Growth hormone responses to repeated maximal cycle ergometer exercise at different pedaling rates

K. A. STOKES,1 M. E. NEVILL,1 G. M. HALL,2 AND H. K. A. LAKOMY1

1Department of Physical Education, Sports Science and Recreation Management, Loughborough University, Loughborough, Leicestershire LE11 3TU; and 2Department of Anaesthesia, St. George’s Hospital Medical School, University of London, London SW17 0RE, United Kingdom

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Growth hormone (GH) is released in a pulsatile manner from the anterior pituitary gland. The release of GH is believed to be regulated by the hypothalamic neuropeptides GH-releasing hormone (GHRH) and somatostatin, with modulation by feedback mechanisms (8).

A number of studies have shown exercise to stimulate increases in blood GH concentrations, although only a few have considered high-intensity or sprint exercise (12, 30). A single 30-s treadmill sprint produces a near-maximal GH response when compared with the results from pharmacological intervention studies, with GH levels remaining elevated for at least 60 min postexercise (30). However, the mechanisms controlling the magnitude of the GH response to exercise are not well understood. Intensity and duration of exercise have been suggested to influence the GH response (34), and the possible roles of blood lactate (6, 19), blood pH (12), and O2 demand and availability (36) have also been considered.

Repeated 30-min bouts of submaximal exercise have shown to elicit an augmented GH response (17). This finding is in contrast to studies showing that repeated administration of pharmacological stimuli results in an attenuated GH response in rats (26, 27) and humans (11). In addition, Cappon et al. (3) found that, in humans, three heavy 10-min exercise bouts resulted in progressive attenuation of the GH response to exercise. At present, therefore, the evidence regarding the GH response to repeated bouts of exercise is equivocal.

The changes in muscle metabolites after maximal cycling at fast (140 rpm) and slow (60 rpm) pedaling rates have been shown to be similar despite greater fatigue being evident with pedaling at 140 rpm (16). In addition, Cherry et al. (5) found no difference in the magnitude of the changes in blood or muscle metabolites with sprinting on a friction-loaded cycle ergometer against different applied resistance, although subjects’ performance appeared to recover more quickly after exercise involving fewer muscle actions. Although it has recently been shown that plasma cortisol levels are elevated during submaximal exercise at slow, but not fast, pedaling rates (7), no studies have yet considered the hormone responses to sprint exercise at different pedaling rates. However, if the metabolic response to exercise determines the magnitude of the GH response, it is likely that the GH response to exercise would be unaffected by the applied resistance.

Therefore, the aim of the present study was to test the hypothesis that repeated bouts of maximal sprint cycling result in an attenuation of the GH response to exercise. In addition, the present study was designed to assess the role of the metabolic response to sprinting in the regulation of the GH response by testing the hypothesis that sprint cycling at different pedaling rates results in similar changes in postexercise GH concentrations.
METHODS

Subjects. Ten healthy male volunteers, aged 21 to 32 yr (24.5 ± 1.1 yr), 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), height ranged from 174.4 to 186.0 cm (179.1 ± 1.6 cm), and body mass index ranged from 21.3 to 27.0 (24.2 ± 0.5).

Equipment. The exercise tests were carried out on a modified friction-loaded cycle ergometer (model 864, Monark), 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 (25) 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 familiarization, the subjects arrived in the laboratory after a 4-h fast on two separate occasions, completing one trial on each visit by using a crossover design. In each trial, the subjects completed two all-out 30-s efforts separated by 1 h of passive recovery. During one trial, the subjects completed both sprints against an applied resistance equal to 7.5% (75 g/kg) of their body mass, whereas, in the other trial, both sprints were completed against a resistance equal to 10% (100 g/kg) 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 standardized submaximal warm-up on the cycle ergometer, consisting of 4 min of pedaling at 60 W, 30 s of pedaling at 80 W, and 30 s of pedaling at 100 W, with 30 s of rest between each intensity. Five minutes after the warm-up, the subjects performed the first of two maximal 30-s sprints from a rolling start (70 rpm against no resistance) on the cycle ergometer. Subjects were instructed to sprint maximally for the duration of each sprint and were encouraged verbally while sprinting.

After 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 ~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 anesthetic (1% lignocaine). Blood samples were taken with the subjects in a seated position at rest (~10 min) and after warm-up (~4 min) before the first sprint and at 10 s and 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. 1) One portion (4–5 ml) was placed into a lithium heparinized tube (LH/5 ml, Sarstedt). Blood pH was measured immediately (ABL5 pH/blood-gas monitor, Radiometer) 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 (29). Further aliquots were removed for the measurement of hematocrit by microcentrifugation (Hawksley) and hemoglobin concentration (by the cyanmethemoglobin method) for the calculation of percent change in plasma volume (9). 2) A further 1.5 ml were placed in a calcium heparinized Eppendorf tube. This was immediately centrifuged, and the plasma was removed and stored at −70°C for the determination of ammonia concentration within 48 h (kit 171-C, Sigma Diagnostics). 3) The remaining blood (4–5 ml) was allowed to clot for 1 h in a plain tube (Serum Z/5 ml, Sarstedt). This was then centrifuged at 3°C for 15 min at a speed of 6,000 rpm (Burkard Koolspin), and the serum was removed and stored at −70°C for the determination of GH, insulin, and cortisol concentrations. Serum GH, insulin, and cortisol were measured by routine ELISA. The GH assay (Medigenix HGH, Biosource) had a sensitivity of 0.11 mU/l, an intra-assay coefficient of variation (CV) of 2.1–3.6%, and an interassay CV of 6.8–7.1%. The insulin assay (Medgenix insulin, Biosource) had a sensitivity of 0.15 mU/l, an intra-assay CV of 3.0–5.3%, and an interassay CV of 5.6–9.8%. The sensitivity of the cortisol assay (DPC cortisol, Milenia) was 8.3 nmol/l with an intra-assay CV of 5.9–8.0% and an interassay 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 GH concentrations [area under the curve (AUC)] and peak values of GH 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 means ± SE.

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 in 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 compared with the slow trial (P < 0.05). The fatigue index, 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 in sprint 2 (Table 1).

Metabolic responses to cycle ergometer sprinting. The blood lactate, blood pH, and plasma ammonia responses are shown in Figs. 1, 2, and 3, respectively. Blood lactate and pH responses did not differ between trials (mean peak blood lactate: 9.69 ± 0.02 vs. 7.08 ± 0.02 for fast vs. slow; mean lowest pH: 7.08 ± 0.02 vs. 7.06 ± 0.02 for fast vs. slow) or sprints (mean peak blood lactate: 10.12 ± 0.52 vs. 10.40 ± 0.62 mmol/l for sprint 1 vs. sprint 2; mean lowest pH: 7.07 ± 0.02 vs. 7.08 ± 0.02 for sprint 1 vs. sprint 2). Plasma
ammonia demonstrated a sprint × time interaction (P < 0.05), reflecting higher peak ammonia concentrations and faster recovery after sprint 1 than after 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.

**Hormone responses to cycle ergometer sprinting.** Figure 4 shows the mean serum GH response to repeated maximal 30-s cycle ergometer sprints. The first sprint resulted in a distinct GH pulse with highest measured mean concentrations of 40.8 ± 8.2 and 20.8 ± 6.1 mU/l 30 min after the sprint in the fast and slow trials, respectively. Serum GH was still elevated 60 min after the first sprint, whereas there was no GH pulse after the second sprint. Serum GH 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 GH response for sprint 1 (mean of individual peaks) during the fast trial was more than twice that during the slow trial (mean peak GH: 37.7 ± 6.0 vs. 17.6 ± 3.7 mU/l for fast vs. slow; P < 0.05).

Mean integrated GH concentrations (AUC) for the 1-h period after each sprint demonstrated a difference between sprints (mean GH AUC: 1,315 ± 243 vs. 729 ± 146 min·mU⁻¹·l⁻¹ for sprint 1 vs. sprint 2; P < 0.01). There was also a trend for a lower GH AUC in the slow trial, with 9 of the 10 subjects following this pattern (mean GH AUC: 1,381 ± 231 vs. 663 ± 162 min·mU⁻¹·l⁻¹ for fast vs. slow; P = 0.06). When the GH AUC for the 60 min of recovery after sprint 1 in the fast and slow trials was compared, there was also a trend for a smaller GH AUC in the slow trial (mean GH AUC after sprint 1: 1,697 ± 367 vs. 933 ± 306 min·mU⁻¹·l⁻¹ for fast vs. slow; P = 0.05). The insulin (Fig. 5) and cortisol (Fig. 6) responses were similar in the two trials, al-

### Table 1. Peak and mean power output, peak and mean pedal rate; and fatigue index for sprints 1 and 2 in the fast and slow trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Sprint 1</th>
<th>Sprint 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO, W</td>
<td>1,178 ± 51</td>
<td>1,132 ± 44</td>
</tr>
<tr>
<td>MPO, W</td>
<td>654 ± 21</td>
<td>660 ± 22</td>
</tr>
<tr>
<td>PPR, rpm</td>
<td>159 ± 4</td>
<td>154 ± 5</td>
</tr>
<tr>
<td>MPR, rpm</td>
<td>117 ± 3</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>Fatigue index, %</td>
<td>61 ± 2</td>
<td>59 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE for 10 subjects. PPO, peak power output; MPO, mean power output; PPR, peak pedal rate; MPR, mean pedal rate. *P < 0.05 main effect: sprint. †P < 0.01 main effect: trial. §P < 0.01 main effect: sprint.

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**Fig. 2. Blood pH at rest and during 1 h of recovery after two 30-s maximal cycle ergometer sprints for the fast (●) and slow (○) trials. Values are means ± SE. †Time main effect, P < 0.01.**

**Fig. 3. 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. Values are means ± SE. †Time main effect, P < 0.01. §Trial × time interaction effect, P < 0.05.**

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**Fig. 1. 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. Values are means ± SE. †Time main effect, P < 0.01.**
though the cortisol response to the first sprint was different from that of the second ($P < 0.05$).

Correlation analysis showed a significant correlation between MPR and GH 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 GH AUC (sprint 1, $r = 0.48$, $P < 0.05$; sprint 2, $r = 0.58$, $P < 0.01$) but not between MPO and GH AUC (sprint 1, $r = 0.31$, not significant (NS); sprint 2, $r = 0.12$, NS). PPO and GH AUC showed a significant correlation during the second sprint but not during the first (sprint 1, $r = 0.41$, NS; sprint 2, $r = 0.47$, $P < 0.05$). When metabolites were considered, integrated lactate and ammonia concentrations showed little correlation with GH AUC (lactate: sprint 1, $r = 0.08$, NS; sprint 2, $r = 0.08$, NS; ammonia: sprint 1, $r = -0.04$, NS; sprint 2, $r = 0.08$, NS). In addition, no significant correlation was found between body mass index and serum GH concentrations expressed as either highest measured GH ($r = -0.38$, NS) or GH AUC ($r = -0.36$).

DISCUSSION

This study demonstrates that a single 30-s sprint is a potent stimulus for GH release. However, if a similar sprint is completed 1 h later, there is no GH response. Furthermore, cycling at fast pedaling rates results in greater increases in postexercise serum GH concentrations than cycling at slow pedaling rates ($P = 0.05$).

A single 30-s treadmill sprint has been shown to stimulate a near-maximal GH response (30). The results of the present study show that 30 s of maximal cycle ergometer exercise elicit a marked GH response, although GH responses in this study were not as large as those found by Nevill et al. (30). This may reflect the fact that sprint cycling, with movement concentrated in the lower limbs, elicits a different GH response when compared with the treadmill sprinting employed in the study by Nevill et al. (30). This is supported by Gordon et al. (12), who showed that a 90-s all-out effort on a cycle ergometer elicited a similar GH 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, GH had not returned to baseline, and, furthermore, a second sprint completed at this time point did not elicit a GH response. This finding is in agreement with studies demonstrating attenuation of spontaneous and GHRH-stimulated GH secretion after exogenous GH administration in rats (26, 27). Studies in humans have also demonstrated a progressively decreasing GH response with repeated GHRH administration (11).

Kanaley et al. (17) demonstrated an augmented GH response to repeated bouts of 30 min of exercise at 70% maximal O2 uptake separated by 60 min of recovery, suggesting that exercise provides sufficient stimulus to overcome the attenuation of the GH response shown using pharmacological interventions. However, Cappon et al. (3) found that the GH response to exercise is attenuated by prior exercise. Their findings are in agreement with those of the present study in which a second bout of exercise did not elicit a GH response, demonstrating that the suppression of the GH 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. (17) may be explained by the nature of the stimulus for GH release, because submaximal exercise is known to induce a lesser GH response than that induced by sprint exercise. However, Ghigo et al. (11), using GHRH as a stimulus, induced an initial GH response similar to that found by Kanaley et al. (17) and yet demonstrated an attenuated GH response to a second stimulus.

The mechanism by which the GH response is attenuated is unclear. The depletion of pituitary stores as a result of the first bout is unlikely because Kanaley et al. (17) found an augmented GH response with repeated submaximal exercise. Furthermore, Jaffe et al. (14) suggested 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. Inhibition of GH by elevated levels of free fatty acids (FFAs) might explain the lack of a GH response to the second sprint, because FFAs can block GH secretion directly at the pituitary gland (4). Although FFA concentrations were not measured in the present study, subsequent work in this laboratory (33) has demonstrated that serum FFA concentrations are not elevated after 60 min of recovery from a single 30-s cycle ergometer sprint (0.29 ± 0.07 vs. 0.15 ± 0.02 mol/l for preexercise vs. 60 min postexercise; NS). On the strength of this evidence, it is unlikely that, in the present study, elevated serum FFA concentrations were responsible for the attenuation of the GH response to the second sprint.

It has also been demonstrated that infusion of recombinant insulin-like growth factor I (IGF-I) suppresses pulsatile and GHRH-stimulated GH secretion in male subjects (15). Serum IGF-I concentrations were not measured in the present study, but Cappon et al. (3) found that acute-exercise-stimulated IGF-I levels had returned to preexercise levels within 60 min of recovery. In addition, subsequent work in this laboratory (33) has provided evidence that serum IGF-I concentrations are not elevated 60 min after a single 30-s cycle ergometer sprint (199 ± 16 vs. 191 ± 13 ng/ml for preexercise vs. 60 min postexercise; NS). If a similar situation is assumed in the present study, it is unlikely that elevated IGF-I was responsible for the suppression of the GH response to the second sprint.

GH can directly inhibit its own release, possibly at the pituitary gland (31). Because, in the present study, GH was still elevated at the start of the second sprint, GH autoinhibition may be responsible for preventing a GH 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 (26) demonstrated that the immunoneutralization of somatostatin prevented the attenuation of spontaneous GH release after GH pretreatment in rats, thus obtaining strong support for a role for somatostatin in GH autoregulation. In a further study, Lanzi and Tannenbaum (27) 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 (2) further supports these findings. Peripheral measurements of GHRH and somatostatin would probably not reflect hypothalamic secretion, but studies employing direct hypothalamic-portal sampling in nonhuman species suggest that hypothalamic discharges of GHRH regulate GH pulses (10), whereas somatostatin may modulate the action of GHRH (35).

In the present study, a GH response to the second bout of exercise was identified in two of the subjects. The GH response to the first sprint in both of these subjects was characterized by moderate increases in serum GH concentrations that had returned to near preexercise levels within 60 min of recovery. GH concentrations in two of the other subjects had, similarly, returned to near preexercise values 60 min after the first sprint, but the increases in serum GH concentrations in these individuals in response to both the first and second sprints were small. The other six subjects displayed moderate to large increases in serum GH concentrations after the first sprint, and GH concentrations were still elevated above preexercise levels after 60 min of recovery. In these six individuals, a second sprint completed 60 min after the first did not result in a further increase in serum GH concentrations. These results provide further evidence that elevated serum GH concentrations might result in the attenuation of the GH response to repeated bouts of exercise.

It has been suggested that the metabolic response to a 30-s sprint has a part to play in GH release (30). The results of the present study do not support this contention because there was a poor correlation between
plasma ammonia and serum GH concentration and between blood lactate and serum GH concentration. When the fast and slow trials were compared, the pH and lactate responses were similar between trials and between sprints, although the highest measured ammonia concentrations were greater in the fast than in the slow trial. Jones et al. (16) demonstrated that high and low pedaling rates on an isokinetic ergometer induced similar metabolic responses in mixed muscle. Previous work in this laboratory (5) also showed that pedaling 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 GH response to a single 30-s cycle ergometer sprint might be greater when pedaling at faster speeds (Fig. 4), although this trend was not found to be significant ($P = 0.05$). This observation indicates that metabolic conditions in muscle and blood might not determine the magnitude of the GH response to all-out sprinting, but that there is another mechanism for GH release.

However, despite a poor correlation, the results of the present study indicate that the plasma ammonia and serum GH responses followed similar trends after sprint exercise at different pedaling rates, with smaller increases in both plasma ammonia and serum GH concentrations after exercise during the slow trial. It has been suggested that blood ammonia produced during exercise might have direct access to the brain, where it has been implicated in the development of central fatigue (1). The most important pathway for ammonia disposal in the brain is synthesis of glutamine from glutamate and ammonia (28), and intravenous injection of ammonium acetate in rats has been demonstrated to decrease both brain glutamine and GABA content (18). Both glutamate and GABA are neurotransmitters, and they may have a role in the control of anterior pituitary secretion.

In the present study, it is possible that smaller increases in plasma ammonia observed after sprinting at slower pedaling rates might result in a smaller reduction in the brain content of GABA, which has been reported to inhibit exercise-induced GH secretion (32). A smaller reduction in brain GABA content might, in turn, result in greater inhibition of GH secretion, when compared with exercise at faster pedaling rates. However, uncertainty over the relationship between plasma ammonia concentrations and brain ammonia content, the effect of ammonia on brain content of glutamate and GABA, and the roles of glutamate and GABA in the regulation of exercise-induced GH secretion means that further work is needed to assess the importance of this potential mechanism for the regulation of GH secretion.

Kozlowski et al. (24) found no causal relationship between blood metabolites and GH but suggested that muscle metabolic receptors may play a role in GH release. The results of the present study do not support this view. However, activity in motor centers may directly stimulate pituitary hormone secretion, including GH, during exercise (21–24). It is possible that, in the present study, there was greater central motor activity with sprinting at faster pedaling rates, resulting in a greater GH response. However, because of the maximal nature of the exercise, it is unlikely that there was any difference in motor center activity between the fast and slow trials.

Other work by Kjaer et al. (20) suggested that hormonal feedback mechanisms and autonomic nervous reflexes, without efferent motor center activity, may exert redundant control of pituitary hormonal responses. Gosselink et al. (13) 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 was a poor correlation between metabolites and hormones but a significant correlation between PPR and serum GH and MPR and serum GH, with smaller GH responses elicited by pedaling at slower speeds. It is possible, therefore, that proprioceptive feedback has a role in the regulation of the GH response to sprint exercise.

In conclusion, this study has demonstrated that a single 30-s cycle ergometer sprint elicits a marked GH response but that a similar sprint completed 60 min later does not result in a GH response. GH autoinhibition may be responsible for the suppression of the GH response to the second sprint, possibly mediated by an increase in somatostatin. The present study has also shown that cycle ergometer sprinting at faster pedaling rates elicits a greater GH response than pedaling at slower pedaling rates, despite similar blood lactate and pH responses. However, the plasma ammonia and serum GH responses followed similar trends after exercise at different pedaling rates, and it is possible that circulating ammonia, in addition to a proprioceptive mechanism, contributes to the regulation of GH release during sprint exercise.

REFERENCES

GROWTH HORMONE RESPONSES TO SPRINT EXERCISE


