**Growth-Hormone Responses to Consecutive Exercise Bouts With Ingestion of Carbohydrate Plus Protein**

James A. Betts, Keith A. Stokes, Rebecca J. Toone, and Clyde Williams

Endocrine responses to repeated exercise have barely been investigated, and no data are available regarding the mediating influence of nutrition. On 3 occasions, participants ran for 90 min at 70% VO$_2$max (R$_1$) before a second exhaustive treadmill run at the same intensity (R$_2$: 91.6 ± 17.9 min). During the intervening 4-hr recovery, participants ingested either 0.8 g sucrose · kg$^{-1}$ · hr$^{-1}$ with 0.3 g · kg$^{-1}$ · hr$^{-1}$ whey-protein isolate (CHO-PRO), 0.8 g sucrose · kg$^{-1}$ · hr$^{-1}$ (CHO), or 1.1 g sucrose · kg$^{-1}$ · hr$^{-1}$ (CHO-CHO). The latter 2 solutions therefore matched the former for carbohydrate or for available energy, respectively. Serum growth-hormone concentrations increased from 2 ± 1 μg/L to 17 ± 8 μg/L during R$_1$ considered across all treatments ($M$ ± SD; $p$ ≤ .01). Concentrations were similar immediately after R$_2$ irrespective of whether CHO or CHO-CHO was ingested (19 ± 4 μg/L and 19 ± 5 μg/L, respectively), whereas ingestion of CHO-PRO produced an augmented response (31 ± 4 μg/L; $p$ ≤ .05). Growth-hormone-binding protein concentrations were unaffected by R$_1$ but increased similarly across all treatments during R$_2$ (from 414 ± 202 pmol/L to 577 ± 167 pmol/L; $p$ ≤ .01), as was the case for plasma total testosterone (from 9.3 ± 3.3 nmol/L to 14.7 ± 4.6 nmol/L; $p$ ≤ .01). There was an overall treatment effect for serum cortisol ($p$ ≤ .05), with no specific differences at any given time point but lower concentrations immediately after R$_2$ with CHO-PRO (608 ± 133 nmol/L) than with CHO (796 ± 278 nmol/L) or CHO-CHO (838 ± 134 nmol/L). Ingesting carbohydrate with added whey-protein isolate during short-term recovery from 90 min of treadmill running increases the growth-hormone response to a second exhaustive exercise bout of similar duration.

**Keywords:** cortisol, IGF-1, testosterone, prolactin, recovery

A single bout of endurance, resistance, or sprint exercise represents a potent stimulus for hypothalamic growth-hormone secretion in humans (Gilbert, Stokes, Hall, & Thompson, 2008). Despite much interest, however, the precise physiological function of the growth-hormone response to exercise remains unclear. Emerging evidence indicates that this variable correlates neither with acute exercise-induced anabolic signaling and muscle protein synthesis nor with chronic training-induced muscle hypertrophy and strength adaptations (West et al., 2010; West et al., 2009). An alternative role for growth hormone in the adaptive response to exercise has been proposed to involve fortification of musculo-tendinous tissue via the stimulation of matrix collagen synthesis (Doessing et al., 2010).

With relevance to short-term postexercise recovery, growth hormone has been implicated as a key regulator of whole-body lipid oxidation over the first few hours after exercise (Pritzlaff et al., 2000), with close associations between growth-hormone response magnitude and the rate of systemic glycerol appearance after submaximal exercise (Wee et al., 2005). Furthermore, intravenous administration of growth hormone increases interstitial femoral and adipose concentrations of nonesterified fatty acids (NEFA) and glycerol in a manner similar to that of submaximal exercise (Gravholt et al., 1999; Mulla, Simonsen, & Bulow, 2000). These effects may operate via increased activity of hormone-sensitive lipase and adipocyte sensitivity to catecholamines (Leung & Ho, 1997) and/or via altered substrate selection in muscle at the level of the mitochondria (Short, Moller, Bigelow, Coenen-Schimke, & Nair, 2008). Maximizing the growth-hormone response to a given exercise stimulus may therefore represent a useful strategy to encourage both mobilization and subsequent disposal of certain metabolic substrates (e.g., lipid/adipose; Wee et al., 2005) while allowing sparing/synthesis of others during either recovery or subsequent exercise (e.g., carbohydrate/glycogen).

In relation to multiple bouts of exercise, an initial brief bout of high-intensity (aerobic or sprint) exercise attenuates or even completely abolishes the growth-hormone response to the same exercise stimulus repeated within 4 hr (Sartorio, Agosti, Marinone, Proietti, & Lafortuna, 2005; Stokes, Nevill, Frystyk, Lakomy, & Hall, 2005). In contrast, repeated exposure to ~30–60 min of submaximal exercise at 3- to 4-hr intervals results in an augmented growth-hormone response to each consecutive exercise.
bouts (Kanaley et al., 1997; Ronsen, Haug, Pedersen, & Bahr, 2001). This reversal of the effect observed with high-intensity exercise may therefore be a consequence of the greater challenge to glucose homeostasis posed by more prolonged exercise. Indeed, consecutive hypoglycemic episodes separated by several hours equally stimulate growth-hormone responses even in the absence of exercise (Jezova, Radikova, & Vigas, 2007), so the progressively attenuated growth-hormone response to repeated exercise may depend on the occurrence of hypoglycemia or glycogen depletion (Galassetti et al., 2001).

While carbohydrate ingestion may therefore play a mediating role in the growth-hormone response to repeated exercise, no previous study has explored growth-hormone responses to repeated exercise bouts in the context of ample carbohydrate ingestion alone or in combination with other nutrients. Resting concentrations of growth hormone are elevated several hours after the ingestion of glucose (after a transient suppression; Bernardi et al., 1999; Frystyk, Grofte, Skaerbaek, & Orskov, 1997) and more promptly with amino acid/protein ingestion (Collier, Casey, & Kanaley, 2005; Suminski et al., 1997; van Vuith, Nieuwenhuizen, Brummer, & Westerterp-Plantenga, 2008) or coingestion of both nutrients (Pallotta & Kennedy, 1968; Rabinowitz, Merimee, Maffezzoli, & Burgess, 1966). However, the magnitude of growth-hormone response to a single bout of exercise appears largely unaffected by ingestion of carbohydrate (Bird, Tarpenning, & Marino, 2006; Cappon, Ipp, Brasel, & Cooper, 1993; Ratamess et al., 2007), amino acids (Bird et al., 2006; Chromiak & Antonio, 2002; Collier, Collins, & Kanaley, 2006; Jacobson, 1990; Ratamess et al., 2007; Suminski et al., 1997), or combined carbohydrate-protein ingestion (Bird et al., 2006; Chandler, Byrne, Patterson, & Ivy, 1994). Notably, every one of the cited studies involved short-duration and resistance-based activities as opposed to prolonged submaximal exercise. Resistance exercise elicits a lower growth-hormone response than does endurance exercise (Gilbert et al., 2008) and may also be less relevant to the proposed primary function of growth hormone in regulating postexercise substrate selection (Doessing et al., 2010; Pritzlaff et al., 2000; Wee et al., 2005; West et al., 2010; West et al., 2009).

With regard to postexercise substrate selection, we previously demonstrated that including protein in a carbohydrate recovery supplement resulted in greater rates of lipid oxidation during recovery and greater rates of carbohydrate oxidation during subsequent exercise but without affecting muscle glycogen metabolism (Betts, Williams, Boobis, & Tsintzas, 2008; Betts, Williams, Duffy, & Gunner, 2007). To gain novel insight regarding the endocrine responses to postexercise nutritional intervention, we undertook further analysis of samples collected during the Betts et al. (2007) study. Measurement of growth hormone provides the first report of this response to sequential bouts of prolonged exercise (i.e., irrespective of nutrition). Of course, systemic growth-hormone concentrations are just one component of a wider endocrine response, acting in concert with other hormones to regulate carbohydrate, lipid, and protein metabolism (e.g., testosterone, IGF-1, and cortisol). In addition, these and other parameters directly mediate the biological actions of growth hormone (e.g., growth-hormone-binding protein, IGF-1, and estradiol), whereas other hormones of related structure or function simply provide a more complete perspective of the broader endocrine response (e.g., prolactin and progesterone). We therefore complemented our primary analysis by also measuring a selected range of these relevant endocrine responses. We thought that ingestion of a carbohydrate-protein solution during a 4-hr recovery from an initial bout of treadmill running would provide useful information to athletes and habitual exercisers who train on multiple occasions daily and need to consume mixed meals between exercise sessions. Moreover, contrasting this response with control solutions matched for either carbohydrate or available energy content would provide novel insight regarding the independent effects of protein and energy content per se on subsequent exercise-induced growth-hormone responses. Based on the available evidence showing limited impact of preexercise nutrition on the growth-hormone response to single bout of exercise, we hypothesized that the growth-hormone response to subsequent exercise should equally be unaffected by the inclusion of protein in a carbohydrate recovery supplement.

### Materials and Methods

#### Participants

Six recreationally active men took part in this study (age 21 ± 3 years, body mass [BM] 72.6 ± 8.4 kg, VO2max 61.4 ± 7.3 ml·kg⁻¹·min⁻¹). These individuals performed 6 ± 2 hr/week of endurance running as part of their habitual training. Each participant was briefed regarding the nature of the study and provided informed consent both verbally and in writing. The study was approved by the Loughborough University Ethical Advisory Committee and was part of a wider project on the impact of nutrition on postexercise recovery, parts of which have been published elsewhere (Betts et al., 2007).

#### Preliminary Measurements

Preliminary tests were conducted to determine each participant’s submaximal and maximal oxygen uptakes (Taylor, Buskirk, & Henschel, 1955) on a motorized treadmill (Technogym, Italy). A subsequent test was then performed 2 weeks before Trial 1 to further familiarize participants with all procedures and also to confirm that calculated running speeds were equivalent to 70% of maximal oxygen uptake (VO2max). All participants continued their habitual training throughout the study period but refrained from strenuous exercise and avoided both alcohol and caffeine consumption during the 48 hr before main trials.
Experimental Design

Participants performed three main trials in a randomized order that were separated by at least 1 week and applied in a double-blind manner. A dietary record was completed for the 48 hr before Trial 1 and was then repeated before subsequent trials (2,497 ± 535 kcal/day; 53% ± 9% CHO, 31% ± 9% fat, 16% ± 3% protein). Main trials involved a 90-min treadmill run at 70% VO_2max_ followed by a 4-hr recovery and then a second treadmill run at the same intensity as the first but performed to the point of volitional exhaustion (i.e., 91.6 ± 17.9 min), with blood samples drawn throughout both exercise sessions. The first exercise session was designed to be prolonged but not exhaustive in nature, and we have previously reported that a run of similar intensity and duration without exogenous carbohydrate ingestion can result in substantially reduced quadriceps glycogen concentrations, specifically localized to the Type I muscle fibers (Tsintzas, Williams, Babbis, & Greenhaff, 1996). During the recovery period, participants rested in the laboratory while consuming a carbohydrate-protein mixture (CHO-PRO trial), a matched amount of carbohydrate alone (CHO trial), or a solution containing a larger amount of carbohydrate (CHO-CHO trial) that matched the CHO-PRO solution for available energy.

Experimental Protocol

Participants arrived at the laboratory between 8 and 8:30 a.m. after a 10-hr overnight fast. After confirming their informed consent to take part in the study, a cannula was inserted into an antecubital vein and a 10-ml resting volume ingested during R1 = 0.5 ± 0.4 l). In subsequent trials (total volume ingested during R2 = 0.8 ± 0.6 l). During R1, venous blood samples were drawn at 10 min and 30 min during exercise to capture the early response and then a final postexercise sample drawn immediately (i.e., within 30 s) on completion of the run. Ambient temperature and humidity were recorded at 30-min intervals throughout all trials using a hygrometer (Zeal, UK) and were not different between trials: 20.6 ± 0.8 °C and 42.5% ± 7.4% in the CHO trial, 21.0 ± 1.0 °C and 40.6% ± 9.3% in the CHO-PRO trial, and 19.9 ± 1.5 °C and 41.5% ± 13.4% in the CHO-CHO trial.

Solution Composition

The rate of carbohydrate (sucrose) ingestion in the CHO and CHO-PRO trials was 0.8 g · kg BM⁻¹ · hr⁻¹, whereas the CHO-CHO solution provided 1.1 g CHO · kg BM⁻¹ · hr⁻¹ (total carbohydrate intake 232 ± 27 g and 320 ± 37 g, respectively). The CHO-PRO solution contained 3.3% of whey-protein isolate in the CHO mixture such that total protein intake was equivalent to 87 ± 10 g (equivalent to an ingestion rate of 0.3 g PRO · kg BM⁻¹ · hr⁻¹); the amino acid profile of this protein is presented in Figure 1. All solutions were provided in equal volumes (581 ± 67 ml/hr), and the higher carbohydrate content of the CHO-CHO solution was therefore achieved by increasing the carbohydrate concentration from 10% to 13.3%. The estimated amount of energy that each solution made available for metabolism was 3.2 kcal · kg BM⁻¹ · hr⁻¹ in the CHO trial and 4.3 kcal · kg BM⁻¹ · hr⁻¹ in both the CHO-PRO and the CHO-CHO trial (total energy intake 929 ± 107 and 1,278 ± 147 kcal, respectively).

Sampling and Analysis

Five milliliters of each whole-blood sample was dispensed into a nonanticoagulant tube where it was left to clot for 45 min at room temperature and then centrifuged at 2,000 g for 10 min at 4 °C (Beckman-Coulter Allegra X-22R, Germany). The serum fraction was then abstracted and stored at −80 °C pending later analyses with a spectrophotometric plate reader (Anthos HTIII, Anthos Labtec International) for growth-hormone-binding protein using a commercially available enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories, Inc.; intra-assay CV ≤ 5.6%). The remaining serum was analyzed using an automated gamma counter (Cobra 5000, Packard Instruments, USA) with commercially available radioimmunoassays to measure serum concentrations of cortisol (MP Biomedicals Ltd., USA; intra-assay CV ≤ 4.8%) and growth hormone (MP Biomedicals Ltd., USA; intra-assay CV ≤ 4.4%). Given that the proportion of different growth-hormone isoforms and fragments in the circulation is known to vary under different circumstances (Trainer, Barth, Sturgeon, & Wieringaon, 2006), we have conducted extensive in-house validation of the radioimmunoassay employed for measurement of growth hormone in this study and have observed excellent correlations (r² ≥ .98) relative to leading enzyme-linked immunosorbent assays (R&D Systems, Inc.; Diagnostic Systems Laboratories, Inc.) using samples collected both during and in recovery from endurance, resistance, and sprint exercise and under a variety of nutritional conditions. The other 5 ml of whole blood was dispensed into tubes containing ethylenediaminetetra-acetic acid before
centrifugation at 2,000 g for 10 min at 4 °C (Beckman-Coulter Allegra X-22R, Germany). The resultant plasma was also stored at ~80 °C and later analyzed using a commercially available enzyme-linked immunosorbent assay for both total and free insulin-like growth factor-1 (IGF-1; Diagnostic Systems Laboratories, Inc.; intra-assay CV ≤ 6.0% and 3.1%, respectively), again with a spectrophotometric plate reader (Anthos HTIII, Anthos Labtec International). Plasma glucose and NEFA concentrations were quantified spectrophotometrically using commercially available assays (Randox, Ireland; WAKO C, USA), and the remaining plasma was analyzed using an automated biochip immunoassay analyzer (Evidence Investigator, Randox, Ireland) with a relevant fertility hormone array (EV3610, Randox, Ireland) to determine plasma concentrations of the following hormones (with intra-assay CV): total testosterone (14.5%), progesterone (10.3%), prolactin (8%), follicle-stimulating hormone (6.3%), luteinizing hormone (5.7%), and estradiol (8.5%).

Five-minute expired-gas samples were collected coincident with all blood samples, with dry-gas fractions of O₂ and CO₂ quantified using paramagnetic and infrared analyzers, respectively (Servomex 1440, UK) and total volumes expired measured using a dry gas meter (Harvard Apparatus, UK). These values were then used to determine oxygen uptake (VO₂) and carbon dioxide production (VCO₂) both for standardization of exercise intensity relative to VO₂max and for determination of respiratory-exchange ratios (i.e., VCO₂:VO₂) as described by Frayn (1983).

Statistical Analyses
A two-way general linear model for repeated measures (Treatment × Time) was used to identify differences over time between experimental conditions, which was then subject to the Greenhouse-Geisser correction for epsilon < .75 and the Huynh-Feldt correction adopted for less severe asphericity. When significant $F$ values were found, the Holm-Bonferroni step-wise method was adopted to determine the location of variance (Atkinson, 2002). These statistical analyses were performed using SPSS for Windows version 14.0 software (Chicago, IL), and all data in text are presented as $M \pm SD$. The variance bars shown on figures are confidence intervals (CI) that have been corrected for between-subjects variation (Masson & Loftus, 2003).
Results

Serum concentrations of growth hormone were elevated substantially during R1 from preexercise values in the region of 1–2 μg/L to postexercise peak concentrations of between 13 and 19 μg/L, with no significant difference between treatments in the magnitude of this response (Figure 2). All three trials also displayed a similar response in that growth-hormone concentrations returned to preexercise levels within the first hour after exercise and were maintained at this level for the remainder of the 4-hr recovery. Conversely, with the onset of exercise subsequent to recovery (i.e., R2), responses to the three treatments began to diverge after 10 min of exercise and were significantly higher in the CHO-PRO trial than in the CHO (p = .01) or CHO-CHO (p = .02) trial at the point of volitional exhaustion. Notably, the peak growth-hormone responses observed after R2 in the CHO and CHO-CHO trials were similar to those observed after R1, while the accentuated growth-hormone response to R2 in CHO-PRO trials was greater than that observed in response to a similar duration of exercise earlier the same day (p < .05). Over this same period, serum growth-hormone-binding protein concentrations did not differ between treatments at any time point (Figure 3).

Serum concentrations of cortisol increased slightly during R1 and decreased during the 4-hr recovery period to below preexercise levels before increasing more markedly by the end of the second exercise bout. No Treatment × Time interaction was apparent for the cortisol response (p = .2), although a main effect of treatment was identified (p ≤ .05). Peak cortisol concentrations reached by the end of R2 showed the reverse pattern to that of growth hormone at the same time point; values were lower with CHO-PRO relative to the similarly elevated responses with CHO and CHO-CHO (Figure 4), although the large CIs on the figure illustrate that this effect was not significant (p = .3 and p = .2, respectively).

Table 1 shows the concentrations of all other selected hormones measured before and after R2. As can be seen, total testosterone, total IGF-1, free IGF-1, progesterone, and prolactin all exhibited significantly elevated concentrations in response to the exercise. However, the magnitude of these responses did not differ between treatments for any parameter. Plasma glucose and NEFA responses throughout trials are illustrated in Table 2. The peak glucose responses after 1 hr of recovery were significantly higher with CHO-CHO than with CHO-PRO (p = .01) and were significantly lower after 10 min (p = .03) and 30 min (p = .004) of the second exercise bout with CHO than with CHO-PRO. NEFA concentrations did not differ significantly between treatments at any time point but were slightly higher throughout R2 with CHO alone, relative to the close agreement between both the

![Figure 2](image-url) — Serum growth-hormone concentrations during Run 1 (R1), recovery, and Run 2 (R2). Participants received CHO, CHO-PRO, or CHO-CHO supplements during recovery. Values are M ± CI. *Time points different CHO-PRO versus both CHO and CHO-CHO (p ≤ .02).
Figure 3 — Serum growth-hormone-binding protein concentrations during Run 1 (R₁), recovery, and Run 2 (R₂). Participants received CHO, CHO-PRO, or CHO-CHO supplements during recovery. Values are $M \pm CI$.

Figure 4 — Serum cortisol concentrations during Run 1 (R₁), recovery, and Run 2 (R₂). Participants received CHO, CHO-PRO, or CHO-CHO supplements during recovery. Values are $M \pm CI$, with a main effect of treatment ($p \leq .05$).
Growth hormone and carbohydrate plus protein trials. Table 2 also includes data showing that the respiratory-exchange ratio was significantly lower during the second (p = .05), third (p = .04), and fourth (p = .005) hr of recovery when CHO-PRO was ingested as opposed to CHO-CHO.

**Discussion**

This is the first study to examine growth-hormone responses to repeated bouts of prolonged exercise. The main findings are that (a) the growth-hormone response did not differ between the bouts when carbohydrate was ingested during the 4-hr recovery and (b) including protein in the recovery supplement potentiated the growth-hormone response to a second exhaustive bout of exercise, both relative to an earlier run of similar but fixed duration (i.e., 90 min) and relative to the carbohydrate- or energy-matched carbohydrate supplements. The latter finding is inconsistent with our initial hypothesis based on all available evidence pertaining to carbohydrate and/or protein ingestion before a single bout of exercise.

Growth hormone promotes lipid mobilization (Gravholt et al., 1999; Mulla et al., 2000; Wee et al., 2005) and oxidation (Short et al., 2008), so it might be reasoned that carbohydrate (glycogen) could be spared in the protein trial, thus accounting for our previous report of improved physical performance (Betts et al., 2007). However, the blood-lipid and whole-body
### Table 2  Plasma Metabolite Concentrations and Respiratory-Exchange Ratios (RER) During Run 1 (R₁), Recovery, and Run 2 (R₂), M ± SD

<table>
<thead>
<tr>
<th>Glucose, mmol/L</th>
<th>Run 1</th>
<th>Recovery</th>
<th>Run 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-R₁</td>
<td>30 min</td>
<td>60 min</td>
<td>Post-R₁</td>
</tr>
<tr>
<td>C</td>
<td>5.08 (0.38)</td>
<td>5.00 (0.56)</td>
<td>4.92 (0.48)</td>
</tr>
<tr>
<td>C-P</td>
<td>4.95 (0.21)</td>
<td>4.89 (0.69)</td>
<td>4.64 (0.61)</td>
</tr>
<tr>
<td>C-C</td>
<td>5.01 (0.21)</td>
<td>4.94 (0.54)</td>
<td>4.72 (0.34)</td>
</tr>
<tr>
<td>NEFA, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.32 (0.17)</td>
<td>0.48 (0.21)</td>
<td>0.69 (0.33)</td>
</tr>
<tr>
<td>C-P</td>
<td>0.32 (0.16)</td>
<td>0.43 (0.24)</td>
<td>0.64 (0.47)</td>
</tr>
<tr>
<td>C-C</td>
<td>0.31 (0.21)</td>
<td>0.43 (0.22)</td>
<td>0.61 (0.34)</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.84 (0.07)</td>
<td>0.89 (0.02)</td>
<td>0.90 (0.02)</td>
</tr>
<tr>
<td>C-P</td>
<td>0.82 (0.08)</td>
<td>0.90 (0.04)</td>
<td>0.90 (0.04)</td>
</tr>
<tr>
<td>C-C</td>
<td>0.82 (0.05)</td>
<td>0.91 (0.04)</td>
<td>0.89 (0.04)</td>
</tr>
</tbody>
</table>

*Note.* C = 0.8 g sucrose · kg⁻¹ · hr⁻¹; C-P = 0.8 g sucrose · kg⁻¹ · hr⁻¹ with 0.3 g · kg⁻¹ · hr⁻¹ whey-protein isolate; C-C = 1.1 g sucrose · kg⁻¹ · hr⁻¹; NEFA = nonesterified fatty acids. Participants received C, C-P, or C-C supplements during recovery.

*Time points different C-P vs. C (p ≤ .03); **Values different C-P vs. C-C (p ≤ .05). Previously published data (Betts et al. 2007).
substrate-oxidation data taken from that study (Table 2) indicate that the added protein increased neither lipolysis nor lipid metabolism, consistent with our separate study reporting no difference in glycogen utilization rates between treatments (Betts et al., 2008). While the added protein is therefore be capable of augmenting the growth-hormone response to repeated exercise, this does not translate into greater lipid oxidation or explain the observed performance benefit, possibly because the wider context of high-dose carbohydrate ingestion and intense exercise limits the ability to substantially increase lipid oxidation irrespective of differences in growth-hormone concentration.

The magnitude of growth-hormone response to each exercise session independent of nutrition (i.e., R1 vs. R2) shows clearly that there was no attenuation of the growth-hormone response with consecutive bouts as observed with high-intensity exercise (Sartorio et al., 2005; Stokes et al., 2005). The negative-feedback response to high-intensity exercise that progressively attenuates sequential growth-hormone release is thought to involve an accumulation of hypothalamic somatostatin during the initial exercise bout (Lanzi & Tannenbaum, 1992b). However, this mechanism is only thought to persist for as long as circulating growth hormone remains elevated and for a maximum of 3–4 hr (Lanzi & Tannenbaum, 1992a), which may partly explain why the effect was absent in the current study.

Notwithstanding this, some degree of attenuation remains even 4 hr after high-intensity exercise (Sartorio et al., 2005; Stokes et al., 2005), whereas prolonged exercise exerts the opposite effect (Kanaley et al., 1997; Ronsen et al., 2001), with the former attributed to elevated concentrations of NEFA acting on the pituitary (Stokes, Tyler, & Gilbert, 2008). The ability of repetitive submaximal exercise to override this autoinhibition and thus augment subsequent exercise-induced growth-hormone responses may therefore depend on both reduced NEFA availability and hypoglycemia (Jezova et al., 2007). This may be why the growth-hormone response was simply maintained between exercise bouts in the current study; high rates of carbohydrate ingestion during recovery would oppose attenuation of subsequent growth-hormone responses by blunting NEFA mobilization but without augmenting the subsequent response by avoiding hypoglycemia (Betts et al., 2007). While the current study was not designed to evaluate the effect of carbohydrate per se on sequential exercise-induced growth-hormone responses, the sustained intake of a concentrated carbohydrate solution nevertheless represents a key difference between this study and those reporting increased growth-hormone responses with each ensuing bout of exercise (Kanaley et al., 1997; Ronsen et al., 2001).

The preceding discussion regarding changes in the magnitude of growth-hormone response over repeated exercise bouts sets the context to consider any potential interactive effects of ingested protein. Inclusion of protein in the recovery supplement did reveal the augmented growth-hormone response to a second bout of submaximal (albeit short-duration) exercise that has been reported by others when participants ingested a light mixed meal (Ronsen et al., 2001) or even remained fasted between exercise bouts (Kanaley et al., 1997). The fact that growth-hormone concentrations after the second bout were higher relative to both the carbohydrate- and available energy-matched supplements further indicates that potentiation of the growth-hormone response to repeated exercise may be protein mediated, as opposed to being a function of the increased energy intake. However, with no clear effect of protein on growth-hormone release even after a single bout of exercise (Bird et al., 2006; Chandler et al., 1994; Chromiak & Antonio, 2002; Collier et al., 2006; Jacobson, 1990; Ratamess et al., 2007; Suminski et al., 1997), the potential for protein to replicate the effect reported here may depend on a variety of protocol-specific factors including exercise intensity (Pritzlaff et al., 1999), recovery duration (Sartorio et al., 2005; Stokes et al., 2005), and individual factors such as nutritional/training status and gender (Mejri, Bchir, Rayana, Hamida, & Slama, 2005; Wideman et al., 2000). Nonetheless, we provide the first evidence that including protein in recovery supplements may be a useful strategy to maximize the growth-hormone response to multiple bouts of exercise. Whether this effect has any value in relation to the proposed roles of growth hormone for collagen synthesis and/or postexercise substrate metabolism remains to be established (Doessing et al., 2010; Pritzlaff et al., 2000; Wee et al., 2005).

The preceding discussion concerning differences in growth-hormone response between bouts or the effect of nutrition should be interpreted with the knowledge that the peak growth-hormone concentrations reported here after the initial run (i.e., 16.6 ± 7.8 μg/L across all trials) are broadly equivalent to previous reports using cycling at the same intensity but more than twice that elicited during resistance and sprint exercises (Gilbert et al., 2008). The primary function and/or importance of growth hormone may therefore vary between different modes of exercise; hence, the pattern and/or importance of findings may be context specific.

It should be noted that the post-R2 blood samples were taken at the point of exhaustion, which varied slightly but significantly between treatments such that the absolute time of measurement was not standardized. However, the CHO trial exhibited the shortest exercise time (84 ± 17 min), and the CHO-CHO trial exhibited the longest (100 ± 20 min) yet produced almost identical growth-hormone responses at the point of fatigue (19 ± 4 and 19 ± 5 μg/L, respectively), whereas growth-hormone concentrations were 50% greater with CHO-PRO (31 ± 4 μg/L) at an intermediate absolute time point (91 ± 16 min). The pattern reported here can therefore be more confidently attributed to a direct effect of the ingested protein rather than an indirect influence of exercise duration. The same reasoning applies to the possibility that diurnal and/or pulsatile rhythms could explain our findings. Notwithstanding that participants all began testing at a standardized time of day (± 30 min), it has been
demonstrated that the acute exercise-induced growth-hormone response is highly reproducible irrespective of even substantial variability in time of day (i.e., 5–18 hr; Kanaley, Weltman, Pieper, Weltman, & Hartman, 2001).

In relation to the range of additional endocrine responses reported here, the results in Table 1 are for the pre- and post-R₂ concentrations of these selected hormones and so only provide information regarding the effect of added protein at those time points, as opposed to the interaction of protein with bout number. It is notable that the response of these hormones to exercise is unaffected by the inclusion of protein in the carbohydrate supplements ingested during the preceding recovery. Again, very limited or no data are available regarding the effects of protein during a repeated bout of exercise, so comparisons can only be made relative to studies that have measured the effects of protein before a single bout of resistance exercise. Similar to the results reported in Table 1, those studies have shown no effect of ingested protein on IGF-1, testosterone, or luteinizing-hormone responses to exercise (Bird et al., 2006; Chandler et al., 1994). One of the studies did demonstrate a reduced cortisol response to exercise with combined carbohydrate-protein ingestion relative to a placebo but not relative to carbohydrate alone (Bird et al., 2006). While a main effect of treatment was noted in the current study for cortisol, this does not of course pertain specifically to the response during the second exercise session. However, although not statistically different, the pattern of response to treatments observed post-R₂ for cortisol is the exact opposite of that observed for growth hormone. The purpose of this balance could relate to the purportedly antagonistic roles of these two hormones for tissue anabolism or, alternatively, may reflect a compensatory reduction in cortisol given the counterregulatory function common to both hormones in response to hypoglycemia.

The current study was designed to examine the hormonal and metabolic responses during repeated bouts of prolonged exercise and clearly demonstrates an effect of nutritional intervention in recovery on growth-hormone and cortisol responses to the second bout. Since these differences were discovered late in the protocol, one limitation is that measurements were not extended into recovery from the second exercise bout. Another limitation is that only 6 participants were tested, which is at the lower end of the range typically employed in studies on this topic involving a crossover design. However, even this limited sample size was adequate to detect the marked (~50%) increase in the primary outcome measure relative to the very tight agreement between the two carbohydrate-only trials. All secondary measures for which no significant effects were detected showed no evidence of meaningful differences between treatments, so we are confident that no important responses have been overlooked simply due to low statistical power.

In conclusion, this study is the first to report growth-hormone responses to repeated bouts of prolonged exercise, with additional novel insight provided by considering the potential mediating influence of nutrition. In contrast to previous reports using short-duration high-intensity exercise, the growth-hormone response to the second exhaustive bout of exercise was neither attenuated nor augmented relative to an earlier run of similar but fixed duration (i.e., 90 min). The inclusion of protein in the recovery supplement augmented the response to the second exercise bout relative to the first bout and to the response observed after ingestion of carbohydrate alone.

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References


