely derived from the liver.

(Fig. 2). This is reflected *in vivo* by an increase in amino acid MCR and the process can be explained quantitatively by a model of structure similar to that shown in Fig. 2. IGF-I also acts directly to stimulate protein synthesis but it has a weaker lipolytic action. GH, IGF-I and insulin thus act in concert to stimulate protein synthesis. Using Schafer's conceptual terminology, GH and IGF-I act in an *autacoid* manner to stimulate protein synthesis while insulin acts in its characteristic *chalone* manner to inhibit protein breakdown. Thus they are synergistic in their powerful anabolic action (Fig. 4). Insulin is essential for the anabolic action of GH. GH administration in the absence of adequate insulin reserves (as during fasting or in Type 1 diabetes) is in fact catabolic and its lipolytic and ketogenic properties can induce diabetic ketoacidosis. Thus GH and insulin are closely linked in normal physiology and it is of great interest to see that athletes have discovered ways in which this normal physiological dependence can be exploited to enhance performance.

Why do athletes 'dope' with GH?

There are several reasons for this. It is well known from surveys amongst elite athletes that they are prepared to take risks in order to win medals. A survey carried out

in 1995 by a prominent sporting magazine in the USA polled a series of elite athletes on a number of questions (Bamberger & Yaeger 1997). One of these was:

'You are offered a banned performance-enhancing substance with two guarantees:

you will not be caught

you will win

Would you take the substance?'

Answer: Yes, 195; No, 3.

Another was:

'You are offered a performance-enhancing substance that comes with two guarantees:

you will not be caught

you will win every competition you enter for the next 5 years and then you will die from the side-effects of the substance

Would you take it?

Answer: Yes >50%.

Even though this may not be a truly representative survey it is clear that the driving force to win is very strong and is behind much of the drug abuse in sport but there are other motives.

Injuries are common in most sports and athletes believe that the prevention or mitigation of these is possible

through judicious use of nutritional supplements and more potent anabolic agents such as steroids and GH. Indeed the original description of GH's action in the 1983 edition of the *Underground Steroid Handbook* stated that GH strengthened tendons such that damage to them by weight and power lifters was much reduced. There is also a view that GH may prevent stress fractures and speeds the healing process – experimental evidence from animal studies indicates that they may well be right!

Perhaps the main reason why GH is such a threat to fair play is, of course, the fact that it is a very potent anabolic agent, readily available in unlimited quantities, pretty safe and completely undetectable. The risks associated with taking it are, in the short-term, minimal. The benefits are potentially very considerable. Although the numbers actually abusing it are likely to be quite small, it does pose a very real threat to the majority of elite athletes who do not abuse it or other performance-enhancing drugs. It is therefore of great importance that not only should it be banned but that there should be a test to catch and disqualify those who use it to cheat.

How can we detect abuse with GH?

Unlike the majority of synthetic anabolic steroids, GH is an 'endogenous substance' and indistinguishable from the naturally occurring hormone. This makes it particularly difficult to detect when used as a drug of abuse. The pituitary gland secretes predominantly a 22 kDa isomer of GH but there are other minor products of gene transcription and post-transcription modification. The most prominent of these is a 20 kDa isomer that is normally present in the circulation at concentrations averaging around 10% of the 22 kDa isomer.

It is possible to distinguish between endogenous GH secretion and exogenous rhGH (but not pituitary-derived GH) administration. If, for example, a blood sample is obtained during a peak of endogenous GH secretion, then 10% of the immunoassayable GH should be 20 kDa GH. Using assays based on a combination of relatively non-specific polyclonal antibodies and specific assays using two monoclonal antibodies, each recognising specific epitopes on the 22 kDa and 20 kDa molecules, Strasburger and colleagues have developed a promising method of detecting rhGH abuse. They have shown that the absence of the 20 kDa isomer when 22 kDa GH is present in substantial amounts can be used as an indicator of exogenous rhGH administration and can thus form the basis of a test of GH abuse (Wu *et al.* 1999). This is one way of tackling the detection of rhGH abuse but it will not, of course, detect administration of exogenous pituitary-derived GH. Its 'window of opportunity' for detecting rhGH abuse will be for less than 24 h after a dose of rhGH since, after then, none will be detectable in the circulation. It could thus best be the basis of an 'out of competition' test when an

unannounced blood sample may well be taken within 24 h of the last rhGH injection. More work is needed to validate this approach however.

Another approach has used the many substances in the body regulated by GH as 'markers' of GH action as the basis of a test for GH abuse. The principle of this approach is that, when given in supra-physiological doses, GH over-stimulates a number of processes normally regulated by GH. Thus it should be possible to detect GH abuse if these GH-dependent markers are present in amounts that greatly exceed the normal physiological range. Clearly this approach will not be able to distinguish the athlete with a GH-secreting pituitary tumour but it should be able to detect normal subjects administered supra-physiological amounts of GH (people with acromegaly have been elite athletes).

This was the main thrust of the international collaborative research project 'GH-2000' jointly funded by the European Union and the IOC, contract number BMH4CT950678.

Since exercise is a powerful stimulus to GH secretion and most samples for 'doping control' are taken immediately post-competition, a pilot 'washout' study investigated the effects of 1 week's rhGH administration and acute exercise on a wide range of substances influenced by GH. The aim of this study was to determine a subgroup of GH-dependent 'markers' that were sensitive to rhGH administration but relatively insensitive to the acute effects of exercise (Wallace *et al.* 1999, 2000).

The results indicated that four markers produced by the liver (IGF-I, IGF-binding protein-2 (IGFBP-2), IGFBP-3 and ALS) and four produced from collagen and bone (osteocalcin, procollagen type III (P-III-P), type I collagen telopeptide and C-terminal propeptide of type I collagen) were suitable to take forward into other studies. These markers and their points of origin are illustrated in Fig. 5.

A 'double-blind placebo-controlled' study of 1 month's rhGH administration at two doses to more than 100 healthy volunteers was carried out in four countries. The eight markers were measured on all the blood samples before, during and for 3 months after the rhGH or placebo administration. Analysis of a subset of the data indicated that the best discrimination between active treatment and placebo was obtained using two of the markers – IGF-I and P-III-P. Using these two markers it was possible to obtain complete separation between the active treatment and placebo, whereas with either one of the markers there was a degree of overlap. Further statistical developments indicated that increased sensitivity and specificity could be obtained by combining more of the markers in the analysis. Analysis of the urine samples showed much weaker discriminating power in separating active treatment from placebo.

There was a need to construct a reference range of these markers in elite athletes from different sporting disciplines.

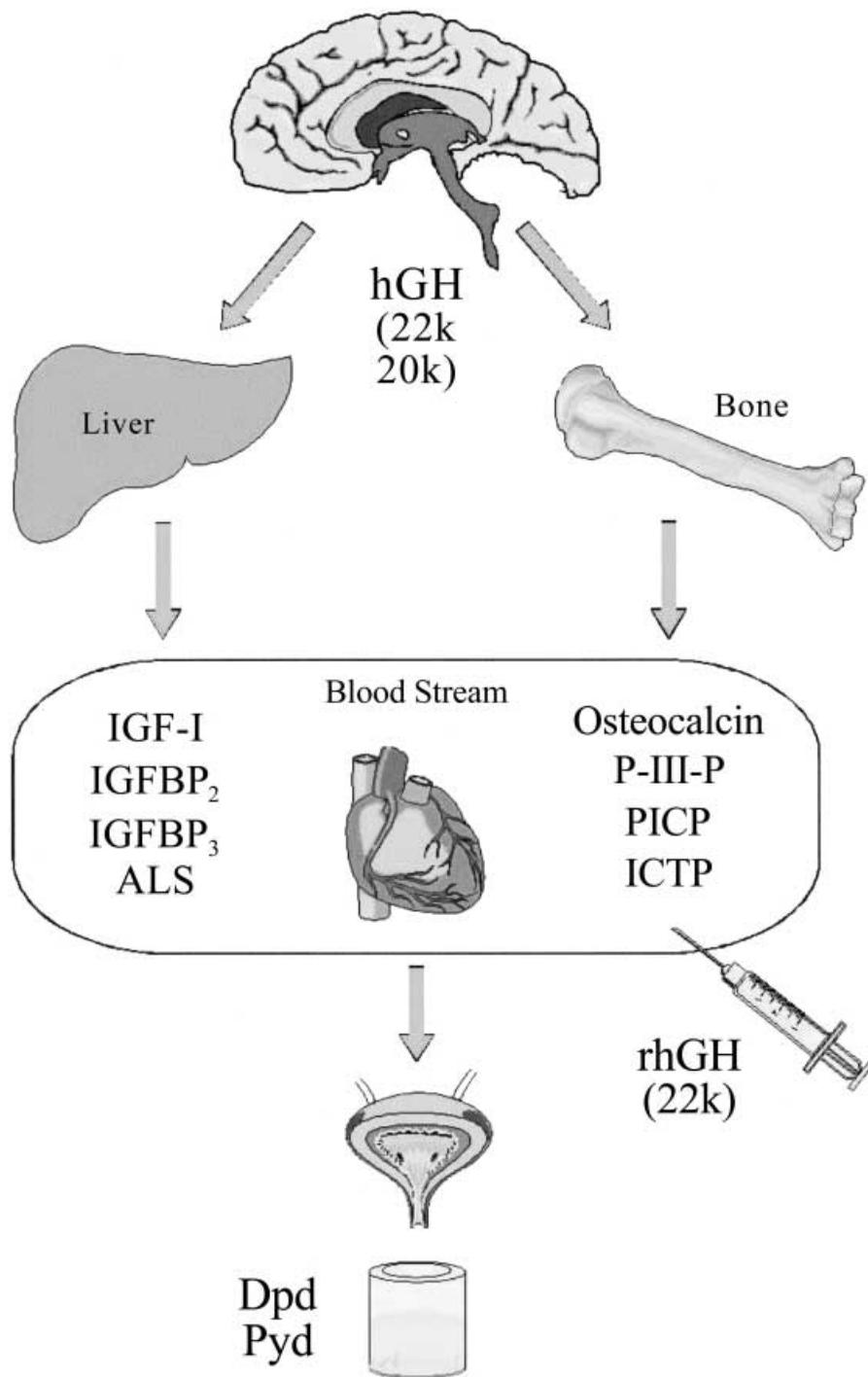


Figure 5 A summary of the potential markers thought to be most useful in developing a test of GH abuse (see text for details). Dpd and Pyd are urinary metabolites of collagen markers.

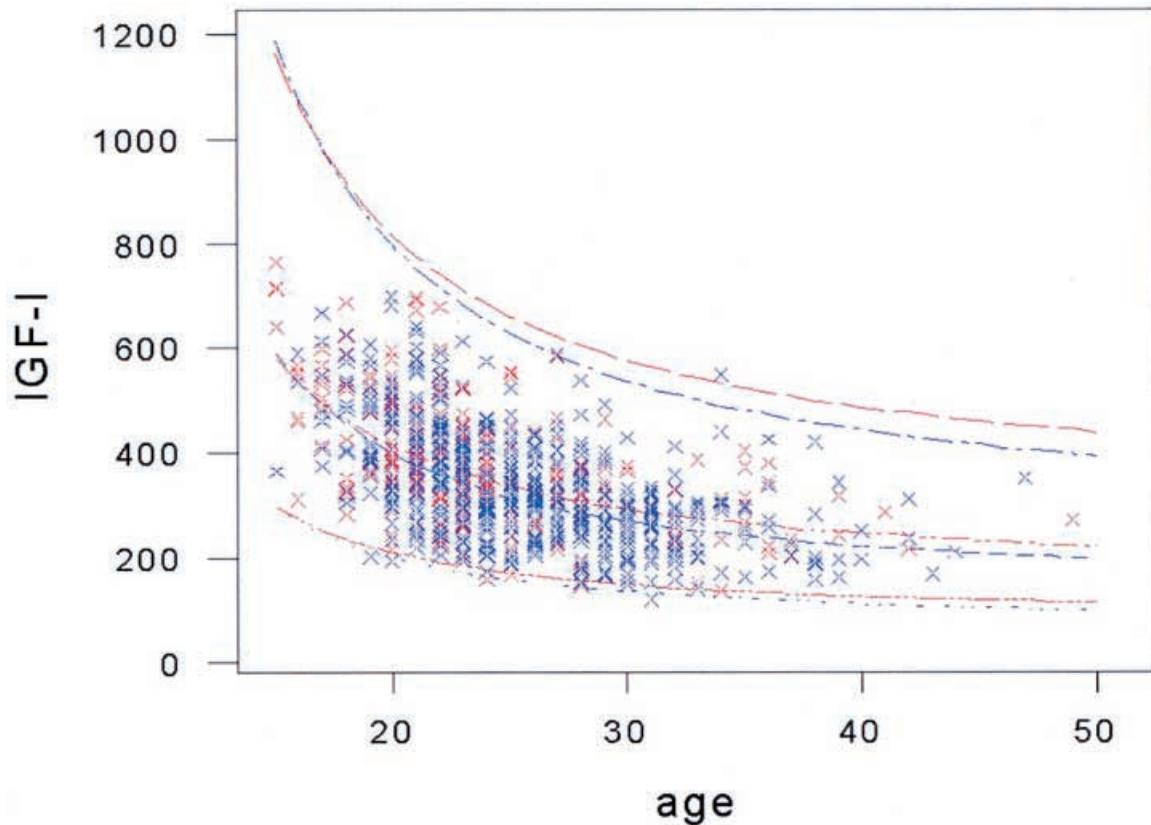


Figure 6 Effects of age on serum IGF-I levels (mcg/l) in 800 elite athletes. Samples were taken within 2 h of the end of competition. There is an exponential fall in IGF-I levels in both male and female elite athletes as in the normal population. Although there are minor differences statistically between men and women these are of marginal importance. Red crosses: female; blue crosses: male.

In order to do this, teams of research workers from the project obtained permission to recruit volunteer elite athletes from a variety of major national and international sporting competitions. During the course of the project, over 800 elite athletes gave explicit written informed consent to a blood sample and for demographic data to be collected immediately post-competition. On as many occasions as possible a sample of urine was also obtained. All eight markers were measured on each blood sample. Analysis showed considerable apparent differences between sports but when the results were adjusted for the effects of age most of these apparent differences disappeared.

The results for all the GH-dependent markers showed a very clear age-related fall. Although this is well recognised for GH and IGF-I it was seen in all the markers. There were gender and ethnic differences in some of the markers but these were relatively minor compared with the age-related effects. The gender effect is well illustrated in Fig. 6 which shows the effects of age on plasma IGF-I in male and female elite athletes. Although there is a significant difference between men and women it is obvious that this is not a major one.

Of importance in developing a test for detecting GH abuse is the stability of the markers over time and in response to exercise, training, injury etc. A subset of those who volunteered for the cross-sectional study used to construct the reference range volunteered to provide samples on other occasions during the year. Many of them also agreed to undertake a simulated extreme exercise event under 'laboratory' conditions. The results from this 'longitudinal study', taken with the information obtained from the placebo-treated group during the double-blind study, showed remarkable stability in the blood concentration of the markers over time. It appears that the blood concentration of the markers is most likely genetically determined and relatively uninfluenced by day-to-day environmental factors.

The effects of injury were examined by looking at the levels of the markers in a group of volunteers from a number of sports injury clinics in the UK. Although the number of subjects studied was too small to form any definitive conclusions, changes were seen after fractures and injuries but, interestingly, although the concentration of the collagen/bone markers rose in most cases, those of

the hepatic IGF-I-related markers tended to fall. The discriminant function based on the combination of IGF-I and P-III-P remained remarkably stable.

Sensitivity and specificity of a test for GH abuse

Using simple discriminant function techniques and adjusting for age, it is possible to obtain reasonable sensitivity in detecting abuse of GH during its administration and for as long as 2 weeks after it has stopped. The exact level of sensitivity depends on the level of specificity that a court of law would demand to uphold a prosecution. Since courts of law do not normally deal in terms of 'scientific probabilities' there is, at present, an important gap between science and the law. In order to have a valid test for GH abuse, this gap must be closed. Lawyers are beginning to realise that the scientific issues of probability must be incorporated into their process but they have quite a long way to go before this issue is resolved satisfactorily. Suffice it to say, a test based on the results of the GH-2000 project has a sensitivity better than 90% of picking up a man taking GH with a probability of less than 1:10 000 of being wrong. This is high degree of scientific certainty but whether or not it is sufficient for a court of law (or the Court of Arbitration in Sport (CAS)) will not be clear until the system is actually tested with a case. It is significant that one member of the CAS present at the workshop, after seeing the data presented by GH-2000, felt sufficiently convinced to remark that he would be prepared to take an athlete caught cheating by these criteria to court '... so long as he was a white European male ...'. This remark highlighted two of the many points that still require further research: firstly that the large majority of volunteers studies in the GH-2000 project were white Europeans and secondly, the gender difference in sensitivity to rhGH is quite marked. Although differences in the criteria for detecting GH abuse between men and women are minimal, the actual sensitivity of the test in detecting GH abuse in women is much lower. On the other hand, the specificity of the test is not lower in women. So why he was less certain about taking a woman to court whose values exceeded the reference range? We need to do further research on the mechanisms behind this gender difference.

When will we have a test for GH abuse?

The project was targeted at providing a test for the Sydney Olympic Games. The final report was handed in to the European Union and the IOC on 21 January 1999, 20 months before the Sydney Olympic Games. The results were presented to the IOC medical commission at the time of the World Doping Conference in Lausanne in February 1999. The IOC convened a special workshop in Rome in March 1999 to review the results. To this they

invited a number of outside experts to review the data critically. It was at this workshop that a distinguished CAS lawyer made the remarks already quoted and the conclusions of the workshop were very much that the project had done a remarkably good job. GH-2000 had shown that the development of a test was feasible and exactly how it should be done. There was, however, the need for some further research to complete the rigorous scientific data that are needed for disqualification of an athlete caught cheating and upholding the decision in the face of an appeal to CAS. There is also a need to publish all the results in peer-reviewed scientific journals in order to provide quality assurance on the research. This has taken a great deal of time as the amount of data collected was enormous and although many papers have been published or are 'in press' it will be another year or two before that is achieved.

GH-2000 was immensely successful – it brought together a team of European endocrinologists and scientists to work together in what turns out to be an exciting new area – sport – and was able to show that what at the outset had appeared to be an impossible task was, in fact, achievable. It has shown the importance of the GH axis in fitness and sport and has revealed that GH has important regulatory control over an even greater range of processes than we had previously understood. It has shown the effects of ageing are not only on the GH-IGF-I axis but on all areas where GH acts and it has shown that even elite athletes who exercise at rates unachievable by most of us still show a decline in GH production as they age. Is this the reason why GH abuse is thought to be so prevalent in sport? Does GH prolong active life? The answer to this is not known but the data indicate that it is a valid question and support the need for much further research work in the area of GH and ageing and the possible therapeutic role of GH in preventing loss of lean tissues with ageing.

Acknowledgements

This paper is based on an invited lecture given to the British Endocrine Society meeting at Imperial College London in November 2000. The results are those of the GH-2000 project, not all these results have yet been published. I would like to thank all those who participated in the project including our volunteer athletes and all the sports organisations who helped us recruit and study our volunteers. Finally, I would like to acknowledge the wonderful statistical support we had from Dr Eryl Bassett and Professor Philip Brown from the University of Kent.

References

- Bamberger M & Yaeger D 1997 Over the edge. *Sports Illustrated* **14** 62–70.

- Boroujerdi MA, Umpleby AM, Jones RH & Sonksen PH 1995 A simulation model for glucose kinetics and estimates of glucose utilization rate in type 1 diabetic patients. *American Journal of Physiology* **268** E766–E774.
- Brown P, Tompkins C, Juul S & Sonksen PH 1978 Mechanism of action of insulin in diabetic patients: a dose-related effect on glucose production and utilisation. *British Medical Journal* **1** 1239–1242.
- Cuneo R, Salomon F, Wiles C, Hesp R & Sonksen PH 1991a Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength. *Journal of Applied Physiology* **70** 688–694.
- Cuneo R, Salomon F, Wiles C, Hesp R & Sonksen PH 1991b Growth hormone treatment in growth hormone-deficient adults. II. Effects on exercise performance. *Journal of Applied Physiology* **70** 695–700.
- Gottesman I, Mandarino L, Verdonk C, Rizza R & Gerich J 1982 Insulin increases the maximum velocity of glucose uptake without altering the Michaelis constant in man. *Journal of Clinical Investigation* **70** 1310–1314.
- Jorgensen JO, Pedersen SA, Thuesen L, Jorgensen J, Ingemann-Hansen T, Skakkebaek NE & Christiansen JS 1989 Beneficial effects of growth hormone in GH-deficient adults. *Lancet* **i** (8649) 1221–1225.
- Randle PJ, Garland PB, Newsholme EA & Hales CN 1965 The glucose fatty acid cycle in obesity and maturity onset diabetes mellitus. *Annals of the New York Academy of Sciences* **131** 324–333.
- Salomon F, Cuneo R, Hesp R & Sonksen PH 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *New England Journal of Medicine* **321** 1797–1803.
- Schafer EA 1916 *The Endocrine Organs – An Introduction to the Study of Internal Secretions*, pp 4–7 and 128–130. London: Longmans, Green and Co.
- Sinha A, Formica C, Tsalamandris C, Panagiotopoulos S, Hendrich E, DeLuise M, Seeman E & Jerums G 1996 Effects of insulin on body composition in patients with insulin-dependent and non-insulin-dependent diabetes. *Diabetic Medicine* **13** 40–46.
- Sonksen PH & Sonksen J 2000 Insulin: understanding its action in health and disease. *British Journal of Anaesthesia* **85** 69–79.
- Thomas SH, Wisner M, Brandenburg D & Sonksen PH 1979 Insulin action on adipocytes, evidence that the anti-lipolytic and lipogenic effects of insulin are mediated by the same receptor. *Biochemical Journal* **184** 355–360.
- Toogood AA, O'Neill PA & Shalet SM 1996 Beyond the somatopause: growth hormone deficiency in adults over the age of 60 years. *Journal of Clinical Endocrinology and Metabolism* **81** 460–465.
- Van Cauter E & Copinschi G 2000 Interrelationships between growth hormones and sleep. *Growth Hormone and IGF Research* **10** (Suppl B) S57–S62.
- Wallace JD, Cuneo RC, Baxter R, Orskov H, Keay N, Pentecost C, Dall R, Rosen T, Jorgensen JO, Cittadini A, Longobardi S, Sacca L, Christiansen JS, Bengtsson B-A & Sonksen PH 1999 Responses of the growth hormone (GH) and insulin-like growth factor axis to exercise, GH administration, and GH withdrawal in trained adult males: a potential test for GH abuse in sport. *Journal of Clinical Endocrinology and Metabolism* **84** 3591–3601.
- Wallace JD, Cuneo RC, Lundberg PA, Rosen T, Jorgensen JOL, Longobardi S, Keay N, Sacca L, Christiansen JS, Bengtsson B-A & Sonksen PH 2000 Responses of markers of bone and collagen turnover to exercise, growth hormone (GH) administration and GH withdrawal in trained adult males. *Journal of Clinical Endocrinology and Metabolism* **85** 124–133.
- Wu Z, Bidlingmaier M, Dall R & Strasburger CJ 1999 Detection of doping with human growth hormone. *Lancet* **353** 895.

Received 18 January 2001

Accepted 10 April 2001