Macronutrient intake and whole body protein metabolism following resistance exercise

BRIAN D. ROY, JONATHON R. FOWLES, ROBERT HILL, and MARK A. TARNOPOLSKY

Departments of Kinesiology, Pathology, and Medicine, McMaster University, Hamilton, Ontario, CANADA

ABSTRACT

ROY, B. D., J. R. FOWLES, R. HILL, and M A. TARNOPOLSKY. Macronutrient intake and whole body protein metabolism following resistance exercise. *Med. Sci. Sports Exerc.,* Vol. 32, No. 8, pp. 1412–1418, 2000. The provision of carbohydrate (CHO) supplements following resistance exercise attenuated muscle protein (PRO) degradation (Roy et al. *J. Appl. Physiol*. 82:1882–1888, 1997). The addition of PRO may have a synergistic effect upon whole body protein balance by increasing synthesis (Biolo et al. *Am. J. Physiol*. 273:E122–E129, 1997). **Purpose:** To determine if the macronutrient composition of a postexercise beverage would alter muscle anabolism and/or catabolism following resistance exercise. **Methods:** We provided isoenergetic CHO (1 $g \cdot kg^{-1}$) and CHO/PRO/FAT supplements and placebo (PL) immediately (t = 0 h) and 1 h (t = +1 h) following resistance exercise (9 exercises/3 sets/80% 1 RM) to 10 young, healthy, resistance-trained males. Whole body leucine turnover was determined from L-[1-^{13}C]leucine kinetics at ~4 h postexercise. **Results:** No differences were observed for urinary 3-methylhistidine or urea nitrogen excretion between the trials. Leucine flux was significantly elevated at ~4 h postexercise for both CHO/PRO/FAT (177.59 ± 12.68 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) and CHO (156.18 ± 7.77 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) versus PL (126.32 ± 10.51 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) ($P < 0.01$). Whole body leucine oxidation was elevated at ~4 h for CHO/PRO/FAT (29.50 ± 3.34 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) versus CHO (16.32 ± 2.33 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) ($P < 0.01$) and PL (21.29 ± 2.54 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) ($P < 0.05$). Nonoxidative leucine disposal (NOLD) was significantly elevated at ~4 h for both CHO/PRO/FAT (148.09 ± 10.37 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) and CHO (139.86 ± 7.02 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) versus PL (105.03 ± 8.97 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) ($P < 0.01$). **Conclusions:** These results suggest that consumption of either CHO or CHO/PRO/FAT immediately and 1 h following a resistance training bout increased NOLD as compared with a placebo. **Key Words:** STABLE ISOTOPES, PROTEIN SYNTHESIS, PROTEIN DEGRADATION, HYPERTROPHY

Resistance exercise has been well documented to result in changes in the rate of protein turnover. Specifically, resistance exercise results in simultaneous increases in muscle protein synthesis and muscle protein degradation, resulting in a more positive, but still negative, net protein balance in individuals in the fasted state (4,23). Because muscle protein balance is determined by both muscle protein synthesis (MPS) and muscle protein degradation (MPD), influencing one or both of these factors change muscle protein balance. Changes in muscle protein balance will ultimately result in changes in muscle size.

Although the presence of insulin is not necessary for exercise induced hypertrophy to occur (13), it may favorably influence rates of protein synthesis and/or degradation. There is evidence supporting an anabolic effect of insulin on protein metabolism through a stimulation of protein synthesis and a decrease in MPD (3,22). Additional studies have observed a decreased MPD with no effect on MPS (25). Thus, it appears that insulin may have a net anabolic effect by decreasing MPD and/or increasing MPS.

In addition, it has been demonstrated that insulin can increase the cellular uptake of amino acids, co-temporal with an increase in MPS. It was concluded that insulin stimulated protein synthesis, but the increase in synthesis is self-limiting due to the decrease in available substrate (circulating amino acids) (3). Also, elevations in the circulating concentrations of amino acids both at rest and postexercise have been observed to increase MPS (2). Therefore, it is possible that insulin would be most beneficial as an anabolic agent when accompanied by an elevated circulating amino acid concentration.

There is a paucity of literature examining the role of nutritional interventions upon insulin response and muscle protein balance following exercise. The administration of a postexercise CHO or CHO/PRO supplement could result in a more positive protein balance by stimulating an increase in circulating insulin (24,33). In addition, a supplement that contained both CHO and PRO could increase PRO synthesis to a greater extent due to the exogenous supply of amino acids (2). Finally, the consumption of nutritional supplements following resistance exercise has been demonstrated to provide a hormonal environment that may be favorable for muscle growth (7).

0195-9131/00/3208-1412/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Submitted for publication August 1998.
Accepted for publication October 1999.

TABLE 1. Distribution of nutritional supplements for each trial.

	Breakfast (0800 h)	Lunch (1100 h)	Snack (1400 h)	Post-Ex
PL	CHO/PRO/FAT + B	L	S	PL
CHO	PRO/FAT + B	L	S	CHO (1 g/kg)
CHO/PRO/FAT	PL + B	L	S	CHO/PRO/FAT

* Post-Ex = supplement given at t = 0 and t = +1 h post-exercise.

The literature has not addressed the potential role of combined nutritional supplementation on protein metabolism following resistance exercise. The possible interaction of these two factors may lead to a more anabolic state due to the combined influence of the insulin (increase in PRO synthesis, decrease in PRO degradation) and the increase in PRO synthesis due to the exercise stimulus and increased amino acid availability.

Thus, the purpose of the current investigation was to determine the influence of the consumption of isoenergetic nutritional supplements of differing macronutrient composition on whole body PRO synthesis and muscle PRO degradation following resistance exercise.

METHODS

Subjects. Ten young (19–21 yr) males were recruited and screened to ensure that they were healthy and had been participating in a resistance training program for at least 2 yr before the investigation (~4 times per week) (mean yr training: 4.4 ± 0.4). They had a mean age of 19.6 ± 0.2 yr and mean percent body fat 12.4 ± 1.3%. Their habitual energy intake was 3030 ± 320 kcal, which consisted of ~50% CHO, ~18% PRO, and ~32% FAT. The experimental procedures, risks, and benefits were explained to each subject and written consent was obtained after approval by the McMaster University Human Ethics Committee.

Design. This study was a placebo controlled, double blind trial. All subjects completed each of the following conditions: a carbohydrate/protein/fat (~66%CHO, ~23%PRO, ~12%FAT) (commercially available sports drink: Mead-Johnson, Canada), a carbohydrate only (CHO) (56% sucrose, 44% glucose polymer from corn syrup solids), and a placebo (PL) (sucralose: sucrose derivative not recognized as CHO by the body) trial. All trials were separated by a minimum of 2 wk. Before the experimental trials, the subjects' one repetition maximum (1 RM) was determined for eight different exercises (see below), and body density was determined by hydrostatic weighing. In addition, subjects collected 4-d diet records, which were analyzed using a nutritional analysis software package (Nutritionist IV, First Data Bank, San Bruno, CA). From this, individual diets were designed for each subject, for both the three days before each trial (diet checklist) and for the trial day itself (prepackaged diet). The diets were isoenergetic, isonitrogenous, and flesh free. The prepackaged diet composition varied according to the postexercise drink that was administered (Table 1). The total daily energy intake remained constant for the three trials. Postexercise energy intake for CHO/PRO/FAT and CHO was the same, whereas only the macronutrient composition varied (Tables 1 and 2). The energy and macronutrients supplied by the predefined formula drinks replaced part of the habitual intake of the subjects. In addition, the amount of energy and macronutrients supplied from predefined formula drinks was the same for each trial day. This was achieved by the administration of a drink with breakfast (Table 1). Therefore, for each trial day the total energy intake was the same, as was the macronutrient composition of the diets.

TABLE 2. Post-exercise energy intake (kcal).

Subject	CHO/PRO/FAT	CHO	Placebo
1	604	603	0
2	615	620	0
3	690	683	0
4	608	604	0
5	840	832	0
6	765	763	0
7	720	720	0
8	685	680	0
9	770	780	0
10	660	657	0
Mean	696*	694*	0
SD	79	78	0

* Significantly greater than Placebo ($p < 0.01$).

The subjects refrained from any resistance exercise for 3 d and any form of exercise for 2 d before each trial. They consumed meals at ~0800 h, 1100 h, and 1400 h (Table 1) and then reported to the laboratory at ~1700 h (t = −1.25 h). A 22-gauge catheter was inserted into an antecubital vein to allow for blood sampling. A blood sample was collected and an expired gas collection was performed (t = ~−1.25 h). The subjects then performed the exercise bout under supervision. Exercise consisted of a full body circuit set workout using a Global gym multi-station training apparatus. Each workout consisted of three sets of each of the following exercises: bench press, sit-ups, knee extension, latissimus pull-downs, biceps-curls, leg press, triceps-press, military press, and an additional series of knee extensions. Subjects performed 20 sit-ups and for all other exercises three sets of ~10 repetitions were performed at ~80% of the individual's 1 RM.

During the exercise bout of the first trial, the subjects were allowed to drink water ad libitum but were required to consume exactly the same quantity for the ensuing two trials. Upon completion of the exercise (t = 0 h), a blood sample was collected and a supplement was given (CHO/PRO/FAT isoenergetic to CHO (1 g·kg^{-1})). Blood samples were then collected every 20 min for the next 2 h and 40 min. Additional blood samples were collected at 4 h, 4.25 h, and 4.5 h postexercise. A second supplement was given at the 1 h postexercise. Expired gas collections were also performed at 4 h, 4.25 h, and 4.5 h post exercise. At 2-h post exercise time point, a second catheter was inserted into a contralateral dorsal hand vein to allow "arterialized" blood sampling (hot box at 65 ± 5 °C) (21). A priming dose of L-[1-^{13}C]leucine (0.949 ± 0.004 mg·kg^{-1}) (99% atom %; C.I.L. Andover, MA) and [^{13}C]sodium bicarbonate (0.310 ± 0.003 mg·kg^{-1}) (99% atom %; Mass Trace, Woburn, MA) was administered over 1 min, followed by a

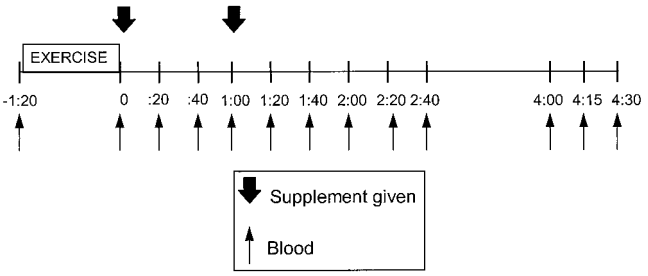

Figure 1—Study design for each testing day.

constant infusion of L-[1-^{13}C]leucine (0.999 ± 0.006 mg·kg^{-1}·h^{-1}) for ~2.5 h (Fig. 1). Under aseptic conditions, the isotopes were diluted into sterile saline immediately before infusion. Before infusion, the infusate was passed through an antibacterial filter (0.2 μm Acrodisc).

All blood samples were collected into prechilled heparinized tubes, centrifuged immediately, and the plasma stored at −50°C for later analysis. Expired gas samples were collected into a computerized closed gas analysis system and then into a 50-L meteorological balloon (CO_2 analyzer: Hewlett Packard 78356A, O_2 analyzer: Ametek S-3A/I, Pneumotachometer: Ametek R-1). Expired O_2, CO_2 concentrations, and ventilatory volumes were determined for subsequent calculations of $\dot{V}O_2$, $\dot{V}CO_2$, and RER. Duplicate samples were drawn from the collected expired gas and injected into evacuated 10 mL Vacutainer tubes (Becton Dickinson, Mississauga, ON). The subjects also collected 24-h urine samples on the day of each trial. Sample collection began the morning of the trial (first urination not collected) and continued through to the following morning (first urination collected).

Analysis

Breath analysis. Within 72 h of collection, the CO_2 of the expired gas samples was cryogenically extracted and sealed in a glass tube for later determination of the $^{13}CO_2/^{12}CO_2$ ratio by gas-isotope ratio mass spectrometry. The CO_2 was separated from the collected breath samples by a series of freeze/thaws in liquid nitrogen and methanol/dry ice slush. It was then condensed into a glass collection tube, which was then sealed by melting the glass. On a later date, the isotopic enrichment of the CO_2 samples was determined by gas-isotope ratio mass spectrometry (VG Isogas, SIRA 10, Cheshire, England). Values are expressed atom percent excess (APE) relative to the baseline samples.

Plasma analysis. Plasma alpha-ketoisocaproic acid ^{13}C enrichment was determined by the method described by Wolf (31) and adapted by Tarnopolsky et al. (27). Briefly, the proteins in 500 mL of plasma were precipitated by the addition of absolute ethanol (5 mL × 2) and then centrifuged (10 min @ 10,000 rpm at 4°C.). The supernatant was evaporated under dry N_2 at 50°C and then resuspended in 1 mL of H_2O; 1 mL of 2% o-phenylenediamine solution was added (2% in 4 N HCl), after which the solution was heated at 100°C for 1 h and then cooled. The derivative was then extracted with 2.5 mL of methylene chloride and centrifuged (2–3 min @ 3000 rpm). The lower layer was removed and placed in a clean tube and the procedure was repeated. The lower layer was then transferred to a screw top tube and evaporated under a stream of dry N_2. Finally, 75 μL of N, O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS) was added, and then the solution was heated at 100°C for 30 min.

The α-KIC enrichment was determined using GC/MS (Hewlett Packard Model 5890 GC; VG-Trio-2 quadrupole mass spectrometer (VG, Cheshire, England)). Approximately 0.3 μL of derivatized plasma was injected directly onto a 30-m fused silica capillary column (DB5 J. W. Scientific, Rancho Verda, CA) with helium as the carrier gas (32 cm·s^{-1}). The GC oven was initially set at 120°C, then increased to 160°C at 8°C·min^{-1}, and then increased to 300°C at a rate of 20°C·min^{-1} and held at 300°C for 3 min to drive off later eluting compounds. Ionization was achieved through electron impact (70 eV, trap current 170, source current 1.6 mA, ion source temp. 200°C). The enrichments of the 233.1/232.1-amu species (enriched/unenriched) were monitored by scanning over a narrow scan range (228–238 amu), and the listing of the mass spectra acquired at the apex of the chromatographic peak was used to determine sample enrichment.

24-h urine sample analysis. The volume of each 24 h collection was determined and three aliquots of urine were taken and frozen at −50°C for later analysis. Urea nitrogen and creatinine were calculated from the 24 h urine collections by colorimetric methods (Sigma Diagnostics, kit nos. 640 and 555, respectively, St. Louis, MO) with intra-assay CV of 2.9% and 1.5%, respectively. Urinary 3-methylhistidine (3-MH) concentration was determined by using an automated amino acid analyzer and was normalized to the 24-h urinary creatinine excretion (Beckman Instruments, Palo Alto, CA).

Calculations

Leucine flux (Q) was calculated using the reciprocal pool model (from [^{13}C] α-KIC values) (15), at isotopic plateau:

$$Q = i(E_i/E_p - 1)$$

where I = L-[13C]leucine infusion rate (μmol·kg^{-1}·h^{-1}), E_I = enrichment of the infused leucine (% ^{13}C), and E_p = enrichment of the plasma α-KIC (APE), and the term "−1" corrects for the contribution of the infused isotope to Q.

Leucine oxidation was calculated from the equation (32)

$$\text{Total leucine oxidation} = [(IECO_2/c)/IE_{\alpha\text{-KIC}}]\cdot VCO_2$$

where $IECO_2$ = enrichment of expired CO_2 (APE), $IE_{\alpha\text{-KIC}}$ = enrichment of plasma α-KIC (APE), $\dot{V}CO_2$ = volume of carbon dioxide evolved (μmol·kg^{-1}·min^{-1}), and c = bicarbonate retention factor. Because exercise is known to affect the retention of CO_2 in the body (31), the bicarbonate retention factor was determined in two subjects 1 wk before the experiment using an identical experimental design with a primed-continuous infusion of [^{13}C]sodium bicarbonate as described by Kien (17):

$$c = VCO_2\cdot(\text{IE bicarbonate } CO_2\cdot F^{-1})$$

where $\dot{V}CO_2$ = volume of carbon dioxide evolved (μmol·kg^{-1}·min^{-1}), IE bicarbonate CO_2 = isotopic enrichment of expired CO_2 at plateau (corrected for background CO_2 enrichment), and F = the infusion rate of the [^{13}C]sodium bicarbonate (μmol·kg^{-1}·min^{-1}). The mean c determined for the two subjects was 0.84, which was similar to what has been previously observed in our lab (27). The individual c was used for each of the two studied subjects and the mean of these values was applied to the other eight subjects in the calculation of leucine oxidation. The influence of the consumption of each of the different supplements and the exercise protocol on background changes in CO_2 enrichment was studied under identical conditions with no isotope infusion, before the study, using techniques previously described (27). The observed changes were used to further correct the leucine oxidation values.

Nonoxidative leucine disposal was used to estimate whole body protein synthesis (19,20,27) according to the following model;

$$Q = S + O$$

where Q = leucine flux, S = synthesis, and O = oxidation (19).

Statistical Analysis

All blood measures were analyzed using repeated measures analysis of variance (time × treatment) (Statistica, V. 5.0, StatSoft, Inc., Tulsa, OK). Urine data was analyzed using a one way repeated measures ANOVA (Statistica, V. 5.0, StatSoft Inc. Tulsa, OK). When a significant interaction occurred, Tukey's *post hoc* analysis was used to locate the pairwise differences. $P < 0.05$ was selected as being indicative of statistical significance. Values are expressed as means ± SE.

RESULTS

Plasma glucose, insulin, and lactate data have been previously reported elsewhere (24).

Twenty-four-hour urinary creatinine excretion was not significantly different between the three conditions (CHO/PRO/FAT: 1.82 ± 0.09 g·24 h^{-1}, CHO: 1.77 ± 0.12 g·24 h^{-1}, PL: 1.62 ± 0.15 g·24 h^{-1}). Because these values were not significantly different, the remainder of the urinary values were expressed relative to the creatinine values; 3-methylhistidine excretion was not significantly different between the three conditions (Fig. 2A) (CHO/PRO/FAT: 84.2 ± 10.4 μmol·g creat^{-1}, CHO: 86.9 ± 8.1 μmol·g creat^{-1}, PL: 95.4 ± 7.8 μmol·g creat^{-1}). Similarly, no differences were observed in urinary urea nitrogen excretion for the three conditions (Fig. 2B) (CHO/PRO/FAT: 7.27 ± 0.36 g·g creat^{-1}, CHO: 6.93 ± 0.39 g·g creat^{-1}, PL: 6.68 ± 0.77 g·g creat^{-1}).

No significant differences over time were observed for ^{13}C-breath enrichment (Fig. 3A) and α-KIC enrichment (Fig. 3B) at 4, 4.25, and 4.5 h postexercise, indicative of an isotopic steady state. Leucine flux (Fig. 4A) was significantly elevated at ~4 h (mean of 4 and 4.25 h) postexercise for both CHO/PRO/FAT (177.59 ± 12.68 μmol·kg^{-1}·h^{-1}) and CHO (156.18 ± 7.77 μmol·kg^{-1}·h^{-1}) versus PL

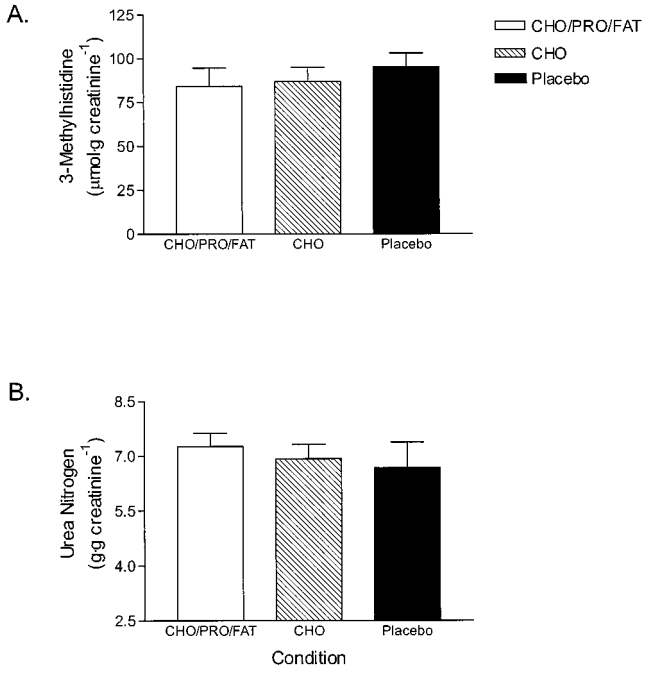

Figure 2—A, 24-h urinary 3-methylhistidine; B, urea nitrogen excretion for CHO/PRO/FAT (*solid bars*), CHO (*open bars*), and PL (*hatched bars*). Values are means ± SE; $N = 10$.

(126.32 ± 10.51 μmol·kg^{-1}·h^{-1}) ($P < 0.01$). Whole body leucine oxidation (Fig. 4B) was elevated at ~4 h for the CHO/PRO/FAT condition (29.50 ± 3.34 μmol·kg^{-1}·h^{-1}) versus both the CHO (16.32 ± 2.33 μmol·kg^{-1}·h^{-1}) and PL (21.29 ± 2.54 μmol·kg^{-1}·h^{-1}) conditions ($P < 0.05$). Nonoxidative leucine disposal (NOLD) (Fig. 4C) was significantly elevated at ~4 h for both the CHO/PRO/FAT

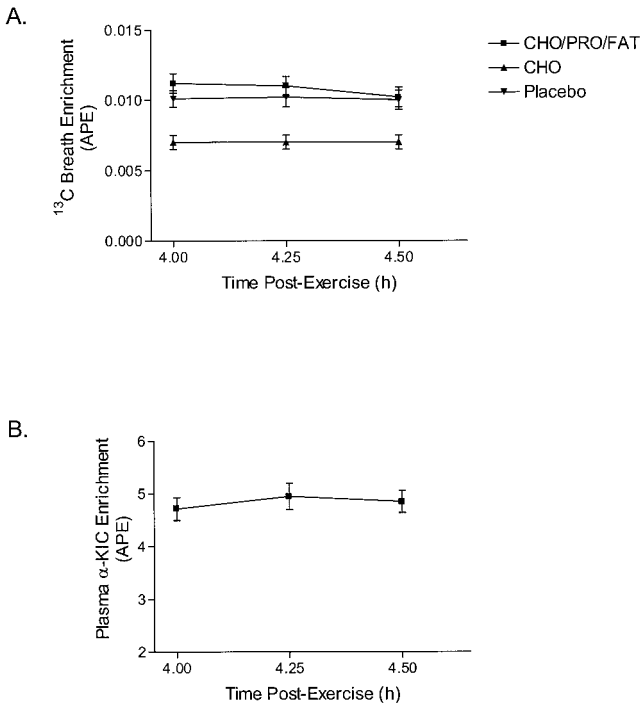

Figure 3—A, ^{13}C breath enrichment for CHO/PRO/FAT (*squares*), CHO (*triangles*), and PL (*inverted triangles*); B, mean plasma α-KIC enrichment for the three conditions. Values are means ± SE; $N = 10$.

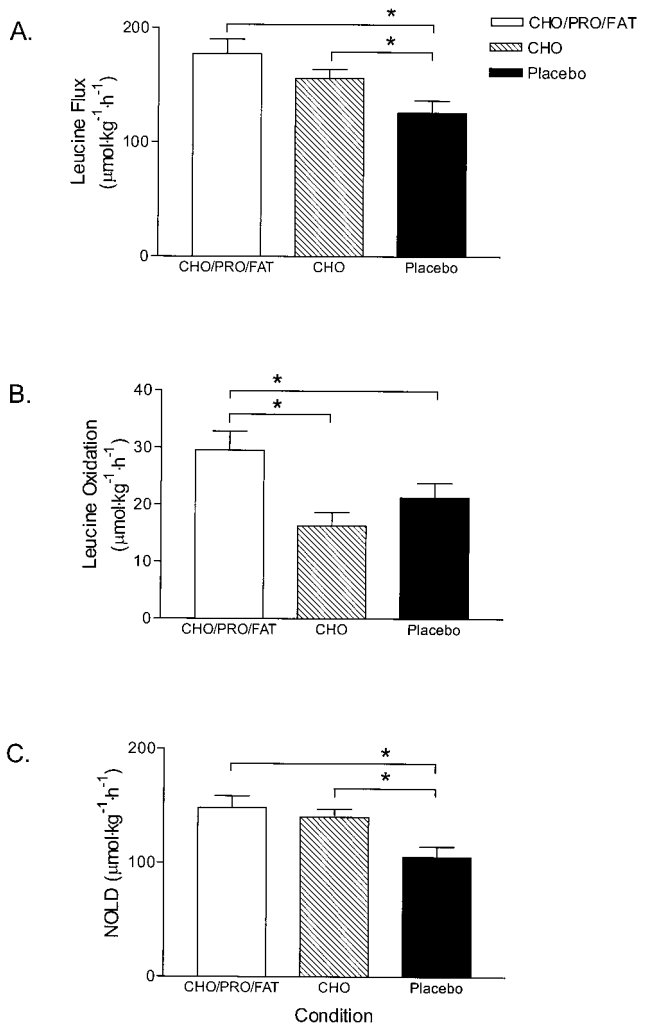

Figure 4—A, Leucine flux; B, leucine oxidation; C, nonoxidative leucine disposal for CHO/PRO/FAT (*solid bars*), CHO (*open bars*), and PL (*hatched bars*). Values are means ± SE; $N = 10$, $*P < 0.05$ between conditions.

(148.09 ± 10.37 μmol·kg^{-1}·h^{-1}) and CHO (139.86 ± 10.37 μmol·kg^{-1}·h^{-1}) conditions versus the PL (105.03 ± 8.97 μmol·kg^{-1}·h^{-1}) condition ($P < 0.01$).

DISCUSSION

The purpose of this investigation was to determine the influence of the consumption of different nutritional supplements on whole body PRO synthesis and muscle PRO degradation following resistance exercise. As previously demonstrated (25), a CHO supplement (1 mg·kg^{-1}, given at 0 h and +1 h postexercise) resulted in significantly increased plasma glucose and insulin (24). In the current study, a supplement of mixed composition (~66%CHO, ~23%PRO, ~12%FAT), CHO/PRO/FAT, led to similar increases in both plasma glucose and insulin (24,25), and we report here that this lead to a significant elevation in NOLD as compared with the PL condition.

Much of the work in the area of the effect of insulin and amino acids on protein turnover have involved the use of insulin and amino acid infusions (1,3,10). Limited work has involved the oral consumption of CHO (25) or amino acids (29) following resistance exercise. The current study is the first report in humans comparing the effect of an oral consumption of a mixed nutritional supplement (CHO/PRO/FAT), a CHO only supplement, and a placebo (PL) upon protein metabolism.

Consumption of both the CHO/PRO/FAT and CHO supplements led to significant increase in NOLD as compared with the PL condition following resistance exercise. NOLD was ~41% and ~33% greater for the CHO/PRO/FAT and CHO conditions as compared with the PL condition respectively. NOLD is considered to provide a directional representation of whole body PRO synthesis (WBPS) (19,20,27). It is unclear whether the exercise protocol *per se* resulted in an increase in WBPS, as this has been previously observed following an isolated bout of endurance exercise (9). However, in the fed state, no increase in WBPS was observed for up to 2 h following a resistance exercise protocol that was similar to the current study (27). Therefore, it is unlikely that the exercise protocol alone resulted in an increase in WBPS, however, postexercise consumption of either the CHO/PRO/FAT or CHO resulted in significant increases in WBPS as compared with a PL. These findings are consistent with reports in the literature that hyperaminoacidemia, and hyperinsulinemia in combination with hyperaminoacidemia significantly increased WBPS (6,28). In addition, the plasma insulin response associated with both the CHO/PRO/FAT and CHO conditions may have contributed to an increase in WBPS. Hyperinsulinemia has been observed to increase amino acid uptake and stimulate muscle protein synthesis (3). Data from the current study suggests that the increase in insulin may be more important with respect to the increase in NOLD, rather than the increase in circulating amino acids. The similar increase in NOLD during the two conditions supports this possibility. We cannot quantitate the contribution of splanchnic protein synthesis or MPS to WBPS using the current methods. However, in a similar previous study we demonstrated a trend toward greater MPS (36% nonsignificant increase) and a lower MPD ($P < 0.05$) for postexercise CHO versus PL supplement (25). Future studies should explain the role of combined supplements upon postexercise MPD and MPS using more direct measurements (i.e., catheterization across muscle and splanchnic beds) (23).

Leucine oxidation was significantly increased ~4 h postexercise during the CHO/PRO/FAT condition as compared with both the CHO and PL conditions. The increased oxidation in this condition likely resulted from the increased availability of amino acids from the PRO source within the drink consumed. Others have observed that with an increased availability of amino acids there is an increase in amino acid oxidation (6,28). We can only assume that there was an increase in the availability of amino acids during the CHO/PRO/FAT condition because no measures of amino acid concentrations were made. Therefore, it appears the provision of amino acids post exercise result in an increase in the amount of leucine oxidation 4 h following the completion of the exercise. There was a trend for the CHO

condition to have a suppression of leucine oxidation. There is evidence that the consumption of CHO may result in the suppression of branch-chain keto acid dehydrogenase (BCKAD) (30). Kasperek and Snider (16) have provided further support for the energy dependent nature of BCKAD. Therefore, a potential mechanism for the role of CHO consumption in the suppression of amino acid oxidation would be the suppression of BCKAD activity.

Consumption of the different nutritional supplements had no significant effect on urinary 3-MH or urea nitrogen excretion. This is not consistent with what has been previously observed with the consumption of a CHO supplement following a bout of resistance exercise (25). The current study involved similar dietary controls (isoenergetic/isonitrogenous/flesh free) and a similar repeated measures design as the earlier study. Such controls are necessary for valid interpretation of changes in 3-MH excretion (5,18,25). It should be noted that the relative differences in 3-MH excretion were similar between the two studies but the intersubject variability was slightly greater in the current study which may have contributed to a type II error. The directional change in 3-MH excretion (lower with supplements) was not statistically significant; however, this change may have clinical relevance, which requires further investigation (23). Furthermore, the absolute level of 3-MH excretion was slightly lower in the current study, even though the total muscle mass used during the exercise was much greater. Because the subjects in the current study were more highly trained than the earlier study (25), this suggests that training status may influence the amount of myofibrillar damage that occurs with an isolated bout of resistance exercise.

It is well documented that previous myofibrillar damage, such as that caused by resistance exercise, causes the muscle to become more resilient to future damage (8). We have observed significantly less myofibrillar damage in highly trained subjects (12) as compared with untrained individuals following a bout of resistance exercise (11). Therefore, the trend toward a decrease in urinary 3-MH excretion during both the CHO/PRO/FAT and CHO conditions as compared with the PL condition may have reached significance if less highly trained subjects had been used. These observations may have clinical relevance since most patients undergoing therapeutic exercise are most often in a relatively untrained state. Therefore, it is possible that nutritional supplementation following exercise in this population may be advantageous by improving net muscle protein balance. Future studies should also determine the effect of nutritional supplements upon muscle fractional breakdown rate (FBR) (23).

Urea nitrogen excretion remained unchanged between the three conditions in the current study. Assuming that sweat and fecal loss of nitrogen was similar for the three conditions, this suggests that whole body PRO turnover was also similar. These findings are consistent with earlier findings within the literature (14) but differ from previous findings in our lab (25). It should be noted that the previous findings from our lab are from a less highly trained group of subjects that performed a different exercise protocol over the collection day. We previously observed that total daily urinary urea nitrogen excretion was decreased with the consumption of a CHO supplement (total 2 $g \cdot kg^{-1}$) following an isolated bout of resistance exercise (25); however, this was not observed in the current study with either the CHO/PRO/FAT or CHO conditions as compared with the PL. The average urinary urea excretion in the current study was lower for all conditions as compared with earlier work in less trained individuals (25). It has been demonstrated that individuals who are initiating a resistance training program require a greater daily intake of PRO as compared with elite strength athletes in order to maintain a positive nitrogen balance (26). Therefore, it is possible that individuals who are initiating a resistance-training program are turning over more PRO. This increased rate of PRO turnover may account for the greater amount of urea nitrogen excretion in our previous study (25). The decreased urea nitrogen excretion observed in the current study further supports the possibility of decreased rates of PRO turnover in highly trained resistance athletes. Therefore, the data from the current study suggests that consumption of either a CHO/PRO/FAT or a CHO supplement following a bout of resistance exercise does not influence urinary urea nitrogen excretion in highly trained resistance athletes.

In summary, our results indicate that consumption of a 1 $g \cdot kg^{-1}$ CHO supplement or a CHO/PRO/FAT supplement (isoenergetic to CHO) immediately and 1 h following completion of a resistance training bout significantly increased NOLD ~4 h after the completion of the exercise. In addition, the same supplements do not appear to decrease myofibrillar PRO degradation as indicated by 3-MH and urea nitrogen excretion in highly trained resistance athletes. However, it appears that an acute bout of resistance exercise in highly trained strength athletes does not increase 3-MH and urea nitrogen excretion to the same extent as observed in untrained individuals. This suggests that the disruptive effect of resistance exercise on myofibrillar PRO may not be as great in highly trained resistance athletes compared with untrained individuals. Finally, consumption of the CHO/PRO/FAT post exercise increased the total leucine oxidation measured at ~4 h postexercise. These findings suggest that nutritional supplementation following exercise may benefit muscle protein balance, which could be beneficial in the rate of lean muscle mass accretion in individuals undergoing therapeutic exercise.

This study was financially supported by a grant from Mead-Johnson, Canada. The authors wish to thank Joan Martin, Scott MacKenzie, and Dan McLennan for their technical assistance and Drs. S. Atkinson, D. MacDougall, and D. Sale for their comments on the manuscript.

Address for correspondence: Dr. M. A. Tarnopolsky, Dept. of Neurology, 4U4, McMaster University Medical Centre, 1200 Main St. W., Hamilton, ON, L8N 3Z5 Canada. E-mail: tarnopol@mcmaster.ca.

REFERENCES

1. Bennet, W. M., A. A. Connacher, C. M. Scrimgeour, R. T. Jung, and M. J. Rennie. Euglycemic hyperinsulinemia augments amino acid uptake by human leg tissues during hyperaminoacidemia. *Am. J. Physiol.* 259:E185–E194, 1990.
2. Biolo, G., K. D. Tipton, S. Klein, and R. R. Wolfe. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am. J. Physiol.* 273:E122–E129, 1997.
3. Biolo, G., R. Y. D. Flemming, and R. R. Wolfe. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J. Clin. Invest.* 95:811–819, 1995.
4. Biolo, G., S. P. Maggi, B. D. Williams, K. D. Tipton, and R. R. Wolfe. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am. J. Physiol.* 268:E514–E520, 1995.
5. Carraro, F., C. S. Stuart, W. H. Hartl, J. Rosenblatt, and R. R. Wolf. Effect of exercise and recovery on muscle protein synthesis in human subjects. *Am. J. Physiol.* 259:E470–E476, 1990.
6. Castellino, P., L. Luzi, D. C. Simpson, M. Haymond, and R. A. Defronzo. Effects of insulin and plasma amino acid concentrations on leucine metabolism in man. *J. Clin. Invest*. Vol. 80:1784–1793, 1987.
7. Chandler, R. M., H. K. Byrne, J. G. Patterson, and J. L. Ivy. Dietary supplements affect the anabolic hormones after-weight training exercise. *J. Appl. Physiol.* 76:839–845, 1994.
8. Clarkson, P. M., K. Nosaka, and B. Braun. Muscle function after exercise-induced muscle damage and rapid adaptation. *Med. Sci. Sport. Exerc.* 24:512–520, 1992.
9. Devlin, J. T., I. Brodsky, A. Scrimgeour, S. Fuller, and D. M. Bier. Amino acid metabolism after intense exercise. *Am. J. Physiol.* 258:E249–E255, 1990.
10. Flakoll, P. J., M. Kulaylat, M. Frexes-Steed, et al. Amino acids augment insulin's suppression of whole body proteolysis. *Am. J. Physiol.* 257:E839–E847, 1989.
11. Gibala, M. J., J. D. Macdougall, M. A. Tarnopolsky, W. T. Stauber, and A. Elorriaga. Changes in human skeletal muscle ultrastructure and force production after acute resistance exercise. *J. Appl. Physiol.* 78:702–708, 1995.
12. Gibala, M. J., S. A. Interisano, M. A. Tarnopolsky, B. D. Roy, and J. D. Macdougall. Myofibrillar disruption following acute resistance exercise in strength trained athletes. *Can. J. Appl. Physiol*. Vol. 20(Suppl.):16P, 1995.
13. Goldberg, A. L. Influence of insulin and contractile activity on muscle size and protein balance. *Diabetes* 28(Suppl. 1):18–24 1979.
14. Hickson, J. F., I. Wolinsky, G. P. Rodriguez, J. M. Pivarnik, M. C. Kent, and N. W. Shier. Failure of weight training to affect urinary indices of protein metabolism in men. *Med. Sci. Sport. Exerc*. 18:563–567, 1986.
15. Horber, F. F., C. M. Harber-Feyder, S. Krayer, W. F. Schwenk, and M. W. Hamond. Plasma reciprocal pool specific activity predicts that of intracellular free leucine for protein synthesis. *Am. J. Physiol.* 257:E385–E399, 1989.
16. Kasperek, G. J., and R. D. Snider. Effect of exercise intensity and starvation on activation of branched-chain keto acid dehydrogenase by exercise. *Am. J. Physiol.* 252:E33–E37, 1987.
17. Kien, C. L. Isotopic dilution of CO_2 as an estimate of CO_2 production during substrate oxidation studies. *Am. J. Physiol.* 257:E296–E298, 1989.
18. Lukaski, H. C., J. Mendez, E. R. Buskirk, and S. H. Cohn. Relationship between endogenous 3-methylhistidine excretion and body composition. *Am. J. Physiol.* 240:E302–E307, 1981.
19. Matthews, D. E., K. J. Motil, D. K. Rohrbaugh, J. F. Burke, V. R. Young, and D. M. Bier. Measurement of leucine metabolism from a primed continuous infusion of L-[1–13C]leucine. *Am. J. Physiol.* 238:E473–E479, 1980.
20. Nair, K. S., D. Halliday, and R. C. Riggs. Leucine incorporation into mixed skeletal muscle protein in humans. *Am. J. Physiol.* 254:E208–E213, 1988.
21. Nauck, M. A., H. Lieb, E. G. Siegel, P. D. Niedmann, and W. Creutzfeldt. Critical evaluation of the "heated-hand-technique" for obtaining "arterialized" venous blood: incomplete arterialization and alterations in glucagon responses. *Clin. Physiol.* 12:537–552, 1992.
22. Newman, E., M. J. Heslin, R. F. Wolf, P. W. T. Pisters, and M. F. Brennan. The effect of systemic hyperinsulinemia with concomitant amino acid infusion on skeletal muscle protein turnover in the human forearm. *Metabolism* 43:70–78, 1994.
23. Phillips, S. M., K. D. Tipton, A. Aarsland, S. E. Wolfe, and R. R. Wolfe. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am. J. Physiol.* 273:E99–E107, 1997.
24. Roy, B. D., and M. A. Tarnopolsky. Influence of differing macronutrient intakes on muscle glycogen resynthesis after resistance exercise. *J. Appl. Physiol.* 84:890–896, 1998.
25. Roy, B. D., M. A. Tarnopolsky, J. D. Macdougall, J. Fowles, and K. E. Yarasheski. Effects of glucose supplement timing on protein metabolism after resistance exercise. *J. Appl. Physiol.* 82:1882–1888, 1997.
26. Tarnopolsky, M. A., S. A. Atkinson, J. D. Macdougall, A. Chesley, S. Phillips, and H. P. Schwarcz. Evaluation of protein requirements for trained strength athletes. *J. Appl. Physiol.* 73: 1986–1995, 1992.
27. Tarnopolsky, M. A., S. A. Atkinson, J. D. Macdougall, B. B. Senor, P. W. R. Lemon, and H. Schwarcz. Whole body leucine metabolism during and after resistance exercise in fed humans. *Med. Sci. Sports. Exerc.* 23:326–333, 1991.
28. Tessari, P., S. Inchiostro, G. Biolo, et al. Differential effects of hyperinsulinemia and hyperaminoacidemia on leucine-carbon metabolism in vivo. *J. Clin. Invest.* 79:1062–1069, 1987.
29. Tipton, K. D., A. A. Ferrando, S. M. Phillips, D. J. Doyle, J. Cortiella, and R. R. Wolfe. Muscle protein metabolism during ingestion of essential amino acids after resistance exercise. *Med. Sci. Sport. Exerc.* . 29(Suppl.):S295, 1997.
30. Vazquez, J. A., H. S. Paul, and S. A. Adibi. Regulation of leucine catabolism by caloric sources. *J. Clin. Invest.* 82:1606–1613, 1988.
31. Wolf, R. R. *Radioactive and Stable Isotope Tracers in Biomedicine: Principals and Practice of Kinetic Analysis.* Toronto: Wiley-Liss, 1992, pp. 471.
32. Wolfe, R. R., R. D. Goodenough, M. H. Wolfe, G. T. Royle, and E. R. Nadel. Isotopic analysis of leucine and urea metabolism in exercising humans. *J. Appl. Physiol.* 52:458–466, 1982.
33. Zawadzki, K. M., B. B. Yaspelkis, and J. L. Ivy. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. *J. Appl. Physiol.* 72:1854–1859, 1992.