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ORIGINAL ARTICLE

Acute regulation of IGF-I by alterations in post-exercise macronutrients

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Abstract This investigation sought to examine the contributions of exercise and nutrient replenishment on in vivo regulation of the insulin-like growth factor-I (IGF-I) axis components. Eight college-aged males completed three high-intensity interval training (HIIT) protocols followed by three post-exercise nutritional protocols: (1) placebo (EX); (2) carbohydrate only (CHO); and (3) essential amino acid/carbohydrate (EAA/CHO). Samples were analyzed for growth hormone (GH), free IGF-I, IGFBP-1, IGFBP-2, insulin, hematocrit, hemoglobin, serum leucine, matrix metalloproteinase-9 (MMP-9) proteolytic activity, and presence of IGFBP-3 protease activity. No evidence for IGFBP-3 proteolysis was observed. Significant increases in [free IGF-I] and [leucine] were observed in the EAA/CHO group only. Significant differences were noted in [IGFBP-1] and [IGFBP-2] across conditions. Significant increases in [GH] and MMP-9 activity were observed in all groups. These results indicate that post-exercise macronutrient ratio is a determinant of [free IGF-I], [IGFBP-1 and -2] and may play a role in modulating the IGF-I axis in vivo.

Keywords IGF-I · HIIT · IGFBP · EAA

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Introduction

Knowledge regarding the complex regulatory dynamics of IGFs has increased significantly since the early observations of Salmon and Daughaday in the 1950s (Salmon and Daughaday 1957). Their original 'somatomedin theory', which posited a GH stimulus eliciting somatomedin secretion and subsequent growth stimulation, has since been progressively complicated with multiple layers of control and regulation. Current evidence supports the premise that GH directly controls the levels of circulating IGFs and some of the IGFBPs (Firth and Baxter 2002; Rajaram et al. 1997), with GH deficiency or surplus being manifest with corresponding IGF deficiency or surplus, respectively (Florini et al. 1996). Indirectly, some evidence suggests that GH might have an acute effect on the control of IGF-I bioavailability (Ketelslegers et al. 1996; Kita et al. 2001).

The IGFs are growth-promoting peptides that share significant structural homology with pro-insulin (Rajaram et al. 1997). Although many tissues in the body have been shown to produce and secrete IGFs, the majority of IGF in the general circulation is believed to originate from hepatic

production (LeRoith et al. 2001; Rajaram et al. 1997). The general consensus in the literature is that basal levels of circulating IGFs are determined largely through the actions of GH on hepatic GH receptors (Huang et al. 2004; Leung et al. 2000). IGFs exert the majority of their biological actions by binding to the Type-1 IGF receptor, which has a high degree of homology to the insulin receptor and is the only IGF receptor to have known IGF-mediated signaling function (Jones and Clemmons 1995).

Unlike insulin, IGFs circulate in plasma complexed to a family of structurally related binding proteins (IGFBPs). Currently, IGFBPs are attributed with three main roles within the IGF system: (1) modulation of IGF action; (2) storage facilitation of IGFs in the extracellular matrix; and (3) exertion of IGF-independent effects (Collett-Solberg and Cohen 1996; Firth and Baxter 2002). In contrast to circulating insulin, a small percentage of circulating IGF-I is present in the free form in the healthy state, with the majority of circulating IGF-I bound to IGFBPs. The IGFBPs regulate the ability of free IGF-I to leave the vasculature and associate with the type-1 IGF receptor (Janssen et al. 2003). Approximately, 95% of circulating IGF-I is bound in a ternary complex consisting of IGF-I, IGFBP-3, and a hepatically produced acid labile subunit (ALS) (Bang et al. 2001; Janssen et al. 2003). IGF-I bound in binary complexes with IGFBP-1, -2, -4, -5, and -6 constitute an additional 4% of circulating IGF-I, with the remainder existing as 'free' IGF-I (Bang et al. 2001). The current theory posits that this free fraction of IGF-I is able to bind to the type 1 IGF receptor and elicit biological effects (Janssen et al. 2003).

There is evidence supporting the premise that all of the IGFBPs have inhibitory roles in the regulation of IGF-I within the systemic vasculature (Lindsley and Rutter 2004; Paye and Forsten-Williams 2006). In addition, there is evidence supporting the role of many of the binding proteins in facilitatory and IGF-I-independent roles within the extracellular matrix (Hwa et al. 1999). The literature supports the fact that IGFBP-1 is primarily regulated by insulin (Frost and Lang 1999; Lee et al. 1993). The majority of the literature suggests that IGFBP-2 may be subject to dual control, with GH and amino acid availability serving as the primary regulators (Rivero et al. 1996; Smith et al. 1995). IGFBP-3 has been shown in some studies to be acutely regulated via proteolysis (Fowlkes et al. 1995; Lamson et al. 1993), but no literature to date has examined the role of exercise-induced MMP activity as being responsible for this proteolysis. There is, however, a significant amount of evidence supporting this premise in the oncology literature; and high-intensity exercise has been shown to be a potent stimulus for peripheral MMP release (Fowlkes et al. 1999; Koskinen et al. 2004).

There is ample evidence supporting the roles of the IGFBPs in regulating IGF-I bioavailability, as well as information regarding their individual regulation dynamics. However, there is a paucity of information regarding the specific integrated regulatory dynamics of IGFBPs in exercising humans. Thus, the purpose of this study was to investigate the differential contributions of IGFBP-1, -2, and -3 on IGF-I bioavailability and to develop additional insight into the individual regulation of these binding proteins in exercising humans. To accomplish this goal, we utilized a group of healthy, recreationally active college-aged males. The participants performed a high-intensity exercise protocol on three separate occasions, with each session followed by one of three nutritional interventions: one session was followed by no nutritional intervention (EX); one was followed by a carbohydrate-only (CHO) nutritional intervention; and one was followed by a mixed essential amino acid (EAA)/CHO supplement.

High-intensity exercise has consistently been shown to elicit significant elevations in post-exercise plasma concentrations of GH (Crewther et al. 2006; Laursen and Jenkins 2002) and MMPs (Rullman et al. 2007; Suhr et al. 2007). Thus, the purposes of the exercise session were twofold: (1) to examine the effect of an increase in plasma GH on [free IGF-I], and (2) to examine the possible effect of an increase in plasma MMP-9 activity on IGFBP-3 proteolysis. As IGFBP-2 has been shown to possibly be under dual control of GH and protein/amino acid availability, the purpose of the exercise session without post-exercise nutrition was to examine the role of GH regulation (in the absence of exogenous amino acid supplementation) on this binding protein. It is well understood that insulin is the primary hormone regulating IGFBP-1 (Lee et al. 1993; Nygren et al. 2001; Paye and Forsten-Williams 2006). Current theory posits that elevated insulin concentrations in the portal vasculature directly inhibit hepatic production of IGFBP-1, with a time-course of roughly 90 min (Hall et al. 1991; Lee et al. 1993). In addition, there is some evidence that elevated insulin concentrations may increase transcapillary movement of IGFBP-1 from the peripheral vasculature (Bar et al. 1990). The other exercise session was followed by mixed EAA/CHO supplementation. The purpose of this protocol was to examine the insulin-induced regulation of IGFBP-1 in combination with the proposed IGFBP-2 regulation via GH and EAA availability.

Materials and methods

Study overview

Eight males completed three identical exercise protocols followed by one of three different post-exercise nutritional supplements: (1) no supplement; (2) CHO-only supplement; and (3) EAA/CHO supplement. The participants

performed the exercise protocol and then immediately ingested the nutritional supplement. Blood samples were obtained for analysis at pre-determined intervals throughout the protocol. Exercise sessions were separated by a minimum of 48 h.

Participants

Male volunteers aged 19–35 meeting the following inclusion criteria were invited to enroll in the study: (1) equal to or above the 50th percentile for age-dependent maximal oxygen uptake (Armstrong et al. 2006); (2) healthy, as determined by Medical History Questionnaire; and (3) currently engaging in at least 3–4 days per week of moderate- to high-intensity strength and/or endurance training for 30–60 min duration (self-reported). Volunteers taking prescription medications with known effects on GH or insulin secretion, or those with known cardiovascular, pulmonary, or metabolic diseases were excluded from the study. The study was approved by the Auburn University Institutional Review Board, and all subjects granted informed consent.

Physiological assessment

Anthropometric measurements including height and weight were obtained, and body density was determined via the manner of Jackson and Pollock (1985). Participants performed a 30-s Wingate maximal anaerobic power test with resistance determined by bodyweight (7.5 g/kg). Participants also performed a graded exercise test on an electrically braked cycle ergometer (Quinton Excalibur, Quinton Instrument Company, Bothell, WA, USA). Each participant pedaled against 200 W for 5 min, 250 W for 3 min, 300 W for 3 min, and then wattage increases of 25 W per minute until volitional exhaustion. Oxygen uptake was measured with an automated metabolic testing system (True Max 2400 Metabolic Testing System, Parvo Medics, Salt Lake City, UT, USA). The highest observed oxygen uptake over three consecutive 15-s averages was considered the peak oxygen consumption (VO_{2peak}).

Experimental procedures

Participants completed three identical exercise protocols, followed by each of three post-exercise nutritional supplements in a randomized order. Participants were instructed to continue normal outside physical activity and dietary practices. On exercise testing days, participants were instructed to refrain from any exercise preceding the testing session and to arrive at the lab after a 2 h fast. Subjects were tested at the same time of day, separated by no less than 48 and no more than 72 h. Upon arrival at the lab, participants provided a urine sample for determination of hydration status. Urine specific gravity was measured utilizing a refractometer (American Optical Corp., Keene, NH, USA), and the study participants were cleared to exercise if urine specific gravity was less than 1.020 g/mL. A venous catheter was placed in a dorsal wrist vein and the participant remained seated for 15 min before the pre-trial blood draw. The participant then performed the exercise session and a post-exercise blood draw was taken. The post-exercise nutritional supplement was consumed within 5 min of the completion of the exercise protocol. Blood draws were then taken, with the participant seated, at 10 min intervals for 80 min post-exercise and at 20 min intervals until 160 min post exercise.

Nutritional supplements

Three different nutritional supplements were utilized in the study: (1) non-caloric placebo; (2) CHO-only supplement; and (3) EAA/CHO supplement. The non-caloric placebo was prepared utilizing a commercially available non-caloric flavoring, and administered in a manner consistent with the other supplements. The CHO-only supplement was prepared using sucrose mixed with water and non-caloric flavoring. It was administered at 0.85 g/kg lean bodyweight (LBW). The EAA/CHO supplement was prepared using an EAA mixture, sucrose and non-caloric flavoring. It was administered at 0.35 g/kg LBW EAA and 0.50 g/kg LBW CHO (Dreyer et al. 2007; Fujita et al. 2007). The participants were blinded to which of the supplements they were receiving.

Composition of the EAA + CHO solutions

The leucine-enriched EAA + CHO solution consisted of essential amino acids in the following proportions: L-histidine, 8%; L-isoleucine 8%; L-leucine, 35%; L-lysine, 12%; L-methionine, 3%; L-phenylalanine, 14%; L-threonine, 10%; and L-valine, 10%; and has been used in previous studies (Dreyer et al. 2007; Fujita et al. 2007). L-leucine is known to activate the mammalian target of rapamycin signaling pathway (Wu 2009) and stimulate protein synthesis in skeletal muscle (Yin et al. 2010). LBW as determined by skinfold analysis was used to calculate the proportion of each EAA (0.35 g/kg LBW) added to the nutrient solution. Similarly, carbohydrate (sucrose) was added at 0.85 g/kg LBW (CHO only) or 0.5 g/kg LBW (EAA/CHO). All ingredients were dissolved in a non-caloric, caffeine-free, flavored beverage to increase palatability.

Exercise conditions

The participants performed a HIIT protocol on an electrically braked cycle ergometer (Quinton Excalibur, Quinton Instrument Company, Bothell, WA, USA). The participants

performed a 20-min protocol, which consisted of 4 min of cycling at 15% of maximum anaerobic power (MAP) followed by 30 s at 90% of MAP. These percentages were based upon pre-trial Wingate tests. This cycle was repeated four times within each protocol, ending with 2 min at 15% MAP. It should be noted here that the cycle ergometer did not transition wattages in a square-wave fashion. Wattage increased/decreased at each power transition at a rate of 150 W per second.

Blood sampling procedures

A venous butterfly catheter (Becton–Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA, 23G x 3/4) was inserted into a dorsal wrist vein and then capped with an intermittent injection port (Kawasumi Laboratories, Inc., Tampa, FL, USA). Blood samples were drawn into 5.0 mL syringes (Becton–Dickinson & Co., Franklin Lakes, NJ, USA) with no additives. Catheter patency was maintained by injection of sodium heparin lock flush (Abbott Laboratories, North Chicago, IL, USA, 10 USP U/mL) following collection of each blood sampling time point and as needed. Whole blood samples were analyzed immediately for hematocrit, hemoglobin, and blood glucose concentrations. The remainder of the sample was allowed to clot at room temperature prior to centrifugation at 1,500×*g* for 10 min for isolation of serum. Serum aliquots were isolated in 2.0 mL ultracentrifuge tubes and stored at -70°C for subsequent analysis.

Hematocrit, hemoglobin, and determination of plasma volume shifts

Hemoglobin and hematocrit were determined immediately following each blood sample using a small portion of the whole blood sample. Hematocrit was determined in duplicate utilizing the microhematocrit method. Hemoglobin was determined utilizing an automated CO-oximeter (Instrumentation Laboratories 682, Lexington, MA, USA). Hemoglobin and hematocrit concentrations from whole blood samples were used to estimate plasma volume shifts resulting from the exercise sessions via the method of Dill and Costill (1974).

Blood glucose and leucine concentrations

Blood glucose concentrations were determined in duplicate via the glucose oxidase method using an automated hand-held analyzer (True Track Smart System, Home Diagnostics, Inc., Fort Lauderdale, FL, USA). Leucine was analyzed in duplicate utilizing high-performance liquid chromatography (Wu and Meininger 2008) and used as a representative marker for changes in serum EAA.

Growth hormone

Serum GH concentrations were determined in duplicate utilizing a coated tube immunoradiometric assay (IRMA) kit (DSL-1900, Diagnostic Systems Laboratories, Webster, TX, USA). Intra-assay CV was 4.5%, and RAM was 102.9%. Assay sensitivity was 0.01 ng/mL.

Insulin

Insulin was determined utilizing a human insulin-specific radioimmunoassay (RIA) kit (HI-14 K, Linco Research, St. Charles, MO, USA). Inter-assay CV was 4.9%, intra-assay CV was 3.3%, and RAM was 97.8%. Assay sensitivity was 2 μU/mL.

Free IGF-I

Free IGF-I concentrations were determined using an active free IGF-I enzyme-linked immunosorbent (ELISA) kit (DSL-10-9400, Diagnostic Systems Laboratories, Webster, TX, USA). Inter-assay CV was 6.3%, intra-assay CV was 4.1%, and RAM was 102%. Assay sensitivity was 0.015 ng/mL.

IGFBP-1 and -2

IGFBP-1 and -2 concentrations were determined using active IGFBP ELISA kits (DSL-10-7800 and DSL-10-7100, Diagnostic Systems Laboratories, Webster, TX, USA). Inter-assay CV was 6.3 and 4.2%, intra-assay CV was 3.1 and 2.85, and RAM was 101 and 96%. Assay sensitivity was 0.25 and 0.017 ng/mL.

MMP-9 activity

MMP-9 activity was determined using a human active MMP-9 fluorescent assay kit (F9M00, R&D Systems, Minneapolis, MN, USA). Inter-assay coefficient of variation was 7.4%, intra-assay coefficient of variation was 3.3%, and RAM was 95.7%. Assay sensitivity was 0.005 ng/mL.

IGFBP-3 proteolysis

IGFBP-3 protease activity was estimated as described by Lamson et al. (1991). IGFBP-3 (human recombinant, Upstate Biotech, 5 μg) was reacted with sodium ^{125}-I in the presence of iodogen (Pierce Chemicals, Rockford, IL, USA, 1.5 μg) for 10 min. Free radioiodine was removed using standard anion exchange chromatography (AG-2 × 8 resin, Bio-Rad, Richmond, CA, USA) and further purified to monomeric ^{125}-I-IGFBP-3 of appropriate MW

(~46 kDa) by Sephadex gel chromatography (G-75, 0.7 × 20 cm column). Serum samples were diluted 1:10 in HPLC-grade water and incubated at a volume equivalent to 10 µl plasma in a final volume of 100 µl buffer containing 50,000 CPM.

^{125}I-IGFBP-3. Buffer consisted of 10 mM TRIS with or without 5 mM calcium chloride or 50 mM EDTA or neither. Samples were incubated 36 h at 37° C in capped 1.5 ml Eppendorf tubes. A control sample was established consisting of serum to which ^{125}I-IGFBP-3 was added but not incubated for the 36 h. Calcium was added to facilitate purported calcium-dependent IGFBP-3 protease activity and EDTA was added to disrupt this activity if present. Aliquots of this matrix (1 µl serum equivalent) were separated electrophoretically by standard PAGE methodology, with the resulting proteins transferred to nitrocellulose. Radioactivity on the resolved protein bands were quantified by phosphoimaging using a Typhoon scanner. Captured images were assessed for radioactivity patterns using the Image-quant software program. Proteolytic activity against IGFBP-3 in serum would reflect a migration of radioactivity at relative molecular weights lower than the intact IGFBP-3.

Statistical analysis

This experiment was a within-subjects design with each participant serving as his own control. Group characteristics were reported as mean ± SE. Multiple three (condition) × ten (time) analysis of variance (ANOVAs) with repeated measures on both condition and time were used to compare GH, blood glucose, amino acid concentrations, insulin, MMP activity, and free IGF-I responses to the different conditions. Significant between-group differences were further explored utilizing a Bonferroni confidence interval adjustment. The a priori significance level for this study was $p < 0.05$. Data analysis was completed with the Statistical Package for the Social Sciences (SPSS, version 16.0, SPSS, Inc., Chicago, IL, USA).

Results

Baseline physiological characteristics

Ten individuals inquired about the study in response to recruiting methods. Two of the individuals were excluded based upon results of preliminary testing. In total, eight volunteers met the study inclusion criteria and agreed to participate in the study. These eight participants began and completed the entire study protocol. Participant baseline physiological characteristics are presented in Table 1.

Table 1 Baseline physiological characteristics

Variable	Mean ± SD	Minimum	Maximum
Age (yrs)	22 ± 2	19	27
Height (cm)	180.5 ± 5.5	173.1	191.2
Weight (kg)	86.0 ± 11.5	77.4	114.5
Body fat %	10 ± 3	6	16
VO_{2peak} (mL/kg/min)	41.3 ± 3.1	36.0	45.2
Wingate (Watts)	906 ± 162	726	1217

All values are presented as mean ± SD and minimum and maximum values

Table 2 Exercise Session Data

Variable	15% MAP	90% MAP
Average power (Watts)	138.4 ± 8.8	832.0 ± 53.5
% VO_{2peak}	46.5 ± 1.8	279.8 ± 11.6
HR (bpm)	145.9 ± 4.7	180.6 ± 4.4
% HR_{peak}	73.6 ± 2.2	91.2 ± 2.2
Total time (min)	18.0	2.0*

All values are presented as mean ± SD

MAP maximum anaerobic power as measured by Wingate; % VO_{2peak}, percentage of peak oxygen uptake at each intensity based on power output at VO_{2peak}; % HR_{peak}, percentage of peak heart rate; *Total time* total exercise time at each intensity for each exercise session

*Wattage increased/decreased to set value at 150 W per second at each power transition

Exercise intervention

All participants were able to complete each of the three exercise conditions. Each of the exercise sessions lasted exactly 20 min, and was identical in power settings and cycle ergometer settings for each participant. Participants completed the exercise protocol following at least a 2 h fast and ingested the post-exercise supplement within 5 min of completing the exercise protocol. The participants completed the exercise protocols within no less than 48 and no more than 72 h. Each of the three exercise protocols was identical within each of the eight participants. Percentage of VO_{2peak} was based upon each participant's power output at VO_{2peak}. Exercise intervention data are presented in Table 2.

Plasma volume shifts

Plasma volume was significantly decreased from pre- to post-exercise ($p < 0.01$), with most participants displaying non-significant post-exercise plasma volume expansion from 20 to 60 min. Thus, all blood and serum measurements were adjusted for plasma volume shifts during this time period. The average post exercise plasma volume

decrease was 12%. There were no significant differences at any time points across the three exercise conditions, so data were pooled.

Growth hormone response to exercise

Serum growth hormone concentrations increased approximately 60-fold from pre- to post-exercise ($p < 0.01$), remaining significantly elevated until 100 min post-exercise. There were no significant differences at any time across the three exercise conditions, so data were pooled. Growth hormone responses to exercise are presented in Fig. 1.

MMP-9 activity

MMP-9 activity significantly increased by 89% from pre- to post-exercise ($p < 0.01$), remaining significantly elevated until 40 min post-exercise ($p < 0.01$). MMP-9 activity again increased significantly at 140 min post-exercise ($p = 0.05$) and remained significantly elevated until 160 min post-exercise ($p = 0.02$). There were no significant differences at any time points across the three exercise conditions, so data were pooled. MMP-9 activity is presented in Fig. 2.

Blood glucose

There were no significant increases or decreases in blood glucose from pre- to post-exercise at any time. However, blood glucose concentrations in the CHO group were significantly elevated above the EAA/CHO group at 40 min post-exercise ($p < 0.01$), remaining significantly elevated until 60 min post-exercise ($p < 0.01$). In addition, blood glucose concentrations in the EX groups were significantly elevated above the CHO group at 100 min post-exercise ($p < 0.01$) and above the EAA/CHO group at 120 min post-exercise ($p < 0.01$). Blood glucose concentrations across the three conditions are presented in Fig. 3.

Insulin

Insulin concentrations for both CHO and EAA/CHO conditions increased sharply beginning at 20 min post-exercise, reaching statistical significance at 40 min ($p = 0.01$, $p = 0.03$), 60 min ($p = 0.01$, $p = 0.04$), and 80 min ($p = 0.03$, $p = 0.02$) post-exercise. There were no statistical differences at any time between the CHO and EAA/CHO conditions. Integrated total area under the curve (tAUC) analysis revealed statistically significant increases

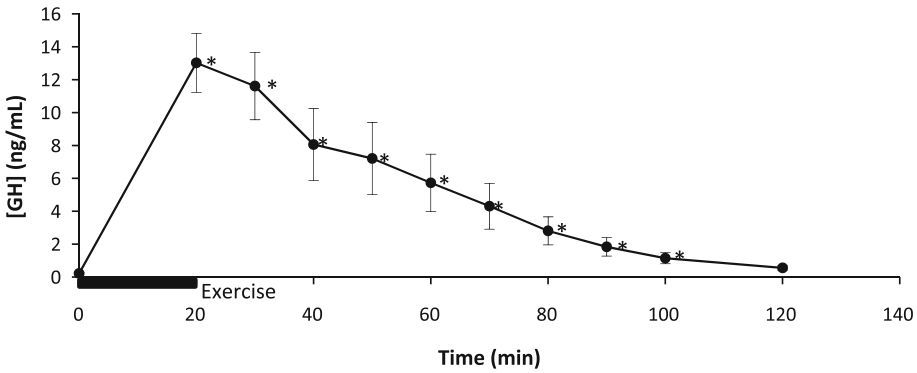

Fig. 1 Growth hormone response to exercise**.** Values are reported as mean ± SE. Means are collapsed across conditions. *Significant increase from pre-exercise value. Serum growth hormone concentrations remained significantly elevated until 100 min post-exercise

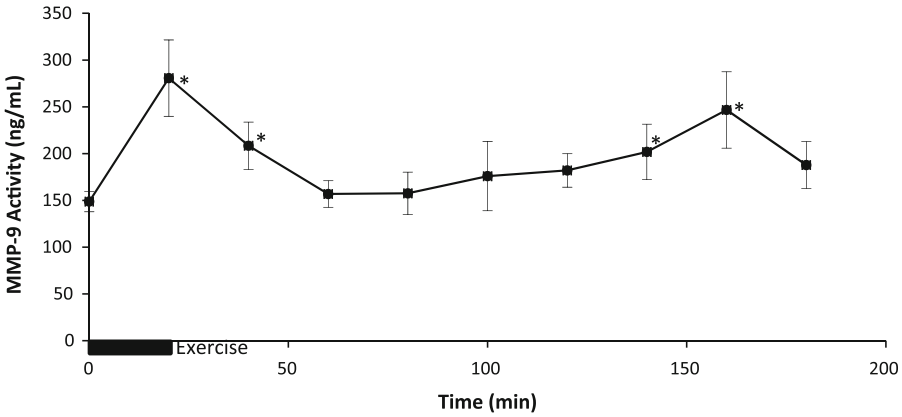

Fig. 2 MMP-9 activity response to exercise. Values are reported as mean ± SE. Means are collapsed across conditions. *Significant difference from pre-exercise value. MMP-9 activity significantly increased from pre- to post-exercise, and again at 120 min post-exercise

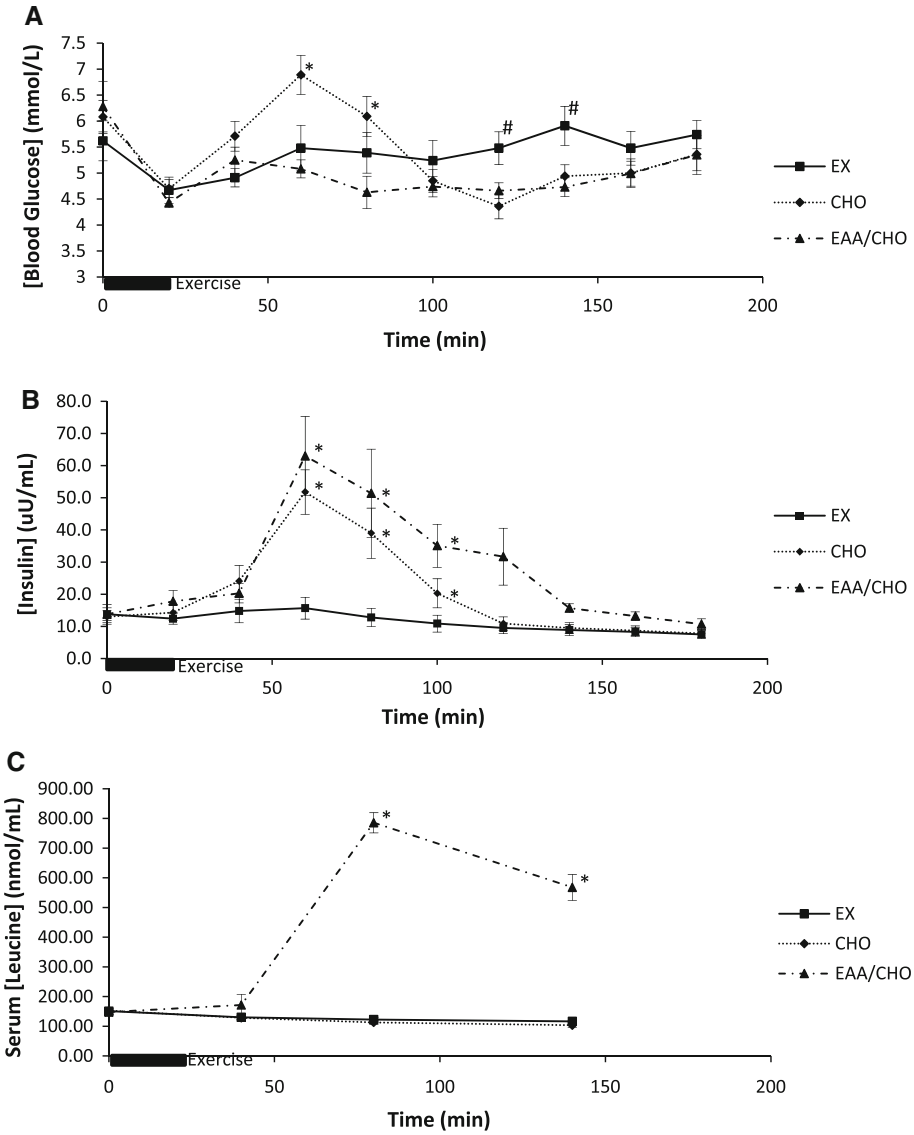

Fig. 3 Blood glucose (**a**), insulin (**b**), and serum leucine (**c**) concentrations across conditions. Values are reported as mean ± SE. **a** *Significant increase from EAA/CHO. #Significant increase from CHO. **b** *Significant increase from EX and pre-exercise value. **c** *Significant increases from EX and CHO, and significantly elevated from baseline

in both CHO and EAA/CHO ($p = 0.03$) compared to EX. Insulin concentrations by time and tAUC across the three conditions are presented in Fig. 3.

Serum leucine concentrations

Serum leucine concentrations were measured at four times: pre-exercise, 20 min post-exercise, 60 min post-exercise, and 120 min post-exercise. Leucine concentration was used as a representative marker for amino acid delivery. Serum leucine concentrations significantly increased in the EAA/CHO group only, beginning 60 min post-exercise ($p < 0.01$) and remained significantly elevated at 120 min post-exercise ($p < 0.01$). Serum leucine concentrations in the EX and CHO groups did not differ significantly from pre-exercise values at any time. Serum leucine concentrations across the three conditions are presented in Fig. 3.

Serum IGFBP-1 concentrations

Serum IGFBP-1 concentrations in all groups increased significantly from pre-exercise to 40 min post-exercise ($p = 0.04$ for EX, $p = 0.03$ for CHO, $p = 0.03$ for EAA/CHO). Following this time point, concentrations in the CHO and EAA/CHO groups trended back towards baseline values. IGFBP-1 concentrations in the EX group remained statistically elevated, relative to baseline, at both 80 and 120 min post-exercise ($p = 0.04$, $p = 0.04$). In addition, IGFBP-1 concentrations in the EX group were significantly elevated relative to both CHO and EAA/CHO conditions, at 120 min post-exercise ($p = 0.03$, $p = 0.02$). Serum

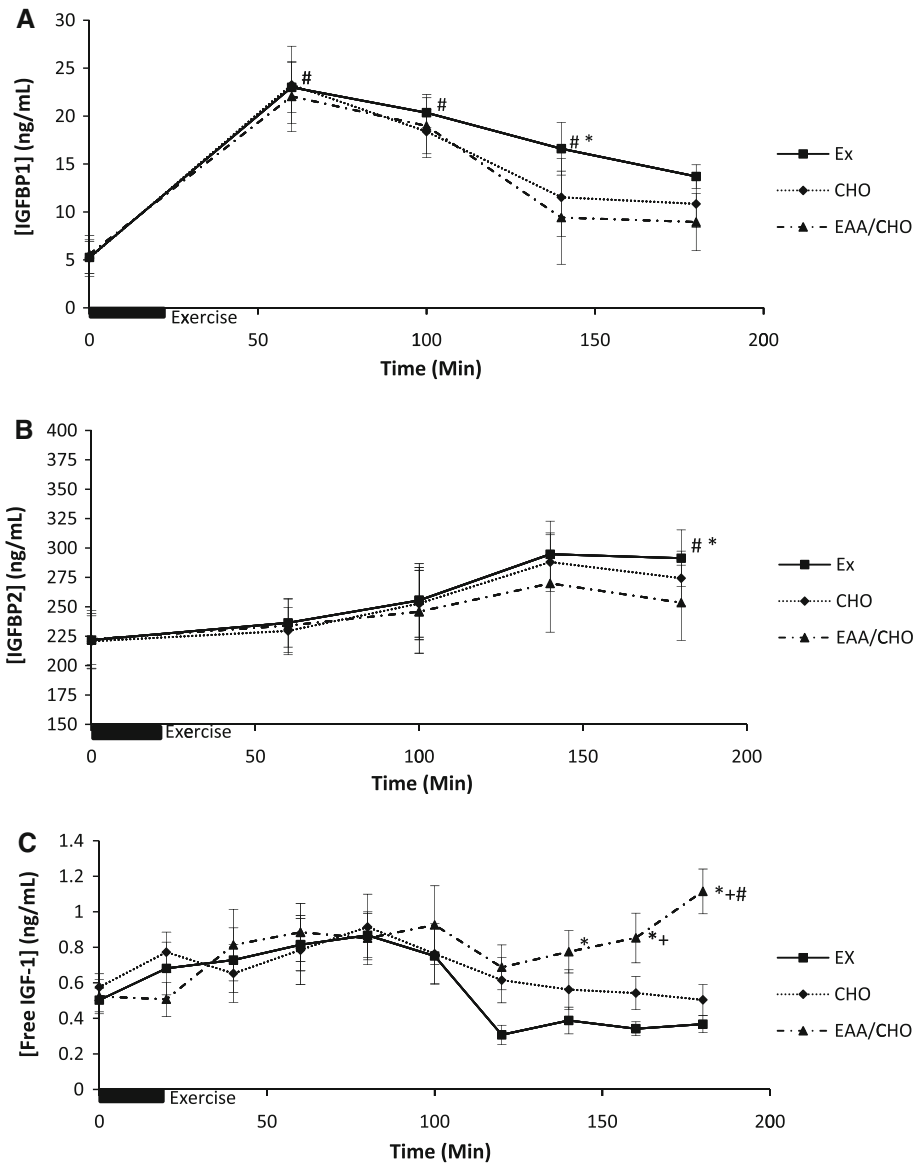

Fig. 4 Serum IGFBP-1 (**a**), IGFBP-2 (**b**), and free IGF-I (**c**) concentrations across conditions. Values are reported as mean ± SE. **a** # Significant increase from pre-exercise value. *Significant increase from CHO and EAA/CHO. **b** # Significant increase from pre-exercise value. *Significant increase from EAA/CHO. **c** # Significant increase from pre-exercise value. *Significant increase from EX. + = significant increase from EX and CHO

IGFBP-1 concentrations across the three conditions are presented in Fig. 4.

Serum IGFBP-2 concentrations

Serum IGFBP-2 concentrations in all groups displayed an increasing trend, relative to baseline, across all time points. IGFBP-2 concentrations in the EX group were significantly elevated, relative to baseline ($p = 0.04$), and were significantly elevated, relative to EAA/CHO, at 160 min post-exercise ($p = 0.04$). Serum IGFBP-2 concentrations across the three conditions are presented in Fig. 4.

Serum free IGF-I concentrations

Serum free IGF-I concentrations in the EAA/CHO condition displayed an increasing trend from pre- to post-exercise values, reaching statistical significance relative to baseline at 160 min post-exercise ($p = 0.02$). Free IGF-I concentrations in the EAA/CHO condition were significantly elevated relative to EX at 120, 140, and 160 min post-exercise ($p < 0.01$, $p < 0.01$, $p < 0.01$); and were significantly elevated relative to CHO at 140 and 160 min post-exercise ($p < 0.01$, $p < 0.01$). Serum free IGF-I concentrations across the three conditions are presented in Fig. 4.

IGFBP-3 proteolysis

IGFBP-3 proteolyis was analyzed pre-exercise and at timepoints displaying the highest MMP-9 activity. There was no evidence for exercise-induced IGFBP-3 proteolysis within this study. Radioligand blot results reflecting IGFBP-3 proteolytic activity are presented in Fig. 5.

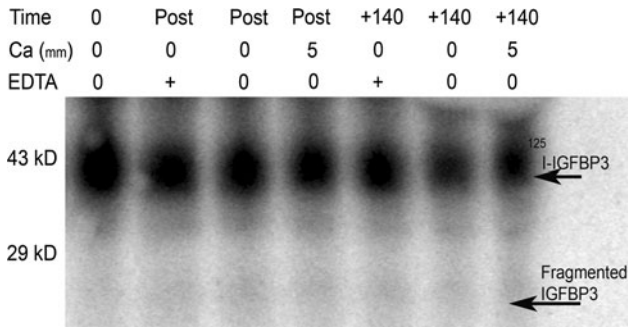

Fig. 5 IGFBP-3 Proteolysis. Western Blot demonstrating no evidence for proteolysis of ^{125}-I-IGFBP-3. Proteolysis was measure pre-exercise, immediate post-exercise, and at 140 min. It should be noted that these time points coincided with highest levels of MMP-9 proteolytic activity

Discussion

The purpose of this study was to determine the effects of macronutrient alteration following high-intensity exercise on the 'free' fraction of plasma IGF-I. In addition, this investigation sought to gain evidence regarding the regulatory dynamics of IGFBP-1, IGFBP-2 and IGFBP-3. This is one of the first studies to demonstrate two important factors regarding IGF-I regulation: (1) post-exercise macronutrient composition directly influences free IGF-I, IGFBP-1, and IGFBP-2 concentrations within the peripheral vasculature; and (2) high-intensity exercise promotes an immediate increase in plasma MMP proteolytic activity. Our data demonstrate that the inclusion of carbohydrate and EAA supplementation following high-intensity exercise promotes significant increases in the free fraction of IGF-I in the peripheral vasculature as well as alterations in IGFBP-1 and IGFBP-2 concentrations.

High intensity exercise of various modes and/or intensities consistently has been shown to elicit significant increases in plasma concentrations of GH (Kraemer et al. 1998; Laursen and Jenkins 2002) and recent evidence has shown that many types of exercise increase plasma MMP concentrations (Rullman et al. 2007; Suhr et al. 2007). This is significant because both of these factors may have implications in the acute regulation of IGF-I by their respective roles in IGFBP regulation and/or proteolysis. The exercise intervention utilized in this investigation was a HIIT protocol very similar to protocols utilized in several recent studies (Burgomaster et al. 2007; Gibala and McGee 2008). HIIT was chosen because preliminary testing revealed that it elicited substantial GH release, in combination with significant post-exercise plasma MMP activity. In recent years, this type of training has been shown to be extremely effective in inducing drastic short-term increases in both aerobic and anaerobic conditioning (Burgomaster et al. 2007; Gibala and McGee 2008). While the GH response to high-intensity exercise has been shown to be extremely robust, the GH response to HIIT is higher than most values seen in the literature for any other type of exercise (Aughey et al. 2007; Burgomaster et al. 2007; Gibala and McGee 2008). In the current investigation, participants displayed 60-fold serum GH concentration increases from pre- to post-exercise, remaining significantly elevated until 80 min post-exercise.

A large body of literature has provided evidence for increased post-exercise and/or post-trauma plasma MMP-9 concentrations (Kirman et al. 2006; Roberts et al. 2006). A growing body of evidence examining MMP activity from these perturbations has emerged, illustrating the importance of not only the quantification of MMP presence, but of MMP activation dynamics and quantification of proteolytic activity (Belizon et al. 2007; Rullman et al. 2007). Thus, the assay utilized within this investigation served to quantify endogenous MMP activity induced from the exercise intervention. The participants in this study displayed significant increases in post-exercise MMP-9 activity, remaining significantly elevated until 20 min post-exercise. These results are not surprising, as they have been seen in similar studies (Nakamura et al. 2005; Rullman et al. 2007). However, the significant increases in MMP-9 activity seen in this study at 120 and 140 min post-exercise were unexpected and belie explanation from nutritional factors, as these increases were mirrored across all conditions. There is a significant body of literature supporting proteolysis of IGFBPs by MMPs, specifically MMP-9 (Belizon et al. 2007; Miyamoto et al. 2004; Mochizuki et al. 2004; Nakamura et al. 2005). While no evidence for changes in IGFBP-3 proteolysis was demonstrated within this study, several other lines of research have shown MMPs to be directly responsible for IGFBP proteolysis (Belizon et al. 2007; Fowlkes 1997; Lamson et al. 1993). Thus, the possibility remains that MMPs are responsible for proteolysis of IGFBP-3 in a more chronic setting or for proteolysis of the other IGFBPs, though this was not explored within the context of this study.

There is a wealth of information regarding individual IGFBP regulation by nutritional (Collett-Solberg and Cohen 1996; Filho et al. 1999; Rivero et al. 1996) and stress-related factors (Fowlkes et al. 1999; Lamson et al. 1993; Munzer et al. 2006). However, no studies to date have examined the combination of exercise-induced factors combined with nutritional intervention on the acute regulation of IGF-I. Thus, this investigation attempted to integrate several bodies of known information into a dynamic setting by combining a high-intensity exercise protocol with different variations of post-exercise nutrition.

Within this investigation, post-exercise insulin spikes were achieved through delivery of CHO and EAA/CHO supplementation, achieving significant increases in insulin

concentrations for both conditions. The exercise session followed by CHO-only supplementation served to examine the role of insulin, without the inclusion of EAAs, on IGFBP-1 regulation. As mentioned previously, insulin has been shown to inhibit hepatic IGFBP-1 production with a time course of roughly 90 min (Hall et al. 1991; Lee et al. 1993). The IGFBP-1 concentrations within this study seem to follow suit with these data by following a similar time-course for inhibition, displaying decreasing concentrations following the CHO and EAA/CHO insulin peaks at approximately 50 min and reaching statistically significant decreases in the EAA/CHO group at time points coinciding with the emergence of significant elevations in [free IGF-I].

In contrast to the abundance of literature regarding IGFBP-1, there is a paucity of definitive information regarding IGFBP-2 regulation. The information that does exist suggests that IGFBP-2 is subject to the dual control of GH and protein/amino acid presence, with IGFBP-2 regulation enacted via hepatic inhibition similar to IGFBP-1 (Clemmons et al. 1991; Smith et al. 1995). The results of this study do seem to support this theory, as free IGF-I concentrations only increased in the EAA/CHO group. In addition, significant differences in IGFBP-2 concentrations between EX and EAA/CHO at 160 min post-exercise were coupled with the highest free IGF-I concentrations in the EAA/CHO group.

Several studies have examined the effects of exercise on serum total IGF-I concentrations, with most studies finding no effect (Berg and Bang 2004; Nguyen et al. 1998; Schwarz et al. 1996). However, these studies assayed total, bound IGF-I instead of free IGF-I. In the current study, a free IGF-I assay kit was utilized, yielding information reflecting the 'bioavailable' portion of the IGF-I pool. This, combined with information regarding the acute regulation of IGFBP-1, IGFBP-2, and IGFBP-3, better illustrates the acute dynamics of the IGF axis. In this context, a theoretical progression of acute IGF-I regulation could be proposed: (1) exercise induces GH release; (2) post-exercise CHO delivery induces insulin release, inhibiting hepatic IGFBP-1 production and possibly increasing clearance; and (3) post-exercise EAA delivery, in combination with exercise-induced GH release, inhibits hepatic IGFBP-2 production. Taken together, we see significantly increased serum concentrations of free IGF-I. While we failed to display evidence of changes in IGFBP-3 proteolysis in concert with increased MMP-9 activity within this study, other studies have demonstrated IGFBP proteolysis with similar exercise protocols (Lalou et al. 1996; Schwarz et al. 1996) while others have shown none (Dall et al. 2001). Regardless, it seems that hepatic regulation of IGFBPs -1 and -2 may play a more important role in acute regulation of IGF-I.

Limitations in this study include the fact that total IGF-I and IGFBP-3 concentrations were not measured. It was originally hypothesized that due to the proportionally large concentrations of IGF-1 in the ternary complex within the peripheral vasculature and the extended half-life of this complex, that these measurements would add little to an acute study such as this. In retrospect, the authors agree that it does make interpretation of these data more difficult. The authors concede that the increases in free IGF-I presented here could be resultant of an overall increase in IGF-I production, though it seems less likely due to the acute course of the study.

This is the first study to demonstrate collectively that free IGF-I, IGFBP-1, and IGFBP-2 concentrations can be manipulated through exercise and dietary alteration. While the importance of exercise and post-exercise nutrition has long been known, this investigation adds valuable insight into the mechanisms of growth factor control and regulation. The precise physiological implications of these findings are as yet unknown, as the specific role of IGF-I in the peripheral vasculature is incompletely understood at this time. Thus while the exact practical implications of these findings are unclear, their main significance lies in the fact that they draw us closer to unraveling the complex regulatory dynamics of the IGF system.

Conflict of interest statement None.

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