# HUMAN GROWTH HORMONE RESPONSES TO SPRINTING

by

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# SUMMARY

A number of studies have shown exercise to stimulate human growth hormone (hGH) secretion, although most of these have considered prolonged submaximal or resistance exercise. Only a few have studied maximal sprint exercise, and these studies have demonstrated considerably elevated circulating hGH concentrations during recovery. However, there is little agreement in the literature regarding the regulation of hGH secretion during and after exercise. This thesis describes a series of experiments considering the hGH response to sprint exercise, with the intention of gaining a better understanding of some of the mechanisms involved in regulating the exercise-induced hGH release.

The time-courses of the hGH response to maximal cycle ergometer sprints of 6 s and 30 s duration are described in the first experimental chapter (Chapter 4). Highest measured mean serum hGH concentrations were more than four and a half times greater following the 30 s sprint than they were following the 6 s sprint (37.0±6.2 vs. 8.0±2.9 mU.l$^{-1}$, $P<0.05$), and remained elevated above pre-exercise levels for longer during recovery, demonstrating that the duration of a sprint has a marked effect on the exercise-induced hGH response. In addition, this chapter highlights the large inter-individual variation in the hGH response to sprinting.

Sprinting at different pedal speeds made it possible to study the effect of the number of muscle actions during a sprint on the hGH response to sprint exercise (Chapter 5). In addition, the effect of performing a second 30 s sprint whilst hGH was still elevated, as a result of the first bout, was considered. There was a trend for serum hGH concentrations to be greater following sprints at faster pedal speeds (mean 2 h integrated hGH concentrations 1381±231 vs. 663±162 mU.l$^{-1}$, $P=0.06$), suggesting a possible role for proprioceptive feedback in the regulation of hGH secretion. It was also observed that there was a marked hGH response to the first sprint in each trial (highest measured mean concentration 40.8±8.2 and 20.8±6.1 mU.l$^{-1}$ for the FAST and SLOW trials, respectively), but that the second sprint in each trial did not elicit a hGH response ($P<0.05$), despite a similar pH and lactate response to the two sprints. Since circulating hGH concentrations were still elevated prior to the second sprint this was likely to be a result of hGH autoinhibition. The plasma ammonia response to the second sprint was also attenuated in these trials.

The effect of sprint training on the hGH response to repeated maximal cycle ergometer sprinting is described in the third experimental chapter (Chapter 6). Six weeks of combined speed and speed-endurance training was found to result in an improvement in sprint performance, but the hGH response to exercise was reduced in all subjects in the training group, resulting in a decrease in highest measured mean hGH concentrations of over 40 % (20.5±6.2 vs. 11.6±5.0 mU.l$^{-1}$, group-training interaction effect, $P<0.05$). Training was also found to result in a reduced ammonia response to sprinting. However, sprint training did not alter the attenuation of the hGH response to repeated sprints.

The fourth experimental chapter (Chapter 7) describes the effect of a longer recovery period between repeated bouts of sprint cycling on the hGH response to the second of

two sprint bouts. After 4 h recovery from a single 30 s sprint, when hGH had returned to pre-exercise concentrations, the hGH response to a second 30 s sprint still showed a tendency to be attenuated ( mean highest measured hGH concentrations $34.1\pm24.8$ vs. $16.8\pm5.0$ mU.l$^{-1}$, $P=0.09$). Serum IGF-I was found to be acutely elevated 5 min after a single sprint, but was not elevated prior to a second sprint completed 4 h later. Therefore, since neither hGH nor IGF-I were elevated prior to the second sprint, an alternative mechanism of feedback, possibly mediated by elevated circulating free fatty acids (FFAs), was probably in operation. In addition, resting IGF-I concentrations were found to be lower the day after exercise, possibly reflecting a change in the pattern of nocturnal hGH secretion.

The studies presented in this thesis have provided evidence of an attenuation of the hGH response to repeated exercise, and also following short-term sprint-training. The mechanism for attenuation with repeated sprinting appears to be negative feedback, probably mediated by circulating hGH in the 2 h after exercise (when hGH is still elevated), but thereafter by another mechanism, possibly elevated circulating FFAs. In addition, the studies in this thesis have provided information regarding the mechanism regulating hGH secretion. It appears that blood pH and lactate do not have an important role in this regulation of hGH release, whilst blood-borne ammonia might have more of an influence. In addition, it is possible that proprioceptive feedback might also modulate the hGH response to exercise.

Some of the experiments described in this thesis have been published as follows:

Stokes KA, Nevill ME, Hall GM, Lakomy HKA, Cherry PW (1999) Growth hormone responses to repeated maximal cycle ergometer exercise at different pedal speeds. J Physiol 515:75P

Stokes KA, Nevill ME, Cherry PW, Hall GM, Lakomy HKA (2000) The effect of 6 weeks of sprint training on the growth hormone response to repeated maximal cycle ergometer exercise. J Physiol 528:51P

# ACKNOWLEDGEMENTS

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# CHAPTER 1

## GENERAL INTRODUCTION

In the past twenty years there has been a rapid growth of interest in the metabolic responses to sprint exercise, which can be defined as an activity in which exercise is performed at a maximal rate from the onset of exercise (Nevill et al., 1996a). The term 'maximal exercise' is often thought of as exercise at an intensity that elicits maximum oxygen uptake ($\dot{V}O_2$max), however, during sprinting of 20-30 s duration, the average power output is approximately 2-3 times higher than that required to elicit $\dot{V}O_2$max (Wooton, 1984).

The development of the 30 s maximal cycle ergometer sprint 'Wingate' test by Bar-Or et al. (1978), which was later modified by Lakomy (1986), in order to take into account the effect of acceleration, allowed high resolution measurement of power output, speed and acceleration during cycle ergometer sprint exercise in the laboratory. The use of sprinting on a friction-loaded cycle ergometer is a very attractive method of exercise testing, since it better represents 'real-life' exercise when compared with isometric contractions, electrical stimulation models and isokinetic cycling, and both the force and velocity components of power output can be considered. The combination of high resolution power output and both blood and muscle biopsy sampling has allowed the study of the relationship between exercise performance and biochemical changes in the body, with a particular view to furthering the understanding of mechanisms involved in fatigue. However, there is very little information available regarding the human growth hormone (hGH) response to exercise, and particularly the hGH response to sprinting.

Human growth hormone is released from the anterior pituitary gland, largely regulated by the balance between two hypothalamic hormones; growth hormone releasing hormone (GHRH) and somatostatin. There are a number of stimuli to hGH secretion, including stress, sleep, hypoglycaemia and exercise, and whilst prolonged exercise has been shown to be a potent stimulus for hGH secretion, very little research has focussed on the hGH response to sprinting. However, Nevill et al (1996b)

reported that a single 30 s treadmill sprint resulted in a 'near maximal' hGH response, when compared with pharmacological intervention studies. Sprint exercise, therefore, provides a very potent stimulus to hGH secretion without the need for extended exercise time and can be used to provide information about the hGH response to exercise, specifically with regard to the mechanisms responsible for the regulation of hGH secretion.

The mechanisms responsible for exercise-induced hGH secretion are not fully understood, despite the attention of a number of studies. The roles of intensity and duration of exercise, blood lactate, blood pH, circulating catecholamines and oxygen demand and availability in the regulation of the magnitude of the hGH response to exercise have all been considered. It is likely that more than one of these factors work together, possibly with a contribution from motor centres in the brain, to regulate hGH secretion, but there is little agreement regarding the relative importance of the different mechanisms. Furthermore, the evidence regarding the hGH response to repeated bouts of exercise remains equivocal, since repeated bouts of exercise have been shown to both augment and inhibit hGH secretion.

Although the actions of hGH, particularly in adulthood, are not fully understood, it is widely accepted that there is a role for hGH in the regulation of lipolysis as well as direct and indirect regulation of protein synthesis. Growth hormone replacement with recombinant (r)hGH is employed in the treatment of a number of disorders where hGH is lacking both as a cause and as a result of the illness. In addition, exogenous GH is used as an illegal performance enhancing agent in athletic competition, a practice which has reportedly increased in popularity following the development of the recombinant form. The study of natural hGH responses to an exercise stimulus and, in particular, repeated exercise might prove useful in the understanding of feedback regulation of hGH secretion, with implications for the therapeutic use of both exercise and rhGH.

The main aim of this thesis is to study the hGH response to sprint exercise on a friction-loaded cycle ergometer with a view to contributing to the understanding of the mechanisms regulating exercise-induced hGH secretion. This thesis is presented in a further seven chapters:

- The review of literature (Chapter 2) provides on overview of fatigue during cycle ergometer sprinting, describing the effects of recovery during repeated sprints, pedalling rate and training on the metabolic responses to sprint exercise. The control of the secretion of hGH is also reviewed, with specific reference to the hGH response to exercise, as well as the importance of feedback in the hGH regulation.

- The general methods (Chapter 3) describe the equipment, methods of analysis and procedures common to the experimental chapters are described.

- The aim of the first experimental chapter (Chapter 4) is to describe the time-course of the hGH response to maximal sprints of different duration.

- The second experimental chapter (Chapter 5) investigates the effect of repeated bouts of maximal sprint exercise on the hGH response, whilst studying the effect of sprint cycling at different pedalling rates on hGH secretion.

- The purpose of the third experimental chapter (Chapter 6) is to examine the effect of 6 wk of sprint training on the performance of, and hGH response to, repeated cycle ergometer sprinting.

- The aim of the fourth experimental chapter (Chapter 7) is to study the effect of the duration of the recovery period between repeated sprints on hGH secretion, and consider the involvement of insulin-like growth factor I (IGF-I) in the regulation of the hGH response to exercise.

- Chapter 8 is a general discussion which draws together the findings of the experimental chapters, explaining some of the possible the mechanisms involved in the regulation of hGH secretion following sprint exercise.

# CHAPTER 2

# REVIEW OF LITERATURE

## 2.1. Introduction

This Chapter is divided into two main sections. The first section provides an overview of the regulation of energy metabolism during maximal, or sprint, exercise, and also highlights relevant literature regarding the causes of fatigue during sprint exercise, the recovery of power output and the effect of training the performance of, and metabolic responses to, sprint exercise. The second main section provides a background to hGH, with particular reference to the regulation of hGH release, and the actions of hGH. In addition, specific reference is made to the regulation of exercise-induced hGH secretion.

## 2.2. Sprint exercise

### 2.2.1. Metabolic changes as a result of sprinting

Sprint exercise results in power production many times that required to elicit maximal oxygen uptake (Spriet, 1995), and this requires very high levels of ATP hydrolysis, catalysed by the activity of three ATPases:

- Actomyosin ATPase, for the dissociation of cross-bridges (Jones and Round, 1990)

- Calcium ($Ca^{2+}$) transport ATPase, for $Ca^{2+}$ reuptake by the sarcoplasmic reticulum (Carafoli, 1991)

- $Na^{+}$-$K^{+}$ ATPase, for restoring membrane ionic balance after each action potential (Horisberger et al., 1991)

$$ATP \xleftrightarrow{\quad ATPases \quad} ADP + P_i + H^+$$
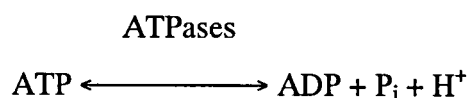
Resting muscle concentrations of ATP are reported to be between ~21 (Jacobs et al., 1982) and ~28 (Cheetham et al., 1986) mmol.kg dry muscle$^{-1}$ which represents a relatively small reserve, and, therefore, during sprint exercise ATP must be

resynthesised at a very fast rate, relying largely on anaerobic metabolism. There are a number of pathways by which ATP can be resynthesised anaerobically:

$$CPK$$
$$PCr + ADP + H^+ \longleftrightarrow ATP + Cr$$

$$Glycogen + 3\ ADP + 3P_i \longrightarrow 3ATP + 2Lactate^- + 2H^+$$

$$adenylate\ kinase$$
$$2ADP \longleftrightarrow ATP + AMP$$

$$AMP\ deaminase$$
$$AMP + H^+ \longleftrightarrow IMP + NH_4^+$$
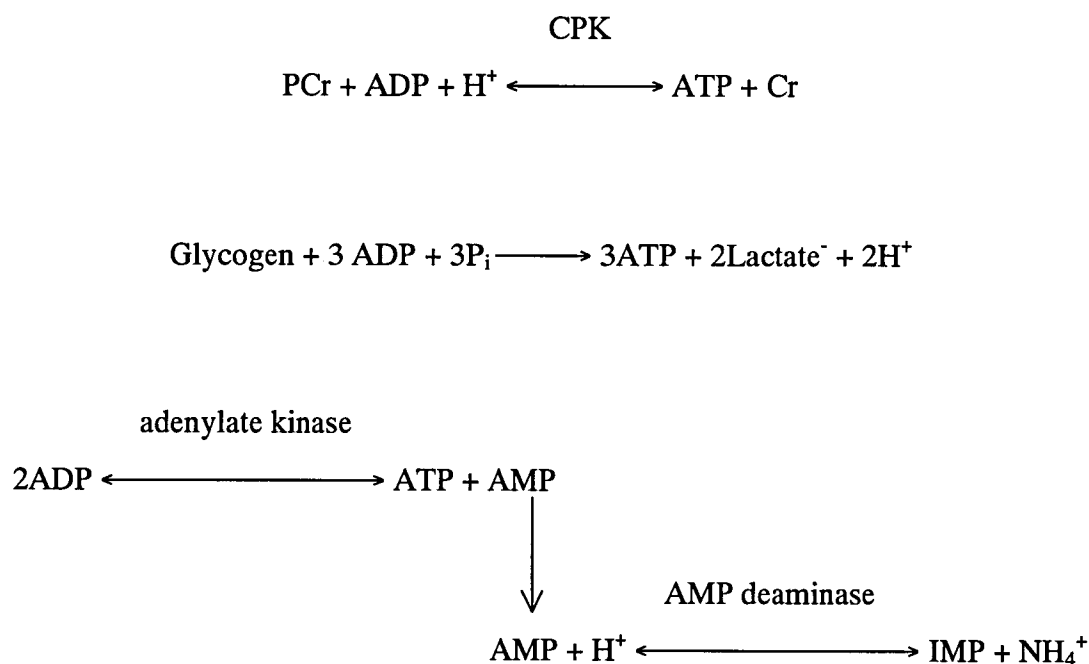
During a 6 s sprint, the total ATP turnover rate from anaerobic sources was reported to be 10.4 mmol.kg dry muscle$^{-1}$.s$^{-1}$ (Boobis, 1987) although Gaitanos et al. (1983) observed ATP production from anaerobic sources to be ~15 mmol.kg dry muscle$^{-1}$.s$^{-1}$ during similar exercise.

**ATP resynthesis from phosphocreatine degradation**

Of all the processes in the cell used in the resynthesis of ATP, the creatine kinase reaction is the most powerful (Sahlin, 1986a), and the muscle content of phosphocreatine (PCr) is 3-4 times higher than that of ATP (Table 2.1). Since the activity of creatine kinase is higher than the activity of ATPase, significant decreases in muscle ATP concentrations only occur when PCr is broken down to 60 % of the resting value (Hultman et al., 1987). At the end of a single 30 s cycle ergometer sprint, PCr was observed to be reduced to as little as ~17 % of resting concentrations (Bogdanis et al., 1996).

The creatine kinase reaction also has a buffering effect due to the involvement of hydrogen ions (H$^+$). Breakdown of PCr absorbs H$^+$, which has the effect of increasing

pH during the first few seconds of maximal contraction. Since the activity of phosphofructokinase (PFK) is pH dependent, alkalisation as a result of PCr breakdown will facilitate the activation of PFK and accelerate glycolysis.

**ATP resynthesis from glycolysis**

Limited muscle PCr content means that the creatine kinase reaction can only supply energy at a high rate for the first few seconds of intense exercise. Therefore, the glycolytic pathway is activated almost immediately after the start of intense contractions (Jacobs et al., 1983), and the findings of Greenhaff et al. (1996) suggest that it takes approximately 3 s to reach its maximal rate. Both muscle glycogen and glucose can be utilised in the glycolytic pathway, however, the intracellular content of free glucose is low and the transport across the cell membrane is a slow process in comparison to the rate of glycogen degradation (Hultman and Sjoholm, 1983). Therefore, muscle glycogen is the main substrate for anaerobic glycolysis during sprinting.

Table 2.1. Muscle metabolite concentrations at rest and after maximal exercise.

| References | | n | | ATP | %↓ ATP | PCr | %↓ PCr | Gly | %↓ Gly | La⁻ post |
|---|---|---|---|---|---|---|---|---|---|---|
| *Cycle ergometer* | | | | | | | | | | |
| Jacobs et al. (1982) | | 9F | 30 s | 20.9 | 34 | 62.7 | 60 | 360 | 23 | 61 |
| Jacobs et al. (1983) | | 15M | 30 s | - | - | - | - | - | - | 74 |
| | | 15M | 10 s | - | - | - | - | - | - | 46 |
| | | 7F | 30 s | - | - | - | - | - | - | 47 |
| | | 7F | 10 s | - | - | - | - | - | - | 25 |
| Boobis et al. (1983) | | 7M | 30 s | 21.2 | 43 | 94.4 | 65 | 266 | 21 | 98 |
| Bogdanis et al. (1995) | | 8M | 30 s | 25.6 | 29 | 77.1 | 80 | 322 | 34 | 119 |
| Bogdanis et al. (1996) | | 8M | 30 s | 27.0 | 27 | 75.2 | 83 | 328 | 30 | 108 |
| Cherry et al. (1998)* | | | | | | | | | | |
| | 7.5% | 8F | 30 s | 21.3 | 9 | 79.6 | 69 | - | - | 110 |
| | 11.0% | 8F | 30 s | 24.0 | 17 | 78.5 | 74 | - | - | 113 |
| Gaitanos et al. (1993) | | 8M | 6 s | 24.0 | 13 | 76.5 | 57 | 317 | 14 | 29 |
| *Non-motorised treadmill* | | | | | | | | | | |
| Cheetham et al. (1986) | | 8F | 30 s | 28.2 | 37 | 87.7 | 64 | 281 | 25 | 78 |
| Nevill et al. (1989) | | 4M,4F | 30 s | 26.7 | 28 | 84.0 | 67 | 317 | 32 | 86 |
| *Isokinetic cycle ergometer* | | | | | | | | | | |
| Jones et al (1985) | | | | | | | | | | |
| | 60 revs.min⁻¹ | 5M | 30 s | 21.3 | 37 | 70.5 | 60 | - | - | 135 |
| | 140 revs.min⁻¹ | 5M | 30 s | 19.1 | 0 | 64.8 | 34 | - | - | 126 |
| McCartney et al. (1986) | | 8M | 30 s | 22.6 | 40 | 62.0 | 70 | 373 | 21 | 126 |

PCr, phosphocreatine; Gly, muscle glycogen; La⁻, muscle lactate; M, male; F, female

*Sprint against an applied resistance of 7.5 % or 11.0 % of the individuals' body mass.

All units are mmol.kg dry muscle⁻¹.

Muscle glycogen content at rest is much larger than that of the high energy phosphates ATP and PCr (Table 2.1). In addition, it has been shown that anaerobic glycolysis is the dominant source of ATP during sprinting with percentage contribution to total anaerobic ATP turnover during a 30 s of 60-70 % (Cheetham et al., 1986), resulting in a reduction in muscle glycogen of ~32 % (Nevill et al., 1996a). The fact that muscle glycogen levels at the end of maximal exercise are still relatively high infers that glycogen availability does not limit exercise performance. However, 3 days on a low carbohydrate (CHO) diet resulted in reduced mean power output during a 30 s cycle ergometer sprint, compared with a moderate CHO diet (Langfort et al., 1997). In this study muscle glycogen was not measured and it is, therefore, not entirely clear whether a low CHO diet actually reduced muscle glycogen content (Maughan and Williams, 1982). Furthermore, even if muscle glycogen levels were depleted, it is possible this acted via an acceleration in PCr degradation, rather than having a direct effect (Hultman, 1967).

**Muscle and blood lactate and pH**

During sprint exercise lactic acid is formed as a result of anaerobic glycolysis. At a physiological pH lactic acid is almost completely dissociated to lactate (La⁻) and $H^+$ (Sahlin, 1986a), and $H^+$ are, therefore, formed in equivalent amounts to lactate. Lactate is considered to be the main origin of $H^+$, and the resulting decrease in pH (Hultman and Sahlin, 1980), and the contribution of lactic acid to total $H^+$ production in the muscle has been estimated to be more than 85 % (Sahlin, 1986a).

A single maximal 30 s cycle ergometer sprint has been shown to result in an increase in muscle lactate concentration from ~9 to ~61 mmol.kg dry muscle$^{-1}$ in females (Jacobs et al., 1982) and to ~74 mmol.kg dry muscle$^{-1}$ in males (Jacobs et al., 1983). Cheetham et al. (1986) reported a good correlation between muscle and blood lactate following a 30 s all-out sprint treadmill sprint, with peak blood lactate concentrations attained 5-6 min into recovery. Cheetham et al. (1986) also found blood lactate concentrations to be highly correlated with peak running speed and peak power output corrected for body mass.

The finding that muscle lactate concentrations increase dramatically during a 10 s cycle ergometer sprint, representing 59 % of the changes observed in a 30 s sprint in

men, and 46 % in women, provides evidence that glycolysis is important in the early part of sprint exercise (Jacobs et al., 1983). Following a single maximal 30 s cycle ergometer sprint muscle lactate was found to have increased from ~4 mmol.kg dry muscle$^{-1}$ at rest to ~119 mmol.kg dry muscle$^{-1}$ (Bogdanis et al., 1995). This was accompanied by an increase in blood lactate concentration from 0.7 mmol.l$^{-1}$ to a highest measured mean concentration of 13.6 mmol.l$^{-1}$ 6 min after the sprint. In addition, blood pH was found to have fallen from 7.38 at rest, to 7.08.

Allsop et al. (1990) measured muscle pH continuously for 30 min after a single 30 s sprint on a non-motorised treadmill. Muscle pH decreased from a resting value of 7.17 to 6.57 immediately after the sprint. Blood pH also decreased from 7.39, at rest, to 7.04 immediately after the sprint, displaying a similar pattern of recovery as muscle pH. A correlation between muscle pH and venous blood pH was identified, although, the predictability of muscle pH from a given blood pH was poor.

Table 2.2. Blood metabolite concentrations at rest and following maximal exercise.

| Reference | | | La$^-$ | | pH | | NH$_4^+$ | |
|---|---|---|---|---|---|---|---|---|
| | | | Pre- | Post- | Pre- | Post- | Pre- | Post- |
| *Cycle ergometer* | | | | | | | | |
| Bogdanis et al. (1995) | 8M | 30 s | 0.7 | 13.6# | 7.38 | 7.08# | 29 | 157# |
| Bogdanis et al. (1996) | 8M | 30 s | - | 12.0† | - | - | - | - |
| Cherry et al. (1998)* | | | | | | | | |
| 7.5 % | 8F | 30 s | - | 11.6‡ | - | - | 26 | 74‡ |
| 11.0 % | 8F | 30 s | - | 10.9‡ | - | - | 23 | 64‡ |
| *Non-motorised treadmill* | | | | | | | | |
| Cheetham et al. (1986) | 8F | 30 s | | | | | | |
| Nevill et al. (1989) | 4M,4F | 30 s | - | 13.0‡ | 7.38 | 7.17 | - | - |
| Allsop et al. (1990) | 7M,3F | 30 s | 0.8 | 15.8‡ | 7.39 | 7.04§ | - | - |
| Nevill et al. (1996a) | 5M,6FST | 30 s | - | 17.9*f* | 7.40 | 7.04§ | - | 251*f* |
| | 6M,6FET | 30 s | - | 12.3*f* | 7.40 | 7.16§ | - | 163*f* |
| *Isokinetic cycle ergometer* | | | | | | | | |
| McCartney et al. (1983) | | | | | | | | |
| 60 revs.min$^{-1}$ | 13M | 30 s | - | 9.7$ | - | - | - | - |
| 100 revs.min$^{-1}$ | 13M | 30 s | - | 10.6$ | - | - | - | - |
| 140 revs.min$^{-1}$ | 13M | 30 s | - | 9.2$ | - | - | - | - |
| Jones et al. (1985) | | | | | | | | |
| 60 revs.min$^{-1}$ | 5M | 30 s | ~2 | ~16# | - | - | - | - |
| 140 revs.min$^{-1}$ | 5M | 30 s | ~1 | ~14# | - | - | - | - |

La$^-$, blood lactate (mmol.l$^{-1}$) (NB. McCartney et al. (1983) and Jones et al. (1985) reported plasma lactate); NH$_4^+$, plasma ammonia (μmol.l$^{-1}$); *Sprint against an applied resistance of 7.5 % or 11.0 % of the individuals' body mass; M, male; F, female; ST, sprint-trained; ET, endurance-trained; § 1 min post-exercise; $ 3min post-exercise; † 4 min post-exercise; ‡ 5 min post-exercise; # 6 min post-exercise; *f* mean peak concentration.

**Oxidative contribution to energy supply during sprinting**

During a 30 s sprint ATP turnover rates are much higher than can be attained by the aerobic energy processes, however, the aerobic contribution might be important for the last part of the sprint, and, particularly during repeated sprinting. McCartney et al. (1986) found that, during the third and fourth of four 30 s bouts of maximal isokinetic cycling, mean power output could be maintained at ~60 % of the values recorded in the first bout, despite evidence of minimal glycolytic flux. It was suggested that this was due to an increased aerobic contribution, and a large increase in plasma glycerol concentrations, reflecting the utilisation of intramuscular triglycerides, provided further support for this suggestion.

An increased aerobic contribution in the last of ten 6 s sprints, separated by 30 s of recovery, has also been reported by Gaitanos et al. (1993). In that study there was no increase in muscle lactate concentration as a result of the last sprint, whilst mean power output was only reduced to ~73 % of that generated in the first sprint. Bogdanis et al. (1996) also considered the aerobic contribution to repeated sprinting, and identified a ~41 % reduction in anaerobic energy in the second of two 30 s cycle ergometer sprints. Despite this, total work done during the second sprint was only reduced by ~18 % and it was suggested that aerobic metabolism compensated for the reduction in anaerobic energy supply, providing ~49 % of energy during the second sprint.

**AMP deamination and ammonia production**

The accumulation of ADP has been suggested to be involved in fatigue (Section 2.2.3) and, therefore the ATP/ADP ratio is kept high via the adenylate kinase reaction. AMP deamination then occurs to promote continued formation of ATP from ADP (Sahlin and Katz, 1988), producing IMP in equivalent amounts to ammonia ($NH_4^+$). IMP remains in the cell, and, since further metabolism of IMP is a relatively slow process, the muscle content of IMP might reflect the extent of energy deficiency (Sahlin, 1992). In contrast, ammonia diffuses into the blood (Graham et al., 1993) where it can be easily measured, and during high intensity exercise almost all ammonia production can be attributed to AMP deamination (Terjung and Tullson, 1992). In addition, Harris et al. (1991) found a significant correlation between plasma

ammonia and the decline in muscle ATP, which might be useful in the evaluation of ATP loss using plasma samples.

A relationship between both blood lactate and plasma ammonia concentrations has been reported (Buono et al., 1984; Itoh and Ohkuwa, 1991). However, it is unlikely that there is a causal link, but rather that there is a coincidental acceleration in the rates of both glycolysis and AMP deamination with intense short-term exercise (Terjung and Tullson, 1992), and it has been demonstrated that ammonia production can occur independent of lactate metabolism in patients with glycolytic enzyme deficiencies (Sahlin et al., 1990).

### 2.2.2. Recovery from sprint exercise

Although studying metabolic responses to a single sprint provides an insight into the importance of different metabolic pathways in energy provision, it does not provide very much information regarding the influence of particular metabolites on fatigue. Instead, using a repeated exercise model allows the examination of the relationships between various metabolites and performance through studying their recovery. The advantage of this approach is that some variables that have been identified as important in fatigue might recover at different rates (Nevill et al., 1996a). Therefore, a large body of research has used repeated exercise models in the study of fatigue.

### Recovery of force and power

Changes in maximal dynamic power with repeated exercise bouts have been studied by McCartney et al. (1986), who employed four 30 s maximal efforts on an isokinetic cycle ergometer at a pedalling rate of 100 rev.min$^{-1}$, with 4 min of recovery between each bout. Highest peak power and average power for a pedal revolution in the initial few seconds of the first exercise bout and were measured at 1626 W and 992 W, respectively. Both were reduced by ~20% in the second bout to 1321 W and 775 W, respectively. A further decline of ~21% was identified in the third bout, but there was no decline in the last exercise period.

The recovery of maximal short term power output was also studied by Hitchcock et al. (1989). Power output was measured during leg extensions on an isokinetic dynamometer following 0, 1, 2, 3, 4 and 8 min of recovery from prior cycle ergometer

exercise equivalent to 60, 80, 100 and 120 % $\dot{V}O_2$max. Short term power output immediately after cycle ergometer exercise decreased to 85, 76, 55 and 45 % of initial values after exercise at 60, 80, 100 and 120 % $\dot{V}O_2$max, respectively, and recovery was found to follow a "two-component exponential pattern" for all intensities of prior exercise. In addition, after prior exercise at intensities equivalent to 60 and 80 % $\dot{V}O_2$max, short term power output had almost fully recovered within 60 s, whereas full recovery did not occur until 4 min after exercise at 100 % $\dot{V}O_2$max and 8 min after exercise at 120 % $\dot{V}O_2$max. It was concluded that most of recovery (50-85 %) occurs within 60 s after exercise, and this time-course was compared with the recovery of PCr stores following exercise.

In a study of the recovery of power output using treadmill sprinting (Holmyard et al., 1994), subjects completed a maximal 30 s sprint followed by passive recovery periods of 15, 45 and 120 s or 30, 60 and 180 s, before the completion of a second maximal sprint of 6 s duration. In a separate trial venous blood samples were taken 60 and 180 s after a single 30 s maximal treadmill sprint. There was an initial rapid recovery of power output, and ~80 % of recovery was complete after 60 s, but only a further 12 % recovery occurred in the second and third minutes after the 30 s sprint. No relationships were found between the recovery of power output and changes in blood metabolites, but recovery was suggested to follow a similar time-course as the resynthesis of intramuscular PCr reported by Harris et al. (1976).

During repeated maximal 30 s sprints on a friction loaded cycle ergometer, it has been demonstrated that peak power output in the second sprint almost recovers to the values seen in the first sprint (77 %) within 90 s of recovery (Bogdanis et al., 1995). However, peak power output, peak pedal speed and mean power output in the first 6 s of the sprint did not reach control values after 6 min of recovery, with peak power outputs after 3 and 6 min of recovery equivalent to 86 and 87 % of the peak power in the first sprint, respectively. Recovery of PCr was found to be significantly correlated with power output restoration for the 90 s and 3 min intervals, but not following 6 min of recovery. In addition, no relationship was identified between muscle lactate

recovery and power output. Once again, these data provide support for the contention that PCr resynthesis plays a dominant role in the recovery of power during repeated bouts of high intensity exercise.

Following 2 min of recovery from a 10 s cycle ergometer sprint, peak power similar to that in the first sprint could be generated despite increased muscle lactate, [$H^+$] and inorganic phosphate, indicating that both the force and contraction velocity components of muscle function were not affected (Bogdanis et al., 1998). However, mean power output in the first 10 s of the sprint could not be reproduced and this was probably due to a reduction in the total rate of ATP resynthesis in this period. Neither peak power output nor mean power output in the first 10 s of the sprint could be reproduced following 2 min of recovery from a 20 s sprint. Phosphocreatine stores were similar before the 30 s sprints in both trials, and oxygen uptake was the same during the sprints, suggesting that the lower work might be related to reduced glycolytic ATP regeneration as a result of the higher muscle acidosis in recovery from the 20 s sprint. However, force has been shown to recover faster than the disappearance of lactic acid in experiments using animal muscle fibres and Sahlin and Ren (1989) found a biphasic pattern of force recovery after isometric knee extension exercise to fatigue. In this study, maximal voluntary force was restored within 2 min of recovery despite high muscle lactate concentrations.

In summary, there appear to be at least two phases of recovery of power output after maximal intensity exercise. Around 80% of the recovery of power output from a 30 s sprint occurs within 60-90 s, but full recovery of peak power output and mean power output for the first 6 seconds of a sprint is not achieved for at least 6 min after a sprint. This pattern of recovery of power output following maximal exercise has been suggested to be associated with the resynthesis of PCr following exercise, and also the recovery of glycolytic ATP generation.

**The ionic imbalance and very rapid recovery**
At the end of high intensity exercise, net $K^+$ uptake by muscle is rapid with a 90-95% recovery of intracellular $K^+$ within 210 s (Lindiger et al., 1995). Cherry (1998) noted that just 2-3 s of recovery from a maximal 30 s cycle ergometer sprint against an applied resistance of either 7.5% or 11% body mass allowed significant recovery of

peak power output in females. The initial rapid phase of recovery was attributed to the restoration of ionic balance. This suggestion is consistent with the finding that inhibition of $Na^+$-$K^+$ pump activity by preincubation of rat muscle with ouabain, resulted in a slower initial rate of force recovery lasting up to 5 min after electrical stimulation at 90 Hz (Harrison et al., 1996). Balog and Fitts (1996) also identified a correlation between rapid initial force recovery and ionic balance restoration following electrical stimulation, as well as a secondary, slower phase which was linked with the recovery of metabolites.

**Recovery of muscle pH after high intensity exercise**
Sahlin et al. (1976) examined muscle lactate concentrations and muscle pH content after cycling exercise calculated to cause exhaustion at ~6 min. From a resting value of 7.08, pH fell to 6.60 at exhaustion and during recovery pH returned to resting levels whilst muscle lactate concentrations remained elevated. These results, therefore, suggest that there is a faster rate of efflux for acid equivalents than lactate during the early part of recovery from high intensity exercise. The changes in muscle metabolites and contraction capacity following isometric knee extensions to fatigue at 66 % of maximum voluntary contraction were studied by Sahlin and Ren (1989). Muscle pH declined from ~7.10 at rest to ~6.50-6.60 at fatigue and remained low for the first 2 min of recovery. This was attributed to a release of $H^+$, as a result of PCr resynthesis, balancing the rate of $H^+$ efflux from the muscle. In contrast, contraction force was completely restored after 2 min of recovery, suggesting that the capacity to generate force *in vivo* is not limited by high intracellular [$H^+$].

Muscle pH has been shown to drop to ~6.70 after a maximal 30 s sprint on a non-motorised treadmill (Cheetham et al., 1986; Cheetham et al., 1987; Nevill et al., 1989) and even to 6.50 following repeated 30 s bouts of maximal sprint exercise (Spriet et al., 1989). Muscle pH has been measured before, and continuously for 30 min after, a single maximal 30 s sprint on a non-motorised treadmill by Allsop et al. (1990). Measurements were made using a needle-tipped pH electrode and thermocouple placed in the vastus lateralis of healthy subjects. Muscle pH was observed to decrease from 7.17 at rest to 6.57 immediately after the sprint, whilst venous blood pH was seen to fall from 7.39 to 7.04. After an initial increase in muscle pH to 6.70 (2 min into recovery), no further recovery was seen in the first 10 min of recovery and 30

min after the sprint muscle pH had recovered to 7.03 and venous blood pH to 7.29, but these values were still significantly different from the resting values.

**Resynthesis of phosphocreatine in muscle**

Bogdanis et al. (1996) considered the contribution of PCr to energy supply during repeated sprint exercise. Subjects completed two cycle ergometer sprints in two main trials. The first sprint was 30 s in duration in both trials, whereas the second sprint was either 10 s or 30 s. Muscle samples were taken at rest, immediately after the first 30 s sprint, after 3.8 min of recovery and immediately after the second, 10 s or 30 s, sprint. At the end of the first sprint muscle PCr was ~17 % of resting concentrations, and muscle pH had dropped to ~6.70. After 3.8 min of recovery, muscle pH had not recovered (~6.80), but muscle PCr concentrations had been restored to ~79 % of the resting value. In the second sprint, PCr was almost completely depleted in the first 10 s. In addition, a relationship between power output recovery and PCr resynthesis was identified, whereas no relationship between power output recovery and any other metabolite was found.

The time course of PCr resynthesis was examined by Harris et al. (1976), who took repeated muscle biopsy samples during recovery from dynamic exercise (8.7 min of cycling at 60 rev.min$^{-1}$) and isometric knee extension (40-55 s at 66% of maximum voluntary force). The data were modelled using a two component exponential equation and fast and slow recovery components were identified. There was no difference in the half times of the fast component of recovery after dynamic or isometric exercise, which were 21 s and 22 s respectively, although the amount of PCr resynthesised after 2 and 4 min of recovery was lower following isometric exercise. Bogdanis (1994) found that the PCr resynthesis was slower following sprint exercise on a cycle ergometer when compared with the dynamic exercise used by Harris et al. (1976). This difference was attributed to reduced blood flow to recovering muscles during passive recovery from sprinting which, in turn, reduced PCr resynthesis.

Sahlin et al. (1979) examined the recovery of muscle PCr following cycling to exhaustion at an intensity calculated to result in fatigue at ~6 min. They observed significant recovery of PCR (68 %) in muscle samples incubated in oxygen for 15 min, but did not see any recovery of PCr in muscle samples incubated in nitrogen.

This highlights the importance of oxygen availability for PCr resynthesis, and Harris et al. (1976) found that occlusion of blood flow to leg muscles during and after exercise to fatigue abolished PCr resynthesis. Further evidence that the initial fast phase of PCr resynthesis is limited by the availability of oxygen is that the initial rate of PCr resynthesis is not affected in patients with glycolytic enzyme deficiencies, who do not produce lactate or $H^+$, but is slower in patients with peripheral vascular disease or mitochondrial myopathies (Chance et al., 1982; Radda et al., 1982; Radda, 1986). However, the slow phase of PCr resynthesis might be limited by the presence of free hydrogen ions (Sahlin et al., 1979).

PCr has been shown to be decreased to a similar extent in both fibre types following a maximal 30 s sprint on a non-motorised treadmill (Greenhaff et al., 1994). However, Tesch et al. (1989) found that PCr recovered to 50 and 68 % of resting levels 60 s after 30 maximal voluntary knee extensions in fast twitch and slow twitch fibres, respectively. In addition, PCr was higher in slow twitch fibres 60 s after ~83 s of electrical stimulation of the quadriceps at 20 Hz (Soderlund and Hultman, 1991). Slower PCr resynthesis in fast, compared with slow, twitch fibres, might be related to the higher mitochondrial density and capillarisation of slow twitch fibres (Soderlund and Hultman, 1991).

The effect of active, rather than passive, recovery on power output during cycle ergometer sprinting was studied by Connell and Maile (1996), who found enhanced recovery of power output when 15 s cycle ergometer sprints were interspersed with 5 min active recovery periods at an intensity of 40 % $\dot{V}O_2$max, compared with 5 min of passive recovery. Similarly, Bogdanis et al. (1996) observed that 4 min of active recovery (cycling at 40 % $\dot{V}O_2$max) resulted in a significantly higher mean power output in the second of two 30 s sprints, compared with a second sprint following passive recovery (603 vs. 589 W). The enhanced recovery of mean power output following active recovery was a result of a 3.1 % higher power generation in the first 10 s of the second sprint, and it was suggested that active recovery enhanced the recovery of power output through increased blood flow to the exercising muscle, resulting in increased ion efflux from the muscle, and also increased oxygen availability for PCr resynthesis.

Trump et al. (1996) studied 3 bouts of isokinetic cycling at 100 rev.min$^{-1}$ with 4 min recovery between bouts and occluded blood flow to one leg for the 4 min period between bouts 2 and 3, to prevent PCr resynthesis. Muscle biopsies taken immediately before and after bout 3 demonstrated no difference in muscle metabolites apart from PCr between the two legs, whilst power output was 15 % lower in the cuffed leg compared with the control leg. These results provide further evidence of a close relationship between the recovery of power output and PCr resynthesis.

In summary, muscle has been shown to recover from fatigue in at least two distinct phases, a rapid phase, complete within 2 min, and a slower phase lasting 30-60 min (Fitts and Holloszy, 1978; Thompson et al., 1992). These distinct phases of recovery might represent at least two distinct mechanisms of fatigue (Cherry, 1997), since different aspects of performance recover with different time-courses. For example, peak power output recovers rapidly as a result of the restoration of ionic balance and an initial rapid phase of PCr resynthesis, whilst mean power output recovers more slowly, probably related to a slower phase of PCr recovery associated with adverse metabolic conditions following a sprint, in addition to reduced inhibition of glycolysis with the recovery of pH.

### 2.2.3. An overview of fatigue in sprinting

Edwards (1981) defined fatigue as, "The inability of the total organism to maintain a required or expected power output", and it is characterised by a rapid decline in mechanical output or tension development in skeletal muscle (Hermansen, 1981). The decline in the contractile force of skeletal muscle that occurs with fatigue is associated with a decrease in muscle PCr and ATP concentrations and an increase in inorganic phosphate ($P_i$) and its acidic fraction ($H_2PO_4^-$), $H^+$ and ADP, with a consequential decrease in free energy available from ATP (Godt and Nosek, 1986). Since ATP rarely drops below ~50 % of the resting value during sprinting (Boobis, 1983), and there is a very low $K_m$ of myosin ATPase for ATP (Glyn and Sleep, 1985) it is unlikely that fatigue is related to reduced energy supply (Jones and Round, 1990). It is more likely that the accumulation of products of the hydrolysis of ATP ($P_i$, ADP, $H^+$) and a decrease in pH due to the contribution of anaerobic glycolysis influence fatigue.

**The role of phosphate**

Increases in inorganic phosphate ($P_i$) have been reported to result in decreases in $Ca^{2+}$ sensitivity and maximal force in skinned skeletal and cardiac muscle preparations (Godt and Nosek, 1985). Similarly, Kentish (1985) observed that the addition of $P_i$ to skinned cardiac muscle of rats greatly decreased force generation. In addition, it was reported that this was neither due to a reduction in the amount of free energy available for work from ATP hydrolysis, nor due to inhibition of creatine kinase activity, but rather that $P_i$ has a direct action on the contractile machinery. The decreases in force generation as a result of the addition of $P_i$ to contracting skinned fibres is probably a result of a slowing of the release of phosphate from the actomyosin complex, leading to an accumulation of cross-bridges in a state where they are unable to develop force (Jones and Round, 1990). However, Cady et al. (1989a) found intact preparations to be much less sensitive to $P_i$ than skinned fibre preparations, and showed that, in intact preparations, the major changes in $P_i$ occur early in exercise, before force begins to decline.

**The role of ADP**

Slowing of relaxation from an isometric contraction is characteristic of acutely fatigued muscle, and is thought to be a result of a reduction in the rate of cross-bridge detachment. The detachment is thought to involve the binding of ATP with the actomyosin complex, and the dissociation of actin and myosin with ATP bound to myosin (Figure 2.1). Phosphate does not influence the rate of these reactions and does not, therefore, influence the force-velocity characteristics. However, ADP accumulation will inhibit the release of ADP, and will therefore reduce the number of cross-bridges which will reach the stage where they can dissociate. This reduces the force at a given velocity and also a reduction in the maximal velocity of shortening (Jones and Round, 1990).

However, the concentration of ADP at which the rate of cross-bridge detachment is reduced (~4 mmol) is outside the normal physiological range (Jones and Round, 1990). In any case, the effect of ADP accumulation on force generation is not entirely clear, since concentrations in excess of the normal physiological range increased force in skinned preparations (Godt and Nosek, 1985), but have been found to result in a decrease in force in intact muscle preparations (Cady et al., 1989a).

vi  ADP Pi  i  ADP Pi  ii  Pi

v  ATP  ADP

ATP  ADP

iv  ATP  iii  ADP
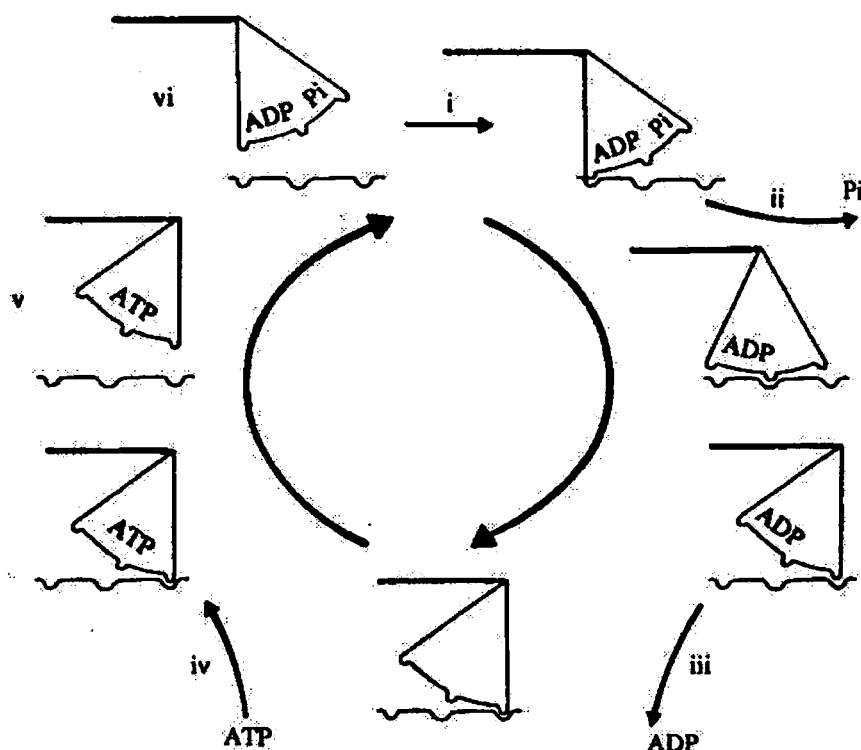
Figure 2.1. Stages in the cross-bridge cycle in relation to the biochemical steps. (i) attachment of actin and myosin giving stiffness to the muscle, (ii) $P_i$ is released from the actomyosin complex initiating rotation, (iii) ADP released, (iv) actomyosin complex binds ATP (v)actin and myosin dissociate with ATP bound to myosin, (vi) ATP is hydrolysed activating the myosin head (in Jones and Round, 1990).

**The role of hydrogen ions**

The activity of PFK, which is a key rate-limiting enzyme in glycolysis, shows a marked pH dependence and, therefore, $H^+$ accumulation might be expected to result in a reduction in glycolysis. However, Sahlin et al. (1975) reported that during isometric contraction to fatigue, the glycolytic rate remained unchanged, despite a decrease in muscle pH from 7.09 at rest to 6.56 at fatigue. Isometric contraction to fatigue was also found to result in increased concentrations of the PFK activators $P_i$, AMP, ADP, fructose 1,6-diphosphate and fructose 6-phosphate (Sahlin et al., 1975) and these probably overcame any pH mediated inhibition of PFK.

It has also been considered that $H^+$ might be involved in the slowing of relaxation. Sahlin et al. (1981) identified a close relation between muscle pH and slowing of relaxation rate in normal rat extensor digitorum longus muscle, but not in muscle poisoned with iodoacetic acid (which stops glycolysis and prevents a drop in pH). In contrast, Cady et al. (1989b) found that slowing of relaxation occurred in both normal subjects and one subject with myophosphorylase deficiency (MPD), who could not produce $H^+$ from glycolysis. These findings indicate that slowing of relaxation can occur independent of $H^+$. However, the recovery of relaxation was faster in the subject with MPD than the normal subjects, suggesting that the recovery of relaxation is due to persisting low pH. Therefore, there may be at least two processes, one due to $H^+$ accumulation and one independent of $H^+$, that cause slow relaxation in fatigue.

Increased concentrations of $H^+$ are also likely to displace the creatine kinase equilibrium so that PCr breakdown increases, thereby reducing high energy phosphate stores (Sahlin et al., 1983). Other mechanisms by which accumulation of $H^+$ might contribute to fatigue include:

- Increased binding, and therefore reduced release, of $Ca^{2+}$ by SR at low pH (Nakamura and Schwartz, 1970)
- Competition with $Ca^{2+}$ for binding sites on troponin C (Palmer and Kentish, 1994)
- Decreased activity of the three ATPases by product inhibition, and therefore slowing relaxation, reducing force and impairing muscle membrane depolarisation.
- Decreased sensitivity of the force generating apparatus (Metzger and Moss, 1987)
- Reduced energy release from ATP breakdown (Cooke and Pate, 1990)

However, force recovery following exercise has been shown to be faster than the restoration of $H^+$ to pre-exercise levels (Section 2.2.2). In addition, although Cady et al. (1989a) observed that in normal subjects loss of force matched increasing $H^+$ concentrations, in one subject with MPD, force loss was independent of $H^+$ accumulation. Sahlin et al. (1981) observed similar results, whereby a similar (~50 %) decline in tension was observed in normal rat extensor digitorum longus muscle and muscle in which glycolysis had been blocked by iodoacetic acid during electrical stimulation. Although tension decline in unpoisoned muscle was closely related to a

decrease in muscle pH, a similar decline in tension in the poisoned muscle suggests another mechanism of fatigue, independent of pH.

**The role of calcium**

There are a number of possible cellular mechanisms which link calcium and muscle fatigue:

- A decline in $Ca^{2+}$ release possibly associated with slow reuptake of $Ca^{2+}$ by SR under adverse metabolic conditions (Cady et al., 1989b) since $Ca^{2+}$ reuptake is an ATP dependent process (Jones and Round, 1990)

- A reduction of tension at saturating $Ca^{2+}$ (Westerblad et al., 1991)

- A decreased sensitivity of the contractile mechanism to $Ca^{2+}$ (e.g. H+-Ca2+ competition for troponin; Palmer and Kentish, 1994)

**The role of potassium**

The excitability of muscle membrane is dependent on the membrane potential, which is, itself, mainly dependent on the $K^+$ gradient across the membrane (Sjogaard, 1987). The rise in extracellular potassium observed during high intensity exercise would eventually cause a depolarisation block, and therefore might be related to fatigue in this type of exercise (Vollestad and Sejersted, 1988). In fact, small increments in extracellular $K^+$ concentration have been shown to result in a reduction of muscle tension during subsequent electrically stimulated contractions in isolated mouse muscle (Juel, 1988). Any loss of intracellular $K^+$ is likely to be due to impairment of the function of the $Na^+$-$K^+$ pumps which might be a result of the inhibition of $Na^+$-$K^+$ ATPase by accumulation of ADP or H+, or it could be the result of energy deficiency.

Bangsbo et al. (1996) studied the effect of acidity on fatigue and found that at the end of intensive exhaustive leg exercise preceded by arm exercise muscle pH was lower than after leg exercise alone. However, fatigue occurred at the same arterial and venous plasma potassium concentrations regardless of whether arm exercise preceded leg exercise or not. These results suggest that increased muscle acidity is not the only cause of fatigue, but that the accumulation of potassium in muscle interstitium might be an important factor. It was suggested that the development of fatigue might be a result of increased release of $K^+$ from the muscle cell due to a decrease in pH.

In summary, there are a number of biochemical changes that have been suggested to play a role in the development of muscle fatigue. The products of hydrolysis of ATP, namely $P_i$, ADP and $H^+$, have been reported to have a direct effect on the contractile apparatus, although conflicting results in skinned and intact preparations make it difficult to assess the importance of these mechanisms. It has also been suggested that the accumulation of $H^+$ is involved in a number of other mechanisms of fatigue, including the inhibition of glycolysis, promotion of PCr breakdown and interference with the normal function of $Ca^{2+}$ at a number of levels. However, fatigue has been found to occur independent of the accumulation of $H^+$ and a role for $K^+$ accumulation in the muscle interstitium has been suggested. It is likely that some, or all, of these mechanisms contribute to fatigue during sprint exercise.

### 2.2.4. The effect of pedalling rate

One of the advantages of using a friction-loaded cycle ergometer to study power output is that both force and velocity components can be measured. Changes in pedalling rate occur throughout a 30 s sprint, but it is possible to modify mean pedal rate during a sprint by altering the applied resistance, since in an all-out sprint, pedalling rate is a function of applied resistance (Cherry, 1997). Mean pedal rate can be used as a good indicator of the number of muscle actions that take place, and therefore, by altering the applied resistance it is possible to manipulate the number of muscle actions during a sprint.

### The effect of pedalling rate on power output

Sargeant and Dolan (1987) studied the effect of the duration of prior exercise on the power output achieved in a 20 s maximal effort under isometric conditions at a pedalling rate of 112 $rev.min^{-1}$. Prior exercise was performed at an intensity equivalent to 98% $\dot{V}O_2max$ and lasted 0.5, 1, 3 or 6 min. With increasing duration of prior exercise, maximal power in the subsequent effort decreased, and 6 min of prior exercise resulted in a reduction in maximal power output of 30 % compared with control. In the same study the effect of the intensity of prior exercise was considered and 6 min bouts of exercise at intensities equivalent to between 32 % and 100 % $\dot{V}O_2max$ preceded a 20 s maximal effort. Six minutes of exercise at power outputs

eliciting 39 % and 56 % $\dot{V}O_2$max resulted in an increase in maximal power of 15.0 % and 10.5 % respectively, compared with control. However, prior exercise at power outputs equivalent to more than 60 % $\dot{V}O_2$max reduced subsequent maximal power compared with control.

The effect of fatigue on maximal power output at a range of different contraction velocities was studied by Beelen and Sargeant (1991). Six healthy male subjects completed 25 s maximal efforts on an isokinetic cycle ergometer at pedalling rates of 60, 75, 90, 105 and 120 rev.min$^{-1}$ immediately after 6 min of cycling exercise at 90 % $\dot{V}O_2$max. These results were compared with a 'control condition' whereby prior exercise was performed at 30 % $\dot{V}O_2$max. Fatiguing prior exercise did not result in significant reductions in maximal power at pedalling rates of 60 and 75 rev.min$^{-1}$, compared with control conditions. However, at the higher pedalling rates (90, 105 and 120 rev.min$^{-1}$) maximal power was reduced by 23 %, 28 % and 25 % compared with control, respectively. These results inferred a velocity dependent effect of fatigue which may reflect selective fatigue of fast, fatigue-sensitive, fibres. In human mixed muscle all fibre types will contribute to power production and the effect of selective fatigue would be small. However, with increasing contraction velocities the contribution of faster fibres will become more important and, therefore, fatigue of these fibres is likely to affect power production at high, rather than slow, contraction velocities. Beelen and Sargeant (1993) studied the effect of prior exercise at different pedalling rates on maximal power. The results showed that 6 min of submaximal cycling exercise performed at a pedal rate of 120 rev.min$^{-1}$ reduced maximal power output during a 25 s maximal effort at 120 rev.min$^{-1}$ more than when the prior exercise was matched for external work production, but was performed at a pedal rate of 60 rev.min$^{-1}$. These results provided further evidence of the greater involvement of fast fatigue-sensitive fibres in exercise at higher pedalling rates.

Cherry et al. (1996) considered the effect of constant paced compared with all-out exercise on a friction loaded cycle ergometer and found that the total work produced during a 30 s all-out sprint could be reproduced during 30 s of constant-paced exercise at pedalling rates equal to 55 % of the peak pedal speed achieved in the 30 s sprint

(CP-55 %). The performance of a 6 s sprint following only 3 s of recovery from the first bout of exercise (all-out sprint or CP-55 %) demonstrated that subjects were less fatigued under CP-55 % conditions, since they performed better in the 6 s sprint. This suggests that the moderate pedalling rates in the CP-55 % condition resulted in a greater contribution of slow type I muscle fibres compared with the all-out sprint, allowing faster fibres to make a greater contribution in the 6 s sprint.

**The effect of pedalling rate on the metabolic response to sprint exercise**

Bergstrom and Hultman (1988) used electrical stimulation at a frequency of 20 Hz to produce contractions with a duration of 0.8 s in one leg and 3.2 s in the other leg. In both experiments the work to rest ratio was 1:1, and the 0.8 s stimulation consisted of 64 contractions giving a total contraction time of 51.2 s. The 3.2 s stimulation gave the same total contraction time and consisted of 16 contractions. Muscle biospies were taken at rest and after 22.4 and 51.2 s of contraction. Despite the observation that force declined more rapidly with contractions of a shorter duration, no significant differences were found in muscle PCr content or muscle lactate concentrations after either 22.4 or 51.2 s of contraction, and calculated ATP utilisation rate did not differ between trials. However, the inclusion of data from Chasiotis et al. (1987), who compared continuous stimulation with 1.6 s intermittent stimulation, resulted in the identification of significant differences in muscle lactate concentrations between the different stimulation protocols, which was observed to increase as the number of muscle actions increased.

Intermittent electrical stimulation (1.6 s contraction, 1.6 s rest) at two frequencies (20 and 50 Hz) for a total of 25 contractions (total contraction time 40 s) resulted in both higher initial force and a greater loss of force at 50 Hz (Hultman and Sjoholm, 1983). However, no differences in the magnitude of changes in concentrations of ATP, ADP, PCr, $P_i$ or lactate were identified. Soderlund et al. (1992) used the same frequencies and stimulation pattern to study changes in muscle metabolites in human type I and type II fibres. Muscle biopsies were taken from the quadriceps femoris muscle at rest and after 10 and 20 s of stimulation (6 and 12 contractions, respectively). The decrease in force, represented as a percentage of the initial force, was similar during electrical stimulation at the two frequencies. The PCr degradation was higher in type II fibres compared with type I fibres at both frequencies. However, there were no

differences in PCr degradation rate at 20 or 50 Hz, and, after 12 contractions, there were no differences in the ATP, PCr or glycogen content in mixed muscle. In contrast, markedly larger increases in mixed muscle lactate, G-6-P and F-6-P concentrations were identified after 12 contractions at 50 Hz compared with 20 Hz.

Lodder et al. (1991) found that, after 40 stimulated contractions of rat extensor digitorum longis muscle at three different shortening velocities (25, 50 and 75 mm.s$^{-1}$), muscle PCr and lactate concentrations were similar. In contrast muscle IMP concentration was significantly higher following stimulation at 75 mm.s$^{-1}$ compared with either 25 or 50 mm.s$^{-1}$ despite the fact that the amount of work produced was not different.

A maximal 30 s effort performed on an isokinetic cycle ergometer at three different pedalling rates (60, 100 and 140 rev.min$^{-1}$) resulted in both greater peak power output and a greater fatigue index with increasing pedalling rate (McCartney et al., 1983). However, no differences in postexercise plasma lactate concentrations were identified. Jones et al. (1985) also examined the changes in muscle metabolite concentrations following 30 s of maximal exercise on an isokinetic cycle ergometer, this time at two different pedalling rates (60 and 140 rev.min$^{-1}$. Peak power output and fatigue index were both greater following exercise at 140 rev.min$^{-1}$, however, maximal exercise at different pedalling rates was observed to induce similar changes in the concentrations of metabolites in mixed muscle. In contrast, plasma lactate concentrations were higher 4 min after exercise at 140 rev.min$^{-1}$ compared with 60 rev.min$^{-1}$.

Cherry et al. (1998) studied the effect of pedalling rate during all-out sprinting on a friction loaded cycle ergometer. In this study pedalling rate was manipulated by altering the applied resistance, since when an all-out sprint is performed on a friction-loaded cycle ergometer, pedalling rate is a function of the applied resistance (Cherry, 1997). The results of Cherry et al (1998) demonstrated that changes in pedalling rate during all-out cycle ergometer sprinting did not result in differences in the magnitude of the subsequent changes in muscle or blood metabolites. Changes in muscle concentrations of PCr, creatine, lactate, ATP and pyruvate were not different following sprints against applied resistances equivalent to 7.5% and 10.1% of

subjects' body mass. In addition, there were no differences in plasma ammonia concentrations, blood pH levels or blood lactate concentrations following sprint exercise at different pedalling rates.

In summary, average pedalling rate during cycle exercise can be regarded as a good indicator of the total number of muscle actions which take place. It is possible to manipulate pedalling rate during sprint exercise on a friction-loaded cycle ergometer by altering the applied resistance. The literature suggests that the manipulation of average pedalling rate, and the associated changes in the speed and number of muscle actions, might alter the metabolic responses to exercise. However, the only available data regarding the metabolic responses to sprint exercise where limbs are free to accelerate, for example on a friction-loaded cycle ergometer, suggest that pedalling rate does not influence the magnitude of changes in either muscle or blood metabolites.

### 2.2.5. Training

Short-term sprint or interval training has been shown to result in small, but significant, improvements in sprint performance (e.g. Boobis et al., 1983; Sharp et al., 1986; Cheetham and Williams, 1987; Nevill et al., 1989; Stathis et al., 1994).

One of the factors involved in the improvement of sprint performance following training might be an increase in the number of contractions during the sprint, which could be facilitated by a decrease in the time to peak tension of fibers after training (Nevill et al., 1989). In fact time to peak tension of the rat soleus muscle has been shown to decrease by 14% following sprint training (Staudte et al., 1973) although no change was observed in the fast-twitch rectus femoris. The activity of myofibrillar ATPase is a very important factor in determining this contractile characteristic and this has been shown to increase by 34% following training (Belcastro et al., 1981). Myofibrillar ATPase activity could be enhanced by an increase in the activity of adenylate kinase, which has been demonstrated to be higher after strength training in humans (Costill et al., 1979). If adenylate kinase activity also increased following sprint training the rate of ADP deamination to IMP and $NH_3$, and the resultant removal of ADP from the contraction site, would rise, thus reducing the inhibition of ATP utilization by product inhibition (Nevill et al., 1989).

**The effect of training on sarcoplasmic reticulum function**

It has also been suggested that decreased contraction time might be due to changes in the functional capacity of the SR (Troup et al., 1986). Fitts et al. (1980) postulated that the SR regulates contraction and relaxation times, observing an association of an increase in SR $Ca^{2+}$ uptake activity with faster contraction and relaxation in thyrotoxic rat soleus. However, Hunter et al. (1999) found that 12 weeks of high-resistance strength training resulted in an increase in SR $Ca^{2+}$ uptake and SR $Ca^{2+}$-ATPase activity in the vastus lateralis of elderly (64-79) but not young (18-27) women and that quadriceps relaxation time did not change in either group as a result of training. Similarly, Ortenblad et al. (2000) did not observe any change in $Ca^{2+}$-ATPase capacity or SR $Ca^{2+}$ uptake rate following 5 weeks of sprint training in young men, despite a 12 % increase in total work performed. However, peak rate of $AgNO_3$-stimulated $Ca^{2+}$ release was found to increase by 9%, probably as a result of enhanced muscle SR content. The fact that there were no changes in the myosin heavy chain (MHC) isoform distribution indicates that there is a different time course in development SR $Ca^{2+}$ regulation properties and MHC isoforms.

**Changes in muscle fibre composition associated with training**

Increases in muscle power might be a result of an increase in the speed of muscle contraction, which has a close association with the expression of different myosin heavy chain (MHC) isoforms (Harridge et al., 1998). Sprint training in humans has been shown to cause an increase in type IIa and a decrease in type IIb fibres (Jacobs et al., 1987). Similarly, Costill et al. (1979) found an increase in the percentage of the muscle's cross-sectional area represented type IIa fibres, along with a decrease in type I fibres, following 7 wk of isokinetic strength training. No changes in the percentage of type I, type IIa and type IIb fibres were observed. Aitken et al. (1989) also found the proportion of type IIa fibres increased by 5.9%, whilst the proportion of type I fibres decreased by 6.3% following sprint training.

Six weeks of sprint training on a cycle ergometer was found to increase pedal revolutions, and therefore power output, achieved during a 3 s sprint test at three different applied resistances (Harridge et al., 1998). However, no changes in MHC isoform or fibre-type distribution were identified, and there were no significant variations in single-fibre contractile characteristics. In fact, in the knee extensors

whole muscle twitch, TPT, a marker of contractile speed, was increased following training, and this change was attributed to a change in $Ca^{2+}$ release and reuptake from SR. The changes in performance during the 3 s sprints were, therefore, attributed to increased strength in the leg muscles and to neural adaptations to cycling. A similar conclusion was drawn by Boobis et al. (1983) who identified an improvement in sprint performance following 8 weeks of sprint training but did not find a concomitant increase in energy provision from glycogenolysis. It was therefore suggested that improvements in performance might be attributable to the recruitment of a larger muscle mass after training.

**The effect of sprint training on enzyme activity**

High intensity training has been shown to decrease contraction time in the rat soleus muscle from 111 to 92 ms (Troup et al., 1986). In addition, the activity of phosphofruktokinase (PFK) increased in the soleus and the deep region of the vastus lateralis following training. Baldwin et al. (1975) observed a close correlation between PFK activity and actomyosin ATPase activity in rats and suggested that the glycogenolytic pathway and actomyosin ATPase activity are regulated in parallel. A correlation has also been demonstrated between actomyosin ATPase activity and speed of shortening suggesting a causal link (Barany, 1967). Staudte et al. (1973) did not see a significant change in PFK activity in rats as a result of a sprint training programme employing shorter intervals of higher speed than the programme of Troup et al. (1986). However sprint and strength training in humans has been shown to result in increased PFK activity both in conjunction with (Costill et al., 1979; Hellsten et al., 1996; Sharp et al. 1986), and in the absence of (Houston et al., 1981; Jacobs et al., 1987), any improvements in performance. Parra et al. (2000) observed increases in PFK activity along with an improvement in sprint performance when training was carried out with two days of recovery between each training sessions but found a larger increase in PFK activity despite finding no improvement in performance when training was carried out every day. This highlights the sensitivity of different training adaptations to rest distribution and indicates that improvements in the performance of maximal exercise may not be dependent on glycolytic enzyme activities. Indeed, it has been estimated that the potential of the enzymes in the glycolytic pathway exceed the demands during maximal exercise and it is not clear as

to why the activity of these enzymes increase with training (Saltin and Gollnick, 1983).

Stathis et al. (1994) found sprint training to result in lower post-exercise muscle IMP and ammonia concentrations after a 30 s sprint reflecting a reduction in the magnitude of ATP depletion. This suggests that the balance between ATP hydrolysis and resynthesis is improved after sprint training. The most likely reason for improved ATP resynthesis is increased glycolytic capacity (Sharp et al., 1986; Jacobs et al., 1987; Nevill et al., 1989). Stathis et al. (1994) also observed plasma ammonia concentrations to be higher in the $2^{nd}$ min of recovery but then tended to be lower by $20^{th}$ min of recovery ($P = 0.06$). Snow et al. (1992) found 7 wks of sprint training to result in a decrease in plasma ammonia concentrations following exercise. Since Nevill et al. (1989) found that sprint training did not result in any changes in ATP depletion during sprint exercise, it was suggested that this decrease in plasma ammonia was unlikely to be a result of lower muscle ammonia accumulation, but rather an attenuation of net efflux of ammonia from the exercising muscles (Snow et al., 1992). However, Nevill et al. (1989) did report an increase in glycolytic flux following sprint training, and it is, therefore, possible that exercise-induced increases in muscle ammonia concentrations were reduced as a result of this.

## The effect of sprint training on the aerobic contribution to sprinting

It has been suggested that sprint training decreases glycolytic rate in rats (Troup et al., 1986) and humans (Harmer et al., 2000) and increases the aerobic contribution to sprinting (MacDougall et al, 1998; Harmer et al., 2000). The contention of Harmer et al. (2000) that sprint training decreases glycolytic rate disagrees with studies employing training protocols utilising very short sprints (Linossier et al., 1993), combined speed and speed endurance training (Nevill et al., 1989), longer sprints (Jacobs et al., 1987) and even studies using a very similar training regime (Stathis et al., 1994; MacDougall et al., 1998). Stathis et al. (1994) reported that there is no conclusive evidence that training enhances muscle oxygen consumption during a single sprint bout, however, MacDougall et al. (1998) saw an increase muscle oxidative enzymes in addition to an increase in PFK activity. Furthermore, it has been argued that decreased glygolysis and glycogenolysis, evidenced by lower glycogen degradation, lower muscle and plasma lactate accumulation, combined with

an improved energy balance, indicated by reduced ATP degradation and IMP accumulation, meant that the aerobic contribution to sprinting must increase (Harmer et al., 2000). However, there was no evidence of an increase in either oxygen consumption or oxygen deficit after training.

An increase in $\dot{V}O_2$max following sprint training was identified by MacDougall et al. (1998), in agreement with the results of Harmer et al. (2000) who observed a similar trend (P=0.07). When the training protocols used in these studies are considered, however, this is not a entirely surprising observation since each session consisted of a high volume of training of the type that would be expected to improve speed endurance rather than explosive speed. Depite this, studies employing the same speed endurance training programme consisting of a progression from four to ten 30 s all-out sprints on a cycle ergometer with 4 min of passive recovery between sprints have shown increases in peak power output in a 30 s sprint of 6-17 % (McKenna et al. 1993; Stathis et al., 1994; Harmer et al., 2000), although MacDougall et al. (1998) did not find any improvement in peak power during a single sprint. The difference in training protocols, as well as different testing procedures probably accounts for much of the disagreement between studies regarding changes in metabolic responses to sprint exercise.

## Changes in buffering capacity associated with sprint training

It has been reported that alterations in substrate levels, glycolytic enzyme activities and fibre composition cannot account for the improvements in performance observed following sprint training (Parkhouse and McKenzie, 1984). Nevill et al. (1989) found that a single 30 s sprint on a non-motorised treadmill resulted in a decrease in blood pH of 0.21 units, and 8 wk of sprint training increased this drop to 0.29 units. It was suggested that training might have resulted in enhanced $H^+$ efflux from exercising muscles. Buffering capacity, as measured using the homogenate technique, was not altered as a result of training, although it was recognised that this method does not take into account transmembrane ionic fluxes. In contrast, when buffering capacity was calculated from changes in lactate concentrations and pH during the 30 s sprint, it was found to increase from 87.9 to 126.7 slykes following training. This increase in buffering capacity would allow a greater anaerobic contribution to energy provision

for a given change in pH, and increased glycolytic flux was attributed to either an increase in PFK activeity, or an increase in $H^+$ efflux from the muscle. These results are similar to those of Sharp et al. (1986), who identified a significant increase in buffering capacity, PFK activity and performance, without any change in pH, following 8 wk of sprint training.

Further evidence for an important role of an increase in buffering capacity as an adaptation during sprint training was provided by Bell et al. (1988), who used one-legged cycling to investigate the effect of 7 wk sprint training on intramuscular pH and nonbicarbonate buffering capacity. Peak and average power outputs during a one-legged 60 s maximal power test were significantly greater following training, as were blood lactate concentrations after exercise. In addition, buffering capacity, defined as the quantity of hydrochloric acid (HCl) required to change the pH of a homogenate of 1 g of muscle by 1 pH unit, increased from 49.9 $\mu$mol HCl.$g^{-1}$.$pH^{-1}$, before training, to 57.8 $\mu$mol HCl.$g^{-1}$.$pH^{-1}$. Although Bell et al. (1988) could not provide a full explanation of the mechanisms for such a training adaptation, the major buffering components are the bicarbonate buffer system, PCr, $P_i$, protein-bound histidine residues and carnosine (Parkhouse and McKenzie, 1984). It is possible that increases in one, or more, of these components might be important in training adaptations associated with improved buffering capacity, but the larger increase in *in vivo* buffering capacity following training, when compared with *in vitro* buffering capacity, identifies the additional importance of enhanced membrane transporter capacity (Juel, 1998).

**Changes in the regulation of ionic balance associated with sprint training**

Potassium has a vital role in muscle function during exercise and has been implicated in fatigue (Section 2.2.3). Active 70 year old men have been found to have a 30-40 % higher [$^3$H]ouabain binding site concentration than age-matched sedentary men, suggesting that $Na^+$-$K^+$ pump concentration can be increased by training (Klitgaard and Clausen, 1989). Furthermore, McKenna et al. (1993) reported increased [$^3$H]ouabain binding site concentration in human skeletal muscle, and improved plasma and skeletal muscle $K^+$ regulation after 7 wk of sprint training, possibly through an increase in $Na^+$-$K^+$-ATPase activity. The improvement in $K^+$ regulation by skeletal muscle following training was accompanied by improvements in sprint

performance. Harmer et al. (2000) took a different approach and found that sprint training reduced plasma $K^+$ concentrations following exercise when the work done in the post-training performance test was matched with that done in the pre-training test. This result provides further support for an improvement in $K^+$ regulation as a result of training, which might play a role in the improvement of sprint performance.

In summary, short-term sprint training is likely to result in a change in the kinetics of $Ca^{2+}$ release and reuptake by SR, and this is likely to precede small, if any, changes in MHC isoform expression. Improvements in performance as a result of a short period of sprint training might also be a result of increased energy provision through enhanced glycolytic flux, or even an increase in the aerobic contribution (particularly in repeated sprints), depending on the focus of training. In addition, increases in muscle buffering capacity as well as improved $K^+$ regulation might be important training adaptations. However, the most likely sources of improvement in performance over a short period are increases in muscle strength in conjunction with neural adaptations.

### 2.3. Human Growth Hormone

### 2.3.1. The biochemistry, production and secretion of human growth hormone

**Biochemistry**

Human growth hormone (hGH), also known as somatotrop(h)in, differs from the growth hormone (GH) of every other species (Merimee, 1979). It is a polypeptide hormone and, although not homogenous, the major one is a single chain of 191 amino acids, stabilised by two intermolecular disulphide bonds (Kutsky, 1973), with half cystines between positions 53 and 65 and between 182 and 189 and a molecular weight of approximately 22 000 daltons (Niall, 1971). Human GH is very similar in structure to prolactin (PL) and chorionic somatomammotropin (HCS) of the placenta suggesting a single progenitor hormone in an earlier stage of evolution (Merimee, 1979).

**Production**

Mature hGH is synthesised and stored in the acidophilic somatotropes of the anterior pituitary. These cells comprise 30-40% of the anterior pituitary (Guyton, 1986) and, in total, contain 5-10 mg hGH, constituting the most abundant hormone in the

pituitary gland (Haynes, 1986). Human GH is a product of translation on ribosomes attached to the endoplasmic reticulum of the somatotropes. It would appear that a hGH precursor is formed (Merimee, 1979) which is then probably transported to the Golgi apparatus by ER transport vesicles where it is compacted and packaged in secretory granules or vesicles. Typical somatotropic cells possess large numbers of these round secretory vesicles in the cytoplasmic compartment (Guyton, 1986) which are 300 to 400 $\mu$m in diameter (Merimee, 1979). The secretory vesicles then store hGH until its release in bursts as a result of stimulation. Secretion of hGH is, therefore, episodic and, at the cellular level, release of hGH appears to involve exocytosis or fusion of secretory vesicles with the plasma membrane followed by solubilisation and diffusion of the vesicle content into the circulation (Merimee, 1979). Production rates of hGH have been reported to be ~0.5 mg/24 h/m$^2$ in females (Thompson et al., 1972), and ~0.4 mg/24 h/m$^2$ in males (Alford et al., 1973).

**Secretion**

Secretion of hGH is largely regulated by the balance between two hypothalamic hormones; one that stimulates hGH release called growth hormone releasing hormone (GHRH) (also known as growth hormone releasing factor/hormone (GRF/GRH) or somatocrinin), and one which inhibits hGH secretion called somatostatin (SS) (also known as growth hormone inhibiting hormone (GHIH) or somatotrophin release inhibiting factor/hormone (SRIF/SRIH)). GHRH and somatostatin are thought to be the main factors mediating the effect of a number of exitatory and inhibitory stimuli including:

Exitatory stimuli:
a) hypoglycaemia
b) physical exercise
c) fasting
d) meals with a high protein content
e) deep sleep
f) stress
g) glucagon
h) L-DOPA

i) clonidine (a central acting α-adrenergic drug)

j) amino acids

Inhibitory stimuli:

a) glucose/hyperglycaemia

b) cortisol

c) hGH

d) free fatty acids

## Growth hormone receptors

After hGH is released into the circulation it is attached to a specific binding protein which is, in fact, the extracellular domain of the cell membrane bound hGH receptors (Rogol, 1989) and is derived from the membrane receptor by cleavage (Spencer et al., 1988). Baumann et al. (1987), using rats, showed that *in vivo* the binding protein (BP) lowers the rate of GH clearance six-fold. In addition, at normal levels of BP, only 25-45% of hGH is bound, so the effect of the BP might be to dampen pulse height and to maintain GH availability in between pulses (Jansson et al., 1985). The hGH-bound complex travels to the liver and other tissues to bind with the specific membrane bound hGH receptors. GH receptors have been identified in many vertebrate cell types including hepatocytes, adipocytes, fibroblasts, chondrocytes, osteoblasts, β-islet cells, macrophages, lymphocytes and ventral prostatic epithelial cells (Waters et al., 1990). The interaction of GH with its receptor leads to the activation of cytoplasmic tyrosine kinases although the GH receptors themselves do not show tyrosine kinase consensus sequences (Waters et al., 1990)

The importance of hGH binding proteins and receptors is highlighted by the fact that children with Laron-type dwarfism are very short , despite high circulating levels of hGH, since they are deficient in both the hGH binding protein and the hGH receptor (Baumann et al., 1987).

## Clearance

Hormones are "cleared" from the plasma through metabolic destruction by tissues, binding with tissues, excretion by the liver into the bile and excretion by the kidneys

into the urine (Guyton, 1986). Human GH is metabolised in the liver and metabolic clearance rate (MCR) is usually constant in individuals, however, there appears to be large amount of variation between individuals (Thompson et al., 1972). In addition, an upright posture has been shown to decrease MCR by 24% (Alford et al., 1973). Hall (1971) demonstrated that hGH has a short half-life since, after intravenous injection, hGH was seen to peak in 1 h before returning to baseline in 3 h. Veldhuis et al. (1995) used deconvolution analysis to produce an estimate for hGH half life of 18 min. Other half-life estimates have been made and George (1996) reports a range of 12-45 min. However, the disappearance rate of hGH from plasma is exponential (Cameron, 1969) and therefore the significance of such half-life estimates is unclear. Various authors have also reported clearance rates of hGH although differing units of measurement make comparison difficult. Franchimont and Burger (1975) reported a clearance rate of 170 l/24 h/m$^2$, whilst Vahl et al. (1997) reported a significant increase in MCR with age from $0.11\pm0.02$ l.min$^{-1}$ in young (27-34 years) subjects to $0.19\pm0.01$ l.min$^{-1}$ in older (47-59 years) subjects.

The short plasma half-life of hGH in addition to the presence of numerous and frequent exitatory and inhibitory stimuli results in marked fluctuations in circulating levels of hGH throughout a 24 h period. However, typical serum concentrations have been reported as $0 - 11.5$ mU.l$^{-1}$ (White and Baxter, 1994). Peak concentrations appear to occur shortly after the onset of sleep (Kern et al., 1995) although average daily hGH concentrations are affected by factors such as sex, age and body composition, amongst others.

## 2.3.2. The regulation of hGH secretion by neuropeptides
### Growth hormone releasing hormone

Three approximately equipotent forms of GHRH have been identified from pancreatic tumours: GHRH 1-44, GHRH 1-40 and GHRH 1-37 corresponding to the number of amino acids in each form (Dieguez et al., 1988). Full biological activity is present in the first 29 amino acids (Lance et al., 1984). Two of these forms, GHRH 1-44 and GHRH 1-40, have been identified in the human hypothalamus (Bohlen et al., 1983). The relative molecular mass of these peptides approximates 13000 daltons (Haynes, 1986). GHRH is inactivated by a plasma dipeptidylaminopeptidase producing the

more stable and biologically less active GHRH 3-44 (Frohman et al., 1986). Immunoreactive GHRH is found mainly in the arcuate nucleus (Arc) although it has also been identified in ventromedial nucleus (VMN) where it is likely to have an exohypophysiotropic role (Muller et al., 1999).

GHRH stimulates GH synthesis and release after binding to specific GHRH $G_s$ protein receptors in the plasma membrane of somatotrope cells, activating adenylate cyclase via $N_s$ which, in turn, activates the cyclic 3',5'-adenosine monophosphate (cAMP) second messenger system. The stimulation of GH secretion occurs through a cAMP and $Ca^{2+}$ dependent mechanism whilst the stimulation of GH synthesis, by increasing the transcription rate of the GH gene, is cAMP but not $Ca^{2+}$ dependent (Baringa et al., 1985).

**Somatostatin**

Somatostatin is a tetradecapeptide that inhibits the secretion of most hormones under physiologic circumstances (Muller et al., 1999). Somatostatin-14 (SS-14) is part of a family of SS-like peptides including somatostatin-28 (SS-28) and a fragment that corresponds to the first 12 amino acids of SS-28 [SS-28-(1-12)] (Muller et al., 1999). The SS-like peptides are synthesised and located in most regions of the brain although somatostatin producing cells are also present throughout the central and peripheral nervous system, the gut and the endocrine pancreas (Muller et al., 1999). In neural tissues, the predominant form is SS-14 with a ratio of 4:1 (SS-14:SS-28) in the hypothalamus and SS-14 is the main form released on $K^+$ depolarisation from slices of rat hypothalamus implying that this is the form responsible for hypothalamic neurotransmission (Muller et al., 1999).

Somatostatin producing cells are present throughout the central and peripheral nervous systems, as well as the gut and endocrine pancreas, and in smaller numbers in other glands and organs. In the hypothalamus, these cells are most common in the periventricular nucleus (PeVN), but they are also found in the Arc and VMN (Muller et al.,1999). Somatostatin binds to its own specific receptors in the plasma membrane of somatotrope cells and inhibits adenylate cyclase activity via inhibitory nitrogen ($N_i$) although it probably has additional actions on calcium fluxes (Dieguez et al.,

1988). In turn this inhibits the accumulation of cAMP in the somatotropes and, therefore, inhibits GH secretion.

## Interactions between GHRH and somatostatin in the regulation of hGH secretion

Growth hormone secretion occurs in an episodic manner punctuated by pulses of relatively short duration which are interspersed by troughs. There has been a great deal of debate concerning the mechanisms that exert an influence on this pulsatile pattern of secretion, largely focussing on the roles of GHRH and somatostatin. However, to assess the relative importance of these hormones is not an easy task since it is not possible to measure GHRH and somatostatin in the peripheral circulation and extrapolate to the hypophyseal portal circulation. This is because circulating somatostatin is likely to be of gastro-pancreatic rather than hypothalamic origin (Patel and Srikant, 1986) and it is possible that GHRH is also of nonhypothalamic origin (Kashio et al., 1987). Much of the data available are therefore from animal studies.

Plotsky and Vale (1985) directly measured concentrations of immunoreactive GHRH and immunoreactive somatostatin in the hypophyseal portal circulation of rats. In addition they took systemic samples from another group of rats. Measurable concentrations of immunoreactive GHRH were only found in samples taken during periods of expected GH secretory activity. In addition, during the collections coinciding with periods of expected GH secretion (i.e. GH pulses), hypophyseal portal plasma immunoreactive somatostatin concentrations decreased by 37±5% in two consecutive samples and then returned to baseline concentrations. It was, therefore, concluded that hypophyseal portal immunoreactive somatostatin and systemic GH are inversely correlated and that GHRH is secreted in a strongly pulsatile fashion. In addition, these results support the contention that GHRH and somatostatin secretion are 180° out of phase (Tannenbaum and Ling , 1984) and suggest that the release immunoreactive GHRH into the hypophyseal portal circulation only occurs when immunoreactive somatostatin secretion is decreased, implying a regulatory role of somatostatin in GHRH secretion. Therefore, each immunoreactive secretory burst was suggested to preceded by, or concurrent with, a reduction in somatostatinergic inhibitory tone. However, the major weakness of the study of Plotsky and Vale

(1985) was that hypophyseal portal measurements of GHRH and somatostatin were taken from a different set of animals to those from which the GH measurements were taken. Although rats display a regular 3-4 h pattern of pulsatile GH secretion, this pattern is not synchronised between animals (Carlsson and Jansson, 1990).

The GHRH/somatostatin/GH axis was also studied by Frohman et al. (1990) using unanaesthetised sheep. Direct measurements of hypothalamic-portal GHRH and somatostatin were made, simultaneous with those of peripheral GH. GHRH was found to have a mean pulse interval of 71 min. Somatostatin was also observed to be pulsatile with a mean pulse interval of 52- 54 min, whilst GH (measured in jugular plasma) had a mean pulse interval of 58-65 min, therefore intermediate between that of GHRH and somatostatin. Whilst there was seen to be considerable concordance between GHRH and GH and considerable discordance between somatostatin and GHRH, no statistically significant results were found. However, a highly significant association was observed between peaks of GHRH simultaneous with or immediately preceding GH peaks. This association explained 62% of GH peaks identified. In contrast no association was seen between somatostatin troughs and GH peaks or between GHRH and somatostatin in hypothalamic-portal plasma. These results suggested that GHRH has a primary role in the regulation of the pulsatile secretory pattern of GH secretion with a lesser role for somatostatin although it was suggested that the intensity of the GH pulse might be modified to some extent by the level of somatostatin at the time of each GHRH pulse. Frohman et al. (1990) also indicated that the fact that GHRH and somatostatin could not explain all of the GH secretory bursts might imply that another hypothalamic factor contributes to the regulation of the pulsatile pattern of GH secretion.

Thomas et al. (1991) continued the study of hypophyseal portal GHRH and somatostatin and their relationship with circulatory GH, this time in ewes after a long period (20 wk) of restricted feeding. Restricted feeding resulted in a significant increase in mean plasma GH in comparison with normal feeding. The secretory pattern of GHRH was pulsatile in all of the ewes with low interpulse concentrations, whereas somatostatin pulses were less regular with generally higher baseline concentrations. The occurrence of GH secretory pulses were highly dependent on GHRH pulses and between 61-70% of GH pulses were coincident with, or

immediately preceded by, a GHRH pulse, whilst 58-61% of GHRH were coincident with, or immediately preceded by, a GH pulse. In contrast, pulsatile secretion of somatostatin appeared to be more random and the concentration of somatostatin at the time of each GHRH was not found to significantly affect the probability of a GH pulse. The earlier suggestion that a decrease in somatostatinergic tone might be the integral factor in the generation of a GH pulse was therefore not supported by the results of this study. The authors did, however, contend that short-term fluctuations in somatostatin might be identified using a shorter (i.e. <10 min) sampling interval. In addition, the higher GH pulse amplitude in the presence of reduced somatostatin concentrations in the underfed sheep suggested that low somatostatin levels might increase the pituitary responsiveness to GHRH.

It must be recognised that there are inter-species differences in the neuroendocrine control of GH secretion and therefore animal data cannot be immediately extrapolated to humans. For example, acute hypoglycaemia results in a decrease in GH secretion in sheep (Frohman et al., 1990) and rats (Tannenbaum et al., 1976), but an increase in GH secretion in humans (Blackard and Waddell, 1969). In fact, whilst the importance of pulsatile GHRH secretion in the regulation of the pulsatile pattern of GH secretion has been demonstrated in sheep (Frohman et al., 1990; Thomas et al., 1991), continuous GHRH infusion has been shown to result in highly pulsatile GHRH secretion in normal humans (Vance et al., 1985). This suggests an important role for somatostatin in the regulation of GH secretory pattern and, in fact, somatostatin withdrawal has been observed to elicit a GH response in humans (Davies et al., 1985).

Jaffe et al. (1993) used $(N\text{-Ac-Tyr}^1,\text{D-Arg}^2)\text{GHRH(1-29)NH}_2$ (GHRH-Ant), a competitive GHRH antagonist at the level of the GHRH receptor, to study the importance of GHRH in the generation of hGH pulses in humans. Exogenous GHRH-stimulated GH secretion was almost completely suppressed by GHRH-Ant. In addition, nocturnal integrated and pulsatile hGH release were both suppressed by GHRH-Ant treatment. Nocturnal hGH profiles displayed a decrease in peak and mean pulse amplitude, but no change in pulse frequency or interpulse hGH concentrations. This provided clear evidence that GHRH plays an important role in the generation of spontaneous hGH secretory pulses in humans, whilst the lack of any

change in the nonpulsatile component of the hGH profile suggests that GHRH is not important in determining interpulse levels of hGH in young men.

The same GHRH antagonist was used to evaluate the role of GHRH in morning (08:30 to 11:30) and evening (20:30 to 23:30) plasma pulsatile hGH secretion (Hanew et al., 1996). Initial plasma hGH concentrations were higher in the evening than in the morning. There was no difference between GHRH-Ant and saline control trials in the morning, however, in the evening plasma hGH decreased during GHRH-Ant infusion and mean 3 h plasma hGH concentration was significantly lower for the GHRH-Ant than for the control trial. It would, therefore, appear that that GHRH maintains evening, but not morning, basal hGH secretion. It is possible that this reflects the fact that evening hGH secretory pattern is dominated by a more pulsatile pattern of hGH secretion than that seen in the morning. If this is the case then the results of this study offered further support for the contention that GHRH regulates pulsatile hGH secretion whilst somatostatin has a greater regulatory influence on interpulse hGH concentrations. However, this study did not give any evidence as to whether the postulated increase in hypothalamic GHRH secretion in the evening was a result of reduced hypothalamic somatostatin. In addition, since GHRH-Ant could not entirely suppress hGH secretion in either the evening or the morning, it is possible that there is another mechanism that plays a role in the secretion of hGH.

Indirect evidence that somatostatin directly modulates the activity of GHRH neurons came from Tannenbaum et al. (1998). It had previously been suggested that somatostatin played a direct regulatory role in GH secretion through its action on the pituitary gland (Brazeau et al., 1973), and also an indirect role through central regulation of GHRH-containing neurons (Tannenbaum, 1994). Of the five subtypes of somatostatin that have been cloned and characterised (Reisine and Bell, 1995), Tannenbaum et al. (1998) colocalised two (sst1 and sst2) in GHRH mRNA-containing neurons in the Arc. This provides strong anatomical evidence that somatostatin is capable of directly modulating the activity of GHRH neurons through interactions with both sst1 and sst2.

It is also possible that other, as yet unidentified, peptides occur in the hypophyseal-portal circulation to stimulate or inhibit GH secretion. For example, a growth

hormone-releasing peptide (GHRP)-receptor has been cloned and does not show homology with other peptide receptors (Howard et al., 1996), strengthening the hypothesis of the existence of a natural GHRP-like ligand (Arvat et al., 1997). In addition, there is evidence suggesting that, in the short term, the ultradian rhythm of GH secretion is independent of GH feedback, but that the generator of this rhythm is, instead, intrinsic to the central nervous system (Willoughby and Kapoor, 1990)

### 2.3.3. The role of neurotransmitters in the regulation of hGH secretion

Although hypothalamic GHRH and somatostatin are thought to have the predominant role in the regulation of GH secretion, other neurotransmitters have a role acting both directly on GH secretion and through the modulation of GHRH and somatostatin release.

**Catecholamines**

*$\alpha_2$-adrenoreceptors*

Clonidine (an $\alpha_2$-adrenergic receptor agonist)-induced GH release in rats was shown to be completely blocked by passive immunisation with GHRH antiserum (Cella et al., 1987), but was not affected by somatostatin antiserum (Eden et al., 1981). These results suggest that GH secretion stimulated by $\alpha_2$-adrenoreceptor activation might be mediated by an increase in hypothalamic GHRH secretion, and not a decrease in hypothalamic somatostatinergic tone. Evidence supporting this contention was provided by the fact that activation of $\alpha_2$-adrenoreceptors, by the administration of clonidine and another $\alpha_2$-adrenergic receptor agonist, guanabenz, resulted in an increased area under the curve for GHRH, with no effect on somatostatin release, in perifused bovine hypothalamic tissue (West et al., 1997). In the same study, administration of idazoxan, an $\alpha_2$-adrenergic receptor antagonist, blocked clonidine-induced secretion of GHRH with no effect on somatostatin.

It is possible, however, that clonidine acts through the inhibition of somatostatinergic pathways. Pre-treatment with GHRH in humans has been shown to abolish the hGH response to a second administration of GHRH, however, the hGH response to clonidine was not altered (Valle et al., 1997). In addition, clonidine administered to rats at a time of spontaneous peak, when somatostatin secretion is low, failed to

stimulate GH release. In contrast, clonidine administration during a trough period increased plasma GH concentrations (Lanzi et al., 1994). It would, therefore, appear that $\alpha_2$-adrenoreceptor agonists enhance GH secretion via both the stimulation of GHRH secretion and the inhibition of somatostatin release.

*$\alpha_1$-adrenoreceptors*

Catecholamines are considered to have a role in the hGH response to hypoglycameia, yet administration of the $\alpha_1$-adrenergic antagonist, prazosin, did not affect the hypoglycaemia-induced hGH response in humans (Tatar and Vigas, 1984). This finding suggests the same mechanism of action, that is inhibition of hypothalamic somatostatin secretion. However, the administration of the $\alpha_1$-adrenergic receptor agonist, methoxamine, has been found to result in a small, not significant, decrease in hGH secretion (Al-Damluji, 1993), and it has been suggested that endogenous catecholamines acting on $\alpha_1$-adrenoreceptors do not play a role in the secretion of hGH (Muller et al., 1999).

*$\beta$-adrenoreceptors*

$\beta$-adrenergic receptors are known to inhibit GH release. The non-specific $\beta$-adrenergic receptor antagonist propranolol has no effect on basal hGH secretion in Caucasians (Blackard and Heidingsfelder, 1968) although it does enhance the hGH response to hypoglycaemia, exercise, glucagon and GHRH (Chihara et al., 1985). In contrast, salbutamol, a $\beta$-adrenergic receptor agonist inhibits the hGH response to GHRH in humans. Activation of pituitary $\beta$-adrenoreceptors *in vitro* stimulate GH secretion, suggesting that $\beta$-adrenergic receptor agonists do not exert their main effect directly at pituitary level (Perkins et al., 1983). It is likely, instead, that the activation of $\beta$-adrenoreceptors *in vivo* is to increase hypothalamic somatostatinergic tone, thus inhibiting GH secretion (Muller et al., 1989).

*Dopamine*

Both stimulatory and inhibitory actions of dopaminergic pathways have been described. Administration of the direct dopamine agonist apomorphine to normal subjects has been shown to cause acute hGH release and increase the hGH response to GHRH (Vance et al., 1987 in Muller). However apomorphine blunts the hGH

response to hypoglycaemia, L-DOPA and arginine when administered by infusion (Woolf et al., 1979; Bansal et al., 1981 in Dieguez). A partial explanation for this is the fact that dopamine has been shown to be capable of stimulating the release of both GHRH and somatostatin from the rat hypothalamus (Kitajima et al., 1989). However, further complication is caused by evidence that dopamine and its agonists inhibit GH release from rat (Cronin et al., 1984) and human (Marcowitz et al., 1982) pituitaries *in vitro*. The role of dopamine in the regulation of hGH secretion, therefore, remains unclear.

**Serotonin**

Serotoninergic pathways appear to provide stimulatory influence for GH release in rats (Arnold and Fernstrom, 1981), although the situation is less clear in humans. The difficulty in assessing the role of serotonin (5-HT) in the regulation of GH secretion is due to the existence of many receptors that mediate the actions of 5-HT, in addition to the lack of specific agonist and antagonist drugs (Valverde et al., 2000). Four main types of 5-HT receptors have been identified in the brain (5-HT1, 5-HT2, 5-HT3 and 5-HT4) with further classification of receptor subtypes. Sumatriptan, a selective 5-HT1D receptor agonist, administration was observed to result in increased spontaneous hGH secretion and also to increase the hGH response to exogenous GHRH in normal prepubertal children (Mota et al., 1995). However, sumatriptan did not change the GH response to clonidine or pyridostigmine. Sumatriptan also induced a GH peak in beagle dogs and potentiated GHRH-induced GH release (Valverde et al., 2000). Atropine (a specific muscarinic cholinergic receptor blocker, which easily crosses the blood-brain barrier) abolished sumatriptan-induced GH secretion, whilst pyridostigmine had no effect. These results suggest that 5-HT1D receptors have a stimulatory effect on GH secretion, possibly through inhibition of hypothalamic somatostatin release. Valverde et al. (2000) also identified a stimulatory role for the 5-HT2C receptor subtype, since a 5-HT2A/C receptor subtype antagonist modified the GHRH-induced GH response whilst a 5-HT2A receptor subtype antagonist failed to do so. Similarly, a 5-HT3 receptor subtype antagonist had no effect on either the basal or GHRH-induced GH response. Thus, different 5-HT receptor subtypes appear to have different roles in the regulation of GH.

It has also been suggested that 5-HT might stimulate the secretion of GH, and other pituitary hormones, acting directly at the pituitary (Balsa et al., 1998). However anterior pituitary cell cultures did not respond to 5-HT, apparently requiring the presence of posterior pituitary cells in coculture in addition to preincubation with corticosterone. These results led the authors to suggest that 5-HT stimulation of GH release is a result of a direct action of 5-HT on the posterior pituitary stimulating release of an unidentified mediator from melanocytes. Further research is required in order to discover whether this mechanism exists, and, if so, to evaluate its importance in the regulation of hGH secretion..

**Acetylcholine**

Casanueva et al. (1984) administered atropine to subjects, and employed three stimuli of hGH secretion: arginine, clonidine and physical exercise. Atropine administration resulted in a complete blockade of hGH secretion. The completeness of the blockade to these three diverse stimuli of hGH secretion indicates the important role of acetylcholine (Ach) in regulating hGH release. Indeed, the results of this study suggest that a cholinergic synapse might be the final common pathway of a variety of different stimuli of hGH release. Atropine has also been demonstrated to suppress pulsatile GH secretion (Casanueva et al., 1983) as well as GH secretion induced by opioids (Casanueva et al., 1980), glucagon (Delitala et al., 1982) and sleep (Mendelson et al., 1978). However, there is evidence that insulin-induced GH secretion (Blackard and Waddell, 1969) as well as GH release in response to surgery (Desborough et al., 1993) are not blocked by cholinergic blockade, indicating that Ach does not mediate all stimuli to GH release.

*In vitro*, Ach has been demonstrated to inhibit somatostatin release from the rat hypothalamus (Richardson et al., 1980). In addition, passive immunisation with anti-somatostatin serum abolished the inhibitory effects of atropine in GHRH-induced GH secretion (Locatelli et al., 1986). These results suggest that cholinergic antagonists enhance somatostatin secretion. However, insulin-induced hypoglycaemia combined with GHRH administration has an additive effect on GH release (Page et al., 1987) suggesting a role for somatostatin in the GH response to hypoglycaemia. This infers that cholinergic antagonists would block the GH response to hypoglycaemia but this is not the case (Blackard and Waddell, 1969). In addition, α-adrenergic blockade

decreases the effect of cholinergic enhancement of GH secretion (Devesa et al., 1991), suggesting that adrenergic pathways might act distally to the cholinergic system in the control of GH secretion (Desborough et al., 1993) disputing the suggestion that a cholinergic synapse is the final common pathway in the control of GH release (Casanueva et al., 1984).

**Glutamate**

The actions of essential amino acids (EAAs), including glutamate, are mediated by a number of receptor subtypes, including N-methyl D-aspartate (NMDA), kainate (KA), 2-amino-3-hydroxy-5 methyl-4-isoxazol proprionic acid (AMPA) and metabotropic receptors. Systemic administration of *N*-methyl-D,L-aspartic acid (NMA), a NMDA receptor agonist, to castrated male sheep (Estienne et al., 1989) has been shown to result in GH release, whilst intravenous injection of boars with NMA increased circulating GH in a dose-dependent manner (Estienne et al., 2000). Kainic acid has also been demonstrated to increase GH secretion in male rats (Pinilla et al., 1986). Furthermore, administration of AMPA to prepubertal rats resulted in an increase in serum GH concentrations, whilst injection of an antagonist of AMPA receptors, 1,2,3,4-tetrahydro-6-nitro-2, 3-dioxo-benzo (f) quinoxaline-7-sulfonamide (NBQX), resulted in the opposite effect, decreasing serum GH levels (Gonzalez et al., 1999). In fact it has been suggested that activation of NMDA, KA and AMPA receptors results in a 'clear-cut' stimulation of GH secretion in animals (Tena Sempere et al., 2000), and taken with the evidence that metabotropic glutamate receptors were found in almost all immunoreactive GHRH neurons of the arcuate nucleus of male rats, providing anatomical evidence of a direct effect of glutamate on GHRH secretion (Kiss et al., 1997), glutamate appears to have an important role in GH secretion. However, there is very little information about the effect of glutamate administration on hGH secretion, and a large oral dose of monosodium glutamate (MSG) had no effect on plasma concentrations of hGH in men (Fernstrom, 2000). This finding probably reflects the fact that diet-derived glutamate does not penetrate hypothalamic regions controlling anterior pituitary function, and extrapolation of the results of animal studies suggests that glutamate probably has a role in the regulation of hGH.

**GABA**

Injection of γ-aminobutyric acid (GABA) into the third ventricle of rats has been shown to result in GH release (McCann et al., 1984). Furthermore, Cavagnini et al. (1980) found that a single oral dose of GABA to 19 subjects resulted in a significant elevation in hGH concentrations, compared with 18 placebo control subjects, with five and a half fold increases in plasma growth hormone 90 min after oral administration. The mechanism for GABA action on GH secretion is unclear, but Willoughby et al. (1986) suggested an action of GABA on the hypothalamus to inhibit somatostatin secretion, and facilitate GH secretion. Conversely, Murakami et al. (1985) suggested that GABAergic stimulation of GHRH release increases basal GH secretion, since passive immunisation with anti-GHRH antibodies abolished this effect. However, Fiok et al. (1981) demonstrated a decrease in plasma GH levels when endogenous GABA levels were increased by pretreatment of rats with GABA-transaminase inhibitors, and identified an increase in plasma GH following a reduction of GABAergic activity by administration of the GABA receptor blocker, bicuculline. In addition, administration of sodium valproate, which is thought to act via a GABA mechanism, increased basal hGH secretion (Steardo et al., 1986), but has been found to inhibit exercise-induced increases in hGH secretion (Steardo et al., 1985; 1986), suggesting dual GABAergic control of hGH secretion.

### 2.3.4. Factors affecting the secretion of hGH

**Sex**

A number of studies have considered the sex-related differences in GH secretion in rats. Jansson et al. (1985) described high amplitude GH pulses with low GH concentrations between pulses in male rats compared with less regular pulses with higher interpulse concentrations in females. The role of GHRH and somatostatin in these different secretory patterns of male and female rats was studied by Painson and Tannenbaum (1991) using passive immunisation with specific antisera. In female rats, a single acute dose of anti-somatostatin serum resulted in increased plasma GH concentrations at all time points for 6 h after administration, as well as an increase in GH peak amplitude, GH nadir levels, and overall mean 6 h GH levels. In contrast, an acute dose of anti-somatostatin serum to male rats increased only GH nadir levels. In addition, administration of an acute dose of anti-GHRH serum raised GH nadir levels

in females but had no effect in males. These findings suggest that the secretory pattern of somatostatin plays an important role in the sexually dimorphic GH secretion patterns in rats.

Women have been identified as secreting more hGH than men (Ho et al., 1987) although the same study showed that higher integrated serum hGH concentrations in young women were determined by serum oestradiol concentrations. Jaffe et al. (1998) compared hGH secretion in women in the early follicular phase of the menstrual cycle, when oestrogen levels are comparable between sexes, with hGH secretion in men and found it to be similar in both groups. In addition, Jaffe et al. (1998) demonstrated a positive correlation between plasma oestradiol concentrations and hGH secretion. These findings suggest that higher oestradial concentrations in women, rather than sex per se, result in greater GH secretion in women than in men. However, the level(s) at which oestradiol exerts its regulatory control is not clear (Muller et al., 1999). The apparent importance of oestradiol in determining average daily hGH secretion means that women using the contraceptive pill, different types of which contain different amounts of oestradiol, will have markedly different levels of daily GH secretion from women not using the contraceptive pill as well as from those using other types of contraceptive pill.

Despite the fact that total secretion rates are similar for males and females matched for age, relative adiposity and oestradiol concentrations, there do appear to be differences in patterns of hGH secretion between men and women (Jaffe et al., 1998). Women were shown to have more hGH pulses with interpulse concentrations twice as high as those of men. The reported higher interpulse hGH concentrations bears a similarity to the differences in GH secretory patterns in male and female rats (Jannson et al., 1985). In addition, Jaffe et al. (1998) found that hGH secretion in men was dominated by large nocturnal pulses with relatively low hGH secretion throughout the rest of the day, in contrast to women who had a much more uniform pulsatile pattern of secretion throughout the day, spending nearly twice as much time in active hGH secretion than men. It is possible that the differences in the pattern of hGH secretion between men and women is attributable to a lesser role of somatostatin in women (Jaffe et al., 1998).

**Age**

"That aging [sic] lowers GH secretion in mammals is almost a tenet of neuroendocrinology." (Muller et al., 1999).

Whilst Muller et al. (1999) report that there is evidence that the pituitary hGH pool is preserved with increased age, daily hGH secretion rate has been negatively correlated with age (Iranmanesh et al., 1991; Veldhuis et al., 1995). This age related fall in hGH secretion appears to be more pronounced in men than in women (Weltman et al., 1994). However, which aspect, or aspects, of secretion rate contribute to the observed changes in secretion rate with age (mass of secretory burst, frequency of secretory burst or basal secretion rate) is not entirely clear. Iranmanesh et al. (1991) observed age to be a major negative statistical determinant of hGH burst frequency and also endogenous hGH half life. However later research found that hGH secretory burst amplitude varied inversely with age, without identifying any significant correlation between age and burst frequency or endogenous hGH half life (Veldhuis et al., 1995). From the results of that study Veldhuis et al. (1995) suggested that the primary impact of age, acting with altered body composition, is to diminish the amount or mass of hGH secreted per burst, possibly mediated by an increase in somatostatinergic inhibitory tone and/or decreased activity of hypothalamic GHRH. In addition, Veldhuis et al. (1995) used an approximate entropy statistic to evaluate the relative degree of serial orderliness or regularity of 24 h serum hGH concentration profiles and observed a reduced regularity of hGH release with age. This suggests that with increasing age there is disruption in the pathways directing hGH secretion, possibly as a result of a reduction in the co-ordination of the release of GHRH and somatostatin.

It has been estimated that for men with a normal body mass index (BMI), an indirect measure of obesity, each decade of increasing age reduces the hGH production rate by 14% and the hGH half-life by 6% (Iranmanesh et al., 1991). Vahl et al. (1997) studied the significance of age on the pharmacokinetics of a single exogenous pulse, mimicking endogenous conditions, in normal adults and found age to be the most important predictor of hGH area under the curve in all subjects along with a greater metabolic clearance rate (MCR) in older individuals.

**Body composition**

Mean (24 h) serum GH concentration has been demonstrated to be negatively correlated with percentage body fat allied with a progressive increase in entropy of 24 h hGH profiles with increasing percentage boy fat (Veldhuis et al., 1995). In addition, intraabdominal fat mass has been shown to be the major determinant of stimulated hGH secretion in healthy non-obese adults (Vahl et al., 1996). Iranmanesh et al. (1991) estimated that each unit increase in BMI, at a given age, reduced the daily secretion rate by 6%.

In addition to sex, age and body composition factors such as sex steroid hormones, nutritional status, physical fitness/exercise training, quality and quantity of sleep and medication use also affect hGH secretion. All of these factors must be considered in the design and interpretation of experimental research.

### 2.3.5. Actions of hGH

The somatomedin-hypothesis (Salmon and Daughaday, 1957) suggests that most growth-promoting actions of GH are mediated by insulin-like growth factor-I (IGF-I). However, GH also exerts metabolic effects either directly or indirectly mediated via IGF-I.

**Glucose metabolism**

GH has both an insulin-like and an anti-insulin effect on glucose metabolism. GH administration in the post-absorptive state result in a decrease in plasma glucose concentrations, suppression of glucose production and an increase in glucose clearance (Adamson et al., 1977), although this insulin agonistic effect is short-lived. In contrast, sustained elevations of GH concentrations has been shown to result in decreased insulin sensitivity of the liver and decreased sensitivity of other tissues to glucose (Moller et al., 1989). It has been suggested that GH reduces glucose metabolism in favour of increased lipid oxidation, reducing the need for protein degradation and gluconeogenesis (Moller et al., 1990). GH also induces increased secretion of IGF-I, which has been shown to have insulin-like effects, but only when IGF-I is in its free form or bound to IGF-binding protein (BP)-3 when it can bind to membrane-bound insulin receptors. *In vivo* the majority of IGF-I circulates bound in a ternary complex and is not free to exert its insulin-like effects in this way.

**Lipid metabolism**

Children (Parra et al., 1979) and adults (Salomon et al., 1989) with GHD display increased body fat compared with healthy individuals which is reduced with GH treatment. Yeh et al., (1994) demonstrated that 16 wk of GH administration suppressed age-related fat gain compared to control in rats. Chronic administration of GH to animals has also been reported to result in depleted adipose stores and an increase in the transfer of lipids to the liver (Merimee, 1979). In addition, GH treatment of GHD increases circulating FFA and glycerol indicating increased lipolysis. This increase in lipolysis is a result of a direct effect of GH in enhancing the activity of hormone sensitive lipase (Dietz and Schwarz, 1991). It has also been suggested that GH might enhance the conversion of FFA to acetyl-CoA in the tissues with subsequent utilisation for energy (Guyton, 1986). IGF-I infusion over several days resulted in increased levels of circulating FFA and increase lipid oxidation, possibly through an IGF-I-induced reduction in insulin secretion (Hussein et al., 1994) but there is no evidence that IGF-I acts directly on adipose tissue *in vivo* (Berneis and Keller, 1996).

**Protein metabolism**

GH treatment has been demonstrated to increase nitrogen retention in patients recovering from thermal injury (Wilmore et al., 1974), and Cuneo et al. (1991a) have demonstrated that exogenous GH increases lean body mass and thigh muscle mass in adults with GHD. In addition, studies using stable isotope-labelled amino acids in post-operative patients treated with recombinant (r)hGH demonstrated an increase in protein synthesis versus protein breakdown of 39% when compared to placebo treated patients (Ward et al., 1987).

It has been suggested that GH has an anabolic effect independent of insulin and IGF-I (Copeland and Nair, 1994) since the administration of rhGH and somatostatin together resulted in an acute inhibition of leucine oxidation, with no changes in plasma insulin, glucagon, cortisol, IGF-I or glucose concentration. Similarly, 6 h of intravenous methionyl-GH infusion after 10 days of underfeeding followed by 10 days of hypocaloric feeding resulted in increased transcription or decreased degradation of myofibrillar protein mRNA in the vastus lateralis of normal men independent of an increase in circulating IGF-I (Fong et al., 1989). In addition,

forearm muscle synthesis has been shown to increase with short term GH infusion and in the absence of any changes in circulating IGF-I concentrations (Fryburg et al., 1991). However, it must be considered that circulating IGF-I levels might not reflect tissue levels of the hormone and that locally produced IGF-I might be mediating the anabolic effect of GH.

However, there is other evidence that GH can have an anabolic effect independent of IGF-I. For example, the actions of GH and IGF-I on protein metabolism may be different, since GH administered to normal subjects resulted in an increase in protein synthesis (Horber and Haymond, 1990) and the predominant anabolic action of IGF-I has been postulated to be an inhibition of proteolysis (Laager and Keller, 1993). There is also evidence that a combination of rhGH and recombinant (r)hIGF-I has a greater anabolic effect than IGF-I alone in calorically restricted humans (Clemmons et al., 1992). These findings support the suggestion that GH might act to promote cell differentiation making cells sensitive to the action IGF-I (Green et al., 1987). Alternatively this may reflect the importance of the IGF-I acid-labile subunit, since both GH and IGF-I have been shown to induce IGF-BP-3 in hypophysectomised rats, yet only GH treatment resulted in the formation of the acid-labile subunit of the complex (Cohick and Clemmons, 1993).

**Water and electrolyte homeostasis**

A common symptom of acromegaly is swelling of soft tissues particularly in the hands and feet and supraphysiological doses of rhGH have been shown to increase body mass due to water retention in addition to increasing sodium retention (Binnerts et al., 1988). Since GH stimulates Na-K-ATPase activity in several tissues, including the kidney (Shimura et al., 1982), it is likely that this mediates GH-induced sodium retention.

**2.3.6. Insulin-like growth factors**

Insulin-like growth factors (IGFs), otherwise known as somatomedins, are polypeptides with a structure similar to insulin. In fact, insulin, IGF-I and IGF-II have approximately 50% of their amino acids in common. IGFs are important in the function of almost every organ in the body (Daughaday and Rotwein, 1989). IGF-I

appears to have the predominant role in regulating growth whilst the physiologic role of IGF-II is less clear.

IGFs are not stored in granules but are continuously synthesised and secreted by most, if not all, tissues in the body (Cooper, 1994). Circulating IGFs are synthesised by the liver although IGFs produced locally by most tissues also act in an autocrine and paracrine manner (Le Roith, 1997). IGFs circulate in nanomolar concentrations (Le Roith, 1997), not in a free form but non-covalently bound to one of six specific binding, or carrier, proteins (IGF-BP-1 to –6, Baxter, 1993). Like the IGFs, IGF-BPs are synthesised both in the liver and in most other tissues (Le Roith, 1997). IGF-BP-3 binds the majority (>95%) of the IGF-I in serum (Le Roith, 1997). This IGF – IGF-BP-3 dimer forms a ternary complex with another protein subunit, the acid-labile subunit (Le Roith, 1997). This stable complex prolongs IGF half life and acts as a reservoir for IGFs. The stability is probably determined by the acid labile subunit since IGF – IGF-BP-3 complexes leave the circulation rapidly (Donaghy and Baxter, 1996). Circulating IGF-BPs also restrict the permeability of IGFs through capillaries and also inhibit their access to membrane receptors, thereby limiting their bioavailability (Daughaday et al., 1980). Once released from the ternary complex, IGF-I leaves the circulation and enters its target tissues assisted by other IGF-BPs.

Acute administration of IGF-I in hypophysectomised rats has been shown to have insulin-like effects including a 35% reduction in blood sugar levels, although longer term infusion does not appear to have this effect (Zapf et al., 1985). It is thought that this is a result of the binding capacity of IGF-BPs being overridden with a single IGF-I injection allowing free IGF-I to circulate and reach tissue receptors and exert its insulin-like effects. In contrast, free IGF-I is not detectable during IGF-I infusion, probably as a result of equilibration with carrier proteins (Zapf et al., 1985).

Both IGF-I and IGF-II are under the control of GH secretion, since growth hormone deficiency (GHD) has been reported to be associated with a fall in both peptides (Rogol, 1989), although IGF-I reductions are of a greater magnitude than IGF-II (Zapf et al., 1981a). In addition, changes in circulating IGF-I with age change in parallel with changes in GH secretion (Le Roith, 1997). Growth hormone induces tissue production of IGF-I and elevations in serum IGF-I concentrations, with most current

evidence indicating that circulating IGF-I is of hepatic origin (Maiter et al., 1988). Marcus et al. (1990) demonstrated an increase in serum IGF-I concentrations several hours after the administration of exogenous rhGH administration in healthy elderly individuals. This rhGH-induced increase in circulating IGF-I required the synthesis of IGF-I and then its transportation into the circulation, accounting for the time-lag between an increase in circulating GH and circulating IGF-I. Hypophysectomised rats have been shown to be deficient in serum IGF-BPs in addition to IGFs themselves, and GH administration stimulates synthesis and secretion of this carrier protein (Zapf et al., 1985).

Whilst hepatic IGF-I synthesis and secretion is under the control of GH (Schwander et al., 1983), diurnal patterns of circulating IGF-I have not been found in man (Vermuelen, 1987) or other mammals (Donaghue et al., 1990), and serum concentrations of IGF-I usually parallel 24 h mean GH concentrations. GH also increases the serum concentrations of both the acid-labile subunit and IGF-BP-3 (Jones et al., 1995). Like insulin, IGF-I and IGF-II specifically bind to membrane bound tyrosine kinase receptors. Insulin binds to its own receptor and, with less affinity, to the IGF-I receptor. IGF-I and IGF-II both activate their own specific receptor, as well as the IGF-I receptor and the insulin receptor (Ruderman et al., 1994). Liver and fat cells express only insulin receptors whilst muscle cells express both insulin and IGF-I receptors (Le Roith, 1997).

IGF-I mediates many, if not most, of the anabolic effects of circulating GH. Six days of IGF-I administration to hypophysectomised rats stimulated three indices of growth in the absence of GH; tibial epiphyseal cartilage width, thymidine-incorporating activity of costal cartilage and body mass (Zapf et al., 1985). IGF-II administration did not increase body mass and stimulated the other two growth indices much less strongly than IGF-I, suggesting that IGF-II mediates growth via IGF-I receptors (Zapf et al., 1985). In humans, patients with IGF-I deficiency as a result of GH insensitivity have been used to test the therapeutic use of recombinant IGF-I. In this disorder, known as Laron dwarfism, IGF-I treatment resulted in increases in circulating IGF-I and an associated stimulation of bone growth as well as an alteration in body composition due to increased protein accretion and a reduction in body fat mass (Laron et al., 1992; Wilton, 1992).

The role of GH in promoting growth was thought to be mediated by circulating IGF-I synthesised in the liver. However, it would appear that locally produced IGF-I, acting in an autocrine or paracrine manner, might play an important role. Whilst experimental increases in IGF-I have resulted in general somatic growth in rats (Bates et al., 1993), direct local infusion of nonsystemic (i.e. doses that avoid generalised somatic growth) doses of IGF-I into the tibialis anterior muscle of rats resulted in muscle hypertrophy with no apparent effect on nearby muscles or the hearts or body mass of the rats (Adams and McCue, 1998). It had previously been demonstrated that IGF-I peptide production increases in the overloaded plantaris muscle of rats (Adams and Haddad, 1997). These results, taken together, suggest a role for locally produced IGF-I in muscle hypertrophy.

Local production of IGF-I is under different control in different tissues. For example, GH, parathyroid hormone and sex steroids regulate the production of IGF-I in bone, whilst sex steroids have the predominant role in production of IGF-I in the reproductive system (Le Roith, 1997). IGF-I also appears to have an essential role in the CNS system with influence over proliferation, differentiation and survival in the developing brain (Muller et al., 1999). IGF-I has a further role in the inhibition of the secretion of GH by the pituitary (Le Roith, 1997), and the infusion of rhIGF-I has been demonstrated to suppress pulsatile and GHRH-stimulated GH secretion in male subjects (Jaffe et al., 1998). This inhibitory effect probably occurs at both the hypothalamic and pituitary levels with an apparent role for somatostatin, since IGF-I has been shown to directly stimulate the acute release of somatostatin from rat hypothalamic fragments in culture (Sheppard et al., 1978).

Physical exercise is a potent stimulus of GH release and Cappon et al. (1994) identified both a GH-dependent and an acute GH-independent, exercise-induced, increase in circulating IGF-I following high intensity exercise. It has also been suggested that the anabolic response to training might involve both GH-dependent increases in hepatic IGF-I production and GH-independent increases in IGF-I in the active muscles since training did not increase hepatic IGF-I mRNA in GH suppressed rats (Zanconato et al., 1994).

Figure 2.2. A comparison of the metabolic effects of GH and IGF-I (from Berneis and Keller, 1996).

* short-term action at relatively high doses of IGF-I
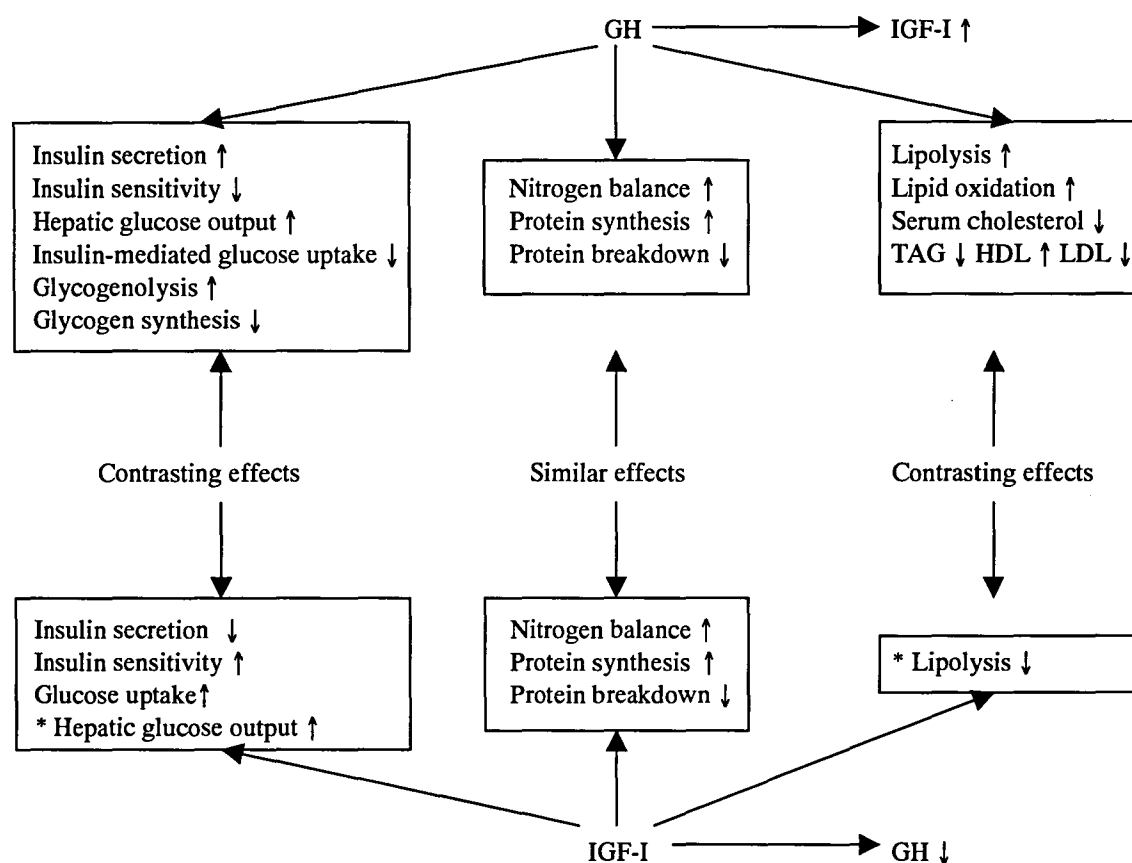


## 2.3.6. Growth hormone use and misuse

### Growth hormone pathophysiology and therapeutic use

There are a number of disease states that are either a consequence or cause of abnormal hGH production and control. When these alterations occur as the result of, rather than being the primary cause of, disease, it is common for hGH production and control to return to normal if the primary disease is treated satisfactorily (Dieguez et al., 1988). However, in instances of growth hormone deficiency it may be necessary to provide exogenous hGH. Originally hGH extracted from human cadavers was employed in hGH replacement therapy, but this was withdrawn due to the potential for the transmission of Creutzfeldt-Jakob disease.

GH has been approved by the US Food and Drug Administration (FDA) for the treatment of GHD in both children and adults, short stature associated with chronic renal insufficiency (CRI) before renal transplantation, short stature in patients with Turner syndrome and human immunodeficiency virus-associated wasting in adults (AACE, 1998).

**Growth hormone deficiency**

Growth hormone deficiency (GHD) as a result of hypothalamic-pituitary disease must be distinguished from the physiologically reduced hGH secretion that occurs with ageing (AACE, 1998). The clinical features of GHD in adults include:

a) increased mass and body fat mass

b) decreased lean body mass

c) decreased exercise capacity

d) decreased muscle mass and strength

e) reduced cardiac performance

f) reduced bone density and increased fracture rate

g) poor sleep

h) impaired sense of well-being

(AACE, 1998)

GHD-related increases in body fat mass also result in increased abdominal fat mass, therefore increasing waist-to-hip ratio and GH-deficient patients are reported to be susceptible to the development of premature cardiovascular disease (AACE, 1998).

It has been demonstrated that rhGH treatment in adults with long standing GHD can increase lean body mass and thigh muscle mass (Cuneo et al., 1991a) and also improve both maximal and submaximal exercise cycling performance with associated subjectively reported improvements in the ease of completion of daily tasks (Cuneo et al., 1991b). In addition, longer term rhGH treatment has been demonstrated to increase both isometric and isokinetic muscle strength (Johannsson et al., 1997) although the combination of exercise and rhGH treatment has been suggested to provide the optimal regimen for improving muscle function in adults with GHD (Grindeland et al., 1994). Another important role for rhGH treatment in hGH-

deficient adults was considered by Christ et al. (1997) who demonstrated that rhGH treatment stimulates the otherwise impaired erythropoiesis in adults with GHD. The observed increases in plasma volume and total blood volume observed with rhGH treatment were suggested to contribute to increased exercise performance in GH-deficient adults (Christ et al., 1997).

The use of hGH treatment to prevent weight loss and reduce recovery time following thermal trauma has also been suggested. Human GH treatment as part of the care for patients recovering from burns was studied by Wilmore et al. (1974). In this study a dose-related improvement in nitrogen retention was identified when hGH treatment was combined with adequate nutrient loading for 7 days in patients with burns from 34 to 76% of the body surface. These results a possible role for hGH therapy in this situation.

Child onset-GHD might result from abnormalities in the hypothalamus, most commonly as a result of deficient hypothalamic GHRH secretion, or from pituitary pathologic conditions such as pituitary tumours (AACE, 1998). The specific cause of GHD is particularly important in determining appropriate treatment to prevent contraindication. Turner syndrome occurs in 1 in 2000 live born girls and is due to abnormalities or absence of an X chromosome (AACE, 1998). This disorder is frequently associated with short stature, which may be ameliorated by GH treatment. Rosenfeld et al. (1988) found methionyl hGH (met-hGH) alone, and in combination with oxandrolone, a weak androgen, to stimulate linear growth in girls with Turner syndrome. However, primary hypogonadism is also associated with this disorder and therefore treatment with estrogens may be required. Delay in the replacement of oestrogen until after the normal age of puberty may help to optimise the effect of GH treatment, but this must be weighed against the need for feminisation (AACE, 1998).

Some children with intrauterine growth retardation (IUGR) have been shown to respond to hGH treatment. Tanner et al. (1971) found that a small number of children without Russell-Silver syndrome responded to hGH treatment. In addition the AACE (1998) report that children with IUGR and those with Russell-Silver syndrome might benefit from GH therapy. Prader-Willi syndrome may result in impaired hGH secretion and consists of hypothalamic obesity, short stature, developmental delay,

hypogonadotropic hypogonadism, small hands and feet and hypotonia. Preliminary studies suggest that some patients with Prader-Willi syndrome might benefit from GH treatment through accelerated growth, reduced hyperphagia, changes in lipolysis and a decrease in obesity (AACE, 1998). GH treatment can also be employed in the treatment of children with a growth delay as a result of abnormalities in the GH-IGF axis caused by Chronic Renal Insufficiency (CRI) (AACE, 1998). Resistance to hGH at the receptor level has also been observed and presents as Laron dwarfism (Laron et al., 1980). Children with Laron dwarfism will not respond to exogenous GHRH or rhGH treatment, although the administration of exogenous IGF-I may be of therapeutic use (Dieguez et al., 1988).

**Excessive hGH secretion**

The effect of excessive hGH secretion is highly dependent on the age of onset. If hGH hypersecretion begins in childhood there is an increase in growth velocity with soft tissue swelling and minimal bone deformity (Haynes, 1986). This is a rare condition known as pituitary gigantism. Acromegaly results from the occurrence of hGH hypersecretion after epiphyseal closure and the onset of this disorder is typically insidious. The aetiology of acromegaly is not clear: ectopic GHRH production and ectopic GH production are both possible causes although both are uncommon (Thorner et al., 1984; Melmed et al., 1985) and it is likely that the most common cause is a primary pituitary disease (Dieguez et al., 1988). The clinical manifestations of acromegaly are numerous, including:

a)  soft tissue swelling of the feet and hands

b)  protruding lips

c)  an increase in coarse body hair

d)  increased size and function of the sebaceous and sweat glands

e)  enlargement of hands and feet

f)  changes in the anatomy of the skull (jaw and nose)

g)  thoracic kyphosis

h)  joint pain (ranging from common arthralgia to degenerative arthritis)

i)  articular cartilage degeneration

j)  deepening of the voice

k)  peripheral neuropathy

l) hypertension

m) diabetes mellitus

In addition, sleep apnoea and daytime somnolence are common and the sleep related hGH peak disappears. There is also a paradoxical glucose-induced rise in hGH concentration (Melmed et al., 1983).

### Incidence of abuse in sport

There appear to be four major abuses of hGH in sport, 1) to increase muscle mass and strength, 2) to increase lean body mass, 3) to improve the 'appearance of musculature' 4) to increase final adult height (George, 1996). It would appear that hGH is used by athletes because it is perceived to be more effective than anabolic steroids in maximising size, strength and performance and because the improved techniques for the detection of anabolic/androgenic steroids increase the risk of disqualification from competition as a result of steroid abuse (Lombardo et al., 1992). In contrast hGH abuse is appealing to some athletes because most drug testing screens cannot detect it (Clarkson and Thompson, 1997). Salva and Bacon (1989) reported that 15 out of 100 physicians surveyed reported a total of 52 inquiries about growth hormone which identifies an interest in GH but not the incidence of use. Other information in this area takes the form of anecdotal reports (Cowart, 1988). It is clear, however, that hGH is being abused and The Underground Steroid Handbook, first published in 1981, touts hGH as:

"...the only drug that can remedy bad genetics as it will make anybody grow. A few side effects can occur however. It may elongate your chin, feet and hands but this is arrested with cessation of the drug...GH is the biggest gamble that an athlete can take, as the side effects are irreversible. Even with all that, we love the stuff."

Previously, the only source of hGH was from human cadavers and supply of the hormone was very limited. However, hGH has become available biosynthetically in unlimited quantities and the recombinant hGH (rhGH) that is produced has an amino acid sequence identical to pituitary derived hGH (Healy and Russell-Jones, 1997). The release of a recombinant form, and the associated increase in availability, has led to increased anecdotal evidence of use of hGH by athletes (Cowart, 1988; Catlin &

Hatton, 1991). Other indications that abuse of hGH is increasing include evidence that a number of burglaries from pharmacies seem to have focussed on obtaining hGH (Healy and Russell-Jones, 1997). In addition, customs officials have seized quantities of hGH from the luggage of competitors and coaches prior to the 1998 Swimming World Championships in Perth and the Sydney 2000 Olympics. Under Australian law anyone taking hGH into the country faces the prospect of a fine of up to £40 000 or even a five year prison sentence.

However, prohibitively high costs currently limit hGH abuse and it is claimed that abusers in the US have had to spend $30 000 per year (£20 000) to obtain "worthwhile effects" (Smith and Perry, 1992). In addition there is evidence that a large quantity of the hGH being supplied to users is either counterfeit, adulterated, of animal origin or some other product (Smith and Perry, 1992). Therefore, abusers are injecting bovine growth hormone stolen from farms (useless in humans), other peptide hormones such as human chorionic gonadotrophin (hCG), which actually mimics the natural stimulation of testosterone production by luteinizing hormone (LH), or even anabolic steroids (Cowart, 1988; Smith and Perry, 1992).

The potential adverse effects of hGH abuse in athletes include the clinical manifestations associated with hGH hypersecretion in addition to the health risks associated with the administration of the hormone. The means of hGH administration is by injection and this means that the risks of transmission of the hepatitis B virus and human immunodeficiency virus (HIV) are increased in the event of needles being shared. In addition several deaths due to Creutzfeldt-Jakob disease have been associated with the administration of hGH derived from human cadavers (Lazarus, 1985).

The side effects from the recommended standard replacement dose of hGH prescribed for hypopituitary patients are reported to be mild and probably only occur after long term usage as a result of the suppression of endogenous hGH secretion (Rogol, 1989). However, it has been suggested that athletes abusing hGH are taking 10 times this dose (Smith and Perry, 1992). Such high doses may result in conditions similar to acromegaly with its potential to disfigure in addition to increased mortality (Rogol, 1989). Long term abusers may suffer from skeletal changes (enlargement of fingers

and toes, growth of the orbit and lengthening of the jaw), enlargement of internal organs (including cardiomegaly, which is one of the causes of death associated with hGH abuse) and thickening of the skin (George 1996). Further reported complications include atherosclerotic cardiovascular disease, often with heart failure and cardiomyopathy, neuropathy, myopathy (especially proximal) in apparently hypertrophied muscles and impaired glucose regulation eventually resulting in diabetes mellitus (Rogol, 1989). Early hGH therapy using doses higher than those currently recommended also resulted in oedema in the extremities, carpal tunnel syndrome, arthralgia and myalgia (AACE, 1998). Increased incidence of leukaemia in hGH-treated patients in Japan, but not in the United States, has also been reported and acromegaly is also associated with and increased risks of malignant lesions (particularly colon cancer) although there is no evidence that this can be extrapolated to hGH-replacement individuals (AACE, 1998) or, therefore, those who abuse hGH. In addition, arthritis and impotence often occur after chronic hGH abuse (Kicman and Cowan, 1992).

## 2.3.7. Exercise as a stimulus of GH release

The mechanisms controlling the magnitude of the hGH response to exercise are not fully understood. The roles of blood lactate (e.g. Karagiorgos et al., 1979; Chwalbinska-Moneta et al., 1996), blood pH (e.g. Gordon et al., 1994) and oxygen demand and availability (e.g. Van Helder et al., 1987) in the regulation of hGH release have been studied. In addition intensity and duration of exercise may influence the hGH response (Sutton et al., 1976).

### Blood Lactate

Sutton et al. (1969) observed a correlation between blood lactate concentrations and hGH concentrations. However, artificial manipulation of blood lactate levels using sodium lactate (Vigas et al., 1974; Sutton et al., 1976) have been shown to have no consistent effect on hGH concentration. The metabolic and hormone responses to continuous and intermittent exercise of equal external work were studied by Karagiorgos et al. (1979). hGH concentrations tended to be higher during and in recovery from intermittent exercise, although the difference did not reach statistical significance until 20 min after exercise, this being the last blood sampling time point. In contrast, blood lactate concentrations were higher in every blood sample after the

resting samples during and in recovery from intermittent exercise. The similar hGH responses in the two trials despite a divergent pattern of blood lactate concentration suggest that the hGH response to exercise is independent of lactate accumulation in the blood.

In contrast, Van Helder et al. (1984a) reported significant positive correlations between plasma lactate concentrations and plasma hGH levels during 20 min of continuous "aerobic" exercise and 20 min of intermittent "anaerobic" exercise of equal external work. The results of this study disagree with those of Karagiorgos et al. (1979), despite similar exercise protocols. The difference in the results was attributed to the fact that "anaerobic" exercise was not employed by Karagiorgos et al. (1979) reflected by higher measured blood lactate concentrations in the study of Van Helder et al. (1984a). Similarly, the serum hGH concentration after arm cranking exercise, leg bicycle exercise and treadmill running at an intensity eliciting similar oxygen consumption in all trials, was found to be positively correlated with blood lactate concentrations (Kozlowski et al., 1983). However, both Van Helder et al. (1984a) and Kozlowski et al. (1983) reflected on the findings of Sutton et al. (1976) and accepted that their results should not be considered to demonstrate a causal link between blood lactate and serum hGH concentrations.

Chwalbinska-Moneta et al. (1996) identified what they considered to be a "hGH-threshold" which occurred at approximately the same work load as that at which they defined the "lactate threshold" during an incremental exercise test to exhaustion. In the same study Chwalbinska-Moneta et al. (1996) also found a significant correlation between blood lactate concentration and plasma hGH concentration. In addition, different weight lifting exercise protocols have also demonstrated a significant correlation between plasma lactate (0-20 min) and plasma hGH concentrations (16-36 min), that is with a 16 minute delay (Van Helder et al., 1984b). However, Kraemer et al. (1990) found no consistent systematic relationship between blood lactate and serum growth hormone concentrations during or after heavy resistance protocols.

**Hydrogen ion concentration**

It has been suggested that any stimulus for hGH release associated with lactate accumulation would be more likely act through hydrogen ion ($H^+$) accumulation

(Gordon et al., 1994). Sutton et al. (1976) studied the role of acid-base balance during exercise in the regulation of the exercise-induced hGH response. Acidotic, alkalotic and control conditions were considered during a ~45 min graded exercise test (last ~5 min at 90% $\dot{V}O_2$max to exhaustion). Although in the first 20 min of the exercise test acidosis resulted in a significantly higher circulating hGH concentration than the other two conditions, this did not extend to the second 20 min or the last 5 min stage. It was therefore concluded that, although exercise is a clear stimulus for hGH release, the stimulatory mechanism acts independently of blood [$H^+$].

However, it was not clear whether the same conclusion could be drawn for short-term, high-intensity exercise. Therefore, male subjects were administered with $NaHCO_3$ or NaCl placebo in a randomised double-blind counterbalanced experiment with a crossover design (Gordon et al., 1994). All-out high intensity exercise of 90 s duration resulted in a larger and faster rise in serum hGH concentrations in the placebo trial in all 10 subjects. Correlational analysis in the placebo trial alone demonstrated significant correlations between highest measured "peak" hGH and both peak [$H^+$] and peak lactate concentrations. Overall correlations, combining data from both trials, showed that highest measured "peak" hGH concentration was correlated with peak [$H^+$] but not peak lactate concentration, suggesting that the hGH response to high intensity exercise is more highly associated with peak venous [$H^+$] than with peak venous lactate concentration. However, it is not clear whether the highest measured hGH concentrations in this study represent the true peak of the hGH response since blood sampling only continued for 30 min after the exercise bout and it may be that, in some subjects at least, hGH concentrations were still rising.

Elias et al. (1996) used combined oral and intravenous administration of either $NaHCO_3$ (base) or NaCl (placebo) to study the effect of acid-base balance on hGH release during an incremental exercise test to exhaustion. Base administration tended to suppress the exercise-induced hGH at rest and during recovery from exercise except for the peak value at 60 min which was similar in the two trials. However, the mechanism by which an acute rise in blood [$H^+$] acts as a stimulus for hGH release is not clear.

**The Oxygen Demand/Availability Ratio**

It is known that hGH levels increase as oxygen demand increases and Lassarre et al. (1974) identified a significant correlation between initial $O_2$ deficit and peak hGH concentrations during 1 h of cycle ergometer exercise. In addition, Raynaud et al. (1981) demonstrated that individuals dwelling at sea level who were non-adapted to hypoxia had a greater hGH response to exercise under acute hypoxic conditions and when exercising at altitude than when exercising under normal conditions. This highlights a possible role for oxygen availability in regulating the hGH response to exercise.

Therefore, it was suggested that the hGH response to exercise should be proportional to the ratio of oxygen demand/availability (Van Helder et al., 1987). As a result of this contention Van Helder et al. (1987) developed an oxygen Demand/Availability ratio and used it to study whether there is a correlation between circulating hGH levels and the D/A ratio using previously published data from their laboratory and from other laboratories. Their results showed a highly significant relationship between the hGH response and the D/A ration for continuous "aerobic" and intermittent "anaerobic" exercise using data previously published by Van Helder et al. (1984a). Perhaps more surprising was the finding that there was a highly significant relationship between hGH and the D/A ration in the data of Karagiorgos et al. (1979). This finding was despite the fact that originally the results of that study identified no significant relationship between blood lactate concentrations and the hGH response to exercise or even between the oxygen deficit in continuous exercise and serum hGH concentrations. The data from these studies, as well as those from other previously published studies (e.g. Sutton et al., 1978; Raynaud et al., 1981), were combined and resulted in the demonstration of a close association between the D/A ratio and the hGH response to exercise for a wide range of "aerobic" and "anaerobic", intermittent and continuous exercise with a duration of between 20 and 60 min. This was proposed to support the suggestion that metabolic receptors exist in the muscle with a regulatory role in the hGH response to exercise.

**Catecholamines**

During progressively incremental exercise, blood catecholamines, adrenaline (A) and noradrenaline (NA) have been shown to rise with increasing exercise intensity

(Weltman et al., 1994). In addition, it has been demonstrated that catecholamines can directly stimulate GH secretion from rat pituitary tissue *in vitro* (13). In exercising humans, Kozlowski et al. (1983) identified a significant positive correlation between plasma [NA] and serum hGH concentrations. These findings were supported by those of Chwalbinska-Moneta et al. (1996) who also demonstrated significant correlations between catecholamine concentrations and serum hGH concentrations during and after an incremental exercise test. In addition, the "hGH-threshold" identified by Chwalbinska-Moneta et al. (1996) during incremental exercise was reported to occur at a similar work load as both the "[A]-threshold" and the "[NA]-threshold", as well as the "lactate threshold". Weltman et al. (2000) demonstrated that peripheral markers of heightened adrenergic outflow, that is [A] and [NA], precede and correlate with exercise-induced hGH concentrations. A time delay between peak-[A] or peak-[NA] and peak-[hGH] of ~20 min was identified and changes in exercise intensity did not alter this interval. In addition, increasing intensity resulted in a linear relationship between the increment (change from baseline to peak) in hGH and the increment in A as well as the increment in NA. Multiple linear regression showed that the dominant relationship was between incremental changes in hGH and NA. These results suggest that higher exercise intensities might drive increased hGH release, at least in part, by central adrenergic activation.

The poor permeability of the blood-brain barrier to catecholamines (Kozlowski et al., 1983) and the contention that only NA in the brain is effective in the control of hGH secretion (Hansen et al., 1971) infer that links between blood catecholamine levels and hGH release are not causal. However, it has been suggested that a decrease in pH in contracting muscles due to accelerated lactate production and associated metabolic changes may stimulate the sympathetic outflow by neural afferent signals from muscle metabolic receptors causing rapid release of catecholamines (McCloskey and Mitchell, 1972; Kjaer et al., 1989a). To take this a step further, catecholamines released following afferent signals from muscle metabolic receptors might, in turn, play a role in the regulation of hGH secretion.

### Afferent signals from muscle metabolic receptors

It has been suggested that a combination of factors related to anaerobic metabolism are involved in controlling hGH release (Kraemer et al., 1990; Nevill et al., 1996b).

However, Karagiorgos et al. (1979) found no correlation between any "anaerobic" metabolite or oxygen deficit and hGH concentration. Even if "anaerobic" metabolites in the blood do not have a role to play in the regulation of exercise-induced hGH release, it would not preclude the possibility that they are detected in the muscle (Kozlowski et al., 1983; Van Helder et al., 1984a; Van Helder et al., 1984b; Van Helder et al., 1987; Nevill et al., 1996b; Scheen et al., 1998; Viru et al., 1998).

It has been suggested that neural afferent signals from muscle metabolic receptors, activated by local changes in lactate concentration, oxygen concentration or pH, might participate in the activation of catecholamine release (McCloskey and Mitchell, 1972; Kjaer et al, 1989). Van Helder et al. (1987) cited the close association between hGH and the D/A ratio as support for the suggestion that similar metabolic receptors occur in the muscle with a role in GH regulation during exercise. Further support was given by the finding of a significant correlation between plasma glucose and plasma hGH concentrations following prolonged exercise (Scheen et al., 1998). Ischaemic exercise, resulting in enhanced accumulation of lactate in the muscle, has been associated with a 2-fold increase in hGH concentrations compared to normal exercise and this was attributed to the activation of muscle receptors (Viru et al., 1998). However, Kjaer et al. (1989b) blocked afferent nerve activity by epidural anaesthesia in order to test the hypothesis that afferent nervous activity from exercising muscle regulates hGH release. Epidural blockade had no effect on the hGH response to exercise although it was not concluded that afferent nervous activity does not have a role to play in the regulation of exercise-induced hGH secretion, since it was postulated that central motor activity might compensate for the reduction in afferent sensory signals.

Furthermore, exercising under hypoxic conditions would be expected to exaggerate metabolic changes in contracting muscle, and, if afferent feedback from muscle metabolic receptors contributes to the regulation of hGH secretion, enhance the hGH response to exercise. Epidural anaesthesia would be expected to blunt this response, yet Kjaer et al. (1999) demonstrated that epidural anaesthesia during leg cycling exercise at ~50% $\dot{V}O_2max$, under hypoxic conditions, enhanced rather than blunted the exercise-induced hGH response. Epidural anaesthesia also reduced muscle

strength and increased perceived exertion, suggesting a role for "central command" in the exercise-induced hGH release, rather than regulation by afferent feedback from receptors in exercising muscle.

**Motor centre activity**

Activity in motor centres may directly stimulate pituitary hormone secretion, including GH, during exercise (Kozlowski et al., 1983; Kjaer et al., 1987; Kjaer et al., 1989b; Kjaer et al., 1996a). Administration of tubocurarine is reported to induce a partial neuromuscular blockade, which increases voluntary effort during exercise and therefore necessitates higher activity in motor centres (Asmussen et al., 1965). Kjaer et al. (1987) administered tubocurarine prior to exercise and demonstrated higher motor activity, through increased rate of perceived exertion, compared to exercise without tubocurarine administration. At the same time exercise with tubocurarine elicited a greater hGH response than exercise alone, suggesting that central motor activity might play a role in the regulation of the hGH response to exercise. In a further study using epidural anaesthesia to block afferent nerve activity, decreased muscle strength and higher rates of perceived exertion during exercise with epidural blockade inferred increased motor centre activity (Kjaer et al., 1989b). Since epidural anaesthesia had no apparent effect on the hGH response to exercise it was suggested that this increase in motor centre activity compensated for the lack of afferent nervous input.

However, Kjaer et al. (1996a) did not observe any decrease in the hGH response to exercise with both afferent sensory blockade by epidural anaesthesia combined with electrically induced cycling, to offset motor centre activity. These results suggested that blood-borne humoral feedback mechanisms and autonomic (i.e. spinal) reflexes are capable of inducing the hGH response to exercise. It was postulated that a decrease in plasma glucose, as observed in this study, was of great importance in the control of the hGH response to exercise. A further study by Kjaer et al. (1996b) compared electrically induced leg cycling in tetraplegic human with voluntary arm cranking at a work rate similar to that achieved during the involuntary leg stimulation trials. It was observed that hGH concentrations increased as a result of voluntary arm exercise, but not with involuntary leg stimulation. These results returned to the suggestion that an intact central nervous system and activity in motor centres as well

as afferent nerves from exercising muscles are needed for the hGH response to exercise. In addition, arm cranking exercise appeared to result in an exaggerated hGH response, probably due to higher motor centre activity relative to work output, since the subjects' arm muscles were weakened by partial paralysis. This finding provides further support for a role of motor centre activity in the regulation of the hGH response to exercise.

Therefore, a proposed model for the regulation of the hGH response to exercise suggests that, at the onset of exercise, impulses in motor centres of the brain elicit a work load dependent increase in increase in hGH. Blood-borne metabolic error signals may then feed back to modulate further hGH secretion (adapted from Kjaer et al., 1987).

**The role of somatostatin and GHRH in the regulation of the hGH response to exercise**

It has been proposed that relatively low intensity exercise induces moderate hGH responses through activation of the central cholinergic system, resulting in a reduction in hypothalamic somatostatin secretion (Maas et al., 2000). However, this is a saturated process and at higher exercise intensities, with complete suppression of hypothalamic somatostatinergic tone, further increases in hGH secretion must be mediated by an increase in GHRH secretion.

The importance of the inhibition of somatostatinergic tone as a result of exercise was demonstrated by Di Luigi et al. (1997), who observed a suppression of the hGH response to treadmill exercise, at 60% $\dot{V}O_2$max, following pretreatment with octreocide, a somatostatin analogue, in humans. Pyridostigmine administration has also been shown to enhance exercise-induced hGH secretion, although it could not reverse the age-related decline in the hGH response to exercise (Marcell et al., 1999). In addition, administration of GHRH at the start of an incremental exercise test lasting 25 min, with an additional stage at 100% $\dot{V}O_2$max until exhaustion, had an additive effect on the hGH response (Maas et al., 2000). All of these results suggest that somatostatin has a role to play in the regulation of hGH secretion during, and in recovery from, exercise. However, co-administration of GHRH and GH-releasing

peptide-2 (GHRP-2) at the start of exercise further potentiated hGH release, possibly via a mechanism potentiating the effect of GHRH (Maas et al., 2000). Since GHRP-2 has been shown to have its own specific receptor (Camanni et al., 1998) it is possible that an endogenous GHRP-2-like ligand exists and exerts its influence on the hGH response to exercise.

**Effect of exercise at different times of day on the hGH response to exercise**

A 20 min incremental treadmill running test, with the last 5 min at 90% $\dot{V}O_2$max, has been shown to induce an increase in hGH regardless of whether the exercise was performed in the morning (between 0700 and 0800) or the afternoon (between 1500 and 1600) in women Galliven et al. (1997). Peak hGH concentrations were measured at the end of each exercise bout and returned to resting levels 20 min after exercise and no changes were identified in either the magnitude or the pattern of the exercise-induced hGH response in the morning or afternoon. These results agree with a study showing no diurnal variation in hGH release in response to insulin-induced hypoglycaemia (Nathan et al., 1979) since it does not seem that there is a diurnal

variation in the pituitary response to exercise at 90% $\dot{V}O_2$max.

Scheen et al. (1998) compared continuous bed-rest with 3 h of mixed high (60% ) and low (40% ) intensity, arm cranking and leg cycling exercise at three different times of day, where exercise was initiated at approximately 0500, 1430 and 2330. The results of this study demonstrated that exercise elicits a clear hGH response regardless of time of day with 5- to 6-fold increases in plasma hGH concentrations. In addition, there was no difference in the magnitude of the exercise-induced hGH response at three different times of day suggesting that there is no diurnal rhythm in the hGH response to prolonged submaximal exercise.

**Effect of exercise on night time hGH release**

Resistance exercise was reported to induce a marked acute hGH response compared to a no-exercise control trial in male trained weight lifters (McMurray et al., 1995). However, there was no difference in the magnitude of the nocturnal hGH response to this resistance exercise session completed in the evening, and the peak hGH responses appeared to occur at about the same time (between 0100 and 0200 hours) as in a

control trial. These results suggest that resistance exercise does not affect nocturnal hGH release.

Kern et al. (1995) compared prolonged low-intensity exercise, comprising of 40 km of bicycle exercise between 1800 and 2030, with prolonged moderate-intensity exercise, comprising of 120-150 km of bicycle exercise between 1600 and 2030 hours, and a no-exercise control trial. Average nocturnal hGH concentrations were not different between each trial, suggesting that endurance exercise does not alter nocturnal hGH secretion. However, the pattern of nocturnal hGH secretion was altered by exercise during the previous day. When the effect of exercise on the previous day was compared with the control trial, both low- and moderate-intensity exercise were shown to suppress hGH secretion in the first part of nocturnal sleep, when hGH concentrations are usually at their peak, and increased hGH secretion in the second part of sleep, when hGH concentrations are normally lower. It is interesting to note that cortisol demonstrated the opposite response resulting in a change from the typical nocturnal secretory pattern whereby the first part of sleep is characterised by high hGH and low cortisol concentrations and the second part of sleep by an inverse relationship between hGH and cortisol concentrations. It would appear, therefore, that although average nocturnal hGH secretion is not altered by daytime exercise, the secretory pattern of hGH may be affected. In addition, different types and intensities of exercise might have an affect on this relationship, as well as the time of day that the exercise bout is completed.

**Growth hormone responses to sprint exercise**

There is relatively little literature available regarding the hGH response to sprint exercise. Some studies report the use of "anaerobic" exercise (e.g. Van Helder et al., 1984a; Weltman et al., 2000), whilst others have considered resistance exercise (e.g. Van Helder et al., 1984b; Kraemer et al., 1990) however, very few have studied the hGH response to sprinting (Gordon et al., 1994; Nevill et al., 1996b). Gordon et al. (1994) employed a 90 s high-intensity cycle exercise test against an opposing force equivalent to 5% of subjects' body mass. Subjects were instructed not to pace themselves during the 90 s, therefore the test was an all-out effort. This exercise protocol induced a marked hGH response, with highest measured hGH concentrations 30 min after the exercise bout. Unfortunately, the 30 min blood sample was the last

so relatively little information was provided regarding the time-course of the hGH response to sprint exercise.

Nevill et al. (1996b) examined the hGH response to sprinting in male and female sprint- and endurance-trained athletes. The subjects completed a single 30 s maximal treadmill sprint which resulted in a marked hGH response with a peak between 20 and 30 min or between 1 and 10 min after the sprint for the sprint- and endurance-trained athletes respectively. Peak hGH concentrations were approximately three times greater in the sprint-trained athletes compared with the endurance-trained athletes and in the sprint-trained group hGH concentrations were still approximately 10 times the baseline value after 60 min of recovery. The response seen in this study was similar to that induced by a combination o exercise and the neuropeptide galanin (Davis et al., 1987), which led the authors to suggest that sprint exercise might induce a "near maximal" hGH response. However, there is no information available regarding the hGH response to sprints of different duration, repeated sprints or sprints where the number of muscle actions have been manipulated by exercising against different applied resistance. In essence, very little is known about the hGH response to sprinting.

**The effect of exercise training on the hGH response to exercise**

A number have studies have concluded that exercise training has no effect on resting hGH concentrations when comparing sedentary individuals and athletes (Bloom et al., 1976; Mikines et al., 1985; Bunt et al., 1986; Barreca et al., 1988), sprint-trained and endurance-trained athletes (Nevill et al., 1996b) and following endurance (Bonifazi et al., 1998) or resistance (Kraemer et al., 1998; McCall et al., 1999) training. However, all of these studies found very low resting hGH concentrations, probably accounting for the lack of any differences between groups. In fact, it is likely that in some cases the assays employed to determine hGH concentrations would have been unable to satisfactorily measure the low levels of hGH associated with human subjects at rest (Veldhuis et al., 1995). However, 14 days of detraining of power athletes has been shown to increase resting hGH concentrations (Hortobagyi et al., 1993) whereas one year of endurance run training has been observed to increase resting hGH concentrations in women (Weltman et al., 1992). The effect that training has on resting hGH concentrations is, therefore, unclear.

Some studies have considered the effect of training state on the hGH response to an acute bout of exercise. There is little agreement between these studies as to whether exercise training increases (Bunt et al., 1986; Bonifazi et al., 1998; McCall et al., 1999), decreases (Bloom et al., 1976; Weltman et al., 1997) or has no effect (Kraemer et al., 1998) on the hGH response to a single exercise bout.

Bunt et al. (1986) identified a significantly greater hGH response to a 30 min run at 60% of $\dot{V}O_2$max in runners (minimum mileage of 40 miles per week) compared with moderately active controls. In agreement with the findings of this study Bonifazi et al. (1998) found that the hGH response to a standard training session (15 x 200 m with 20 s rest between sets) was enhanced in 9 top-level male endurance swimmers (national team members). There is also evidence that high volume resistance training elicits an increase in the hGH response to resistance exercise in young men with recreational resistance training experience (McCall et al., 1999). In addition, it has also been shown that endurance-trained athletes have a greater hGH response to insulin-induced hypoglycaemia than untrained individuals (Mikines et al., 1985).

However, other studies have identified a lower hGH response to exercise in well-trained compared with untrained cyclists (Bloom et al., 1976) and an attenuated hGH response to a 20 min constant load cycle ergometer test exercise following training (Weltman et al., 1997). Weltman et al. (1997) suggested that reduced exercise-induced hGH concentrations following training may be a result of a combination of reduced hGH secretion and enhanced hGH clearance. In support of this contention is the suggestion that the half-life of endogenous hGH is shorter in exercising than resting individuals (Thompson et al., 1993). On the other hand, Kraemer et al. (1998) did not find any change in the exercise-induced hGH response following resistance training, although it was postulated that other hGH variants might adapt differently to training and that different variants are different in their biological activity. The effect that exercise training has on the hGH response to exercise is, therefore, not entirely clear and might depend on a number of factors including the type, frequency and duration of the training period. In addition, only three of the studies mentioned (Bloom et al., 1976; Bunt et al., 1986; McCall et al., 1999) compared the response of

trained individuals with untrained control subjects and of these only McCall et al (1999) completed longitudinal study incorporating a control group. It would appear, therefore, that further research is required in this area.

Weltman et al. (1992) observed an increase in 24 h integrated hGH concentrations following one year of run training in women. It has also been reported that serum IGF-I concentrations, taken as a measure of integrated hGH secretion because it is hGH dependent, increase following two weeks of endurance training (Roelen et al., 1997). A similar trend was described by Weltman et al. (1997) although this increase was not found to be significant. It is, therefore, possible that 24 h hGH concentrations are elevated following training independent of the acute hGH response to exercise.

## 2.3.8. Human GH negative feedback mechanisms

There is a great deal of evidence suggesting that GH, like a number of other hormones (Guyton, 1986), regulates its own secretion via a negative feedback mechanism, although the nature of this autoregulation is not entirely clear. A number of possibilities exist and the role of GH autofeedback, increased somatostatinergic tone and/or decreased GHRH release, increased circulating FFA and modulation by IGF-I have all been considered.

Lanzi and Tannenbaum (1992a) found spontaneous GH release to be inhibited within 1 to 2 h after a single subcutaneous (sc) injection of rhGH in rats, and it remained completely suppressed for up to 4 h after the rhGH injection. They also demonstrated no difference in the duration or magnitude of attenuation of the GH response according to an acute (single sc injection) or chronic (5 day) injection regimen. Passive immunisation with specific somatostatin antiserum reversed the rhGH-induced blunting of the spontaneous GH response by restoring the amplitude of the GH secretory bursts. The fact that immunoneutralisation of somatostatin prevented the attenuation of spontaneous GH release after GH pre-treatment provides strong support for a role for somatostatin in GH autoregulation.. However, since the normal pattern of pulsatile GH secretion was not restored by passive immunisation with somatostatin antiserum, the possibility of a GH-induced inhibitory effect on hypothalamic GHRH cannot be discounted.

In a further study Lanzi and Tannenbaum (1992b) also demonstrated a role for somatostatin in the attenuation of exogenous GHRH-induced GH release in rats. Serial injections of GHRH at 2 h intervals elicited 4- to 6-fold increases in GH release when GHRH was administered at times of peak spontaneous GH secretion, but only a minimal GH response was observed during trough periods. There was no evidence of desensitisation of somatotropes since high GH responsiveness to exogenous GHRH was maintained at a time of spontaneous secretory episode following a previous exogenous GHRH challenge during a trough period. These results demonstrate the importance of the cyclical increase in endogenous hypothalamic somatostatin secretion in preventing desensitisation of the pituitary to GHRH. In the same study a single subcutaneous rhGH injection 3 h prior to GHRH administration severely attenuated the GHRH-induced GH response. Passive immunisation with specific somatostatin antiserum reversed the blunted GH response and completely restored GH responsiveness to GHRH. This was consistent with the contention that GH feedback is exerted, at least in part, by somatostatin. In addition, the understanding that GH receptor mRNA is colocalized in somatostatin-positive neurons in the Periventricular nucleus of the rat hypothalamus (Burton et al., 1991) further supports these findings.

In normal adults repeated GHRH administration has been shown to result in an attenuated hGH response to the second stimulus (Ghigo et al., 1991). However, the administration of arginine, which acts to suppress somatostatin release, with the second bolus of GHRH restored the responsiveness of the somatotroph and, in fact, even potentiated the hGH response. This suggests that the attenuation of the hGH observed using repeated boluses of GHRH alone was not due to a GHRH-induced reduction in the size of the pool of hGH available for release. In addition, the fact that arginine administration reinstated the GHRH-induced hGH response following the second stimulation implies an important role for somatostatin in hGH autoregulation.

The GH response to repeated bouts of exercise has also been studied. Somewhat surprisingly, Kanaley et al. (1997) demonstrated an augmented hGH response to repeated bouts of 30 min exercise at 70% $\dot{V}O_2$max separated by either 60 min or 210 min of recovery. Each exercise bout resulted in a distinct hGH pulse and the apparent progressive increase in hGH response tended to be greater with a longer (210 min)

recovery period. The augmented response with repeated bouts of exercise provided evidence that the depletion of pituitary stores with repeated stimuli to hGH release is unlikely. Jaffe et al. (1993) supported this viewpoint, suggesting that pituitary GH content far exceeds the amount of GH released in their study and yet they demonstrated a suppression of the GH response to repeated GHRH administration. Kanaley et al. (1997) concluded that exercise provides sufficient stimulus to overcome the autonegative feedback demonstrated using pharmacological interventions.

In contrast, Cappon et al. (1994) demonstrated the hGH response to 10 min of constant power cycling exercise, at an intensity corresponding to 50% of the difference between the lactate threshold and $\dot{V}O_2max$, to be dramatically attenuated as a result of previous exercise bouts. In addition, they demonstrated an acute, hGH independent, exercise-induced increase in IGF-I. A purified IGF-I preparation has been shown to inhibit GH release from pituitary cells in culture (Berelowitz et al., 1982), and the infusion of rhIGF-I has also been demonstrated to suppress pulsatile and GHRH-stimulated GH secretion in male subjects (Jaffe et al., 1998). There is an apparent role for somatostatin in this long-loop feedback, since Berelowitz et al. (1982) also observed IGF-I to stimulate a dose-related release of somatostatin from hypothalamic explants. However intraventricular IGF infusion in ewes had no effect on GH secretion, whereas intrapituitary infusion resulted in the inhibition of GH release (Fletcher et al., 1995) providing strong evidence for a direct effect of IGF-I at the level of the pituitary.

However, Cappon et al. (1994) found that exercise-induced IGF-I levels were not significantly higher than baseline within 30 min of recovery, whilst the recovery between exercise bouts was 50 min, suggesting that IGF-I did not play a role in the attenuation of the hGH response to exercise in their study. In addition, it would appear that exercise-induced IGF-I would not have a role in the regulation of the exercise-induced hGH response in exercise bouts separated by more than 30 min. Lanzi and Tannenbaum (1992a) also measured IGF-I and did not observe an increase in plasma IGF-I concentration, but reported hGH release to be suppressed for 4 h after rhGH administration in rats. This implies that the GH negative feedback loop can

function independently of IGF-I, although there does remain the possibility that locally synthesised IGF-I in the pituitary gland might play a role.

Cappon et al. (1994) also considered hGH autoinhibition, whereby hGH feeds back on itself directly, however, this seemed unlikely as GH was only slightly elevated at the end of each recovery period. Alternatively, an increase in FFA as a result of the first exercise bout might have blocked hGH secretion directly at the pituitary level, as demonstrated by Casanueva et al. (1987). The potential role of FFA in the hGH feedback loop was studied by Pontiroli et al. (1991). Infusion of methionyl-GH (met-GH) blocked the response to exogenous GHRH and administration of acipimox, an antilipolytic agent, and pyridostigmine, to block hypothalamic somatostatin release, did not restore the hGH response to GHRH. This indicates that inhibition of the hGH response to GHRH can occur independently of circulating plasma FFA levels and hypothalamic somatostatin release and was probably mediated by hGH autofeedback at the pituitary gland.

# CHAPTER 3

# GENERAL METHODS

## 3.1. Introduction

The methods common to all studies presented in this thesis (Chapters 4, 5, 6 and 7) are described in this chapter. Each testing protocol was approved by the Ethical Advisory Committee of Loughborough University (see Appendix A). This Chapter describes the equipment, instruments and calibration routines used, gives information about subjects, familiarisation procedures and standardised testing procedures, including performance variables measured and describes the procedures employed to collect, handle and analyse blood samples. Further details of blood metabolite and hormone assays can be found in Appendix B. The present chapter also includes a description of calculations done and statistical techniques used throughout this thesis. The final four sections of this chapter describe four pilot studies. Sections 3.9 and 3.10 show the results of using two different methods of assessing the repeatability of two key performance measures, peak (PPO) and mean (MPO) power output; firstly by calculating the coefficients of variation for both PPO and MPO, and, secondly, using the method described by Bland and Altman (1986). Section 3.11 considers the effect of the insertion of a cannula on resting serum concentrations of hGH and cortisol, and the final section of this chapter is a storage study which determines how long serum samples can be stored for without a significant change in serum hGH concentrations at the time of analysis.

## 3.2. Equipment

In all of the studies in this thesis a modified friction-loaded cycle ergometer (Monark, model 864), interfaced with a microcomputer (BBC, model B), was used in order to calculate "corrected" instantaneous power generated during maximal sprint exercise (Lakomy, 1986). This method corrects for the inertia characteristics and instantaneous changes in angular velocity of the flywheel. Lakomy (1986) found that employing the protocol of Bar-Or (1978) resulted in a 32% underestimation of peak power output when compared with the "corrected" method with an averaging period of 1 s. Time to reach peak power was also reduced, as it was reached before peak

pedal speed. In contrast, there was no difference in total work over an entire 30 s sprint when using the different methods.

In order to obtain the "corrected" power output values, a high speed data collection system was used (Lakomy, 1986). An electric generator was attached to the frame of the ergometer such that it was driven by the ergometer flywheel, giving an analogue signal proportional to the angular velocity of the flywheel. The signal was logged by the microcomputer via an analogue-to-digital (A-D) converter, combined with a timing signal derived from the computer's internal jiffy clock. The sampling rate was 20Hz.

The important factor included in the "corrected" method developed by Lakomy (1986) was the "acceleration balancing load" which was the force required at any instant to stop the subject from accelerating the flywheel. Instantaneous power output could then be calculated as the product of the speed of the flywheel and the "effective" load (resistive load + acceleration balancing load). The values for instantaneous speed and power were averaged over 1 s intervals. At the conclusion of the test the computer was programmed to calculate, display and print both the "corrected" and "uncorrected" results. Figure 3.1 shows a typical power output and pedal speed profile for a maximal 30 s sprint.

Before and at the end of each study the relationship between the angular velocity of the flywheel and the output from the generator and the A-D converter was calibrated. This calibration was also checked prior to each individual trial. The ergometer was pedalled by one of the experimenters for approximately 100 s at a pedal speed of 60 rev.min$^{-1}$, and the number of pedal revolutions was counted. In addition, the deceleration time of the flywheel at three different loads was determined by setting the flywheel in motion, pedalling in excess of 120 rev.min$^{-1}$ and ceasing pedalling. The microcomputer recording the time it took the flywheel to stop and calculated a linear regression equation of load vs. flywheel deceleration enabling the "acceleration balancing load" to be calculated (for a full explanation see Lakomy, 1988).

Figure 3.1. Power output and pedal speed profiles generated during a 30 s sprint on the modified cycle ergometer
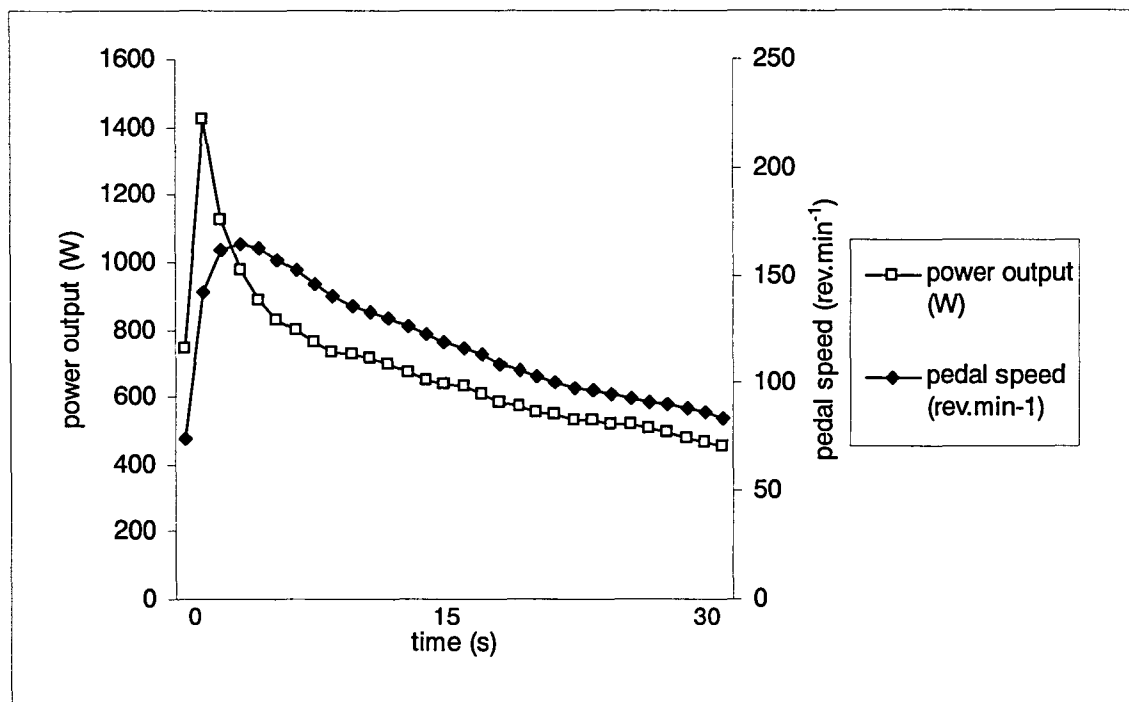


The magnitude of the measured power output may be influenced by a number of factors in addition to those that the experiments in this thesis attempted to manipulate. Such factors include crank length, degree of pelvic tilt and saddle height (Nordeen-Snyder, 1977; Yoshihuku and Herzog, 1996). In order to eliminate the effect of different crank length, as well as any other confounding variables associated with the choice of ergometer, the same cycle ergometer was used throughout each study. In fact one cycle ergometer was used for the study described in chapter 5 whilst the studies described in chapters 4, 6 and 7 were all carried out using another ergometer (same model, Monark, model 864). On the first visit to the laboratory optimal saddle height was determined for each subject whereby slight knee flexion occurred at the bottom of the pedal stroke. Subjects' then used this saddle height on all further visits to the laboratory.

In order to restrict exercise as much as possible to the lower limbs and prevent subjects from rising out of the saddle, a wide restraining harness was passed around the subject's waist. The harness was positioned so that it did not interfere with the subject's breathing and did not place uncomfortable pressure on the subject's

stomach. Two side-straps on the harness were fixed to a metal rail bolted to the floor behind the cycle ergometer. A number of holes were drilled in the metal rail so that the tension of the straps could be adjusted for different subjects; the same setting was used for all trials.

The effects of the restraining harness on power output during maximal sprint cycling have been examined by Bogdanis (1991). It was suggested that the use of a restraining harness does not affect peak power output (Bogdanis 1991), although it is possible that peak power output is increased slightly. This may be due to the fact that the harness holds the subject on the seat allowing most of the power generated in the legs to be applied to the flywheel rather than to lifting the subject off the saddle.

During each trial toe clips (reinforced with adhesive tape) were used to secure the subjects' feet on the pedals.

Figure 3.2. The ergometer and restraining harness used in the studies in this thesis.
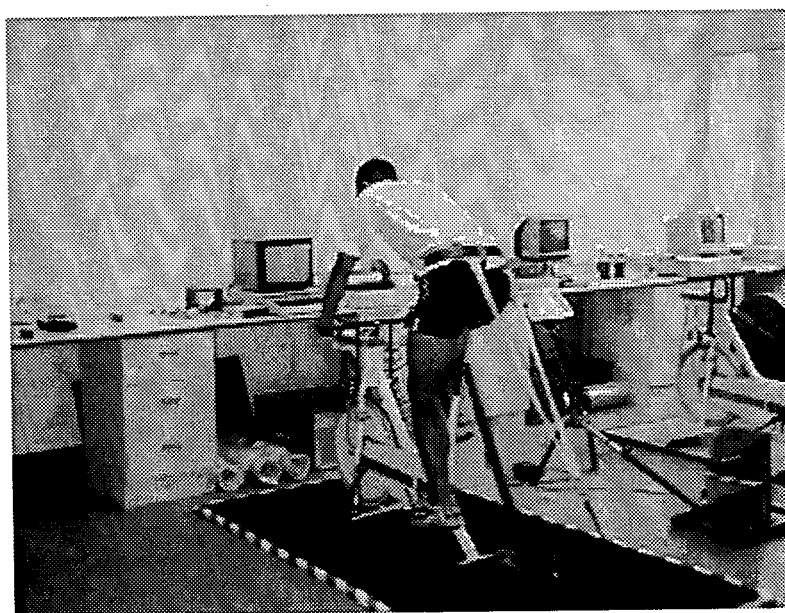
## 3.3. Subjects

Due to the demanding nature of the exercise performed in the studies described in this thesis, only physically active individuals, many of whom were involved in regular exercise training and sport, were recruited. All subjects were informed in verbal and written form about the purposes and requirements of the study and any known risks or discomforts associated with the protocol. It was also clearly stated that all subjects had the right to terminate their participation at any time, with no obligation to give a reason for their decision. Subjects expressed their understanding by signing a statement of informed consent (Appendix A). A short questionnaire was attached to the statement of informed consent in order to obtain information about the sporting/exercise activities of each individual. A medical history questionnaire (Appendix A) was also completed in the presence of an experimenter (to provide clarification and assistance), and subjects with medical conditions that posed potential problems were excluded. Participation was voluntary and no remuneration was given to any of the subjects, although food and drink was provided after each trial.

## 3.4. Familiarisation

Prior to any experimental testing each subject completed 3-6 (typically 4) sprinting practice sessions when optimal seat height and restraining harness arrangement were determined for future tests. Body height (Holtain stadiometer) and body mass (Avery 3306 ABV balance) measurements were also taken during these visits. The purpose of the familiarisation sessions was so that subjects could:

- get accustomed to all-out sprinting on the cycle ergometer
- get accustomed to sprinting whilst wearing a restraining harness
- learn to accelerate maximally on the command "3-2-1-GO"
- get accustomed to maintaining maximal effort throughout the full 30 s of a sprint

Typically the first 1-2 familiarisation sessions consisted of a warm-up, at least 5 (but up to 10 depending on how comfortable the subject was) sprints of brief duration (~6 s) followed by a 20 s all-out sprint. Later familiarisation sessions consisted of a warm up and 2 all-out 30 s sprints separated at least 10 min passive recovery. All results for

the 30 s sprints were recorded and when performance improvements had stopped subjects were deemed to be ready to start the experimental tests.

Following familiarisation the mean coefficient of variation for peak power in a 30 s all-out sprint on a cycle ergometer from a standing start, against an applied resistance equivalent to 7.5% of the subjects' bodymass, was 5.5±1.5% (Cherry, 1997). Section 3.9 Describes a pilot study that determines the coefficients of variation for two performance parameters measured in this thesis.

### 3.5. Standardised testing procedures

On the day preceding each main trial subjects were asked to refrain from alcohol and physical training or heavy exertion and to consume their normal diet. Subjects arrived at the laboratory on the day of each trial following an overnight fast or, if this were not possible, at least 4 h postprandial.

Ambient (Ball et al., 1997) and muscle (Sargeant, 1994) temperature have been reported to influence power output in maximal exercise. Although it was not possible to accurately control ambient temperature in the laboratory, all experiments in this thesis were conducted in a thermoneutral environment (18-23°C). In addition, each testing protocol incorporated a standardised warm-up and subjects completed their trials at a similar time of day, so the inter-trial variation in muscle temperature was probably not too great. The standardised warm-up consisted of 4 min pedalling at 60W, 30 s pedalling at 80 W and 30 s pedalling at 100W, with 30 s rest between each intensity. The warm-up was followed by 5 min of rest on the cycle ergometer while a blood sample was taken and the subject prepared for the sprint.

Following the 5 min rest, subjects completed a sprint from either a rolling start of 70 rev.min$^{-1}$ against no resistance (Chapter 4) or a stationary start (Chapters 5, 6 and 7). In both cases a countdown (3-2-1) was given, followed by the command "GO". On this command the subjects completed the all-out sprint. Subjects were instructed to sprint maximally throughout each sprint and to avoid pacing. The importance of attaining maximum speed as soon as possible after the start of the sprint was emphasised and strong verbal encouragement was given during each sprint. Sprints

were separated by passive recovery seated upright on a couch maintaining approximately the same body position as on the cycle ergometer. On a few occasions subjects found it necessary to lie flat on the couch for a few minutes following the sprint to overcome feelings of nausea, however, they were encouraged to return to an upright seated position as soon as they felt they had recovered.

## 3.6. Performance variables

The following performance parameters were recorded for each sprint from the computer print out:

- Peak power output (PPO) – highest 1 s average power output
- Peak power output corrected for each subject's bodymass (PPO-corr) – PPO/bodymass
- Mean power output for the whole 30 s sprint (MPO$_{30}$)
- Mean power output corrected for each subject's bodymass (MPO-corr) – MPO$_{30}$/bodymass
- Mean power output for the first 6 s (MPO$_6$)
- Peak pedal revolutions (PPR) – highest 1 s average pedal speed
- Mean pedal revolutions for the whole 30 s sprint (MPR$_{30}$)
- Mean pedal revolutions for the first 6 s (MPR$_6$)
- Total work completed during the 30 s sprint
- Fatigue index (FI) – the percentage decline from peak to end power output

$$FI = \frac{PPO - \text{end power output}}{PPO} \times 100$$

## 3.7. Collection, storage and analysis of blood samples

Venous blood samples were obtained from an antecubital forearm vein via an indwelling cannula (Venflon 2, 18 guage) inserted under local anaesthetic (0.5 ml of 1% lignocaine). The cannula was placed while the subjects rested on an examination couch. Repeated sampling was facilitated by a 3-way stop cock and tubing (Connecta®) connected to the cannula and strapped securely with surgical tape. The

Figure 3.3. Summary of the procedures for dispensing venous blood samples.

~10 ml venous blood

~1.5 ml blood into eppendorf containing Ca/hep

~5.5 ml blood into orange plasma tube containing Li/hep (Sarstedt® LH/5 ml)

~ 5 ml blood into white serum tube Sarstedt® serum Z/5 ml)

Centrifuge (Eppendorf 5412) for 3 min at 13000rpm

Remove supernatant and place in plain eppendorf. Freeze at -70°C for later determination of **plasma ammonia concentrations** (Appendix B)

Immediately measure **pH** (Radiometer blood gas analyser, ABL/5)

Remove 2 x 20 µl aliquots, dispense into 5 ml 'Drabkins' solution for subsequent **haemoglobin** determination (Appendix B)

Leave to clot for at least 45 min, centrifuge (Burkard Koolspin) for 15 min at 6000 rpm

Remove supernatant and place in 2 x plain eppendorfs. Freeze at -70°C for later analysis of **hGH, insulin, cortisol** and **IGF-I** concentrations (Appendix B)

Nearly fill 3 ammonia/hep micro-hamatocrit tubes (Scientific Instruments), mix then seal one end with plasticine (Miniseal) for later measurement of **haematocrit** (Appendix B)

Remove 2 x 20 µl aliquots, dispense into2 x 200ml of 2.5% perchloric acid, mix and centrifuge (Eppendorf 5412) for 3 min at 13000 rpm. Freeze at -20°C for later analysis of **lactate concentrations** (Appendix B)

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first (resting) blood sample was taken at least 15 min after the cannula was inserted. Patency was maintained by displacing the blood contained in the cannula with sterile isotonic saline. Further blood samples were obtained at pre-determined times either seated on the cycle ergometer or in an upright seated position on the couch.

Venous blood samples were dispensed into three tubes, as summarized in Figure 3.3:

(i)     One portion (4 to 5 ml) was placed into a lithium heparinised tube (Sarstedt LH/5 ml). Blood pH was measured immediately (Radiometer ABL5 pH/blood gas monitor) and thereafter, 20 µl aliquots of blood were removed, deproteinized in 2.5% perchloric acid and stored at -20°C for later determination of blood lactate concentrations (Maughan, 1982). Further aliquots were removed for the measurement of haematocrit by microcentrifugation (Hawksley Ltd.) and haemoglobin concentration (by the cyanmethaemoglobin method) for the calculation of percentage change in plasma volume (Dill and Costill, 1974).

(ii)    A further 1.5 ml was placed in a calcium heparinised eppendorf tube. This was immediately centrifuged (Eppendorf Centrifuge 5415C) and the plasma removed and stored at -70°C for the determination of ammonia concentration within 48 h (for procedure see Appendix B). A pilot study on the effect of freezing and storage conditions on plasma ammonia concentrations conducted in this laboratory (Tzintzas and Wilson; unpublished observations) showed that plasma samples could be stored at -70°C for up to 48 h without any significant change in ammonia concentration. The ammonia assay (Sigma Diagnostics, kit 171 - C) had an intra-assay coefficient of variation (cv) of 1.7-4.2% and an inter-assay cv of 2.1-3.3%.

(iii)   The remaining blood (4 to 5 ml) was allowed to clot for 1 h in a plain tube (Sarstedt Serum Z/5 ml). This was then centrifuged at 3°C for 15 min at a speed of 6000 rev.min$^{-1}$ (Burkard Koolspin) and the serum was removed and stored at -70°C for the determination of serum hGH (Chapters 4, 5, 6 and 7), insulin (Chapter 5), cortisol (Chapters 4, 5, 6 and 7) and IGF-I (Chapter 7)

concentrations by routine ELISA (for procedures see Appendix B). The GH assay (Medgenix HGH-Biosource) had a sensitivity of 0.11 mU.l$^{-1}$, an intra-assay cv of 2.1-3.6% and an inter-assay cv of 6.8-7.1%. The insulin assay (Medgenix insulin-Biosource) had a sensitivity of 0.15 mU.l$^{-1}$, an intra-assay cv of 3.0-5.3% and an inter-assay cv of 5.6-9.8%. The sensitivity of the cortisol assay (Milenia, DPC cortisol) was 8.3 nmol.l$^{-1}$ with an intra-assay cv of 5.9-8.0% and an inter-assay cv of 8.3-9.0%. The IGF-I assay (R&D Systems Europe) had a sensitivity of 2.6 mg.ml$^{-1}$, and intra-assay cv of 3.5-4.3% and an inter-assay cv of 7.5-8.3%.

## 3.8. Calculations and statistical analysis

Mean integrated serum hGH concentrations (area under the curve – AUC) were calculated using the trapezium method (Parsons and Dawson, 1980) whereby the area was divided into a number of strips and the points where the ordinates meet the curve were joined by a straight line to form trapezia. The area of each trapezium was calculated using the equation:

$$Area = \frac{(y_1 + y_2) \times d}{2}$$

Figure 3.4. The trapezium method of calculating area under the curve.
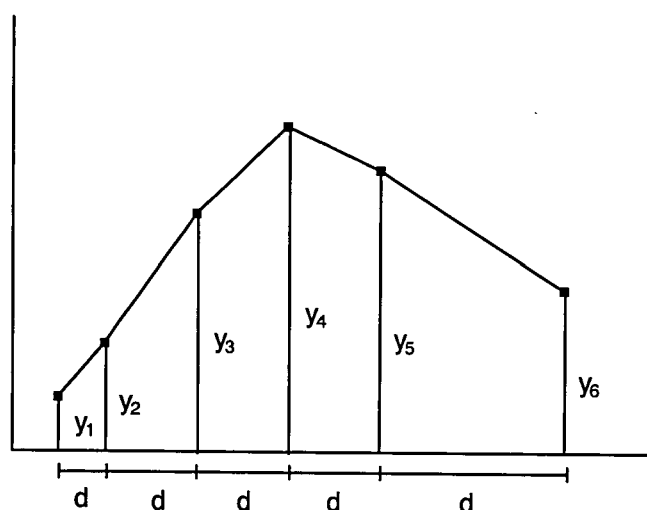
The mean integrated serum hGH concentration was calculated as the sum of the area of all trapezia.

A commercially available statistical computer package (Statistica release 5.0, StatSoft Inc.) was used to analyse data using $t$ tests and two-way, three-way and four-way analyses of variance (ANOVA) with repeated measures where appropriate. Relationships between variables were examined by calculating the Pearson product moment correlation coefficient (r). Statistical significance was accepted at the $P < 0.05$ level. All results are expressed as mean ± standard error of the mean (SEM).

### 3.9. The coefficients of variation for peak and mean power output during a 30 s cycle ergometer sprint.

**Methods**

Ten physically active male subjects volunteered to take part in this study. Following familiarisation they completed 10 trials on separate visits to the laboratory. Each trial consisted of a standardised submaximal warm-up, followed by 5 min rest, and then a single 30 s sprint from either a rolling (subjects A-E) or a stationary (subjects F-J) start (see section 3.5).

The coefficients of variation (V) for peak power output (PPO) and mean power output (MPO) were calculated for each subject using the following formula:

$$V = \frac{100\ \text{S.D.}}{M_s}\ \%$$

Where, S.D. is the standard deviation and $M_s$ is the mean of the sample (Cohen and Holliday, 1982).

**Results**

The individual data are depicted in Tables 3.1-3.4, and the coefficients of variation are summarised in Table 3.5. There were no differences in the coefficients of variation for PPO and MPO, or between a stationary and a standing start.

**Discussion**

The coefficients of variation for PPO from both a rolling start and a stationary start in the present study are similar to that reported by Cherry et al. (1997) who found that, following familiarisation, the mean coefficient of variation for PPO in a 30 s all-out cycle ergometer sprint from a stationary start was 5.5±1.5 %. Both the results in the present study and those of Cherry et al. (1997) are higher than the test-retest value of 2.6% reported by Winter et al. (1996). However, for the group as a whole, a coefficient of variation of 4.8 % for both PPO and MPO suggests that, once subjects are familiarised, there is not much variation in PPO and MPO measured during cycle ergometer sprinting on separate occasions, and, therefore, measurements of

performance can be considered to be representative of the subjects' mean performance.

Table 3.1. Peak power output (PPO) achieved by 5 subjects from a rolling start (70 rev.min$^{-1}$ against no applied resistance) on 10 separate occasions.

| | SUBJECT | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| 1 | 904 | 1164 | 1074 | 1092 | 1305 |
| 2 | 842 | 1046 | 1048 | 1068 | 1194 |
| 3 | 878 | 1122 | 1103 | 1132 | 1268 |
| 4 | 878 | 1176 | 1105 | 1198 | 1311 |
| 5 | 859 | 1134 | 985 | 1123 | 1248 |
| 6 | 950 | 1107 | 1026 | 1126 | 1217 |
| 7 | 865 | 1159 | 1017 | 1156 | 1367 |
| 8 | 985 | 1072 | 1066 | 1230 | 1521 |
| 9 | 961 | 1131 | 1073 | 1159 | 1291 |
| 10 | 925 | 1170 | 1052 | 1080 | 1185 |
| Mean | 905 | 1128 | 1055 | 1136 | 1291 |
| SD | 48 | 43 | 38 | 51 | 99 |
| CV (%) | 5.4 | 3.8 | 3.6 | 4.5 | 7.7 |

Table 3.2. Mean power output (MPO) achieved by 5 subjects from a rolling start (70 rev.min$^{-1}$ against no applied resistance) on 10 separate occasions.

|  | SUBJECT | | | | |
|---|---|---|---|---|---|
|  | A | B | C | D | E |
| 1 | 623 | 574 | 570 | 617 | 755 |
| 2 | 573 | 555 | 584 | 612 | 739 |
| 3 | 583 | 570 | 560 | 621 | 772 |
| 4 | 606 | 567 | 544 | 648 | 763 |
| 5 | 600 | 655 | 616 | 604 | 752 |
| 6 | 609 | 613 | 584 | 663 | 744 |
| 7 | 620 | 638 | 549 | 664 | 744 |
| 8 | 653 | 642 | 616 | 651 | 836 |
| 9 | 659 | 658 | 603 | 675 | 836 |
| 10 | 663 | 569 | 590 | 649 | 829 |
| Mean | 619 | 604 | 582 | 640 | 777 |
| SD | 31 | 41 | 26 | 25 | 40 |
| CV (%) | 5.0 | 6.8 | 4.4 | 3.9 | 5.2 |

Table 3.3. Peak power output (PPO) achieved by 5 subjects from a stationary start on 10 separate occasions.

| | SUBJECT | | | | |
|---|---|---|---|---|---|
| | F | G | H | I | J |
| 1 | 1403 | 1819 | 1670 | 1561 | 1397 |
| 2 | 1383 | 1720 | 1570 | 1551 | 1389 |
| 3 | 1487 | 1615 | 1674 | 1722 | 1274 |
| 4 | 1304 | 1754 | 1693 | 1643 | 1256 |
| 5 | 1361 | 1752 | 1609 | 1664 | 1458 |
| 6 | 1451 | 1673 | 1641 | 1624 | 1382 |
| 7 | 1409 | 1800 | 1577 | 1661 | 1297 |
| 8 | 1397 | 1580 | 1620 | 1645 | 1308 |
| 9 | 1438 | 1802 | 1646 | 1690 | 1229 |
| 10 | 1640 | 1645 | 1515 | 1593 | 1345 |
| Mean | 1427 | 1716 | 1622 | 1635 | 1334 |
| SD | 90 | 84 | 55 | 54 | 73 |
| CV (%) | 6.3 | 4.9 | 3.4 | 3.3 | 5.5 |

Table 3.4. Mean power output (MPO) achieved by 5 subjects from a stationary start on 10 separate occasions.

|  | SUBJECT | | | | |
|---|---|---|---|---|---|
|  | F | G | H | I | J |
| 1 | 676 | 873 | 819 | 696 | 765 |
| 2 | 685 | 789 | 815 | 684 | 697 |
| 3 | 682 | 724 | 799 | 726 | 706 |
| 4 | 702 | 793 | 813 | 702 | 790 |
| 5 | 744 | 822 | 821 | 709 | 818 |
| 6 | 735 | 750 | 868 | 779 | 697 |
| 7 | 685 | 797 | 779 | 738 | 677 |
| 8 | 688 | 754 | 791 | 725 | 741 |
| 9 | 717 | 761 | 782 | 730 | 719 |
| 10 | 700 | 792 | 757 | 724 | 747 |
| Mean | 701 | 786 | 804 | 721 | 736 |
| SD | 23 | 42 | 31 | 26 | 45 |
| CV (%) | 3.3 | 5.3 | 3.8 | 3.7 | 6.1 |

Table 3.5. Summary of the coefficients of variation for peak power output and mean power output

|  | PPO | MPO |
| --- | --- | --- |
| Rolling start (A-E) | 5.0±0.7 % | 5.1±0.5 % |
| Stationary start (F-J) | 4.7±0.6 % | 4.5±0.5 % |
| All subjects (A-J) | 4.8±0.4 % | 4.8±0.4 % |

## 3.10. The repeatability of sprinting on a cycle ergometer.

### Methods

The results of 16 of the physically active male volunteers that took part in the studies in this thesis were used in this study. Following familiarisation (see section 3.4), they completed two trials on separate days. Each trial consisted of a standardised submaximal warm-up, followed by 5 min rest and then a sprint from either a rolling or a stationary start. Ten subjects performed sprints from a rolling start during both trials, whilst eight subjects completed two sprints during both trials (two subjects completed the trials from both a rolling and a stationary start). The method described by Bland and Altman (1986) was then used to assess the repeatability of the peak (PPO) and mean (MPO) power output of the sprints.

### Results

The individual data are depicted in Figures 3.5-3.8, and the coefficients of repeatability are summarised in Table 3.6. Table 3.6 shows that, when using linear data, the coefficients of repeatability for PPO and MPO were 8.9 to 5.8 % of average PPO and MPO, respectively, for cycle ergometer sprints from a rolling start, and 6.7 to 6.3 % for cycle ergometer sprints from a stationary start.

### Discussion

The coefficients of repeatability calculated for the linear data, when expressed as a percentage of average power output, were slightly higher than the coefficients of variation for PPO and MPO reported in Section 3.9. However, heteroscedasticity in the measurement of power output during sprint tests (Nevill et al., 1995) lends itself to the use of log-transformed data when applying the Bland and Altman method of assessing repeatability (Bland and Altman, 1986). When the coefficients of repeatability were calculated for log-transformed data, and expressed as a percentage of the average log-transformed power output, they were around 1 % (Table 3.7). This infers good repeatability in measurements of both PPO and MPO during sprinting on a cycle ergometer, both from a rolling start and from a stationary start.

Figure 3.5. The relationship between mean PPO, for sprint 1 and sprint 2, and the absolute difference between sprint 1 and sprint 2, for sprints from a rolling start.
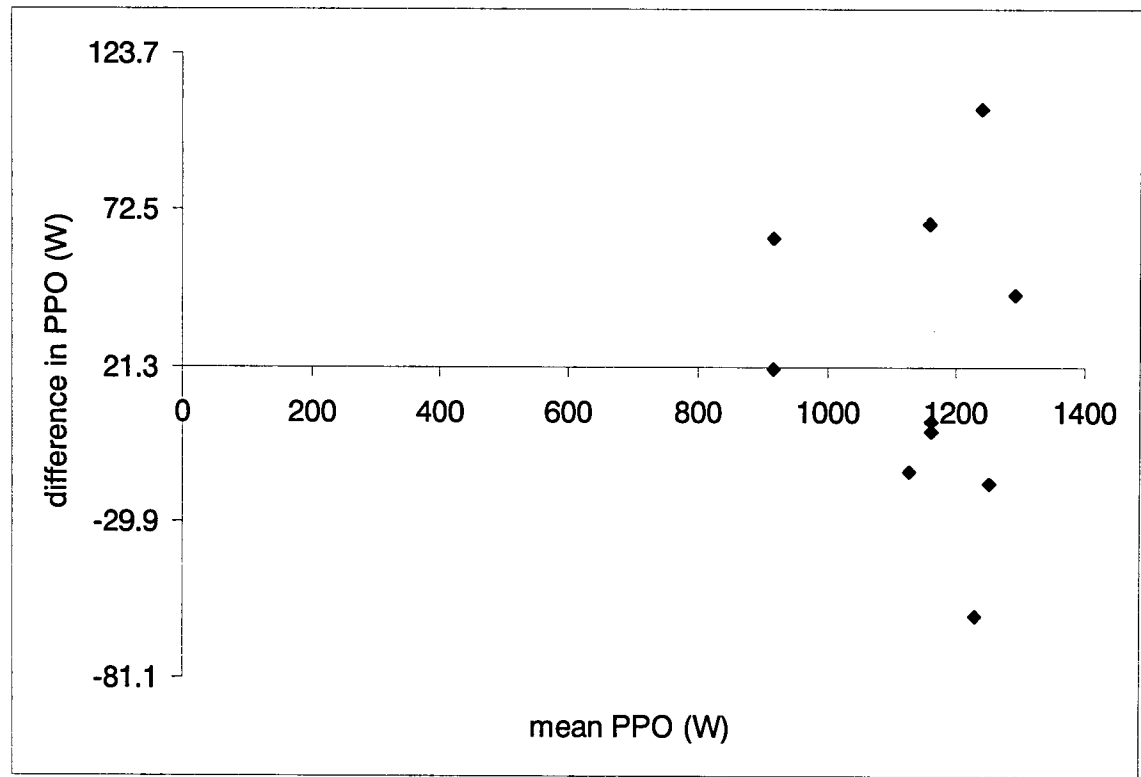


N.B. The y-axis crosses the x-axis at the point of mean difference between sprint 1 and sprint 2 (i.e. the bias). The upper and lower limits of the y-axis represent the bias ± the coefficient of repeatability (calculated as 2 x the standard deviation of the differences between sprint 1 and sprint2).

Figure 3.6. The relationship between mean MPO, for sprint 1 and sprint 2, and the absolute difference between sprint 1 and sprint 2, for sprints from a rolling start.
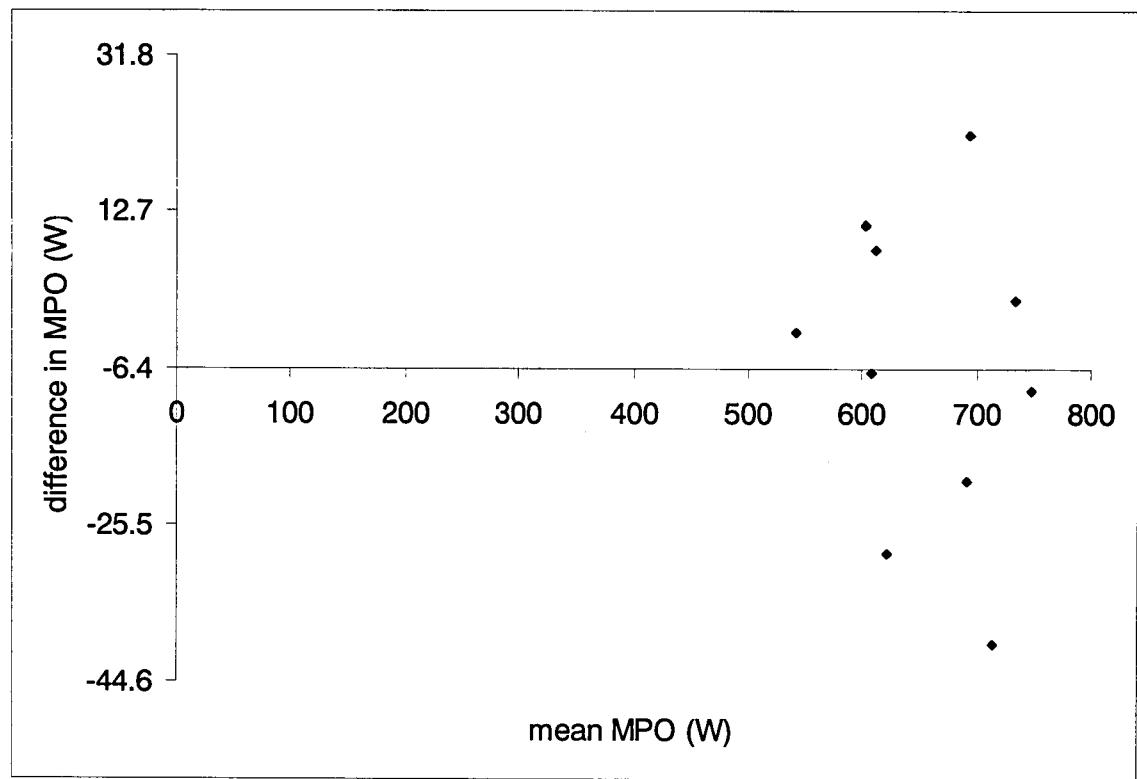


N.B. The y-axis crosses the x-axis at the point of mean difference between sprint 1 and sprint 2 (i.e. the bias). The upper and lower limits of the y-axis represent the bias ± the coefficient of repeatability  (calculated as 2 x the standard deviation of the differences between sprint 1 and sprint2).

Figure 3.7. The relationship between mean PPO, for sprint 1 and sprint 2, and the absolute difference between sprint 1 and sprint 2, for sprints from a stationary start.
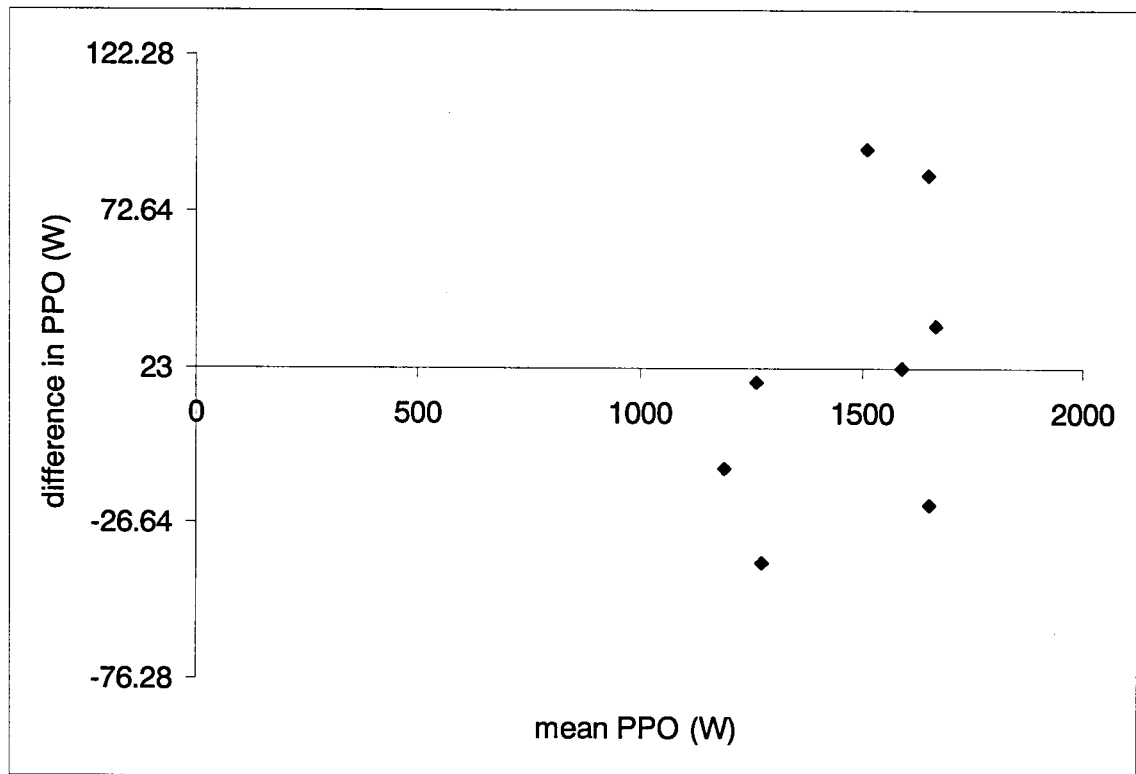


N.B. The y-axis crosses the x-axis at the point of mean difference between sprint 1 and sprint 2 (i.e. the bias). The upper and lower limits of the y-axis represent the bias ± the coefficient of repeatability (calculated as 2 x the standard deviation of the differences between sprint 1 and sprint2).

Figure 3.8. The relationship between mean MPO, for sprint 1 and sprint 2, and the absolute difference between sprint 1 and sprint 2, for sprints from a stationary start.
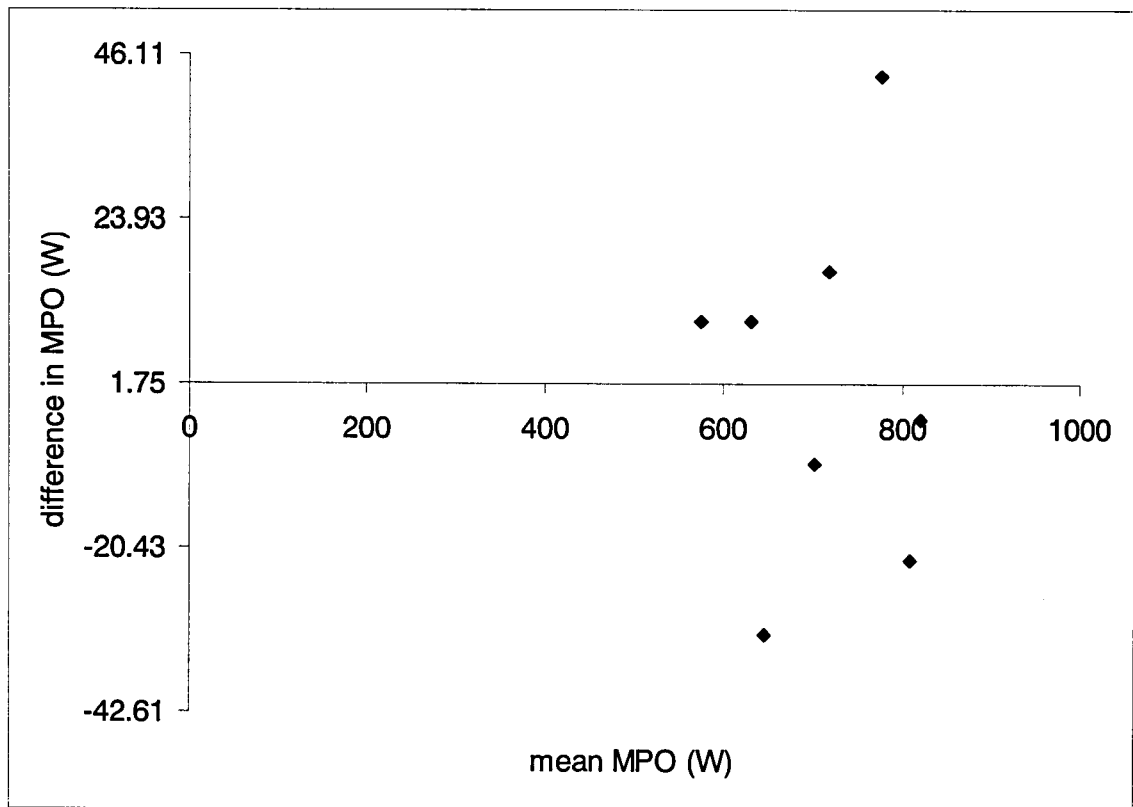


N.B. The y-axis crosses the x-axis at the point of mean difference between sprint 1 and sprint 2 (i.e. the bias). The upper and lower limits of the y-axis represent the bias ± the coefficient of repeatability (calculated as 2 x the standard deviation of the differences between sprint 1 and sprint2).

Table 3.6. Summary of the coefficients of repeatability, expressed as the calculated value and as the % of average power output.

| | | Average power output (W) | Average difference from mean | Coefficient of Repeatability | % of average power output |
|---|---|---|---|---|---|
| Rolling start | PPO | 1146 | 21.3 | 102 | 8.9 |
| | MPO | 657 | -6.4 | 38 | 5.8 |
| Stationary start | PPO | 1476 | 23.0 | 99 | 6.7 |
| | MPO | 710 | 1.8 | 44 | 6.3 |

Table 3.7. Summary of the coefficients of repeatability for the log-transformed data, expressed as the calculated value and as the % of average power output.

| | | Coefficient of Repeatability | % of average power output |
|---|---|---|---|
| Rolling start | PPO | 0.04 | 1.27 |
| | MPO | 0.02 | 0.87 |
| Stationary start | PPO | 0.03 | 0.90 |
| | MPO | 0.03 | 0.94 |

## 3.11. The effect of the insertion of a cannula on resting concentrations of hGH and cortisol

### Introduction

It has been suggested that hypodermic puncture might elicit an increase in both serum hGH concentration and serum cortisol concentration. It is important to determine that exercise itself is the stimulus for hGH and cortisol secretion that is being evaluated in the experimental chapters of this thesis. Therefore, the purpose of the present study was to determine whether the insertion of a cannula results in a detectable increase in serum hGH and cortisol concentrations in resting subjects.

### Methods

*Subjects*

Six healthy male volunteers aged 20 to 27 years (24.0±1.3 years) gave their written informed consent for this study which had the approval of the Loughborough University Ethical Committee. Body mass ranged from 73.8 to 87.0 kg (80.2±2.9 kg) and height ranged from 170.5 to 186.1 cm (177.8±2.5 cm).

*Protocol*

Following an overnight fast, subjects rested for 2 h whilst seated on a couch (maintaining approximately the same body position as they would on a cycle ergometer) whilst venous blood samples were collected.

*Blood sampling and analysis*

Venous blood samples were taken via a cannula inserted into an antecubital forearm vein under local anaesthetic (1% lignocaine). Patency was maintained by displacing the blood contained in the cannula with isotonic saline. Blood samples were taken in a seated position at rest 15, 30, 35, 45, 55, 65, 85, 115 and 145 min after the insertion of the cannula. These times corresponded to the resting sample (-10 min) and then what would be 5, 10, 20, 30, 40, 60, 90 and 120 min after a sprint in the protocols used in the experimental chapters of this thesis.

Samples were dispensed into three tubes: (i) One portion (4 to 5 ml) was placed into a lithium heparinised tube (Sarstedt LH/5ml). Blood pH was measured immediately (Radiometer ABL5 pH/blood gas monitor) and thereafter, 20µl aliquots of blood were removed, deproteinized in 2.5% perchloric acid and stored at -20°C for later determination of blood lactate concentrations (Maughan, 1982). Further aliquots were removed for the measurement of haematocrit by microcentrifugation (Hawksley Ltd.) and haemoglobin concentration (by the cyanmethaemoglobin method) for the calculation of percentage change in plasma volume (Dill and Costill 1974). (ii) A further 1.5 ml was placed in a calcium heparinised eppendorf tube. This was immediately centrifuged and the plasma removed and stored at -70°C for the determination of ammonia concentration within 48 h (Sigma Diagnostics, kit 171 - C). (iii) The remaining blood (4 to 5 ml) was allowed to clot for 1 h in a plain tube (Sarstedt Serum Z/5ml). This was then centrifuged at 3°C for 15 min at a speed of 6000 rev.min$^{-1}$ (Burkard Koolspin) and the serum was removed and stored at -70°C for the determination of hGH concentrations. Serum GH and cortisol were measured by routine ELISA. The GH assay (Medigenix HGH-Biosource) had a sensitivity of 0.11 mU.l$^{-1}$, an intra-assay coefficient of variation (cv) of 2.1-3.6% and an inter-assay cv of 6.8-7.1%. The sensitivity of the cortisol assay (Milenia, DPC cortisol) was 8.3 nmol.l$^{-1}$ with an intra-assay cv of 5.9-8.0% and an inter-assay cv of 8.3-9.0%.

*Statistical analysis*

A one-way analysis of variance with repeated measures was used to discover any changes over time. Statistical significance was accepted at the $P<0.05$ level. All results are reported as mean±SEM.

**Results**

No significant changes in blood lactate concentrations, pH or plasma ammonia concentrations were identified during the study (Table 3.8). Similarly, there were no significant differences over time in serum hGH (Figure 3.9) or serum cortisol (Figure 3.10) concentrations. Only two of the six subjects showed a marked increase in serum hGH concentrations at any time throughout the trial, with one subject demonstrating a peak hGH concentration of 27.81 mU.l$^{-1}$. Highest measured mean serum hGH concentration was 5.9±4.5 mU.l$^{-1}$ and was identified 85 min after insertion of the

cannula. This corresponds to 60 min after a sprint in the protocols used in the experimental chapters in this thesis. However, four of the six subjects did not demonstrate any clear increase in hGH concentration during the trial. In addition, two of the six subjects maintained undetectable serum hGH concentrations throughout the trial, and a further subject's hGH levels were undetectable until 145 min after the insertion of the cannula (the last blood sampling time point).

Highest measured mean serum cortisol concentration was identified 15 min after the insertion of the cannula as $451.8 \pm 81.3$ nmol.l$^{-1}$. Throughout the rest of the trial serum cortisol concentrations steadily declined with a lowest measured mean concentration of $321.5 \pm 85.2$ nmol.l$^{-1}$ 145 min after insertion of the cannula. There was no significant change in plasma volume during the study. The greatest mean decrease in plasma volume was $2.7 \pm 1.3\%$.

Table 3.8. Blood lactate concentrations, blood pH and plasma ammonia concentrations during 120 min following the insertion of a cannula (n=6).

| Time (min) | 0 | 5 | 10 | 20 | 30 | 40 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|---|---|---|
| Blood Lactate (mmol.l$^{-1}$) | 0.17± 0.03 | 0.14± 0.05 | 0.12± 0.04 | 0.15± 0.05 | 0.15± 0.05 | 0.14± 0.04 | 0.12± 0.03 | 0.18± 0.03 | 0.12± 0.03 |
| Blood pH | 7.37± 0.01 | 7.38± 0.00 | 7.38± 0.00 | 7.38± 0.00 | 7.37± 0.00 | 7.37± 0.01 | 7.37± 0.00 | 7.36± 0.00 | 7.36± 0.00 |
| Plasma Ammonia (µmol.l$^{-1}$) | 15.1± 3.7 | 22.1± 7.9 | 18.7± 4.5 | 17.0± 5.9 | 15.1± 3.9 | 23.1± 5.6 | 18.6± 3.2 | 18.2± 4.3 | 16.8± 2.5 |

Figure 3.9. Resting serum hGH concentrations following the insertion of a cannula at 0 min.
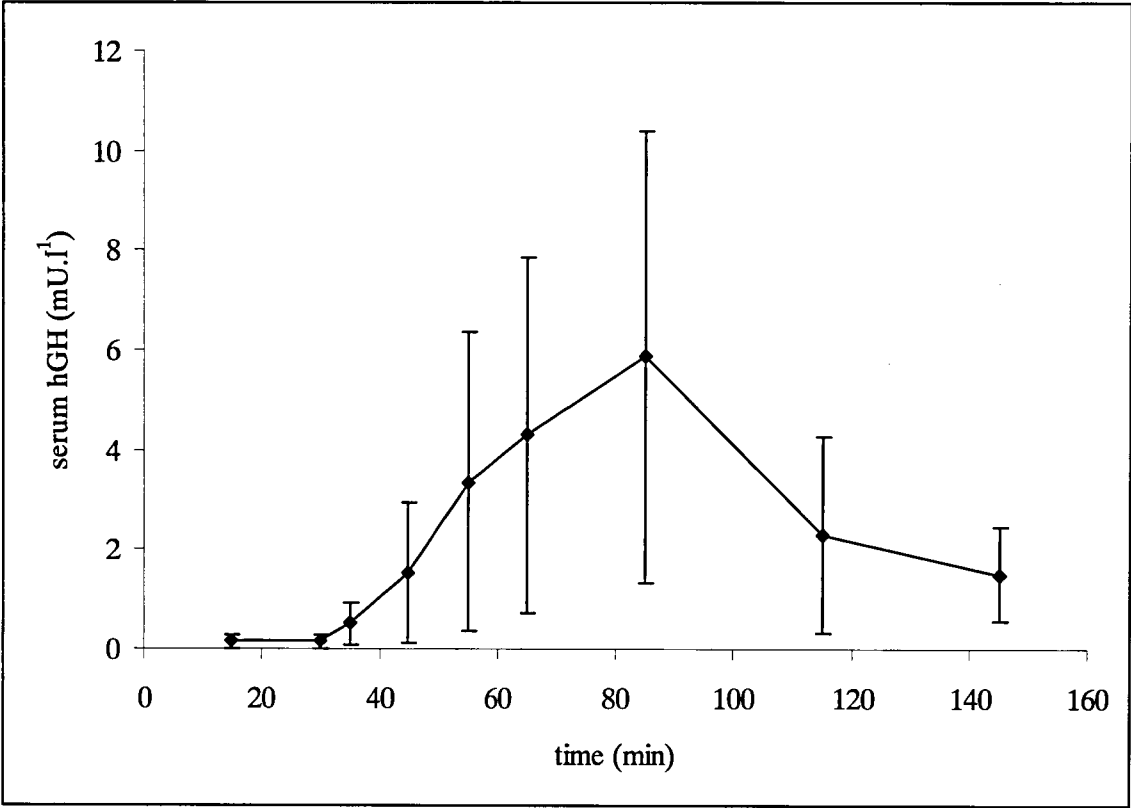
Figure 3.10. Resting serum cortisol concentrations following insertion of a cannula at 0 min.
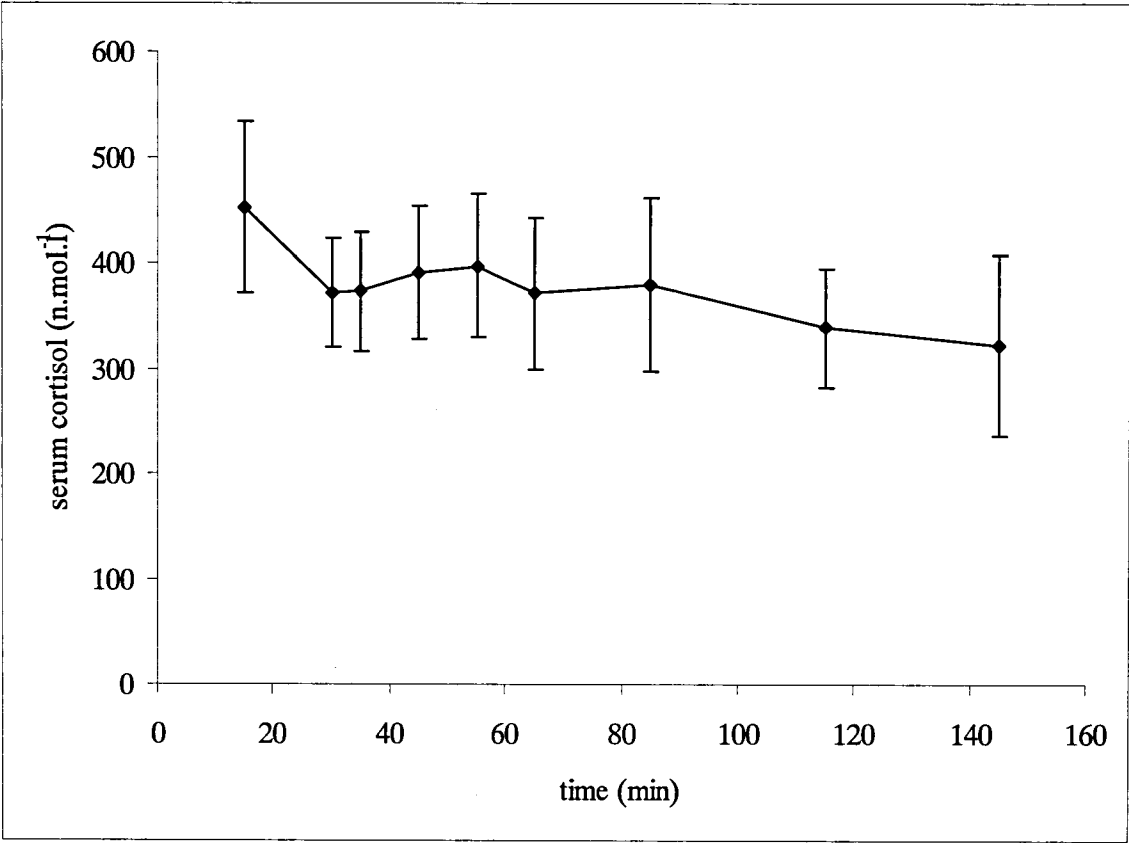
**Discussion**

The main result of this study was that the insertion of a cannula does not elicit a significant increase in serum hGH concentrations. However, there is variation between individuals since, in three subjects, hGH levels were undetectable using the assay employed in the present study for the majority of the trial, whilst, in one subject, serum hGH concentrations increased dramatically. The responses of the other two subjects were intermediate between these extremes. The hGH concentrations measured in the present study compare favourably with resting samples taken in other studies. For example, Barreca et al. (1988) reported resting concentrations of ~4 mU.l$^{-1}$ in both athletes and sedentary subjects. Similarly, Mikines et al. (1985) reported resting levels of hGH of 5±3 mU.l$^{-1}$ and 4±2 mU.l$^{-1}$ in trained and untrained men. In addition, the highest measured mean serum hGH concentrations observed in the present study are much lower than highest measured mean hGH concentrations of 59.8±13.3 mU.l$^{-1}$ following a single 30 s treadmill sprint in men (Nevill et al., 1996b).

It is possible that the reason for the interindividual differences in the hGH response to the insertion of a cannula was a variation degrees of anxiety or stress experienced by each subject as a result of this procedure. In addition, it might be that anxiety associated with being in the laboratory environment under experimental conditions caused an increase in circulating hGH levels. However, this seems unlikely on the basis that the individual who demonstrated by far the highest response was, himself, experienced in working in the laboratory environment both as experimenter and subject, in contrast to some of the other participants.

The serum cortisol concentrations measured in the present study are similar to the resting samples taken in other studies. For example, Barreca et al. (1988) measured resting cortisol concentrations prior to physical exercise of ~550 nmol.l$^{-1}$ in blood drawn from a cannula. Resting concentrations of 493±44 nmol.l$^{-1}$ were also reported by Del Corral et al., 1998). Mean resting cortisol levels of 193.1 nmol.l$^{-1}$ for healthy men aged 28-56 reported by Cornil et al. (1965) were much lower than those observed in the present study. However, Cornil et al. (1965) observed a small decrease, to 184.9 nmol.l$^{-1}$, in plasma cortisol concentration over 50 min of rest, in agreement with the findings of the present study. If cortisol is taken as a marker of anxiety, as

considered by Follenius and Brandenburger (1975), then the insertion of a cannula did not appear to result in any apprehension in the present study, unless serum cortisol concentrations were elevated within 15 min of the insertion of the cannula. Alternatively, if apprehension due to being under experimental conditions were a factor, cortisol concentrations may have been elevated even before the cannula was inserted.

However, since only two of the subjects demonstrated an increase (n.s.) in hGH concentrations, and no significant changes in serum cortisol concentrations were identified, it would appear that any anxiety felt by the subjects was not reflected in the circulating hormone levels measured in this study. The marked increases in serum hGH concentrations in one of the subjects remains unexplained, although it may be that this reflects the inter-individual variation in the growth hormone response to a stimulus for hGH secretion.

### 3.12. Storage Study

### Methods

One physically active male subject completed a single 30 s cycle ergometer sprint, and 30 min later, a 20 ml venous blood sample was taken from an antecubital forearm vein by venepuncture. The blood was allowed to clot in a plain tube and then centrifuged at 3°C for 15 min at 6000 rev.min$^{-1}$. The serum was removed and ~0.5 ml aliquots were dispensed into 40 Eppendorf tubes for storage at –70°C. Serum concentrations of hGH were determined by routine ELISA (for procedures see Appendix B) as soon as was possible (1 week), 1 month, 4 months and 18 months after the trial.

### Results

The serum hGH concentrations for the four analysis runs are shown in Table 3.9. No statistically significant differences were found in the serum hGH concentrations measured at any time.

### Discussion

The results of this pilot study show that the analysis of serum for the determination of hGH concentrations can be undertaken up to 18 months after blood sample collection without any significant changes in the concentrations measured.

Table 3.9. Serum hGH concentrations measured 1 week, 1 month, four months and 18 months after the collection of a post-exercise blood sample.

|  | Time to analysis | | | |
|---|---|---|---|---|
|  | 1 week | 1 month | 4 months | 18 months |
| 1 | 10.8 | 11.0 | 11.8 | 10.8 |
| 2 | 9.0 | 12.4 | 10.5 | 12.6 |
| 3 | 10.7 | 12.1 | 10.7 | 11.7 |
| 4 | 12.4 | 11.8 | 11.1 | 10.2 |
| 5 | 10.7 | 10.0 | 11.0 | 11.5 |
| 6 | 12.2 | 11.5 | 11.7 | 11.8 |
| 7 | 12.1 | 11.9 | 10.8 | 10.6 |
| 8 | 10.7 | 11.8 | 10.4 | 11.6 |
| 9 | 11.4 | 12.4 | 9.8 | 10.8 |
| 10 | 11.3 | 10.4 | 11.2 | 9.3 |
| Mean | 11.1 | 11.5 | 10.9 | 11.1 |
| SEM | 0.3 | 0.3 | 0.2 | 0.3 |
| CV (%) | 9.0 | 7.1 | 5.6 | 8.5 |

# CHAPTER 4

# THE TIME-COURSE OF THE HUMAN GROWTH HORMONE RESPONSE TO A 6 S AND A 30 S CYCLE ERGOMETER SPRINT

## Introduction

A number of studies have shown exercise to be a potent stimulus for the release of human growth hormone (hGH). Most of these studies have considered prolonged submaximal exercise whilst only a few have studied high intensity or maximal sprint exercise (Gordon et al. 1994; Nevill et al. 1996b).

Gordon et al. (1994) found considerably elevated hGH concentrations following 90 s of high intensity exercise. However, blood samples were only taken for 30 min following the exercise bout, at which time hGH concentrations were higher than at any other time point. Nevill et al. (1996b) studied metabolic and hormonal responses for a 60 min period following a 30 s treadmill sprint, again demonstrating a significant hGH response to high intensity exercise. In this study the blood lactate and plasma ammonia concentrations and blood pH had returned to resting levels within 60 min of recovery, however, hGH was still elevated. There is, therefore, a need to measure hGH concentrations for longer than 60 min after a bout of high intensity exercise to establish the full time-course of the hGH response.

The mechanisms controlling the magnitude of the hGH response to exercise are not fully understood. It has been suggested that blood lactate (Karagiorgos et al., 1979; Chwalbinska et al., 1996), blood pH (Gordon et al., 1994) and oxygen demand and availability (Vanhelder et al., 1987) may have roles in controlling hGH release. In addition intensity and duration of exercise may influence the hGH response (Sutton et al., 1976). No study has considered the effect that the duration of a bout of maximal sprint exercise has, either directly or mediated by other factors, on the hGH response to a sprint. Therefore the purpose of this study was to determine the time-course of the hGH response to a 6 s and a 30 s maximal cycle ergometer sprint.

## Methods

### Subjects

Nine healthy male volunteers aged 18 to 28 years (23±1 year) gave their written informed consent for this study which had the approval of the Loughborough University Ethical Committee. Body mass ranged from 72.5 to 88.0 kg (82.1±1.9 kg) and height ranged from 175.1 to 186.1 cm (180.8±1.4 cm).

### Equipment

The exercise tests were carried out on a modified friction-loaded cycle ergometer (Monark 864), which was interfaced to a microcomputer (BBC). This allowed instantaneous power output, corrected for flywheel acceleration, to be monitored and recorded accurately. Performance data were averaged over 1 s intervals. Lakomy (1986) has described the equipment used in detail. A restraining harness was also placed around the subjects' waists in order to prevent them from rising out of the saddle, thereby concentrating movement in the lower limbs. The same harness setting and saddle height were used for each trial. Toe-clips and tape held the subjects' feet securely in the pedals.

### Protocol

After familiarisation, the subjects arrived in the laboratory after a 4 h fast on two separate occasions, completing one trial on each visit using a crossover design. During one trial subjects completed a single all out 6 s effort and during the other trial they completed a single all out 30 s effort.

Before the first sprint of each trial all subjects completed a standardised sub-maximal warm-up on the cycle ergometer, consisting of 4 min pedalling at 60W, 30 s pedalling at 80W and 30 s pedalling at 100W. Five minutes after the warm-up, the subjects performed the maximal sprint from a stationary start on the cycle ergometer against an applied resistance equal to 7.5% (75g.kg$^{-1}$) of their body mass. Subjects were instructed to sprint maximally for the duration of the sprint and were encouraged verbally whilst sprinting. Following the sprint subjects were seated in an upright position on a couch (maintaining approximately the same body position as on the

ergometer). They remained on the couch and blood samples were obtained for 3 h after the sprint.

Three of the subjects completed an additional control trial on another occasion, arriving at the laboratory after a 4 h fast. During this trial the subjects did not perform any exercise but remained seated in an upright position on the couch for 2 h whilst blood samples were obtained.

**Blood sampling and analysis**

Venous blood samples were taken via a cannula inserted into an antecubital forearm vein under local anaesthetic (1% lignocaine). In the exercise trials blood samples were taken in a seated position at rest (-10 min) and post warm-up (-4 min) prior to the first sprint and 5, 10, 20, 30, 40, 60, 90, 120, 150 and 180 min after the sprint. The first blood sample was taken at least 15 min after the cannula was inserted. Patency was maintained by displacing the blood contained in the cannula with isotonic saline. In the control trial the first blood sample was taken at least 15 min after the cannula was inserted and then at 5, 10, 20, 30, 40, 60, 90 and 120 min after the first sample

Samples were dispensed into three tubes: (i) One portion (4 to 5 ml) was placed into a lithium heparinised tube (Sarstedt LH/5ml). Blood pH was measured immediately (Radiometer ABL5 pH/blood gas monitor) and thereafter, 20µl aliquots of blood were removed, deproteinized in 2.5% perchloric acid and stored at -20°C for later determination of blood lactate concentrations (Maughan, 1982). Further aliquots were removed for the measurement of haematocrit by microcentrifugation (Hawksley Ltd.) and haemoglobin concentration (by the cyanmethaemoglobin method) for the calculation of percentage change in plasma volume (Dill and Costill 1974). (ii) A further 1.5 ml was placed in a calcium heparinised eppendorf tube. This was immediately centrifuged and the plasma removed and stored at -70°C for the determination of ammonia concentration within 48 h (Sigma Diagnostics, kit 171 - C). (iii) The remaining blood (4 to 5 ml) was allowed to clot for 1 h in a plain tube (Sarstedt Serum Z/5ml). This was then centrifuged at 3°C for 15 min at a speed of 6000 rev.min$^{-1}$ (Burkard Koolspin) and the serum was removed and stored at -70°C

for the determination of hGH concentrations. Serum GH was measured by routine ELISA. The GH assay (Medigenix HGH-Biosource) had a sensitivity of 0.11 mU.l$^{-1}$, an intra-assay coefficient of variation (cv) of 2.1-3.6% and an inter-assay cv of 6.8-7.1%.

**Statistical analysis**

A paired $t$ test was used to ascertain whether there were any differences in performance between the 6 s trial and the first 6 s of the 30 s trial. A two-way analysis of variance with repeated measures was used to discover any differences in biochemical responses between 6 s and 30 s trials (main effect - trial) and the response of each subject with respect to time (main effect - time). Statistical significance was accepted at the $P < 0.05$ level. All results are expressed as mean ±SEM.

<u>Results</u>

**Performance**

Table 4.1 summarises the performances achieved by the subjects in this study. There was no difference in peak power output (PPO) or mean power output during the first 6s (MPO-6) between the 6 s and 30 s trials. Similarly, peak pedal revolutions (PPR) and mean pedal revolutions over the first 6 s (MPR-6) showed no difference between trials. However, total work done in the 30 s trial was more than three times greater than that done in the 6 s trial (20903±480 vs. 6179±155 J, $P<0.05$).

Table 4.1. Peak power output (PPO), mean power output (MPO), peak pedal rate (PPR), mean pedal rate (MPR), work done and fatigue index for the 6 s and the 30 s sprints. [a] $P<0.05$

| | 6 s | 30 s |
|---|---|---|
| **PPO (W)** | 1494±43 | 1468±42 |
| **MPO-30 (W)** | - | 697±16 |
| **MPO-6 (W)** | 1030±26 | 1010±25 |
| **PPR (rev.min$^{-1}$)** | 160±4 | 158±4 |
| **MPR-30 (rev.min$^{-1}$)** | - | 118±3 |
| **MPR-6 (rev.min$^{-1}$)** | 139±4 | 137±4 |
| **Work (J)** | 6179±155 | 20903±480 [a] |
| **Fatigue Index (%)** | 68±1 | 42±1 |

**Metabolic responses to cycle ergometer sprinting**

The blood lactate, blood pH and plasma ammonia responses are shown in Figures 4.1, 4.2 and 4.3. Highest measured mean blood lactate concentrations, observed 5 min post-exercise in both exercise trials, were more than three times greater following the 30 s sprint than they were following the 6 s sprint ($11.81\pm0.52$ vs. $3.59\pm0.32$ mmol.l$^{-1}$, $P<0.05$). Blood lactate concentrations returned to resting levels between 40 and 60 min after the 6 s sprint but not until between 90 and 120 min following the 30 s sprint (trial-time interaction effect, $P<0.05$). In the control trial mean blood lactate concentrations did not exceed 0.20 mmol.l$^{-1}$.

Lowest measured mean blood pH was observed 5 min following the sprint in each exercise trial. Following the 6 s sprint blood pH reached 7.32 compared with 7.16 following the 30 s sprint ($P<0.05$). Blood pH had returned to resting levels between 20 and 30 min after the 6 s sprint compared with between 30 and 40 min following the 30 s sprint (trial-time interaction effect, $P<0.05$). During the control trial mean blood pH remained at 7.37 for the duration of the trial.

Highest measured mean plasma ammonia concentrations were almost three times greater following the 30 s sprint than they were following the 6 s sprint ($166.8\pm26.3$ vs. $56.7\pm9.8$ µmol.l$^{-1}$, $P<0.05$). Plasma ammonia concentrations also demonstrated a trial-time interaction effect as the concentrations returned to resting levels 30 to 40 min after the 6 s sprint compared with approximately 120 min after the 30 s sprint. During the control trial the highest measured mean plasma ammonia concentration was 25.7 µmol.l$^{-1}$.

Figure 4.1. Mean blood lactate concentrations at rest and during 3 h of recovery after a single 6 s or a single 30 s sprint and for 2 h in the CON trial (n=3). Exercise trial statistics: Trial main effect $P<0.05$, time main effect $P<0.05$, trial-time interaction effect $P<0.05$.
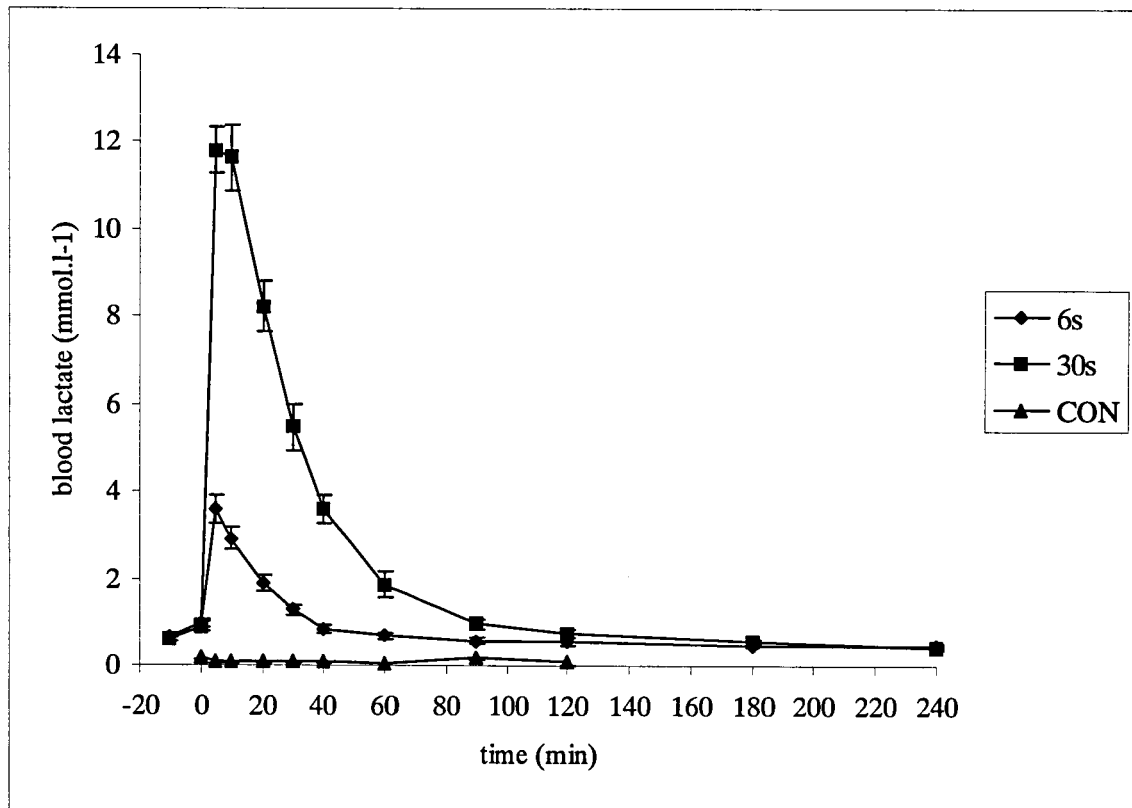
Figure 4.2. Mean blood pH at rest and during 3 h of recovery after a single 6 s or a single 30 s sprint and for 2 h in the CON trial (n=3). Exercise trial statistics: Trial main effect $P<0.05$, time main effect $P<0.05$, trial-time interaction effect $P<0.05$.

Figure 4.3. Mean plasma ammonia concentrations at rest and during 3 h of recovery after a single 6 s or a single 30 s sprint and for 2 h in the CON trial (n=3). Exercise trial statistics: Trial main effect $P<0.05$, time main effect $P<0.05$, trial-time interaction effect $P<0.05$.
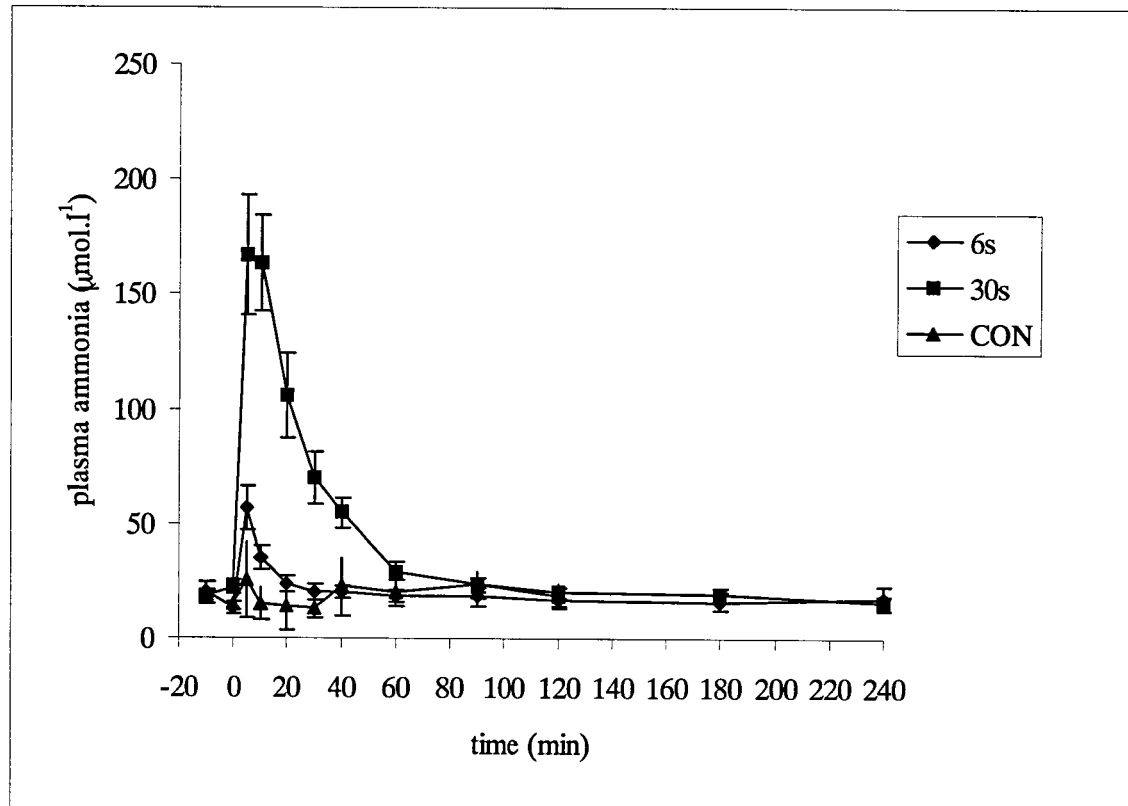
**Hormone responses to cycle ergometer sprinting**

Figure 4.4 shows the mean serum hGH response to a 6 s or a 30 s maximal cycle ergometer sprint. In this study, a 30 s sprint resulted in a distinct hGH pulse with highest measured mean concentrations of $37.01\pm6.19$ mU.l$^{-1}$ 40 min after the sprint. This represents a 530% increase in mean serum hGH concentration above resting levels. Serum hGH remained elevated for between 90 and 120 min after the 30 s sprint. In contrast a 6 s sprint resulted in a smaller hGH response with a highest measured mean hGH concentration of $8.01\pm2.90$ mU.l$^{-1}$ 40 min after the sprint, representing an increase of 217% over resting levels. hGH concentrations returned to resting levels less than 60 min after the 6 s sprint. Thus, highest measured mean hGH concentrations were more than four and a half times greater following a 30 s sprint than they were following a 6 s sprint ($P<0.05$). In the control trial mean serum hGH concentrations did not exceed 0.30 mU.l$^{-1}$.

The GH response to a 30 s sprint was greater than the response to a 6 s sprint in all subjects. However, Figure 4.5 demonstrates the large inter-individual variation in serum hGH concentration following a 30 s sprint. Highest measured concentrations ranged from 4.5 mU.l$^{-1}$ to 79.3 mU.l$^{-1}$ and time to reach the highest measured concentration ranged from 20 min in three subjects to 60 min in one subject. Because of these variations it is more appropriate to consider the hGH response as an integrated value rather than concentrations at specific time points. Figure 4.6 shows the hGH response as a mean area under the curve (AUC); the hGH response to a 30 s sprint was more than three and a half times greater than the response to a 6 s sprint ($3615\pm180$ vs. $963\pm294$ mU.l$^{-1}$, $P<0.05$).

**Changes in plasma volume**

The estimated changes in mean plasma volume at 5-min postexercise were $-5.1\pm1.4$ % and $-14.8\pm1.0$ % for the 6 s sprint and 30 s sprint respectively ($P<0.05$). Estimated changes in plasma volume had returned to baseline by 20-min postexercise in the 6 s trial and by 40 min postexercise in the 30 s trial.

Figure 4.4. Mean serum hGH concentrations at rest and during 3 h of recovery after a single 6 s or a single 30 s sprint and for 2 h in the CON trial (n=3). Exercise trial statistics: Trial main effect $P<0.05$, time main effect $P<0.05$, trial-time interaction effect $P<0.05$.
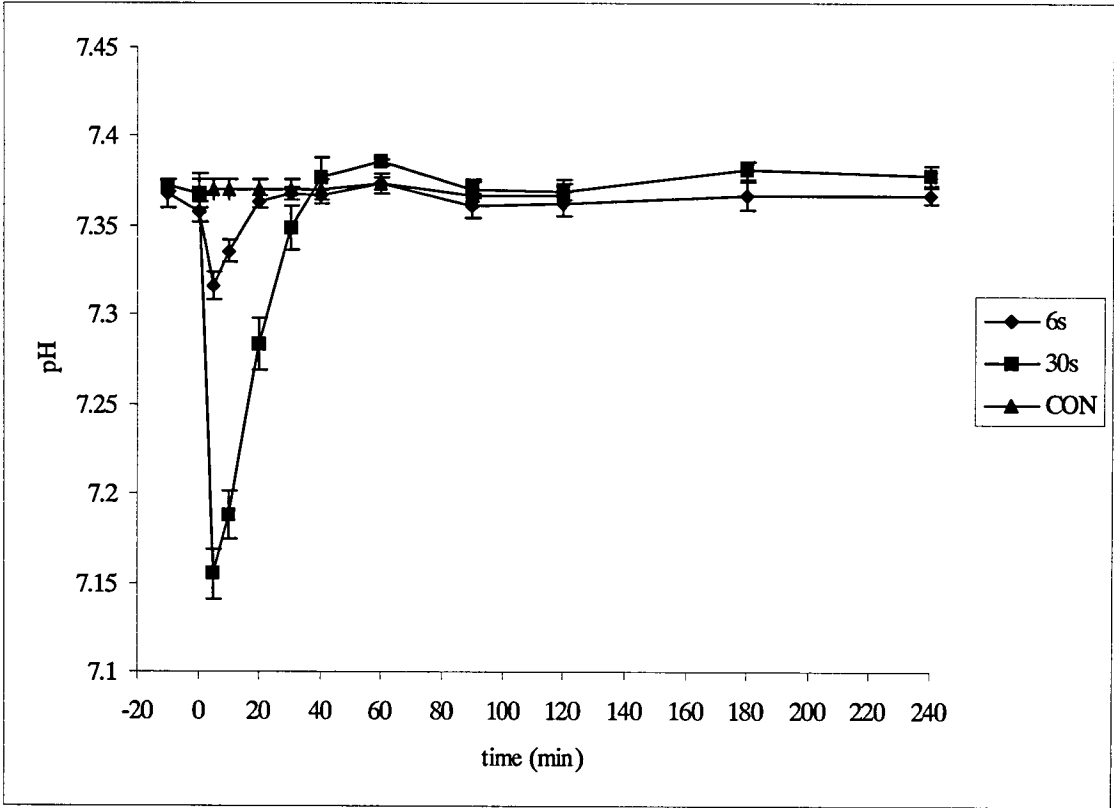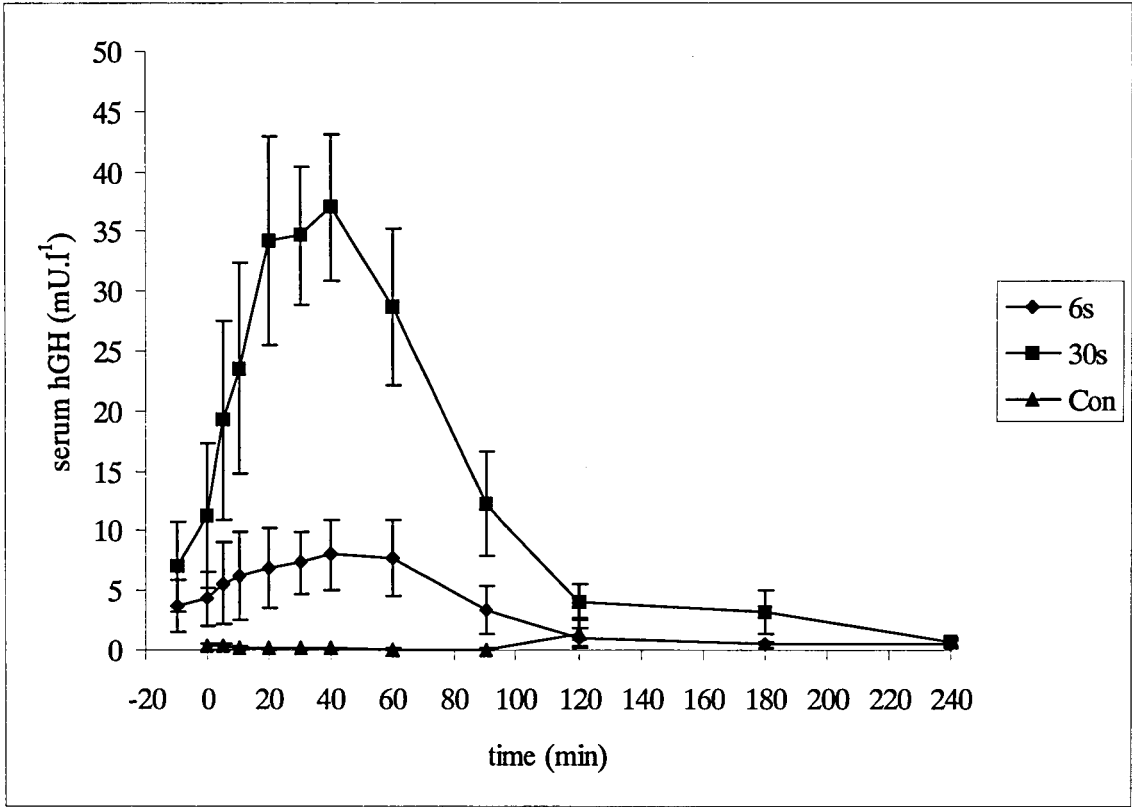
Figure 4.5. Individual serum hGH concentrations at rest and during 3 h recovery after a single 30 s sprint.
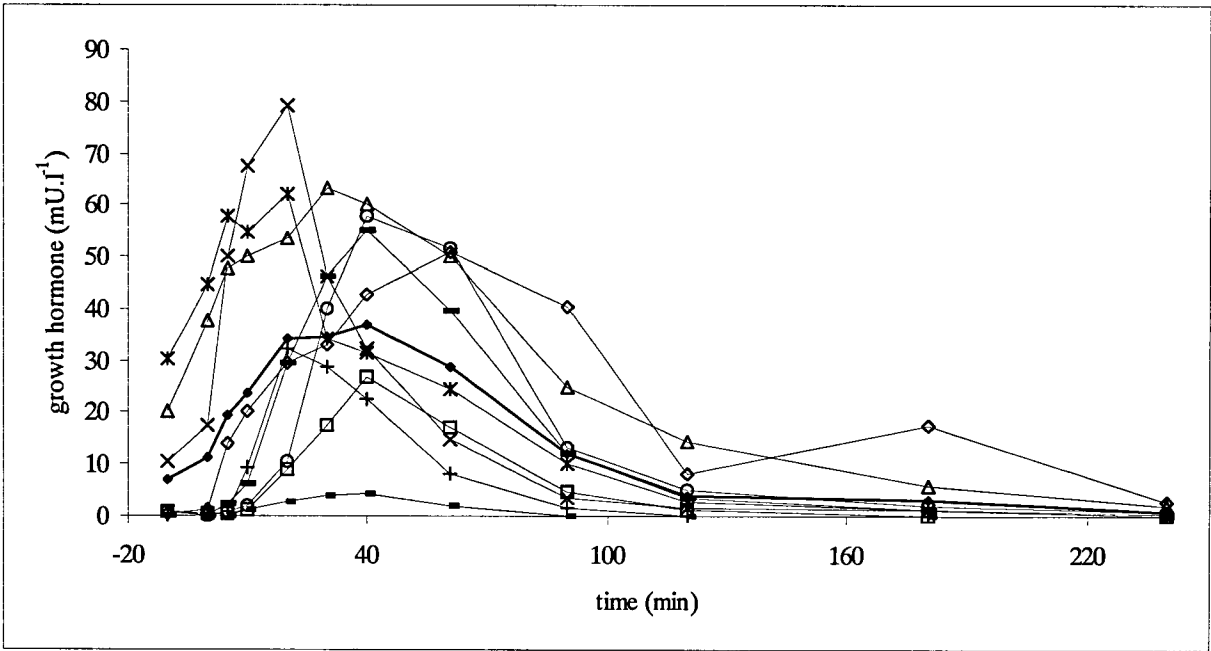
Figure 4.6. Mean integrated 3 h serum hGH concentration (AUC) during recovery after a single 6 s or a single 30 s sprint. [a] $P<0.05$
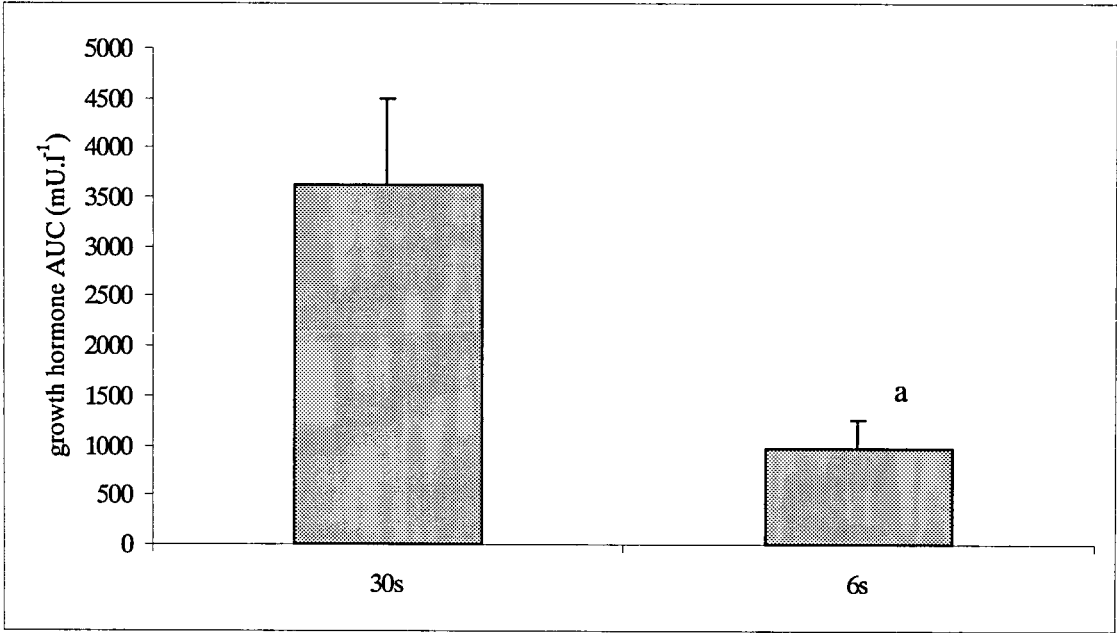
**Discussion**

This study shows that the duration of a bout of maximal sprint exercise determines, either directly or indirectly, the magnitude of the growth hormone response. A single 30 s sprint elicits a marked increase in serum hGH concentration when compared with a single 6 s sprint. This study also highlights the inter-individual variability in the hGH response to an exercise stimulus.

The amount of work done in a 30 s sprint is much greater than that completed in a 6 s sprint, however, in this study the first 6 s of the 30 s sprint was similar to the 6 s sprint itself. Therefore the differences observed in the metabolic and hormonal responses between the two sprints were likely to be due to the total amount of work done in the sprints as opposed to the work done in the first few seconds of the sprints. It is possible that the amount of external work per se determined the hGH concentrations during recovery, or it may be that factors such as the different metabolic responses to the sprints had a controlling effect on the hGH response. The results for the three subjects that completed the control trial indicate that, in these individuals, the hGH response seen in the exercise trials was not caused by the procedure of inserting the cannula since mean serum hGH concentrations did not exceed 0.30 mU.l$^{-1}$ in the control trial.

Activity in motor centres may directly stimulate pituitary hormone secretion, including hGH, during exercise (Kozlowski et al. 1983; Kjaer et al. 1987; Kjaer et al. 1989; Kjaer et al. 1996a). It is likely that in the present study there was greater central motor activity over the duration of the 30 s sprint than there was over the 6 s sprint, which could have resulted in a greater hGH response. Takarada et al. (2000) found a larger hGH response to low intensity exercise with vascular occlusion than to the same amount of exercise without occlusion. When exercise was completed with vascular occlusion the relative integrated electromyogram (iEMG) was approximately 1.8 times greater than when exercise was completed without occlusion. It is possible that this reflects the fact that in the hypoxic intramuscular environment motor units of more muscle fibres are activated to maintain the same level of force generation (Moritani et al., 1992). Miller et al. (1996) have also demonstrated that accumulation of lactate promotes recruitment of additional motor units in seriously fatigued muscles. In the present study it is likely that there was greater activation level of

muscle in the 30 s sprint than in the 6 s sprint since a higher fatigue index was seen and a higher blood lactate concentration, reflecting higher muscle lactate concentration, was observed. This may have influenced the magnitude of the hGH response to the sprints.

Other work by Kjaer et al. (1996b) suggests that humoral feedback mechanisms and autonomic nervous reflexes, without efferent motor centre activity, may exert redundant control of pituitary hormonal responses. Gosselink et al. (1998) found evidence of a proprioceptive mechanism for the regulation of bioassayable GH but not of immunoassayable GH in rats. However, they also showed that stimulation of a large muscle mass resulted in elevated immunoassayable GH. It was suggested that this might be due to metabolic perturbations, although metabolites were not measured. In the present study there would have been more proprioceptive feedback during the 30 s sprint than there was during the 6 s sprint and it is possible that this proprioceptive feedback played a role in the regulation of the hGH response.

Although Sutton et al. (1969) found a correlation between blood lactate concentrations and hGH concentrations, artificial manipulation of lactate levels using sodium lactate (Vigas et al., 1974) and lactic acid (Sutton et al., 1976) have been shown to have no consistent effect on hGH concentration. This does not, however, preclude lactate as a regulator of hGH concentrations as it may be that lactate is detected in the muscle and is therefore not related to blood lactate concentrations. Other authors have suggested that a combination of factors related to anaerobic metabolism are involved in controlling hGH release (Kraemer et al., 1990; Nevill et al., 1996b). However, Karagiorgos et al. (1979) found no correlation between any anaerobic metabolite or oxygen deficit and hGH concentration.

There is relatively little literature regarding the time course of the hGH response to exercise. Raynaud et al. (1981) considered the time course of plasma growth hormone during exercise in humans at altitude. Their results only extend for 90-120 min post-exercise at which point hGH was still slightly elevated above baseline concentrations. Other studies considering the hGH response have similarly taken measurements for 2 h post-exercise or less (Prange Hansen, 1973; Raynaud et al., 1983; Kraemer et al., 1990). The results of the present study show that in response to

a 6 s sprint serum hGH concentrations return to pre-exercise levels less than 60 min post-exercise. In contrast following a 30 s sprint serum hGH concentrations remain above baseline values for between 90 and 120 min post-exercise and that in some individuals (Figure 4.5) hGH levels remain elevated for even longer. This is in agreement with the findings of Raynaud et al. (1983) who employed a number of different types of work but this is the first time it has been demonstrated using sprint exercise.

The highest measured mean concentrations of hGH occurred at around 40 min of recovery from both the 6 s and the 30 s sprint. However there was some interindividual variation in time to highest measured concentration with a range from 20 to 60 min after the sprint. Raynaud et al. (1981) suggested that the time sequence of hGH should be characterised by parameters other than maximal value, giving mean concentration over the period of observation as an example. In view of this, in the present study the hGH response was reported using AUC as an integrated measure in addition to considering individual time-points. Using this method it was found that the hGH response to the 30 s sprint was more than three and a half times greater than the response to a 6 s sprint. This is slightly less of a difference than the four and a half times greater following a 30 s compared with a 6 s sprint using highest measured mean concentrations but is still significant and is, perhaps, a better indicator of the overall hGH response to a sprint.

There was greater variation in the time to highest measured concentration following the 30 s sprint than there was following the 6 s sprint. Figure 4.5 shows the large inter-individual variation in the serum hGH concentration following a 30 s sprint. It is important to note that there was no apparent relationship between any performance variable and hGH concentration following a 30 s sprint in these subjects. Therefore the large interindividual differences are not a result of differences in performance. Raynaud et al. (1983) described this intersubject variability in the hGH response to exercise and suggested that care should be taken when drawing conclusions from averaged hGH results when there is inherent variability between individuals' responses. Previous studies have described their results by dividing subjects into groups based on the magnitude of their hGH responses to a stimulus (Raastad et al., 2000). However, as Figure 4.6 shows, in the present study it is difficult to determine

distinct groups of subjects with similar responses, but rather there is a range of highest measured concentrations as well as time taken to reach highest measured concentration.

In conclusion this study has determined that the duration of a bout of maximal sprint exercise affects the magnitude of the hGH response. The mechanism for this effect is not clear, it may be the total amount of work done during the sprint per se or may be mediated by other factors. Nevertheless a single 30 s sprint elicits a significant hGH response when compared to a 6 s sprint. This study also highlights the inter-individual variation in the hGH response to a bout of sprint exercise and illustrates the care that must be taken when drawing conclusions from data with such variability.

# CHAPTER 5

# GROWTH HORMONE RESPONSES TO REPEATED MAXIMAL CYCLE ERGOMETER EXERCISE AT DIFFERENT PEDAL SPEEDS

## Introduction

Growth Hormone (hGH) is released in a pulsatile manner from the anterior pituitary gland. The release of hGH is believed to be regulated by the antagonistic effects of the hypothalamic neuropeptides growth hormone releasing hormone (GHRH) and somatostatin, with modulation by feedback mechanisms (Dieguez et al., 1988).

A number of studies, have shown exercise to stimulate hGH release although only a few have considered high intensity or sprint exercise (Gordon et al., 1994; Nevill et al., 1996b). A single 30 s treadmill sprint produces a near maximal hGH response when compared with the results from pharmacological intervention studies, with hGH levels remaining elevated for at least 60 min post-exercise (Nevill et al., 1996b). However, the mechanisms controlling the magnitude of the hGH response to exercise are not well understood. Intensity and duration of exercise have been suggested to influence the hGH response (Sutton et al., 1976), whilst the possible roles of blood lactate (Karagiorgos et al., 1979; Chwalbinska-Moneta et al., 1996), blood pH (Gordon et al., 1994) and $O_2$ demand and availability (Vanhelder et al., 1987) have also been considered.

Repeated 30 min bouts of submaximal exercise have been shown to elicit an augmented hGH response (Kanaley et al., 1997). This finding is in contrast to studies showing that repeated administration of pharmacological stimuli results in an attenuated GH response in rats (Lanzi and Tannenbaum, 1992a; Lanzi and Tannenbaum, 1992b) and in humans (Ghigo et al., 1991). In addition, Cappon et al. (1994) found that, in humans, three heavy 10 min exercise bouts resulted in progressive attenuation of the hGH response to exercise. At present, therefore, the evidence regarding the hGH response to repeated bouts of exercise is equivocal.

The muscle metabolic responses to maximal cycling at fast (140 rev.min$^{-1}$) and slow (60 rev.min$^{-1}$) pedal speeds have been shown to be similar despite greater fatigue being evident when pedalling at 140 rev.min$^{-1}$ (Jones et al., 1985). In addition, Cherry et al. (1998) found no difference in blood or muscle metabolites when sprinting on a friction loaded cycle ergometer against different applied resistance, although subjects' performance appeared to recover more quickly following exercise involving fewer muscle actions. No studies have yet considered the hormonal responses to exercise at different pedal speeds yet if the metabolic response to exercise determines the magnitude of the hGH response it is likely that the hGH response to exercise would be unaffected by the applied resistance.

Therefore, the aim of this study was firstly to test the hypothesis that repeated bouts of maximal sprint cycling result in an attenuation of the hGH response whilst secondly studying, for the first time, the effect of sprint cycling at different pedal speeds on hGH release.

## Methods

### Subjects

Ten healthy male volunteers aged 21 to 32 years (24.5 ± 1.1) gave their written informed consent for this study, which had the approval of the Loughborough University Ethical Committee. Body mass ranged from 68.2 to 84.5 kg (77.1 ± 1.8) and height ranged from 174.4 to 186.0 cm (179.1 ± 1.6).

### Equipment

The exercise tests were carried out on a modified friction-loaded cycle ergometer (Monark 864), which was interfaced to a microcomputer (BBC). This allowed instantaneous power output, corrected for flywheel acceleration, to be monitored and recorded accurately. Performance data were averaged over 1 s intervals. Lakomy (1986) has described in detail the equipment used. A restraining harness was also placed around the subjects' waists to prevent them from rising out of the saddle, thereby concentrating movement in the lower limbs. The same harness setting and saddle height were used for each trial. Toe-clips and tape held the subjects' feet securely in the pedals.

**Protocol**

After familiarisation, the subjects arrived in the laboratory after a 4 h fast on two separate occasions, completing one trial on each visit using a crossover design. In each trial the subjects completed two all-out 30 s efforts separated by one hour of passive recovery. During one trial the subjects completed both sprints against an applied resistance equal to 7.5% (75 g.kg$^{-1}$) of their body mass, whereas in the other trial both sprints were completed against a resistance equal to 10% (100 g.kg$^{-1}$) of the subject's body mass. The purpose of this intervention was to manipulate the number of muscle actions through altering the pedal rate in each trial; the 7.5% body mass (FAST) trial would result in a higher average pedal rate than the 10% body mass (SLOW) trial.

Before the first sprint of each trial all subjects completed a standardised sub-maximal warm-up on the cycle ergometer, consisting of 4 min pedalling at 60 W, 30 s pedalling at 80 W and 30 s pedalling at 100 W, with 30 s rest between each intensity. Five min after the warm-up, the subjects performed the first of two maximal 30 s sprints from a rolling start (70 rev.min$^{-1}$ against no resistance) on the cycle ergometer. Subjects were instructed to sprint maximally for the duration of each sprint and were encouraged verbally whilst sprinting.

Following the first sprint subjects remained on the ergometer for 3 min to allow blood samples to be taken. They were then seated in an upright position on a couch (maintaining approximately the same body position as on the ergometer). Subjects remained on the couch and further blood samples were obtained until approximately 55 min after the sprint. Subjects then returned to the cycle ergometer where a further blood sample was taken 60 min after the first sprint. The subjects then performed a second maximal 30 s sprint from a rolling start. Subjects remained on the ergometer for 3 min and were then seated in an upright position on the couch until 60 min after the second sprint for the remainder of the blood samples.

**Blood sampling and analysis**

Venous blood samples were taken via a cannula inserted into an antecubital forearm vein under local anaesthetic (1% lignocaine). Blood samples were taken in a seated position at rest (-10 min) and post warm-up (-4 min) before the first sprint and 10 s, 2, 4, 10, 30 and 60 min after each sprint. The first blood sample was taken at least 15 min after the cannula was inserted. Patency was maintained by displacing the blood contained in the cannula with isotonic saline. Samples were dispensed into three tubes: (i) One portion (4 to 5 ml) was placed into a lithium heparinised tube (Sarstedt LH/5 ml). Blood pH was measured immediately (Radiometer ABL5 pH/blood gas monitor) and thereafter, 20 μl aliquots of blood were removed, deproteinized in 2.5% perchloric acid and stored at -20°C for later determination of blood lactate concentrations (Maughan, 1982). Further aliquots were removed for the measurement of haematocrit by microcentrifugation (Hawksley Ltd.) and haemoglobin concentration (by the cyanmethaemoglobin method) for the calculation of percentage change in plasma volume (Dill and Costill, 1974). (ii) A further 1.5 ml was placed in a calcium heparinised eppendorf tube. This was immediately centrifuged and the plasma removed and stored at -70°C for the determination of ammonia concentration within 48 h (Sigma Diagnostics, kit 171 - C). (iii) The remaining blood (4 to 5 ml) was allowed to clot for 1 h in a plain tube (Sarstedt Serum Z/5 ml). This was then centrifuged at 3°C for 15 min at a speed of 6000 rev.min$^{-1}$ (Burkard Koolspin) and the serum was removed and stored at -70°C for the determination of hGH, insulin and cortisol concentrations. Serum hGH, insulin and cortisol were measured by routine ELISA. The hGH assay (Medigenix HGH-Biosource) had a sensitivity of 0.11 mU.l$^{-1}$, an intra-assay coefficient of variation (cv) of 2.1-3.6% and an inter-assay cv of 6.8-7.1%. The insulin assay (Medgenix insulin-Biosource) had a sensitivity of 0.15 mU.l$^{-1}$, an intra-assay cv of 3.0-5.3% and an inter-assay cv of 5.6-9.8%. The sensitivity of the cortisol assay (Milenia, DPC cortisol) was 8.3 nmol.l$^{-1}$ with an intra-assay cv of 5.9-8.0% and an inter-assay cv of 8.3-9.0%.

**Statistical analysis**

A two-way analysis of variance was used to ascertain whether there were any differences in performance between the FAST and SLOW trials (main effect - trial) and between the first and second sprints in each trial (main effect - sprint). A three-

way analysis of variance was used to discover any differences in biochemical responses between FAST and SLOW trials (main effect - trial), between first and second sprints in each trial (main effect - sprint) and the response of each subject with respect to time (main effect - time). A two-way analysis of variance was used to find any differences in integrated hGH concentrations (area under the curve - AUC) and peak values of hGH between trials and sprints. A Pearson product moment correlation was also employed. Statistical significance was accepted at the $P < 0.05$ level. All results are expressed as mean ±SEM.

## Results

### Performance

There was no difference in peak (PPO) or mean (MPO) power output in the FAST and SLOW trials, however PPO was found to be greater in sprint 1 than sprint 2 ($P$ <0.05). Similarly, peak pedal revolutions (PPR) were found to be greater in sprint 1 than in sprint 2. Both PPR and mean pedal revolutions (MPR) were greater in the FAST trial when compared with the SLOW trial ($P$ <0.05). Fatigue, as measured by drop in power from PPO to power output at the end of the sprint, was found to be greater in sprint 1 than sprint 2 (Table 5.1).

Table 5.1. Peak (PPO) and mean (MPO) power output, peak (PPR) and mean (MPR) pedal rate and Fatigue index for sprints1 and 2 in the FAST and SLOW trials.

|  | FAST | | SLOW | | |
|---|---|---|---|---|---|
|  | Sprint 1 | Sprint 2 | Sprint 1 | Sprint 2 | |
| PPO (W) | 1178 ± 51 | 1132 ± 44 | 1124 ± 59 | 1099 ± 70 | * |
| MPO (W) | 654 ± 21 | 660 ± 22 | 707 ± 32 | 702 ± 34 | |
| PPR (rev.min$^{-1}$) | 159 ± 4 | 154 ± 5 | 130 ± 5 | 126 ± 5 | † ‡ |
| MPR (rev.min$^{-1}$) | 117 ± 3 | 118 ± 3 | 95 ± 4 | 95 ± 4 | † |
| Fatigue Index (%) | 61 ± 2 | 59 ± 2 | 56 ± 3 | 54 ± 3 | ‡ |

* $P$<0.05 main effect sprint

† $P$<0.01 main effect trial

‡ $P$<0.01 main effect sprint

**Metabolic responses to cycle ergometer sprinting**

The blood lactate, blood pH and plasma ammonia responses are shown in Figures 5.1, 5.2 and 5.3. Blood lactate and pH responses did not differ between trials (mean peak blood lactate, FAST 9.69 ± 0.63 compared with SLOW 9.86 ± 0.58 mmol.l$^{-1}$; mean lowest pH, FAST 7.08 ± 0.02 compared with SLOW 7.06 ± 0.02) or sprints (mean peak blood lactate, sprint 1 10.12 ± 0.52 compared with sprint 2 10.40 ± 0.62 mmol.l$^{-1}$; mean lowest pH, sprint 1 7.07 ± 0.02 compared with sprint 2 7.08 ± 0.02). Plasma ammonia demonstrated a sprint-time interaction ($P$ <0.05) reflecting higher peak ammonia concentrations and faster recovery following sprint 1 than sprint 2, and a trial-time interaction ($P$ <0.05) reflecting higher peak ammonia and faster recovery in the FAST trial than in the SLOW trial.

Figure 5.1. Mean blood lactate concentrations at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints for the FAST and SLOW trials. Sprint main effect n.s., trial main effect n.s., time main effect $P<0.01$, sprint-trial interaction effect n.s., sprint-time interaction effect n.s., trial-time interaction effect n.s., sprint-trial-time interaction effect n.s.

Figure 5.2. Mean blood pH at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints for the FAST and SLOW trials. Sprint main effect n.s., trial main effect n.s., time main effect $P<0.01$, sprint-trial interaction effect n.s., sprint-time interaction effect n.s., trial-time interaction effect n.s., sprint-trial-time interaction effect n.s.

Figure 5.3. Mean plasma ammonia concentrations at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints for the FAST and SLOW trials. Sprint main effect n.s., trial main effect n.s., time main effect $P<0.01$, sprint-trial interaction effect n.s., sprint-time interaction effect n.s., trial-time interaction effect $P<0.05$, sprint-trial-time interaction effect n.s.
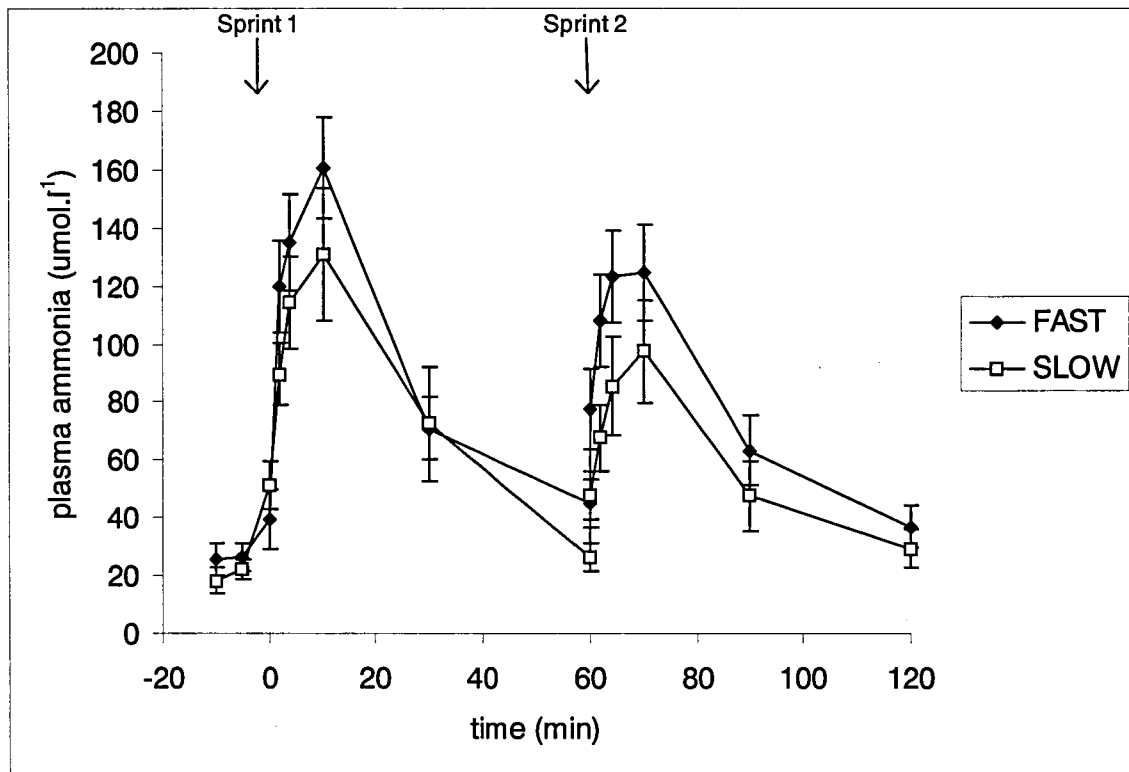
**Hormone responses to cycle ergometer sprinting**

Figure 5.4 shows the mean serum hGH response to repeated maximal 30-s cycle ergometer sprints. The first sprint resulted in a distinct hGH pulse with highest measured mean concentrations of 40.8 ± 8.2 and 20.8 ± 6.1 mU.l$^{-1}$ 30 min after the sprint in the FAST and SLOW trials respectively. Serum hGH was still elevated 60 min after the first sprint whereas there was no hGH pulse after the second sprint. Serum hGH did not show a trial effect ($P$ = 0.08), but there was a sprint effect ($P$ <0.05), a time effect ($P$ <0.05) and a sprint-time interaction ($P$ <0.05). The peak hGH response for sprint 1 (mean of individual peaks) during the FAST trial was more than twice that during the SLOW trial (mean peak hGH, FAST 37.7 ± 6.0 compared with SLOW 17.6 ± 3.7 mU.l$^{-1}$, $P$ <0.05).

Mean integrated hGH concentrations (AUC) for the 1 h period following each sprint demonstrated a difference between sprints (mean hGH AUC, sprint 1 1315 ± 243 compared with sprint 2 729 ± 146 min.mU$^{-1}$.l$^{-1}$, $P$ <0.01). There was also a trend for a lower hGH AUC in the SLOW trial, with nine of the ten subjects following this pattern (mean hGH AUC, FAST 1381 ± 231 compared with SLOW 663 ± 162 min.mU$^{-1}$.l$^{-1}$, $P$ = 0.06). The insulin (Figure 5.6) and cortisol (Figure 5.7) responses were similar in the two trials, although the cortisol response to the first sprint was different to that of the second ($P$ <0.05).

Correlation analysis showed a significant correlation between MPR and hGH AUC in both sprints (sprint 1, $r$ = 0.59, $P$<0.01; sprint 2, $r$ = 0.61, $P$<0.01). A significant correlation was also found between PPR and hGH AUC (sprint 1, $r$ = 0.48, $P$ <0.05; sprint 2, $r$ = 0.58, $P$ <0.01), but not between MPO and hGH AUC (sprint 1, $r$ = 0.31, n.s.; sprint 2, $r$ = 0.12, n.s.). PPO and hGH AUC showed a significant correlation during the second sprint, but not the first (sprint 1, $r$ = 0.41, n.s.; sprint 2, $r$ = 0.47, $P$ <0.05). When considering metabolites, integrated lactate and ammonia concentrations showed little correlation with hGH AUC (Lactate, sprint 1, $r$ = 0.08, n.s.; sprint 2, $r$ = 0.08, n.s.; Ammonia, sprint 1, $r$ = - 0.04, n.s.; sprint 2, $r$ = 0.08, n.s.)

Figure 5.4. Mean serum growth hormone concentrations at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints for the FAST and SLOW trials. Sprint main effect $P<0.05$, trial main effect $P=0.08$ (n.s.), time main effect $P<0.01$, sprint-trial interaction effect n.s., sprint-time interaction effect $P<0.01$, trial-time interaction effect n.s., sprint-trial-time interaction effect n.s.
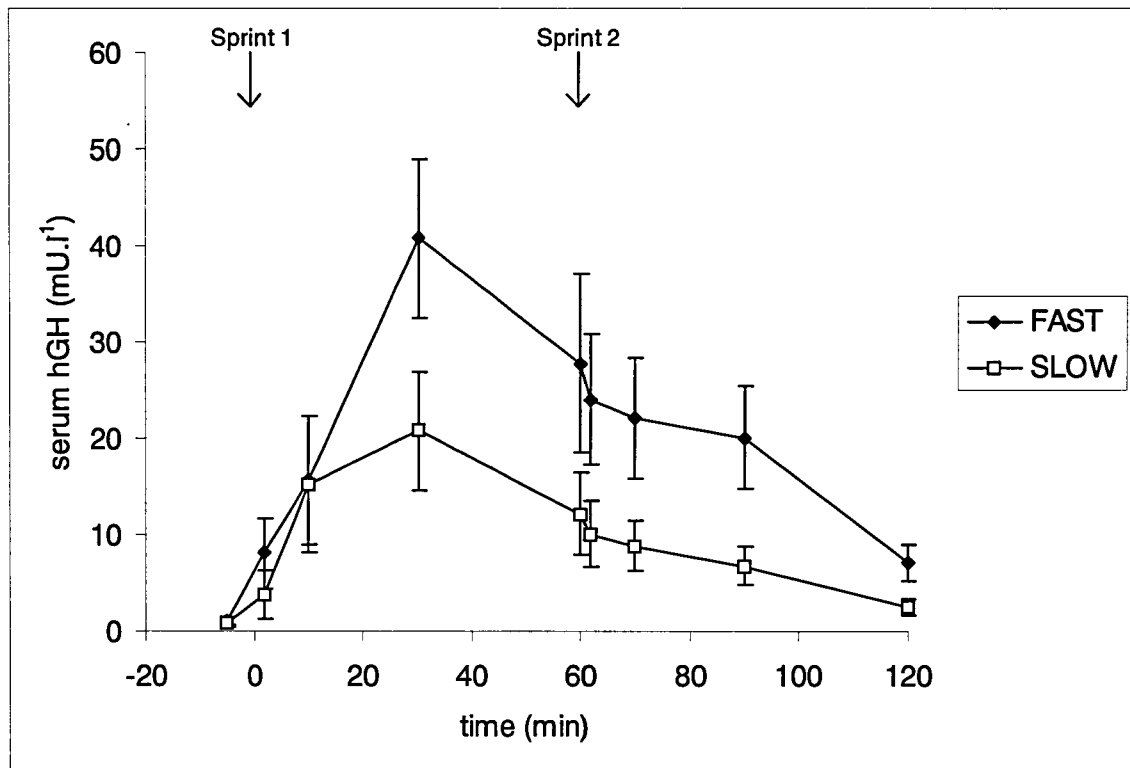
Figure 5.5. Mean serum insulin concentrations at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints for the FAST and SLOW trials. Sprint main effect n.s., trial main effect n.s., time main effect n.s., sprint-trial interaction effect n.s., sprint-time interaction effect n.s., trial-time interaction effect n.s., sprint-trial-time interaction effect n.s.
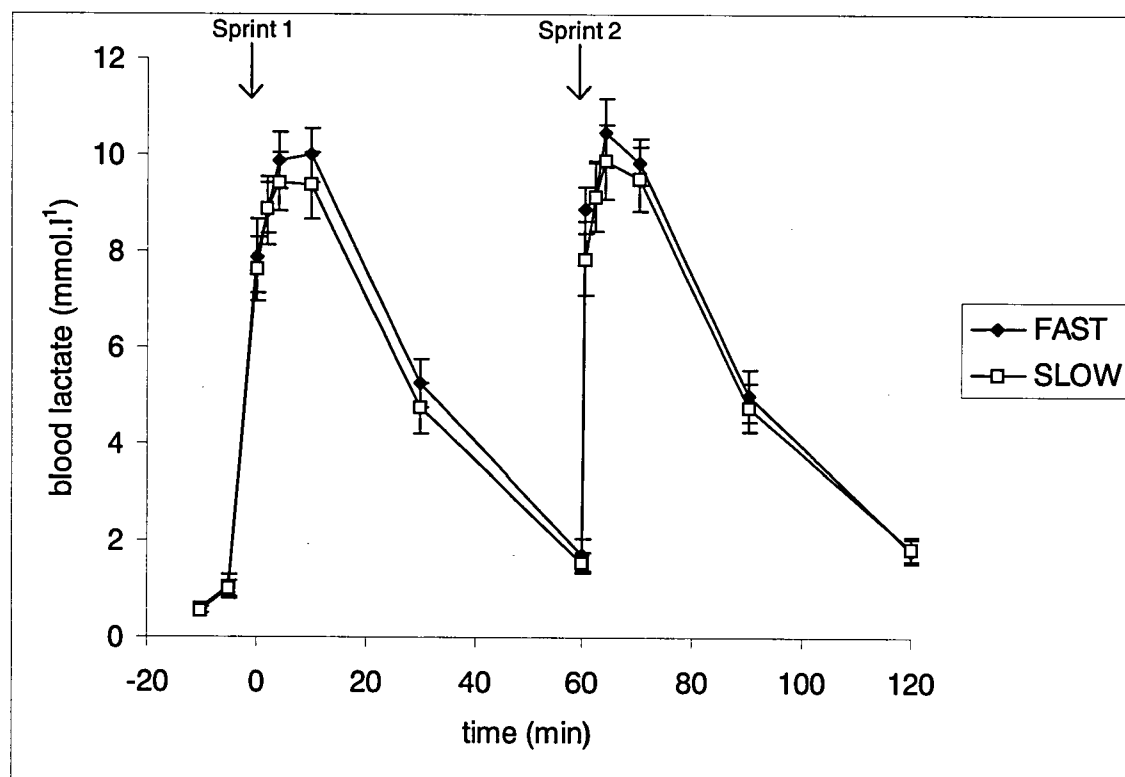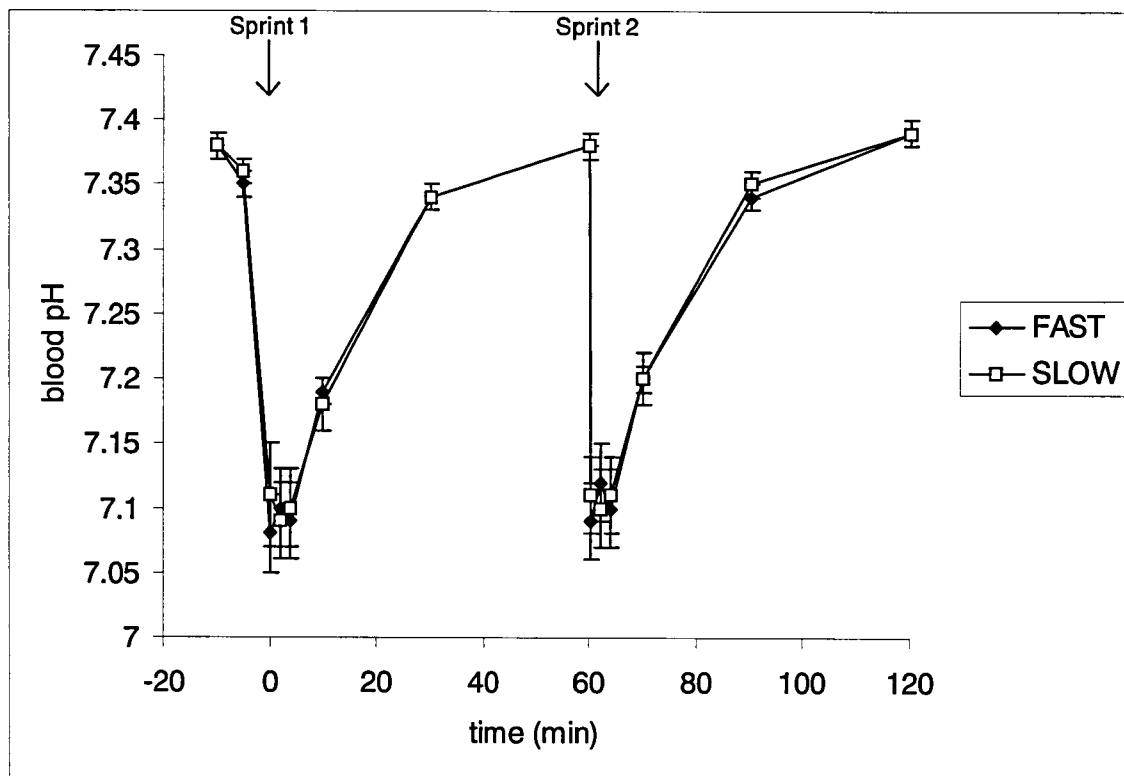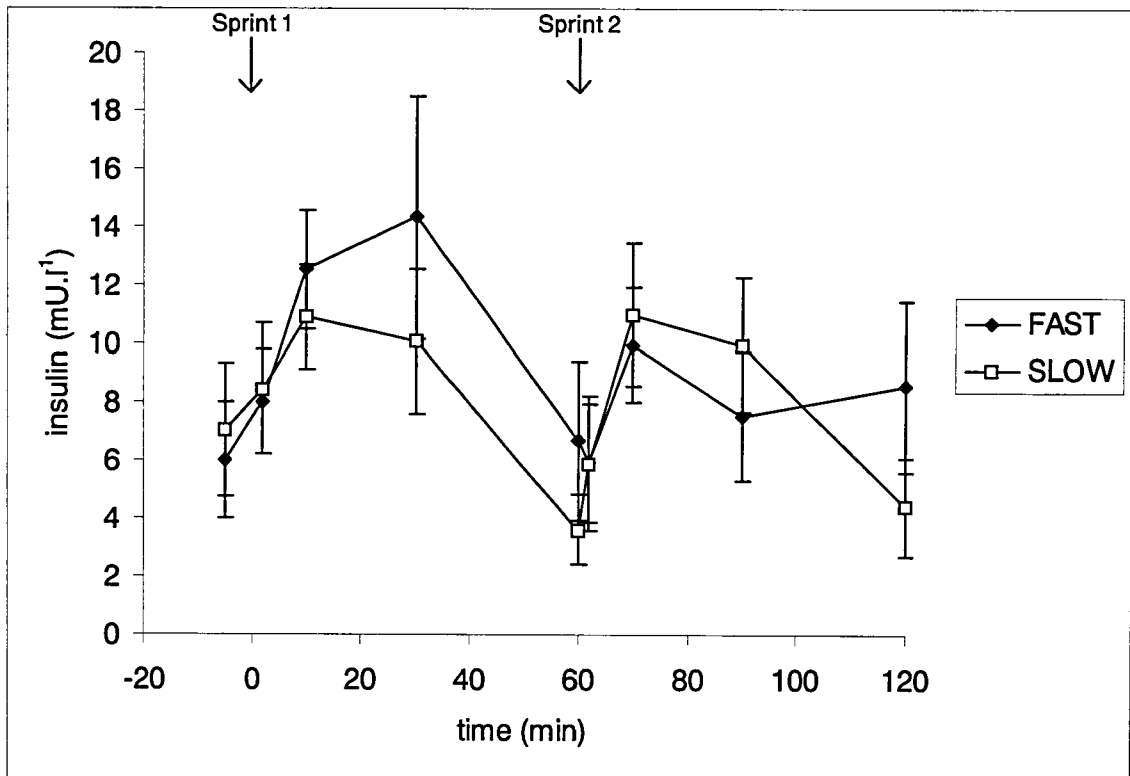
Figure 5.6. Mean serum cortisol concentrations at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints for the FAST and SLOW trials. Sprint main effect $P$ <0.05, trial main effect n.s., time main effect n.s., sprint-trial interaction effect n.s., sprint-time interaction effect n.s., trial-time interaction effect n.s., sprint-trial-time interaction effect n.s.
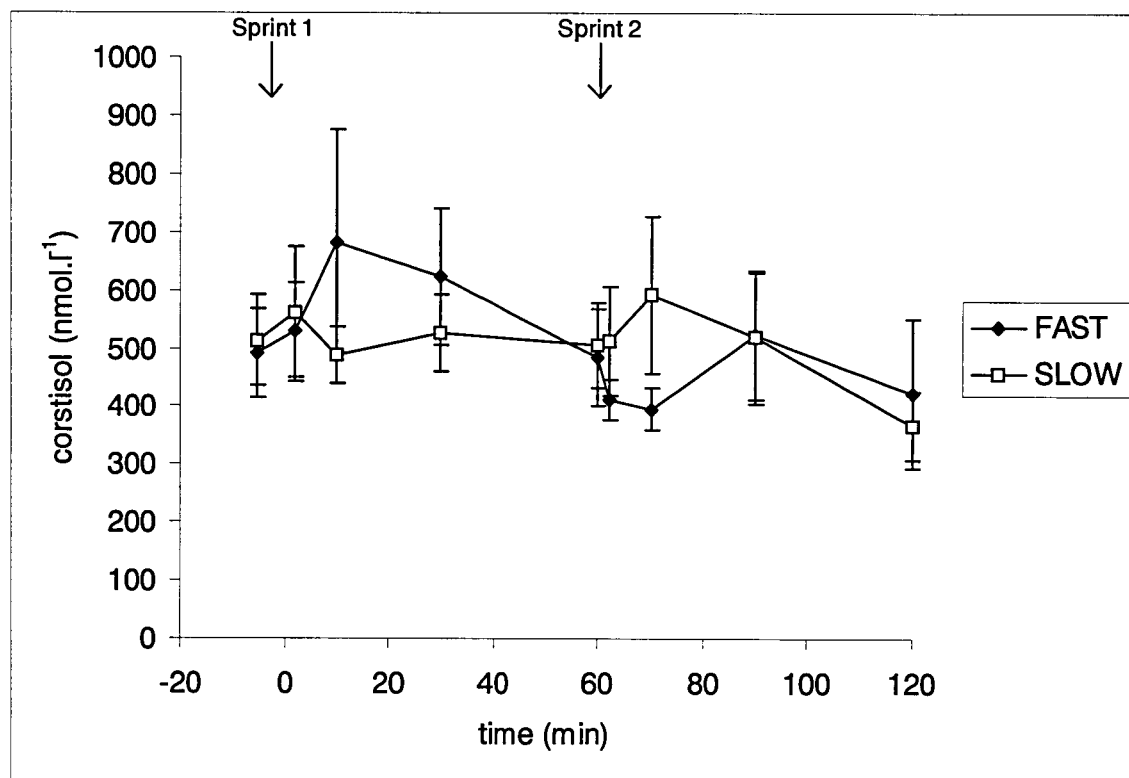
**Changes in plasma volume**

The estimated changes in mean plasma volume at 2 min post-exercise were -17.0 ± 0.6% and -13.7 ± 0.8 % for sprint 1 and sprint 2 and -15.2 ± 0.9% and -15.4 ± 0.7% for FAST and SLOW trials (sprint 1 compared to sprint 2, n.s.; FAST compared to SLOW, n.s.). All values had returned to baseline by 30 min post-exercise.

## Discussion

This study demonstrates that a single 30 s sprint is a potent stimulus for hGH release. However, if a similar sprint is completed 1 h later there is no hGH response. Furthermore, cycling at fast pedal speeds results in greater hGH release than cycling at slow pedal speeds ($P = 0.06$).

A single 30 s treadmill sprint has been shown to stimulate a near maximal hGH response (Nevill et al., 1996b). The results of the present study show that 30 s of maximal cycle ergometer exercise elicits a marked hGH response, although hGH responses in this study were not as large as those found by Nevill et al. (1996b). This may reflect the fact that sprint cycling, with movement concentrated in the lower limbs, elicits a different hGH response when compared with the treadmill sprinting employed in the study by Nevill et al. (1996b). This is supported by Gordon et al. (1994) who showed that a 90 s all-out effort on a cycle ergometer elicited a similar hGH response to that seen in the present study.

One hour after the first sprint in each trial blood metabolites had returned to near baseline. A second sprint completed at this time resulted in a metabolic response over 60 min similar to that after the first sprint. In contrast, after 60 min of recovery from a single sprint, hGH had not returned to baseline and, furthermore, a second sprint completed at this time point did not elicit a hGH response. This finding is in agreement with studies demonstrating attenuation of spontaneous and GHRH-stimulated GH secretion after exogenous GH administration in rats (Lanzi and Tannenbaum, 1992a and 1992b). Studies in humans have also demonstrated a progressively decreasing hGH response with repeated GHRH administration (Ghigo et al., 1991).

Kanaley et al. (1997) demonstrated an augmented hGH response to repeated bouts of 30 min exercise at 70% $\dot{V}O_2$max separated by 60 min of recovery suggesting that exercise provides sufficient stimulus to overcome the attenuation of the hGH response shown using pharmacological interventions. However, Cappon et al. (1994) found that the hGH response to exercise is attenuated by prior exercise. These findings are in agreement with those of the present study where a second bout of exercise did not elicit a hGH response, demonstrating that the suppression of the hGH response with repeated stimuli is not limited to studies employing pharmacological stimuli, but that it is also a physiological response. The discrepancy between the findings of the present study and those of Kanaley et al. (1997) may be explained by the nature of the stimulus for hGH release, since submaximal exercise is known to induce a lesser hGH response than that induced by sprint exercise. However, Ghigo et al. (1991), using GHRH as a stimulus, induced an initial hGH response similar to that found by Kanaley et al. (1997), and yet demonstrated an attenuated hGH response to a second stimulus.

The mechanism by which the hGH response is attenuated is unclear. The depletion of pituitary stores as a result of the first bout is unlikely since Kanaley et al. (1997) found an augmented hGH response with repeated submaximal exercise. Furthermore, Jaffe et al. (1993) suggested that pituitary hGH content far exceeds the amount of hGH released in their study and yet they demonstrated a suppression of the hGH response to repeated GHRH administration.

Inhibition of hGH by elevated levels of insulin-like growth factor (IGF-I) is a possible explanation for the lack of a hGH response to the second sprint. Jaffe et al. (1998) demonstrated that infusion of rhIGF-I suppressed pulsatile and GHRH-stimulated hGH secretion in male subjects. However, IGF-I concentration was not measured in the present study, and it is not known, therefore, whether IGF-I was elevated at the onset of the second sprint. Cappon et al. (1994) found that acute, exercise stimulated IGF-I levels had returned to pre-exercise levels within 60 min of recovery. If this occurred in the present study, elevated IGF-I was unlikely to be responsible for the suppression of the hGH response.

Growth hormone can directly inhibit its own release, possibly at the pituitary gland (Pontiroli et al., 1991). Since, in the present study, hGH was still elevated at the start of the second sprint, hGH autoinhibition may be responsible for preventing a hGH response to the second sprint.

Alternatively, it has been suggested that this auto-negative feedback occurs at the level of the hypothalamus, mediated by an increase in somatostatin release and/or a decrease in the release of GHRH. Lanzi and Tannenbaum (1992a) demonstrated that the immunoneutralization of somatostatin prevented the attenuation of spontaneous GH release after GH pre-treatment in rats, thus obtaining strong support for a role for somatostatin in GH autoregulation. In a further study Lanzi and Tannenbaum (1992b) also demonstrated a role for somatostatin in the attenuation of exogenous GHRH induced GH release. The understanding that GH receptor mRNA is colocalized in somatostatin neurons in the rat hypothalamus (Burton et al., 1991) further supports these findings. Peripheral measurements of GHRH and somatostatin would probably not reflect hypothalamic secretion but studies employing direct hypophysial-portal sampling in nonhuman species suggest that hypothalamic discharges of GHRH regulate GH pulses (Frohman et al., 1990) whilst somatostatin may modulate the action of GHRH (Thomas et al., 1991).

It has been suggested that the metabolic response to a 30 s sprint has a part to play in hGH release (Nevill et al. 1996b). However, the results of the present study do not support this contention since there was a poor correlation between plasma ammonia and serum hGH concentration and blood lactate and serum hGH concentration. When comparing FAST and SLOW trials the pH and lactate responses were similar between trials and between sprints although the highest measured ammonia concentrations were greater in the FAST than the SLOW trial. Jones et al. (1985) demonstrated that high and low pedalling rates on an isokinetic ergometer induced similar metabolic responses in mixed muscle. Previous work in this laboratory (Cherry et al. 1998) also showed that pedalling rate during sprinting on a friction loaded cycle ergometer has little effect on the magnitude of the subsequent metabolic response in muscle and blood. The results of the present study suggest that the hGH response to a single 30 s cycle ergometer sprint might be greater when pedalling at faster speeds (Figure 5.4), although this trend was not found to be significant ($P = 0.06$). This observation

indicates that metabolic conditions in muscle and blood might not determine the magnitude of the hGH response to all-out sprinting, but that there is another mechanism for hGH release.

Kozlowski et al. (1983) found no causal relationship between blood metabolites and hGH but suggested that muscle metabolic receptors may play a role in hGH release. The results of the present study do not support this view. However, activity in motor centres may directly stimulate pituitary hormone secretion, including hGH, during exercise (Kozlowski et al., 1983; Kjaer et al., 1987; Kjaer et al., 1989; Kjaer et al., 1996a). It is possible that in the present study there was greater central motor activity when sprinting at faster pedal speeds, resulting in a greater hGH response. However, due to the maximal nature of the exercise it is unlikely that there was any difference in motor centre activity in the FAST and SLOW trials.

Other work by Kjaer et al. (1996b) suggested that humoral feedback mechanisms and autonomic nervous reflexes, without efferent motor centre activity, may exert redundant control of pituitary hormonal responses. Gosselink et al. (1998) found evidence of a proprioceptive mechanism for the regulation of bioassayable hGH but not of immunoassayable hGH in rats. However, they also showed that stimulation of a large muscle mass resulted in elevated immunoassayable hGH. It was suggested that this might be due to metabolic perturbations although metabolites were not measured. In the present study there was a poor correlation between metabolites and hormones but a significant correlation between PPR and serum hGH and MPR and serum hGH, with smaller hGH responses elicited by pedalling at slower speeds. It is possible, therefore, that proprioceptive feedback plays an important role in the regulation of hGH during sprint exercise.

In conclusion, this study has demonstrated that a single 30 s cycle ergometer sprint elicits a marked hGH response, but that a similar sprint completed 60 min later does not result in an increase in hGH concentrations. Human GH autoinhibition may be responsible for the suppression of the hGH response to the second sprint, possibly mediated by an increase in somatostatin. The present study has also shown that cycle ergometer sprinting at faster pedal speeds elicits a greater hGH response than pedalling at slower pedal speeds, despite a similar metabolic response. This

highlights the potential importance of a proprioceptive mechanism for the regulation of hGH release during sprint exercise.

CHAPTER 6

THE EFFECT OF SIX WEEKS OF SPRINT TRAINING ON THE GROWTH
HORMONE RESPONSE TO REPEATED MAXIMAL CYCLE ERGOMETER
EXERCISE

**Introduction**

A number of studies have identified an increase in human growth hormone (hGH)
concentration in response to acute exercise, although only a few have considered high
intensity or sprint exercise (Gordon et al., 1994; Nevill et al., 1996b). The
mechanisms controlling the magnitude of the GH response to exercise are not well
understood but it has been suggested that intensity and duration of exercise (Sutton et
al., 1976), metabolic responses to the exercise bout (Karagiorgos et al., 1979; Gordon
et al., 1994; Chwalbinska-Moneta et al., 1996) and training state may have a role to
play.

The results of Chapters 4 and 5 show that a single 30 s cycle ergometer sprint elicits a
distinct increase in serum hGH concentration in healthy young men with levels
remaining elevated for at least 60 min post-exercise. These results support the
findings of Nevill et al. (1996b) who demonstrated a near maximal GH response to a
single 30 s treadmill sprint when compared with the results from pharmacological
intervention studies, although the hGH response to sprint cycling was smaller than the
response to treadmill sprinting.

Some studies have considered the effect of training state on the hGH response to an
acute bout of exercise. There is little agreement between these studies as to whether
exercise training increases (Bunt et al., 1986; Bonifazi et al., 1998; McCall et al.,
1999), decreases (Bloom et al., 1976; Weltman et al., 1997) or has no effect on
(Kraemer et al., 1998) the hGH response to a single exercise bout. However only
three of these studies (Bloom et al., 1976; Bunt et al., 1986; McCall et al., 1999)
compared the response of trained individuals with untrained control subjects and no
study has considered the effect of a supervised sprint training regimen on the hGH
response to a single bout of sprint exercise. In addition, Nevill et al. (1996b) found

that sprint-trained individuals had a larger hGH response to a single 30 s sprint than endurance-trained individuals. It was suggested that these results could be attributed to changes in the metabolic responses to, and the power output achieved during, a sprint. Both of these factors may be expected to be altered by a short period of sprint training (Nevill et al., 1989), and it is likely, therefore, that hGH secretion will be augmented following six weeks of sprint training.

Cappon et al. (1994) found that, in humans, repeated heavy 10 min exercise bouts resulted in progressive attenuation of the hGH response to the exercise. The results of Chapter 5 also demonstrate an attenuated hGH response to repeated 30 s cycle ergometer sprinting, however, the effect of a period of sprint training on the hGH response to repeated sprint exercise has not yet been considered.

It has been demonstrated that hGH can directly inhibit its own release either at the level of the pituitary gland (Pontiroli et al., 1991) or the hypothalamus (Lanzi and Tannenbaum, 1992a; Lanzi and Tannenbaum, 1992b). These findings, and the understanding that hGH concentrations remain elevated for at least 60 min following a single 30 s sprint (Nevill et al., 1996b; Chapters 4 and 5) suggest that, if hGH autoregulation is the dominant mechanism for the attenuation of the hGH response to repeated exercise, the proposed increases in exercise-induced hGH secretion following training will ensure that attenuation of the hGH response will persist.

Therefore, the purpose of the present study was to test two hypotheses. First, that six weeks of participation in a prescribed sprint training programme will improve sprint performance and modify the metabolic response to exercise, and that these changes will contribute to greater exercise-induced hGH secretion. Second, that training will not modify the attenuation of the hGH response to repeated bouts of cycle ergometer sprinting.

## Methods

### Subjects

Sixteen healthy male volunteers gave their written informed consent for this study which had the approval of the Loughborough University Ethical Committee. Volunteers were randomly assigned to either a training group (n=8), who were

prescribed a sprint training program, or a control group (n=8), who continued with regular daily activities. Table 6.1 summarises the physical characteristics of the subjects in each of the groups.

Table 6.1. Physical characteristics of the subjects in the training and control groups.

| | Age (years) | | Height (cm) | | Mass (kg) | | | |
| | | | | | Pre- | | Post- | |
| | mean | SEM | mean | SEM | mean | SEM | mean | SEM |
| Training | 24.0 | 1.1 | 179.2 | 2.6 | 78.5 | 2.6 | 77.7 | 2.5 |
| Control | 25.4 | 1.4 | 178.3 | 1.2 | 80.3 | 2.0 | 80.2 | 1.9 |

## Equipment

The exercise tests were carried out on a modified friction-loaded cycle ergometer (Monark 864), which was interfaced to a microcomputer (BBC). This allowed instantaneous power output, corrected for flywheel acceleration, to be monitored and recorded accurately. Performance data were averaged over 1 s intervals. Lakomy (1986) has described the equipment used in detail. A restraining harness was also placed around the subjects' waists in order to prevent them from rising out of the saddle, thereby concentrating movement in the lower limbs. The same harness setting and saddle height were used for each trial. Toe-clips and tape held the subjects' feet securely in the pedals.

## Protocol

Before any main trials were completed subjects carried out at least three familiarisation sessions where they practised sprint starts and became accustomed with all-out sprinting on the cycle ergometer.

Each subject completed two main trials, one before the training period and one following the training period. The protocol for these trials was identical. Subjects were asked to arrive at the laboratory in a post-absorptive state having refrained from heavy exercise for a day prior to each trial.

The subjects then completed a standardised sub-maximal warm-up consisting of 4 min pedalling at 60W, 30 s pedalling at 80W and 30 s pedalling at 100W. Subjects then rested on the ergometer for 5 min before completing an all-out 30 s sprint from a stationary start against an applied resistance equivalent to 7.5% ($75N.kN^{-1}$) of their body weight. Subjects then rested whilst seated on a couch (maintaining approximately the same body position as on the ergometer) for 60 min before repeating the same warm-up routine followed by a second all-out 30 s sprint, against the same applied resistance, 5 min later. Subjects then rested on a couch for a further 60 min. Subjects were instructed to sprint maximally for the duration of each sprint and were encouraged verbally whilst sprinting.

**Blood sampling and analysis**

Venous blood samples were taken via a cannula inserted into an antecubital forearm vein under local anaesthetic (1% lignocaine). Blood samples were taken in a seated position at rest (-10 min) and post warm-up (-4 min) prior to the first sprint and 5, 10, 20, 30, 40 and 60 min after each sprint. The first blood sample was taken at least 15 min after the cannula was inserted. Patency was maintained by displacing the blood contained in the cannula with isotonic saline. Samples were dispensed into three tubes: (i) One portion (4/5 ml) was placed into a lithium heparinised tube (Sarstedt LH/5ml). Blood pH was measured immediately (Radiometer ABL5 pH/blood gas monitor) and thereafter, 20 μl aliquots of blood were removed, deproteinized in 2.5% perchloric acid and stored at -20°C for later determination of blood lactate concentrations (Maughan, 1982). Further aliquots were removed for the measurement of haematocrit by microcentrifugation (Hawksley Ltd.) and haemoglobin concentration (by the cyanmethaemoglobin method) for the calculation of percentage change in plasma volume (Dill and Costill, 1974). (ii) A further 1.5 ml was placed in a calcium heparinised eppendorf tube. This was immediately centrifuged and the plasma removed and stored at -70°C for the determination of ammonia concentration within 48 h (Sigma Diagnostics, kit 171 - C). (iii) The remaining blood (4/5 ml) was allowed to clot for 1 h in a plain tube (Sarstedt Serum Z/5 ml). This was then centrifuged at 3°C for 15 min at a speed of 6000 $rev.min^{-1}$ (Burkard Koolspin) and the serum was removed and stored at -70°C for the determination of GH, insulin and cortisol concentrations. Serum GH and cortisol were measured by routine ELISA.

The GH assay (Medigenix HGH-Biosource) had a sensitivity of 0.11 mU.l$^{-1}$, an intra-assay coefficient of variation (cv) of 2.1-3.6% and an inter-assay cv of 6.8-7.1%. The sensitivity of the cortisol assay (Milenia, DPC cortisol) was 8.3 nmol.l$^{-1}$ with an intra-assay cv of 5.9-8.0% and an inter-assay cv of 8.3-9.0%.

**Training**

After the first main trial had been completed subjects completed a six week training period. During this time 9 subjects completed three supervised speed/speed-endurance sessions per week in the laboratory (Training group) whilst 9 subjects did not have to report to the laboratory. Both groups were asked to continue with normal activity and training levels throughout the training period so that the training group completed the sprint-cycling training in addition to normal activity whilst the control group simply maintained normal activity levels. Table 6.2 summarises the training sessions completed by the training group. Subjects in the training group completed three supervised sessions per week for the six week training period. In most cases supervised sessions were separated by at least one day. The first and third session of each week was prescribed as a "speed session" and the second session was a "speed endurance session". Every session started with a standard warm-up consisting of a 5 min period of cycling against an applied resistance of 1 kg, 2 min cycling at 60 W and 30 s at 90 W followed by 5 min of stretching.

Speed sessions were designed to improve speed and strength particularly over the early part of a 30 s sprint. The early sessions (Weeks 1 and 2) were intended to facilitate the development of good technique and start to develop strength. Sessions against the lighter resistance of 4% (40 N.kN$^{-1}$) body weight were designed for the development of pure speed whilst those against the heavier resistance of 11% (110N.kN$^{-1}$) body weight were designed to develop strength. The middle sessions (Weeks 3 and 4) were designed to build on speed and strength improvements and increase the intensity of the training sessions by increasing the number of sprints completed and reducing recovery time between sprints. The final sessions (Weeks 5 and 6) used the same resistance of 7.5% (75N.kN$^{-1}$) as that of the main trial. During these sessions the number of sprints completed per session were reduced and recovery between sprints increased.

The training group completed one speed endurance session per week. The purpose of these sessions was to facilitate the maintenance of a high pedal speed throughout a 30 s sprint, thereby increasing the total amount of work performed. During these sessions subjects were asked to perform paced efforts rather than maximal sprints. In each session subjects were asked to perform two sets of 4 paced efforts achieving 60-70-70-60% of the work done in their 30 s sprint. Pedal speed was set at 110% of mean pedal rate achieved during the subjects' 30 s sprints. Applied resistance was then calculated at that pedal speed in order to achieve 60% and 70% of work done in their sprints. In weeks 3 and 5 subjects completed a maximal 30 s sprint in place of the first set of 4 paced efforts. If any improvement in work done compared with the pre-training trial, the 60% and 70% values were recalculated in order that absolute work done during the training sessions was increased.

Table 6.2. Summary of the 18 sessions completed by the training group over the 6 week period of training.

| SPEED SESSIONS | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEK | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| SESSION | 1 | 3 | 4 | 6 | 7 | 9 | 10 | 12 | 13 | 15 | 16 | 18 |
| LOAD | 4% | 11% | 4% | 11% | 4% | 11% | 4% | 11% | 4% | 11% | 4% | 11% |
| SPRINTS | 2 sets of 8 X 6 s | 2 sets of 8 X 6 s | 2 sets of 8 X 6 s | 2 sets of 8 X 6 s | 2 sets of 10 X 6 s | 2 sets of 10 X 6 s | 2 sets of 10 X 6 s | 2 sets of 10 X 6 s | 2 sets of 10 X 6 s | 2 sets of 10 X 6 s | 2 sets of 8 X 6 s | 2 sets of 8 X 6 s |
| RECOVERY | | | | | | | | | | | | |
| Between sprints | 60 s | 60 s | 50 s | 50 s | 45 s | 45 s | 30 s | 30 s | 60 s | 60 s | 90 s | 90 s |
| Between sets | 120 s | 120 s | 90 s | 90 s | 120 s | 120 s | 90 s | 90 s | 120 s | 120 s | 180 s | 180 s |

| SPEED ENDURANCE SESSIONS | | | | | | |
|---|---|---|---|---|---|---|
| WEEK | 1 | 2 | 3 | 4 | 5 | 6 |
| SESSION | 2 | 5 | 8* | 11 | 14* | 17 |
| EFFORTS | 2 sets of 4 X 30 s | 2 sets of 4 X 30 s | 1 set of 4 X 30 s | 2 sets of 4 X 30 s | 1 set of 4 X 30 s | 2 sets of 4 X 30 s |
| RECOVERY | | | | | | |
| Between efforts | 120 s | 90 s | 75 s | 60 s | 75 s | 120 s |
| Between sets | 300 s | 300 s | - | 300 s | - | 300 s |

## Statistical analysis

A three-way analysis of variance with repeated measures was used to ascertain whether there were any differences in performance between Training and Control groups (main effect – group), between pre- and post-training (main effect – training) and between the first and second sprints in each trial (main effect – sprint). A four-way analysis of variance with repeated measures was also used to discover any differences in biochemical responses between Training and Control groups (main

effect – group), between pre- and post-training (main effect – training), between the first and second sprints in each trial (main effect – sprint) and the response of each subject with respect to time (main effect – time). Statistical significance was accepted at the $P < 0.05$ level. All results are expressed as mean ±SEM.

## Results

### Performance

A summary of the performance variables pre- and post-training in the TR and CON groups is shown in Table 6.3. Peak power output (PPO) and peak power output corrected to the subjects' body mass (PPO-corr) displayed no significant differences between Sprint 1 and Sprint 2. A group-training interaction effect was found in PPO ($P<0.05$) and PPO-corr ($P<0.05$) with a 6% improvement in PPO (1385±56 W to 1468±51 W) in the TR group after training and a 7% improvement in PPO-corr (17.9±0.5 W.kg$^{-1}$ to 19.1±0.5 W.kg$^{-1}$), while in the CON group PPO and PPO-corr fell by 6% over the training period.

Mean power output (MPO) and mean power output corrected to the subjects' body mass (MPO-corr) were greater in Sprint 2 than Sprint 1 ($P<0.05$). A group-training interaction effect was also found in MPO ($P<0.05$) and MPO-corr ($P<0.05$) with a 5% improvement in MPO (665±27 W to 696±21 W) in the TR group after training and a 6% improvement in MPO-corr (8.6±0.2 W.kg$^{-1}$ to 9.1±0.1 W.kg$^{-1}$). In the CON group MPO and MPO-corr fell by 6% over the training period.

Peak pedal rate (PPR) demonstrated a group-training interaction effect ($P<0.05$) and a group-training-sprint interaction effect ($P<0.05$). PPR was 2% faster in the TR group after training (161±4 rev.min$^{-1}$ to 165±2 rev.min$^{-1}$) whereas PPR in the CON group was 3% slower after the training period.

Mean pedal rate (MPR) was faster in Sprint 2 than Sprint 1 ($P<0.05$). There was also a group-training interaction effect ($P<0.05$) with a 5% increase in MPR in the TR group (118±3 rev.min$^{-1}$ to 125±1 rev.min$^{-1}$) after training and a 5% decline in MPR in the CON group after the training period. Fatigue index showed no significant changes in either the TR or the Con group over the training period.

Table 6.3. Peak power output (PPO), PPO corrected for each subject's body mass (PPO-corr), mean power output (MPO), MPO corrected for each subject's body mass (MPO-corr), peak (PPR) and mean (MPR) pedal revolutions and fatigue index for the training and control groups before and after the 6 week training period. [a] $P<0.05$ group-training interaction effect, [b] $P<0.05$ group-training-sprint interaction effect.

| | PRE- | | | | POST- | | | | |
| | Training | | Control | | Training | | Control | | |
| | Sprint 1 | Sprint2 | Sprint 1 | Sprint2 | Sprint 1 | Sprint2 | Sprint 1 | Sprint2 | |
|---|---|---|---|---|---|---|---|---|---|
| PPO (W) | 1395(83) | 1376(81) | 1398(91) | 1390(96) | 1470(73) | 1465(76) | 1287(68) | 1322(81) | a |
| PPO-corr (W) | 18.0(0.8) | 17.8(0.7) | 17.5(1.0) | 17.4(1.0) | 19.2(0.6) | 19.1(0.7) | 16.2(0.9) | 16.6(0.9) | a |
| MPO (W) | 656(40) | 674(38) | 715(39) | 727(44) | 692(29) | 700(31) | 670(31) | 687(31) | a |
| MPO-corr (W) | 8.5(0.3) | 8.7(0.2) | 8.9(0.3) | 9.1(0.4) | 9.0(0.1) | 9.1(0.2) | 8.4(0.3) | 8.6(0.3) | a |
| PPR (rev.min$^{-1}$) | 160(6) | 163(5) | 158(6) | 158(6) | 165(3) | 165(3) | 151(5) | 153(6) | a,b |
| MPR (rev.min$^{-1}$) | 117(5) | 120(3) | 122(5) | 124(5) | 124(2) | 126(2) | 115(4) | 118(4) | a |
| Fatigue Index (%) | 69(2) | 67(1) | 62(3) | 62(2) | 69(1) | 68(1) | 62(3) | 63(3) | |

## Metabolic responses to cycle ergometer sprinting

Figure 6.1 shows the blood pH response to Sprint 1 and Sprint 2 in the TR and CON groups before and after training. Blood pH was lower in the TR group than the CON group (mean lowest measured pH $7.12\pm0.01$ compared with $7.17\pm0.01$, $P<0.05$) and lower after Sprint 1 than Sprint 2 (mean lowest measured pH $7.13\pm0.01$ compared with $7.16\pm0.01$, $P<0.05$). The training period did not result in any significant changes in pH levels following sprinting although there was a trend for higher pH levels during recovery in the training group (group-training interaction effect, $P=0.07$).

The blood lactate response is shown in Figure 6.2. Blood lactate concentrations were higher in the TR group than the CON group (mean highest measured blood lactate $13.8\pm0.5$ compared with $10.4\pm0.4$ mmol.l$^{-1}$, $P<0.05$) and the blood lactate response also showed a group-time interaction effect ($P<0.05$). Training resulted in a change in the blood lactate response with regard to time, with an increase in highest measured mean blood lactate concentrations following the first sprint, but lower blood lactate

concentrations for the remainder of recovery from the first sprint and following sprint 2 (group-training-time interaction effect, $P<0.05$).

Figure 6.3 shows the plasma ammonia response to Sprint 1 and Sprint 2 in the TR and CON groups before and after the training period. Plasma ammonia concentrations were higher after Sprint 1 than after Sprint 2 (mean highest measured plasma ammonia 182.2±12.5 $\mu$mol.l$^{-1}$ compared with 139.0±9.2 $\mu$mol.l$^{-1}$, $P<0.05$). Plasma ammonia was also lower in the TR group after training (mean highest measured plasma ammonia 184.1±9.8 $\mu$mol.l$^{-1}$ compared with 137.0±11.7 $\mu$mol.l$^{-1}$, $P<0.05$) whilst plasma ammonia concentrations in the CON group did not change over the training period. Plasma ammonia concentration also demonstrated a group-training-time interaction effect ($P<0.05$) and a group-training-sprint-time interaction effect ($P<0.05$).

Figure 6.1. Mean blood pH at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints (Sprint 1 and Sprint 2) for the TR (top) and CON (bottom) groups pre- and post-training. Main effect group $P<0.05$, main effect sprint $P<0.05$, training-sprint interaction effect $P<0.05$.

Figure 6.2. Mean blood lactate concentrations at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints (Sprint 1 and Sprint 2) for the TR (top) and CON (bottom) groups pre- and post-training. Main effect group $P<0.05$.

Figure 6.3. Mean plasma ammonia concentrations at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints (Sprint 1 and Sprint 2) for the TR (top) and CON (bottom) groups pre- and post-training. Main effect sprint $P<0.05$, group-training interaction effect $P<0.05$.
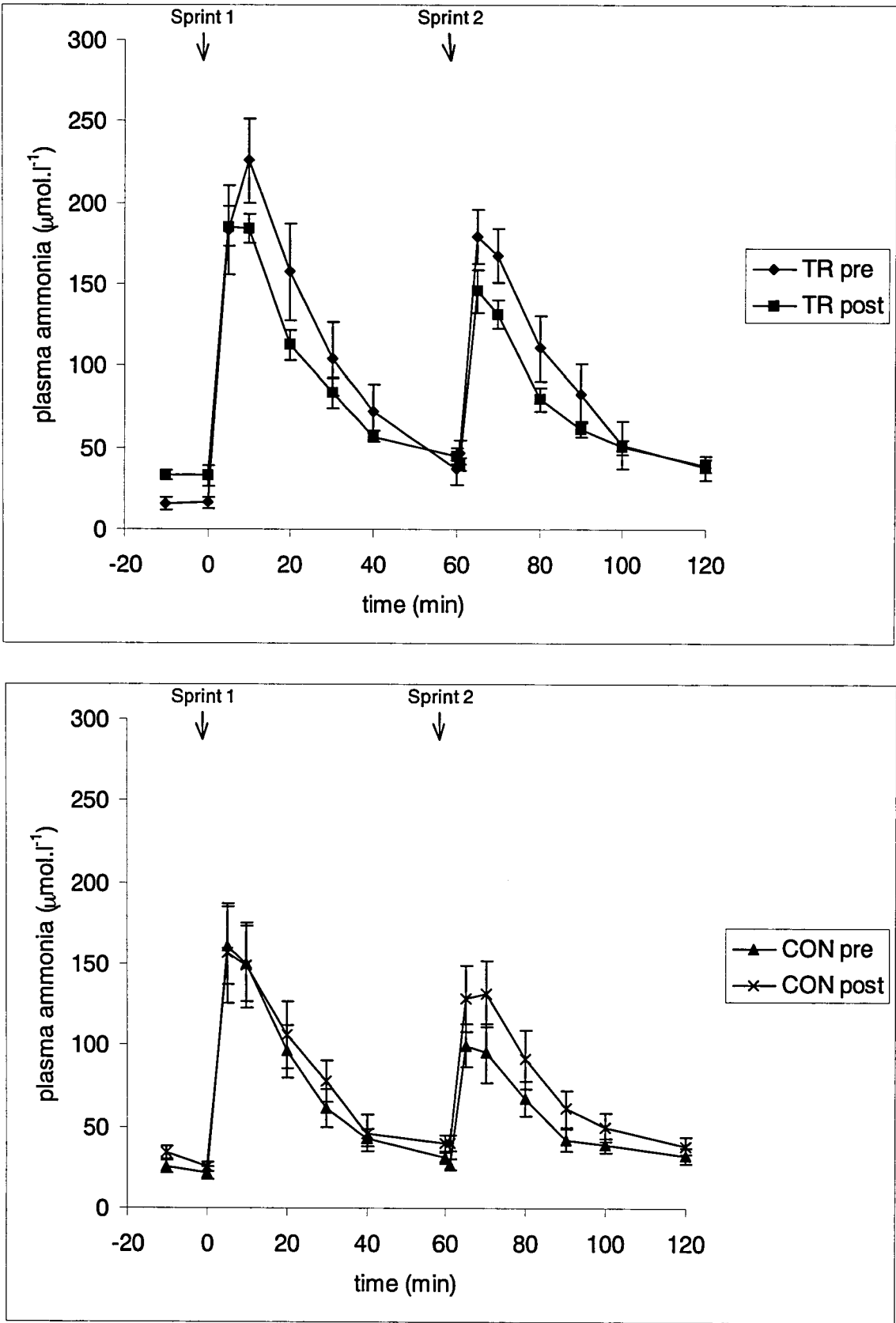
**Hormone responses to cycle ergometer sprinting**

Figure 6.4 shows the mean serum hGH response to Sprint 1 and Sprint 2 before and after the training period in both the TR and CON groups. There were no differences in resting hGH concentrations between groups or before and after the training period. In all trials Sprint 1 resulted in a distinct hGH pulse with highest measured mean concentrations of $20.5\pm6.2$ mU.l$^{-1}$ in the TR group and $11.9\pm4.7$ mU.l$^{-1}$ in the CON group between 20 and 40 min after the sprint before the training period. In each case hGH was still elevated 60 min after Sprint 1. In contrast, there was no hGH pulse following Sprint 2 in any of the trials and there was a significant difference between the response to Sprint 1 and Sprint 2 with highest measured mean concentrations of $13.4\pm2.4$ mU.l$^{-1}$ and $4.6\pm1.0$ mU.l$^{-1}$ respectively ($P<0.05$).

There was also a group-training interaction effect for hGH ($P<0.05$) with highest measured mean concentrations lower in all subjects in the TR group after the training period resulting in a mean decrease in highest measured mean concentration of over 40% over the 6 weeks of training ($20.5\pm6.2$ mU.l$^{-1}$ vs. $11.6\pm5.0$ mU.l$^{-1}$, $P<0.05$) with no change in the control group ($11.9\pm4.7$ mU.l$^{-1}$ vs. $13.0\pm5.1$ mU.l$^{-1}$, n.s.). hGH also demonstrated a training-time interaction effect ($P<0.05$). Mean integrated serum hGH concentrations (Area under the curve – AUC) are shown in Figure 6.5. These were 55% lower in the TR group after the training period with no differences in the CON group (TR, $1133\pm316$ vs. $512\pm241$; CON, $741\pm298$ vs. $896\pm297$, $P<0.05$).

Figure 6.6 shows the mean serum concentration of cortisol following Sprint 1 and Sprint 2 before and after the training period in both the TR and CON groups (n=8). There were no differences in the serum cortisol response between groups and no differences were seen between pre- and post-training serum cortisol concentrations.
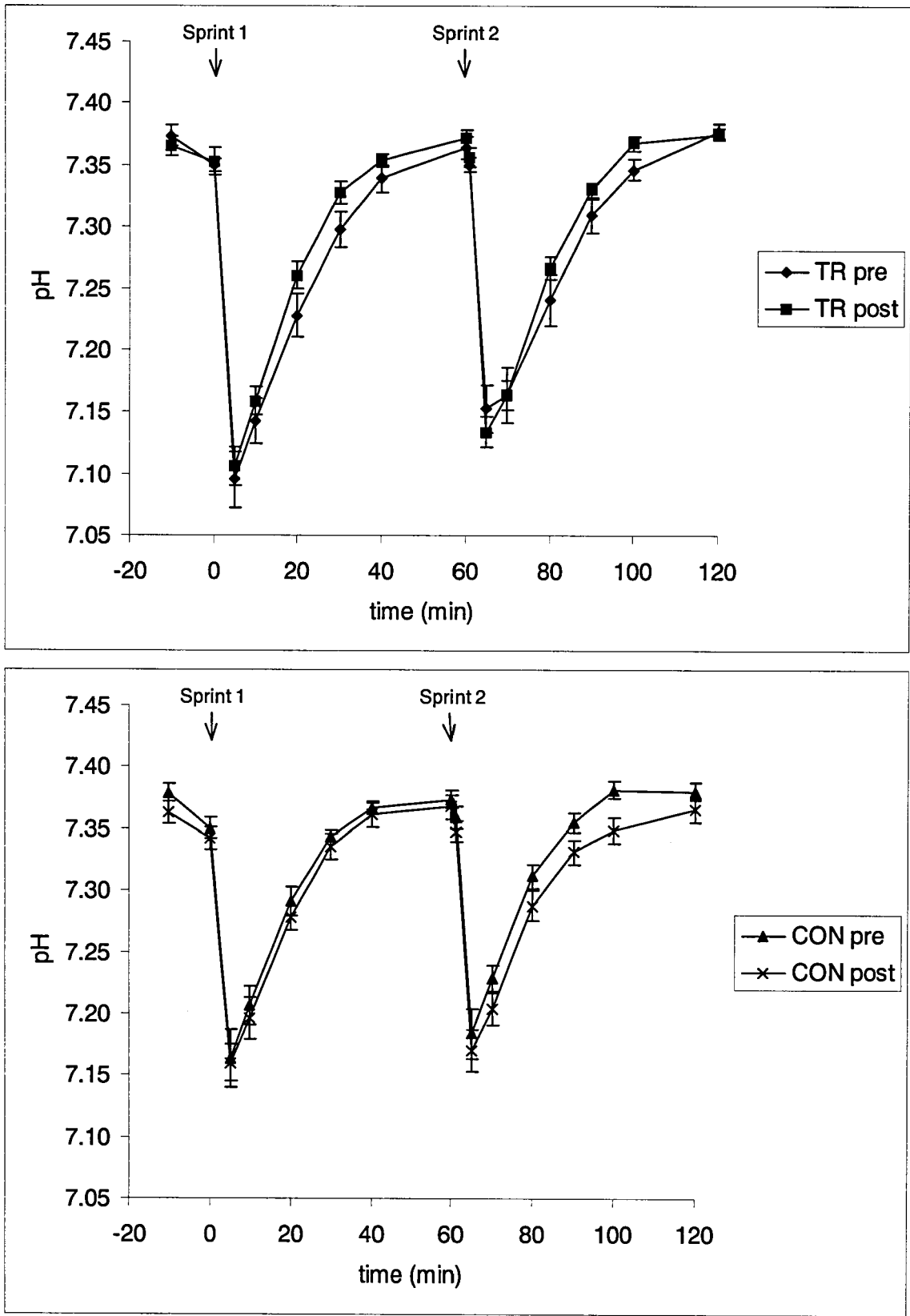
Figure 6.4. Mean serum hGH concentrations at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints (Sprint 1 and Sprint 2) for the TR (top) and CON (bottom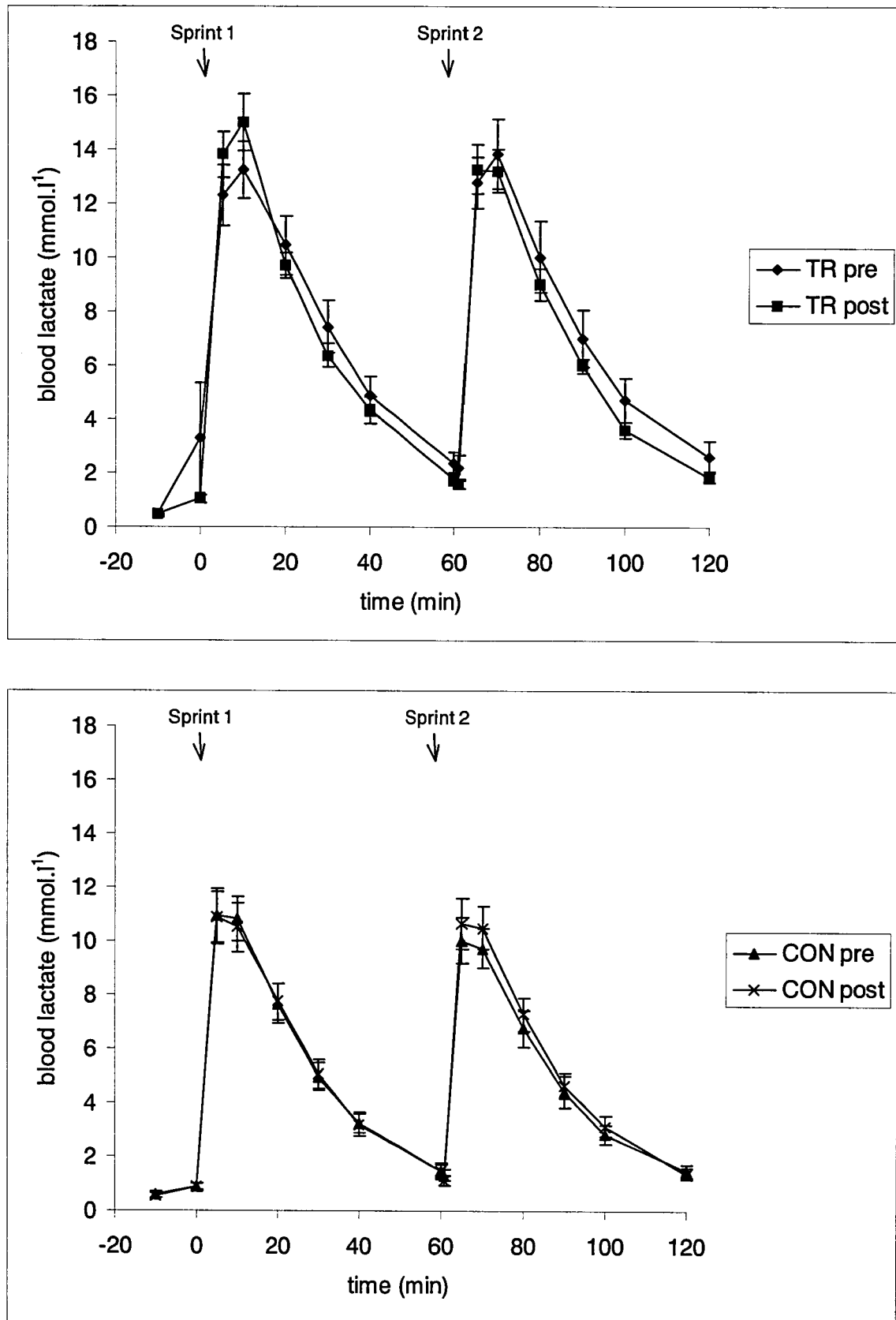) groups pre- and post-training. Main effect sprint $P<0.05$, group-training interaction effect $P<0.05$, training-time interaction effect $P<0.05$.
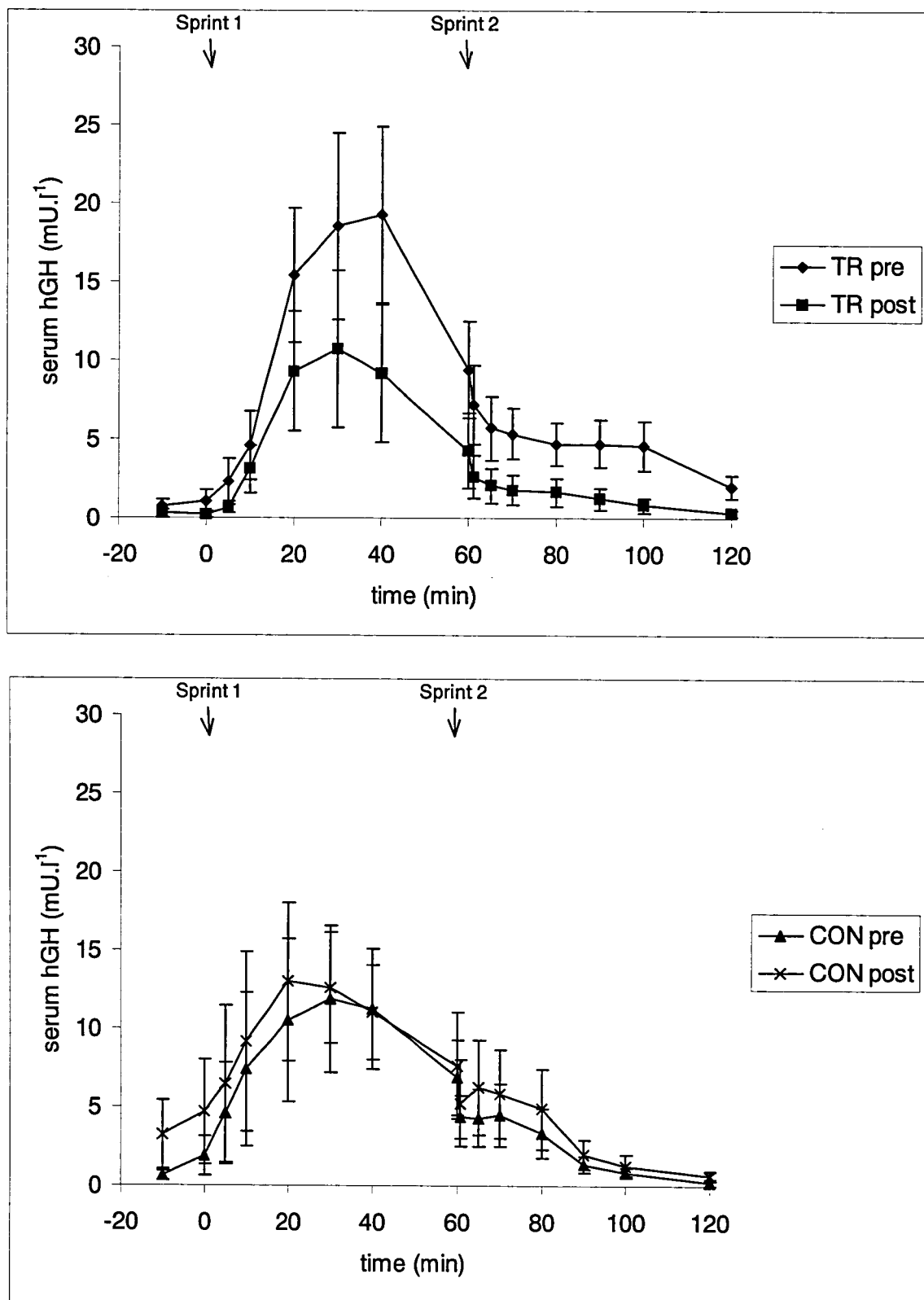
Figure 6.5. Mean integrated 2 h serum hGH concentrations (AUC) for the TR and CON groups pre- and post-training. Group-training interaction effect $P<0.05$.
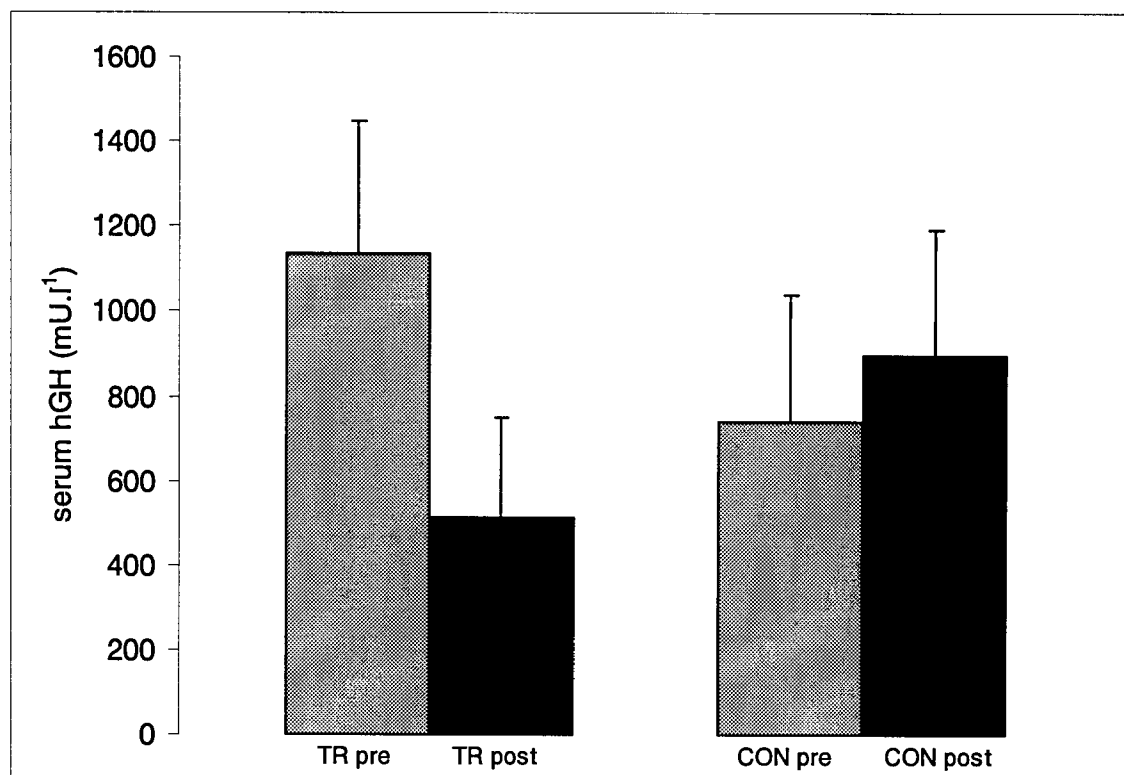
Figure 6.6. Mean serum cortisol concentrations (n=8) at rest and during 1 h of recovery after two 30 s maximal cycle ergometer sprints (Sprint 1 and Sprint 2) for the TR (top) and CON (bottom) groups pre- and post-training.
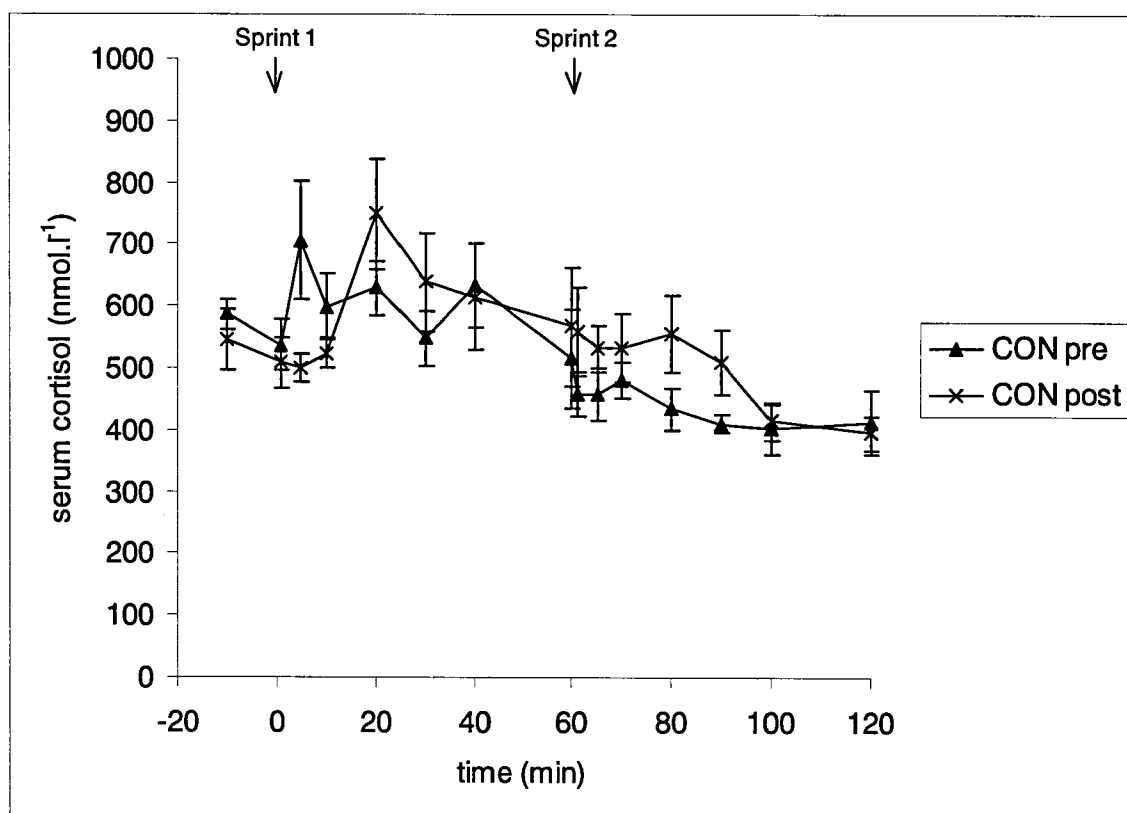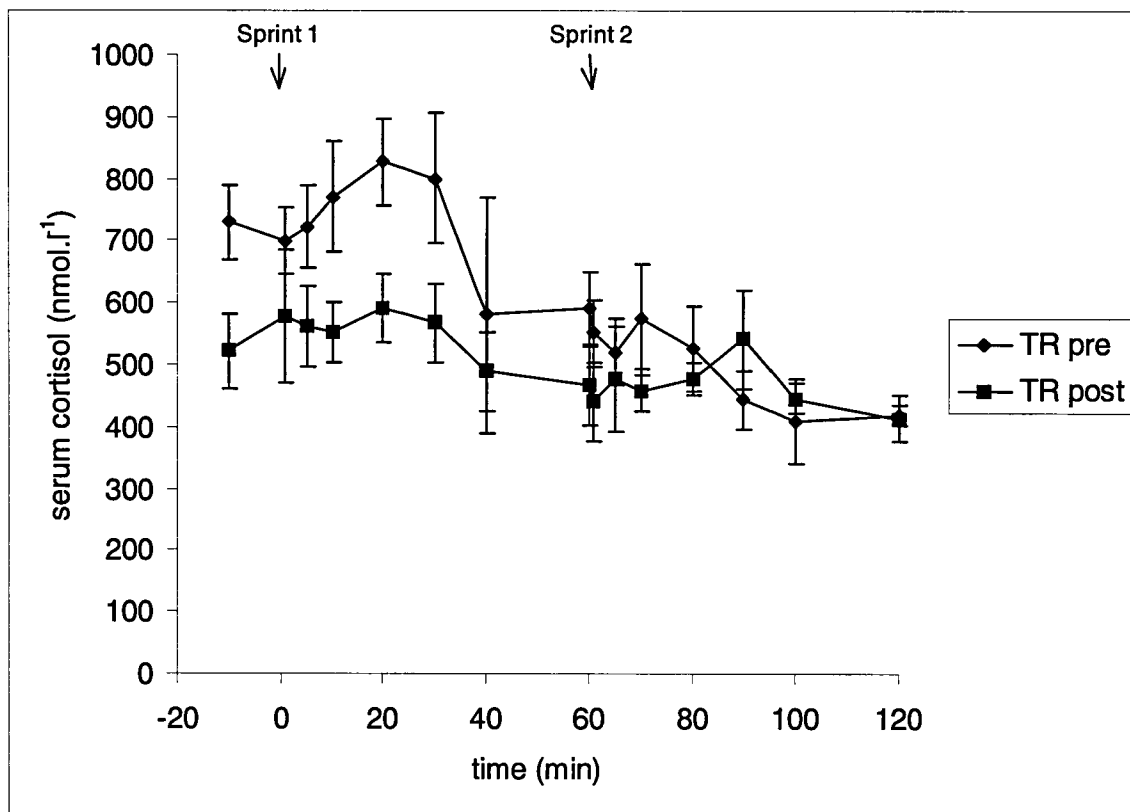
**Changes in plasma volume**

The estimated changes in mean plasma volume were greater in Sprint 1 than Sprint 2 ($-5.7\pm0.5\%$ and $-3.9\pm0.5\%$, $P<0.05$). At 5 min postexercise mean estimated changes in plasma volume were $-17.2\pm1.4\%$ and $-13.9\pm1.7\%$ for Sprint 1 and Sprint 2 for the TR group before training and $-16.7\pm1.1\%$ and $-13.8\pm1.2\%$ for the TR group after training with similar changes in the control group (group main effect n.s., group-training interaction effect n.s). All values had returned to baseline by 40-min postexercise.

## Discussion

The results of the present study have demonstrated that a short period of sprint training results in a blunted hGH response to repeated maximal cycle ergometer sprinting despite an improvement in sprint performance. In addition, following training, the hGH response to a second sprint, completed 60 min after the first, was still attenuated indicating that sprint training did not have any effect on hGH autonegative feedback.

In the present study the TR group demonstrated small, but significant, improvements in performance, which is in agreement with other studies employing short-term sprint or interval training (Boobis et al., 1983; Sharp et al., 1986; Cheetham and Williams, 1987; Nevill et al., 1989; Stathis et al., 1994). The fact that an improvement in performance was seen in the present study demonstrates that a 30 s cycle ergometer sprint is sensitive enough to demonstrate a training effect after a short period of sprint training despite the concerns of Jacobs et al. (1987).

It is possible that the mechanism for improved power output was that the number of contractions during the 30 s sprint increased following training. Indeed the TR group demonstrated an increase in both PPR and MPR after the training period. An increase in the number of contractions during the sprint could be facilitated by a decrease in the time to peak tension of fibres after training (Nevill et al., 1989). Sprint training has been found to reduce time to peak tension of rat soleus muscle by 14 % (Staudte et al., 1973), whilst high intensity training reduced contraction time in rat soleus muscle from 111 to 92 ms (Troup et al., 1986). In addition, Troup et al. (1986)

identified an increase in the activity of phosphofructokinase (PFK) following training, which has been correlated with actomyosin ATPase activity in rats (Baldwin et al., 1975), suggesting that the glycogenolytic pathway and actomyosin ATPase activity are regulated in parallel. Furthermore a correlation between actomyosin ATPase activity and speed of shortening (Barany, 1967) makes it possible that, in the present study, sprint training elicited an increase in glycogenolytic rate in parallel with increased actomyosin ATPase activity, resulting in the greater PPR and MPR.

In the present study, training resulted in an increase in highest measured mean post-exercise blood lactate concentrations in association with an improvement in sprint performance, suggesting that, if blood lactate concentrations reflect the accumulation of lactate in the muscle, there was an increase in anaerobic energy provision. Boobis et al. (1983) identified an improvement in sprint performance following 8 weeks of sprint training but did not find a concomitant increase in energy provision from glycogenolysis, whilst it has also been suggested that sprint training decreases glycolytic rate in rats (Troup et al., 1986) and humans (Harmer et al., 2000) and increases the aerobic contribution to sprinting (MacDougall et al., 1998; Harmer et al., 2000). However, studies employing training protocols utilising very short sprints (Linossier et al., 1993), longer sprints (Jacobs et al., 1987), speed-endurance training (Stathis et al., 1994; MacDougall et al., 1998) and combined speed and speed endurance training (Nevill et al., 1989), have all reported an increase in glycolytic rate. Therefore, in the present study, if measurements of blood lactate concentration can be extrapolated to muscle lactate accumulation, it is likely that glycolytic rate was enhanced following training resulting in an improvement in sprint performance.

Another possible mechanism for the improved performance and increase in both PPR and MPR identified in the present study is an increase in the activity of myofibrillar ATPase, since the activity of this enzyme is a very important factor in determining this contractile speed (Belcastro et al., 1981). Furthermore, Belcastro et al. (1981) observed a 34% increase in myofibrillar ATPase following training. Myofibrillar ATPase activity could be enhanced by an increase in the activity of adenylate kinase, which has been demonstrated to be higher after strength training in humans (Costill et al., 1979). If adenylate kinase activity also increased following sprint training, the removal of ADP from the contraction site would be enhanced, via deamination of

ADP to IMP, thus reducing the inhibition of ATP utilisation by product inhibition (Nevill et al., 1989).

However, increases in adenylate kinase activity following training might be expected to result in greater plasma $NH_3$ concentrations, yet in the present study plasma ammonia concentrations were lower in the TR group following training with no change in the CON group. Stathis et al. (1994) found sprint training to result in lower post-exercise muscle IMP and ammonia concentrations after a 30 s sprint reflecting a reduction in the magnitude of ATP depletion, and observed a tendency for plasma ammonia concentrations to be lower after 20 min of recovery ($P = 0.06$) despite being higher 2 min after exercise. Snow et al. (1992) also found sprint training to reduce exercise-induced increases in plasma ammonia concentrations. A decrease in the plasma ammonia response to exercise might reflect reduced muscle ammonia concentrations due to an improved balance between ATP hydrolysis and resynthesis following training, and this is most likely to be due to increased glycolytic rate (Sharp et al., 1986; Jacobs et al., 1987; Nevill et al., 1989).

In the present study the blood lactate response to exercise was observed to be greater following training, suggesting an increase in glycolysis. It is also possible that the decrease in the plasma ammonia response to exercise following training might reflect an attenuation of the net efflux of ammonia from exercising muscles (Snow et al., 1992) rather than reduced muscle ammonia accumulation. However, evidence from the present study, and other studies, of increased glycolytic flux following sprint training (Jacobs et al., 1987; Nevill et al., 1989; Linossier et al., 1993; Stathis et al., 1994; MacDougall et al., 1998) suggest that the lower exercise-induced plasma ammonia concentrations actually reflect reduced muscle ammonia concentrations. Sprint training might not, therefore, result in an increase in adenylate kinase activity in the present study, and, furthermore, another training adaptation might be responsible for any reduction in the time to peak tension of the fibres.

Sprint training has been reported to improve buffering capacity following 8 wk of sprint training (Sharp et al., 1986). In addition, Nevill et al. (1989) reported that buffering capacity calculated from changes in lactate concentration and pH during a 30 s treadmill sprint, showed a tendency to increase following training, and it was

suggested that training might result in enhanced efflux from exercising muscles. The results of the present study, however, show a trend for a higher blood pH levels following training, that is a decrease in the magnitude of the changes in blood pH, and do not, therefore, support this suggestion. It is possible, however, that intracellular buffering might have improved following training through increases in the major buffering components; the bicarbonate buffer system, PCr, $P_i$, protein-bound histidine residues and carnosine (Parkhouse and McKenzie, 1984). Improvements in intramuscular pH and nonbicarbonate buffering capacity have been shown to occur in association with improvements in performance in one-legged sprint exercise following training (Bell et al., 1988), and may account for the improvements in sprint performance identified in the present study. However, it is likely that the improvements in both peak and mean power output are attributable to the recruitment of a larger muscle mass (Boobis et al., 1983) in addition to neural adaptations (Harridge et al., 1998) as a result of cycle ergometer sprint training.

In the present study sprint training did not result in any changes in resting hGH concentrations. This has previously been identified when comparing sedentary individuals and athletes (Bloom et al., 1976; Mikines et al., 1985; Bunt et al., 1986; Barreca et al., 1988), sprint-trained and endurance-trained athletes (Nevill et al., 1996b) and following endurance (Bonifazi et al., 1998) or resistance (Kraemer et al., 1998; McCall et al., 1999) training. In all of these studies resting hGH concentrations are very low, probably accounting for the lack of any differences between groups. In contrast, 14 days of detraining of power athletes has been shown to increase resting hGH concentrations (Hortobagyi et al., 1993) whereas one year of endurance run training has been observed to increase resting hGH concentrations in women (Weltman et al., 1992). It is, therefore, unclear what effect training has on resting hGH concentrations but the results of the present study suggest that they are not significantly affected by sprint training.

However, in the present study, the hGH response to cycle ergometer sprinting is attenuated as a result of sprint training. This is in agreement with Bloom et al. (1976) and Weltman et al. (1997) who suggested that the reduced exercise-induced hGH concentrations following training may be a result of a combination of reduced hGH secretion and enhanced hGH clearance. In support of this contention is the suggestion

that the half-life of endogenous hGH is shorter in exercising than resting individuals (Thompson et al., 1993). On the other hand, Kraemer et al. (1998) did not find any change in the exercise-induced hGH response following resistance training, although it was postulated that other hGH variants might adapt differently to training and that different variants are different in their biological activity.

Other studies have demonstrated an increase in the hGH response to endurance (Bunt et al., 1986; Bonifazi et al., 1998) and resistance (McCall et al., 1999) exercise and insulin-induced hypoglycaemia (Mikines et al., 1985) following training. McCall et al. (1999) carried out the only study employing a similar design as the present study, that is a short term, longitudinal study with a control group. The increases in post-exercise hGH concentrations observed by McCall et al. (1999) following training are at odds with the result of the present study. It is possible that the reason for this difference might be the different types of exercise employed, both during training and in performance tests. The present study is the first to consider the exercise-induced hGH response following sprint training and it would appear that sprint training results in an attenuated hGH response to exercise. This is supported by the fact that detrained power athletes have increased resting hGH concentrations (Hortobagyi et al., 1993). However, further study of all types of exercise training is required in order to improve the understanding of the resulting hGH adaptation.

It is possible that the decrease in post-exercise hGH concentrations in the present study are a result of a sudden increase in training volume for the subjects. Although all of the subjects were previously involved in athletic training, an extra three sessions per week of the intensity employed in the present study may have resulted in overtraining and the changes in exercise-induced hGH concentrations may reflect this. Urhausen et al. (1998) observed that pituitary function was impaired in overtrained endurance cyclists, including lower exercise-induced hGH concentrations. Resting hGH concentrations were unchanged in the overtrained state and there was no change in the performance of a 30 s cycle ergometer sprint, although exercise duration to exhaustion in a stress test was decreased. In contrast, Fry et al. (1998) measured a 12% decrement in 1-RM strength performance, but no concomitant decrease in exercise-induced hGH concentrations as a result of high intensity resistance overtraining. It is, therefore, possible that resistance over-training does not influence

resting or exercise-induced hGH concentrations. However, Fry et al. (1998) only measured hormone concentrations for 5 min after the exercise bout whereas a longer period of measurement might identify changes in the hGH response to exercise. There are no data currently available on endocrine responses to sprint overtraining and it is therefore difficult to assess whether the results in the present study are a manifestation of overtraining. However, other studies have shown a decreased exercise-induced hGH response as a result of training and it is unlikely that in all of these cases this response was due to overtraining.

Although there is an attenuated hGH response to exercise after training in the present study, it is possible that 24 h secretion of hGH is increased after training. Weltman et al. (1992) observed an increase in 24 h integrated hGH concentrations following one year of run training in women. It has also been reported that serum IGF-I concentrations, taken as a measure of integrated hGH secretion because it is hGH dependent, increase following two weeks of endurance training (Roelen et al., 1997). A similar trend was described by Weltman et al. (1997) although this increase was not found to be significant. It is, therefore, possible that 24 h hGH concentrations are elevated following training despite the blunted acute hGH response to exercise.

The mechanism for hGH release in response to exercise is not fully understood. It has been suggested that hGH release is, at least in part, regulated by blood lactate concentration (Sutton et al., 1969). However, artificial manipulation of lactate levels using sodium lactate (Vigas et al., 1974) and lactic acid (Sutton et al., 1976) have shown blood lactate concentrations to have no consistent effect on hGH concentration. In addition the hGH response to intermittent exercise has been shown to be similar to the response to continuous exercise, despite a greater blood lactate response to the intermittent exercise (Karagiorgos et al., 1979). Weltman et al. (1997) found that 6 weeks of endurance training resulted in a continuous decrease in blood lactate concentration, but observed a different time course for the alteration of hGH concentration which appeared to be complete by the third week of training. In the present study highest measured blood lactate concentrations following exercise increased as a result of training in contrast to the serum hGH response which was attenuated. It is possible that training altered the relationship between blood lactate and hGH concentrations. However, it is more likely to be further evidence that blood

lactate concentration, whilst it may play a part, is not the prime regulator of the hGH response to sprint exercise.

It has been suggested that there is a close link between sympathetic activity and hGH secretion (Weltman et al., 1997). Kozlowski et al. (1983) observed serum hGH and plasma noradrenaline to be positively correlated at the end of exercise whilst Kjaer et al. (1997) suggested that impulses from motor centres directly stimulate both sympathoadrenal and pituitary secretion. The relationship between peripheral catecholamine concentrations and hGH concentrations are not entirely clear, however, since only noradrenaline released in the brain is effective in control of hGH secreton. Participation of blood noradrenaline in neuroendocrine control is, therefore, unlikely because of the poor permeability of the blood-brain barrier to catecholamines. Brooks et al. (1985) observed that the plasma catecholamine response to a 30 s sprint is reduced following endurance training whilst Nevill et al. (1989) found the plasma catecholamine response to a 30 s sprint to increase following sprint training. In addition, higher post-exercise catecholamine concentrations have been identified in sprint-trained athletes when compared with endurance-trained athletes (Nevill et al., 1996b). Although plasma catecholamines were not measured in the present study it is reasonable to assume that the response to sprint training would be similar to that observed in the study by Nevill et al. (1989), that is they would increase. The hGH response to sprint exercise was, however, attenuated in the present study suggesting that peripheral catecholamines might not regulate hGH secretion.

Central sympathetic tone may, however, be an important regulator of hGH release since α-adrenergic blockade has been shown to suppress the hGH response to exercise (Sutton & Lazarus, 1974). In addition, clonidine is a specific activator of central adrenergic receptors and it's oral administration results in hGH release in humans (Casanueva et al., 1984). However the administration of both clonidine and atropine, a muscarinic cholinergic receptor blocker which easily crosses the blood-brain barrier, resulted in complete hGH secretory blockade (Casanueva et al., 1984). Administration of atropine in conjunction with physical exercise also resulted in complete hGH sectretory blockade suggesting that cholinergic tone is very important

in regulating hGH secretion and that a cholinergic synapse is a final common pathway of a variety of different stimuli to hGH release (Casanueva et al., 1984).

Thompson et al. (1993) demonstrated that administration of a cholinergic agonist, pyridostigmine, potentiated the increase in hGH concentration following exercise probably through the suppression of somatostatin release from the hypothalamus. However, it is also possible that cholinergic tone may modulate $\alpha_2$-adrenergic neurons. Therefore links seen between sympathetic activity, as measured by peripheral catecholamine concentrations, and hGH release may have some relevance and it may be that increased cholinergic tone potentiates the hGH response to norepinephrine (Thompson et al., 1993) released in response to motor center activity (Kjaer et al., 1987).

At low exercise intensities, however, it is thought that hGH increased central cholinergic tone results in suppression of somatostatin release from the hypothalamus, producing a weak stimulus for hGH release (Casanueva et al., 1984; Maas et al., 2000; Thompson et al., 1993). However, Maas et al. (2000) suggest that there is complete suppression of somatostatin release at relatively low exercise intensities and that when exercise intensity is increased another mechanism must contribute to the stimulation of hGH secretion. This mechanism might be an increase in GHRH, possibly with the help of a secondary releasing factor which either stimulates different receptors to either somatostatin or GHRH or works as an activator for an unknown hypothalamic factor (Maas et al., 2000).

In the present study the subjects performed sprint exercise and therefore, by definition, exercise intensity was maximal. Post-exercise highest measured mean concentrations of hGH were much lower ($11.9\pm4.7 - 20.5\pm6.2$ mU.l$^{-1}$) than those observed at the end of an incremental exhaustive exercise test performed on a cycle ergometer ($116.8\pm18.6$ mU.l$^{-1}$) (Maas et al., 2000) and were more similar to those measured after a 20 min constant load cycle ergometer test carried out at an intensity above the lactate threshold ($\sim10$ mU.l$^{-1}$, one subject reported) (Weltman et al., 1997). However, in Chapter 4, the same exercise protocol as that in the present study elicited hGH responses twice as great ($46\pm10$ mU.l$^{-1}$) as those reported here, indicating that

there is a need for further study to elucidate the mechanisms regulating the magnitude of the hGH response to sprint exercise.

Repeated GHRH administration (Ghigo et al., 1991) and repeated exercise bouts (Cappon et al., 1994; Chapter 4) have been demonstrated to result in an attenuated hGH response. The results of the present study support these earlier findings since, before the training period, 60 min after a single sprint, GH had not returned to baseline and, furthermore, a second sprint completed at this time point did not elicit a GH response. Since, in the present study, hGH was still elevated at the start of the second sprint, hGH autoinhibition may be responsible for preventing a GH response to the second sprint. hGH can directly inhibit its own release, possibly at the pituitary gland (Pontiroli et al., 1991) or at the hypothalamus, mediated by an increase in somatostatin and/or a decrease in GHRH (Lanzi and Tannenbaum, 1992a; Lanzi and Tannenbaum, 1992b). In the presnt study the attenuated hGH response to the second sprint was still in evidence following the six week training period suggesting that hGH autonegative feedback is not affected by training.

In summary, this study is the first to consider the effect of sprint training on the exercise-induced hGH response. A six week period of combined speed and speed-endurance training resulted in a blunted hGH response to a 30 s maximal cycle ergometer sprint despite an improvement in sprint performance. In addition, sprint training did not alter the regulation of hGH in response to repeated sprinting, probably mediated by hGH autonegative feedback.

# CHAPTER 7

# HUMAN GROWTH HORMONE RESPONSES TO REPEATED BOUTS OF MAXIMAL SPRINT CYCLING WITH DIFFERENT RECOVERY PERIODS BETWEEN BOUTS

## Introduction

Exercise has been shown to be a potent stimulus for hGH secretion and the previous studies in this thesis have demonstrated that a single 30 s cycle ergometer sprint elicits a marked hGH response. Furthermore, hGH release is under the control of a number of feedback pathways and Chapters 5 and 6 have shown that repeated bouts of sprinting separated by 60 min of recovery result in an attenuated hGH response. It is likely that this inhibition of hGH secretion is a result of hGH autoinhibition, possibly at the level of the pituitary since hGH levels are still elevated 60 min after a single 30 s sprint, and it has been shown that inhibition of the GH response to GHRH can occur independently of circulating plasma FFA and somatostatin release (Pontiroli et al., 1991).

However, it is not known whether the attenuation of the hGH response to sprint exercise continues beyond the return of hGH concentrations to resting levels. In contrast to the results of the previous studies in this thesis, Kanaley et al. (1997) demonstrated an augmentation of the hGH response with repeated bouts of submaximal exercise, and gave evidence for greater augmentation of hGH release with an increased recovery period. Chapter 4 shows that serum hGH concentrations remain elevated for between 90 and 120 min after a single 30 s cycle ergometer sprint. The magnitude of the hGH response to a second 30 s sprint performed more than 120 min after the first sprint has not been studied.

In addition, it has been suggested that both acute hGH dependent IGF-I release and hGH independent IGF-I release with a longer time course can occur following high intensity exercise (Cappon et al., 1994). However, increases in IGF-I immediately after exercise have not been found in all studies (Wilson and Horowitz, 1987; Hagberg et al., 1988) or following different types of exercise (Nguyen et al., 1998).

Marcus et al. (1990) demonstrated an acute increase in IGF-I several hours after rhGH administration and it is expected that an exercise-induced increase in circulating hGH concentrations will have a similar effect. IGF-I is known to participate in the GH negative feedback system (Tannenbaum et al., 1983), and highly purified IGF-I has been shown to stimulate somatostatin secretion from rat hypothalamus cells in culture (Berelowitz et al., 1981). However, the role of both direct and indirect exercise-induced IGF-I synthesis and secretion in the regulation of the hGH response to repeated sprints is unclear.

It has been suggested that circulating IGF-I levels might remain elevated for more than 24 h after exercise and Yan et al. (1993) found that IGF-I immunoreactivity did not increase significantly until 4 days after 192 eccentric muscle contractions in rats. There is no information available regarding IGF-I concentrations the day after sprint exercise. In addition, the potential influence that variations in resting serum IGF-I concentrations might have on the hGH response to exercise performed on consecutive days has not been studied.

Therefore, the aim of the present study was to test three hypotheses. First, that a single 30 s cycle ergometer sprint will result in an increase in serum concentrations of both hGH and IGF-I, and that IGF-I will have returned to resting levels within 60 min of recovery, whereas hGH levels will remain elevated. The elevated hGH concentration will result in an attenuation of the hGH response to the second sprint. Second, that by 240 min of recovery hGH levels will have returned to baseline, and will not, therefore, moderate the hGH response to a second sprint. However, at this time there might be a hGH-induced increase in serum IGF-I concentrations, which will attenuate the hGH response to the second sprint. Third, that IGF-I concentrations might be elevated the day after sprint exercise, which would attenuate the hGH response to a 30 s sprint under these conditions. Should IGF-I levels not be elevated, the exercise-induced hGH response will be the same as that observed on the previous day.

## Methods

### Subjects

Eight healthy male volunteers aged 19 to 26 years (23±1 year) gave their written informed consent for this study which had the approval of the Loughborough University Ethical Committee. Body mass ranged from 66.3 to 97.4 kg (82.7±4.1 kg) and height ranged from 170.1 to 189.1 cm (180.2±2.4 cm).

### Equipment

The exercise tests were carried out on a modified friction-loaded cycle ergometer (Monark 864), which was interfaced to a microcomputer (BBC). This allowed instantaneous power output, corrected for flywheel acceleration, to be monitored and recorded accurately. Performance data were averaged over 1 s intervals. Lakomy (1986) has described the equipment used in detail. A restraining harness was also placed around the subjects' waists in order to prevent them from rising out of the saddle, thereby concentrating movement in the lower limbs. The same harness setting and saddle height were used for each trial. Toe-clips and tape held the subjects' feet securely in the pedals.

### Protocol

After familiarisation, the subjects arrived in the laboratory after a 4 h fast on three separate occasions, completing one trial on each visit. The subjects then completed a standardised sub-maximal warm-up consisting of 4 min pedalling at 60W, 30 s pedalling at 80W and 30 s pedalling at 100W. Subjects then rested on the ergometer for 5 min before completing an all-out 30 s sprint from a standing start against an applied resistance equivalent to 7.5% (75N.kN$^{-1}$) of their body weight. Subjects then rested whilst seated on a couch (maintaining approximately the same body position as on the ergometer) whilst venous blood samples were collected. In Trial A, subjects rested on the couch for 60 min before repeating the same warm-up routine followed by a second all-out 30 s sprint, against the same applied resistance. Subjects then rested on a couch for a further 60 min. In Trial B, subjects rested on the couch for 240 min following the first sprint, before repeating the same warm-up routine followed by a second all-out 30 s sprint, against the same applied resistance. They then rested on the couch for a further 90 min whilst further blood samples were taken.

Trial C was carried out the day after Trial B and followed the same procedure as the other two trials. However, in Trial C, subjects only completed one cycle ergometer sprint before resting on the couch for 120 min whilst blood samples were taken. Subjects were instructed to sprint maximally for the duration of each sprint and were encouraged verbally whilst sprinting.

**Blood sampling and analysis**

Venous blood samples were taken via a cannula inserted into an antecubital forearm vein under local anaesthetic (1% lignocaine). The first blood sample was taken at least 15 min after the cannula was inserted. Patency was maintained by displacing the blood contained in the cannula with isotonic saline. In Trial A blood samples were taken in a seated position at rest (-10 min) and then post warm-up (-4 min), prior to, and 5, 10, 20, 30, 40 and 60 min after each sprint. In trial B, blood samples were taken at rest and then post warm-up, prior to, and 5, 10, 20, 30, 40, 60, 90, 120, 180 and 240 min after the first sprint and post warm-up, prior to, and 5, 10, 20, 30, 40, 60 and 90 min after the second sprint. In Trial C, blood samples were taken at rest and then post warm-up, prior to, and 5, 10, 20, 30, 40, 60, 90 and 120 after the single sprint.

Samples were dispensed into three tubes: (i) One portion (4 to 5 ml) was placed into a lithium heparinised tube (Sarstedt LH/5ml). Blood pH was measured immediately (Radiometer ABL5 pH/blood gas monitor) and thereafter, 20µl aliquots of blood were removed, deproteinized in 2.5% perchloric acid and stored at -20°C for later determination of blood lactate concentrations (Maughan, 1982). Further aliquots were removed for the measurement of haematocrit by microcentrifugation (Hawksley Ltd.) and haemoglobin concentration (by the cyanmethaemoglobin method) for the calculation of percentage change in plasma volume (Dill and Costill 1974). (ii) A further 1.5 ml was placed in a calcium heparinised eppendorf tube. This was immediately centrifuged and the plasma removed and stored at -70°C for the determination of ammonia concentration within 48 h (Sigma Diagnostics, kit 171 - C). (iii) The remaining blood (4 to 5 ml) was allowed to clot for 1 h in a plain tube (Sarstedt Serum Z/5ml). This was then centrifuged at 3°C for 15 min at a speed of 6000 rev.min$^{-1}$ (Burkard Koolspin) and the serum was removed and stored at -70°C

for the determination of hGH, cortisol IGF-I and FFA concentrations. Serum GH, serum cortisol and serum IGF-I were measured by routine ELISA. The GH assay (Medigenix HGH-Biosource) had a sensitivity of 0.11 mU.l⁻¹, an intra-assay coefficient of variation (cv) of 2.1-3.6% and an inter-assay cv of 6.8-7.1%. The sensitivity of the cortisol assay (Milenia, DPC cortisol) was 8.3 nmol.l⁻¹ with an intra-assay cv of 5.9-8.0% and an inter-assay cv of 8.3-9.0%. The IGF-I assay (R&D Systems Europe) had a sensitivity of 0.026 ng.ml⁻¹, and intra-assay cv of 3.5-4.3% and an inter-assay cv of 7.5-8.3%.

**Statistical analysis**

One-way analysis of variance with repeated measures was used to discover any differences in sprint performance. Two-way analysis of variance with repeated measures was used to discover any differences in the blood lactate (n=7), blood pH, plasma ammonia (n=7) and serum hGH and cortisol responses for 60 min following sprint 1 and sprint 2 in Trial A and in Trial B as well as when comparing the first sprint in Trial B (day 1) with the sprint in Trial C (day 2). One-way analysis of variance was also used to discover any differences in the serum IGF-I concentrations pre-exercise and 5 min after sprints 1 and 2 in Trial A and in Trial B, and the sprints performed on day 1 and day 2. A paired *t* test was used to discover any differences between serum FFA concentrations prior to sprints 1 and 2 in Trial A and in Trial B, and the sprints performed on day 1 and day 2. Statistical significance was accepted at the *P* < 0.05 level. All results are expressed as mean ±SEM.

<u>**Results**</u>

**Performance**

Table 7.1 is a summary of the performance variables in all of the trials. Peak power output (PPO) was similar in both sprints in trial in Trial A and in both sprints in Trial B. However, PPO was significantly greater in Trial B sprint 1 compared with a sprint completed 24 h later (Trial C). There were no other differences in sprint performance variables either between sprints in Trial A and Trial B, or between the first sprint in Trial B (day 1) and the sprint in Trial C (day 2).

Table 7.1. Peak (PPO) and mean (MPO) power output, peak (PPR) and mean (MPR) pedal revolutions, total work done and fatigue index in sprints 1 and 2 in Trial A and Trial B and the sprint completed in Trial C (n=8).

| | Trial A | | Trial B | | Trial C |
|---|---|---|---|---|---|
| | Sprint 1 | Sprint 2 | Sprint 1 | Sprint 2 | |
| PPO (W) | 1538±65 | 1494±70 | 1528±85 | 1487±75 | 1464±67[a] |
| MPO (W) | 719±30 | 725±32 | 711±32 | 699±31 | 709±32 |
| PPR (rev.min$^{-1}$) | 165±4 | 164±4 | 163±5 | 162±5 | 162±4 |
| MPR (rev.min$^{-1}$) | 122±3 | 123±3 | 120±3 | 119±3 | 120±3 |
| Work Done (J) | 21488±944 | 21757±959 | 21311±959 | 20980±940 | 21265±953 |
| Fatigue Index (%) | 70±2 | 68±2 | 70±2 | 70±1 | 68±2 |

a $P<0.05$ vs. Trial B sprint 1

**Trial A (60 min recovery)**

**Metabolic responses to sprinting**

Figure 7.1a shows the blood lactate responses to sprint 1 and sprint 2 in Trial A (n=7). There were no differences in the blood lactate responses to the sprints, with highest measured mean blood lactate concentrations of 11.8±0.6 mmol.l$^{-1}$ following sprint 1 and 11.5±0.8 mmol.l$^{-1}$ following sprint 2 (n.s.). There was, however, a sprint-time interaction effect ($P<0.05$) reflecting faster recovery of blood lactate concentrations towards pre-exercise values following sprint 2. There were no differences in the blood pH responses to sprint 1 and sprint 2 in Trial A (Figure 7.1b). Similarly, there were no significant differences in the plasma ammonia responses to the sprints in Trial A (Figure 7.1c, n=7), although there was a trend for a reduced highest measured mean plasma ammonia concentration, and for a slower recovery towards pre-exercise concentrations following the second sprint (sprint-time interaction effect, $P=0.06$, n.s.). Pre-exercise serum FFA concentrations (Table 7.2, n=7) were not significantly different prior to sprint 1 and sprint 2.

**Hormone and growth factor responses to sprinting**

Figure 7.2a shows the serum hGH responses to sprint 1 and sprint 2 in Trial A. Sprint 1 resulted in a significant elevation of serum hGH concentrations with highest measured mean hGH concentrations of $27.3 \pm 6.9$ mU.l$^{-1}$ 40 min after the sprint. Serum hGH concentrations were still elevated above pre-exercise levels after 60 min of recovery. Sprint 2 did not induce a further increase in serum hGH concentrations. Human GH demonstrated a sprint-time interaction effect ($P<0.05$), whilst the area under the curve for hGH (hGH AUC – $919 \pm 246$ vs. $451 \pm 129$) and mean highest measured hGH concentrations (hGH peak – $28.8 \pm 6.8$ vs. $14.1 \pm 3.4$ mU.l$^{-1}$) were both significantly higher in recovery from sprint 1 compared with sprint 2 (both $P<0.05$).

Serum IGF-I responses to sprint 1 and sprint 2 in Trial A are shown in Figure 7.2b (n=3). Both sprints resulted in an acute increase in circulating IGF-I level, although serum IGF-I concentrations did not remain elevated for longer than 10 min. Table 7.3 (n=7) shows that serum IGF-I concentrations were significantly elevated above pre-exercise concentrations 5 min after both sprint 1 and sprint 2 (sprint 1, $199 \pm 16$ vs. $238 \pm 22$ mg.ml$^{-1}$, sprint 2, $191 \pm 13$ vs. $227 \pm 23$ mg.ml$^{-1}$, time main effect, $P<0.05$) and there was no difference in the responses between sprints. Serum cortisol concentrations (Figure 7.2c) increased following sprint 1 with highest measured mean levels of $656.6 \pm 63.5$ nmol.l$^{-1}$ 20 min into recovery. After this point serum cortisol concentrations fell and there was no further increase following the second sprint (sprint main effect, $P<0.05$).

Figure 7.1. Mean blood lactate concentrations (a, n=8), mean blood pH (b, n=8) and mean plasma ammonia concentrations (c, n=7) at rest and during 60 min of recovery from sprint 1 and sprint 2 in Trial A.

(a) time main effect ($P<0.05$), sprint-time interaction effect ($P<0.05$).

(b) time main effect ($P<0.05$).

(c) time main effect ($P<0.05$), sprint-time interaction effect ($P=0.06$, n.s.)
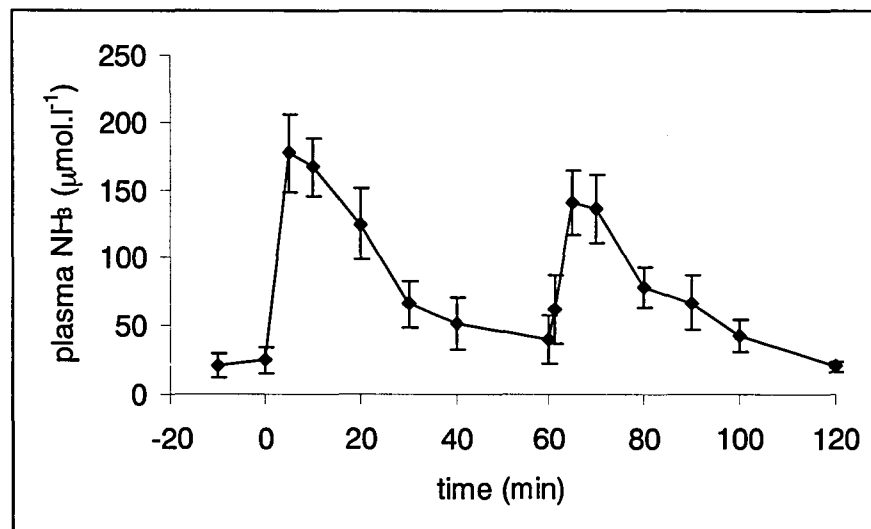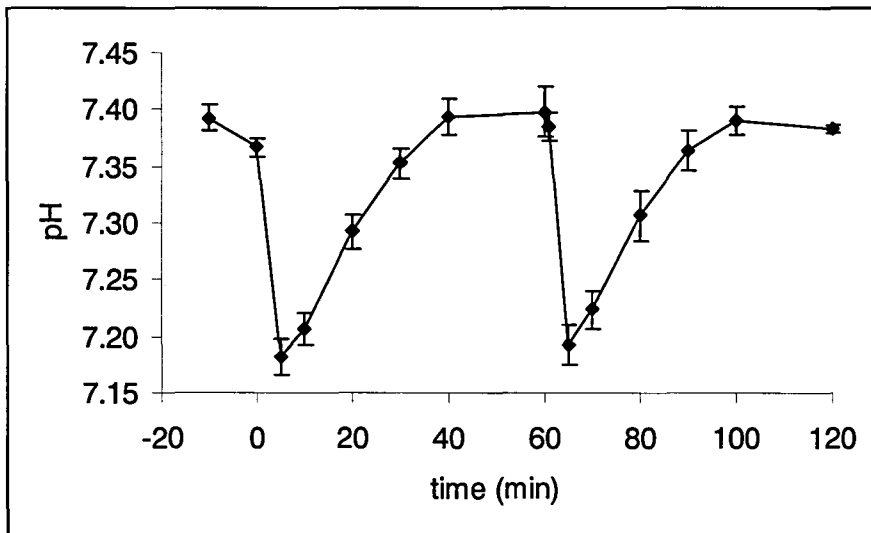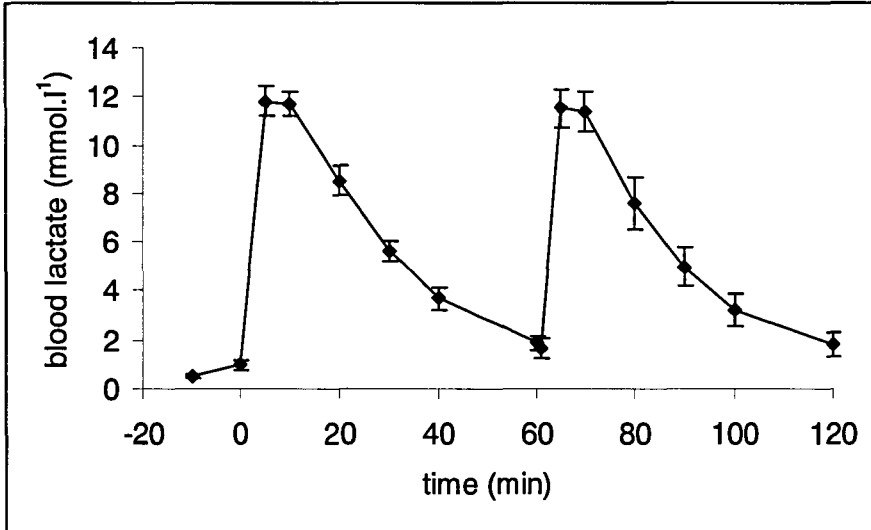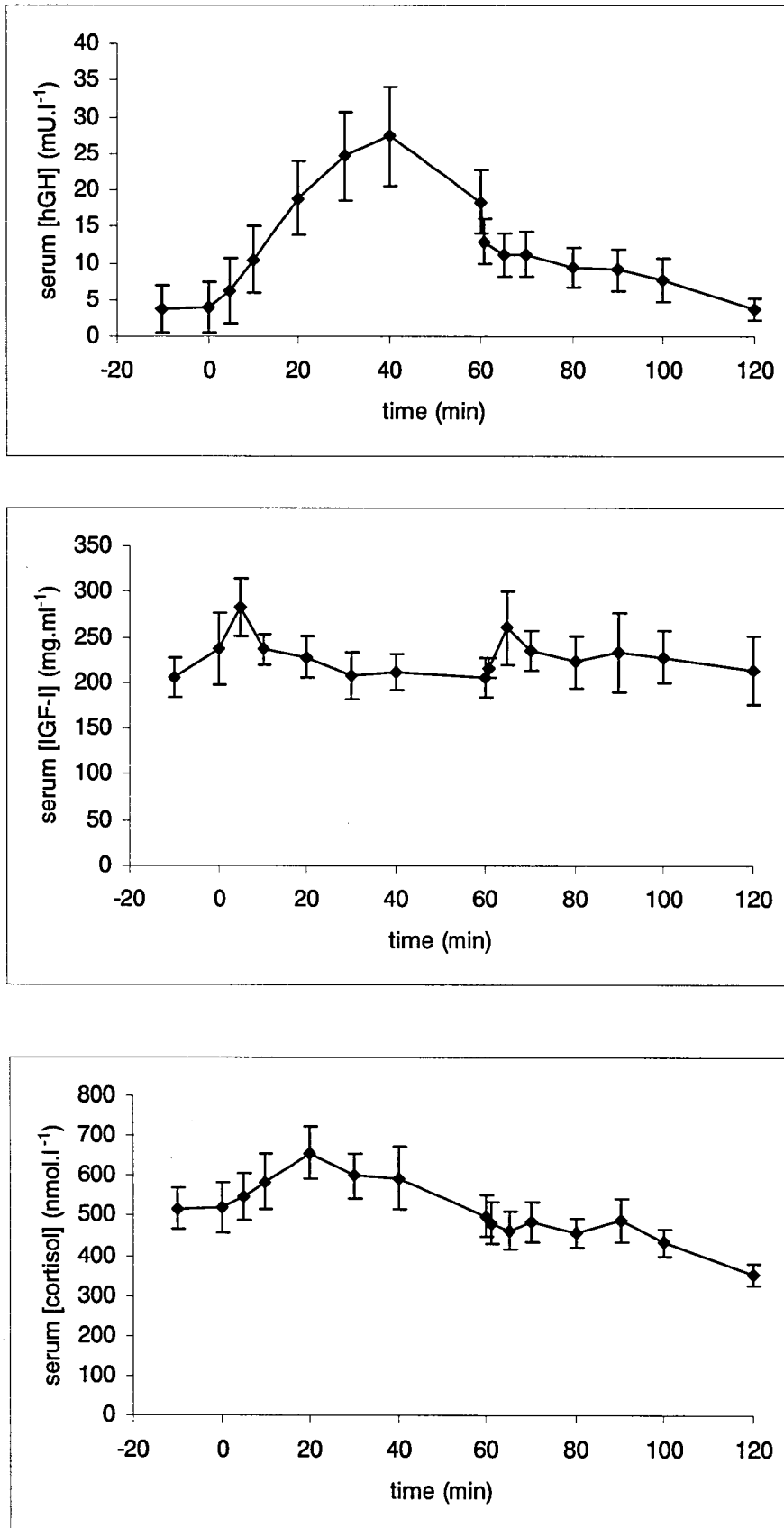
Figure 7.2. Mean serum hGH concentrations (a, n=8), mean serum IGF-I concentrations (b, n=3) and mean serum cortisol concentrations (c, n=8) at rest and during 60 min of recovery from sprint 1 and sprint 2 in Trial A.

(a) time main effect ($P<0.05$), sprint-time interaction effect ($P<0.05$).

(c) sprint main effect ($P<0.05$), time main effect ($P<0.05$

**Trial B (240 min recovery)**

**Metabolic responses to sprinting**

Figure 7.3a shows the blood lactate responses to sprint 1 and sprint 2 in Trial B (n=7). Both sprints resulted in an increase in blood lactate concentrations, but the blood lactate response to the first sprint was greater than that following the second sprint, with highest measured mean blood lactate concentrations of $10.9 \pm 0.9$ mmol.$l^{-1}$ and $10.1 \pm 0.8$ mmol.$l^{-1}$ following sprint 1 and sprint 2, respectively (main effect sprint, $P<0.05$). There was also a statistically significant difference between the blood pH responses following the two sprints with lowest measured mean pH of $7.20 \pm 0.01$ following sprint 1 and $7.21 \pm 0.02$ following sprint 2 (main effect sprint, $P<0.05$). Plasma ammonia demonstrated a difference between sprints as well as a sprint-time interaction effect (Figure 7.3c, n=7). The response to the second sprint was lower than that to the first sprint with highest measured mean plasma ammonia concentrations of $182.3 \pm 25.9$ and $143.8 \pm 24.0$ $\mu$mol.$l^{-1}$ after sprint 1 and sprint 2, respectively (main effect sprint, $P<0.05$). Table 7.2 (n=7) shows that pre-exercise serum FFA concentrations were higher before sprint 2 than before sprint 1 ($P<0.05$).

**Hormone and growth factor responses to sprinting**

The serum hGH responses to sprint 1 and sprint 2 in Trial B are shown in Figure 7.4a. Sprint 1 resulted in a marked hGH response with highest measured mean serum hGH concentrations of $25.3 \pm 7.4$ mU.$l^{-1}$ 40 min into recovery. Serum hGH levels returned to pre-exercise concentrations within 120 min following sprint 1 and remained low for the rest of the 240 min recovery period. Sprint 2 resulted in a further hGH response, although there was a tendency for the exercise-induced increases in hGH levels to be smaller than those seen after sprint 1 with highest measured mean hGH concentrations of $13.2 \pm 3.9$ mU.$l^{-1}$ 40 min after sprint 2 (sprint main effect, $P=0.10$, n.s.). There was also a trend for lower hGH AUC ($10223 \pm 280$ vs. $483 \pm 134$, $P=0.09$, n.s.) and hGH peak ($34.1 \pm 10.0$ vs. $16.8 \pm 5.0$ mU.$l^{-1}$, $P=0.09$, n.s.) following sprint 2.

Figure 7.4b shows the serum IGF-I responses to sprint 1 and sprint 2 for 3 subjects. Serum IGF-I concentrations did not increase following sprint 1, and although serum IGF-I appeared to be elevated above pre-exercise levels 180 min after sprint 1, this elevation did not extend to 240 min of recovery. Table 7.3 (n=7) shows that serum

IGF-I concentrations were not elevated 5 min after sprint 1 (208±21 vs. 208±23 mg.ml$^{-1}$) and that IGF-I concentrations were not elevated 240 min after sprint 1. In addition, despite a small acute increase in serum IGF-I concentrations following sprint 2, there were no significant differences between the IGF-I responses to sprint 1 and sprint 2. There was no acute increase in serum cortisol concentrations following sprint 1 and from 40 min of recovery serum cortisol levels decreased to 331.1±24.8 nmol.l$^{-1}$ immediately prior to the second sprint (240 min of recovery). Sprint 2 resulted in an acute increase in serum cortisol concentrations to 427.6±52.4 nmol.l$^{-1}$, and there was a difference in the cortisol response compared to sprint 1 (main effect sprint, $P<0.05$).

Figure 7.3. Mean blood lactate concentrations (a, n=8), mean blood pH (b, n=8) and mean plasma ammonia concentrations (c, n=7) at rest and during 240 min and 90 min of recovery from sprint 1 and sprint 2, respectively, in Trial B.

(a) sprint main effect ($P<0.05$), time main effect ($P<0.05$).

(b) sprint main effect ($P<0.05$), time main effect ($P<0.05$).

(c) sprint main effect ($P<0.05$), time main effect ($P<0.05$), sprint-time interaction effect ($P<0.05$).
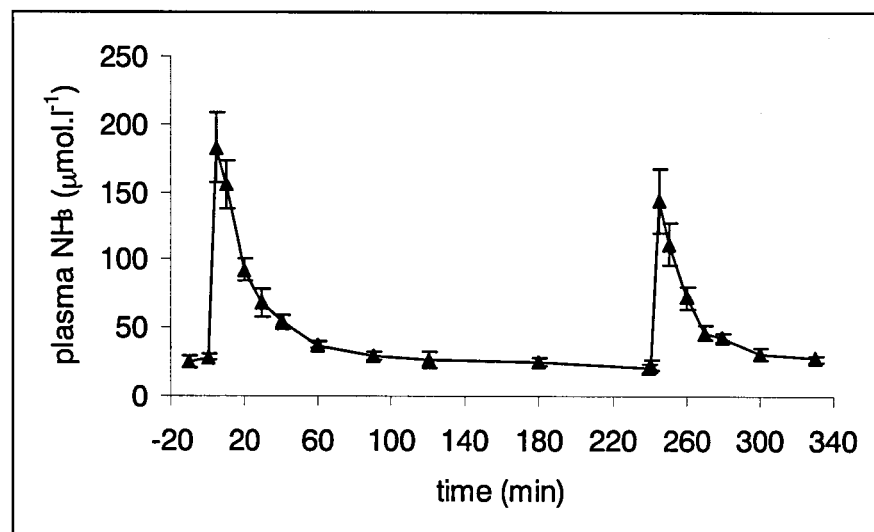
Figure 7.4. Mean serum hGH concentrations (a, n=8), mean serum IGF-I concentrations (b, n=3) and mean serum cortisol concentrations (c, n=8) at rest and during 240 min and 90 min of recovery from sprint 1 and sprint 2, respectively, in Trial B.

(a) sprint main effect ($P$=0.10, n.s.), time main effect ($P$<0.05).

(c) sprint main effect ($P$<0.05), time main effect ($P$<0.05).
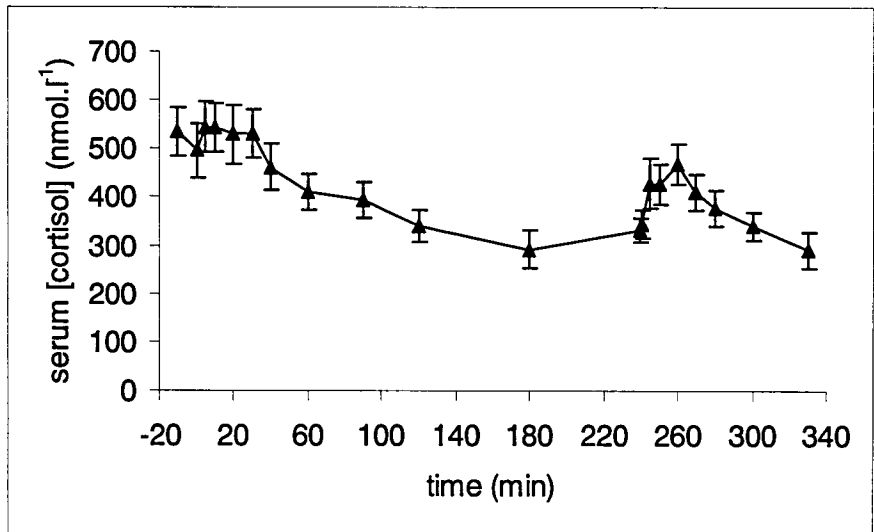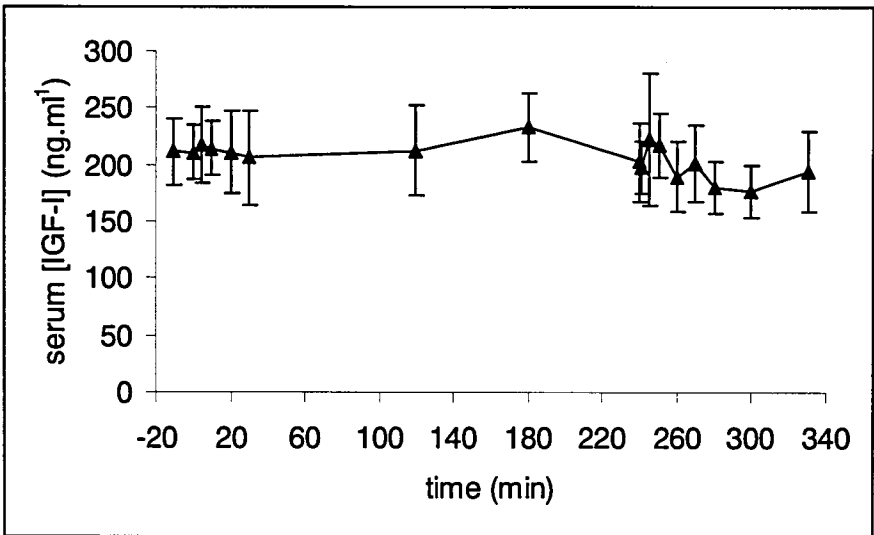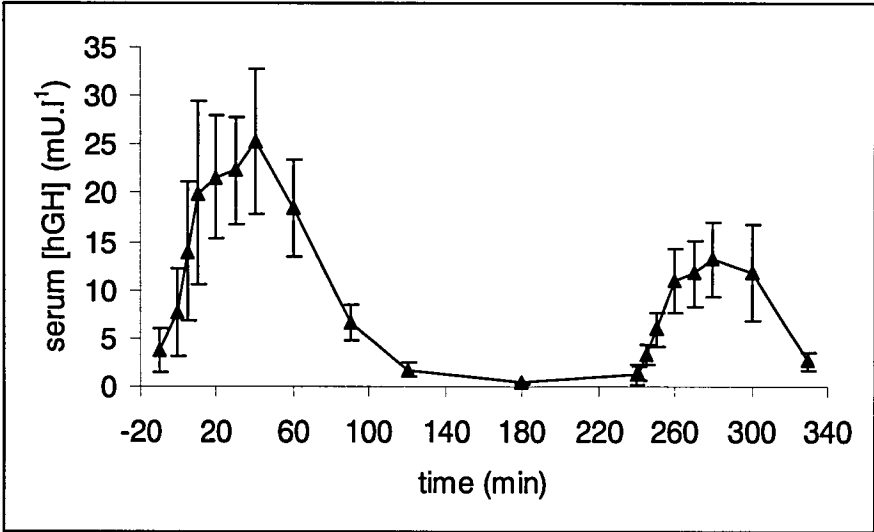
**Trial C (24 h recovery)**

**Metabolic responses to sprinting**

There were no significant differences in the blood lactate (Figure 7.5a, n=7) and blood pH (Figure 7.5b) responses to sprint 1 in Trial B (day 1) and the sprint completed 24 h later, in Trial C (day 2). However, plasma ammonia responses to the sprint on day 2 were smaller than those observed following the first sprint on day 1 (Figure 7.5c, n=7) with highest measured mean plasma ammonia concentrations of $182.3 \pm 25.9 \ \mu mol.l^{-1}$ 5 min after the sprint on day 1 and $143.9 \pm 12.7 \ \mu mol.l^{-1}$ 5 min after the sprint on day 2 (main effect sprint, $P<0.05$). Table 7.2 shows that pre-exercise serum FFA concentrations were higher on day 2 than on day 1 ($P<0.05$).

**Hormone and growth factor responses to sprinting**

Figure 7.6a shows the hGH responses to sprint 1 in Trial B (day 1) and the sprint in Trial C (day 2). As described above, the sprint on day 1 induced a marked increase in serum hGH concentrations with highest measured mean hGH concentrations of $25.3 \pm 7.4 \ mU.l^{-1}$. There was no difference between the hGH responses to this sprint and the sprint on day 2, although highest measured mean serum hGH concentrations were $27.1 \pm 9.7 \ mU.l^{-1}$ 20 min after the sprint on day 2, 20 min earlier than the highest measured mean serum hGH concentrations following the sprint on day 1. Serum IGF-I concentrations following the sprints on day 1 and day 2 are shown in Figure 7.6b (n=3). Table 7.3 (n=7) shows that pre-exercise serum IGF-I concentrations were higher on day 1 than on day 2 ($208 \pm 21$ vs. $186 \pm 21 \ mg.ml^{-1}$, sprint-time interaction effect, $P<0.05$). In addition, there was an acute increase in serum IGF-I levels following the sprint on day 2, which was not evident following the sprint on day 1, although this only resulted in serum IGF-I concentrations similar to those found on day 1 ($208 \pm 23$ vs. $211 \pm 25 \ mg.ml^{-1}$). There were no differences in the serum cortisol responses to the sprints on day 1 and day 2.

Figure 7.5. Mean blood lactate concentrations (a, n=8), mean blood pH (b, n=8) and mean plasma ammonia concentrations (c, n=7) at rest and during 120 min of recovery from sprint 1 in Trial B (day 1) and the sprint in Trial C (day 2).
(a) time main effect ($P<0.05$).
(b) time main effect ($P<0.05$).
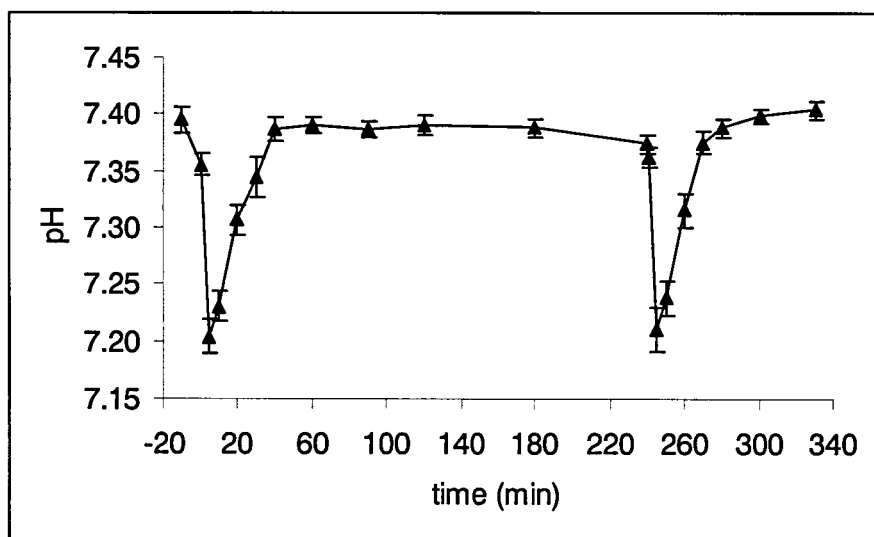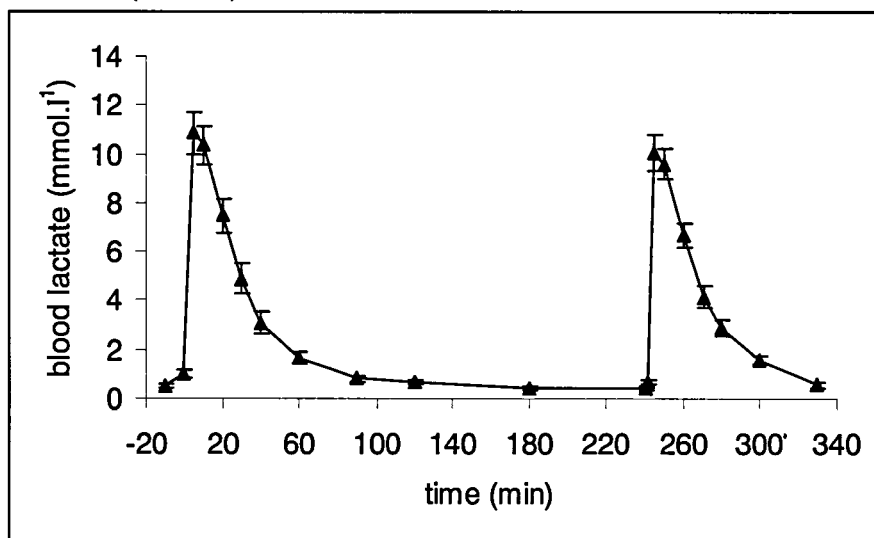(c) sprint main effect ($P<0.05$), time main effect ($P<0.05$).
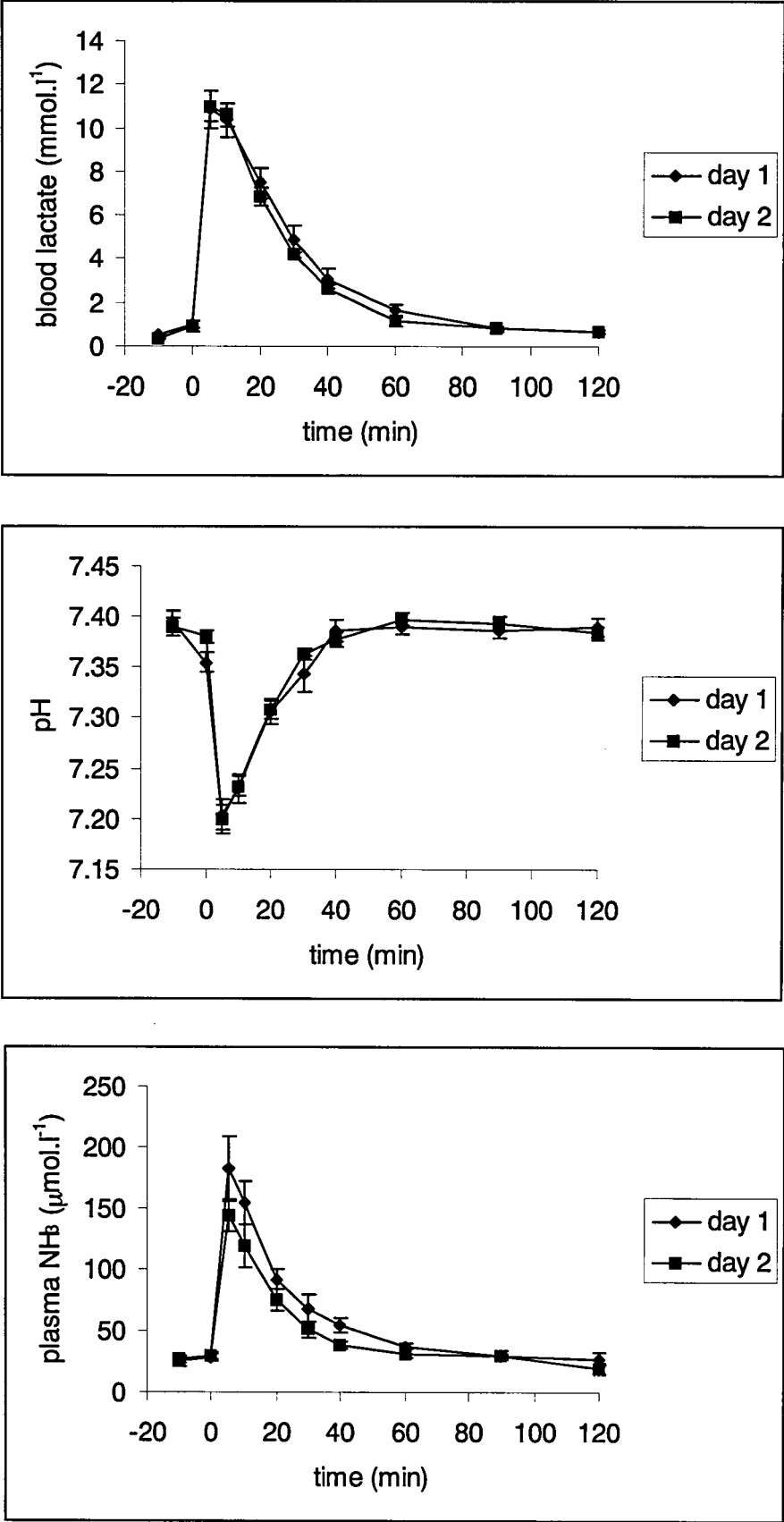
Figure 7.6. Mean serum hGH concentrations (a, n=8), mean serum IGF-I concentrations (b, n=3) and mean serum cortisol concentrations (c, n=8) at rest and during 120 min of recovery from sprint 1 in Trial B (day 1) and the sprint in Trial C (day 2).
(a) time main effect ($P<0.05$).
(c) time main effect ($P<0.05$).
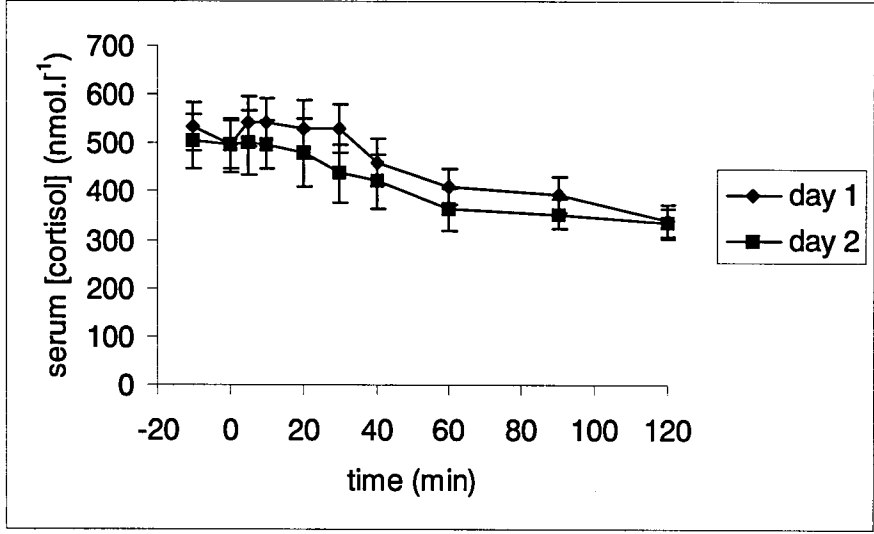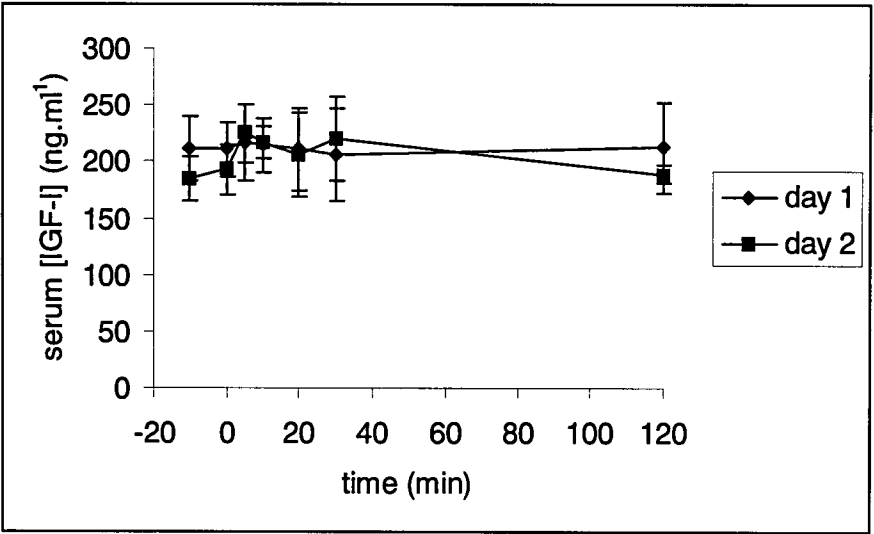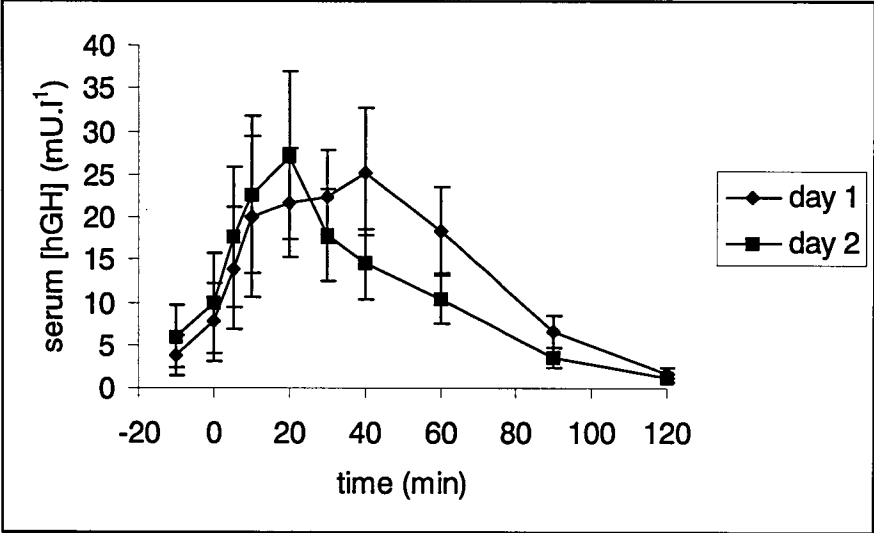
Table 7.2. Mean serum FFA concentrations (mol.l$^{-1}$) before sprint 1 and sprint 2 in trals A, B and C (n=7)

| Trial | Sprint 1 | Sprint 2 | |
|-------|----------|----------|---|
| A | 0.29±0.07 | 0.15±0.02 | |
| B | 0.33±0.09 | 0.76±0.11 | a |
| C | 0.33±0.09 | 0.46±0.11 | a |

a, sprint 1 vs. sprint 2, $P<0.05$

**Changes in plasma volume**

All of the sprints in Trials A, B and C resulted in a change in plasma volume with mean estimated changes in plasma volume of -17.3±1.2, -14.1±1.1, -13.4±1.2, -10.0±1.7 and −14.8±0.8% 5 min post-exercise in Trial A, sprint 1 and sprint 2, Trial B, sprint 1 and sprint 2, and Trial C respectively. There were no differences in estimated change in plasma volume following the two sprints in Trial A, or between Trial B sprint 1 and the sprint in trial C. However, the mean estimated changes in plasma volume were greater in the first sprint than the second sprint in Trial B (-3.9±1.0 vs. 0.2±1.1%, main effect sprint, $P<0.05$).

Table 7.3. Mean serum IGF-I concentrations (ng.ml$^{-1}$) at rest and 5 min after sprint 1 and sprint 2 in trials A, B and C (n=7).

| Trial | Sprint 1 | | Sprint 2 | | |
|-------|----------|---------|----------|---------|---|
| | Pre-ex | Post-ex | Pre-ex | Post-ex | |
| A | 199±16 | 238±22 | 191±13 | 227±23 | a |
| B | 208±21 | 208±23 | 190±20 | 210±31 | |
| C | 208±21 | 208±23 | 186±21 | 211±25 | b |

a, time main effect, $P<0.05$
b, sprint-time interaction effect, $P<0.05$

**Discussion**

The results of the present study demonstrate an attenuation of the hGH response to repeated bouts of cycle ergometer sprinting separated by 1 h of passive recovery. The hGH response to exercise was not fully restored within 4 h of recovery. However, the hGH responses to sprints completed at the same time on consecutive days were similar. A single 30 s cycle ergometer sprint has also been shown to induce an acute increase in IGF-I.

As shown in previous chapters, this study demonstrates that a single 30 s cycle ergometer sprint elicits a marked hGH response, and serum hGH concentrations were still elevated 60 min after the sprint. Furthermore, the results show once again that a second sprint, completed 60 min after the first, does not stimulate further hGH secretion since both mean highest measured hGH concentrations and hGH AUC were significantly lower following the second sprint. These results add further support for a role of hGH autofeedback, possibly at the level of the pituitary.

The results of the present study also demonstrate an acute increase in serum IGF-I concentrations following both sprints in Trial A. Highest measured serum IGF-I concentrations were identified 5 min after each sprint and were significantly different from resting concentrations, however, they had returned to close to pre-exercise levels within 10 min of recovery. Previously, conflicting results have been reported regarding the effect of exercise on IGF-I. Neither Wilson and Horowitz (1987) nor Hagberg et al. (1988) identified an increase in IGF-I following exercise. However, Bang et al. (1990) reported a 26% increase in IGF-I 10 min into a 30 min exercise bout and suggested a rapid time course for the exercise-induced hGH response. In agreement with these findings, Cappon et al. (1994) identified an increase in circulating IGF-I of ~14 % after high intensity submaximal exercise, whilst Weller et al. (1999) observed increased levels of circulating IGF-I immediately, but not 1 h, after an incremental exercise test to exhaustion. The results of the present study support these findings, demonstrating a rapid time course for the acute IGF-I response to sprint exercise.

This acute increase in serum IGF-I concentrations appears to be independent of serum hGH concentrations, since, in the present study, highest measured mean IGF-I

concentrations occurred ~35 min before the highest measured mean hGH concentrations following the first sprint in Trial A. Cappon et al. (1994) came to the same conclusion since they demonstrated that the time courses of the IGF-I and hGH responses to 10 min of exercise at ~70% $VO_2$max were similar. In contrast, it took several hours before an increase in IGF-I concentrations was detected following rhGH administration in healthy elderly individuals (Marcus et al., 1990). Further evidence for a hGH independent increase in IGF-I levels following exercise in humans is the finding that the magnitude of the IGF-I response to high intensity submaximal exercise was not altered despite the attenuation of the exercise-induced hGH response by a high fat meal (Cappon et al., 1994).

However, the mechanism for the acute exercise-induced increase in circulating IGF-I levels is unclear. Most current evidence suggests that circulating IGF-I is of hepatic origin (Maiter et al., 1988) but as explained in the previous paragraph, hepatic IGF-I synthesis and secretion takes longer than 5 min (the time taken to reach mean highest measured IGF-I in the present study). It has been shown that IGF-I peptide concentrations are elevated in muscles undergoing hypertrophy (Adams and Haddad., 1996) and that hGH might have a role in this local IGF-I production (Adams and McCue, 1998). Zanconato et al. (1994) found 4 wk of treadmill exercise training in female rats to result in an increase in hepatic and skeletal muscle IGF-I mRNA. However, additional GH suppression, with GHRH antisera, inhibited IGF-I mRNA to a greater extent in the liver than in skeletal muscle suggesting a less important role of hGH in the regulation of muscle IGF-I production than hepatic IGF-I production. It is, therefore, possible that exercise results in an acute increase in local production of IGF-I in exercising muscle by an, as yet, unidentified mechanism. Furthermore, acute exercise-induced increases in circulating IGF-I levels, rather than being of hepatic origin, might reflect an efflux of locally produced IGF-I from exercising muscles.

Since serum IGF-I concentrations in the present study were not elevated following 60 min of recovery from a single sprint but, in fact, returned to pre-exercise levels within 10 min of recovery, it seems that circulating IGF-I does not play a role in the attenuation of the hGH in repeated bouts of sprint exercise separated by 1 h of recovery. These results do not, however, preclude the possibility that IGF-I produced

locally in the pituitary gland is involved in the regulation of the hGH response to repeated exercise.

The increased serum hGH concentrations following a single sprint were found to have returned to pre-exercise levels within 2 h of recovery and remained at this resting concentration until 4 h of recovery (when the second sprint was completed in Trial B). The second sprint in Trial B was observed to elicit a hGH response although there was a trend for a decreased hGH AUC ($P$=0.09) and a lower mean highest measured hGH ($P$=0.09). These results suggest that there is still a degree of inhibition of hGH release, but that it is not as great as that seen in Trial A with 60 min of passive recovery. However, the fact that serum hGH concentrations are at resting levels immediately prior to the second sprint in Trial B suggests that hGH does not play a direct role in the inhibition of its own secretion in this trial.

In contrast to both sprints in Trial A, the first sprint in Trial B did not result in an acute increase in circulating IGF-I concentrations. The explanation for this is unclear, but it does suggest that more research is required to further the understanding exercise-induced IGF-I synthesis and release. However, it was expected that there would be an increase in circulating IGF-I levels in response to the increase in serum hGH concentrations following the first sprint in Trial B, since Marcus et al. (1990) demonstrated an acute increase in IGF-I several hours after rhGH administration. In addition, Bengtsson et al. (1993) identified a dose-dependent increase in circulating IGF-I concentrations following rhGH treatment of hGH deficient adults. In the present study there does appear to be a small elevation in IGF-I concentrations 180 min after the first sprint in 3 subjects, however, by 240 min of recovery (when the second sprint was performed in Trial B), serum IGF-I concentrations were not significantly different from levels prior to sprint 1. In fact, in 6 out of the 7 subjects for which IGF-I was measured 240 min after sprint 1, serum IGF-I concentrations were lower than they were before sprint 1. These findings, in addition to the observation that IGF-I immunoreactivity only started to increase 2 days after, and were not significantly elevated until 4 days after, a single bout of 192 eccentric contractions in rats (Yan et al., 1993), suggest that hGH-induced increases in circulating IGF-I following exercise take longer than 4 h to become apparent. It is unlikely, therefore, that IGF-I played a significant role in the attenuation of the hGH

response to the second sprint, and, therefore, that another mechanism is responsible for the tendency for an attenuated hGH response in this trial.

The results of the present study demonstrate that serum FFA concentrations are elevated 4 h after a single 30 s sprint. Administration of rhGH has been shown to induce a significant increase in lipid intermediates, with concentrations of NEFA, 3-hydroxybutyrate and glycerol remaining elevated for at least 300 min (Vahl et al., 1997), which is 60 min longer than the recovery period in the present study. In addition, Casanueva et al. (1987) demonstrated that FFA can block hGH secretion directly at the pituitary gland. In the present study it is, therefore, possible that hGH secretion induced by the first sprint elicited an increase in lipolysis and, consequently, increased circulating FFA levels. In turn these FFA might participate in the attenuation of the hGH response to a second sprint performed after 4 h of recovery from the first sprint.

Another possibility is that the smaller hGH response to sprint 2 compared with sprint 1 in Trial B is a reflection of the smaller metabolic response, measured by lower blood lactate and plasma ammonia concentrations and higher pH levels, to sprint 2. A number have studies have suggested that afferent signals from metabolic receptors in the muscles might have a role in the regulation of the hGH response to exercise (Kozlowski et al., 1983; Nevill et al., 1996b; Viru et al, 1998). However, Kjaer et al. (1996a) used electrically induced cycling exercise with afferent sensory blockade by epidural anaesthesia and found no decrease in the hGH response compared with voluntary cycling at the same $VO_2$ as in electrically induced exercise. Exercising under hypoxic conditions, which enhances the metabolic response in exercising muscle, would be expected to increase both afferent feedback from muscle metabolic receptors and blood-mediated afferent signalling. Kjaer et al. (1999) demonstrated that in subjects cycling under hypoxic conditions, epidural anaesthesia did not blunt the hGH response. These results infer that afferent feedback from muscle metabolic receptors is not as important as a blood-borne feedback mechanism in the regulation of the hGH response to exercise.

However, previous studies in this thesis have provided evidence that blood lactate concentrations and blood pH do not play an important role in the regulation of hGH

secretion, since there was a divergence between both blood lactate and blood pH responses and the hGH response to exercise at different pedal speeds (Chapter 5). In addition, following 6 weeks of sprint training there is an attenuation of the hGH response to sprint exercise, with no significant differences in the blood pH response to sprinting, and increased highest measured post-exercise blood lactate concentrations (Chapter 6). However, the relationship between plasma ammonia and serum hGH concentrations is less clear, and in Trial B there is a tendency for both to be lower following sprint 2. It is, therefore, possible that plasma ammonia might play a role in the regulation of the hGH response to exercise, and that lower plasma ammonia concentrations following sprint 2, rather than any feedback effects, are responsible for the smaller hGH response to the second bout of sprinting.

It is possible that when the second sprint was completed in Trial B, exercise-induced hGH release was still attenuated, but that this was overcome by the subjects' fasted states since they had not eaten for ~18 h at the onset of the second sprint. However, it is unlikely that fasting alone increased serum hGH concentrations in the present study since Galbo et al. (1981) did not identify any change in resting hGH concentrations following a 59 h fast. Conversely, Galbo et al. (1981) measured higher plasma hGH concentrations during exercise in healthy men following a 59 h fast compared with an overnight fast. It is, therefore, possible that there was an interaction between fasting and exercise, a possibility that was discussed by Kanaley et al. (1997) as an explanation for an augmented hGH response to repeated submaximal exercise. It is also possible that the time of day that each sprint was completed affected the hGH response since, in Trial B, the first sprint was completed between 0930 and 1000, and the second sprint between 1330 and 1400 by all subjects. However, Galliven et al. (1997) and Scheen et al. (1998) did not observe any changes in the magnitude of the hGH response to exercise at different times of day. Therefore, the tendency for a reduced hGH response to the second sprint in Trial B is probably a result of feedback inhibition rather than the effect of time of day.

Resting serum IGF-I concentrations in Trial C were lower than resting concentrations in Trial B, the previous day. This might reflect a change in the pattern of nocturnal hGH secretion as a result of exercise on the previous day. Kern et al. (1995) found that moderate intensity exercise altered the temporal pattern of nocturnal hGH

secretion without affecting total hGH secretion. Specifically, peak hGH was diminished during early sleep. Since IGF-I concentration correlates better with pulsatile than basal GH secretion (Maiter et al., 1988) this might reduce the circulating concentration of IGF-I the following morning.

In contrast to the first sprint in Trial B, the 30 s cycle ergometer sprint in Trial C did induce an acute IGF-I response, although there was no significant difference between the trials because of the lower resting serum IGF-I concentrations in Trial C. These lower resting IGF-I levels in Trial C might be expected to result in an augmented hGH response to sprinting due to reduced inhibition by IGF-I, whilst, conversely, higher pre-exercicse serum FFA concentrations day 2 than on day 1 might be expected to attenuate the hGH response to exercise. However, there was no difference in the hGH response to the sprint in Trial C, compared with the first sprint in Trial B. In contrast, there was a lower plasma ammonia response to the sprint performed on day 2 compared with the sprint performed on day 1. This dissociation between the hGH response to exercise and the plasma ammonia response to exercise suggests that increased plasma ammonia concentrations following sprint exercise might not play a major role in the regulation of the exercise-induced hGH response. The PPO attained during the sprint performed on day 2 was also lower than that in the sprint on day 1. This did not significantly alter the hGH response to the exercise bout and suggests that PPO is not an important factor in the regulation of the hGH response to sprint exercise. It appears, therefore, that sprint exercise on the previous day does not affect the hGH response to sprint exercise via an IGF-I mediated, or any other, mechanism.

In conclusion, this study has provided further evidence for direct hGH autoinhibition when exercise bouts are separated by 1 h of passive recovery. However, a longer recovery period does not fully restore the hGH response to sprint exercise despite a return of serum hGH concentrations to resting levels. This divergence between the hGH response and pre-exercise hGH levels suggests the presence of another mechanism for the inhibition of hGH in response to repeated exercise. Since IGF-I concentrations were not elevated immediately prior to a second sprint performed 4 h after the first, it is possible that this mechanism is a hGH-induced increase in circulating FFA. There is a tendency for resting IGF-I levels to be lower following

exercise on the previous day, possibly as a result of a change in the temporal pattern of nocturnal hGH secretion. However, the hGH response to cycle ergometer sprinting is not altered by these changes and the hGH response to sprint exercise on consecutive days is similar.

# CHAPTER 8

## GENERAL DISCUSSION

The aim of this chapter is to draw together the findings of the experimental studies in this thesis, to provide an explanation for these findings and to consider how the results of the four studies have provided information regarding the mechanisms regulating the release of hGH as a result of exercise.

### 8.1. Main findings

- Chapter 4 describes the time course of the hGH response to a single cycle ergometer sprint of either 6 s or 30 s duration. A single 30 s sprint resulted in a marked (530 %) increase in serum hGH concentrations with highest measured mean serum hGH concentrations more than four and a half times greater than those seen when a single 6 s sprint was performed. A 30 s sprint also resulted in the exercise-induced elevation in hGH concentrations persisting for between 90 and 120 min, compared with less than 60 min following the 6 s sprint. In addition, this study highlights the inter-individual variation in the hGH response to sprint exercise, identifying differences in both the magnitude of, and time taken to reach highest measured hGH concentrations across the 8 subjects taking part in the study.

- Chapter 5 investigates the effect of repeated sprinting on the hGH response to exercise, and also considers the influence that manipulating pedalling rate, and therefore the number of muscle actions, during a sprint has on hGH secretion following sprinting. As in Chapter 4, a single 30 s sprint was found to be a potent stimulus for hGH release, and hGH concentrations remained elevated for at least 60 min. However, when a second sprint was completed 60 min after the first, hGH concentrations were not elevated any further, that is there was no hGH response to the second sprint. In addition, cycle ergometer sprinting at a faster pedalling rate (more muscle actions) resulted in a hGH response twice as great as that following a sprint performed at a slower pedalling rate, despite a similar metabolic response.

- Chapter 6 considers the effect of a short-term sprint-training programme on the hGH response to repeated sprint exercise. Six weeks of combined speed and speed-endurance training resulted in a blunting of the hGH response to a maximal cycle ergometer sprint compared with pre-training values, despite improvements in sprint performance. In addition, in both pre- and post-training repeated sprint tests, the first sprint resulted in a marked hGH response whilst the hGH response to the second sprint, completed 60 min later, was attenuated (as in Chapter 5).

- Chapter 7 examines the effect of the length of the recovery period between sprints on the attenuation of the hGH response to the second sprint, with particular reference to the possible role of IGF-I. Consistent with the results in Chapters 5 and 6, there was a marked increase in serum hGH concentrations following a single 30 s sprint, but when a second sprint was completed 60 min later there was no further hGH response. Extending the recovery period to 4 h did not result in complete restoration of the hGH response to the second sprint, however, 24 h of recovery was sufficient to allow full recovery of the hGH response to a further 30 s sprint.


## 8.2. The time course of the hGH response to a single sprint

Relatively few studies have considered the time course of the hGH response to exercise, and none have previously described the entire time course of the hGH response to sprint exercise. The study that is described in Chapter 4 required subjects to complete a single 6 s sprint in one trial, and a single 30 s sprint in another. A single 6 s sprint resulted in an increase serum hGH concentrations of more than 200 % over resting levels, and levels remained elevated for 60-90 min after exercise.

In all four experimental chapters a single 30 s sprint resulted in a marked hGH response with highest measured mean serum hGH concentrations ranging from 11.9 to 37.7 mU.l$^{-1}$ observed between 20 and 40 min after exercise. The results of the study in Chapter 4 demonstrate highest measured mean hGH concentrations to be more than four and a half times greater following a 30 s sprint than following a 6 s sprint. Furthermore, in all four experimental chapters hGH concentrations remained elevated for at least 60 min of recovery. In Chapters 5 and 6, and in one trial in Chapter 7, subjects completed a second 30 s sprint at this time and therefore it was not

possible to study the entire time course of the hGH response to sprint exercise. However, in Chapters 4 and 7 serum hGH concentrations following exercise were studied for 3 h and 4 h, respectively. Serum hGH concentrations were found to elevated for between 90 and 120 min in both studies, although, the results in Chapter 4 show that whilst hGH concentrations had returned to pre-exercise values within 120 min of recovery in most individuals, they remained elevated for a longer period in some subjects. Kraemer et al. (1990) found that various resistance exercise protocols resulted in different hGH responses, however, in each case hGH concentrations had returned to pre-exercise values within 2 h. Raynaud et al. (1983) studied the time course of the hGH response to different types of work, but only measured hGH concentrations for 60-90 min post-exercise, at which point hGH levels were still elevated in some, but not all, subjects.

The results of the study reported in Chapter 4 show inter-subject variability in both the magnitude of the hGH response to sprint exercise, and the time taken to reach highest measured concentrations (Figure 4.5). These findings are consistent with those of Raynaud et al. (1983), and this inter-subject variability provides some explanation for the difficulty encountered in the identification of relationships between hGH concentrations and other variables when attempting to determine the mechanisms responsible for exercise-induced hGH secretion. Furthermore, the variation between subjects in the results of these studies highlights the difficulty of reporting data of this nature, and supports the suggestion of Raynaud et al. (1983) that care must be taken when drawing conclusions from averaged hGH results when there is inherent variability between the responses of different individuals. It has been suggested that it is possible to determine distinct groups of individuals with similar patterns of hGH response to exercise (Raastad et al., 2000), however, such distinctions could not be made in the study in Chapter 4. This finding suggests that there is a continuum covering the inter-subject variation in the magnitude of the hGH response to sprint exercise which cannot be clearly divided into sub-groups according to subjects' 'responsiveness'.

In summary, the results in this thesis show that sprint exercise results in a marked increase in serum hGH concentrations. In addition, both the magnitude and duration of the hGH response to sprint exercise is determined, directly or indirectly, by the

length of the sprint. Furthermore, the results of this thesis have highlighted the inter-individual variation in the hGH response to exercise, identifying that care must be taken when drawing conclusions from data with such inherent variability.

## 8.3. The effect of repeated exercise on the hGH response to sprinting

In the studies reported in Chapters 5, 6 and 7 the same protocol was used in at least one of the trials, whereby subjects completed a single sprint followed by 60 min of recovery, at which time a second 30 s sprint was performed. The results of all three studies identified a marked increase in serum hGH concentrations after the first, but not the second, sprint. Similarly, all three studies found that 60 min after the first sprint, that is immediately before the performance of a second sprint, hGH concentrations were still above pre-exercise concentrations. This finding is consistent with the observation in Chapter 4 that serum hGH concentrations remain elevated for between 90 and 120 min in most subjects, and even longer in some.

Other studies have identified an attenuation of spontaneous and GHRH-stimulated GH secretion after exogenous GH administration in rats (Lanzi and Tannenbaum 1992a; 1992b), whilst repeated GHRH administration in humans has been shown to result in a progressively decreasing hGH response (Ghigo et al., 1991). However, the evidence regarding the effect of repeated bouts of exercise on the hGH response is less equivocal. Kanaley et al. (1997) demonstrated an augmented hGH response to repeated bouts of 30 min exercise at 70 % $VO_2max$, whilst Cappon et al. (1994) found that the hGH response to 10 min of constant power cycling, at an intensity corresponding to 50 % of the difference between lactate threshold and $\dot{V}O_2max$, was attenuated by prior exercise. The results of the studies in this thesis support the results of Cappon et al. (1994), suggesting that repeated high intensity exercise will result in an attenuation of the hGH response.

The mechanism by which the hGH response is attenuated is not entirely clear. Jaffe et al. (1993) suggested that pituitary stores of hGH far exceed the amount released as a result of a single GHRH stimulus, yet they identified a suppression of the hGH response to repeated GHRH administration. Roles for circulating hGH, IGF-I and FFA have all been suggested in the attenuation of the hGH response to repeated

stimuli. In Chapters 5, 6 and 7 it was evident that hGH was elevated as a result of the first sprint, and was still high after 60 min of recovery, when the second sprint was performed. Growth hormone has the ability to inhibit its own release, possibly at the level of the pituitary gland (Pontiroli et al., 1991), or at the hypothalamus. Immunoneutralisation of somatostatin has been shown to reverse rhGH-induced attenuation of spontaneous and GHRH-induced GH release in rats (Lanzi and Tannenbaum, 1992a; 1992b). These results and the finding of Burton et al. (1991), who demonstrated a colocalisation of GH receptor mRNA in somatostatin neurons in the rat, provide strong evidence of a role for somatostatin in hGH-induced negative feedback, however, a possible role for GHRH secretion cannot be excluded (Lanzi and Tannenbaum, 1992a).

The results of the study described in Chapter 7 show that 4 hr of recovery from a single 30 s cycle ergometer sprint allows time for exercise-induced elevations in hGH to return to pre-exercise levels. This finding is consistent with the results in Chapter 4, which demonstrate that hGH concentrations return to pre-exercise levels within 120 min of recovery in most subjects. However, when a second sprint was performed after 4 h of recovery from the first sprint there was a tendency for the hGH response to be attenuated. Therefore hGH secretion was still inhibited, but it is unlikely that this is a result of hGH inhibition of its own release.

It might be expected that there would be an increase in circulating IGF-I concentrations within 4 h as a result of the hGH response to the first sprint, since Marcus et al. (1990) demonstrated an acute increase in IGF-I several hours after rhGH administration. In addition, an increase in circulating IGF-I might inhibit hGH secretion, since a purified IGF-I preparation has been shown to inhibit GH release from rat pituitary cells in culture, and to stimulate a dose-dependent release of somatostatin release from hypothalamic explants (Berelowitz et al., 1982). The infusion of rhIGF-I has also been shown to suppress pulsatile and GHRH-stimulated hGH secretion in male subjects (Jaffe et al., 1998). However, in the study in Chapter 7, serum IGF-I concentrations increased immediately after exercise, probably as a result of increased local production of, and increased efflux from exercising muscles of, IGF-I, but returned to pre-exercise levels within 10 min. Whilst there appeared to be an increase in circulating IGF-I concentrations 3 h after the first sprint, IGF-I had

returned to pre-exercise levels by 4 h, when the second sprint was performed. Therefore inhibition of hGH secretion cannot be explained by increased circulating IGF-I concentrations in this trial. In contrast, the results of the study reported in Chapter 7 demonstrate elevated FFA concentrations after 4 h of recovery after a single 30 s sprint. It is possible, therefore, that a hGH-induced increase in FFA might explain the inhibition of hGH release after 4 h of recovery, since Casanueva et al. (1987) demonstrated that FFA can block hGH secretion directly at the pituitary gland.

In summary, in the studies in this thesis it is likely that, where hGH levels are elevated as a result of prior exercise, further hGH release is inhibited either directly at the pituitary or at the level of the hypothalamus, through an increase in somatostatin release and/or a decrease in GHRH secretion. With a longer period of recovery, which allows exercise-induced elevations in hGH to return to pre-exercise values, the attenuation of the hGH response to a second bout is still apparent, possibly as a consequence of elevated FFA concentrations. It is likely that increased circulating IGF-I concentrations might also have a role in the inhibition of hGH secretion, but the failure to raise serum IGF-I levels in the study in Chapter 7, means that it is not possible to comment on this mechanism from the results of this thesis.

### 8.4. The effect of training on the hGH response to sprinting

A number of studies have considered the effect that exercise training has on the hGH response to exercise, but there is little agreement between the results of these studies. Concentrations of hGH following exercise have been found to increase (Bunt et al., 1986; Bonifazi et al., 1998; McCall et al., 1999), decrease (Bloom et al., 1976; Weltman et al., 1997), and not to change (Kraemer et al., 1990) as a result of training. The results of the study presented in Chapter 6 show that 6 wk of sprint training resulted in an attenuation in the hGH response to sprint exercise. This finding is not consistent with the only other short-term, longitudinal training study that includes a control group (McCall et al., 1999), which found that resistance training resulted in an augmentation of the hGH response to exercise. The reason for the conflicting findings of these two studies is unclear, but it might reflect the different exercise performed during both training and testing.

It is possible that the attenuation of the exercise-induced hGH response in the study in Chapter 6 is a result of a sudden increase in training volume for the subjects. Overtrained endurance cyclists have been found to have impaired pituitary function (Urhausen et al., 1998), although Fry et al. (1998) did not identify a decrease in circulating hGH concentrations after exercise following high intensity resistance overtraining. It is, therefore, not clear whether overtraining might have occurred in the study reported in this thesis. However, when the results of this study and those of Bloom et al. (1976) and Weltman et al. (1997), who also observed a decrease in exercise-induced hGH secretion, are taken together, it is unlikely that in all of these cases an attenuation of the hGH response was a manifestation of overtraining.

The results of the study described in Chapter 6 also demonstrate that 6 wk of sprint training does not alter resting hGH concentrations, and this finding is in agreement with training studies employing short-term endurance (Bonifazi et al., 1998) and resistance exercise (Kraemer et al., 1998; McCall et al., 1999). However, Weltman et al. (1992) found that a longer period (1 yr) of endurance training resulted in an increase in resting hGH concentrations in women. In addition, it has been shown that resting IGF-I concentrations, which have been used as measure of integrated hGH secretion since it is hGH dependent, increase after two weeks of endurance training (Roelen et al., 1997). From the results of the study in Chapter 6 it appears that short-term sprint training does not alter resting hGH concentrations, but it might be that this is due to the fact that resting hGH levels are so low that detection is difficult. In order to satisfactorily determine the effect of training on hGH concentrations at rest, more than one resting sample would have to be taken. In fact, it would be interesting to determine the effect of exercise training on integrated 24 h hGH secretion, which would provide a much clearer picture regarding the effect of training on hGH regulation.

## 8.5. Possible mechanisms regulating hGH secretion following sprint exercise

### The roles of lactate and pH

The study described in Chapter 5 demonstrates that manipulating pedalling rate, and therefore the number of muscle actions, during sprint exercise has no effect on the blood lactate or blood pH response to that exercise. This finding is in agreement with

studies manipulating pedalling rate during maximal isokinetic cycling (Jones et al., 1985) and sprinting on a friction loaded cycle ergometer (Cherry et al., 1998). However, the results in Chapter 5 show that there is a greater hGH response to sprinting against an applied resistance of 7.5 %, compared with 10 %, of the subjects' body mass, that is there was a greater hGH response when pedalling at faster pedal speeds. The divergence in the blood pH and blood lactate and the serum hGH responses suggests that blood lactate and blood pH do not regulate the secretion of hGH during exercise.

The findings of the study reported in Chapter 6 demonstrate that 6 wk of sprint training results in an attenuation of the hGH response to exercise, despite an increase in highest measured mean blood lactate concentrations, and no change in blood pH responses. These findings are consistent with those of Weltman et al. (1997) who described a different time course for the alteration of hGH and blood lactate concentrations as a result of exercise, following 6 wk of endurance training. The results of these studies suggest that blood lactate and pH do not regulate exercise-induced hGH secretion.

Whilst significant correlations have been found between blood lactate and muscle lactate (Cheetham et al., 1986), and between blood pH and muscle pH (Allsop et al., 1990), poor predictability of muscle pH from a given value of blood pH has also been reported (Allsop et al., 1990). If the blood lactate concentrations and blood pH levels found in Chapters 5 and 6 in this thesis reflect muscle lactate concentrations and muscle pH levels, it would appear that there is neither a role for blood-borne regulation of hGH secretion by lactate and pH, nor a role for metabolic receptors in skeletal muscle. Kjaer et al. (1999) blocked afferent nerve activity using epidural anaesthesia, and found that this did not blunt the hGH response to leg cycling exercise, suggesting that exercise is not modulated by afferent feedback from muscle metabolic receptors. This finding supports the conclusions of the studies in this thesis.

**Proprioceptive feedback**

It has been suggested that activity in motor centres might directly stimulate pituitary hormone secretion during exercise (Kozlowski et al., 1983; Kjaer et al., 1987; Kjaer et

al., 1989b; Kjaer et al., 1996b). In the study in Chapter 5 it is possible that sprinting at faster pedalling rates was associated with greater motor centre activity, resulting in a larger hGH response. However, the sprints performed in these trials were, by definition, maximal, making a higher level of motor centre activity at faster pedalling rates unlikely. Another possibility is that proprioceptive feedback from exercising muscles provides a stimulus for hGH release. Kjaer et al. (1996b) suggested that humoral feedback mechanisms and autonomic nervous reflexes might exert redundant control of pituitary hormonal responses, whilst Gosselink et al. (1998) found evidence of a proprioceptive mechanism for the regulation of bioassayable, but not immunoassayable, GH in rats. Significant correlations between both PPR and MPR and serum hGH concentrations in the study in Chapter 5 suggest that a proprioceptive mechanism might also play a role in the regulation of immunoassayable hGH during sprint exercise.

**A possible role for plasma ammonia**

In Chapter 5 it was found that the plasma ammonia response to sprint exercise at faster pedalling rates was greater than the response to sprinting at slower pedalling rates. Although no significant correlations were found, the plasma ammonia response to sprinting at different pedalling rates followed the same trend as the serum hGH responses. In addition, Chapter 6 identified a decrease in both the plasma ammonia response to sprint exercise and the serum hGH response to exercise following 6 wk of sprint training. In Chapter 7, when a second sprint was completed 240 min after the first, there was a tendency for both the plasma ammonia and the serum hGH responses to be lower than they were following the first sprint, although a 24 h recovery period resulted in a dissociation between the hGH response and plasma ammonia response to the sprint on the second day. However, the results of all of these studies, taken together, indicate that there might be an association between circulating ammonia concentrations and hGH secretion.

It has been proposed that blood ammonia produced during exercise might have direct access to the brain, and, since it is neurotoxic, it has been implicated as having a role in the development of central fatigue (Mutch and Banister, 1983; Banister and Cameron, 1990). Increases in brain ammonia stimulates glutamine synthesis, which is the most important alternate pathway for ammonia disposal in the brain (Lockwood et

al., 1979). The increased synthesis of glutamine from glutamate and ammonia is catalysed by an increase in the activity of glutamine synthase, which is found mainly in the glial cells. Furthermore, glutamine synthesis results in decreased brain glutamate content, and, as glutamate is a precursor for GABA synthesis, enhanced glutamine synthesis as a result of increased brain ammonia levels might also decrease GABA levels (Cooper and Plum, 1987). Intravenous injection of ammonium acetate in rats has been found to result in a decrease in the brain content of glutamate and GABA between 15 and 30 min after administration (Kanamatsu and Tsukada, 1999). In addition, exercise to exhaustion was found to result in increased brain glutamine in both trained and untrained rats, whilst brain glutamate were slightly decreased only in trained rats, which ran more than four times longer than control rats and had ~50% higher brain ammonia after exercise (Guezzenec et al., 1998).

The use of $^{13}$C labelling in rats has demonstrated that the anaplerotic pathway contributes to γ-aminobutyric acid (GABA) and glutamate synthesis through trafficking of glutamine from glia to neuron (Kanamatsu and Tsukada, 1999). In the same study, intravenous injection of ammonium acetate was found to increase the rate of contribution of the anaplerotic pathway to brain amino acid synthesis, probably due to an increase in pyruvate carboxylase (PC) activity (Kanamatsu and Tsukada, 1999). Lapidot and Gopher (1994) reported that the anaplerotic pathway could account for 16 % of GABA synthesis, whilst Kanamatsu and Tsukada (1999) estimated a 13 % contribution after glucose administration, and a 23 % contribution when glucose administration was combined with that of ammonium acetate. It seems, therefore, that blood ammonia produced as a result of exercise might cross the blood-brain barrier, resulting in increased activity of glutamine synthase and a consequent decrease in brain glutamate and GABA content. However, it is likely that an additional increase in PC activity maintains TCA pool intermediates allowing continued synthesis of glutamate via α-keto glutarate.

Both glutamate and GABA are known to act as neurotransmitters. Glutamate is an exitatory amino acid (EAA), whilst GABA is an inhibitory amino acid, and it has been suggested that there might be a role for both in the control of anterior pituitary hormone secretion. In this thesis, smaller increases in plasma ammonia

concentrations and serum hGH concentrations were observed following sprinting at slower pedalling rates compared with faster pedalling rates (Chapter 5), and following a 6 wk training period (Chapter 6). It is possible that the smaller plasma ammonia responses to sprint exercise at slower pedal speeds and following training might result in a smaller reduction of brain content of glutamate and GABA. Since glutamate is thought to stimulate hGH release, a smaller reduction in brain glutamate content as a result of reduced ammonia production might be expected to result in a greater hGH response. However, this is not consistent with the findings of the studies in this thesis which identify smaller increases in plasma ammonia concentrations to be accompanied by smaller hGH responses to exercise. Therefore, it is possible that reduced GABAergic inhibitory tone is more important in regulating the magnitude of the hGH response to exercise, and the smaller increases in plasma ammonia concentrations observed following sprinting at slower pedalling rates, and following a 6 wk training period, might result in a smaller reduction of brain content of GABA. The resulting higher brain content of GABA might, in turn, inhibit exercise-induced hGH secretion, resulting in lower serum hGH concentrations.

It is possible, therefore, that increased plasma ammonia during exercise has a role to play in the regulation of the hGH response to exercise, since increases in plasma ammonia might cross the blood-brain barrier and alter brain content of the neurotransmitters glutamate and GABA via a change in the rate of glutamine synthesis. However, uncertainty over the effect of increases in circulating ammonia concentrations on brain glutamate and GABA content, in addition to the need for greater understanding of the role of both glutamate and GABA in the regulation of hGH secretion means that further work must be done to establish the importance of this potential mechanism for exercise-induced hGH secretion.

**Summary of mechanisms of hGH release during sprint exercise**

From the results of the studies in this thesis it is possible to comment on various potential mechanisms involved in the regulation of exercise-induced hGH secretion. Previous studies have considered the roles of blood lactate and blood pH, but the results of the studies described in Chapters 5 and 6, indicate that neither blood lactate nor blood pH are the prime regulators of the hGH response to exercise. Similarly, if it is assumed that blood lactate concentrations and blood pH levels reflect the situation

in exercising muscles, these results also infer that the proposed afferent signalling from muscle metabolic receptors do not regulate the magnitude of the hGH response to exercise.

Although it has not been possible to study the level of motor activity during exercise as part of the studies in this thesis, it is likely that motor centre activity plays an important role in hGH release. In addition, it is possible that proprioceptive feedback signals might provide a mechanism for the modulation of hGH secretion. In addition, blood-borne afferent signalling might be important, since Chapters 5 and 6 identified similar changes in the plasma ammonia responses and the serum hGH responses to the sprint exercise at different pedalling rates, and following a short-term training programme, respectively. It is possible that increased circulating ammonia concentrations alter amino acid synthesis in the brain, resulting in a change in the brain content of GABA, which, in turn, has a role in the regulation of hGH secretion.

## 8.6. Possibilities for future research

From the results of the studies in this thesis it appears that blood pH and blood lactate concentrations do not have a principal role in the regulation of the hGH response to exercise. It is inferred from these findings that accumulation of metabolites in muscle, sensed by metabolic receptors in the muscle, does not determine the magnitude of the hGH response, however, direct measurement of muscle metabolites using the muscle biopsy technique would provide further evidence that this is the case. In addition, the results of the studies in this thesis have suggested that there is a possible link between ammonia production during sprint exercise and the resulting hGH response. Manipulation of circulating ammonia concentrations both at rest and during exercise would provide further information about this potential axis for hGH regulation. Alternatively, phosphorylase deficient individuals, who have been observed to have abnormally elevated ammonia production during exercise, or AMP deaminase deficient individuals, who have an inability to deplete adenine nucleotides to IMP and ammonia during cellular conditions of energy imbalance, could be studied to compare the hGH response to exercise in these conditions to that of healthy individuals.

There is still some doubt as to the relative importance of somatostatin and GHRH in regulating the hGH response to exercise, and it has been proposed that other GHRPs occur in the body. Therefore, further study is required to better understand the mechanisms by which the hGH response to exercise is regulated. The effect of the administration of octreocide (a somatostatin analogue), GHRH or other GHRPs prior to exercise on hGH secretion has been considered in some studies. However, further studies increasing somatostatinergic tone using octreocide, or decreasing somatostatinergic tone using pyridostigmine might elucidate the importance of this hypothalamic factor, whilst administration of GHRH and/or GHRPs, or a GHRH-antagonist would provide further information about the importance of these peptides in regulating pituitary hGH secretion.

It has been identified that there is a feedback loop inhibiting the hGH response to repeated stimuli. It is possible that this is mediated by elevated circulating levels of hGH itself, but the fact that the hGH response to exercise did not fully recover after 4 h of recovery, when serum hGH had returned to pre-exercise levels suggests that another mechanism is important. It would be interesting to discover the length of time that is required for the recovery of the hGH response to exercise, as well as to determine the mechanisms involved. A role for increased circulating FFA concentrations in inhibiting hGH secretion has been postulated in this thesis, and the importance of FFA in the attenuation of hGH secretion with repeated exercise warrants further study. One method by which the role of FFA might be investigated is by blocking lipolysis with acipimox. A full understanding of the refractory period of the hGH response to exercise could provide important information in the maximisation of the anabolic effect of exercise.

Chapter 6 of this thesis considered the effect of a short-term sprint training programme on the hGH response to exercise. The influence that different types of training (endurance, resistance, sprint), over both the short-term and the longer-term, have on the hGH response to exercise warrants further study, since there is little agreement between the results of studies that are currently available. In addition, the effect of training on integrated 24 h hGH and IGF-I concentrations would provide an interesting focus for further study, particularly in children, adolescents, frail elders or those in chronic disease states, since the anabolic role of both hGH and IGF-I

secretion following exercise might have important clinical implications. Furthermore, the effect of a combination of short-term training and hGH administration in both normal and GH deficient subjects would provide information regarding the efficacy of this combined approach to the treatment of GHD, in addition to providing evidence of the effects of GH abuse on exercise performance. At present there is very little scientific evidence that administration of GH to healthy individuals improves performance, and it might be that training alone is the best stimulus for improved performance in healthy individuals, without any benefit of hGH abuse.

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# APPENDIX A

**SUBJECT DOCUMENTS:**
    **HEALTH HISTORY QUESTIONNAIRE**
    **RETURN FORM**

**ETHICAL ADVISORY COMMITTEE DOCUMENTS:**
    **EXAMPLE RESEARCH PROPOSAL**
    **CLEARANCE NOTES**

# HEALTH SCREEN FOR STUDY VOLUNTEERS    Name or Number    ...............

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

**Please complete this brief questionnaire to confirm fitness to participate:**

1. **At present, do you have any health problem for which you are:**
   (a) on medication, prescribed or otherwise ................. Yes ☐    No ☐
   (b) attending your general practitioner ...................... Yes ☐    No ☐
   (c) on a hospital waiting list ................................. Yes ☐    No ☐

2. **In the past two years, have you had any illness which require you to:**
   (a) consult your GP ......................................... Yes ☐    No ☐
   (b) attend a hospital outpatient department ................. Yes ☐    No ☐
   (c) be admitted to hospital ................................. Yes ☐    No ☐

3. **Have you ever had any of the following:**
   (a) Convulsions/epilepsy ..................................... Yes ☐    No ☐
   (b) Asthma .................................................. Yes ☐    No ☐
   (c) Eczema .................................................. Yes ☐    No ☐
   (d) Diabetes ................................................ Yes ☐    No ☐
   (e) A blood disorder ........................................ Yes ☐    No ☐
   (f) Head injury ............................................. Yes ☐    No ☐
   (g) Digestive problems ...................................... Yes ☐    No ☐
   (h) Heart problems .......................................... Yes ☐    No ☐
   (i) Problems with bones or joints .......................... Yes ☐    No ☐
   (j) Disturbance of balance/coordination ..................... Yes ☐    No ☐
   (k) Numbness in hands or feet .............................. Yes ☐    No ☐
   (l) Disturbance of vision ................................... Yes ☐    No ☐
   (m) Ear / hearing problems ................................. Yes ☐    No ☐
   (n) Thyroid problems ....................................... Yes ☐    No ☐
   (o) Kidney or liver problems ............................... Yes ☐    No ☐

If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived. insignificant or well controlled.) ........................................

**Additional questions for female participants**
   (a) are your periods normal/regular? ........................ Yes ☐    No ☐
   (b) are you on "the pill"? ................................... Yes ☐    No ☐
   (c) could you be pregnant? ................................. Yes ☐    No ☐
   (d) are you taking hormone replacement therapy (HRT)? Yes ☐    No ☐

Thank you for your cooperation!

## RETURN SLIP:

I am interested in participating in the "recovery from sprint exercise" project:

NAME: _____ AGE: _____

ADDRESS: _____

_____

_____

TELEPHONE:(daytime) _____(evening) _____

- Do you think you are better in:
    - activities requiring **endurance**     [ ]
    - activities requiring **power/speed**   [ ]

Please tick (√) the appropriate box.

- **If you are an active sportsman**, please indicate the sport and level of performance (e.g. recreational, varsity, national etc.)

SPORT: _____

LEVEL OF PERFORMANCE: _____

- **How often do you train** at present? (please tick (√) the appropriate box):

| not at all | 1-3 times/week | 4-6 times/week | more |
|:---:|:---:|:---:|:---:|
| [ ] | [ ] | [ ] | [ ] |

## STATEMENT OF INFORMED CONSENT:

I have read the above outline of procedures which are involved in this investigation, and I understand what will be required of me. I have had the opportunity to ask for further information and for clarification of the demands of each of the procedures. I am aware that I have the **right to withdraw** from the study at any time, **with no obligation to give reasons for my decision**.

I agree to take part in the "sprint cycling study".

SIGNED: _____ DATE: _____

WITNESSED BY: _____

# LOUGHBOROUGH UNIVERSITY
## ETHICAL ADVISORY COMMITTEE
## RESEARCH PROPOSAL FOR HUMAN BIOLOGICAL INVESTIGATIONS

This application should be completed after reading the Code of Practice paying particular attention to the advice given in Section 6.3.

### (i) Applicants:

Dr. Mary E. Nevill and Mr. Keith A. Stokes

### (ii) Project Title:

Human growth hormone response to repeated bouts of maximal sprint cycling.

### (iii) Aims and Outline of the Project:

During a single bout of exercise a number of metabolic and hormonal changes take place concurrently. It is difficult to discriminate which of these changes are important in regulating the human Growth Hormone (hGH) response to sprint exercise. However, these changes recover at different rates following exercise. Therefore if a second sprint is completed during the recovery period it may be easier to discover the factors which are important in regulating the hGH response to sprint exercise.

Serum hGH concentrations remain elevated one hour after a single 30-s sprint (Nevill et al., 1996, Stokes et al., 1999). Furthermore recent work in this department has demonstrated that if two 30-s sprints are completed separated by one hour of recovery, hGH release is attenuated following the second sprint (Stokes et al., 1999). It may be, therefore, that hGH has a direct negative feedback effect on its own release. However, the effect of repeated bouts of sprint exercise on the hGH response when hGH levels have returned to baseline before the second sprint has not been considered.

It has also been postulated that Insulin like Growth Factors (IGF) have a negative feedback effect on hGH release. IGF are released a few hours after an exercise bout, stimulated by elevated hGH levels in the blood. The IGF response to a single 30-s maximal sprint is not known and, therefore, neither is the effect that this may have on the hGH response to repeated bouts of maximal sprinting.

This proposed study therefore seeks to build on the findings of current work and it is hoped that the information gained will further the understanding of the mechanisms regulating hGH release.

### (iv) Names and status of investigators:

Dr. Mary E. Nevill - Senior Lecturer, Dept. of P.E., S.S. & R.M., Loughborough University
Dr. Henryk K.A. Lakomy - Lecturer, Dept. of P.E., S.S. & R.M., Loughborough University
Mr. Keith A. Stokes - Research Student

**(v)    Subjects (see Section 6.3e):**

The subjects will be largely student volunteers (aged 18-35 years) from the Department of PE, SS&RM. Ten physically active male subjects will be recruited. All subjects will have the study explained to them in verbal and written form (requirements, possible risks and discomforts), and will sign a voluntary consent form which clearly states that they may withdraw from the study at any time without giving any reason.

Subjects will also be required to complete a medical questionnaire in the presence of an experimenter (to provide clarification and assistance) prior to any test. Any subject with a known history of cardiovascular or coagulation/bleeding disorders or metabolic disease will be excluded.

All subjects will be thoroughly familiarised with the equipment and procedures of the study during 3-4 familiarisation visits.

Although information will be kept on a computer, each subject will be entered as a number rather than a name, and will not be identifiable. This is in accordance with the data protection act.

**(vi)    Location (any special facilities to be used):**

The work will be carried out in the Sports Science laboratories of the Department of Physical Education, Sports Science and Recreation Management at Loughborough University.

**(vii)    Duration (including demand on subject's time):**

Prior to any experimental testing the subjects will be asked to complete at least three practice sessions (30 min each) in order to become familiarised with all-out sprinting on the cycle ergometer.

The main tests will involve four visits to the laboratory. One trial will consist of a single visit lasting approximately three hours. One trial will consist of a single visit lasting approximately seven hours. One trial will consist of two visits, on consecutive days, each visit lasting approximately three hours.

**(viii)    Reasons for undertaking the study (e.g. contract, student research):**

This is a staff and Ph.D. research project examining the human Growth Hormone response to sprint exercise.

## (ix)    Methodology (a brief outline of research design):

Ten male subjects will attend at least three practice sessions before completing three main trials in a randomised order.  On the day of each trial subjects will report to the laboratory in the morning in a rested and post-absorptive state.  Trial A will be completed in a single visit to the laboratory and will consist of two 30-s sprints separated by one hour of passive recovery.  Blood samples will be drawn via a venous cannula during the hour between the sprints and for two hours following the second sprint.  Trial B will be completed in a single visit consisting of two 30-s sprints separated by four hours of passive recovery.  Blood samples will be drawn via a venous cannula during the four hours between the two sprints and for two hours following the second sprint.  Trial C will be completed in two visits to the laboratory on consecutive days.  On each day subjects will complete a single 30-s sprint.  Blood samples will be drawn via a venous cannula for two hours following each of the sprints.

## (iv)    Procedures and measurements (for experimental and control subjects)

Sprinting will be performed on a modified cycle ergometer, described by Lakomy (1986). Power output will be measured during each sprint.

Practice sessions – During the 3-4 practice sessions subjects will be thoroughly familiarised with the equipment to be used and will have the opportunity to practice sprinting on a cycle ergometer.

Main tests –
(A) Subjects will complete a standardised submaximal warm-up followed by a maximal 30-s sprint.  They will then rest on a couch for one hour before repeating the warm-up and sprint.  Venous blood samples will be drawn via a cannula at rest, after each warm-up and 5, 10, 20, 30, 40 and 60 minutes after each sprint and 90 and 120 minutes after the second sprint.
(B) Subjects will complete a standardised submaximal warm-up followed by a maximal 30-s sprint.  They will then rest on a couch for four hours before repeating the warm-up and sprint.  Venous blood samples will be drawn via a cannula at rest, post warm-up and 5, 10, 20, 30, 40, 60, 90, 120, 180 and 240 minutes after the first sprint and post warm-up and 5, 10, 20, 30, 40, 60, 90 and 120 minutes after the second sprint.
(C) On day one subjects will complete a standardised submaximal warm-up followed by a maximal 30-s sprint.  They will then rest on a couch for three hours.  Venous blood samples will be drawn at rest, post warm-up and 5, 10, 20, 30, 40, 60, 90, 120 and 180 minutes after the sprint.  On day two exactly the same protocol will be followed.

The total blood volume drawn during trial A will be approximately 150ml, during trial B approximately 200ml and during trial C approximately 150ml per visit (i.e. 300ml over two days).  The total blood volume drawn for each subject over the whole study will therefore be approximately 650ml.  Blood samples will be analysed for lactate, ammonia, pH, Hct, Hb, hGH, insulin, cortisol, IGF and catecholamines.  All blood sampling, handling and analysis will be done according to the University" Code of Practice for handling biological fluids.

**(xi)    Possible risks, discomforts and/or distress (see Section 6.3k):**

The experiment involves maximal sprint exercise and is therefore demanding. However there will be an opportunity to practice sprinting on the cycle ergometer in order to become accustomed with this form of exercise.

The cannula will be placed under local anaesthetic (lignocaine), and therefore discomfort will be minimised. Blood sampling via a cannula may cause minor bruising and carries an extremely small risk of plastic or air embolism. However, good practice minimises this risk. Dr. M.E. Nevill, who will perform the cannulations, has the approval of the Committee for carrying out these procedures. Blood samples will only be drawn by individuals approved by the ethical committee. All procedures will be carried out in accordance with the Code of Practice for Workers having Contact with Body Fluids.

**(xii)   Procedures for taking measurements and for chaperoning and supervision of subjects during investigations:**

Throughout the preliminary and main trials study participants will be supervised by at least two investigators.

**(xii)   Names of investigators and personal experience of proposed procedures and/or methodologies:**

Dr. Mary E. Nevill – 16 years experience of similar investigations in this laboratory. Trained in venous cannulation by the Leicestershire Ambulance Service. Dr. Nevill has the approval of the Ethical Committee to perform cannulations.
Dr. Henryk K.A. Lakomy – 16 years experience in sports science research. Dr. Lakomy has interfaced the cycle ergometer with a microcomputer and has written the necessary computer programmes.
Mr. Keith A. Stokes – Has been trained in, and is responsible for, withdrawing blood via the cannula by the Leicestershire Ambulance Service.

**(xiv)   Details of any payments to be made to the subjects**

None.

**(xv)** Do any investigators stand to gain from a particular conclusion of the research project:

No.

**(xvi)** Whether the University's Insurers have indicated that they are content for the University's Public Liability Policy to apply to the proposed Investigation (Committee use only):

**(xvii)** Whether the insurance cover additional to (xv) has been arranged by the Investigator (see Section 6.3o):

No.

**(xviii)** In the case of studies involving new drugs or radioisotopes, written approval for the study must be obtained from the appropriate national body and submitted with the protocol. State if applicable:

Not applicable.

## (xix) Declaration

> I have read the University's Code of Practice on Investigations on Human Subjects and completed this application.

**Signature of applicant:** ................................................................

**Signature of Head of Department:** ................................................................

**Date:** ................................................................

# Human growth hormone response to repeated bouts of maximal sprint cycling.

**Investigator:** Keith Stokes*
**Supervisors:** Dr. M.E. Nevill, Dr. H.K.A. Lakomy
*Department of P.E., Sports Science and Recreation Management, Loughborough University, Loughborough, Leics., LE11 3TU.*
*Research (Ph.D.) Student
**Dates:** September to December 1999

## INTRODUCTION
In the light of increasing **Growth Hormone** abuse by sports performers it is important to understand the pattern of natural growth hormone release after exercise. A number of studies have considered the growth hormone response to prolonged submaximal exercise but very few have looked at the growth hormone response to sprint exercise.

Previous work in this laboratory has shown that a single 30-s sprint results in elevated growth hormone concentrations for at least 60-min after exercise. Further work has shown that if two 30-s sprints are completed with 60-min of recovery in between, there is no apparent growth hormone response to the second sprint. It is possible that the growth hormone circulating as a result of the first sprint prevents the release of further growth hormone after the second sprint. No one has yet considered the growth hormone response to repeated sprint exercise when growth hormone levels have reached resting levels before the second sprint.

## METHODS
Sprinting will be performed on a cycle connected to a microcomputer which calculates instantaneous power output by counting flywheel revolutions.

Ten male subjects will be recruited for this study. You will complete 3-4 practice sessions during which time height and weight measurements will be taken, the resistance to be applied to the ergometer will be calculated (7.5% of your body weight) and you will have an opportunity to practice cycle ergometer sprinting. You will then complete 2 main trials in a random order. Trial A will be completed in a single visit to the laboratory and will consist of two 30-s sprints separated by one hour of passive recovery. Trial B will be completed over a two-day period. On the first day you will complete two 30-s sprints separated by four hours of passive recovery. The following day you will complete a single 30-s sprint. During each main trial venous blood samples will be taken via a cannula at rest and after each sprint in each trial.

## TIME COMMITMENT AND REQUIREMENTS
You will be asked to report to the laboratory on the day of each main trial in a rested state following an overnight fast.

Practice sessions will require 3-4 separate visits to the laboratory. Each session will last approximately 40 minutes. Trial A will consist of a single visit to the laboratory lasting approximately 3 hours. Trial B will consist of two visits to the laboratory on consecutive days, the visit on the first day lasting approximately 7 hours and the visit on the second day lasting approximately 3 hours.

## LOCATION

The work will be carried out in the Sports Science laboratories of the Department of Physical Education, Sports Science and Recreation Management at Loughborough University (Old Sports Hall building (RR) where the swimming pool is).

## POSSIBLE RISKS AND DISCOMFORTS

The experiment involves maximal sprint exercise and is therefore demanding. However there will be an opportunity to practice sprinting on the cycle ergometer in order to become accustomed with this form of exercise.

The cannula will be placed under local anaesthetic (lignocaine), and therefore discomfort will be minimised. Blood sampling via a cannula may cause minor bruising and carries an extremely small risk of plastic or air embolism. However, good practice and the experience of Dr. M.E. Nevill, who will perform the cannulations, minimise these risks.

The investigators are at all times vigilant in their observations of subjects performing under the prescribed exercise conditions, and are ready to terminate any test should you report, or appear, to be unduly stressed.

If at any time you feel that you wish to withdraw from the study then you will be free to do so without any obligation to give any reason for your decision.

## CONFIDENTIALITY

Although information will be stored on a computer, each subject will be entered as a number rather than a name and will not be identifiable. This is in accordance with the Data Protection Act.

You will be provided, on request, with a full record of your performance data.

## FURTHER INFORMATION

Any questions about this study or future studies in this area are welcome. If you have any doubts or questions, please ask for further explanation by contacting **Keith Stokes** in the Sports Hall Balcony office, RR105 (at the end of the corridor and up the stairs, telephone – 228183) or by e-mail: k.a.stokes@lboro.ac.uk

# Memorandum

**Loughborough University**

To:  Dr M E Nevill, PE, SS, RM
     Mr K A Stokes, PE, SS, RM

Date: 1 October 1999

cc.  Dr R H Hooper, Chair, EAC
     Mr D Massey, University Insurance Officer

From: Wendy Clarke
      Secretary to the Ethical Advisory Committee

**Subject - Ethical Clearance for Research Proposal**

**Research Protocol:**     Human growth hormone response to repeated bouts of maximal sprint cycling

**Reference No.**     R99/P11

I write to confirm that the above research proposal has been cleared by the University's Ethical Advisory Committee and may now proceed under your direction.

For your information I enclose the Committee's cover sheet which gives the conditions on which clearance is granted. This document and the proposal you submitted to the Committee is the approved protocol which will be lodged in the Committee's files. Thank you for assisting the Committee with its work.

# LOUGHBOROUGH UNIVERSITY
# ETHICAL ADVISORY COMMITTEE

# HUMAN BIOLOGICAL INVESTIGATION
# RESEARCH PROPOSAL

Title:              Human growth hormone response to repeated bouts of maximal
                    sprint cycling

Applicants:         Dr M E Nevill, PE, SS, RM
                    Mr K A Stokes, PE, SS, RM

Departments:        PE, Sports Science and Recreation Management

Date of clearance:  1 October 1999

Comments of the Committee:      The Committee was content to issue clearance after
                                receiving a satisfactory response to their comments.

Investigators:      Mr K A Stokes, PE, SS, RM

# APPENDIX B

**BLOOD ASSAYS:**
   HAEMOGLOBIN
   HAEMATOCRIT
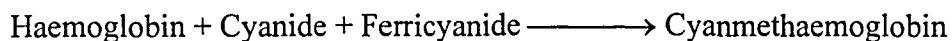   BLOOD LACTATE
   PLASMA AMMONIA
   SERUM HUMAN GH
   SERUM INSULIN
   SERUM CORTISOL
   SERUM IGF-I

## B.1. Haemoglobin (Cyanmethaemoglobin Method)

**Principle**

Haemoglobin + Cyanide + Ferricyanide ⟶ Cyanmethaemoglobin

(Van Kampen and Zijlstra, 1961)

**Reaction mixture**

The reaction mixture ('Drabkins') was made from a kit (Boehringer Mannheim) by diluting with distilled water (1000ml) and contained phosphate buffer, potassium cyanide, potassium ferricyanide and detergent. It was then stored in a brown bottle at between +15°C and +20°C.

**Procedure**

1. In duplicate, 20μl of whole blood was added to 5.0 ml of the reaction mixture in a plastic tube and mixed.
2. The tubes were left to stand for at least 5 min, but not longer than 24 hr. The exact time varied between runs but in most cases samples were analysed at the end of a trial and the time samples were left to stand did not exceed6 hr.
3. The absorbance of the sample was measured using a spectrophotometer (Cecil Instruments) at a wavelength of 546 nm in a cuvette with a 1.0cm light path. The reaction mixture on its own was used as a blank in order to zero the spectrophotometer. Relative absorbance (A) (absorbance of sample − absorbance of blank) was therefore taken as the reading on the spectrophotometer.
4. The haemoglobin concentration of the sample was then calculated from the following equation:

Haemoglobin concentration (g.100 ml$^{-1}$) = (37.2 x A) + 0.06

(Wintrobe, 1956)

## B.2. Heamatocrit (% Cell Volume)

### Procedure

1.  In triplicate, haematocrit tubes (Scientific Instruments) were approximately three-quarters filled with whole blood.  The blood was then run up and down the tube to mix it.
2.  The tubes were sealed at one end with plasticine by placing them in a 'miniseal' tray.  They were then left for analysis at the end of the trial.  Samples were not left for more than 7 hr before they were analysed.
3.  Tubes were placed in a microcentrifuge (Hawksley Ltd.) with the sealed end towards the outside and centrifuged for 14 min.
4.  Percentage cell volume was calculated by placing each tube in a reader (Hawksley Ltd.) where the base (bottom) line of the reader was aligned with the base of the red cells and the top line intersected the top of the plasma.  The middle line was then adjusted so that it intersected the top of the red cells and the percentage cell volume read from the scale.
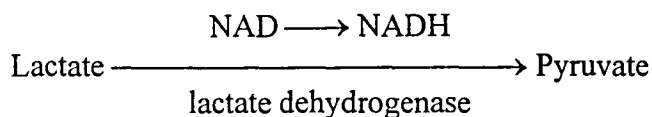
### Plasma Volume

Once haemoglobin and haematocrit values were known, plasma volume could be calculated using the method of Dill and Costill (1974).

## B.3. Blood Lactate

## Principle

The fluorimetric assay for lactate is based on that described by Maughan (1982).

$$Lactate \xrightarrow[\text{lactate dehydrogenase}]{NAD \longrightarrow NADH} Pyruvate$$

The lactate concentrations reported in Chapters ? and ? were obtained by dispensing the samples and reagents by hand and analysing using a fluorimeter (Locarte) (non-automated analyser protocol) whilst those in Chapters ? and ? were obtained using an automated analyser (Cobas Bio, Roche Diagnostics) protocol. In essence, these two methods are the same but both procedures will be explained in full.

## Non-automated analyser protocol

### Reaction mixture

2.0 mg NAD
10.0 $\mu$l LDH
per 1.0 ml of hydrazine buffer (pH ~9.4)

### Standards

In addition to a blank of 2.5% perchloric acid, working standards of 1, 2.5, 5, 10, 15 and 20 mM were prepared from 1.0 mM Sodium L-Lactate stock solution.

### Protocol (each sample was analysed in duplicate)

1. After being allowed to thaw at room temperature, samples were mixed (Whirlimix) and centrifuged for 3 min at 13000 rev.min$^{-1}$ (Eppendorf Centrifuge 5415C).
2. 20 $\mu$l of either standard or supernatant was pipetted into a glass fluorimeter tube and 200 $\mu$l of reaction mixture was added.
3. The tubes were mixed thoroughly (Whirlimix), covered, and left to incubate at room temperature for 30 min.
4. 1.0 ml of lactate diluent (0.07M HCL) was added to each tube and they were mixed thoroughly (Whirlimix) in order to stop the reaction.
5. The fluorescence of the blanks, standards and samples were measured (Locarte).
6. A linear regression plot of the standards was made and the lactate concentration of the samples was calculated.

## Automated analyser protocol

### Reaction mixture

1.7 mg NAD

7.0 µl LDH
per 1.0 ml of hydrazine buffer (pH ~9.4)

## Standards

The Cobas Bio automated analyser automatically blanks itself. The reagent tray can only accommodate three standards and so standards with concentrations over the appropriate range were used. For the studies described in this thesis standards of 5, 10 and 15 mM were chosen. In addition a commercially available quality control (Sigma) at a concentration of 2.1 mM was run with each batch of samples. Both standards and quality control were diluted in 2.5% perchloric acid in the same ratio as the samples.

## Procedure

1. The automated analyser (Cobas Bio, Roche Diagnostics) was switched on and the self-check was completed.
2. The test code for the lactate assay was selected on the keyboard.
3. The standards, quality control and samples were mixed thoroughly (Whirlimix) and centrifuged for 3 min at 13000 rev.min$^{-1}$ (Eppendorf Centrifuge 5415C).
4. The reagent tray was filled with the three standards and the reaction mixture. Pressing the 'START' button initiated the programme on the analyser. When the assay was complete, the concentrations of the standards were printed both in units and as a percentage of their expected values (i.e. 5, 10 and 15 mM). This step was repeated until all standards were 99-101% of their expected concentrations.
5. Approximately 100 µl of sample or quality control was placed into each of the 25 cups comprising the sample disc. During each run two of the cups (the first and last) contained the quality control. The sample cups were pressed firmly into position on the disc and it was placed on the turntable.
6. The reagent tray was re-filled with reaction mixture, if necessary, and the assay was run. The concentration of each sample and quality control was printed in mM.

## B.4. Plasma Ammonia

### Blood Collection

When the blood was drawn ~1 ml whole blood was dispensed into a calcium heparinised eppendorf tube (prepared using the method described below). Samples were centrifuged inmmediately for 3 min at 13000 rev.min$^{-1}$ (Eppendorf Centrifuge 5415C). The plasma was then removed and pipetted into a plain eppendorf tube and immediately snap frozen until the end of the trial and then stored at –70$^{\circ}$C until it was analysed, not more than 48 hr later. The time from blood being drawn to freezing was kept to a minimum in order to prevent contamination from external nitrogen sources and was always less than 15 min.

### Preparation of Ca/Hep tubes

1. The required amount of Ca/Hep was calculated:

> 1 mg (or ml) of Ca/Hep contains 183 units; 15 units are needed per ml of whole blood and each eppendorf tube would contain ~1.5 ml of whole blood. Therefore, each eppendorf tube required ~30 units of Ca/Hep (rounded up to allow for wastage).

> E.g. for a batch of 300 eppendorf tubes, 9000 units (300 x 30) would be required. This is equivalent to ~50 mg (9000 ÷ 183) of Ca/Hep which would be added to 1.2 ml (4 x 300) of distilled water.

2. 4$\mu$l of this solution was then be pipetted into each eppendorf tube.

### Principle

The spectrophotometric assay is based on the methods described by Neeley and Phillipson (1968) and van Anken and Schiphorst (1974):

$$\text{2-Oxoglutarate} + NH_3 + NADPH \xrightarrow{\text{GLDH}} \text{Glutamate} + NADP$$
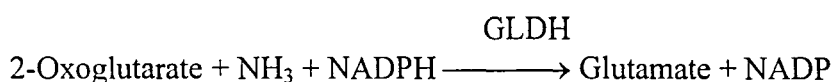
The decrease in absorbance at 340 nm, due to the oxidation of NADPH, is proportional to the plasma ammonia concentration.

### Reaction mixture

The reagents are found in bottles found in a commercially available kit (Sigma Diagnostics).

*Reagent solution*
The reagent solution was reconstituted with the volume of distilled water indicated on each vial label. Each reagent then contained:

2-Oxoglutarate 3.4 mmol.L$^{-1}$
NADPH 0.23 mmol.L$^{-1}$

In addition the reagent mixture contained buffer, stabilisers and nonreactive fillers.

*Enzyme solution*

L-Glutamate dehydrogenase 1200 U/ml
Glycerol 50 % (v/v)
Phosphate buffer pH 7.4

## Standards

Each kit included an ammonia control solution at a concentration of 294 $\mu$mol.L$^{-1}$. In addition control solutions at ammonia concentrations of 29.5 and 118 $\mu$mol.L$^{-1}$ were used (Sigma Diagnostics).

## Procedure

1. A series of 1.5 ml disposable plastic cuvettes with a lightpath of 1cm were set up for BLANK, STANDARD 1 (29.5 $\mu$mol.L$^{-1}$, in duplicate), STANDARD 2 (118 $\mu$mol.L$^{-1}$, in duplicate), STANDARD 3 (294 $\mu$mol.L$^{-1}$, in duplicate) and SAMPLES.
2. 0.5 ml of the reagent solution was added to each cuvette.
3. 150 $\mu$l of distilled water was added to the BLANK cuvette, 100 $\mu$l of distilled water and 50 $\mu$l of ammonia control solution to each of the STANDARD cuvettes and 150 $\mu$l of plasma to each of the SAMPLE cuvettes.
4. Cuvettes were covered with sealing film (Nescofilm, Bando Chemical Ind. Ltd., Kobe, Japan) and mixed by gentle inversion.
5. Cuvettes were allowed to equilibrate for approximately 3 min at room temperature.
6. INITIAL absorbance of each cuvette was read (Cecil Instruments) and recorded at 340 nm.
7. 5 $\mu$l of enzyme solution was added to each cuvette, cuvettes were mixed by gentle inversion and left to stand for approximately 5 min at room temperature for the reaction to complete.
8. FINAL absorbance of each cuvette was read and recorded at 340 nm.
9. The ammonia concentration was calculated using the following equations:

$$\Delta A = \text{INITIAL A} - \text{FINAL A}$$

$$\text{STANDARD Ammonia } (\mu\text{mol.l}^{-1}) = ((\Delta A_{\text{STANDARD}} - \Delta A_{\text{BLANK}}) \times 35.8) \times 58.8$$

$$\text{SAMPLE Ammonia } (\mu\text{mol.l}^{-1}) = ((\Delta A_{\text{SAMPLE}} - \Delta A_{\text{BLANK}}) \times 11.93) \times 58.8$$

Where the factor 58.8 converts $\mu$g.ml$^{-1}$ to $\mu$mol.l$^{-1}$.

## Serum human growth hormone, cortisol and insulin.

### Principle

Commercially available kits were used for the determination of serum human growth hormone (hGH), insulin (both Medgenix, Biosource) and cortisol (Milenia, DPC) concentrations by routine ELISA. Microtitration plates pre-coated with specific monoclonal antibodies are supplied into which serum samples and standards are pipetted. Standards and samples containing hGH, cortisol or insulin (according to the assay) react with capture antibodies coated on the wells of the microtitation plates (Mabs 1) and with monoclonal antibodies (Mabs 2) labelled with horse radish peroxydase (HRP). During incubation a sandwich forms (coated Mabs 1 – hormone – Mabs 2 – HRP). The microtitation plate is washed to remove any unbound enzyme labelled antibodies. A revelation solution is added and incubated before a stopping agent is added and the microtitation plate is read at the appropriate wavelength. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is proportional to the hormone concentration. The procedures for each of the assays are similar but they will be described in full.

### B.5. Serum Human Growth Hormone (hGH)

Medgenix-hGH-EASIA, Biosource.

### Reagents

- Microtitration plate
- Standard 0 mU.l$^{-1}$ in sheep serum, sodium merthiolate
- Standards 1, 5, 15, 100 mU.l$^{-1}$ in sheep serum, sodium merthiolate
- Controls 1 and 2 in human serum, sodium merthiolate
- Anti-hGH-HRP conjugate in Tris-HCl buffer with bovine serum albumin and preservatives
- Tween 20, 20% (washing solution)
- Chromogen TMB (Tetramethylbenzidine)
- Substrate buffer, $H_2O_2$ in acetate/citrate buffer
- $H_2SO_4$ 1.8 N (stopping reagent)

### Procedure

1. Reagents were prepared according to the instructions in each kit.
2. 50μl of each standard, control or sample was pipetted into the appropriate wells.
3. 50μl of Anti-hGH-HRP conjugate was pipetted into each well.
4. The standards, controls and samples were incubated for 1 hr at room temperature on a horizontal shaker (Automix III, Heidolph) set at 700 ± 100 rpm.
5. The plate was washed using an automated washer (Denley Wellwash 4 mk 2) by aspirating the liquid from each well, dispensing 0.4 ml of washing solution into each well, aspirating the content of each well, dispensing 0.4 ml of washing solution into each well for a second time and aspirating the content of each well.

6. 200μl of freshly prepared revelation solution (chromogen TMB and substrate buffer) was pipetted into each well within 15 min following washing.
7. The standards, controls and samples were incubated for 15 min at room temperature on a horizontal shaker (Automix III, Heidolph) set at $700 \pm 100$ rpm, avoiding direct sunlight.
8. 50μl of stopping reagent was pipetted into each well.
9. Absorbance was measured within 1 hr using an ELISA plate reader (Anthos htII microplate reader, Anthos Labtec Instruments) and the results printed.

## B.6. Serum Insulin

Medgenix-INS-EASIA, Biosource.

### Reagents

- Microtitration plate
- Standard 0 mU.l$^{-1}$ in human serum, sodium merthiolate
- Standards 5, 15, 50, 150, 500 mU.l$^{-1}$ in human serum, sodium merthiolate
- Controls 1 and 2 in human serum, sodium merthiolate
- Anti-insulin-HRP conjugate in Tris-HCl buffer with bovine serum albumin and preservatives
- Tween 20, 20% (washing solution)
- Chromogen TMB (Tetramethylbenzidine)
- Substrate buffer, $H_2O_2$ in acetate/citrate buffer
- $H_2SO_4$ 1.8 N (stopping reagent)

### Procedure
1. Reagents were prepared according to the instructions in each kit.
2. 50µl of each standard, control or sample was pipetted into the appropriate wells.
3. 50µl of Anti-insulin-HRP conjugate was pipetted into each well.
4. The standards, controls and samples were incubated for 30 min at room temperature on a horizontal shaker (Automix III, Heidolph) set at $700 \pm 100$ rpm.
5. The plate was washed using an automated washer (Denley Wellwash 4 mk 2) by aspirating the liquid from each well, dispensing 0.4 ml of washing solution into each well, aspirating the content of each well, dispensing 0.4 ml of washing solution into each well for a second time and aspirating the content of each well.
6. 200µl of freshly prepared revelation solution (chromogen TMB and substrate buffer) was pipetted into each well within 15 min following washing.
7. The standards, controls and samples were incubated for 15 min at room temperature on a horizontal shaker (Automix III, Heidolph) set at $700 \pm 100$ rpm, avoiding direct sunlight.
8. 50µl of stopping reagent was pipetted into each well.
10. Absorbance was measured within 1 hr using an ELISA plate reader (Anthos htII microplate reader, Anthos Labtec Instruments) and the results printed.

### B.7. <u>Serum Cortisol</u>

Milenia-Cortisol, DPC

### Reagents

- Ligand-labelled cortisol
- Cortisol antiserum (containing rabbit anti-cortisol antibodies)
- Cortisol enzyme-labelled anti-ligand (containing horseradish peroxidase-labelled anti-ligand)
- Cortisol calibrators (0, 1, 5, 10, 20, 50 mU.l$^{-1}$ in processed human serum)
- Second antibody-coated microplate
- TMB/substrate solution
- Buffered wash solution concentrate
- Stop solution

### Procedure

1. Reagents were prepared according to the instructions in each kit.
2. 25 μl of each calibrator, control or sample was pipetted into the appropriate wells.
3. 100 μl of ligand labelled cortisol was pipetted into each well.
4. 100 μl of cortisol antiserum was pipetted into each well.
5. The standards, controls and samples were incubated for 1 h at room temperature on a horizontal shaker (Automix III, Heidolph) set at 1300 ± 100 rpm.
6. 25 μl of cortisol enzyme-labelled anti-ligand was pipetted into each well.
7. The standards, controls and samples were incubated for 30 min at room temperature on a horizontal shaker (Automix III, Heidolph) set at 1300 ± 100 rpm.
8. Each well was aspirated and washed a total of four times using an autowasher (Denley Wellwash 4 mk 2) by dispensing 300 μl of Wash Buffer into each well and aspirating the contents completely. After the last wash any remaining buffer was removed by aspirating or decanting and then inverting the plate and blotting it against clean paper towelling.
9. 200 μl of TMB/substrate solution was pipetted into each well.
10. The plate was incubated at room temperature in the dark without shaking for 30 min.
11. 50μl of stop solution was pipetted into each well.
12. Absorbance was measured at 450 nm within 15 min using an ELISA plate reader (Anthos htII microplate reader, Anthos Labtec instruments) and the results printed.

## B.8. Serum Insulin-like Growth Factor I (IGF-I)

Quantikine® IGF-I Immunoassay, R&D Systems Europe.

### Principle

A commercially available kit was used for the determination of serum insulin-like growth factor-I (R&D Systems Europe). The assay employs the quantitative sandwich enzyme immunoassay technique. A micro titration plate pre-coated with monoclonal antibodies specific for IGF-I is supplied. Standards and pre-treated samples are pipetted into the appropriate wells and any IGF-I present is bound by the immobilised antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IGF-I is added to the wells. A further wash removes any unbound antibody-enzyme reagent and then a substrate solution is added to the wells and colour develops in proportion to the amount of IGF-I bound in the initial step. The colour development is stopped and the intensity of the colour measured.

### Reagents

- IGF-I microplate coated with a murine monoclonal antibody against IGF-I
- IGF-I conjugate – polyclonal antibody against IGF-I conjugated to horse radish peroxidase, with preservative.
- IGF-I standard – recombinant human IGF-I in a buffer with preservative.
- Assay diluent RD1-53 – a buffer with preservative.
- Calibrator diluent RD5-22 – a buffer with blue dye and preservatives.
- Pretreatment A – an acidic dissociation solution.
- Pretreatment B – buffered protein with a blue dye and preservatives.
- Wash Buffer Concentrate – a 25-fold concentrated solution of buffered surfactant with concentrated solution.
- Colour reagent A – stabilised hydrogen peroxide.
- Colour reactant B – stabilised chromogen (tetramethylbenzidine).
- Stop solution – 2 N sulphuric acid.

### Procedure

1. All reagents, working standards and samples were prepared as directed in the kit instructions.
2. 150 μl of Assay Diluent RD1-53 was pipetted into each well.
3. 50 μl of standard or pre-treated sample was pipetted into the appropriate wells. The plate was covered with the adhesive strips provided in the kit and incubated at 2-8 °C for 2 hr.
4. Each well was aspirated and washed a total of four times using an autowasher (Denley Wellwash 4 mk 2) by dispensing 400 μl of Wash Buffer into each well and aspirating the contents completely. After the last wash any remaining buffer was removed by aspirating or decanting and then inverting the plate and blotting it against clean paper towelling.

5. 200 μl of cold IGF-I conjugate (stored at 2-8 °C until use) was pipetted into each well. The plate was covered with a new adhesive strip and incubated at 2-8 °C for 1 hr.

6. The aspiration/wash cycle (step 4) was repeated.

7. 200 μl of Substrate Solution (Colour Reagents A and B) was pipetted into each well and the plate was incubated at room temperature, whilst being protected from light, for 30 min.

8. 50 μl of Stop Solution was pipetted into each well and the plate was tapped gently to ensure thorough mixing.

9. Optical density was determined within 30 min using a microplate reader (Anthos htII microplate reader, Anthos Labtec Instruments) set at 450 nm. With wavelength correction set at 540 nm.