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## Endocrine Responses During Overnight Recovery From Exercise: Impact of Nutrition and Relationships With Muscle Protein Synthesis

James A. Betts, Milou Beelen, Keith A. Stokes, Wim H.M. Saris, and Luc J.C. van Loon

Nocturnal endocrine responses to exercise performed in the evening and the potential role of nutrition are poorly understood. To gain novel insight, 10 healthy men ingested carbohydrate with (C+P) and without (C) protein in a randomized order and double-blind manner during 2 hr of interval cycling followed by resistance-type exercise and into early postexercise recovery. Blood samples were obtained hourly throughout 9 hr of postexercise overnight recovery for analysis of key hormones. Muscle samples were taken from the vastus lateralis before and after exercise and then again the next morning (7 a.m.) to calculate mixed-muscle protein fractional synthetic rate (FSR). Overnight plasma hormone concentrations were converted into overall responses (expressed as area under the concentration curve) and did not differ between treatments for either growth hormone ( $1,464 \pm 257$  vs.  $1,432 \pm 164$  pg/ml  $\cdot$  540 min) or total testosterone ( $18.3 \pm 1.2$  vs.  $17.9 \pm 1.2$  nmol/L  $\cdot$  540 min, C and C+P, respectively). In contrast, the overnight cortisol response was higher with C+P ( $102 \pm 11$  nmol/L  $\cdot$  540 min) than with C ( $81 \pm 8$  nmol/L  $\cdot$  540 min;  $p = .02$ ). Mixed-muscle FSR did not differ between C and C+P during overnight recovery ( $0.062\% \pm 0.006\%$  and  $0.062\% \pm 0.009\%$ /hr, respectively) and correlated significantly with the plasma total testosterone response ( $r = .7, p < .01$ ). No correlations with FSR were apparent for the response of growth hormone ( $r = -.2, p = .4$ ), cortisol ( $r = .1, p = .6$ ), or the ratio of testosterone to cortisol ( $r = .2, p = .5$ ). In conclusion, protein ingestion during and shortly after exercise does not modulate the endocrine response or muscle protein synthesis during overnight recovery.

**Keywords:** carbohydrate, growth hormone, cortisol, testosterone

Tissue repair and regeneration after exercise and subsequent physiological adaptation depend on muscle protein synthesis in response to an exercise stimulus. Net protein balance will remain negative during the acute response to exercise unless nutrients are ingested (Rennie & Tipton, 2000). Transition from a negative to a positive net protein balance can be achieved by ingesting carbohydrate and protein, which reduce protein breakdown and stimulate muscle protein synthesis (Beelen, Koopman, et al., 2008; Koopman et al., 2004; Koopman et al., 2005; Rasmussen, Tipton, Miller, Wolf, & Wolfe, 2000). The acute muscle protein synthetic response to individual exercise sessions may therefore translate into lean tissue accrual during more prolonged exercise training (Rennie & Tipton, 2000).

It has been shown that long-term accrual of lean tissue mass with resistance training can occur even without marked endocrine responses during acute postexercise recovery (Wilkinson, Tarnopolsky, Grant, Correia, & Phillips, 2006). Moreover, these acute endocrine responses have recently been reported to predict neither the muscle

protein synthetic response over the first 4 hr of postexercise recovery (West et al., 2009) nor long-term gain in muscle mass or strength (West et al., 2010). Such findings are intriguing because they appear incompatible with the established view that acute postexercise elevations in the systemic concentration of certain hormones are critical to tissue growth (Kraemer & Ratamess, 2005). Given such inconsistencies, it becomes understandable that emerging studies have begun to explore the extended time course of muscle protein synthesis beyond the immediate postexercise recovery period (i.e., overnight recovery; Beelen, Tieland, et al., 2008), thus complementing the few existing studies that have documented overnight endocrine responses to daytime exercise (Adamson, Hunter, Ogunremi, Oswald, & Percy-Robb, 1974; Hackney, Ness, & Schrieber, 1989; Kern, Perras, Wodick, Fehm, & Born, 1995; McMurray, Eubank, & Hackney, 1995; Tuckow et al., 2006; Zir, Smith, & Parker, 1971).

Previously, Beelen, Tieland, et al. (2008) reported no differences in overnight muscle protein synthesis rates after exercise performed in the evening when participants ingested a carbohydrate-protein mixture relative to water only. Based on these data, it would not be anticipated that ingesting this carbohydrate-protein mixture would stimulate overnight muscle protein synthesis any more effectively than the carbohydrate fraction alone. Here we

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report these responses, alongside the nocturnal endocrine responses to evening exercise. The latter have not previously been investigated with these nutritional interventions, nor has the potential relationship between each hormone and overnight mixed-muscle protein fractional synthetic rate (FSR).

The examination of recovery from exercise performed after a full day of dietary standardization is highly relevant to most people who generally exercise in the evening while in a fed state. Given this practical value, it is notable that so many fundamental questions remain to be answered in relation to exercise under such conditions. The primary aim of this study was to explore the effect of ingesting carbohydrate with and without additional protein on overnight endocrine responses after exercise performed in the evening. A secondary aim was to examine whether these responses are correlated with mixed-muscle protein FSR assessed over the same time period. Coingestion of dietary protein with carbohydrate does not seem to modulate plasma growth hormone, cortisol, or testosterone responses over the first few hours after exercise (Bird, Tarpenning, & Marino, 2006a; Bird, Tarpenning, & Marino, 2006c; Chandler, Byrne, Patterson, & Ivy, 1994), although supplementing with dietary protein has been associated with lower resting testosterone and cortisol concentrations over multiple days of exercise (Bird, Tarpenning, & Marino, 2006b; Volek, Kraemer, Bush, Incledon, & Boetes, 1997). We hypothesized that protein coingestion during and shortly after exercise, though unlikely to alter muscle protein synthesis over an entire overnight recovery, might modulate the overnight endocrine response.

## Methods

### Participants

Ten healthy men volunteered to participate in this experiment (age  $20 \pm 2$  years, total body mass [BM]  $69.4 \pm 7.4$  kg, fat mass  $8.4 \pm 3.0$  kg, fat-free mass  $61.1 \pm 6.8$  kg, body-fat percentage  $12\% \pm 4\%$ , leg volume  $8.2 \pm 1.4$  L;  $M \pm SD$ ). All subjects were recreationally active, defined as participating in various forms of exercise on an irregular basis for approximately 2–4 hr/week and with no substantial history of dedicated endurance or resistance training. Participants had not undergone any dietary intervention or supplementation regimen in the 3 months before this experiment. All participants were fully informed of the nature and possible risks of the experimental procedures before providing written consent. The study was approved by the medical ethics committee of the Academic Hospital Maastricht, The Netherlands, and is part of a larger project on the impact of nutrition on postexercise recovery (Beelen, Koopman, et al., 2008).

### Pretesting

All participants completed two screening sessions separated by at least 5 days. In the morning after an overnight fast, body composition was determined by hydrostatic

weighing. Body-fat percentage was calculated using Siri's (1956) equation, and leg volume was measured by anthropometry (Jones & Pearson, 1969). Thereafter, participants were familiarized with the exercise equipment and exercise procedures. Proper lifting technique was demonstrated and practiced for each of the upper body exercises (chest press, shoulder press, and lateral pull-down) and the two lower limb exercises (leg press and leg extension). Thereafter, maximum strength for the two leg exercises was estimated using the multiple-repetition testing procedure originally described by Mayhew et al. (1995).

In the second screening session, participants' one-repetition maximum (1RM) was determined for both the leg-press ( $217 \pm 33$  kg) and leg extension ( $122 \pm 13$  kg) exercises to confirm the previously estimated maximal-strength values using an extended protocol (Kraemer & Fry, 1995). In addition, participants performed an incremental exhaustive exercise test (Kuipers, Verstappen, Keizer, Geurten, & Vankranenburg, 1985) on an electronically braked cycle ergometer (Lode Excalibur) to measure their maximal oxygen uptake ( $\dot{V}O_{2\max}$ :  $50.3 \pm 8.0$  ml  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) and workload capacity ( $W_{\max}$ :  $305 \pm 36$  W).

### Experimental Design

Participants took part in two experimental days each involving a different nutritional intervention, applied in a randomized order and a double-blind manner. During the experimental days, all participants received the same standardized diet (breakfast, lunch, dinner, and snacks). Apart from the standardized diet, they went about their normal daily activities and reported to the hospital in the evening. Subsequently, they performed a 2-hr endurance- and resistance-type exercise session during which they ingested either carbohydrate (C) or a mixture of carbohydrate and protein hydrolysate (C+P). Participants received two additional boluses of the test drink during early recovery and remained overnight at the hospital. Plasma samples were collected every 15 min during exercise, every 30 min during the first 2 hr of postexercise recovery, and every hour during overnight sleep. Muscle biopsies from the vastus lateralis were taken before and immediately after exercise and in the morning after 9 hr of postexercise recovery (7 a.m.). Tests were designed to determine mixed-muscle protein FSR by incorporating L-[ring-<sup>13</sup>C<sub>6</sub>]-phenylalanine in the mixed-muscle protein pool of the collected tissue samples.

### Standardization Procedures

All participants received a standardized diet the evening before the experimental day consisting of 3.7 MJ, with 62% of this energy in the form of carbohydrate, 22% in the form of fat, and a further 16% protein, and during the experimental day they received  $0.16 \pm 0.01$  MJ/kg BM, with this total energy intake comprising  $62\% \pm 0.4\%$  carbohydrate,  $26\% \pm 0.4\%$  fat, and  $12\% \pm 0.2\%$  protein. These meals were ingested at realistic intervals

throughout the experimental day (i.e., breakfast at 8:30 a.m., snack at 10:30 a.m., lunch at 12:30 p.m., snack at 3 p.m., and dinner at 5 p.m.), with the final meal composed of foods likely to be acceptable to all participants (i.e., tomato soup, bread with marmalade, and orange juice) and unlikely to interfere with the exercise performed 3 hr after the commencement of feeding. Participants' energy requirements were calculated with Harris and Benedict's (1918) equation, with a physical activity index of 1.7 (Plasqui & Westerterp, 2004). Although the precise macronutrient composition of the prescribed diet may not have accurately reflected the habitual intake of every participant, a protein intake of 1.1 g/kg BM meets current recommendations and should be considered more than adequate based on previous work in similar populations (Lejeune, Westerterp, Adam, Luscombe-Marsh, & Westerterp-Plantenga, 2006; Veldhorst, Westerterp-Plantenga, & Westerterp, 2009). The investigator provided the participants with measured amounts of all foods, and participants were instructed to take all meals and snacks at predetermined times. All volunteers were instructed to refrain from any strenuous physical activity and to keep their diet as constant as possible over the 2 days before the experimental day.

## Experimental Protocol

The experimental protocol is illustrated in Figure 1. At 6:30 p.m., participants reported to the laboratory, where a Teflon catheter was inserted into an antecubital vein for the primed, continuous infusion of isotopically labeled phenylalanine (priming dose 2  $\mu\text{mol/kg BM}$  L-[ring- $^{13}\text{C}_6$ ]-phenylalanine, infusion rate 0.05  $\mu\text{mol} \cdot \text{kg BM}^{-1} \cdot \text{min}^{-1}$  L-[ring- $^{13}\text{C}_6$ ]-phenylalanine). Another Teflon catheter was inserted into a contralateral hand vein, which was placed in a hotbox for arterialized blood sampling. After a background blood sample was collected ( $t = -180$  min;  $\sim 7$  p.m.), continuous tracer infusion was started (thus 2 hr after participants' last meal) and participants rested in

a supine position for 1 hr. Before the exercise protocol ( $t = -120$  min;  $\sim 8$  p.m.), the first muscle biopsy was collected, after which participants ingested the first bolus of test drink (4.5 ml/kg BM). During exercise, participants received subsequent boluses (1.5 ml/kg BM) of the test drink every 15 min. The exercise protocol consisted of an interval-cycling program followed by (whole-body) resistance-type exercise. This exercise protocol was designed to mimic a practical fitness-training session. At 10 p.m., immediately after the end of the exercise protocol ( $t = 0$  hr), an arterialized blood sample from the heated hand's vein and a second muscle biopsy from the vastus lateralis were obtained. Participants rested supine during the remainder of the evening and were provided with two beverages (4 ml/kg BM) after 30 and 90 min of postexercise recovery. This was followed by 7 hr of sleep, after which participants were awoken at 7 a.m. for a third muscle biopsy. The total postexercise recovery time was 9 hr. Blood samples (8 ml) were taken from the arterialized hand vein at  $t = -180, -120, -105, -90, -75, -60, -45, -30, -15, 0, 30, 60, 90,$  and 120 min and  $t = 3, 4, 5, 6, 7, 8,$  and 9 hr during sleep. Blood samples at  $t = 3, 4, 5, 6, 7, 8,$  and 9 hr during sleep were not arterialized, because sleeping would have been impossible with the hand in a hotbox.

## Exercise Protocol

After 10 min of warming up on a cycle ergometer (50%  $W_{\text{max}}$ ), participants cycled  $4 \times 5$  min at 65%  $W_{\text{max}}$ , alternated by  $4 \times 2.5$  min at 45%  $W_{\text{max}}$ . After a 5-min rest period, they commenced the resistance-exercise protocol, consisting of an upper body and lower body workout. The upper body workout was performed with a workload set at 40% of the total body weight, in which participants completed five sets of 10 repetitions on three upper body machines (chest press, shoulder press, and lat pull-down). A rest period of 1 min between sets was allowed. Given that FSR was

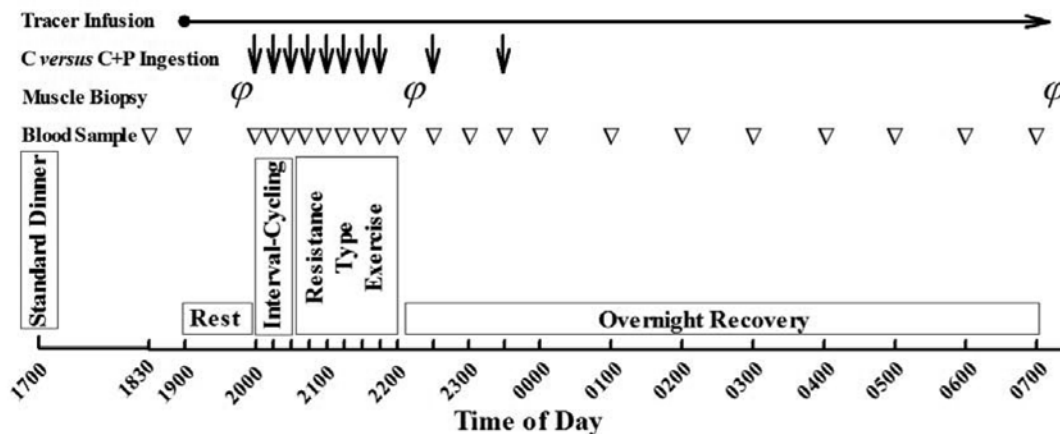


Figure 1 — Schematic of the experimental protocol.

assessed in the vastus lateralis, this upper body workout served primarily as an ecologically valid extended warm-up and was not therefore performed relative to 1RM, as were the lower leg exercises. This was followed by a lower limb workout, which consisted of nine sets of 10 repetitions on the horizontal leg-press machine (Technogym BV) and nine sets of 10 repetitions on the leg-extension machine (Technogym). On both machines, three sets were completed at 55% of participants' 1RM, three at 65% 1RM, and three at 75% 1RM, with 2-min rest periods between sets. Finally, participants performed two sets of 30 abdominal crunches. All were verbally encouraged during the exercise regimen to complete the entire protocol within ~120 min.

### Supplement Composition

Participants received either carbohydrate alone (C) or a mixture of carbohydrate and protein hydrolysate (C+P) in solution at a volume of 1.5 ml/kg BM every 15 min during exercise and 4 ml/kg BM 30 and 90 min after cessation of exercise. This resulted in carbohydrate ingestion rates of  $0.15 \text{ g} \cdot \text{kg BM}^{-1} \cdot \text{hr}^{-1}$  during both C and C+P trials, both in the form of 50% glucose and 50% maltodextrin. The C+P solution also provided  $0.15 \text{ g} \cdot \text{kg BM}^{-1} \cdot \text{hr}^{-1}$  of protein hydrolysate, thus providing 0.6 g/kg BM in total over the first 4 hr after the onset of exercise and increasing 24-hr protein intake from  $1.1 \pm 0.1$  to  $1.7 \pm 0.1 \text{ g/kg BM}$ . The preexercise bolus was provided in a volume of 4.5 ml/kg BM to stimulate gastric emptying and, as such, to allow a more continuous supply of glucose and amino acids from the gut during exercise. Glucose and maltodextrin were obtained from AVEBE (Veendam, The Netherlands). The casein protein hydrolysate (PeptoPro; 85.3% protein) was prepared by DSM Food Specialties as described previously (Beelen, Koopman, et al., 2008). To make the taste comparable, all solutions were flavored by adding 0.05 g/L sodium saccharinate, 0.9 g/L citric acid, and 5.0 g/L cream vanilla flavor (Quest International).

### Sampling and Analysis

Whole-blood samples were collected into tubes containing ethylenediaminetetra-acetic acid and centrifuged at 1,000 g for 10 min at 4 °C. Aliquots of plasma were frozen in liquid nitrogen and stored at -80 °C until analysis. Plasma glucose concentrations were analyzed using the COBAS-FARA semiautomatic analyzer (Uni Kit III, 07367204, La Roche), and plasma insulin was analyzed via radioimmunoassay (Linco, Human Insulin RIA kit, Linco Research). Enzyme-linked immunosorbent assays (ELISA) with a spectrophotometric plate reader (Anthos HTIII, Anthos Labtec International) were used to determine plasma concentrations of total testosterone (R&D Systems, Inc.), cortisol (Diagnostic Systems Laboratories, Inc.) and human growth hormone (R&D Systems, Inc.), with coefficients of variation for each

parameter of 2.9%, 3.7%, and 2.6%, respectively, across the full range of samples analyzed. We also adhered to the most recent consensus statement for the standardization of growth hormone assays (Trainer, Barth, Sturgeon, & Wieringaon, 2006) in that data were generated as SI units from an immunoassay using a highly purified *E. coli*-expressed recombinant human growth hormone calibrated by the manufacturer to International Reference Preparation 98/574 (R&D Systems, Inc.). Moreover, the antibody used in this assay was raised against the most abundant full-length 22 kDa growth hormone isoform, for which our in-house validation of this ELISA (R&D Systems, Inc.) has shown excellent correlations with both radioimmunoassay ( $r^2 = .98$ ; MP Biomedicals Ltd.) and other ELISA ( $r^2 = .99$ ; Diagnostic Systems Laboratories, Inc.). Given that the proportion of different growth hormone isoforms in the circulation is known to vary under different circumstances (Wallace et al., 2001), it is relevant that these validation data pertain to samples collected both during and in recovery from various modes of exercise.

Plasma and muscle L-[ring- $^{13}\text{C}_6$ ]-phenylalanine enrichment were measured as described previously (Beelen, Tieland, et al., 2008). Plasma L-[ring- $^{13}\text{C}_6$ ]-phenylalanine enrichment was used as the preferred precursor pool because the multiple plasma samples obtained throughout overnight sleep provide a more accurate representation of the overnight fluctuations in precursor enrichment. This study is part of a larger project on the impact of nutrition on overnight recovery, from which the tracer and FSR data during exercise have been published previously (Beelen, Koopman, et al., 2008).

### Statistical Analyses

A two-way general linear model for repeated measures (Treatment  $\times$  Time) was used to identify differences in the endocrine responses over time both within and between experimental conditions, with the Greenhouse-Geisser correction applied for epsilon  $< .75$  and the Huynh-Feldt correction adopted for less severe asphericity. When significant *F* values were found, the Holm-Bonferroni stepwise method was adopted to determine the location of variance (Atkinson, 2002). Total area under the concentration curve for plasma concentrations of growth hormone, cortisol, total testosterone, insulin, and glucose during both exercise and overnight recovery were calculated using the method recommended by Wolever (2004). This method was deemed most appropriate because it reflects the total exposure to each hormone, as opposed to the incremental area under curve, for which the area below an arbitrary baseline is subtracted from the total area under the curve. The intraindividual differences between treatments for such non-time-dependent variables were examined using a Shapiro-Wilk test, with normally distributed data analyzed for treatment differences using a paired *t* test and nonnormally distributed

data analyzed nonparametrically using a Wilcoxon's test. Data are presented as  $M \pm SEM$  unless otherwise stated. All statistical analyses were performed using SPSS for Windows version 14.0 (Chicago, IL), with significance set at an alpha level of .05.

## Results

### Plasma Growth Hormone

Marked elevations in plasma growth hormone concentration were observed both during the exercise protocol and during overnight recovery (with peaks occurring midexercise and at approximately 2–3 a.m.; effect of time,  $F = 7.1, p < .001$ ). Neither the time course nor the magnitude of this response differed between treatments (Figure 2A). The overall response (expressed as area under the concentration curve) did not differ between treatments during exercise ( $479 \pm 84$  vs.  $511 \pm 107$  pg/ml  $\cdot$  120 min) or during postexercise overnight recovery ( $1,464 \pm 257$  vs.  $1,432 \pm 164$  pg/ml  $\cdot$  540 min in C and C+P, respectively).

### Plasma Cortisol

Plasma cortisol exhibited a marked response to exercise (effect of time:  $F = 25.1, p < .001$ ), with concentrations peaking at the midexercise time point and remaining significantly different from baseline until 2 a.m. and then again from 6 a.m. onward (Figure 2B;  $p \leq .05$ ). The subsequent overnight increase observed from 3 to 7 a.m. displayed a slight divergence in responses between treatments and resulted in a significant overall effect of treatment ( $F = 6.5, p = .03$ ). This effect is supported by a significant difference between overnight cortisol responses (expressed as area under the concentration curve) between treatments, with a higher overnight response to C+P than to C ( $102 \pm 11$  and  $81 \pm 8$  nmol/L  $\cdot$  540 min, respectively,  $p = .02$ ).

### Plasma Total Testosterone

Figure 2C illustrates plasma total testosterone concentrations during exercise and the ensuing overnight recovery. A gradual increase was apparent during exercise, which continued at a similar rate throughout recovery (effect of time:  $F = 13.3, p < .001$ ), reaching peak values between 2 and 7 a.m. across different individuals and remaining significantly elevated relative to baseline from the postexercise time point onward ( $p \leq .01$ ). This pattern did not differ between C and C+P, and overall responses (expressed as area under the concentration curve) were similar between treatments ( $3.1 \pm 0.2$  vs.  $2.9 \pm 0.2$  nmol/L  $\cdot$  120 min during exercise and  $18.3 \pm 1.2$  vs.  $17.9 \pm 1.2$  nmol/L  $\cdot$  540 min during overnight recovery for C and C+P, respectively).

### Plasma Insulin and Glucose

There were no significant differences in plasma insulin concentrations between treatments at any specific time

point, although a trend was apparent for concentrations to be higher in C+P than in C (treatment:  $F = 4.5, p = .07$ ; Figure 3A). Similarly, we observed a trend for higher plasma glucose concentrations after C+P than C (treatment:  $F = 4.8, p = .06$ ), and there were no significant differences between treatments at any given time point (Figure 3B).

### Plasma Tracer Enrichment and Mixed-Muscle Protein FSR

The ingestion of C+P during exercise and during the first stages of postexercise recovery significantly elevated plasma phenylalanine concentrations by the end of exercise, with values remaining different between treatments for the first 2 hr of recovery (Figure 4A). Figure 4B shows the reverse pattern between treatments in relation to plasma L-[ring- $^{13}\text{C}_6$ ]phenylalanine tracer enrichment. The mean plasma L-[ring- $^{13}\text{C}_6$ ]phenylalanine tracer enrichments and mixed-muscle protein FSR are presented in Table 1.

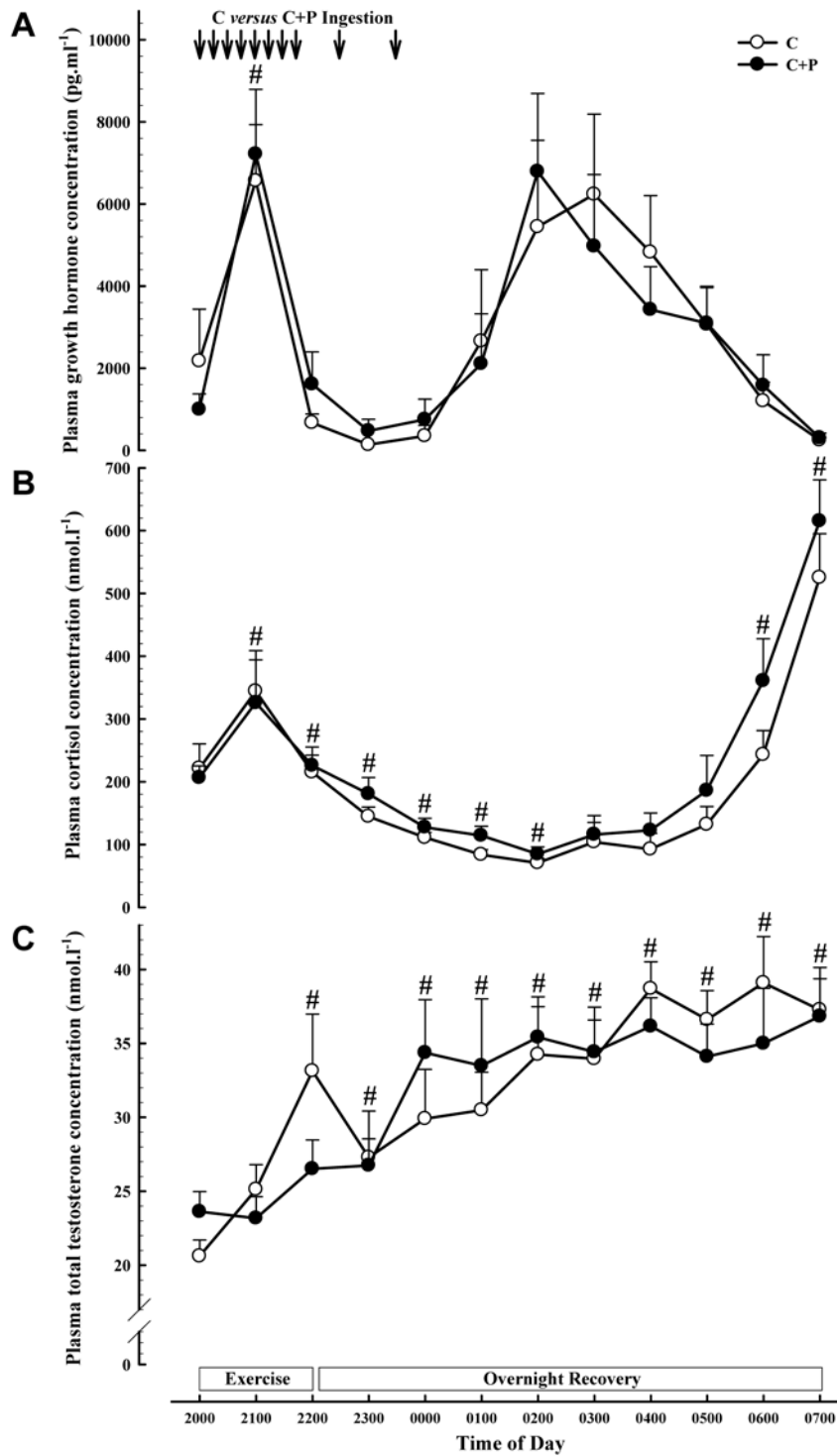
### Correlations

Rates of mixed-muscle protein fractional synthesis during both exercise and subsequent overnight recovery were correlated with all endocrine responses expressed both in terms of the peak concentration of each hormone measured in each individual and in terms of the overall exposure to each hormone during that time frame (i.e., area under the concentration curve). We observed a significant correlation between the mixed-muscle FSR recorded during overnight recovery and the associated overnight plasma total testosterone response (Figure 5) both within each treatment (C,  $r = .7, p = .04$ , and C+P,  $r = .7, p = .03$ ) and when combined ( $r = .7, p < .01$ ). In contrast, no significant correlations were observed between overnight mixed-muscle FSR and growth hormone (C,  $r = -.1, p = .9$ , and C+P,  $r = -.4, p = .3$ ), cortisol (C,  $r = -.02, p = 1.0$ , and C+P,  $r = .2, p = .6$ ), or the ratio of total testosterone to cortisol (C,  $r = .35, p = .4$ , and C+P,  $r = .05, p = .9$ ).

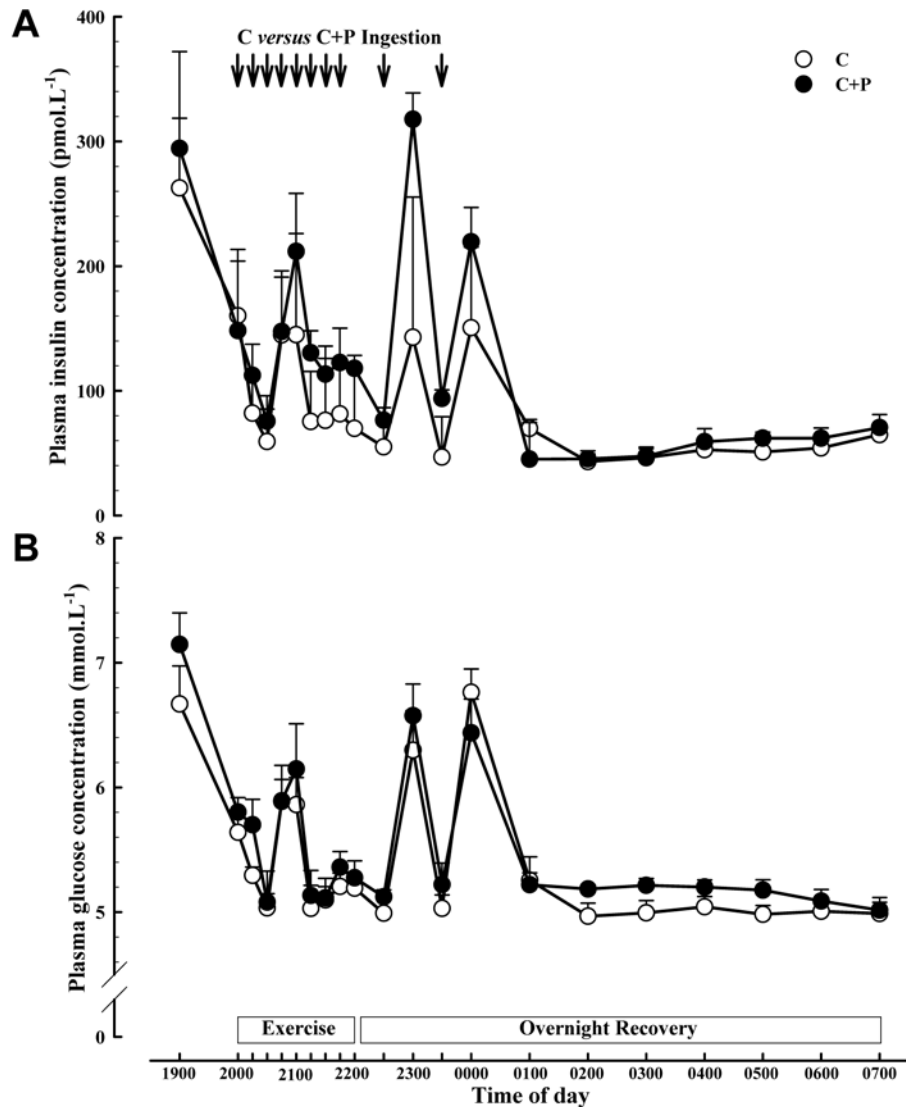
## Discussion

The main findings of this study were that the overnight responses of plasma growth hormone and total testosterone after a bout of combined endurance- and resistance-type exercise did not differ when participants ingested carbohydrate with or without additional protein during and immediately after exercise. In contrast, plasma cortisol concentrations were shown to be significantly higher when dietary protein was coingested during and after exercise. The plasma total testosterone response showed a significant positive correlation with mixed-muscle protein FSR during overnight recovery.

The nocturnal endocrine response during postexercise recovery has not been well studied. Few studies have documented plasma growth hormone, cortisol, or testosterone responses during overnight postexercise recovery, with many of them reporting discrepant findings



**Figure 2**—Plasma (A) growth hormone, (B) cortisol, and (C) total testosterone concentrations during exercise performed in the evening and during subsequent overnight recovery,  $M \pm SEM$ . Participants received either carbohydrate (C) or C plus protein (C+P) supplements during exercise and after 30 and 90 min of postexercise recovery. #Time points significantly different from baseline ( $p \leq .05$ ), with no significant differences between treatments at any given time point but a main effect of treatment across all time points for cortisol ( $F = 6.5, p = .03$ ).



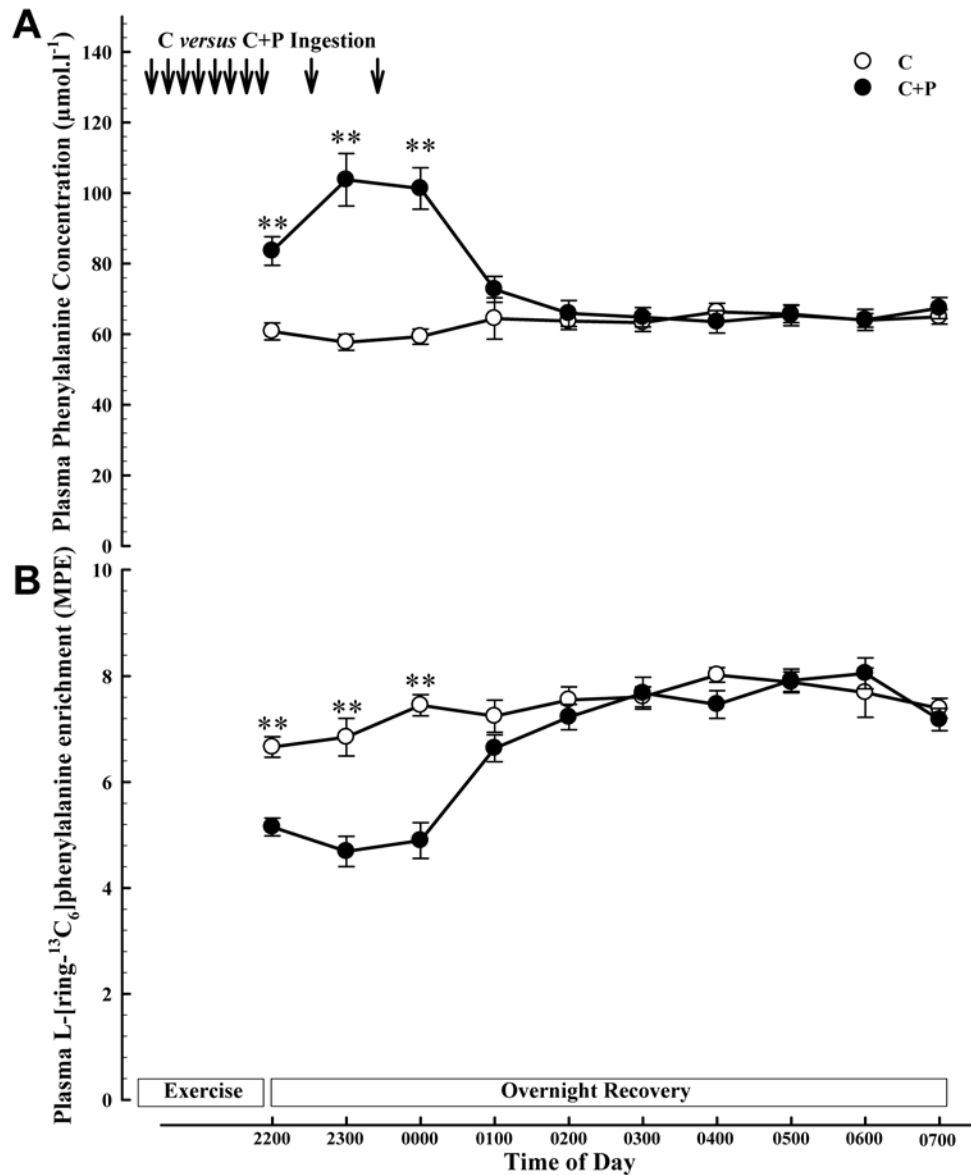
**Figure 3**—Plasma (A) insulin and (B) glucose concentrations during exercise performed in the evening and during subsequent postexercise overnight recovery,  $M \pm SEM$ . Participants received either carbohydrate (C) or C plus protein (C+P) supplements during exercise and after 30 and 90 min of postexercise recovery. There were no significant differences between treatments at any given time point, but there were trends for main effects of treatment across all time points (insulin,  $F = 4.5$ ,  $p = .07$ ; glucose,  $F = 4.8$ ,  $p = .06$ ).

(Adamson et al., 1974; Hackney et al., 1989; Kern et al., 1995; McMurray et al., 1995; Tuckow et al., 2006; Zir et al., 1971). Overnight growth hormone concentrations have generally been reported to be unaffected by exercise performed on the preceding day (Kern et al., 1995; McMurray et al., 1995; Tuckow et al., 2006; Zir et al., 1971), although there have been reports of plasma growth hormone responses being either augmented (Adamson et al., 1974) or attenuated (Hackney et al., 1989). Equally, some studies have reported no effect of daytime exercise on the subsequent overnight responses of plasma cortisol (Kern et al., 1995; McMurray et al., 1995) or testosterone concentrations (Hackney et al., 1989; Kern et al., 1995), whereas others have reported attenuated

nocturnal cortisol/corticosteroid (Adamson et al., 1974; Hackney et al., 1989) or testosterone responses (McMurray et al., 1995).

The reason for the apparent inconsistency may be related to variance in training status across the populations studied, which would modulate the overnight endocrine response (Gouarne, Groussard, Gratas-Delamarche, Delamarche, & Duclos, 2005; Weltman et al., 1992). Alternatively, results may vary according to the precise stage or time point of sleep measured in each study (Kern et al., 1995), with hormone secretory bursts showing an irregular frequency, duration, and amplitude over an entire night's recovery after exercise (Tuckow et al., 2006). Another possibility is that differences in nutritional



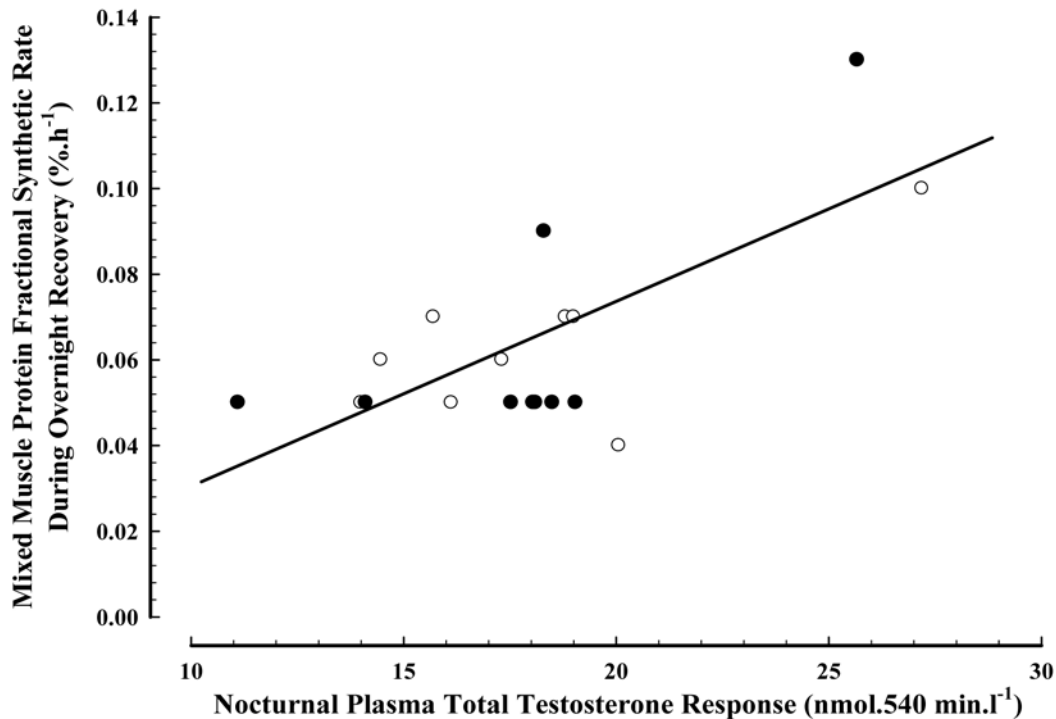


**Figure 4**—Plasma (A) phenylalanine concentrations and (B) L-[ring- $^{13}\text{C}_6$ ]phenylalanine tracer enrichment during overnight recovery from exercise performed in the evening,  $M + SEM$ . Participants received either carbohydrate (C) or C plus protein (C+P) supplements during exercise and after 30 and 90 min of postexercise recovery. \*\*Time points significantly different between treatments ( $p < .01$ ).

**Table 1** Plasma and Muscle L-[Ring- $^{13}\text{C}_6$ ]Phenylalanine Tracer Enrichments and Mixed-Muscle Protein Fractional Synthetic Rate (FSR) During Overnight Recovery From Exercise Performed in the Evening,  $M + SEM$

	C	C+P
Plasma L-[ring- $^{13}\text{C}_6$ ] phenylalanine tracer enrichment	$0.0813 \pm 0.0018$	$0.0727 \pm 0.0026^*$
Mixed-muscle protein FSR (%/hr)	$0.062 \pm 0.006$	$0.062 \pm 0.009$

Note. Participants received either carbohydrate (C) or C plus protein (C+P) supplements during exercise and after 30 and 90 min of postexercise recovery. \*Values significantly different between treatments ( $p < .01$ ).



**Figure 5** — Correlation between overnight mixed-muscle protein fractional synthetic rates and the plasma total testosterone response during overnight recovery (expressed as area under the concentration curve). Trend line denotes the significant positive correlation between variables ( $r = .7$ ,  $p < .01$ ), with carbohydrate (C) represented by open circles ( $r = .7$ ,  $p = .04$ ) and C plus protein (C+P) represented by closed circles ( $r = .7$ ,  $p = .03$ ).

status between studies modulated the overnight hormonal response. In particular, amino acid ingestion is thought to affect the exercise-induced response of certain hormones (Chromiak & Antonio, 2002; Jacobson, 1990), with this response most evident in altered basal concentrations during acute (i.e., 60 min) postexercise recovery (Suminski et al., 1997). The current study is the first to directly examine the effect of protein coingestion during and immediately after exercise performed in the evening on the overnight endocrine response. Clearly, the observed effect of coingesting protein during and for a short time after exercise on overnight cortisol response (Figure 2B) may account for some of the discrepancy between previous studies (Hackney et al., 1989; Kern et al., 1995; McMurray et al., 1995).

The importance of documenting nocturnal endocrine responses to various stimuli is underlined by the proposed role of certain hormones in the skeletal muscle's adaptive response to exercise. For example, elevations in systemic growth hormone and testosterone have been proposed to stimulate tissue anabolism (Kraemer & Ratamess, 2005), and the combination of low cortisol and elevated insulin would be expected to attenuate protein catabolism (Fryburg, Jahn, Hill, Oliveras, & Barrett, 1995). It might therefore be hypothesized that the extent of muscle hypertrophy and strength gains can also be modulated by the overnight endocrine response. However, although the current study was not designed to examine whether such endocrine responses are causally related to muscle protein

synthesis, it is notable that muscle protein synthesis rates differed between treatments only during exercise (Beelen, Koopman, et al., 2008), when no differences were apparent in plasma hormone levels between treatments. This observation is consistent with recent work showing that the acute hormonal response to an exercise bout is not translated to greater postexercise muscle protein synthesis rates, muscle hypertrophy, or strength gains (West et al., 2010; West et al., 2009; Wilkinson et al., 2006). Indeed, even 2 weeks of high-dose recombinant human growth hormone administration exerts no impact on myofibrillar protein synthesis in young healthy men, with the anabolic influence of this hormone evident solely in connective tissue via stimulation of collagen synthesis (Doessing et al., 2010).

Further to the previous discussion, similar comparisons can be made between the nocturnal endocrine responses recorded during overnight recovery in this study and rates of mixed-muscle protein synthesis measured over the same time period. Given that acute increases in protein synthesis over the first 3 hr after exercise are suggested to accurately reflect the 24-hr response (Tipton, Borsheim, Wolf, Sanford, & Wolfe, 2003) and that postexercise protein coingestion results in a more positive protein balance during the acute stages of postexercise recovery (Beelen, Koopman, et al., 2008; Koopman et al., 2004; Koopman et al., 2005; Rasmussen et al., 2000), it seems logical to assume that protein coingestion during and/or immediately after each exercise bout would

improve the skeletal muscle's adaptive response to more prolonged resistance-type exercise training (Esmarck et al., 2001). However, recent evidence indicates that this is not always the case. Verdijk et al. (2009) showed no further increase in muscle mass or strength after protein supplementation before and immediately after each exercise session in a 12-week resistance-type exercise training program in healthy, elderly men. The observation in the current study that neither overnight endocrine responses nor overnight muscle protein synthesis rates differed between treatments may explain this in that the effects of such nutritional intervention are short-lived and do not necessarily translate into greater muscle protein accretion during a more prolonged recovery period.

Notwithstanding this interpretation, it is interesting that overnight protein synthesis did not differ between treatments in this study, given that Tipton et al. (2003) showed the acute effects of nutrition after exercise to be reflective of the overall 24-hr response. One possible explanation is that the results of each study were influenced by the different times of day at which exercise was performed. This may partly be a direct influence of circadian variability in endocrine responses, because both cortisol and testosterone exhibit naturally more elevated absolute plasma concentrations in the morning but with greater potential for exercise-induced responses in the evening (Bird & Tarpenning, 2004; Kanaley, Weltman, Pieper, Weltman, & Hartman, 2001). Equally, this time-of-day effect could operate indirectly via diurnal variability in daily meal patterns; that is, evening exercise precedes a period of fasting during sleep, whereas the morning exercise model employed by Tipton et al. (2003) incorporated a subsequent meal later in the day. If supported, such an explanation would emphasize the importance of continued intake of nutrients later into recovery to allow acute responses to translate into long-term adaptations.

The fact that any acute effect of combined carbohydrate-protein ingestion on protein synthesis had returned to the postabsorptive levels for most of the night (similar to water; Beelen, Tieland, et al., 2008) also makes it interesting that it was during this extended period that protein synthesis correlated with the nocturnal testosterone response. Although this finding must be balanced against the general lack of such correlations for growth hormone or cortisol, it could be speculated that a sustained increase in basal total testosterone concentrations (particularly at the upper end of the physiological range—see Figure 5) may play a more important role than acute endocrine responses to an exercise session in dictating the overall skeletal-muscle adaptive response to exercise training. Future investigations are therefore warranted to directly examine the causal significance of nocturnal endocrine responses in the regulation of protein synthesis over more extended recovery periods, as has recently been explored in relation to acute postexercise recovery (West et al., 2010).

In conclusion, coingestion of protein with carbohydrate during and immediately after combined endurance- and resistance-type exercise does not modulate the

plasma growth hormone or total testosterone response during exercise or subsequent overnight recovery. These data are consistent with the view that acute endocrine responses to exercise do not regulate muscle protein synthesis rates during exercise or during acute postexercise recovery.

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