Postexercise protein intake enhances whole-body and leg protein accretion in humans

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ABSTRACT

LEVENHAGEN, D. K., C. CARR, M. G. CARLSON, D. J. MARON, M. J. BOREL, and P. J. FLAKOLL. Postexercise protein intake enhances whole-body and leg protein accretion in humans. *Med. Sci. Sports Exerc.*, Vol. 34, No. 5, pp. 828—837, 2002. **Purpose:** Exercise increases the use of amino acids for glucose production and stimulates the oxidation of amino acids and other substrates to provide ATP for muscular contraction, and thus the availability of amino acids and energy for postexercise muscle protein synthesis may be limiting. The purpose of this study was to determine the potential of postexercise nutrient intake to enhance the recovery of whole-body and skeletal muscle protein homeostasis in humans. **Methods:** Primed-continuous infusions of L-[1-^{13}C]leucine and L-[ring-$^{2}H_5$]phenylalanine were initiated in the antecubital vein and blood was sampled from a femoral vein and a heated (arterialized) hand vein. Each study consisted of a 30-min basal, a 60-min exercise (bicycle at 60% $\dot{V}O_{2max}$), and a 180-min recovery period. Five men and five women were studied three times with an oral supplement administered immediately following exercise in random order: NO = 0, 0, 0; SUPP = 0, 8, 3; or SUPP+PRO = 10, 8, 3 g of protein, carbohydrate, and lipid, respectively. **Results:** Compared to NO, SUPP did not alter leg or whole-body protein homeostasis during the recovery period. In contrast, SUPP+PRO increased plasma essential amino acids 33%, leg fractional extraction of phenylalanine 4-fold, leg uptake of glucose 3.5-fold, and leg and whole-body protein synthesis 6-fold and 15%, respectively. Whereas postexercise intake of either NO or SUPP resulted in a net leg release of essential amino acids and net loss of whole-body and leg protein, SUPP+PRO resulted in a net leg uptake of essential amino acids and net whole-body and leg protein gain. **Conclusions:** These findings suggest that the availability of amino acids is more important than the availability of energy for postexercise repair and synthesis of muscle proteins. **Key Words:** WHOLE-BODY PROTEIN HOMEOSTASIS, LEG PROTEIN HOMEOSTASIS, AMINO ACIDS, PROTEIN SYNTHESIS, PROTEIN BREAKDOWN, MUSCLE GROWTH, DEPOSITION

During exercise, metabolic processes are focused toward the support of muscle contraction at the expense of events not involved in muscle movement. Modifications in neural activity, shifts in blood flow, alterations in subcellular events, and changes in the activities of hormonal mediators, growth factors, and cytokines are important in diverting energy to support muscle contraction. The tremendous energy required by muscle contraction leaves less energy available for protein synthesis within the muscle. Furthermore, there is evidence that amino acid oxidation, in particular skeletal muscle branched-chain amino acid oxidation (36), and conversion of gluconeogenic amino acids to glucose (43) both increase during exercise, thereby resulting in a reduced availability of amino acids for protein synthesis. The increased diversion of energy and amino acids toward events of muscle contraction leads to the hypothesis that the availability of substrates may limit postexercise muscle protein repair and synthesis.

Exercise promotes the breakdown of glycogen, providing a readily available source of energy for the muscle. The extent of glycogen depletion is dependent upon the duration and intensity of the exercise performed (9,15,22,23,39). The mechanical events of exercise also result in skeletal muscle structural and membrane protein damage (3,14,25), which translates into an increased need for postexercise repair and synthesis of skeletal muscle proteins. Although maximal postexercise synthetic rates of muscle glycogen and protein are essential for optimal skeletal muscle recovery from exercise, it is possible to hypothesize that postexercise glycogen and protein synthesis may be limited because of a potential deficit in available sources of energy, glucose, and amino acids.

While many studies have demonstrated the importance of postexercise carbohydrate supplementation in the stimulation of glycogenesis (8,9,22,23,37,39,40), data are limited regarding the role of postexercise nutrient supplementation on the components of muscle protein homeostasis. Therefore, the purpose of this study was to determine the potential of postexercise nutrient intake to enhance the recovery of whole-body and skeletal muscle protein homeostasis in humans. Simultaneously, the effects of postexercise nutrient supplementation on net uptake and release of glucose and amino acids by the leg and on whole-body energy expen-

0195-9131/02/3405-0828/$3.00/0
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Submitted for publication April 2001.
Accepted for publication October 2001.

diture, substrate oxidation, lipolysis, and glucose utilization were examined.

SUBJECTS AND METHODS

Subject selection. Ten healthy adult subjects (5 men and 5 women) 20–41 yr of age and within 25% of ideal body weight based on Metropolitan Life Insurance Company tables (New York, NY) were selected and screened for participation in a metabolic study at the Vanderbilt University Medical Center, Nashville, TN. Each subject was provided with an explanation of the study, and informed written consent was obtained for procedures to be performed at the General Clinical Research Center (GCRC). The experimental protocols and procedures were approved by the Institutional Review Board of Vanderbilt University Medical Center.

Each subject underwent a complete physical examination and provided a full medical history. None of the subjects had any apparent hepatic, pulmonary, thyroid, renal, or metabolic dysfunction. Female subjects were not pregnant, as determined by a pregnancy test, and were premenopausal with regular menstrual cycles. Women were studied between 3 and 10 d after the onset of menses (follicular phase) to reduce experimental variability. Each subject's body density was determined by hydrostatic weighing, and body fat and lean masses were calculated using equations for either Caucasians or African Americans, as previously described (28). A maximal exercise test was conducted on each subject using a recumbent stationary cycle (Ergometrics 800, Ergoline, Bandhagen, Sweden) to perform incremental (+25 W per min) exercise until exhaustion. A respiratory exchange ratio of >1.0 and an increase in O_2 uptake ($\dot{V}O_2$) of <0.2 $L \cdot min^{-1}$ over the previous work rate were the criteria used for $\dot{V}O_{2max}$. Energy expenditure (EE) was determined during rest and during exercise using indirect calorimetry (Sensormedics 2900 Metabolic Cart, Yorba Linda, CA).

Metabolic study protocol. For the three days before the metabolic studies, subjects received their meals from the dietary kitchen of the GCRC to maintain consistency of preexercise body nutrient stores. Energy intake was kept at maintenance levels based upon the Harris-Benedict equation and each subject's gender, height, weight, and activity level.

On metabolic study days, subjects were admitted to the GCRC following an overnight fast (>12 h). To obtain samples of venous blood draining from the leg, a 5-French sheath was introduced into the femoral vein under local anesthesia (1% xylocaine infiltration), and the distal tip of the sheath was positioned using fluoroscopy in the external iliac vein, a few centimeters above the inguinal ligament. Indwelling catheters were also placed in a heated superficial hand vein for arterialized blood sampling and in the antecubital vein of the nondominant arm for infusion of stable isotopic tracers. The catheterized hand was placed in a heated thermoplastic box with the temperature adjusted automatically to 55°C for complete arterialization of blood samples (1).

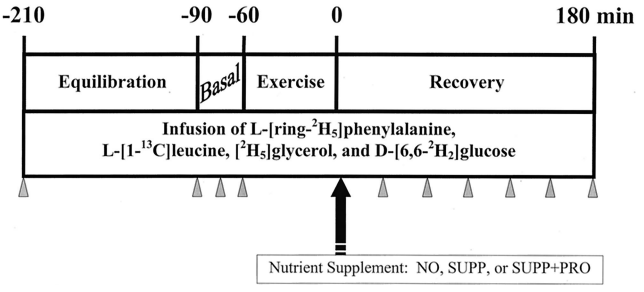

FIGURE 1—The experimental design consisted of equilibration, basal, exercise, and postexercise recovery periods. During the recovery period isotopic tracers of phenylalanine, leucine, glucose, and glycerol were infused to measure leg and whole-body kinetics. Each subject was studied three times and one of three oral nutrient supplements was administered immediately after exercise: 1) NO: no nutrients; 2) SUPP: oral administration of 0 g protein, 8 g carbohydrate, and 3 g lipid; or 3) SUPP+PRO: oral administration of 10 g protein, 8 g carbohydrate, and 3 g lipid. Blood and breath samples (▲) were taken during the basal period and for 3 h after nutrient intake.

Following the collection of blood and breath samples to determine isotopic backgrounds (−210 min), a bolus infusion of [^{13}C]$NaHCO_3$ (2.9 $\mu mol \cdot kg^{-1}$), D-[6,6-$^2H_2$]glucose (20 $\mu mol \cdot kg^{-1}$), L-[1-^{13}C]leucine (14.4 $\mu mol \cdot kg^{-1}$), and L-[ring-$^2H_5$]phenylalanine (3.6 $\mu mol \cdot kg^{-1}$) was given to prime the carbon dioxide, glucose, leucine, and phenylalanine pools, respectively. Subsequently, a continuous infusion of D-[6,6-$^2H_2$]glucose (0.33 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), L-[1-^{13}C]leucine (0.24 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), L-[ring-$^2H_5$]phenylalanine (0.06 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), and [$^2H_5$]glycerol (0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was initiated and continued throughout the study (isotopes from Cambridge Isotopes Laboratory, Andover, MA). Each metabolic study consisted of four periods (Figure 1): 1) a 120-min equilibration period; 2) a 30-min basal sampling period; 3) a 60-min exercise period; and 4) a 180-min recovery period. During the 60-min exercise period, subjects exercised on a recumbent bicycle at 60% of $\dot{V}O_{2max}$ as determined by indirect calorimetry and heart rate measures.

Arterial and venous blood samples were taken every 15 min during the basal period and every 30 min for 180 min after postexercise nutrient intake for determination of hormone and metabolite concentrations, as well as isotopic enrichments. Simultaneously, breath samples were collected from each subject in a Douglas bag, and duplicate 20-mL samples were placed into nonsiliconized evacuated glass tubes for the determination of breath $^{13}CO_2$ enrichment. Leg blood flow measurements were determined by plethysmography (Model 2560 with URI/CP software v 3.0, UFI, Morro Bay, CA) (34). Rates of plasma flow were determined from the product of one minus the hematocrit (Hct) and blood flow. Finally, carbon dioxide production, oxygen consumption, and energy expenditure were determined throughout each period by indirect calorimetry (Sensormedics 2900 Metabolic Cart, Palo Alto, CA).

Experimental protocol. Each subject was studied three times during which one of three nutrient supplements was administered immediately following the conclusion of exercise: 1) NO: no nutrients were consumed during the

three hours immediately following exercise; 2) SUPP: oral administration of 8 g carbohydrate and 3 g lipid without protein; or 3) SUPP+PRO: oral administration of 10 g protein, 8 g carbohydrate, and 3 g lipid (Jogmate®, Pharmavite Corporation, Mission Hills, CA). In SUPP, the carbohydrates were derived from regular sugar and the fat was derived from milk. In SUPP+PRO, protein, carbohydrate, and fat were derived from casein, regular sugar, and milk fat, respectively. A 4-wk "washout" period was maintained between each metabolic study to allow isotopic tracer clearance and to ensure that the female participants were in the follicular phase of their menstrual cycle. The order of treatment administration for the three metabolic studies was random. Subjects were instructed to maintain consistent daily exercise activity, dietary intake, and body weight for 2 wk before each test day so that they remained similar between treatments.

Analytical procedures. Blood samples were collected into Venoject tubes containing 15 mg Na_2EDTA (Terumo Medical Corp, Elkton, MD). A 1-mL aliquot of whole blood from each sampling site was deproteinized with 3 mL of 4% perchloric acid for determination of whole blood lactate, glycerol, and glutamine concentrations using enzymatic methods (30,31). In addition, 3 mL of blood was transferred to a tube containing EDTA and reduced glutathione with the plasma stored at −80°C for later measurement of plasma epinephrine and norepinephrine concentrations by HPLC (18). The remaining blood was spun in a refrigerated (4°C) desktop centrifuge (Beckman Instruments, Fullerton, CA) at 2,000 g for 10 min to obtain the plasma, which was stored at −80°C for later analysis. Plasma glucose concentrations were determined by the glucose oxidase method (Model II Glucose Analyzer, Beckman Instruments). Plasma urea concentration was determined using a Beckman BUN Analyzer 2 (Beckman Instruments).

Immunoreactive insulin was determined in plasma with a double-antibody system (32). Plasma aliquots for glucagon determination were placed in tubes containing 25 kallikrein-inhibitor units of aprotinin (Trasylol; FBA Pharmaceutical, New York, NY) and were later measured by established radioimmunoassay with a double-antibody system modified from the method of Morgan and Lazarow (32) for insulin. Insulin and glucagon antisera and standards, as well as [^{125}I] labeled hormones, were obtained from RL Gingerich (Linco Research, St. Louis, MO). Clinical Assays Gammacoat Radioimmunoassay kit (Travenol-GenTech, Cambridge, MA) was used to measure plasma cortisol concentrations. IGF-1 was measured in a subset of subjects ($N = 3$) for each protocol using a commercial IGF-1 kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). Plasma amino acid concentrations were determined by reversed-phase HPLC after derivatization with phenylisothiocyanate (21). The data from the amino acid analyses were assessed individually and by specific groups. The groups included were branched chain amino acids (BCAA), the sum of leucine, isoleucine, and valine; essential amino acids (EAA), the sum of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; total amino acids (TAA), the sum of all individual amino acids; and nonessential amino acids (NEAA), the difference between TAA and EAA.

Plasma enrichments of [^{13}C]leucine, [^{13}C]ketoisocaproate (KIC), and [ring-$^2H_5$]phenylalanine were determined using gas chromatography/mass spectrometry (GC/MS). Plasma was deproteinized with 4% perchloric acid, and the supernatant was passed over a cation exchange resin to separate the keto and amino acids. The keto acids were further extracted with methylene chloride and 0.5 M ammonium hydroxide (33). After drying under nitrogen gas, both the keto and amino acid fractions were derivatized (38) with N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide containing 1% t-butyldimethylchlorosilane (MTBSTFA + 1% t-BDMCS; Regis Technologies, Inc, Morton Grove, IL). The derivatized samples were then analyzed with GC/MS (Hewlett-Packard 5890A GC and 5970 MS, San Fernando, CA) for plasma leucine and KIC enrichments. Following deproteinization with $Ba(OH)_2$ and $ZnSO_4$ and elution over cation and anion resins, plasma D-[6,6-$^2H_2$]glucose enrichment was determined by GC/MS according to the method of Bier (4), and plasma [$^2H_5$]glycerol enrichment was determined by GC/MS by the method of Flakoll et al. (13). Breath $^{13}CO_2$ enrichment was measured by isotope ratio-mass spectrometry (Metabolic Solutions, Nashua, NH).

Calculations. Net leg balances of glucose and amino acids were derived from the concentration gradient of leg venous versus arterialized plasma multiplied by corresponding leg plasma flow. A positive value denotes net leg uptake, and a negative number denotes net leg release of glucose or amino acids.

Net skeletal muscle protein dynamics were determined by measuring the dilution of phenylalanine enrichment across the hind limb as described by Gelfand and Barrett (17). Because phenylalanine is neither synthesized nor metabolized by skeletal muscle, the rate of appearance (Ra) of unlabeled phenylalanine reflects muscle protein breakdown, whereas the rate of disappearance (Rd) of labeled phenylalanine is an estimate of muscle protein synthesis (17). Phenylalanine Rd was calculated by multiplying the fractional extraction of the labeled phenylalanine (based on plasma arterial and venous phenylalanine enrichments and concentrations) by the arterial phenylalanine concentration and leg plasma flow (17,44). Net phenylalanine Ra was calculated by subtracting the net arteriovenous balance of phenylalanine across the hind limb from the phenylalanine Rd (17,44). Rates of skeletal muscle protein breakdown and net synthesis were determined from the phenylalanine Rd and Ra, assuming that 3.8% of skeletal muscle protein is comprised of phenylalanine (16).

The steady-state rates of whole-body leucine Ra (an estimate of whole-body protein breakdown) were calculated by dividing the [^{13}C]leucine infusion rate by the plasma [^{13}C]KIC enrichment (44). Plasma KIC provides a better estimate of intracellular leucine enrichment than does plasma leucine enrichment because KIC is derived from intracellular leucine metabolism (44). Endogenous leucine Ra was calculated by subtracting the exogenous leucine Ra

from the total leucine Ra. Exogenous leucine Ra from the nutrient supplement was calculated with the assumptions that the protein consumed was 7.8% leucine (16), there was a 26% splanchnic clearance of exogenous leucine (6), and the plasma leucine Ra from the exogenous supplement was constant (24.44 μmol·min^{-1}) over the entire 3-h recovery period. This assumption was supported by orally administering 100 mg of L-[$^2H_8$]valine with the protein supplement and measuring enrichment with time. While valine enrichment was relatively constant across the 3-h period for SUPP+PRO (0.19 ± 0.01%), enrichment fell and was not detectable in blood taken from two subjects 30 min after the 3-h period, suggesting that the appearance of amino acids from exogenous protein was negligible after the 3-h period. Breath $^{13}CO_2$ production was determined by multiplying the total CO_2 production rate by the breath $^{13}CO_2$ enrichment (44). The rate of whole-body leucine oxidation was calculated by dividing breath $^{13}CO_2$ production by 0.8 (correction factor for the retention of $^{13}CO_2$ in the bicarbonate pool) (2) and by the plasma KIC enrichment. The nonoxidative leucine Rd (an estimate of whole-body protein synthesis) was determined indirectly by subtracting leucine oxidation from total leucine Ra. Rates of whole-body protein breakdown, amino acid oxidation, and protein synthesis were calculated from the endogenous leucine Ra, the leucine oxidation rate, and the nonoxidative leucine Rd, respectively, assuming that 7.8% of whole-body protein is comprised of leucine (16).

Whole-body glucose Rd was calculated by dividing the D-[6,6-$^2H_2$]glucose infusion rate by the plasma [$^2H_2$]glucose enrichment (44). With this method, the deuterium label is lost during the phosphoenolpyruvate cycle, is diluted into the total body water pool, and is not recycled. Whole-body glycerol Ra was calculated by dividing the [$^2H_5$]glycerol infusion rate by the plasma glycerol enrichment (44). Because glycerol released during lipolysis cannot be reincorporated into triacylglycerol in adipose cells because of the lack of glycerol kinase activity, glycerol Ra was multiplied by 3 to determine rates of whole-body lipolysis (13).

Rates of whole-body amino acid, carbohydrate, and lipid oxidation were determined from indirect calorimetry in combination with the leucine oxidation data. The energy expended because of amino acid oxidation was subtracted from the total energy expenditure, and the net rates of carbohydrate and lipid oxidation were calculated based on the nonprotein respiratory quotient (24). The assumptions and limitations of calculating net substrate oxidation based on indirect calorimetry measurements have been reviewed previously (24). Net whole-body nutrient balances were calculated by subtracting whole-body nutrient oxidation from nutrient intake.

Statistical analysis. For each protocol, mean variables for each 180-min recovery period were calculated. Values presented in the text and figures are means ± SEM for each variable during the 180-min recovery period. No differences were noted between treatments during the basal periods, and thus, the data for this period will not be reported. Because each subject was studied three times, differences between the mean values obtained during the postnutrient intake periods were assessed using a repeated-measures analysis of variance with a model of treatment within gender (Statistical Analysis System for Windows, 1996, Release 6.12, SAS Institute, Cary, NC). A P value of <0.05 was required to reject the null hypothesis of no difference between the means.

TABLE 1. Arterial metabolite and hormone concentrations.

	NO	SUPP	SUPP + PRO
Glucose (mg·dL^{-1})	87.3 ± 1.3	92.2 ± 1.4	92.0 ± 2.1
Urea (mg·L^{-1})	11.0 ± 1.4	9.0 ± 0.7	10.9 ± 0.7
Lactate (μmol·L^{-1})	606 ± 86	711 ± 70	746 ± 106
Glycerol (μmol·L^{-1})	94.3 ± 9.1	90.4 ± 13.0	78.6 ± 12.2
Insulin (μU·L^{-1})	2.7 ± 0.5	4.3 ± 0.7	5.1 ± 1.0
Glucagon (ng·L^{-1})	46.4 ± 2.4[a]	47.2 ± 2.7[a,b]	55.7 ± 4.4[b]
Cortisol (nmol·L^{-1})	9.4 ± 1.4	7.4 ± 0.7	10.2 ± 1.4
Growth hormone (ng·mL^{-1})	2.2 ± 0.7	1.3 ± 0.5	1.8 ± 0.4
IGF-1 (ng·mL^{-1})	129.2 ± 22.6	159.8 ± 23.0	134.7 ± 35.6
Epinephrine (pmol·L^{-1})	44.8 ± 9.8	41.2 ± 15.8	46.0 ± 11.9
Norepinephrine (nmol·L^{-1})	197 ± 43	189 ± 49	139 ± 30

Values are the mean ± SEM for each post-exercise period. Differing letters denotes significance at $P < 0.05$. The time points for the 3-h recovery period were averaged for each subject, and then the overall period mean ± SEM was calculated. $N = 10$ for each variable except for IGF-1 ($N = 3$). One of three oral nutrient supplements was administered immediately after exercise: 1) NO: no nutrients; 2) SUPP: 0 g protein, 8 g carbohydrate, and 3 g lipid; or 3) SUPP + PRO: 10 g protein, 8 g carbohydrate, and 3 g lipid.

RESULTS

Subject characteristics. The 10 healthy adult subjects (5 women and 5 men) were 31.5 ± 2.2 yr of age, 172.7 ± 3.0 cm tall, and 76.0 ± 4.3 kg. Weight did not significantly change after screening in comparison to the three study days (Screening 76.0 ± 4.3 kg, NO 75.0 ± 4.2 kg, SUPP 74.5 ± 4.2 kg, and SUPP+PRO 74.9 ± 4.5 kg). Subjects were recreational athletes with an average $\dot{V}O_{2max}$ of 33.2 ± 4.0 and 44.9 ± 2.9 mL·kg^{-1}·min^{-1} for women and men, respectively. Average body fat was 30.5 ± 3.4% and 17.0 ± 2.5% for women and men, respectively.

Metabolite & hormone concentrations. Circulating glucose, urea, lactate, and glycerol concentrations were not different between the three postexercise nutrient supplement treatments (Table 1). In addition, the concentrations of plasma insulin, IGF-1, cortisol, growth hormone, epinephrine, and norepinephrine did not differ between protocols. In contrast, plasma glucagon concentration was 20% greater when protein was added to the postexercise nutrient supplement in comparison with no postexercise supplement.

Plasma individual and grouped plasma amino acid concentrations were not significantly altered from NO by the intake of SUPP (Table 2). Conversely, the concentrations of histidine, isoleucine, leucine, lysine, ornithine, phenylalanine, proline, tryptophan, tyrosine, and valine were increased by at least 20% for SUPP+PRO versus either NO or SUPP. Plasma threonine with SUPP+PRO was increased by 36% versus NO. For the groups of amino acids, there were increases with SUPP+PRO versus NO for concentrations of branched chain amino acids (BCAA; 47%),

TABLE 2. Plasma arterial amino acid concentrations (μmol·L^{-1}).

	NO	SUPP	SUPP + PRO
1-Methylhistidine	3.5 ± 0.7	7.5 ± 1.6	5.8 ± 1.4
3-Methylhistidine	5.6 ± 1.8	7.6 ± 1.5	7.3 ± 0.9
Alanine	214.1 ± 22.2	251.5 ± 21.9	257.8 ± 21.9
Arginine	75.6 ± 4.9	74.8 ± 7.1	79.6 ± 5.0
Asparagine	57.6 ± 6.6	59.6 ± 5.0	70.4 ± 4.3
Aspartate	6.24 ± 0.6	6.9 ± 1.2	7.2 ± 0.7
Citrulline	33.0 ± 2.7	35.8 ± 3.0	31.5 ± 2.1
Glutamate	52.1 ± 4.9	58.2 ± 5.3	55.3 ± 5.6
Glutamine	437.0 ± 29.1	492.5 ± 54.9	456.4 ± 26.5
Glycine	206.4 ± 15.9	225.4 ± 19.8	219.3 ± 22.0
Histidine	61.0 ± 2.8[a]	57.5 ± 3.1[a]	72.7 ± 3.1[b]
Hydroxyproline	5.8 ± 0.5	6.9 ± 0.9	6.0 ± 0.6
Isoleucine	38.0 ± 1.9[a]	34.4 ± 2.9[a]	70.9 ± 6.7[b]
Leucine	152.7 ± 5.6[a]	145.8 ± 6.6[a]	204.4 ± 11.2[b]
Lysine	138.1 ± 4.8[a]	136.2 ± 7.4[a]	166.1 ± 8.4[b]
Methionine	20.6 ± 2.3	21.4 ± 3.1	26.2 ± 1.9
Ornithine	41.0 ± 2.2[a]	42.4 ± 2.1[a]	56.1 ± 2.2[b]
Phenylalanine	55.8 ± 2.7[a]	52.6 ± 3.3[a]	74.7 ± 2.8[b]
Proline	136.8 ± 7.4[a]	146.4 ± 9.4[a]	197.0 ± 12.7[b]
Serine	86.6 ± 3.1	91.8 ± 3.3	101.0 ± 6.3
Taurine	100.6 ± 15.1	109.3 ± 18.3	96.4 ± 11.8
Threonine	100.7 ± 10.1[a]	116.9 ± 10.0[a,b]	139.4 ± 11.8[b]
Tryptophan	35.6 ± 2.3[a]	32.6 ± 1.8[a]	43.0 ± 2.4[b]
Tyrosine	46.4 ± 4.4[a]	45.6 ± 3.7[a]	68.0 ± 4.2[b]
Valine	155.0 ± 7.6[a]	151.0 ± 12.2[a]	232.0 ± 12.9[b]
BCAA	345.7 ± 14.0[a]	331.3 ± 20.6[a]	507.4 ± 28.9[b]
EAA	833.2 ± 28.1[a]	823.3 ± 35.8[a]	1109.2 ± 33.2[b]
NEAA	1432.7 ± 45.6[a]	1587.8 ± 67.2[a,b]	1635.5 ± 41.4[b]
TAA	2265.9 ± 62.3[a]	2411.0 ± 65.3[a]	2744.7 ± 59.4[b]

Values (μmol·L^{-1}) are the mean ± SEM for each post-exercise period. Differing letters denotes significance at $P < 0.05$.
The time points for the 3-h recovery period were averaged for each subject, and then the overall period mean ± SEM was calculated. One of three oral nutrient supplements was administered immediately after exercise: 1) NO: no nutrients; 2) SUPP: 0 g protein, 8 g carbohydrate, and 3 g lipid; or 3) SUPP + PRO: 10 g protein, 8 g carbohydrate, and 3 g lipid.
BCAA, branched-chain amino acids: sum of leucine, isoleucine, and valine.
EAA, essential amino acids: sum of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.
NEAA, nonessential amino acids: difference between total amino acids (TAA) and EAA.
TAA: sum of all amino acids.

essential amino acids (EAA; 33%), nonessential amino acids (NEAA; 14%), and total amino acids (TAA; 21%). In addition, there were increases for SUPP+PRO versus SUPP for BCAA (53%), EAA (35%), and TAA (14%).

Net glucose and amino acid balance across the leg. Mean leg blood flows were not significantly different between the three treatment groups (5.4 ± 0.6 mL·min^{-1}·100cc^{-1} for NO, 5.1 ± 0.6 mL·min^{-1}·100cc^{-1} for SUPP, and 6.0 ± 0.9 mL·min^{-1}·100cc^{-1} for SUPP+PRO). Similarly, Hcts were not different between the groups (39.3 ± 0.7% for NO, 38.9 ± 0.5% for SUPP, and 39.4 ± 0.4% for SUPP+PRO). Net leg glucose uptake was 32.8 μg·min^{-1}·100cc^{-1} when no nutrients were given after exercise (Figure 2). SUPP did not significantly increase net leg glucose uptake versus NO. However, when protein was added to the oral postexercise supplement, net leg glucose balance was 3.5 times greater compared with NO.

During the NO and SUPP treatments, there was a net release of BCAA, EAA, NEAA, and TAA from the leg (Table 3). Only the individual amino acids aspartate, glutamate, hydroxyproline, ornithine, and taurine had a net uptake from the leg during NO and SUPP. However, most individual amino acids had reduced net release when SUPP+PRO was compared with either NO or SUPP. The individual amino acids isoleucine, leucine, phenylalanine, proline, and valine switched from a net release in the NO and SUPP treatments to a net uptake with SUPP+PRO. In addition, with SUPP+PRO there was a net uptake across the leg for BCAA, EAA, NEAA, and TAA. The values for BCAA, EAA, and TAA were significantly different from those encountered in the NO and SUPP protocols.

Leg fractional extraction of phenylalanine also was significantly affected by the content of the postexercise nutrient supplement. Leg phenylalanine fractional extraction with the SUPP+PRO treatment (17.0 ± 2.4%) was approximately four-fold greater than NO (4.3 ± 0.9%) and nearly three-fold greater than SUPP (6.6 ± 2.2%).

Leg protein kinetics. The addition of carbohydrate and fat without protein (SUPP) did not significantly alter leg protein kinetics in comparison with NO (Figure 3). However, leg protein synthesis and net protein balance were both significantly increased by the addition of protein to the oral postexercise nutrient supplement. Leg protein synthesis was approximately 6 times greater for SUPP+PRO compared with NO and 4 times greater compared with SUPP. Leg protein breakdown, however, was not significantly different between treatments. Thus, there was a net gain of protein in the leg for SUPP+PRO, but a net loss of leg protein for both NO and SUPP. The net absolute difference in accumulation of leg muscle protein was 105 μg·min^{-1}·100cc^{-1} (+60.5 versus −44.6 μg·min^{-1}·100cc^{-1}) for SUPP+PRO versus SUPP and 112 μg·min^{-1}·100cc^{-1} (+60.5 versus −51.3 μg·min^{-1}·100cc^{-1}) for SUPP+PRO versus NO.

Whole-body protein kinetics. Whole-body proteolysis, as measured by endogenous leucine Ra, was not significantly affected by the content of the postexercise nutrient supplement (Figure 4). Furthermore, SUPP did not increase whole-body protein synthesis. However, there was a trend for whole-body protein synthesis to be increased by 15% for SUPP+PRO versus NO. Finally, there was a slight net accretion of protein by the body (0.02 ± 0.15 mg·kg^{-1}·min^{-1}) when protein was added to the postexercise supplement compared with a net loss of protein by the body for NO (−0.41 ± 0.04 mg·kg^{-1}·min^{-1}) or SUPP (−0.53 ± 0.12 mg·kg^{-1}·min^{-1}). The net absolute difference in accumulation of whole-body protein was 0.55 mg·kg^{-1}·min^{-1} (+0.02 versus −0.53 mg·kg^{-1}·min^{-1}) for SUPP+PRO versus SUPP and was 0.43 mg·kg^{-1}·min^{-1} (+0.02 versus −0.41 mg·kg^{-1}·min^{-1}) for SUPP+PRO versus NO.

Whole-body glucose & glycerol kinetics. Whole-body glucose utilization for SUPP (2.80 ± 0.47 mg·kg^{-1}·min^{-1}) was 44% greater than NO (1.95 ± 0.10 mg·kg^{-1}·min^{-1}) and 16% greater than SUPP+PRO (2.40 ± 0.11 mg·kg^{-1}·min^{-1}). Whole-body glycerol flux was unaffected by the content of the postexercise supplement (6.79 ± 1.04, 8.09 ± 2.65, and 7.82 ± 1.18 μmol·kgFat^{-1}·min^{-1}) for NO, SUPP, and SUPP+PRO, respectively).

Energy expenditure and substrate oxidation. There was no change in the total energy expenditure, normalized to body weight, in NO (0.86 ± 0.05 kcal·kg^{-1}·h^{-1}),

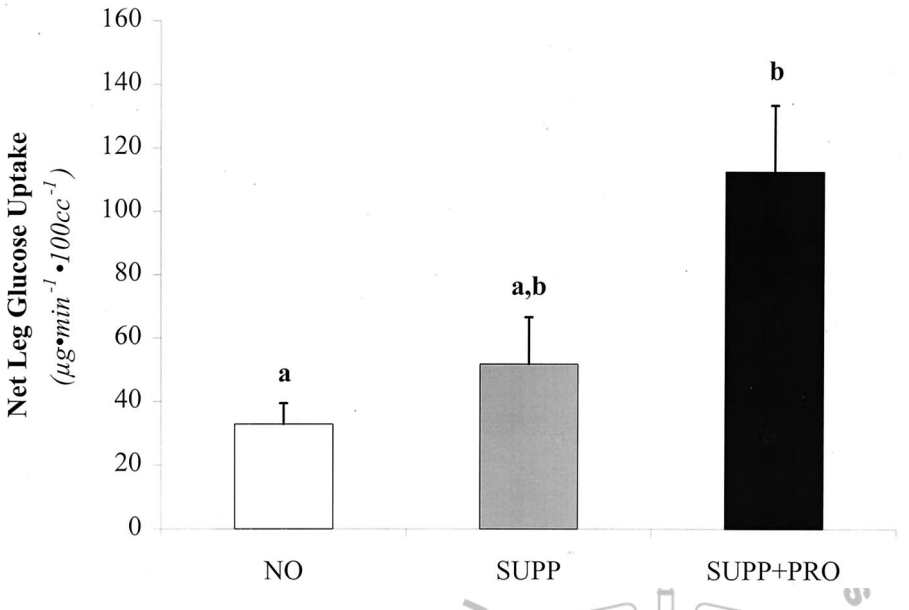

FIGURE 2—Net leg glucose uptake for 10 subjects given one of three oral nutrient supplements immediately after exercise: 1) NO: no nutrients; 2) SUPP: oral administration of 0 g protein, 8 g carbohydrate, and 3 g lipid; or 3) SUPP+PRO: oral administration of 10 g of protein, 8 g carbohydrate, and 3 g lipid. Differing letters denote significance at $P < 0.05$.

SUPP (0.89 ± 0.04 kcal·kg^{-1}·h^{-1}), and SUPP+PRO (0.94 ± 0.04 kcal·kg^{-1}·h^{-1}). Respiratory quotient (RQ) values were not different between NO (0.74 ± 0.00), SUPP (0.74 ± 0.01) and SUPP+PRO (0.74 ± 0.01). The proportion of energy from carbohydrate or lipid was not different between treatments (Figure 5). In the group receiving postexercise supplementation of carbohydrate and fat only, oxidation rates of carbohydrate and fat were not significantly increased. However, in the group receiving protein with the postexercise supplement, amino acid oxidation was greater than either SUPP or NO.

DISCUSSION

The purpose of the present experiment was to test the hypothesis that the substrates required for protein synthesis in healthy adults may limit muscle protein synthesis. The approach used was to maintain carbohydrate and fat intake constant between the two treatments, enabling us to study the independent effect of postexercise protein supplementation. When a protein-devoid supplement was provided in the form of 8 g of carbohydrates and 3 g of fat, the effects of energy supplementation alone could be assessed. Energy supplementation without protein did not alter circulating amino acid concentrations or their uptake by the leg when compared with providing no supplement at all. Furthermore, energy intake alone did not have an impact on whole-body and leg protein synthesis or breakdown. There was a net loss of leg and whole-body protein during the 3 h after exercise when no supplement was provided. In spite of the increased energy intake, net losses in leg and whole-body protein also were found when the carbohydrate/fat supplement was provided. In contrast, the additional intake of 10 g of protein with 8 g of carbohydrate and 3 g of fat significantly increased the concentration of circulating amino acids, stimulated the fractional extraction and uptake of amino acids by the leg, and enhanced leg protein synthesis. Moreover, the net leg and whole-body protein accretion observed when the supplement containing protein was provided was in direct contrast with the net losses found when no nutrients or when nutrients without protein were provided. While these data

TABLE 3. Amino acid balance across the leg (nmol·100cc leg volume^{-1}·min^{-1}).

	NO	SUPP	SUPP + PRO
1-Methylhistidine	0.8 ± 0.4	1.9 ± 0.8	1.1 ± 1.4
3-Methylhistidine	0.6 ± 0.6	−0.4 ± 1.1	0.6 ± 1.1
Alanine	−99.6 ± 17.8	−175.7 ± 44.4	−36.6 ± 66.4
Arginine	−31.1 ± 4.2	−36.6 ± 7.4	−17.3 ± 12.3
Asparagine	−20.0 ± 3.6	−20.6 ± 5.3	−5.9 ± 9.1
Aspartate	2.8 ± 2.4	3.6 ± 0.8	4.9 ± 2.0
Citrulline	−0.7 ± 2.1	−11.3 ± 9.8	2.2 ± 3.0
Glutamate	91.2 ± 19.2	84.4 ± 18.7	68.5 ± 34.0
Glutamine	231.6 ± 40.1^{a}	−99.2 ± 46.8^{a,b}	−258.8 ± 59.1^{b}
Glycine	−72.6 ± 8.2	−80.5 ± 14.0	−36.2 ± 35.6
Histidine	−16.0 ± 3.4	−13.4 ± 10.2	−8.6 ± 7.1
Hydroxyproline	3.2 ± 0.7	3.1 ± 0.6	3.5 ± 1.3
Isoleucine	−7.4 ± 1.5^{a}	−10.5 ± 1.6^{a}	6.4 ± 3.6^{b}
Leucine	−9.2 ± 5.0^{a}	−19.3 ± 4.1^{a}	16.1 ± 6.4^{b}
Lysine	−26.9 ± 7.2	−24.6 ± 7.8	−0.4 ± 10.0
Methionine	−4.0 ± 1.6^{a,b}	−7.9 ± 1.3^{a}	−0.6 ± 2.7^{b}
Ornithine	6.2 ± 1.7	8.3 ± 2.6	12.4 ± 5.0
Phenylalanine	−10.3 ± 1.8^{a}	−11.7 ± 3.3^{a}	13.9 ± 2.5^{b}
Proline	−24.1 ± 4.2^{a}	−31.4 ± 10.2^{a}	11.5 ± 6.9^{b}
Serine	1.4 ± 3.7	4.3 ± 5.1	12.1 ± 4.4
Taurine	100.3 ± 20.8	87.8 ± 15.5	91.7 ± 33.8
Threonine	−34.2 ± 7.7	−38.9 ± 8.7	−9.9 ± 15.5
Tryptophan	1.1 ± 1.6	1.4 ± 4.0	1.0 ± 2.7
Tyrosine	−10.4 ± 2.0^{a}	−8.3 ± 5.3^{a,b}	1.4 ± 4.4^{b}
Valine	−9.6 ± 3.7^{a}	−10.3 ± 11.3^{a}	18.7 ± 5.9^{b}
BCAA	−26.2 ± 9.4^{a}	−40.1 ± 13.8^{a}	41.3 ± 15.6^{b}
EAA	−147.5 ± 26.4^{a}	−171.8 ± 37.1^{a}	19.5 ± 55.5^{b}
NEAA	−15.6 ± 27.4^{a,b}	−131.7 ± 61.4^{a}	137.1 ± 73.6^{b}
TAA	−163.1 ± 43.6^{a}	−304.0 ± 87.1^{a}	156.6 ± 118.0^{b}

Values (nmol·100cc^{-1}·min^{-1}) are the mean ± SEM for each post-exercise period. Differing letters denotes significance at $P < 0.05$.

The time points for the 3-h recovery period were averaged for each subject, and then the overall period mean ± SEM was calculated. A positive balance indicates a net uptake of amino acids; a negative balance indicates a net release of amino acids across the leg.

One of three oral nutrient supplements was administered immediately after exercise: 1) NO: no nutrients; 2) SUPP: 0 g protein, 8 g carbohydrate, and 3 g lipid; or 3) SUPP + PRO: 10 g protein, 8 g carbohydrate, and 3 g lipid.

BCAA, branched-chain amino acids: sum of leucine, isoleucine, and valine.

EAA, essential amino acids: sum of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

NEAA, nonessential amino acids: difference between total amino acids (TAA) and EAA.

TAA: sum of all amino acids.

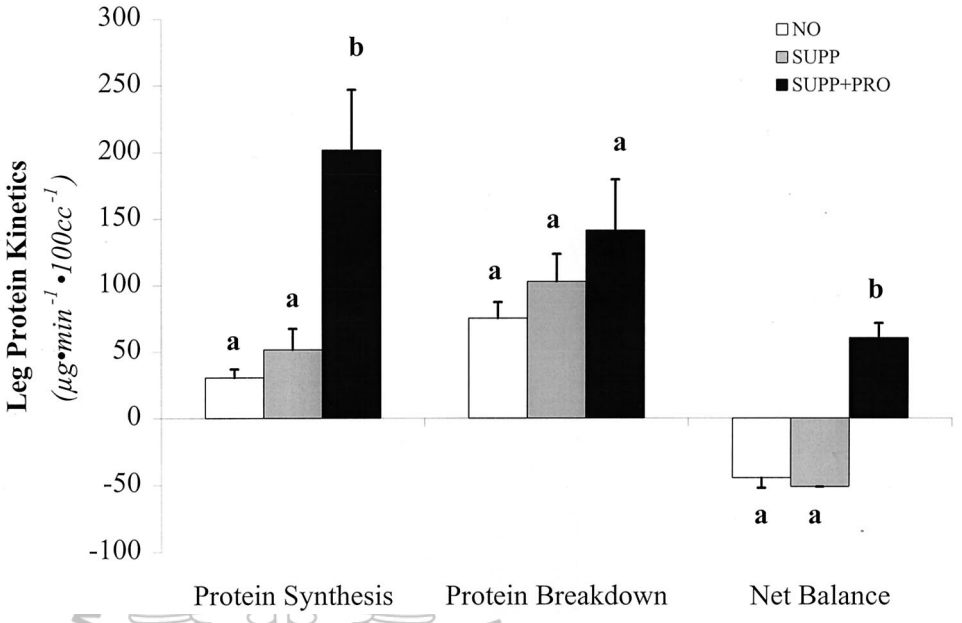

FIGURE 3—Rates of leg protein kinetics for 10 subjects given one of three oral nutrient supplements immediately after exercise: 1) NO: no nutrients; 2) SUPP: oral administration of 0 g protein, 8 g carbohydrate, and 3 g lipid; or 3) SUPP+PRO: oral administration of 10 g protein, 8 g carbohydrate, and 3 g lipid. Differing letters denote significance at $P < 0.05$.

support the hypothesis that postexercise leg muscle protein synthesis is limited by the availability of amino acids, they do not support the hypothesis that energy availability *per se* controls postexercise muscle protein synthesis. Hence, amino acid availability appears to be more limiting than energy for muscle protein synthesis following exercise.

It could be argued that the addition of protein to the nutrient supplement was effective only because it provided additional energy. While conclusive evidence is impossible to obtain from the present study as protein intake provides both amino acids and energy, there are two lines of evidence suggesting that the energy component of the protein supplement was of lesser importance than the amino acid component for the postexercise synthesis of proteins. First, the nutrient supplement with only carbohydrate and fat contained 59 kcal of energy and had no effect on the events of protein homeostasis. The additional energy provided by the protein was only 40 kcal, and thus, it is not likely that this energy would influence protein dynamics. Second, the proportion of amino acids utilized by oxidative pathways versus those directed toward events of protein synthesis remained constant between the nutrient supplement intakes with and without protein (12% vs 11%), demonstrating that the proportion of protein used as energy did not increase. It must be emphasized, however, that further study is required to determine if there is an amount of postexercise energy that would alter protein homeostasis.

Previously, Lemon (26) suggested that athletes have greater protein requirements than sedentary individuals and proposed that the current RDA of 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ be

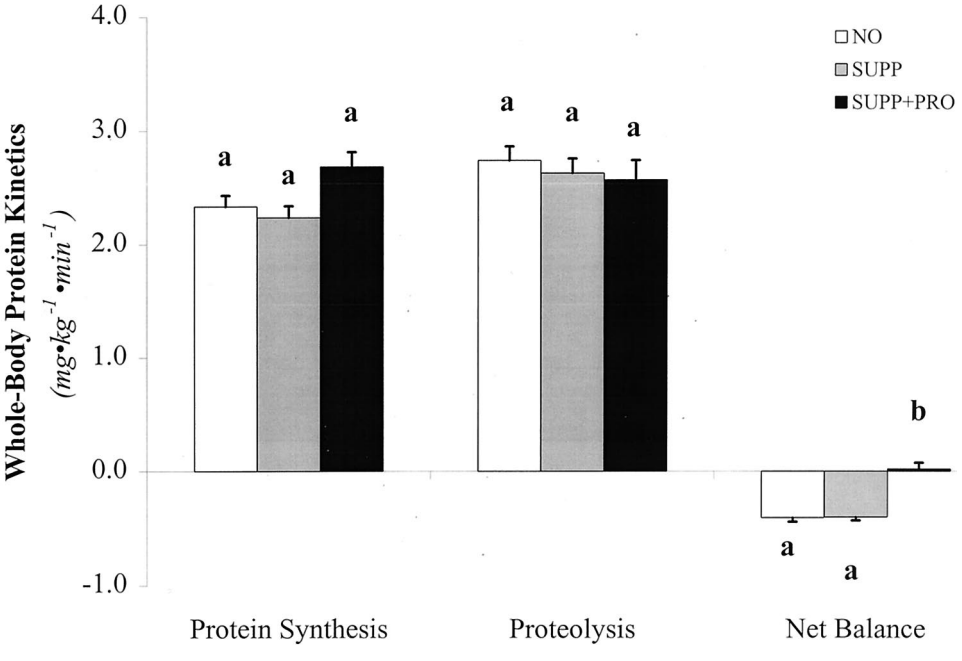

FIGURE 4—Rates of whole-body protein kinetics for 10 subjects given one of three oral nutrient supplements immediately after exercise: 1) NO: no nutrients; 2) SUPP: oral administration of 0 g protein, 8 g carbohydrate, and 3 g lipid; or 3) SUPP+PRO: oral administration of 10 g protein, 8 g carbohydrate, and 3 g lipid. Differing letters denote significance at $P < 0.05$.

increased to between 1.4 and 1.8 in strength athletes and to between 1.2 and 1.4 in endurance athletes (26). Additional protein needs have been suggested for several types of strength and endurance exercise (7,26,27). In the present study, significant gains in net protein accretion were detected for the treatment receiving protein, even with what would amount to a moderate increase in protein intake (0.13 $g \cdot kg^{-1}$). If added to the normal RDA, this amount of protein would result in a 17% increase in daily protein intake. However, it is important to acknowledge that this was an acute study and that it is impossible to speculate as to what happened to subsequent protein intake throughout the remainder of the day or even the next day. Furthermore, alterations in the timing of protein intake in relationship to exercise may be more important than overall protein requirements. Acute (29) and chronic studies (10) in humans support this hypothesis.

Protein intake in the current study may not reflect maximal effects on protein accretion following exercise. In previous studies with humans undergoing resistance exercise, Tipton et al. demonstrated that intake of a drink with 40 g of amino acids increased net muscle protein balance similar to the present study (41). However, future studies are necessary to determine the dose-responsive effects on postexercise muscle deposition. Furthermore, it may be speculated that protein supplementation in athletes promotes uremia because of a potential increased need for nitrogen clearance. However, plasma urea concentrations were not different between treatments in the present study, suggesting that the subjects were able to adequately clear any excess nitrogen produced from the supplement containing 10 g of protein.

Physical activity at varying intensities causes catabolism of amino acids (35,36). Amino acid oxidation, and in particular leucine oxidation, increases during exercise. Furthermore, exercise promotes conversion of amino acids to glucose via gluconeogenic pathways, and as a result, the rate of urea production is stimulated (43). The amino acids utilized during exercise are derived from increased proteolysis and decreased protein synthesis (35). The decrease in protein synthesis is related to the diversion of amino acids and energy away from the events supporting protein synthesis toward the events supporting muscle contraction. Therefore, it is after exercise that rates of protein synthesis have the potential to be accelerated beyond the rates of proteolysis, allowing protein balance to become positive. Data from the present study suggest that, without postexercise protein supplementation, protein synthesis may be limited because of the decreased availability of amino acids.

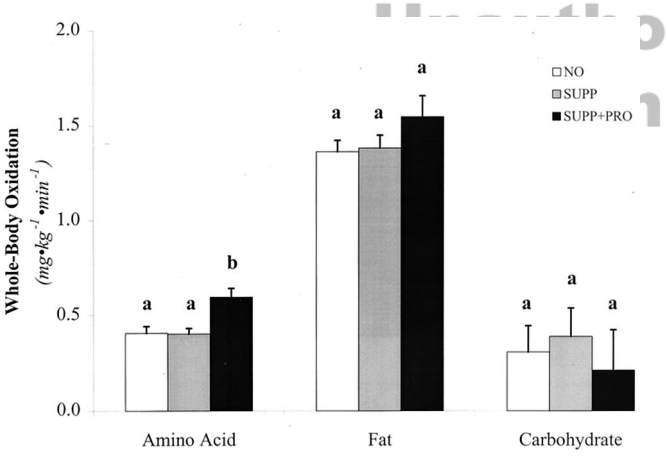

FIGURE 5—Rates of whole-body amino acid, carbohydrate, and fat oxidation for 10 subjects given one of three oral nutrient supplements immediately after exercise: 1) NO: no nutrients; 2) SUPP: oral administration of 0 g protein, 8 g carbohydrate, and 3 g lipid; or 3) SUPP+PRO: oral administration 10 g of protein, 8 g carbohydrate, and 3 g lipid. Differing letters denote significance at $P < 0.05$.

Insulin is a very important regulator of protein homeostasis (5,12). Previous studies have demonstrated that the infusion of amino acids during hyperinsulinemia provides a stimulus for increased whole-body and muscle protein synthesis (12,17). In the present study, the anabolic hormones insulin, insulin-like growth factor I, and growth hormone did not significantly change with the intake of either nutrient supplement. Sensitivity and responsiveness to the events controlled by insulin are enhanced by exercise (42). However, exercise-enhanced insulin action should be similar between treatments. In fact, plasma glucagon was the only metabolic hormone to differ between groups. However, the increased glucagon in the group receiving supplemental protein most likely would not increase whole-body or skeletal muscle protein synthesis, as the main role for glucagon in the regulation of protein homeostasis is to stimulate hepatic uptake of amino acids and increase gluconeogenesis (11). Because circulating concentrations of metabolic hormones other than glucagon were not different between treatments, the differences observed for the treatment group receiving protein are attributable to changes in substrate rather than hormone concentrations.

After exercise there also is a need for nutrients to be directed toward glycogen deposition and repletion. An abundance of studies has demonstrated that carbohydrate intake after exercise increases muscle glycogen repletion and deposition (9,15,22,23,39). Previously, Ivy et al. (23) demonstrated that 1.5 g of glucose per kilogram body weight increased muscle glycogen deposition after exercise, but that 3.0 g of glucose per kilogram body weight provided no additional benefit. In the present study, glucose was administered at 0.1 $g \cdot kg^{-1}$ body weight, a dose considerably below that for maximal glycogen deposition. Although we did not directly measure muscle glycogen storage, we did measure the uptake of glucose across the leg and found that the supplement containing 8 g of carbohydrate and 3 g of fat did not significantly increase leg glucose uptake compared with no supplement. However, the addition of 10 g of protein to the nutrient supplement did significantly increase leg glucose uptake, either by providing more gluconeogenic substrates or by sparing glucose oxidation. This is consistent with previous findings demonstrating that the energetic content of a supplement is the major determinant to the restoration of glycogen stores (8,37,40).

Previously, Halseth et al. (19) demonstrated in canines that exercise results in a significant loss of protein from

tissues other than muscle, specifically from gastrointestinal tract (gut) tissues and the liver. Therefore, while it is important to consider how tissues other than skeletal muscle may respond to the overall uptake of supplemented protein, a paucity of this information exists. We did not directly measure components of protein homeostasis in nonskeletal muscle tissues in this study. However, leg protein synthesis and net protein deposition were altered to a greater magnitude than were whole-body protein synthesis and net protein deposition, suggesting that the magnitude of change may be greater in the leg than in other tissues. Recently, Hamada et al. (20) found that there was a net leg release of phenylalanine and a net gut and liver release of leucine in canines during infusions of saline or glucose for 60 min before and 150 min during treadmill exercise. When amino acids were infused in addition to glucose, there was a net leg uptake of phenylalanine and a net gut and liver uptake of leucine, suggesting that exercise-induced protein losses in muscle, gut, and liver were reduced. However, the magnitude of impact for amino acid availability appeared to be greater in the gut and liver than in the leg in these canine studies. While further study is necessary for definitive conclusions, these data lead to the speculation that increased amino acid availability during exercise may be preferentially directed toward splanchnic tissues, while increased amino acid availability after exercise may be directed toward insulin-sensitive tissues, such as muscle.

In conclusion, whereas the postexercise intake of either no nutrients or a nutrient supplement without protein resulted in a net postexercise loss of whole-body and leg protein, the addition of protein to the nutrient supplement resulted in net whole-body and leg protein gain. This finding suggests that the availability of amino acids is more important than the availability of energy for postexercise repair and synthesis of muscle proteins. Further studies are warranted to determine the optimal protein level required for maximal net synthesis of whole-body and muscle protein stores under these conditions. In addition, future studies are necessary to determine the interactions of postexercise protein supplementation with factors such as the type, intensity, and duration of exercise performed, the prior exercise training status, protein and energy intake before exercise, and the type and form of the nutrient supplements. (12,30,38).

The aid of the personnel and use of the facilities at the General Clinical Research Center (RR00095), Clinical Nutrition Research Unit (DK-26657), and Diabetes Research and Training Center (DK-20593) at Vanderbilt University are greatly appreciated. In addition, we thank the Pharmavite Corporation (Mission Hills, CA) for their support. We appreciate the outstanding technical expertise of the personnel in the Cardiac Catheterization Laboratory at Vanderbilt University Medical Center in completing this research. Furthermore, we deeply appreciate the expert technical and analytical assistance of Suzan Vaughan, Mu Zheng, Donna Schot, and Wanda Snead. Finally, we want to thank the subjects for their loyal compliance and participation.

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