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ORIGINAL ARTICLE

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Independent and combined effects of liquid carbohydrate/essential amino acid ingestion on hormonal and muscular adaptations following resistance training in untrained men

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Abstract This investigation examined chronic alteration of the acute hormonal response associated with liquid carbohydrate (CHO) and/or essential amino acid (EAA) ingestion on hormonal and muscular adaptations following resistance training. Thirty-two untrained young men performed 12 weeks of resistance training twice a week, consuming ~675 ml of either, a 6% CHO solution, 6 g EAA mixture, combined CHO + EAA supplement or placebo (PLA). Blood samples were obtained pre- and post-exercise (week 0, 4, 8, and 12), for determination of glucose, insulin, and cortisol. 3-Methylhistidine excretion and muscle fibre cross-sectional area (fCSA) were determined pre- and post-training. Post-exercise cortisol increased ($P < 0.05$) during each training phase for PLA. No change was displayed by EAA; CHO and CHO + EAA demonstrated post-exercise decreases ($P < 0.05$). All groups displayed reduced pre-exercise cortisol at week 12 compared to week 0 ($P < 0.05$). Post-exercise insulin concentrations showed no change for PLA; increases were observed for the treatment groups ($P < 0.05$), which remained greater for CHO and CHO + EAA ($P < 0.001$) than PLA. EAA and CHO ingestion attenuated 3-methylhistidine excretion 48 h following the exercise bout. CHO + EAA resulted in a 26% decrease ($P < 0.01$), while PLA displayed a 52% increase ($P < 0.01$). fCSA increased across groups for type I, IIa, and IIb fibres ($P < 0.05$), with CHO + EAA displaying the greatest gains in fCSA relative to PLA ($P < 0.05$). These data indicate that CHO + EAA ingestion enhances muscle anabolism following resistance training to a greater extent than either CHO or EAA consumed independently. The synergistic effect of CHO + EAA ingestion maximises the anabolic response presumably by attenuating the post-exercise rise in protein degradation.

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Introduction

Resistance exercise stimulates acute changes in the rate of muscle protein turnover, resulting in an increase in both protein synthesis and protein degradation (Chesley et al. 1992; Biolo et al. 1995; Phillips et al. 1997). However, in the absence of nutritional intake, net muscle protein balance (i.e., the difference between protein synthesis and degradation) remains negative in the early stages of recovery (Biolo et al. 1995). Furthermore, myofibrillar protein degradation, as determined by 3-methylhistine (3-MH) excretion, has been shown to remain elevated for 48 h post-exercise following an acute bout of resistance exercise (Bird et al. 2005). Collectively, these findings indicate that during post-exercise recovery from resistance exercise, catabolic events predominate in determining net protein turnover status. This imbalance between protein synthesis and degradation is likely facilitated by the antagonistic relationship between the hormones insulin and cortisol.

The resultant physiological stress associated with resistance exercise protocols designed to maximally stimulate the major muscle groups (i.e., whole-body; moderate volume, high intensity) often produces hypersecretion of cortisol (CORT) > 500 nmol l^{-1} (Tarpenning et al. 2001; Bird et al. 2005; Kraemer et al. 2005), while insulin (INS) levels remain unchanged (Roy et al. 1997) or decrease (Raastad et al. 2000). Infusion studies have shown that elevated systemic cortisol results in net muscle protein catabolism (Simmons et al. 1984; Gore et al. 1993). Simmons et al. (1984) confirmed that not only did an increase in CORT within the physiologic range increase leucine concentration, but also such an increase occurred in the absence of any change in INS levels. Since the only source of leucine in the post-absorptive state is tissue protein, the authors concluded that an increase in CORT within the physiologic range

stimulates proteolysis independently of changes in INS (Simmons et al. 1984).

If an acute increase in protein degradation is the primary response of skeletal muscle to resistance exercise during the initial phases of recovery (Biolo et al. 1995; Bird et al. 2005), attenuating catabolic mediators, such as exercise-induced CORT release may be an essential component necessary for enhancing the skeletal muscle hypertrophic response to resistance training. The importance of such a contention, as discussed by Laurent and Millward (1980), implicates protein degradation as an event that may inhibit skeletal muscle growth rather than being an integral component of the hypertrophy process. Therefore, reductions in the CORT response and hormone-mediated protein degradation are potential mechanisms by which protein accretion occurs, that of stimulating muscle growth by suppressing muscle breakdown (Goldberg 1969; Seene and Viru 1982).

Recently, Paddon-Jones et al. (2003) demonstrated that ingestion of 15 g of essential amino acid (EAA) can stimulate muscle protein synthesis and maintain net protein balance for more than 90 min in the presence of acute hypercortisolemia. Furthermore, additional research (Paddon-Jones et al. 2005) confirmed that such responses may be synergistically potentiated by the addition of carbohydrates (CHO). CHO + EAA supplementation enhanced net muscle protein synthesis despite acute hypercortisolemic challenges administered in association with 28 days of bed rest. Therefore, the potential role of a combined CHO + EAA nutritive intervention to increase both extracellular amino acid availability and insulin release, as well as suppressing exercise-induced CORT secretion, should provide a potent stimulator for positive protein balance. Such a strategy remains a realistic potential mechanism aimed at conservation of myofibrillar protein, thereby enhancing skeletal muscle growth by suppressing myofibrillar protein degradation. This provides the basis for the hypothesis that CHO + EAA ingestion during an acute bout of resistance exercise will provide a transient decrease in hormone-mediated protein degradation, and when repeated, will result in greater gains in muscle fibre size following chronic resistance training.

Methods

Subjects

As a companion study to a previous investigation (Bird et al. 2005), 32 healthy male volunteers were recruited to participate in this investigation. The subjects were physically active but considered untrained as none had been involved in any regular exercise or resistance training for at least 6 months prior to the start of the study. The mean (±SD) age, height, and body mass for all subjects were 21.0 (2.4) years, 182.7 (6.9) cm, and 79.6 (12.1) kg, respectively. Further descriptive data are reported elsewhere (Bird et al. 2005). After a full explanation of all procedures and possible risks of the investigation, written informed consent was obtained before testing began. None of the subjects had any history of endocrine disorders and none was taking any medication or nutritional supplementation. All experimentation was approved by the Ethics in Human Research Committee of the University.

Experimental design

The study used a randomised, double-blind repeated measures design in which subjects were allocated into one of four groups, either CHO group ($n=8$), EAA group ($n=8$), combined CHO + EAA group ($n=8$), or placebo (PLA) group ($n=8$) and participated in 12 weeks of resistance training. Subjects visited the Exercise and Sport Sciences Laboratory, on five occasions during the 14-week experimental period, once for equipment familiarisation (resistance training equipment) and four for data collection. On each occasion the time of day was standardised, with times held constant for each subject. Prior to laboratory tests, all participants were required to refrain from all strenuous activity, alcohol use, caffeine, and sexual activity, and were notified to maintain normal nocturnal sleep habits (i.e., approximately 8 h per night).

Body composition, muscle fibre area, and myofibrillar protein degradation were assessed pre-training (week −1) and post-training (week 13). Body composition was determined using dual energy X-ray absorptiometry (DEXA). Muscle biopsies were obtained from the right vastus lateralis muscle and analysed for muscle fibre cross-sectional area (fCSA). Myofibrillar protein degradation was assessed from 3-MH excretion using high-performance liquid chromatography (HPLC). Muscular strength and blood sampling were performed at week 0, 4, 8, and 12. Maximal strength was assessed for each of the eight selected exercises in the resistance training protocol by completing a one-repetition maximum (1-RM) test, while peak force of the knee extensors and knee flexors of the dominant leg was assessed using a KIN-COM isokinetic dynamometer. Blood samples were collected prior to exercise, and during the post-exercise period for analysis of glucose (GLU), CORT, and INS. The experimental timeline is illustrated in Fig. 1.

Dietary control

Food intake diaries were used to control for 3 days prior to, and concluding the experimental period. Dietary assessments were performed at week −1 and 13, to assess whether there were differences in energy intake and macronutrient composition between groups, but on days separate from those of the lacto-ovo-vegetarian

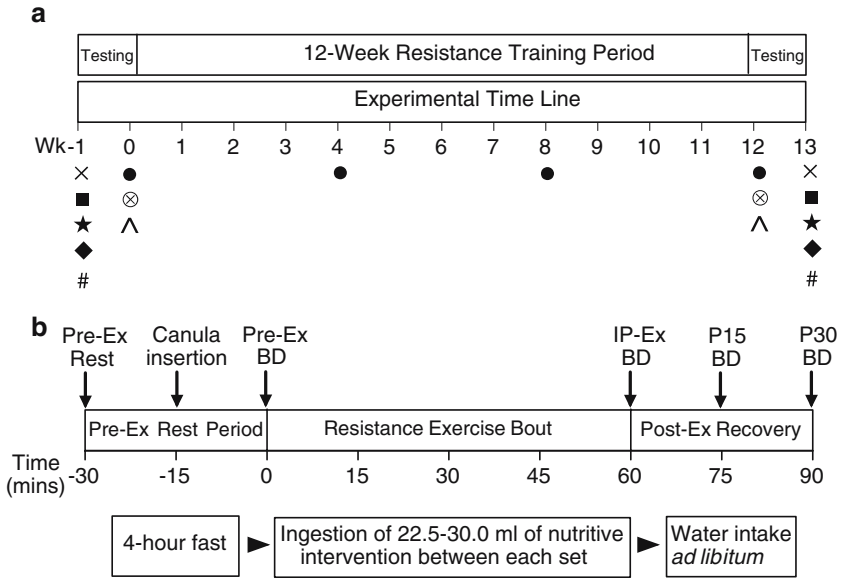

Fig. 1 **a** Experimental timeline depicting when measurements were performed. *Times* muscle biopsy, *filled square* DEXA, *filled circle* blood sampling, *times within circle* urine collection, *filled star* isokinetic strength testing, *filled diamond* 1-RM testing, # dietary assessment, *cap* lacto-ovo-vegetarian diet. **b** The acute exercise bout protocol illustrating when blood sampling was performed. *RM* repetition maximum, *Ex* exercise, *BD* Blood draw, *IP-Ex* immediate post-exercise, *P15* 15 min post-exercise, *P30* 30 min post-exercise

(meat-free) diet. Before beginning the study, each subject met with an Accredited Practicing Dietitian (Dietitians Association of Australia), where they were provided with detailed instructions on recording all food items and portion sizes consumed for three designated days (2 × weekdays and 1 × weekend days), with subjects instructed to consume their normal diet during this period. Nutritional analysis was performed using the Serve Nutrition Management System (Serve v2.0, St. Ives, Australia). Each item was entered onto a personal computer and the program provided the total energy consumption and macronutrient composition on average over the 3 days. If a nutrient value was missing, information from other food tables (English and Lewis 1991) or information provided by food manufacturers was obtained.

Nutritive intervention

Following commencement of the exercise bout, subjects consumed either a CHO (Gatorade, Quaker Oats, Inc., Chicago, IL, USA), a EAA (Musashi, Notting Hill, Australia), a combined CHO + EAA supplement, or a PLA (aspartame and citrus flavouring, Quaker Oats, Inc.), dissolved in water at a fluid volume of 8.5 ml kg body mass^{-1} (an average of 675 ml of solution). The total volume of fluid was divided by 25 servings, allowing for between 22.5 and 30.0 ml depending on body size to be ingested between each set of resistance exercise. The EAA composition consisted of histidine (0.65 g), isoleucine (0.60 g), leucine (1.12 g), lysine (0.93 g), methionine (0.19 g), phenylalanine (0.93 g), threonine (0.88 g), and valine (0.70 g), which have been shown to enhance muscle anabolism following resistance training (Rasmussen et al. 2000).

Body composition

Body composition was determined by DEXA. The subjects were scanned, in the morning, after an overnight fast and after voiding. A DPX-IQ scanner (Lunar Corp., Madison, WI, USA) was used, with scans performed at baseline and after 12 weeks of training. A medium scanning procedure (25 min) was chosen, with the principal investigator performing all scans. The same technician analysed all scans, using the extended research analysis software (Lunar v4.6C; Lunar Corp.). In the analysis, total body scan was divided into three regions: arms, legs, and trunk. Total and regional scans were further divided into three compartments: fat free mass (FFM), fat mass (FM), and bone mineral content (BMC). Daily calibrations were performed prior to all scans using a calibration block provided by the manufacturer. Estimation of precision was determined by duplicate scans for eight subjects. The correlations between duplicate scans ranged from 0.91 and 0.98, and the coefficient of variation (CV) for FFM was 1.2%.

Muscular strength measures

Maximal strength was assessed for each of the eight selected resistance exercises by completing a 1-RM test (i.e., the heaviest load that could be correctly performed once). Warm-up consisted of one set of 5–10 repetitions at 40–60% of perceived maximum. Subjects then rested for 1 min, performing light stretching. This was followed by 3–5 repetitions performed at 60–80% of perceived maximum. Thereafter, 3–4 subsequent attempts were made to determine the 1-RM, with the weight increased progressively until the subject failed at the given load. Three minutes of rest was allocated between lifts. By

definition, 1-RM is the maximum amount of weight that could be lifted one time through a full range of motion, using good form at a tempo of 2:0:2 (2 s eccentric; 2 s concentric).

Peak force of the knee extensors and knee flexors of the dominant leg was assessed using a KIN-COM isokinetic dynamometer (Chattanooga Group Inc., Hixon, TN, USA) both prior to, and at the completion of the study. The dominant leg was defined as the leg that the subject would use if asked to kick a ball. Leg and body positions were adjusted so that the axis of rotation for the dynamometer was visually aligned with the lateral epicondyle of the femur with the lower leg attached to the lever arm at the level of the lateral malleolus. To minimise extraneous body movement subjects were restrained by leg, waist, and upper body straps. Once correctly positioned subjects performed a standardised warm-up and familiarisation protocol, which consisted of three sub-maximal isokinetic knee extension and knee flexion exercises through a range of 15°–80° with the reference point (0°) being full extension. Following which, three trials of maximal isokinetic (60° per second) contractions were performed for each movement, with a 5-s rest between trials. Reproducibility was assessed by testing peak force of the knee extensors and knee flexors on eight subjects, 1 week apart. The CV for knee extension and knee flexion peak torque was 2.2 and 2.6%, respectively.

Resistance training protocol

All subjects followed the same supervised, resistance training protocol two times per week for 12 weeks. A progressive resistance training principle was employed, with the load increased as necessary to maintain a training intensity at approximately 75% of each individuals 1-RM (i.e., 8–10 repetitions per set), with subjects performing each set to failure (Staron et al. 1990). The resistance training protocol used for this investigation was that previously used by Bird et al. (2005), which has been shown to influence hormonal concentrations. Briefly, the resistance training protocol consisted of a complete body workout, with a combination of machine equipment (Panatta Sports, Apiro MC, Italy) and free weight (York Barbell, York, PA, USA) exercises, including in order: leg press, leg curl, leg extension, shoulder press, lat pulldown, bench press, barbell bicep curl, and supine tricep extension. The subject's goal was to complete three sets of 8–10 repetitions at approximately 75% of their 1-RM. One minute of rest between each set and 2 min between each exercise was allowed for recovery. The resistance exercise session lasted approximately 60 min and was preceded by a 10-min warm-up and concluded with a 10-min warm-down period. Staff trained in the principles associated with resistance training supervised all sessions. All training sessions were performed between 1500 and 1700 h to minimise the influence of diurnal variations on hormone release, with times held constant for each subject. Subjects were informed that missing two training sessions resulted in disqualification from the study. Additionally, all aerobic exercise was controlled during the experimental period with only 2 h per week allowed.

Blood collection and biochemical analysis

Blood sampling was undertaken on four occasions, week 0, 4, 8, and 12. Briefly, following a 4-h fast, subjects reported to the laboratory and sat quietly for 15 min, after which a teflon-indwelling cannula (Saf-T-Intima; Becton Dickinson Inc., Sandy, UT, USA) was inserted into an antecubital forearm vein. Subjects sat quietly for a further 15-min period prior to blood collection to minimise hormonal fluctuations related to anticipatory responses (Kraemer et al. 1990). Using a vacutainer assembly and serum separator tubes (Monovette, Sarstedt, Numbrecht, Germany), 5-ml blood samples were drawn pre-exercise (Pre-Ex), immediately post-exercise (IP), and 30 min post-exercise (P30). To assist keeping the line clear, and prevent clotting 1 ml of saline solution was injected into the cannula line between each blood draw. Blood samples were gently inverted five times, and allowed to stand at room temperature for a minimum of 20 min. Samples were then centrifuged for 10 min at 3,000 rpm, with the supernatant removed and placed into plastic storage containers and frozen at −20°C until analysed.

Before analysis the serum was allowed to reach room temperature, and mixed gently via inversion. GLU was determined via an enzymatic spectrophotometric method (Dimension Xpand, Dade Bearing Inc., Newark, DE, USA). CORT concentrations were determined by a competitive immunoassay technique using chemiluminescent technology (VITROS ECi, Ortho-Clinical Diagnostics Inc., Rochester, NY, USA) with detection limits of < 3.0 nmol l^{-1}. INS concentrations were determined by a solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA, USA), with detection limits of 2 μl U ml^{-1}. To avoid inter-assay variations, all samples for each subject were assayed in the same assay run. Analyses of GLU, CORT, and INS showed intraassay CV of 5.9, 2.7, and 3.1%, respectively. Serum hormone concentrations were not corrected for plasma volume shifts, thus all statistical analyses were performed on hormone values based on actual measured circulating concentrations.

Urine collection and 3-methylhistidine analysis

Subjects provided timed and complete 24-h urine collections from at 0700 h (Kies 1977) for the measurement of 3-MH, an index of myofibrillar protein degradation (Young and Munro 1978; Ballard and Tomas 1983) during week 0 and 12. The designated urine collection procedure was that previously described by Bird et al.

(2005). Briefly, subjects were instructed to discard the product of the first urination upon waking in the morning and then collect all samples for the following 24 h, including the first one upon waking up the next morning. Urine samples were returned to the laboratory where the urine volume was recorded and aliquots of each urine sample were drawn off from the 24-h collection and stored at −20°C until analysed. Before sampling, urine collections were acidified to 1% of volume with 12 N hydrochloric acid (Kies 1977). 3-MH was assessed via HPLC (HPLC 1100 B, Hewlett-Packard, Palo Alto, CA, USA) using a Synergi 4μ Hydro-RP 80A column (Phenomenex, Torrance, CA, USA), with the amount in a 24-h sample of urine expressed relative to lean body mass per day (μmol LBM^{-1} day^{-1}).

Meat sources of protein have been shown to increase 3-MH excretion and falsely represent total protein breakdown values (Kies 1977), and at least 3 days of a meat-free diet are required to return urine concentrations of 3-MH to baseline levels (Lukaski et al. 1981). An Accredited Practicing Dietitian (Dietitians Association of Australia) designed 7-day lacto-ovo-vegetarian sample menus for each subject. A non-animal protein supplement (P40 protein powder; Musashi, Notting Hill, Australia) was provided to help ensure adequate protein intake. Diets were administered on the basis of body mass, with energy intake set at the RDI of 9.8–13.7 MJ day^{-1} based on height and body mass (NHMRC 1991) with 15% of energy from PRO, 55% of energy from CHO, and 30% of energy from fat, in the form of three daily meals and small snacks.

Muscle biopsy sampling and histochemical analysis

Muscle biopsies were obtained pre- and post-training (week −1 and 13) from the right vastus lateralis muscle, at mid-thigh level (Blomstrand and Ekblom 1982), using the percutaneous needle biopsy technique (Bergstrom 1962). To ensure adequate sample sizes, biopsies were obtained using a double-chop method (Staron et al. 1994) combined with manual suction (Evans et al. 1982). Post-training biopsies were obtained 48 h after completion of the last training session using the same incision site as that for the first. Muscle samples (25–50 mg) were immediately orientated cross-sectionally on a cork block, mounted in optimal cutting temperature (OCT) compound (Tissue-Tek; Miles Inc., Elkhart, IN, USA), frozen in liquid nitrogen, and transferred to pre-chilled vials for storage at −80°C until histochemical analysis. The same surgeon performed all biopsies.

Serial cross-sections (10-μm thick) of each muscle sample were cut on a cryostat (HM500OM, Microm, Heidelberg, Germany) at −20°C and stained for myofibrillar adenosine triphosphatase (ATPase) activity using pre-incubation mediums at pH 4.3, 4.6, and 10.4 (Brooke and Kaiser 1970). This allows fibres to be classified as either type I, Ic, IIc, IIa, IIab, or IIb based on their staining intensities (Staron et al. 2000). However, hybrid fibres (Ic, IIc, and IIab) were not quantified in this analysis as they have been deemed to represent an insignificant proportion of the total fibre population (Staron et al. 2000; Hakkinen et al. 2003). To avoid interassay variance all tissue samples were stained simultaneously 72 h following the second biopsy. fCSA was determined on at least 50 fibres (McCall et al. 1998) per major type (I, IIa, and IIb) per biopsy. Computerised images of the histochemical preparations using the 4.6 pH pre-incubation were analysed from computer-generated images (100× magnification) using public-domain Scion Image software (Scion Corporation, Frederick, MD, USA). These were used in combination with the other histochemical preparations (pre-incubation pH values of 4.3 and 10.4) to determine total fibre number in each biopsy.

Statistical analysis

Analysis included standard descriptive statistics, paired t tests to compare within group differences between pre- and post-training, one-way ANOVA to examine changes occurring with training for each group, and two-way ANOVA (group × time) with repeated measures. The source of significant differences was located using Tukey's HSD post hoc procedure. Regression analysis determined associations between selected variables. Analysis was performed using the Statistical Package for Social Sciences (SPSS v11.5, Chicago, IL, USA). Significance was accepted when $P < 0.05$. Values are expressed as mean (±SEM).

Results

Body composition

There were no significant differences between the PLA and treatment groups (CHO, EAA, and CHO + EAA) at baseline (week −1) for any measures of body composition. Consequent to 12 weeks of progressive resistance training, an increase in body mass was recorded by the treatment groups only ($P < 0.05$). Further, the CHO + EAA group displayed greater gains in body mass relative to the PLA group [2.8 (0.8) compared to 0.4 (0.3) kg; $P < 0.05$). FFM increased across all groups ($P < 0.05$), with CHO + EAA consumption resulting in higher increases in FFM relative to the PLA group [4.1 (0.6) compared to 1.8 (0.3) kg; $P < 0.05$]. Although FM decreased across all groups ($P < 0.05$), no significant differences between groups were reported (Fig. 2).

Muscle fibre CSA

Figure 3 shows the changes in type I, type IIa, and type IIb fCSA following 12 weeks of progressive resistance training. Analysis determined no significant difference at baseline in fCSA between groups for the three major

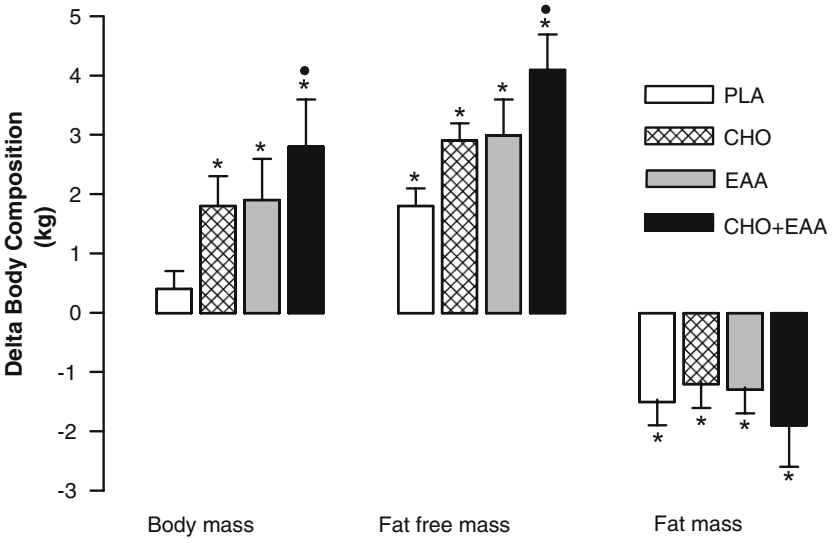

Fig. 2 Body composition changes following 12 weeks of resistance training. Significant difference ($P < 0.05$) from baseline value, *. Treatment group pre- to post-training change is significantly different ($P < 0.05$) from PLA (*filled circle*)

fibre types. Following 12 weeks of progressive resistance training, a hypertrophic effect was observed for all three fibre types across groups. CHO + EAA demonstrated the greatest relative increase in type I fCSA of 23.4 (2.8) % compared to PLA and EAA [7.1 (1.3) and 13.1 (1.6) %, respectively; $P < 0.01$]. CHO consumption resulted in a significant increase in type I fCSA [18.3 (1.5) %; $P < 0.01$] relative to PLA only. Changes in type II fCSA exhibited a similar trend to that of type I. CHO + EAA ingestion resulted in the greatest gain in type IIa fCSA of 27.1 (2.7) % relative to all groups, while EAA and CHO demonstrated a significant increase [17.1 (1.3) and 16.3 (2.1) %, respectively; $P < 0.05$] in type IIa fCSA relative to PLA [8.5 (0.9) %]. CHO + EAA and EAA ingestion resulted in significantly greater increases in type IIb fCSA relative to PLA [20.4 (4.0) and 18.2 (3.2) %, respectively, compared to 7.0 (1.5) %; $P < 0.01$].

Muscular strength

There were no significant differences at baseline for 1-RM strength between groups. Consequent to 12 weeks of progressive resistance training increases in 1-RM strength were recorded across all groups ($P < 0.05$), pre- to post-training. CHO + EAA ingestion resulted in greater increases in 1-RM leg press strength relative to the PLA group [210.0 (16.4) compared to 151.3 (12.2) kg; $P < 0.05$). Additionally, the PLA and treatment groups displayed a somewhat different time course for 1-RM strength development. The treatment groups exhibited increases ($P < 0.05$) in 1-RM strength during each of the 4-week training blocks (weeks 0–4, 4–8, 8–12). Conversely, 1-RM strength for the PLA group displayed no significant change following the final training block (weeks 8–12). No significant difference was observed at baseline for isokinetic strength between groups. Consequent to 12 weeks of progressive resistance training, there was an increase in isokinetic strength across all groups ($P < 0.01$), pre- to post-training, however no group × time interactions were present.

Biochemical responses

Biochemical responses during 12 weeks of resistance training are presented in Table 1. PLA and EAA displayed no significant change in GLU concentration,

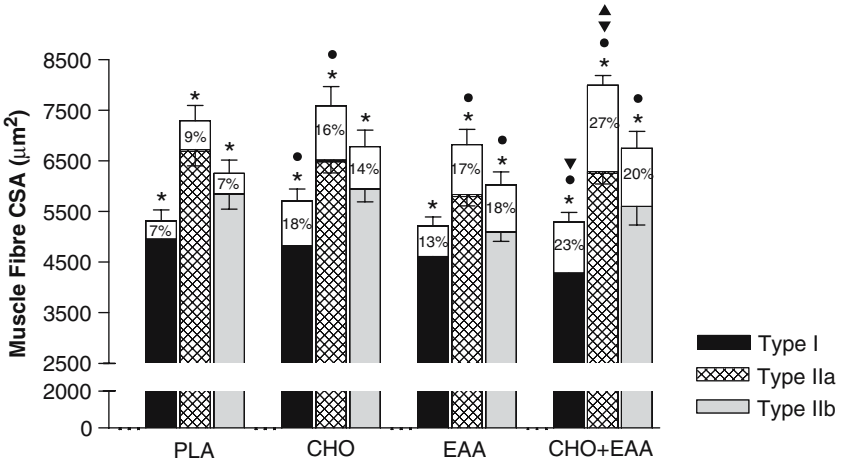

Fig. 3 Muscle fibre CSA of type I, IIa, and IIb before (*solid bars*) and after (*open bars*) 12 weeks of resistance training. *Post-training muscle fibre CSA is significantly different ($P < 0.05$) from pre-training. Treatment group change in muscle fibre CSA is significantly different ($P < 0.05$) from PLA (*filled circle*), CHO (*up filled triangle*), and EAA (*down filled triangle*)

Table 1 Biochemical responses during 12 weeks of resistance training

Variable	PLA group			CHO group			EAA group			CHO + EAA group		
	Pre	IP	P30	Pre	IP	P30	Pre	IP	P30	Pre	IP	P30
Glucose (mmol l^{-1})												
Week 0	4.6 (0.1)	4.9 (0.2)	4.2 (0.1)	4.3 (0.1)	5.6 (0.2)* [ab]	5.1 (0.1)* [a]	4.5 (0.1)	4.6 (0.2)	4.2 (0.2)	4.4 (0.1)	6.1 (0.1)* [ab]	5.5 (0.4)* [ab]
Week 4	4.6 (0.1)	4.9 (0.2)	4.1 (0.1)	4.3 (0.1)	5.6 (0.1)*	5.3 (0.2)*	4.7 (0.2)	4.9 (0.2)	4.3 (0.2)	4.5 (0.1)	6.3 (0.2)* [ab]	5.8 (0.6)* [ab]
Week 8	4.3 (0.1)	4.2 (0.2)	3.9 (0.1)	4.3 (0.1)	5.6 (0.2)* [a]	5.4 (0.2)* [a]	4.5 (0.1)	4.8 (0.2)	4.6 (0.1)	4.3 (0.1)	5.7 (0.2)* [ab]	5.3 (0.3)* [a]
Week 12	4.4 (0.1)	4.8 (0.2)	4.1 (0.2)	4.4 (0.1)	5.9 (0.1)* [ab]	5.3 (0.3)*	4.3 (0.1)	4.6 (0.1)	4.4 (0.2)	4.8 (0.1)	6.9 (0.3)* [abc]	6.8 (0.4)* [abc]
Insulin (μl U ml^{-1})												
Week 0	8.8 (1.2)	10.0 (1.0)	8.1 (0.8)	7.9 (0.7)	16.0 (1.6)* [a]	20.3 (2.5)* [a]	8.3 (1.1)	12.7 (1.5)	14.0 (2.2)*	7.6 (0.6)	17.3 (0.8)* [a]	22.0 (4.8)* [a]
Week 4	9.1 (1.6)	10.1 (1.6)	8.6 (1.3)	9.3 (0.7)	17.6 (1.9)*	20.8 (3.3)*	9.8 (1.5)	16.6 (2.4)	16.1 (3.7)	9.2 (0.8)	20.2 (2.6)* [a]	23.9 (5.1)* [a]
Week 8	10.1 (1.1)	10.7 (1.0)	7.1 (0.8)	9.2 (1.1)	19.0 (1.9)*	21.3 (4.7)*	11.9 (2.0)	18.8 (3.3)	18.9 (5.2)	9.8 (0.9)	22.6 (2.9)* [a]	25.3 (5.8)* [a]
Week 12	11.7 (1.4)	12.7 (1.7)	10.7 (1.1)	10.0 (1.3)	20.0 (2.4)*	25.7 (3.8)* [a]	10.7 (2.0)	16.5 (2.4)	19.4 (4.0)*	10.1 (1.1)	25.6 (3.8)* [a]	35.7 (5.0)* [a]
Cortisol (nmol l^{-1})												
Week 0	294 (31)	602 (60)*	452 (69)*	303 (46)	267 (46) [a]	222 (46)* [a]	294 (33)	429 (47)	345 (64)	317 (28)	296 (31) [a]	245 (23)* [a]
Week 4	248 (27)	507 (43)*	384 (58)*	280 (32)	238 (27) [a]	169 (19)* [a]	272 (41)	342 (37) [a]	213 (41) [a]	303 (25)	289 (33) [a]	228 (24)* [a]
Week 8	215 (27)	458 (44)*	345 (50)*	256 (21)	200 (25)* [ab]	149 (18)* [a]	261 (30)	328 (26) [a]	249 (25)	272 (33)	244 (24) [a]	205 (21)* [a]
Week 12	201 (27)	385 (40)*	328 (54)*	220 (19)	179 (11)* [a]	146 (10)* [a]	190 (21)	234 (47) [a]	167 (28) [a]	216 (26)	187 (31) [a]	144 (19)* [a]
Testosterone (nmol l^{-1})												
Week 0	10.1 (1.2)	11.6 (1.4)	9.2 (1.3)	12.2 (1.0)	12.4 (1.3)	10.2 (1.0)*	9.3 (1.0)	11.6 (1.5)*	9.2 (1.2)	12.9 (1.8)	13.3 (1.8)	11.0 (1.3)
Week 4	10.6 (1.2)	12.3 (1.1)*	10.4 (1.0)	12.8 (1.4)	13.7 (1.7)	12.1 (1.7)	11.6 (1.6)	14.1 (1.6)*	10.8 (1.5)	15.9 (1.8)	16.4 (2.0)	13.2 (1.5)*
Week 8	11.7 (1.4)	13.6 (1.9)*	11.0 (1.7)	12.8 (1.3)	13.8 (1.2)	11.5 (0.8)	10.2 (1.2)	13.0 (1.7)*	10.8 (1.3)	12.6 (1.2)	13.2 (1.0)	10.3 (1.2)*
Week 12	12.9 (1.0)	15.8 (1.5)*	13.6 (1.4)	15.0 (2.2)	15.8 (1.4)	12.8 (1.2)	12.1 (1.7)	16.1 (2.4)*	12.5 (1.8)	19.3 (2.6)	20.2 (2.8)	15.6 (2.2)*
Growth hormone (ml U l^{-1})												
Week 0	1.0 (0.4)	24.4 (12.8)	9.8 (6.5)	1.4 (0.5)	35.2 (15.2)*	13.1 (7.6)	1.3 (0.3)	41.7 (8.9)*	9.8 (3.0)	2.0 (0.7)	30.4 (8.3)*	8.5 (2.3)
Week 4	2.3 (1.2)	41.7 (16.4)*	16.6 (10.1)	4.7 (2.7)	24.3 (8.9)	7.5 (3.2)	2.6 (1.6)	32.7 (7.1)*	7.6 (2.2)	3.8 (2.4)	38.9 (16.0)*	12.2 (5.4)
Week 8	7.3 (3.3)	44.1 (11.2)*	13.6 (4.8)	5.2 (3.0)	31.1 (6.1)*	8.1 (2.2)	2.8 (1.8)	25.4 (7.6)*	5.2 (2.3)	7.6 (2.8)	27.1 (7.3)	5.1 (1.2)
Week 12	2.4 (1.4)	44.3 (8.6)*	15.0 (5.6)	5.3 (3.6)	24.3 (4.6)*	6.3 (1.4)	3.4 (1.5)	33.2 (6.6)*	9.1 (2.2)	8.9 (1.9)	37.8 (11.7)	10.3 (3.1)

Data are given as mean (± SEM)
Pre pre-exercise, *IP* immediately post-exercise, *P30* 30 min post-exercise
*Significant difference from pre-exercise value
[a]Significant difference from PLA
[b]Significant difference from EAA
[c]Significant difference from CHO

either in response to the exercise bout, or as an adaptation with training. Conversely, the CHO and CHO + EAA displayed increases in IP ($P < 0.01$) and P30 ($P < 0.01$), which were not attenuated with training. Further, the pre- to post-exercise change in GLU concentration observed by CHO and CHO + EAA remained higher ($P < 0.01$) than PLA and EAA throughout the 12-week training period (Fig. 4). INS responses are presented in Fig. 5. INS concentrations IP ($P < 0.05$) and P30 ($P < 0.01$) were higher for CHO and CHO + EAA at all time points measured, and remained greater ($P < 0.05$) than PLA throughout the 12-week training period. Additionally, CHO + EAA ingestion resulted in greater ($P < 0.05$) INS concentrations than EAA at week 0 and 12. Pre-Ex INS concentrations were greater for EAA at week 4 and 8 ($P < 0.05$), and week 12 ($P < 0.05$) for CHO + EAA. CORT responses are presented in Fig. 6. CORT increases above Pre-Ex values were observed IP ($P < 0.01$) and P30 ($P < 0.05$) during all training phases for PLA. EAA displayed no significant change in CORT concentrations in response to the exercise bout. Conversely, CHO displayed reductions ($P < 0.05$) in CORT IP at week 8 and 12, as well as all time points for P30 ($P < 0.05$). CHO + EAA ingestion resulted in reductions in CORT at P30 ($P < 0.05$) throughout the study. The pre- to post-exercise change in CORT concentration observed by the treatment groups remained lower from that of PLA at week 0, 4, and 8 ($P < 0.05$), with CHO and CHO + EAA lower at week 12 ($P < 0.05$). As an adaptation to training, reductions in Pre-Ex CORT concentrations were recorded across groups at week 12 compared to week 0 ($P < 0.05$). A negative correlation was observed between the change in fCSA following the 12-week training period and change in CORT response (averaged for week 0, 4, 8, and 12) to an acute bout of resistance exercise (pre- to post-exercise), when all data were combined (type I: $r = -0.70$, $P < 0.001$; type IIa: $r = -0.59$, $P < 0.001$; and type IIb: $r = -0.48$, $P < 0.01$).

3-Methylhistidine excretion

For 3-MH excretion (Fig. 7), PLA displayed a striking rise throughout the 48-h post-exercise period, with an average increase of 52.3 (10.4) % ($P < 0.01$) 48-h

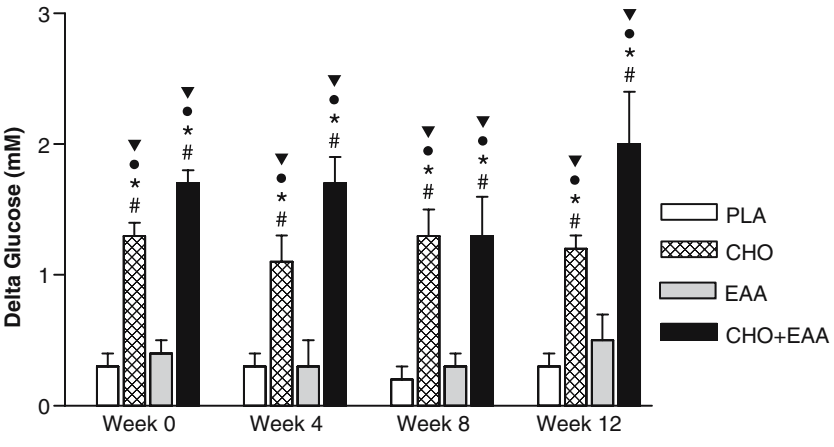

Fig. 4 Change in serum glucose concentration in response to resistance exercise. Immediate post-exercise glucose concentration is significantly different ($P < 0.05$) from pre-exercise value, *. 30 min post-exercise glucose concentration is significantly different ($P < 0.05$) from pre-exercise value, #. Treatment group change in glucose concentration is significantly different ($P < 0.05$) from PLA (*filled circle*) and EAA (*down filled triangle*)

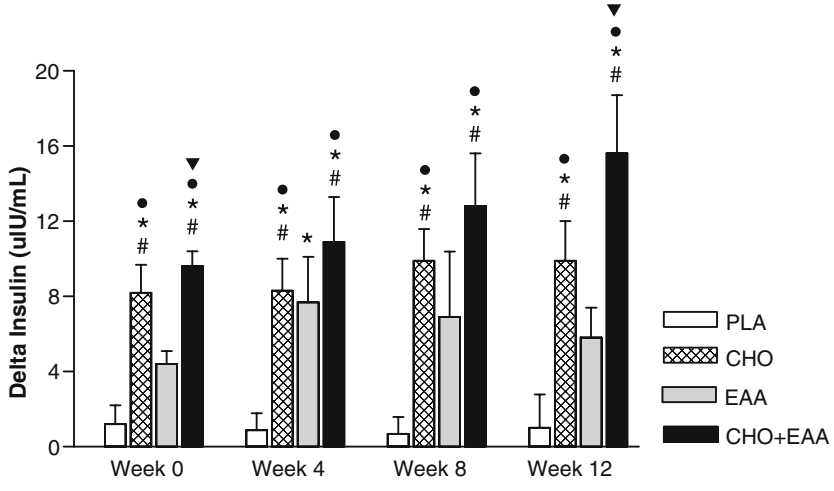

Fig. 5 Change in serum insulin concentration in response to resistance exercise. Immediate post-exercise insulin concentration is significantly different ($P < 0.05$) from pre-exercise value, *. 30 min post-exercise insulin concentration is significantly different ($P < 0.05$) from pre-exercise value, #. Treatment group change in insulin concentration is significantly different ($P < 0.05$) from PLA (*filled circle*) and EAA (*down filled triangle*)

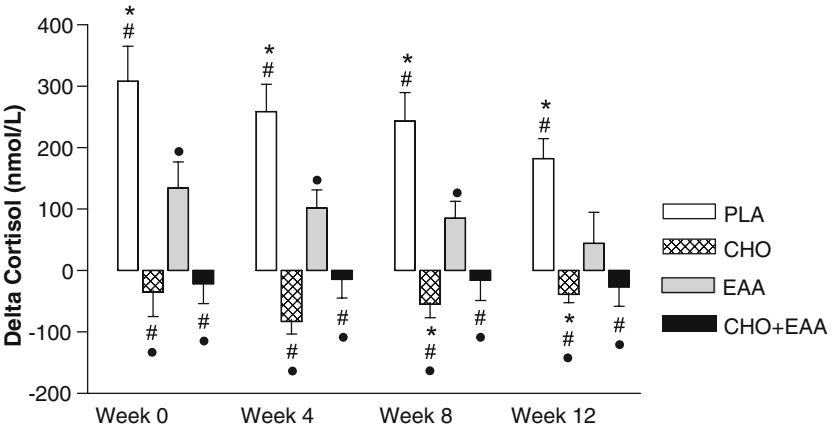

Fig. 6 Change in serum cortisol concentration in response to resistance exercise. Immediate post-exercise cortisol concentration is significantly different ($P < 0.05$) from pre-exercise value, *. 30 min post-exercise cortisol concentration is significantly different ($P < 0.05$) from pre-exercise value, #. Treatment group change in cortisol concentration is significantly different ($P < 0.05$) from PLA (*filled circle*)

post-exercise, over the 12-week training period. The EAA and CHO groups showed no significant change in 3-MH excretion in the 48-h post-exercise period, displaying an average decrease of 4.7 (3.4) and 13.1 (5.5) %, respectively. Conversely, CHO + EAA ingestion resulted in a decrease of 25.8 (7.5) %, ($P < 0.05$) in 3-MH excretion throughout the 48-h post-exercise period. The 48-h post-exercise change in 3-MH excretion displayed by the treatment groups remained lower ($P < 0.05$) than that of PLA throughout the 12-week training period. No training-induced adaptations were observed. A negative correlation was observed between the change in 3-MH excretion following an acute bout of resistance exercise (pre- to 48-h post-exercise), during week 0, and type II fCSA following 12 weeks of resistance training, when all data were combined (type IIa: $r = -0.53$, $P < 0.01$; and type IIb: $r = -0.39$, $P < 0.05$).

Discussion

The primary findings from this investigation indicate that progressive resistance training performed with CHO or EAA ingestion during the exercise bout suppresses exercise-induced CORT release, with this response sustained throughout the 12-week training period. The hormonal milieu displayed by the treatment groups was accompanied by a reduction in hormone-mediated protein degradation, which was associated with significantly greater gains in fCSA. Furthermore, such responses are augmented when the treatments are combined, with the CHO + EAA group exhibiting the greatest relative decrease in 3-MH excretion, and the greatest relative gains in fCSA. This is in contrast to significantly higher exercise-induced CORT release and hormone-mediated protein degradation shown by the PLA group.

Previous research indicates that the hypertrophic response of skeletal muscle following resistance training is associated with differences in resting and exercise-induced hormonal adaptations (Staron et al. 1994; Kraemer et al. 1999; Tarpenning et al. 2001). In a study examining skeletal muscle adaptations during the early phase of heavy-resistance training, Staron et al. (1994) report positive changes in skeletal muscle size with reductions in the resting CORT concentrations in young men. Not only were CORT levels significantly lower at week 7 and 9 than at week 1, a significant inverse correlation ($r^2 = 0.47$; $P < 0.05$) was reported for changes in fCSA of type IIb fibres (increased) and resting CORT concentrations (decreased). Additionally, Kraemer et al. (1999) report that in young men the CORT response to

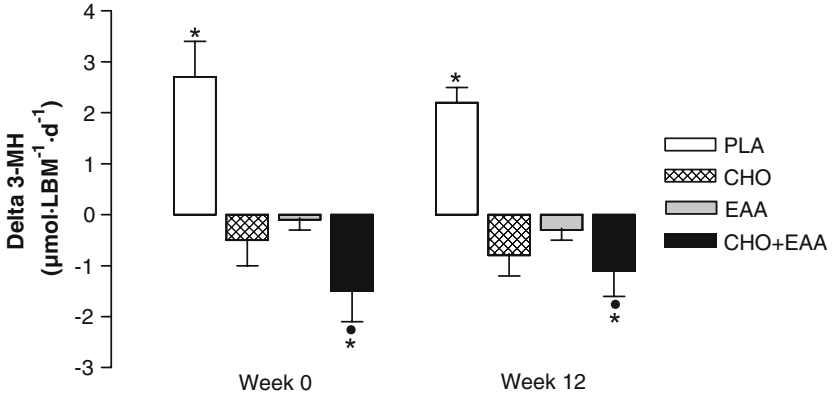

Fig. 7 Change in urinary 3-MH excretion in response to resistance exercise. Values expressed as micromoles per kilogram of lean body mass per day (μmol LBM^{-1} day^{-1}). 48-h post-exercise value is significantly different ($P < 0.05$) from pre-exercise value, *. Treatment group change in urinary 3-MH excretion is significantly different ($P < 0.05$) from PLA (*filled circle*)

resistance exercise tends to increase less as training progresses, while total testosterone (TT), growth hormone (hGH), and insulin-like growth factor-1 (IGF-I) responses remain unchanged following 10 weeks of resistance training. Interestingly, this hormonal response was accompanied by a 10.1 (3.7) % increase in thigh muscle CSA. Collectively, these findings indicate that the hypertrophic response of skeletal muscle appears to be mediated, at least to some extent, by reductions in exercise-induced CORT response.

The CORT response in the current investigation demonstrated an antagonistic relationship to that of glucose. That is, when glucose levels were unchanged, CORT concentration increased, and when glucose levels increased, CORT concentration decreased. At the completion of 12 weeks of resistance training, the PLA group displayed a mean increase IP of 103.2% in CORT concentration, which corresponded with no significant change in glucose levels. In contrast, CHO and CHO + EAA consumption was associated with a mean decrease in IP CORT concentrations of 16.7 and 8.8%, respectively. This corresponded with a mean increase in glucose levels of 31.1 and 38.8%, respectively. All groups displayed a significant reduction in Pre-Ex CORT concentrations at week 12 compared to week 0, an adaptation to resistance training that is consistent with previous reports (Staron et al. 1994; Kraemer et al. 1999; Tarpenning et al. 2001). The implications of repeated modification of the acute exercise-induced CORT response may be inferred from the work of Kraemer et al. (2005), who suggest that an accumulated reduction in CORT concentrations results in reduced total tissue exposure to CORT, thereby influencing the subsequent phase of recovery by modulating anabolic and catabolic processes (Kraemer et al. 1998b). In the current investigation, changes in fCSA were significantly correlated with the change in CORT response, during week 0, when all data were combined. The above findings are in agreement with Tarpenning et al. (2001), who recently reported that CHO-induced blunting of the CORT response was associated with significantly greater gains in both type I ($r = -0.86$, $P < 0.01$) and type II ($r = -0.72$, $P < 0.05$) fCSA. Furthermore, we have previously reported (Bird et al. 2005) that marked increases in protein degradation 48-h post-exercise significantly correlate ($r = 0.75$, $P < 0.05$) with CORT area under the curve concentrations. Therefore, chronic reductions in the exercise-induced CORT response associated with CHO and/or EAA ingestion can positively impact the skeletal muscle hypertrophic adaptation to resistance training via reductions in hormone-mediated protein degradation.

Several studies indicate that CHO + EAA ingestion produces strong insulinotropic effects (Rasmussen et al. 2000; van Loon et al. 2000; Tipton et al. 2001). However, Borsheim et al. (2004) delineate that the effect of INS on skeletal muscle growth is complicated by the fact that INS enhances the uptake of amino acids from the blood resulting in a decrease in extracellular amino acids availability. Such a response could potentially counteract the direct action of INS on promoting protein synthesis; however, the effect of CHO is mediated, in part, by the INS response. Although post-exercise CHO ingestion has been shown to increase INS concentrations, this was not associated with an increase in muscle fractional synthetic rate (Roy et al. 1997). Thus, a key signalling event in the hypertrophic response of skeletal muscle is an increased rate of amino acid transport (Tipton et al. 2001), and this directly corresponds to muscle contractile activity and INS concentrations (Goldberg 1979). In the current investigation, consumption of a liquid CHO or EAA beverage resulted in a substantial increase in the acute INS response to resistance exercise. Furthermore, such responses are augmented when the treatments are combined (CHO + EAA). This is in contrast to the non-significant change displayed by the PLA group. These findings are in agreement with recent reports indicating that inclusion of EAA with CHO acts in a synergistic fashion to enhance the acute INS response (Williams et al. 2001; Koopman et al. 2005). While the PLA and CHO groups showed no significant change in resting INS concentrations throughout the study, unexpectedly there were significantly higher resting concentrations at week 8 and 12, for the EAA and CHO + EAA groups, respectively. Thus, it appears that the adaptive response of INS following resistance training may be influenced by EAA consumption. However, the significance of such adaptations is yet to be elucidated.

The physiological importance of INS action in the control of hormone-mediated protein degradation remains unclear. Although post-exercise hyperinsulinemia has been shown to decrease mixed muscle protein degradation (Biolo et al. 1999), the release of 3-MH from skeletal muscle was unaffected by hyperinsulinemia (Moller-Loswick et al. 1994). Such a differential effect has been addressed by Kettelhut et al. (1988), who suggest that INS decreases lysosomal proteolytic activity, but does not mediate the ubiquitin-proteasome pathway, which is responsible for the bulk of myofibrillar proteolysis (Rock et al. 1994). In the current investigation urinary 3-MH excretion was used as an index of myofibrillar protein degradation, as 3-MH is known to be a constituent amino acid of actin and myosin, and upon catabolism, the only fate of 3-MH is that of excretion in urine (Ballard and Tomas 1983). Regression analysis revealed no significant associations between changes in 3-MH excretion 48-h post-exercise, and the exercise-induced INS response following resistance exercise. These findings lend support to the hypothesis that INS ability to regulate skeletal muscle growth by inhibiting hormone-mediated protein degradation appears to be related to conservation of non-contractile elements, such as sarcoplasmic rather than myofibrillar proteins (Tarpenning et al. 2001).

A number of studies indicate that resistance training combined with nutritive intervention results in significant changes in body composition (Gater et al. 1992; Rozenek et al. 2002; Chromiak et al. 2004). Gater et al.

(1992) reported that following 10 weeks of resistance training, supplementation with a mixed macronutrient beverage resulted in significant increases in both body mass (3.8 kg) and FFM (3.6 kg) compared to PLA (1.6 and 2.1 kg, respectively). Interestingly, Rozenek et al. (2002) observed similar increases in both body mass and FFM for subjects consuming either a liquid CHO + PRO supplement or an isocaloric CHO beverage, which were significantly greater compared to a resistance training only control group. In agreement, Chromiak et al. (2004) reported that post-exercise consumption of a recovery drink containing whey PRO, CHO, amino acids, and creatine, did not promote greater gains in body mass or FFM compared with a CHO beverage. However, caution is warranted with interpretation of the above results (Rankin et al. 2004), as it is difficult to ascertain whether the reported benefits are due to additional energy intake or the specific macronutrient mix of the supplements.

The progressive resistance training protocol used in the current investigation resulted in significant changes in body composition, and this was most pronounced in the treatment groups. Interestingly, gains in body mass and FFM were remarkably similar between the CHO and EAA groups. CHO ingestion resulted in an increase of 1.8 (0.5) and 2.9 (0.3) kg, respectively; this was matched by the EAA group with an increase of 1.9 (0.7) and 3.0 (0.6) kg, respectively. It is difficult to determine the reason for the similarity in changes in body composition; however, it is conceivable that the independent effects of CHO and EAA ingestion may stimulate similar pathways. For instance, Gater et al. (1992) suggest that when nutritive interventions meet the body's energy needs around the time of exercise; the relative amount of energy provided via gluconeogenesis may be reduced. Therefore, the diminished need for glucose, via an increase in the exogenous glucose load (CHO group) or a transient rise in amino acid availability (EAA group), might activate the same negative feedback sequence responsible for suppressing protein degradation. Consistent with this notion, during the current investigation, both CHO and EAA ingestion resulted in a blunted CORT response and suppression of hormone-mediated protein degradation. Such mechanism(s) may contribute to increases in body mass and FFM. Further, the effects appear to be additive when treatments are combined, with significantly greater gains in body mass and FFM displayed by the CHO + EAA group relative to the PLA group. Resistance training resulted in significant decreases in FM across all groups; however, no significant differences were recorded between groups. These findings further support the contention that resistance training provides a potent stimulus for fat loss, regardless of nutritive intervention (Gater et al. 1992).

Muscle fibre hypertrophy is a common adaptation that has been found to occur as a result of resistance training (Staron et al. 1994; Kraemer et al. 1999; Ahtiainen et al. 2003), with recent studies reporting greater gains in fCSA following supplementation with CHO and PRO (Tarpenning et al. 2001; Andersen et al. 2005). In the present study resistance training resulted in a significant increase in fCSA in both type I and type II fibres, across groups, with the treatment groups displaying significantly greater hypertrophy than PLA. Although both type I and type II muscle fibres hypertrophied, the increase in size was more pronounced in the type II subtypes, which demonstrate a greater capacity for hypertrophy (McCall et al. 1996). Further, the type II subtypes were more varied in their size and were larger than type I fibres both pre- and post-training. The relative increase in hypertrophy between fibre types appears to be mediated, at least to some extent, by the independent effects of CHO and EAA ingestion. For example, in the current investigation CHO ingestion resulted in a significant decrease of 16.7% in the CORT response IP, which corresponded with a 12.1% greater gain in type I fCSA, compared to the PLA group. Since CORT is a primary regulator of hormone-mediated protein degradation (Bird et al. 2005), reductions in the CORT response appear to enhance the hypertrophic response of type I muscle fibres, with the reduction in protein degradation rather than increase in protein synthesis as the primary mechanism (Kraemer et al. 1998a). Alternatively, hypertrophy of type II muscle fibres may be more reliant on increases in protein synthesis. Previous research indicates that amino acid concentrations dramatically increase following ingestion of EAA (Tipton et al. 1999), with protein synthesis modulated by extracellular amino acid availability (Bohe et al. 2003). Such mechanisms may explain the significantly greater gains in type IIa [17.1 (1.3) %] and type IIb [18.2 (3.2) %] fCSA displayed by the EAA group, compared to the PLA group [8.5 (0.9) and 7.0 (1.5) %, respectively]. This is consistent with the view that protein synthesis and net protein balance are greater when amino acid availability is increased following resistance exercise.

However, the synergistic effect of combined CHO + EAA ingestion is suggested to reflect the sum of their individual effects (Miller et al. 2003); this was most evident by the dramatic increases in hypertrophy demonstrated by the CHO + EAA group. Type I fCSA exhibited a relative increase of 23.4 (2.8) % for this group, which was 16.4% greater than PLA, and 10.3% greater than the EEA group. Furthermore, increases in fCSA were most pronounced for type IIa, with a relative increase of 27.1 (2.7) %, this was 18.6% greater than PLA, 10.8% greater than CHO, and 10.3% greater than the EAA group. Finally, type IIb fCSA increased by 20.4 (4.0) %, which was 13.5% greater than the PLA group. These findings indicate that CHO + EAA ingestion enhances skeletal muscle anabolism following 12 weeks of progressive resistance training to a greater extent than either CHO or EAA consumed independently.

Andersen et al. (2005) outline that for muscle hypertrophy to occur the rate of protein synthesis must exceed protein degradation, thus inducing positive net protein balance. Yet, in the absence of nutrient intake net muscle

protein balance remains negative following resistance exercise (Biolo et al. 1995). Although net muscle protein balance was not determined in the current investigation, in theory the significant hypertrophy shown by the CHO + EAA group is the product of an "anti-catabolic effect", as suggested by Tarpenning et al. (2001). However, the current data indicates that such a response is mediated by the interaction of two separate mechanisms. The first involves a transient decrease in hormone-mediated protein degradation, via suppressing exercise-induced CORT release. The second is a transient increase in protein synthesis, via increased extracellular amino acid availability. This contention is supported, in part, by Tipton et al. (2001), who report that ingestion of 6 g EAA + 35 g sucrose, either pre- or post-exercise, not only inhibits protein breakdown (as assessed by phenylalanine R_a), but also stimulates protein synthesis. These authors suggest that the stimulation of protein synthesis by EAA, in addition to inhibition of the normal post-exercise rise in protein degradation, is likely to be accounted for by the synergistic effect of the drink (Tipton et al. 2001).

In agreement with previous work (Carroll et al. 1998; Hakkinen et al. 2003) a training frequency of 2 day - week^{-1} in untrained individuals, following the progressive overload principle (i.e., periodisation), is effective in promoting muscular adaptations in novice trainers. Exercise-induced changes in strength expression appear to be mediated by the complex interplay between neural-induced adaptations and the hypertrophic response of skeletal muscle, with early phase (first 6–8 weeks) strength gains primarily due to neural adaptations, while hypertrophic responses begin to occur at the latter phases (12–26 weeks) of training (Moritani and deVries 1979; Sale 1988). In the current investigation significant increases in 1-RM strength for the leg press and bench press occurred across all groups, with the CHO + EAA group displaying the greatest increases in 1-RM strength, and this was significant for the leg press, relative to PLA. This is consistent with previous reports of strength gains following resistance training of similar durations (Gater et al. 1992; Kraemer et al. 1999; Chromiak et al. 2004).

The observed disparity in the degree of hypertrophy between the PLA and treatment groups resulted in differences in strength expression over the 12-week training period. The treatment groups displayed significantly greater gains in muscle fibre CSA than PLA, and this was accompanied by significant increases in 1-RM strength during each 4-week training block throughout the study. Conversely, the PLA group displayed the smallest relative gains in muscle fibre CSA, which was accompanied by no change in 1-RM strength following the final 4-week training block. The lack of change in 1-RM strength shown by the PLA group following the final 4 weeks of training supports the theory that neural adaptations are the predominant mechanism for increases in muscular strength in the early phase of resistance training (Moritani and deVries 1979; Sale 1988). Therefore, differences in 1-RM strength reported in the current investigation are attributed to the hypertrophic response of skeletal muscle, as it is well established that muscular strength is proportional to fCSA (Maughan et al. 1983).

Interestingly, all groups demonstrated similar increases in peak torque, with no significant differences reported between groups for isokinetic strength. In agreement with Andersen et al. (2005), there appears to be a discrepancy between gains in muscle size and velocity-specific contraction strength. For example, in the current investigation, the significantly greater gains in fCSA in the treatment groups did not result in proportionally greater increases in isokinetic strength compared with the PLA group. Explanation for such a response, as discussed by Andersen et al. (2005), suggests that neural-induced adaptations have the potential to overrule the effect of muscle hypertrophy on velocity-specific contraction strength. However, this remains speculative at this time.

In summary, the major findings of the present investigation were that CHO + EAA ingestion enhances muscular and hormonal adaptations following 12 weeks of progressive resistance training to a greater extent than either CHO or EAA consumed independently. These findings provide a link between acute changes in the pathway of adaptation to the chronic adaptations of resistance training, as proposed by Volek (2004), demonstrating that the exercise-induced hormonal profile can be influenced by nutritive interventions toward a profile more favourable for anabolism, therefore optimising the skeletal muscle hypertrophic adaptations associated with resistance training. Furthermore, these data provide evidence that skeletal muscle anabolism is mediated, at least to some extent, by the "anti-catabolic effect". Such a regulatory role is an important component in the pathway of adaptation, linking nutritive interventions to the acute and chronic responses of resistance exercise.

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References

Ahtiainen JP, Pakarinen A, Alen M, Kraemer WJ, Hakkinen K (2003) Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. Eur J Appl Physiol 89:555–563

Andersen LL, Tufekovic G, Zebis MK, Crameri RM, Verlaan G, Kjaer M, Suetta C, Magnusson P, Aagaard P (2005) The effect of resistance training combined with timed ingestion of protein on muscle fiber size and muscle strength. Metabolism 54:151–156

Ballard FJ, Tomas FM (1983) 3-methylhistidine as a measure of skeletal muscle protein breakdown: the case for its continued use. Clin Sci 65:209–215

Bergstrom J (1962) Muscle electrolytes in man. Scand J Clin Lab Invest 14:S11–S14

Biolo G, Maggi SP, Williams BD, Tipton KD, Wolfe RR (1995) Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. Am J Physiol Endocrinol Metab 268:E514–E520

Biolo G, Williams BD, Fleming RY, Wolfe RR (1999) Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. Diabetes 48:949–957

Bird SP, Tarpenning KM, Marino FE (2005) Liquid carbohydrate/essential amino acid ingestion during an acute bout of resistance exercise suppresses myofibrillar protein degradation. Metabolism (in press) DOI:10.1016/j.metabol.2005.11.011

Blomstrand E, Ekblom B (1982) The needle biopsy technique for fibre type determination in human skeletal muscle—a methodological study. Acta Physiol Scand 116:437–442

Bohe J, Low A, Wolfe RR, Rennie MJ (2003) Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose–response study. J Physiol 552:315–324

Borsheim E, Aarsland A, Wolfe RR (2004) Effect of an amino acid, protein, and carbohydrate mixture on net muscle protein balance after resistance exercise. Int J Sport Nutr Exerc Metab 14:255–271

Brooke MH, Kaiser KK (1970) Muscle fiber types: how many and what kind? Arch Neurol 23:369–379

Carroll TJ, Abernethy PJ, Logan PA, Barber M, McEniery MT (1998) Resistance training frequency: strength and myosin heavy chain responses to two and three bouts per week. Eur J Appl Physiol Occup Physiol 78(3):270–275

Chesley A, MacDougall JD, Tarnopolsky MA, Atkinson SA, Smith K (1992) Changes in human muscle protein synthesis after resistance exercise. J Appl Physiol 73:1383–1388

Chromiak JA, Smedley B, Carpenter W, Brown R, Koh YS, Lamberth JG, Joe LA, Abadie BR, Altorfer G (2004) Effect of a 10-week strength training program and recovery drink on body composition, muscular strength and endurance, and anaerobic power and capacity. Nutrition 20(5):420–427

English R, Lewis J (1991) Australia New Zealand Food Authority: nutritional values of Australian foods. Australian Government Publishing Service, Canberra

Evans WJ, Phinney SD, Young VR (1982) Suction applied to a muscle biopsy maximizes sample size. Med Sci Sports Exerc 14:101–102

Gater DR, Gater DA, Uribe JM, Bunt JC (1992) Impact of nutritional supplements on body composition, strength and insulin-like growth factor-1. J Appl Sport Sci Res 6(2):66–76

Goldberg AL (1969) Protein turnover in skeletal muscle. Part II: effects of denervation and cortisone on protein catabolism in skeletal muscle. J Biol Chem 244:3223–3229

Goldberg AL (1979) Influence of insulin and contractile activity on muscle size and protein balance. Diabetes Suppl 1:18–24

Gore DC, Jahoor F, Wolfe RR, Herndon DN (1993) Acute response of human muscle protein to catabolic hormones. Ann Surg 218:679–684

Hakkinen K, Alen M, Kraemer WJ, Gorostiaga E, Izquierdo M, Rusko H, Mikkola J, Hakkinen A, Valkeinen H, Kaarakainen E, Romu S, Erola V, Ahtiainen J, Paavolainen L (2003) Neuromuscular adaptations during concurrent strength and endurance training versus strength training. Eur J Appl Physiol 89:42–52

Keis CV (1977) Techniques in human nitrogen balance studies. In: Bodwell CE (ed) Evaluations of proteins for humans. AVI, Westport, pp 162–176

Kettelhut IC, Wing SS, Goldberg AL (1988) Endocrine regulation of protein breakdown in skeletal muscle. Diabetes Metab Rev 4:751–772

Koopman R, Wagenmakers AJ, Manders RJ, Zorenc AH, Senden JM, Gorselink M, Keizer HA, van Loon LJ (2005) Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. Am J Physiol Endocrinol Metab 288:E645–E653

Kraemer WJ, Marchitelli L, Gordon SE, Harman E, Dziados JE, McCurry D, Fleck SJ (1990) Hormonal and growth factor responses to heavy resistance exercise protocols. J Appl Physiol. 69:1442–1450

Kraemer WJ, Staron RS, Hagerman FC, Hikida RS, Fry AC, Gordon SE, Nindl BC, Gotshalk LA, Volek JS, Marx JO, Newton RU, Hakkinen K (1998a) The effects of short-term resistance training on endocrine function in men and women. Eur J Appl Physiol 78:69–76

Kraemer WJ, Volek JS, Bush JA, Putukian M, Sebastianelli WJ (1998b) Hormonal responses to consecutive days of heavy-resistance exercise with or without nutritional supplementation. J Appl Physiol 85:1544–1555

Kraemer WJ, Hakkinen K, Newton RU, Nindl BC, Volek JS, McCormick M, Gotshalk LA, Gordon SE, Fleck SJ, Campbell WW, Putukian M, Evans WJ (1999) Effects of heavy-resistance training on hormonal response patterns in younger vs. older men. J Appl Physiol 87:982–992

Kraemer WJ, French DN, Spiering BA, Volek JS, Sharman MJ, Ratamess NA, Judelson DA, Silvestre R, Watson G, Gomez A, Maresh CM (2005) Cortitrol supplementation reduces serum cortisol responses to physical stress. Metabolism 54:657–668

Laurent GJ, Millward DJ (1980) Protein turnover during skeletal muscle hypertrophy. Fed Proc 39:42–47

van Loon LJ, Saris WHM, Verhagen H, Wagenmakers AJ (2000) Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. Am J Clin Nutr 72:96–105

Lukaski HC, Mendez J, Buskirk ER, Cohn SH (1981) Relationship between endogenous 3-methylhistidine excretion and body composition. Am J Physiol Endocrinol Metab 240:E302–E307

Maughan RJ, Watson JS, Weir J (1983) Strength and cross-sectional area of human skeletal muscle. J Physiol 338:37–49

McCall GE, Byrnes WC, Dickinson A, Pattany PM, Fleck SJ (1996) Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. J Appl Physiol 81:2004–2012

McCall GE, Byrnes WC, Dickinson AL, Fleck SJ (1998) Sample size required for the accurate determination of fiber area and capillarity of human skeletal muscle. Can J Appl Physiol 23:594–599

Miller SL, Tipton KD, Chinkes DL, Wolf SE, Wolfe RR (2003) Independent and combined effects of amino acids and glucose after resistance exercise. Med Sci Sports Exerc 35:449–455

Moller-Loswick AC, Zachrisson H, Hyltander A, Korner U, Matthews DE, Lundholm K (1994) Insulin selectively attenuates breakdown of nonmyofibrillar proteins in peripheral tissues of normal men. Am J Physiol Endocrinol Metab 266:E645–E652

Moritani T, deVries HA. 1979, Neural factors versus hypertrophy in the time course of muscle strength gain. Am J Phys Med 58(3):115–130

National Health and Medical Research Council (1991) Recommended dietary intakes for use in Australia. Australian Government Publishing Service, Canberra

Paddon-Jones D, Sheffield-Moore M, Creson DL, Sanford AP, Wolf SE, Wolf RR, Ferrando AA (2003) Hypercortisolemia alters muscle protein anabolism following ingestion of essential amino acids. Am J Physiol Endocrinol 284:E946–E953

Paddon-Jones D, Sheffield-Moore M, Urban RJ, Aarsland A, Wolfe RR, Ferrando AA (2005) The catabolic effects of prolonged inactivity and acute hypercortisolemia are offset by dietary supplementation. J Clin Endocrinol Metab 90:1453–1459

Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR (1997) Mixed protein synthesis and breakdown after resistance exercise in humans. Am J Physiol Endocrinol Metab 273:E99–E107

Raastad T, Bjoro T, Hallen J (2000) Hormonal responses to high- and moderate-intensity strength exercise. Eur J Appl Physiol 82:121–128

Rankin JW, Goldman LP, Puglisi MJ, Nickolas-Richardson SM, Earthman CP, Gwazdauskas FC (2004) Effect of post-exercise supplement consumption on adaptationsto resistance training. J Am Coll Nutr 23(4):322–330

Rasmussen BB, Tipton KD, Miller SL, Wolf SE, Wolfe RR (2000) An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. J Appl Physiol 88:386–392

Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, Hwang D, Goldberg AL (1994) Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. Cell 78:761–771

Roy BD, Tarnopolsky MA, MacDougall JD, Fowles J, Yarasheski KE (1997) Effect of glucose supplement timing on protein metabolism after resistance training. J Appl Physiol 82:1882–1888

Rozenek R, Ward P, Long S, Garhammer J (2002) Effects of high-calorie supplements on body composition and muscular strength following resistance training. J Sports Med Phys Fitness 42(3):340–347

Sale DG (1988) Neural adaptation to resistance training. Med Sci Sports Exerc 20(5):S135–S145

Seene T, Viru A (1982) The catabolic effect of glucocorticoids on different types of skeletal muscle fibers and its dependence upon muscle activity and interaction with anabolic steroids. J Steroid Biochem 16:349–352

Simmons PS, Miles JM, Gerich JE, Haymond MW (1984) Increased proteolysis. An effect of increases in plasma cortisol within the physiologic range. J Clin Invest 73:412–420

Staron RS, Malicky ES, Leonardi MJ, Falkel JE, Hagerman FC, Dudley GA (1990) Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women. Eur J Appl Physiol 60:71–79

Staron RS, Karapondo DL, Kraemer WJ, Fry AC, Gordon SE, Falkel JE, Hagerman FC, Hikida RS (1994) Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. J Appl Physiol 76:1247–1255

Staron RS, Hagerman FC, Hikida RS, Murray TF, Hostler DP, Crill MT, Ragg KE, Toma K (2000) Fiber type composition of the vastus lateralis muscle of young men and women. J Histochem Cytochem 48:623–629

Tarpenning KM, Wiswell RA, Hawkins SA, Marcell TJ (2001) Influence of weight training exercise and modification of hormonal response on skeletal muscle growth. J Sci Med Sports 4:431–446

Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, Wolfe RR (1999) Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol Endocrinol Metab 276:E628–E634

Tipton KD, Rasmussen BB, Miller SL, Wolf SE, Owens-Stovall SK, Petrini BE, Wolfe RR (2001) Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. Am J Physiol Endocrinol Metab 281:E197–E206

Volek JS (2004) Influence of nutrition on responses to resistance training. Med Sci Sports Exerc 36:689–696

Williams AG, van den Oord M, Sharma A, Jones DA (2001) Is glucose/amino acid supplementation after exercise an aid to strength training? Br J Sports Med 35:109–113

Young VR, Munro HN (1978) N^{τ}-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. Fed Proc 37:2291–2300