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Liquid carbohydrate/essential amino acid ingestion during a short-term bout of resistance exercise suppresses myofibrillar protein degradation

Stephen P. Bird*, Kyle M. Tarpenning, Frank E. Marino

School of Human Movement Studies, Charles Sturt University, Bathurst, NSW 2795, Australia

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In memory of the late Dr Kyle Tarpenning.

Abstract

A number of physiological events including the level of contractile activity, nutrient status, and hormonal action influence the magnitude of exercise-induced skeletal muscle growth. However, it is not the independent action of a single mechanism, but the complex interaction between events that enhance the long-term adaptations to resistance training. The purpose of the present investigation was to examine the influence of liquid carbohydrate (CHO) and essential amino acid (EAA) ingestion during resistance exercise and modification of the immediate hormonal response on myofibrillar protein degradation as assessed by 3-methylhistidine (3-MH) excretion. After a 4-hour fast, 32 untrained young men (18-29 years) performed a single bout of resistance exercise (complete body; 3 sets × 10 repetitions at 75% of 1-repetition maximum; 1-minute rest between sets), during which they consumed a 6% CHO (n = 8) solution, a 6-g EAA (n = 8) mixture, a combined CHO + EAA (n = 8) supplement, or placebo (PLA; n = 8) beverage. Resistance exercise performed in conjunction with CHO and CHO + EAA ingestion resulted in significantly elevated ($P < .001$) glucose and insulin concentrations above baseline, whereas EAA ingestion only increased the postexercise insulin response ($P < .05$). Time matched at 60 minutes, the PLA group exhibited a peak cortisol increase of 105% ($P < .001$) with no significant change in glucose or insulin concentrations. Conversely, the CHO and CHO + EAA groups displayed a decrease in cortisol levels of 11% and 7%, respectively. Coinciding with these hormonal response patterns were significant differences in myofibrillar protein degradation. Ingestion of the EAA and CHO treatments attenuated 3-MH excretion 48 hours after the exercise bout. Moreover, this response was synergistically potentiated when the 2 treatments were combined, with CHO + EAA ingestion resulting in a 27% reduction ($P < .01$) in 3-MH excretion. In contrast, the PLA group displayed a 56% increase ($P < .01$) in 3-MH excretion. These data demonstrate that not only does CHO and EAA ingestion during the exercise bout suppress exercise-induced cortisol release; the stimulatory effect of resistance exercise on myofibrillar protein degradation can be attenuated, most dramatically when the treatments are combined (CHO + EAA). Through an "anticatabolic effect," this altered balance may better favor the conservation of myofibrillar protein.

1. Introduction

Resistance exercise stimulates immediate changes in the rate of muscle protein turnover, resulting in an increase in both protein synthesis and protein degradation [1-3]. Any imbalance between the rate of protein synthesis and rate of protein degradation will lead to a change in the size of the protein tissue pool [4]. Thus, for protein accretion to occur, the rate of synthesis must exceed the rate of degradation. However, in the absence of nutritional intake, net muscle protein balance (ie, the difference between protein synthesis and protein degradation) remains negative in the early stages of recovery [2]. A number of studies support the notion that hormonal events play critical roles in controlling protein turnover [5-7], with the response of insulin and cortisol receiving much attention, as they are intimately involved in this cyclical process. Although the ability to alter the anabolic hormonal milieu and the influence that such adjustments have on modifying protein synthesis are well documented [8-10], the ability to alter the catabolic hormonal environment and the impact that such a change might have on affecting protein degradation have received considerably less attention.

* Corresponding author. Tel.: +61 612 6338 4155; fax: +61 612 6338 4065.

E-mail address: sbird@csu.edu.au (S.P. Bird).

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Urinary 3-methylhistidine (3-MH) excretion has been used as an index of myofibrillar protein degradation [11-14]. 3-Methylhistidine 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 [14]. Estimates indicate that ~90% of total human 3-MH is located in skeletal muscle [15], validating its use as an index of myofibrillar protein degradation [14,16]. Yet, the clinical use of 3-MH has been met with criticism. A substantial limitation appears to be the effect of protein intake on 3-MH excretion [17]. Lukaski et al [18] determined that meat consumption increases 3-MH excretion and that 3 days of a meat-free diet are required to return urinary 3-MH to baseline levels. However, with careful dietary and exercise controls, qualitative inferences can be made concerning skeletal muscle metabolism [14,16,19].

A variety of resistance exercise protocols result in an immediate increase in cortisol [20-22]. However, Goldberg and Goodman [23] reported that elevated cortisol levels are the primary factor stimulating exercise-induced increases in protein degradation. Therefore, long-term elevations in cortisol associated with resistance training may have a negative impact on skeletal muscle hypertrophic adaptations [21]. Such a contention implicates the glucoregulatory action of cortisol. Therefore, if an individual ingested a carbohydrate (CHO) solution during the exercise bout, the exogenous glucose load would raise blood glucose levels. This response may modify biochemical signals and attenuate the stimulus for the adrenal cortex to secrete cortisol to catabolize cellular protein for gluconeogenic purposes. Moreover, addition of essential amino acids (EAAs) may augment this response, as recent reports demonstrate that protein feeding [24] and leucine ingestion [25] rapidly reduce the release of 3-MH. These findings indicate that, at least to some extent, myofibrillar protein degradation may be controlled by extracellular amino acid availability.

Previous work has demonstrated that liquid CHO ingestion during a short-term bout of resistance exercise can decrease exercise-induced cortisol release [21]. Resistance exercise with CHO ingestion blunted exercise-induced cortisol release both during and after the exercise bout. This response is in contrast to significantly elevated cortisol levels occurring with resistance exercise alone (99%) [21]. Thus, modification of exercise-induced cortisol release could influence protein turnover by altering the balance between hormone-mediated anabolic and catabolic activities [26]. A reduction in the cortisol response and hormone-induced protein degradation are potential mechanisms by which protein accretion occurs, that of enhancing skeletal muscle growth by suppressing myofibrillar protein degradation [21]. Furthermore, such responses may be synergistically potentiated by the addition of EAA (CHO + EAA). Therefore, the purpose of the present investigation was to examine the influence of liquid CHO and EAA ingestion during resistance exercise and modification of the immediate hormonal response on myofibrillar protein degradation as assessed by 3-MH excretion.

2. Materials and methods

2.1. Subjects and study design

After a full explanation of all procedures and possible risks of the investigation, we obtained written informed consent from 32 untrained young men (mean [±SD]; age, 21.0 ± 2.4 years; height, 182.7 ± 6.9 cm; body mass, 79.6 ± 12.1 kg) who volunteered to participated 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 before the start of the study. After a full explanation of all procedures and possible risks of the investigation, we obtained written informed consent before testing began. Subjects completed a health history questionnaire; all were apparently healthy and had no medical contraindications or history of any endocrine disorders that might influence their responsiveness to a short-term bout of resistance exercise. None of the subjects were taking any medication or nutritional supplementation; all were nonsteroid users and nonsmokers. All experimentation was approved by the ethics in human research committee of the university.

A matched, randomized, double-blind, placebo-controlled design was used, in which subjects visited the Exercise and Sports Science Laboratory on 4 occasions during the 10-day experimental timeline. On all occasions, the time of day was standardized, with times held constant for each subject. All sessions were performed between 3:00 and 5:00 PM to minimize the influence of diurnal variations on exercise performance and hormonal response [27]. Subjects were required to refrain from all strenuous activity, alcohol use, caffeine, sexual activity, and meat consumption, and were notified to maintain normal nocturnal sleep habits (ie, approximately 8 hours per night) throughout the experimental timeline.

During the initial laboratory session (day 1), anthropometric measurements including height, body mass, and body composition were recorded. Height was measured to the nearest 0.1 cm using a stadiometer (Len Blayden, Lugarno, Australia), and body mass was measured to the nearest 0.01 kg using an electronic precision balance scale (HW-100KAI, GEC, Avery Ltd, Miranda, Australia). Body composition was determined by dual-energy x-ray absorptiometry with a total-body scanner (DPX-IQ, Prodigy, Lunar, Madison, WI). At the completion of the session, an accredited practicing dietitian (Dietitians Association of Australia) instructed subjects with procedures for reporting detailed dietary intake and eating habits for a 3-day period.

The second session (day 2) consisted of equipment familiarization and 1-repetition maximum (1-RM) strength testing for all exercises in the resistance exercise protocol. On day 3, subjects began a lacto-ovo-vegetarian diet (meat-

free) up to 2 days after the resistance exercise session and commenced timed and complete 24-hour urine collections each day of the study at 7:00 AM [28]. During the third session (day 7), subjects performed a short-term bout of resistance exercise during which they consumed 1 of 4 nutritive interventions (CHO group, n = 8; EAA group, n = 8; combined CHO + EAA group, n = 8; or placebo [PLA] group, n = 8). Blood samples were collected every 15 minutes throughout the exercise bout and postexercise period. On day 10, subjects concluded the meat-free diet and final timed 24-hour urine collection.

2.2. Nutritive intervention

After commencement of the exercise bout, subjects consumed a 6% CHO (Gatorade, Quaker Oats, Chicago, IL), a 6-g EAA (Musashi, Notting Hill, Australia), a combined CHO + EAA, or a PLA (aspartame and citrus flavoring, Quaker Oats). 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 has been previously shown to enhance skeletal muscle anabolism [29]. All treatments were dissolved in water at a fluid volume of 8.5 mL/kg body mass ($\approx$675 mL of solution), with the total volume of fluid 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.

2.3. Dietary control

Food intake diaries were used to control for 3 days before, and throughout the experimental period, but on days separate from those of the meat-free diet. This was done to assess whether there were any differences in energy intake and macronutrient composition between groups. Before the beginning of 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 the 3 designated days (2 weekdays and 1 weekend day). Subjects were 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 [30] or information provided by food manufacturers was obtained.

An accredited practicing dietitian (Dietitians Association of Australia) designed 7-day lacto-ovo-vegetarian sample menus for each subject. Meat sources of protein were not to be ingested, as they have been shown to increase 3-MH excretion and falsely represent total protein breakdown values [27]. A non-animal protein supplement (P40 protein powder, Musashi, Notting Hill, Australia) was provided to help ensure adequate protein intake. Energy intake was set at the recommended dietary intake of 9.8-13.7 MJ/d based on height and body mass [31] with 15% of energy from protein, 55% of energy from CHO, and 30% of energy from fat in the form of 3 daily meals and small snacks.

2.4. One-repetition maximum strength test

Maximal strength was assessed for each of the 8 selected resistance exercises by completing a 1-RM test, (ie, the heaviest load that could be correctly performed once). Warm-up consisted of 1 set of 5 to 10 repetitions at 40% to 60% of perceived maximum. Subjects then rested for 1 minute, performing light stretching. This was followed by 3 to 5 repetitions performed at 60% to 80% of perceived maximum. Thereafter, 3 to 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 1 time through a full range of motion, using good form at a tempo of 2:0:2 (2 seconds eccentric; 2 seconds concentric).

2.5. Resistance exercise protocol

The resistance exercise protocol used for this investigation was that previously used by Bird and Tarpenning [22], which has been shown to influence hormonal concentrations. Briefly, the resistance training protocol consisted of a complete-body workout and included the following exercises in order: leg press, leg curl, leg extension, shoulder press, lat pulldown, bench press, barbell bicep curl, and supine tricep extension. Three sets of 8 to 10 repetitions per set were performed at approximately 75% of the subject's 1-RM. One minute of rest between each set and 2 minutes between each exercise were allowed for recovery. The exercise bout lasted approximately 60 minutes and was preceded by a 10-minute warm-up and concluded with a 10-minute warm-down period. Staff trained in the principles associated with resistance training supervised all sessions.

2.6. Blood sampling and hormone analysis

After a 4-hour fast, subjects reported to the laboratory and sat quietly for 15 minutes. A 20-gauge 1.00-in Teflon-indwelling cannula (Saf-T-Intima, Becton Dickinson, Sandy, UT) was inserted into an antecubital forearm vein, after which subjects sat quietly for a further 15-minute period before blood collection to minimize hormonal fluctuations related to anticipatory responses [32]. Using a vacutainer assembly and serum separator tubes (Monovette, Sarstedt, Numbrecht, Germany), we collected 5-mL blood samples before exercise; after the second, fourth, sixth, and eighth exercises (approximately every 15 minutes); and 15 and 30 minutes postexercise. One milliliter of saline solution was injected into the cannula line between each blood draw to assist keeping the line clear and prevent clotting. Blood samples were gently inverted 5 times and allowed to stand at room temperature for a minimum of 20 minutes. Samples

were then centrifuged for 10 minutes at 3000 rpm, with the supernatant removed and placed into plastic storage containers and frozen at −20°C until analyzed.

Before analysis the serum was allowed to reach room temperature and mixed gently via inversion. All samples for each subject were assayed in the same assay run to avoid interassay variation. Glucose was determined via an enzymatic spectrophotometric method (Dimension Xpand, Dade Behring, Newark, DE), with an intra-assay coefficient of variation (CV) of 5.9%. Cortisol concentrations were determined by a competitive immunoassay technique using chemiluminescent technology (VITROS ECi, Ortho-Clinical Diagnostics, Rochester, NY). The sensitivity of the cortisol assay was less than 3 nmol/L and the intra-assay CV was 2.7%. Insulin concentrations were determined by a solid-phase, 2-site chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA). The sensitivity of the insulin assay was 2 μIU/mL and the intra-assay CV was 3.1%. 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.

2.7. Urinary collection and 3-MH analysis

For the measurement of 3-MH, an index of myofibrillar protein degradation [11-14], subjects provided timed and complete 24-hour urine collections from day 4 to day 10 of the study at 7:00 AM [28]. For the designated urine collection procedure, subjects were instructed to discard the product of the first urination upon waking in the morning and then collect all samples for the after 24 hours, 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-hour collection and stored at −20°C until analyzed. Before analysis, urine collections were acidified to 1% of volume with 12 N hydrochloric acid [28]. 3-Methylhistidine was assessed in duplicate via high-performance liquid chromatography (HPLC 1100 B, Hewlett-Packard, Palo Alto, CA) using a Synergi 4μ Hydro-RP 80A column (Phenomenex, Torrance, CA), with the amount in a 24-hour sample of urine expressed relative to lean body mass per day (μmol/lean body mass per day).

2.8. Statistical analysis

Descriptive data were generated for all variables and expressed as mean ± SEM. Analysis was performed using the Statistical Package for Social Sciences (v11.5, SPSS, Chicago, IL). Statistical analysis involved a 2-way analysis of variance (group × time) with repeated measures. The source of significant differences was located using Tukey honestly significant difference post hoc procedure. Significant interactions were analyzed by simple main effects. Area under the curve (AUC) analyses were calculated using standard trapezoidal statistical methods. Regression analysis determined associations between AUC for specific hormones (insulin and cortisol) and myofibrillar protein degradation (3-MH) for each condition. Significance was accepted when $P < .05$.

3. Results

3.1. Physical characteristics, 1-RM performance data, and daily dietary intake

The physical characteristics, 1-RM performance data, and daily dietary intake of the research subjects are shown in Table 1. The 4 groups did not significantly differ with respect to their physical characteristics and 1-RM performance data. Thus, the groups were matched for these variables. Nutritional analysis of daily dietary intake revealed that diets were consistent between groups. The macronutrient composition of the dietary intake was also similar between groups.

Table 1
Physical characteristics, 1-RM performance data, and daily intake of dietary energy and nutrients

	PLA group (n = 8)	CHO group (n = 8)	EAA group (n = 8)	CHO + EAA group (n = 8)
Physical characteristics				
Age (y)	20.3 ± 0.8	22.3 ± 1.2	21.3 ± 0.7	20.6 ± 0.5
Height (cm)	182.6 ± 1.8	184.4 ± 2.3	181.2 ± 2.9	183.4 ± 2.6
Weight (kg)	80.4 ± 4.5	79.3 ± 4.4	79.7 ± 4.6	79.3 ± 3.9
Fat mass (kg)	18.7 ± 3.2	12.7 ± 1.5	18.3 ± 3.2	13.8 ± 2.8
Fat-free mass (kg)	58.3 ± 1.9	63.1 ± 3.2	58.1 ± 1.9	61.7 ± 2.9
1-RM performance data				
45° Leg press (kg)	141.9 ± 16.6	145.9 ± 7.8	138.8 ± 12.0	142.5 ± 16.4
Bench press (kg)	58.1 ± 6.7	67.8 ± 5.2	65.6 ± 3.1	63.1 ± 6.0
Dietary intake				
Energy (J)	11314.2 ± 2324.6	12116.0 ± 2669.3	11835.7 ± 2614.6	11480.7 ± 2994.8
Protein (g)	127.3 ± 13.0	113.6 ± 9.0	139.1 ± 11.8	113.9 ± 9.5
CHO (g)	299.7 ± 20.9	343.6 ± 22.9	298.1 ± 30.0	338.3 ± 33.8
Fat (g)	108.6 ± 8.7	113.0 ± 9.5	117.6 ± 10.0	100.3 ± 14.0

Data are given as mean ± SE. No significant differences were found in any parameter.

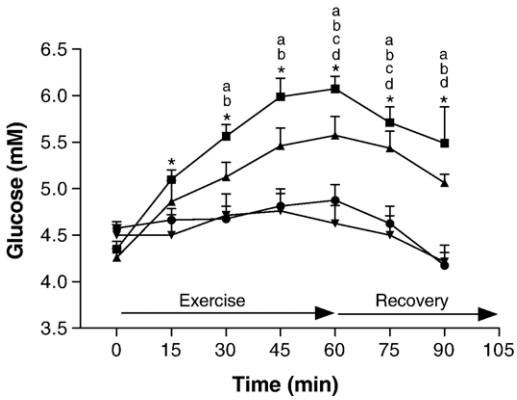

Fig. 1. Serum glucose concentrations for PLA (•), CHO (▲), EAA (▼), and CHO + EAA (■). Significantly different from preexercise value (*$P < .05$): CHO vs 30, 45, 60, 75, and 90 minutes; CHO + EAA vs 30, 45, 60, 75, and 90 minutes. Significant difference between treatments ($P < .05$): [a]CHO from PLA; [b]CHO + EAA from PLA; [c]CHO from EAA; [d]CHO + EAA from EAA.

3.2. Glucose

Both the PLA and EAA groups showed no significant change in serum glucose concentration to the exercise bout from baseline. Conversely, CHO and CHO + EAA ingestion resulted in significantly elevated serum glucose levels of 31% and 40%, respectively, with all time points significantly higher compared with preexercise levels. Carbohydrate ingestion resulted in significantly higher glucose levels than PLA and EAA at 60, 75 and 90 minutes, and 60 and 75 minutes, respectively. In addition, CHO + EAA ingestion resulted in significantly higher glucose levels than PLA and EAA at 30, 45, 60, 75, and 90 minutes (Fig. 1).

3.3. Insulin

The PLA group showed no significant change in serum insulin concentration to the exercise bout from baseline. Essential amino acid ingestion resulted in significant increases at 75 and 90 minutes. Both CHO and CHO + EAA consumption significantly increased insulin concentration at 60, 75, and 90 minutes. It is noteworthy that insulin appeared to still be increasing for the CHO + EAA group after this time point. CHO and CHO + EAA subjects had significantly higher insulin levels than PLA at 60, 75, and 90 minutes (Fig. 2).

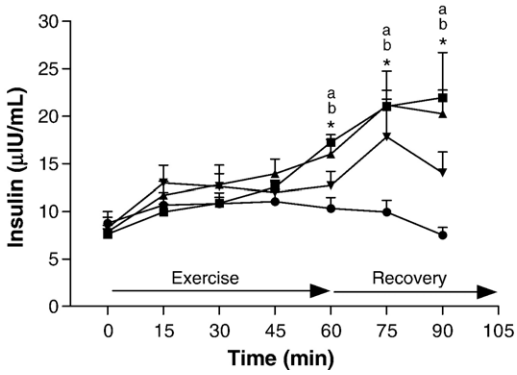

Fig. 2. Serum insulin concentrations for PLA (•), CHO (▲), EAA (▼), and CHO + EAA (■). Significantly different from preexercise value (*$P < .05$): CHO vs 60, 75, and 90 minutes; EAA vs 75 and 90 minutes; CHO + EAA vs 60, 75, and 90 minutes. Significant difference between treatments ($P < .05$): [a]CHO from PLA; [b]CHO + EAA from PLA.

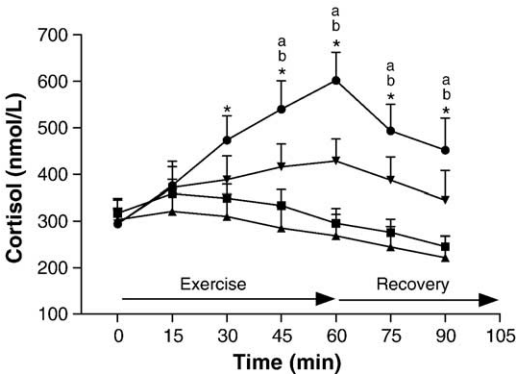

Fig. 3. Serum cortisol concentrations for PLA (•), CHO (▲), EAA (▼), and CHO + EAA (■). Significantly different from preexercise value (*$P < .05$): PLA vs 30, 45, 60, 75, and 90 minutes; CHO vs 90 minutes; CHO + EAA vs 90 minutes. Significant difference between treatments ($P < .05$): [a]CHO from PLA; [b]CHO + EAA from PLA.

3.4. Cortisol

Ingestion of the PLA beverage resulted in a significant increase in cortisol at 30 minutes, with values for all remaining time points significantly higher compared with baseline. The EAA group showed no significant change in serum cortisol concentration to the exercise bout from baseline. However, consequent to the CHO and CHO + EAA treatments, the exercise-induced cortisol response was blunted, resulting in a significant decrease pre- to postexercise. This blunted response was associated with significantly lower cortisol levels than PLA at 45, 60, 75, and 90 minutes (Fig. 3).

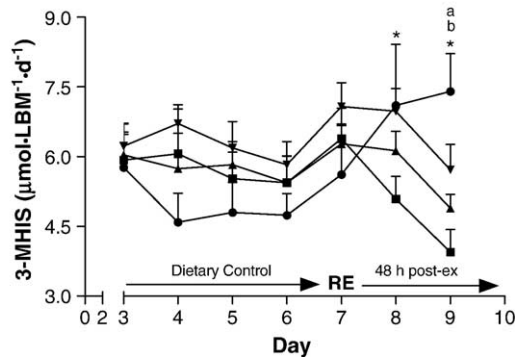

Fig. 4. Mean daily 24-hour urinary 3-MH excretion values for PLA (•), CHO (▲), EAA (▼), and CHO + EAA (■), expressed as micromoles per kilogram of lean body mass (LBM) per day. Significantly different from preexercise (day 6) value (*$P < .05$): PLA vs days 8 and 9; CHO + EAA vs day 9. Significant difference between treatments ($P < .05$): [a]CHO from PLA; [b]CHO + EAA from PLA.

3.5. 3-Methylhistidine excretion

Fig. 4 shows 3-MH excretion in the 48-hour postexercise period. A washout for dietary 3-MH was evident after 72 hours of dietary restriction, as indicated by 2 consecutive 24-hour urine collections (days 5 and 6). The PLA group exhibited significantly elevated 3-MH excretion was on day 8, with a peak increase of 56% recorded on day 9. Alternatively, the EAA and CHO groups showed no change in 3-MH excretion in the 48-hour postexercise period from baseline. CHO + EAA ingestion resulted in no change in 3-MH excretion on day 8, with a significant decrease of 27% at day 9. Furthermore, CHO and CHO + EAA consumption resulted in significantly lower 3-MH excretion than PLA on day 9.

Regression analysis revealed a positive linear association for the PLA group ($r = 0.75$, $P < .05$), with 57% of the variance of change in myofibrillar protein degradation accounted for by cortisol AUC concentration (Fig. 5A). Conversely, a negative linear association was observed for the CHO + EAA group ($r = -0.77$, $P < .05$), with 59% of the variance of change in myofibrillar protein degradation accounted for by cortisol AUC concentration (Fig. 5B). No significant associations were reported between the cortisol response and myofibrillar protein degradation for either CHO or EAA. In addition, regression analysis revealed no significant relationships between changes in 3-MH excretion 48 hours postexercise and insulin AUC concentrations after a short-term bout of resistance exercise.

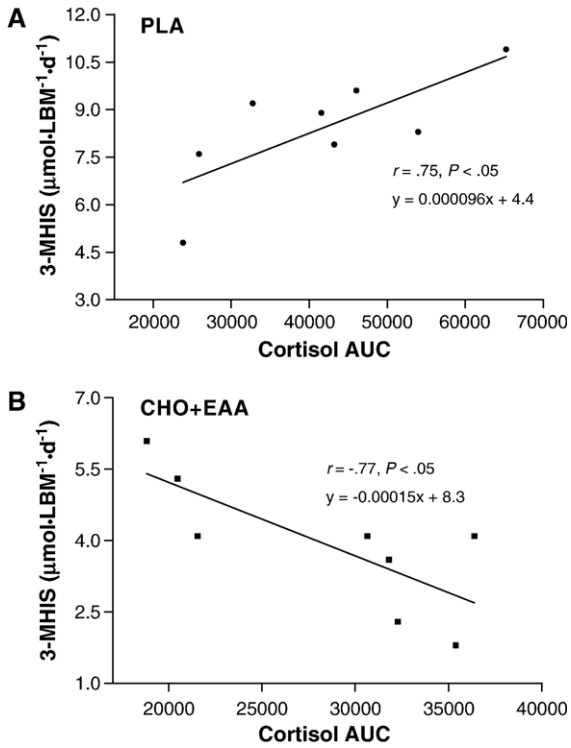

Fig. 5. Association between cortisol AUC concentrations after a short-term bout of resistance exercise and mean daily 24-hour urinary 3-MH excretion values 48 hours postexercise. A, PLA. B, CHO + EAA.

4. Discussion

The primary findings from this investigation were that resistance exercise performed in conjunction with liquid CHO ingestion significantly affected the immediate hormonal response to exercise, resulting in significantly elevated insulin levels and a significant decrease in cortisol concentration during and after the exercise bout. Moreover, the addition of EAA (CHO + EAA) potentiated these responses. This altered hormonal response was associated with attenuated myofibrillar protein degradation (as assessed by 3-MH). Although the EAA and CHO groups displayed a nonsignificant decrease in 3-MH excretion compared with preexercise values, the CHO + EAA group displayed a significant reduction in 3-MH excretion. These results are in contrast to the PLA group, where insulin concentrations showed no change, whereas cortisol levels were significantly elevated. The hormonal milieu displayed by PLA was accompanied by significantly elevated 3-MH excretion. These data indicate that at least to some extent, the stimulatory effect of resistance exercise on myofibrillar protein degradation can be overcome by EAA, CHO, or CHO + EAA administration.

A short-term bout of resistance exercise performed in conjunction with liquid CHO ingestion results in significantly elevated insulin levels [21,33,34]. The implication of insulin's action in skeletal muscle growth may be inferred from the work of Roy et al [34] who report that supplementation with liquid CHO (1 g/kg glucose) immediately and 1 hour after a short-term bout of single leg knee extensor resistance exercise significantly decreased myofibrillar protein breakdown (as assessed by 3-MH) and urinary urea excretion, resulting in a more positive body protein balance. The authors proposed that the reduction in protein breakdown was the result of insulin's ability to inhibit myofibrillar protein degradation. However, insulin's ability to inhibit myofibrillar protein degradation may be limited, with several lines of research indicating that myofibrillar protein degradation remains unaffected by insulin [35-38]. In healthy human subjects, insulin did not change 3-MH fluxes across either the leg or the forearm [37,38]. Moller-Loswick et al [38] confirmed that although a postabsporptive increase in plasma insulin improved the overall protein balance entirely by decreasing protein breakdown, release of 3-MH was totally unaffected by hyperinsulinemia. The authors concluded that insulin selectively attenuated the degradation rate of nonmyofibrillar proteins [38]. Explanation for the absence of an inhibitory effect of insulin on myofibrillar proteolysis as discussed by Kettelhut et al [39] indicates that insulin decreases lysosomal proteolytic activity, but does not mediate the ubiquitin-proteasome pathway, which is responsible for the bulk of myofibrillar proteolysis [40]. Although insulin can reduce whole-body protein breakdown, such an effect has not been demonstrated on myofibrillar protein. Consequently, insulin's mechanism(s) of action on skeletal muscle growth remains unclear.

In the present investigation, the PLA group showed no change in insulin levels in response to resistance exercise, whereas the treatment groups displayed significant increases in insulin. However, no significant associations were found for the treatment groups between the changes in 3-MH excretion 48 hours postexercise and insulin AUC concentrations after a short-term bout of resistance exercise. These data further support the contention that insulin does not play a major role in the regulation of myofibrillar protein degradation in skeletal muscle. Although Roy et al [34] are of the opinion that the decrease in myofibrillar protein breakdown reported in their study was regulated by insulin, it is possible that CHO-induced suppression of cortisol release contributed to the reduction in 3-MH excretion. Interestingly, cortisol values were not reported in this study.

Previous reports indicate that the myofibrillar proteolytic rate is regulated to a large extent by glucocorticoids [41], with suppression of glucocorticoid activity shown to inhibit protein degradation [42,43]. Furthermore, glucocorticoid excess has been reported to antagonize insulin's antiproteolytic action [44]. Total tissue exposure to cortisol, as determined by AUC, was significantly greater for the PLA group, and this corresponded with a substantial increase in 3-MH excretion 48 hours after the exercise bout. Furthermore, a positive linear association was observed between the change in myofibrillar protein degradation and cortisol AUC for this group. Conversely, the treatment groups exhibited a blunted cortisol response, which coincided with significant differences in myofibrillar protein degradation. Although ingestion of the EAA and CHO treatments produced attenuation of 3-MH excretion 48 hours after the exercise bout, this response was synergistically potentiated when the 2 treatments were combined. Not only did CHO + EAA ingestion suppress exercise-induced cortisol, this treatment resulted in significantly decreased 3-MH excretion 48 hours after the exercise bout. This would suggest that CHO + EAA ingestion during resistance exercise results in conservation of myofibrillar protein, potentially enhancing skeletal muscle growth by suppressing myofibrillar protein degradation. However, this study is limited as it only examined the immediate response. Future studies should further examine the impact of long-term manipulation of the exercise-induced cortisol response on skeletal muscle growth.

In conclusion, ingestion of a liquid CHO, EAA, or CHO + EAA solution during the exercise bout blunted the exercise-induced cortisol response, with CHO + EAA consumption resulting in significantly lower 3-MH excretion (an index of myofibrillar protein degradation). This is in contrast to significantly elevated cortisol levels and 3-MH excretion occurring with PLA. The importance of such a finding, as discussed by Goldberg [26], implicates the modification of the cortisol response on protein turnover, thereby altering the balance between hormone-mediated anabolic and catabolic activities. Through an "anticatabolic effect," this altered balance may better favor the conservation of myofibrillar protein, thereby enhancing skeletal muscle growth by suppressing muscle breakdown [21]. The decrease in 3-MH excretion reported in the current investigation supports this position. However, the importance of myofibrillar protein degradation as a regulatory process and whether suppression of myofibrillar protein degradation can occur to enhance skeletal muscle growth are yet to be elucidated.

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