Am J Physiol Endocrinol Metab
280: E982–E993, 2001.

Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis

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Received 30 August 2000; accepted in final form 14 February 2001

Levenhagen, Deanna K., Jennifer D. Gresham, Michael G. Carlson, David J. Maron, Myfanwy J. Borel, and Paul J. Flakoll. Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. *Am J Physiol Endocrinol Metab* 280: E982–E993, 2001.—Although the importance of postexercise nutrient ingestion timing has been investigated for glycogen metabolism, little is known about similar effects for protein dynamics. Each subject ($n = 10$) was studied twice, with the same oral supplement (10 g protein, 8 g carbohydrate, 3 g fat) being administered either immediately (EARLY) or 3 h (LATE) after 60 min of moderate-intensity exercise. Leg blood flow and circulating concentrations of glucose, amino acids, and insulin were similar for EARLY and LATE. Leg glucose uptake and whole body glucose utilization (D-[6,6-$^2H_2$]glucose) were stimulated threefold and 44%, respectively, for EARLY vs. LATE. Although essential and nonessential amino acids were taken up by the leg in EARLY, they were released in LATE. Although proteolysis was unaffected, leg (L-[*ring*-$^2H_5$]phenylalanine) and whole body (L-[1-^{13}C]leucine) protein synthesis were elevated threefold and 12%, respectively, for EARLY vs. LATE, resulting in a net gain of leg and whole body protein. Therefore, similar to carbohydrate homeostasis, EARLY postexercise ingestion of a nutrient supplement enhances accretion of whole body and leg protein, suggesting a common mechanism of exercise-induced insulin action.

synthesis; deposition; amino acids; exercise

SYNTHESIS OF GLYCOGEN AND PROTEIN is essential for skeletal muscle recovery from the catabolic events of exercise. Glycogen is broken down and used during exercise as energy for muscle contraction. Because of the damage of muscle proteins and the diversion of amino acids and energy away from the events of protein synthesis during exercise, there also is a need for increased postexercise protein repair and synthesis. It has been well established that the timing of carbohydrate intake after exercise significantly influences postexercise carbohydrate homeostasis and recovery (22, 43). For example, when carbohydrate supplements were provided to twelve male cyclists several minutes after exercise, muscle glycogen storage was more rapid than when the same supplement was consumed 2 h after exercise (22). Although the importance of timing for carbohydrate intake after exercise has been demonstrated, little information exists as to the influence of timing for postexercise protein intake.

Exercise induces a greater sensitivity and responsiveness to the events controlled by insulin (44). In vitro and in vivo studies have demonstrated increased insulin-mediated glucose uptake in response to muscle contraction (6, 9, 10, 28, 34, 35, 48). Furthermore, exercise has been shown to benefit patients with non-insulin-dependent diabetes mellitus by lowering postexercise blood glucose, lowering basal and postprandial insulin concentrations, improving insulin sensitivity, and reducing glycosylated hemoglobin levels (10). These observations potentially could be explained by increased blood flow (9) enhancing nutrient availability to tissues for the same magnitude of insulin. However, even at constant receptor and nutrient concentrations, insulin-stimulated glucose and amino acid transport have been shown to be increased by muscular contraction (48).

A vital role also has been demonstrated for insulin in the regulation of protein dynamics (12). Therefore, if exercise stimulates insulin sensitivity and responsiveness, the timing of postexercise nutrient supplementation may alter protein dynamics. However, the influence of postexercise nutrient supplementation on protein dynamics in humans is undefined. Therefore, the objective of this investigation was to examine how the timing of postexercise nutrient ingestion affects whole body and leg protein dynamics in healthy adults.

SUBJECTS AND METHODS

Subject selection. Ten healthy adult subjects (5 males and 5 females), aged 20–41 yr and within 25% of ideal body weight based on Metropolitan Life Insurance Company tables (27), were selected (see Table 1) 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

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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. Females were studied between 3 and 10 days 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 (24). A maximal exercise test was conducted in which each subject used a recumbent stationary cycle (Ergometrics 800, Ergoline, Bandhagen, Sweden) to perform incremental (+25 W/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/min over the previous work rate were the criteria used for maximal $\dot{V}O_2$ ($\dot{V}O_{2\,max}$). Energy expenditure (EE) was determined during rest and during exercise using indirect calorimetry (Sensormedics 2900 Metabolic Cart, Yorba Linda, CA).

Metabolic study protocol. For 3 days before the metabolic studies, subjects received their meals from the dietary kitchen of the GCRC to maintain consistency between preexercise body nutrient stores. Energy intake was kept at maintenance levels on the basis of the Harris Benedict equation and each subject's gender, height, weight, and activity level.

On metabolic study days, subjects were admitted to the GCRC after 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 also were 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 to 55°C for complete arterialization of blood samples (1).

After a collection of blood and breath samples to determine isotopic backgrounds (−210 min), a bolus infusion of [^{13}C]$NaHCO_3$ (0.24 mg/kg), D-[6,6-$^2H_2$]glucose (3.6 mg/kg), L-[1-^{13}C]leucine (14.4 μmol/kg), and L-[*ring*-$^2H_5$]phenylalanine (3.6 μmol/kg) was given to prime the carbon dioxide, glucose, leucine, and phenylalanine pools, respectively. Subsequently, a continuous infusion of D-[6,6-$^2H_2$]glucose (0.06 mg·kg^{-1}·min^{-1}), L-[1-^{13}C]leucine (0.24 μmol·kg^{-1}·min^{-1}), L-[*ring*-$^2H_5$]phenylalanine (0.06 μmol·kg^{-1}·min^{-1}), and [$^2H_5$]glycerol (0.12 μmol·kg^{-1}·min^{-1}) was initiated and continued throughout the study. Each metabolic study consisted of four periods (Fig. 1): *1*) a 120-min equilibration period; *2*) a 30-min basal sampling period; *3*) a 60-min exercise period; and *4*) either a 180-min or 360-min recovery period. During the 60-min exer-

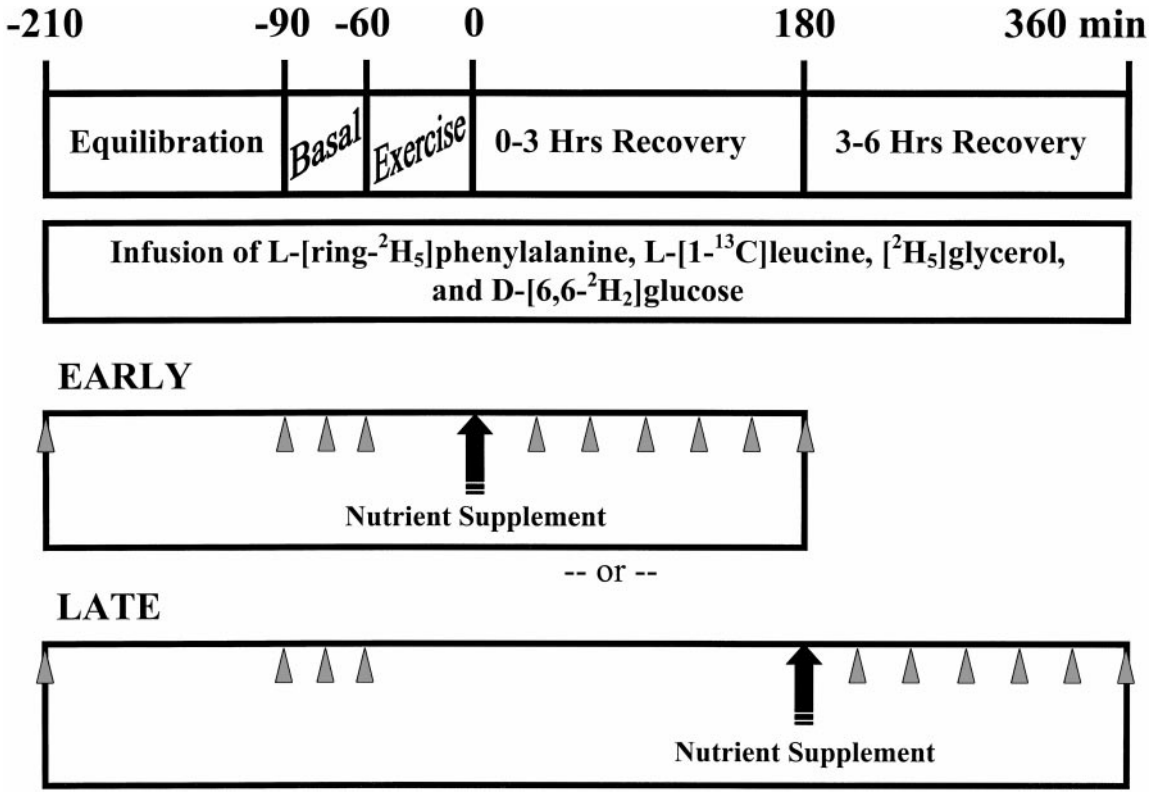

Fig. 1. The experimental design consisted of equilibration, basal, exercise, and postexercise recovery periods. During the recovery period of either 180 or 360 min, isotopic tracers of phenylalanine, leucine, glucose, and glycerol were infused to measure leg and whole body kinetics. Each subject was studied twice, and an oral nutrient supplement (10 g protein, 8 g carbohydrate, and 3 g fat) was administered either immediately after exercise (EARLY) or 3 h after exercise (LATE). Blood and breath samples (▲) were taken during the basal period and for 3 h after nutrient intake.

cise period, subjects exercised on a recumbent bicycle at 60% of $\dot{V}O_{2\,max}$ 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 version 3.0; UFI, Morro Bay, CA) (31). Finally, carbon dioxide production, $\dot{V}O_2$, and EE were determined throughout each period by indirect calorimetry (Sensormedics 2900 Metabolic Cart, Palo Alto, CA).

Experimental design. Each subject was studied twice. A nutrient supplement containing 10 g protein, 8 g carbohydrate, and 3 g lipid (Jogmate; Pharmavite, Mission Hills, CA) was administered either immediately (EARLY) or 3 h after the conclusion of exercise (LATE). The majority of protein, carbohydrate, and fat in the nutrient supplement was derived from casein, regular sugar, and milk fat, respectively. The order of treatment administration was random. A 4-wk "washout" period was maintained between metabolic studies to allow isotopic tracer clearance and to ensure that the female participants were in the follicular phase of their menstrual cycle. Subjects were instructed to maintain daily exercise activity, dietary intake, and a constant 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, Elkton, MD). A 1-ml aliquot of whole blood from each sampling site was deproteinized with 3 ml of 4% perchloric acid for determination with enzymatic methods of whole blood lactate, glycerol, and glutamine concentrations (25, 26). In addition, 3 ml of blood were 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 (17). The remaining blood was spun in a refrigerated (4°C) desktop centrifuge (Beckman Instruments, Fullerton, CA) at 3,000 rpm 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, Fullerton, CA).

Immunoreactive insulin was determined in plasma with a double-antibody system (29). 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 (29) 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). A Clinical Assays Gammacoat Radioimmunoassay kit (Travenol-GenTech, Cambridge, MA) was used to measure plasma cortisol concentrations. Plasma amino acid concentrations were determined by reversed-phase HPLC after derivatization with phenylisothiocyanate (20). Individual amino acids were also placed into groups for analysis purposes. These groups included 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.

After 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 gas chromatography-mass spectrometry (GC-MS) according to the method of Bier et al. (5). Plasma enrichments of [^{13}C]leucine, [^{13}C]ketoisocaproate (KIC), and [*ring*-$^2H_5$]phenylalanine were determined using 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 (30). After drying under nitrogen gas, both the keto and amino acid fractions were derivatized (37) with *N*-methyl-*N*-(*t*-butyldimethylsilyl)-trifluoroacetamide containing 1% *t*-butyldimethylchlorosilane (MtBSTFA + 1% *t*-BDMCS; Regis Technologies, 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. For determination of [$^2H_5$]glycerol enrichment, plasma was deproteinized with 4% perchloric acid, and the supernatant was passed over cation and anion exchange resins. The eluate was dried overnight at 50°C, the glycerol fraction was derivatized with MtBSTFA + 1% *t*-BDMCS, and the derivatized samples were analyzed by GC-MS for determination of plasma glycerol enrichment (13). Breath $^{13}CO_2$ enrichment was measured by isotope ratio-mass spectrometry (Metabolic Solutions, Nashua, NH) (38).

Calculations. Net skeletal muscle protein balance (synthesis-breakdown) was determined by dilution and enrichment of phenylalanine across the hindlimb as described by Gelfand and Barrett (16). Because phenylalanine is neither synthesized nor metabolized by skeletal muscle, the appearance rate (R_a) of unlabeled phenylalanine reflects muscle protein breakdown, whereas the rate of disappearance (R_d) of labeled phenylalanine estimates muscle protein synthesis (16). Phenylalanine R_d 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 (16, 47). Net phenylalanine R_a was calculated by subtracting the net arteriovenous balance of phenylalanine across the hindlimb from the phenylalanine R_d (16, 47). Rates of skeletal muscle protein breakdown and net synthesis were determined from the phenylalanine R_d and R_a, with the assumption that 3.8% of skeletal muscle protein is comprised of phenylalanine.

Steady-state rates of whole body glucose disappearance (R_d) were calculated by dividing the D-[6,6-$^2H_2$]glucose infusion rate by the plasma [$^2H_2$]glucose enrichment (47). With this method, the deuterium label is lost during the phospho*enol*pyruvate cycle, is diluted into the total body water pool, and is not recycled. The steady-state rates of whole body leucine appearance (R_a; an estimate of whole body protein breakdown) were calculated by dividing the [^{13}C]leucine infusion rate by the plasma [^{13}C]KIC enrichment (47). Plasma KIC provides a better estimate of intracellular leucine enrichment than does plasma leucine enrichment because KIC is derived from intracellular leucine metabolism (47). The endogenous leucine R_a was calculated by subtracting the exogenous leucine R_a from the total leucine R_a. Exogenous leucine R_a from the nutrient supplement was calculated with the assumption that the protein consumed was 7.8% leucine and that the plasma leucine R_a from the exogenous supplement was constant (33.03 μmol/min) over the entire 3-h

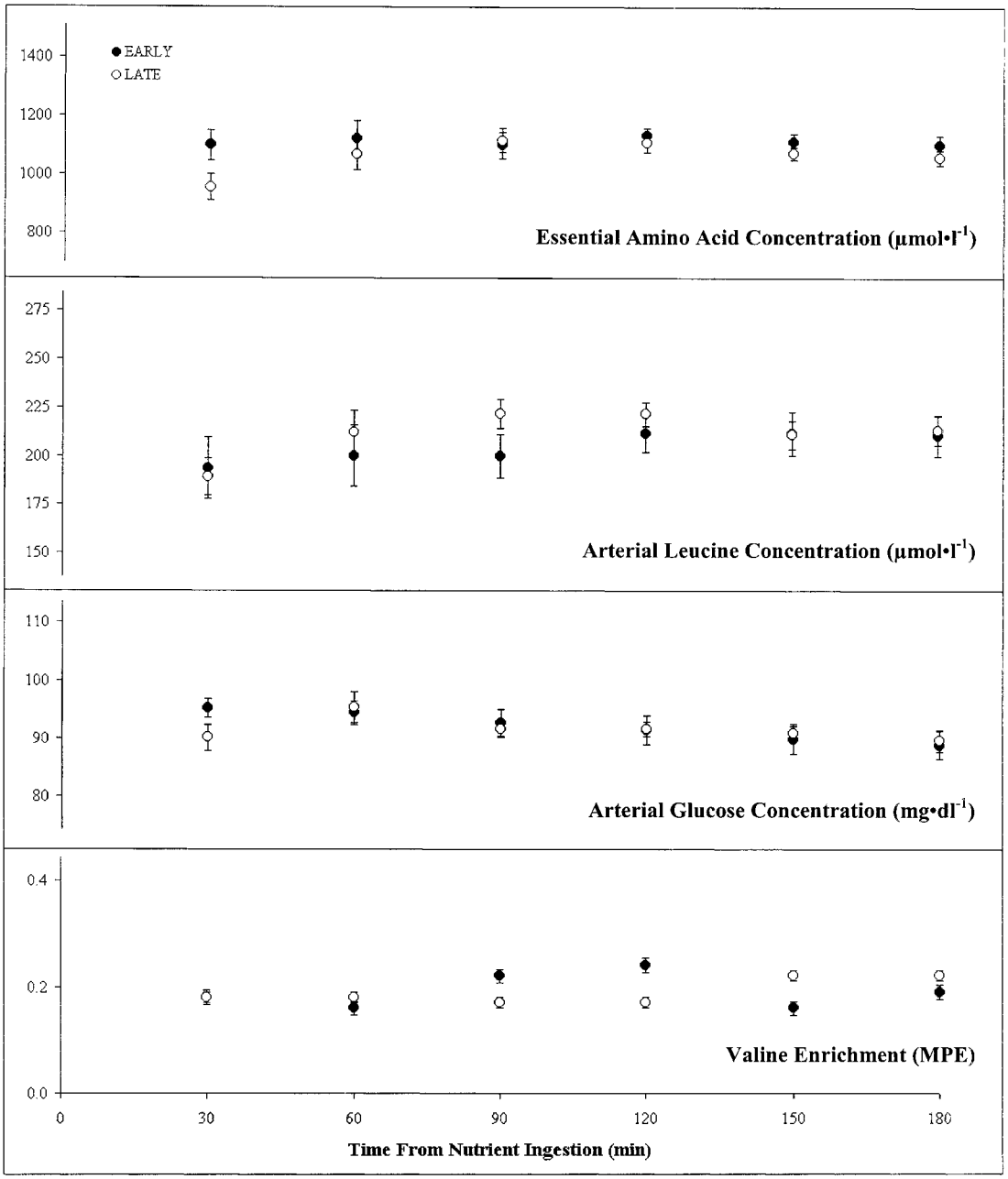

Fig. 2. Point-by-point timing of arterial enrichment of valine and arterial concentrations of glucose, leucine, and essential amino acids for the 3 h after postexercise nutrient ingestion. MPE, mole percent excess.

period in both EARLY and LATE. This assumption was validated by two lines of evidence. First, when 100 mg of L-[$^2H_8$]valine were administered with the supplement, arterial L-[$^2H_8$]valine enrichments were similar at corresponding time points in both EARLY and LATE (mean = 0.19 ± 0.01 vs. 0.19 ± 0.01%). A second line of evidence deals with the concentration of hormones and metabolites. For example, plasma leucine increased within 30 min after supplement intake and remained similarly elevated for 3 h after intake in both EARLY and LATE (Fig. 2). Breath $^{13}CO_2$ production was determined by multiplying the total CO_2 production rate by the breath $^{13}CO_2$ enrichment (47). 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 R_d, an estimate of whole body protein synthesis, was determined indirectly by subtracting leucine oxidation from total leucine R_a. Rates of whole body protein breakdown, amino acid oxidation, and protein synthesis were calculated from the endogenous leucine R_a, the leucine oxidation rate, and the nonoxidative leucine R_d, respectively, assuming that 7.8% of whole body protein is comprised of leucine (15). Because glycerol released during lipolysis cannot be reincorporated into triacylglycerol in the adipose cell because of the lack of glycerol kinase activity, the R_a of endogenous glycerol multiplied by 3 was used to determine rates of whole body lipolysis (13). The endogenous glycerol R_a was calculated by dividing the [$^2H_5$]glycerol infusion rate by the plasma glycerol enrichment and subtracting the exogenous glycerol infusion rate (47).

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 due to amino acid oxidation was subtracted from the total EE, and the net rates of carbohydrate and lipid oxidation were calculated on the basis of the nonprotein respiratory quotient (23). The assumptions and limitations of calculating net substrate oxidation on the basis of indirect calorimetry measurements have been reviewed previously (23). 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 period (basal and the 3-h postexercise intake periods) were calculated. Values presented in the text and Figs. 1–7 are means ± SE for each period. No differences were noted between treatments during the basal periods, and thus the data for this period will be reported as the overall means ± SE. Because each subject was studied twice, 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 <0.05 was required to reject the null hypothesis of no difference between the means. Because gender groups responded to treatments similarly, data are reported as means of all subjects combined.

RESULTS

Subject characteristics. The ten healthy adult subjects (5 females and 5 males) were 20–41 yr of age and within 25% of ideal body weight (Table 1). Subjects were recreational athletes with an average $\dot{V}O_{2\,max}$ of 33.2 ± 4.0 and 44.9 ± 2.9 ml·kg^{-1}·min^{-1} for females and males, respectively. Whereas body mass index (BMI) was similar between females and males, average body fat was 30.5 ± 3.4 and 17.0 ± 2.5% for females and males, respectively.

Metabolite and hormone concentrations. Circulating glucose, lactate, and glycerol concentrations were not different whether the nutrient supplement was given immediately after exercise (EARLY) or 3 h after exercise (LATE; Table 2). In addition, the concentrations of plasma insulin, glucagon, growth hormone, epinephrine, and norepinephrine did not differ between EARLY and LATE. In contrast, plasma cortisol concentration was 18% greater during EARLY than during LATE.

Whereas amino acid concentrations increased after the supplement was given in both instances, the concentrations of the individual plasma amino acids were unaffected by the timing of the intake of the nutrient supplement containing protein, with the exception of glutamine. In the case of glutamine, the concentration was 19% greater when the supplement was given EARLY vs. LATE. In addition, although the plasma concentrations of BCAA and EAA were not different between EARLY and LATE, the significant difference in glutamine concentrations causes similar differences in NEAA and TAA between EARLY and LATE (Table 3).

Table 2. *Arterial metabolite and hormone concentrations*

	Basal	EARLY	LATE
Glucose, mg/dl	89.7 ± 1.2	92.0 ± 2.1	91.5 ± 1.6
Lactate, μmol/l	662 ± 69	746 ± 106	564 ± 68
Glycerol, μmol/l	78.4 ± 6.9	78.6 ± 12.2	78.7 ± 10.0
Insulin, μU/ml	3.7 ± 0.4	5.1 ± 1.0	4.2 ± 0.5
Glucagon, ng/l	43.4 ± 1.6	55.7 ± 4.4	47.9 ± 2.7
Cortisol, nmol/l	8.5 ± 1.0	10.2 ± 1.4	6.2 ± 1.0*
Growth hormone, ng/ml	1.4 ± 0.4	1.8 ± 0.40	2.8 ± 0.9
Epinephrine, pmol/l	38.6 ± 8.0	46.0 ± 11.9	47.6 ± 10.7
Norepinephrine, nmol/l	200 ± 35	139 ± 30	188 ± 34

An oral nutrient supplement was administered either immediately after exercise (EARLY) or 3 h postexercise (LATE). Time points for each period were averaged for each subject before the overall period mean ± SE was calculated. Values are reported as means ± SE for each period. Basal measurements are overall means for both protocols, because there were no significant differences between basal measurements by treatment. *$P < 0.05$, EARLY vs. LATE.

Net glucose and amino acid balance across the leg. The mean leg blood flow measurements (ml·min^{-1}·100 ml^{-1}) during basal (6.1 ± 0.8), EARLY (6.0 ± 0.9), or LATE (5.2 ± 0.4) were not significantly different. Net leg glucose uptake was 32.5 μg·min^{-1}·100 ml^{-1} during the basal period. When the oral postexercise supplement was given 3 h after the completion of exercise (LATE), there was not a significant increase from basal for net leg glucose uptake (Fig. 3). However, when the supplement was given immediately after exercise (EARLY), net leg glucose uptake was 3.5 times greater than basal or LATE.

During the basal period, there was a net uptake of aspartate, glutamate, hydroxyproline, ornithine, serine, and taurine by the leg and a net release of BCAA, EAA, NEAA, and TAA (Table 4). Net balance was significantly more positive during EARLY, compared with LATE, for isoleucine, leucine, lysine, phenylalanine, proline, valine, BCAA, EAA, NEAA, and TAA. Therefore, as a result of these changes in individual amino acid balance, there was a net leg uptake of BCAA, EAA, NEAA, and TAA with EARLY, which contrasted with a net release of BCAA, EAA, NEAA, and TAA in LATE.

Leg fractional extraction of phenylalanine also was significantly affected by the timing of the oral postex-

Table 1. *Subject data*

		Age, yr	Weight, kg	Height, cm	BMI, kg/m^2	Body Fat, %	$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}
Total	10	31.5 ± 2.2	76.0 ± 4.3	172.7 ± 3.0	25.4 ± 1.1	23.7 ± 3.0	39.1 ± 3.0
Females	5	32.8 ± 3.6	69.1 ± 3.6	166.1 ± 3.1	25.1 ± 1.7	30.5 ± 3.4	33.2 ± 4.0
Males	5	30.2 ± 3.0	80.8 ± 6.8	179.2 ± 2.8	25.7 ± 1.5	17.0 ± 2.5	44.9 ± 2.9

Values are means ± SE. BMI, body mass index; $\dot{V}O_{2max}$, maximal oxygen consumption.

Table 3. *Plasma arterial amino acid concentrations*

	Basal	EARLY	LATE
1-Methylhistidine	5.1 ± 1.1	5.8 ± 1.5	3.4 ± 0.7
3-Methylhistidine	8.3 ± 1.0	7.3 ± 0.9	9.3 ± 2.3
Alanine	250.4 ± 17.4	257.8 ± 21.9	216.1 ± 23.4
Arginine	77.4 ± 3.0	79.6 ± 5.0	84.1 ± 4.9
Asparagine	65.0 ± 3.9	70.4 ± 4.3	68.2 ± 7.1
Aspartate	5.9 ± 0.4	7.2 ± 0.7	6.9 ± 0.6
Citrulline	33.6 ± 1.4	31.5 ± 2.1	32.6 ± 2.0
Glutamate	45.3 ± 3.0	55.3 ± 5.6	51.2 ± 5.4
Glutamine	433.4 ± 19.3	456.4 ± 26.5	382.8 ± 20.0*
Glycine	229.2 ± 14.2	219.3 ± 22.0	211.4 ± 18.2
Histidine	65.1 ± 2.1	72.7 ± 3.1	72.6 ± 3.0
Hydroxyproline	5.8 ± 0.4	6.0 ± 0.6	5.3 ± 0.5
Isoleucine	44.8 ± 2.5	70.9 ± 6.7	67.7 ± 3.2
Leucine	149.5 ± 5.2	204.4 ± 11.2	211.3 ± 6.2
Lysine	156.6 ± 6.4	166.1 ± 8.5	165.5 ± 6.7
Methionine	22.4 ± 1.3	26.2 ± 1.9	25.8 ± 2.1
Ornithine	49.5 ± 2.1	56.1 ± 2.2	51.3 ± 2.6
Phenylalanine	55.5 ± 2.1	74.7 ± 2.8	68.1 ± 3.3
Proline	154.7 ± 6.9	197.0 ± 12.7	175.0 ± 5.8
Serine	96.0 ± 3.8	101.0 ± 6.3	98.8 ± 3.8
Taurine	77.8 ± 6.7	96.4 ± 11.8	89.6 ± 11.0
Threonine	124.3 ± 7.2	139.4 ± 11.8	120.4 ± 10.9
Tryptophan	35.4 ± 1.7	43.0 ± 2.5	41.6 ± 2.3
Tyrosine	47.3 ± 2.8	68.0 ± 4.2	57.9 ± 4.5
Valine	183.6 ± 7.4	232.0 ± 13.0	205.8 ± 9.6
BCAA	377.9 ± 14.1	507.4 ± 28.9	484.7 ± 17.3
EAA	914.6 ± 25.4	1,109.2 ± 33.2	1,062.6 ± 28.6
NEAA	1,507.0 ± 30.6	1,635.5 ± 41.4	1,459.7 ± 39.3*
TAA	2,421.6 ± 45.4	2,744.7 ± 59.4	2,522.3 ± 55.7*

Values (μmol/l) are means ± SE for each period. 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. Basal measurements are overall means for both protocols, because there were no significant differences between basal measurements by treatment. *$P < 0.05$, EARLY vs. LATE.

Table 4. *Amino acid balance across the leg*

	Basal	EARLY	LATE
1-Methylhistidine	0.8 ± 0.5	1.1 ± 1.4	0.0 ± 0.6
3-Methylhistidine	−1.0 ± 1.5	0.6 ± 1.1	−0.2 ± 0.7
Alanine	−147.7 ± 24.7	−36.6 ± 66.4	−92.6 ± 13.1
Arginine	−40.3 ± 4.4	−17.3 ± 12.3	−28.8 ± 4.4
Asparagine	−23.6 ± 3.2	−5.9 ± 9.1	−18.2 ± 2.3
Aspartate	2.9 ± 1.1	4.9 ± 2.0	3.0 ± 1.1
Citrulline	−6.7 ± 2.1	2.2 ± 3.0	5.2 ± 7.4
Glutamate	82.0 ± 14.6	68.5 ± 34.0	76.0 ± 17.6
Glutamine	−200.2 ± 34.8	−258.8 ± 59.1	−184.4 ± 26.7
Glycine	−92.3 ± 9.5	−36.2 ± 35.6	−70.8 ± 9.4
Histidine	−18.2 ± 2.8	−8.6 ± 7.1	−11.3 ± 2.9
Hydroxyproline	2.8 ± 0.6	3.5 ± 1.3	1.6 ± 0.4
Isoleucine	−17.4 ± 2.3	6.4 ± 3.6	−3.6 ± 1.9*
Leucine	−27.9 ± 4.0	16.1 ± 6.4	−7.5 ± 4.4*
Lysine	−35.6 ± 5.4	−0.4 ± 10.0	−22.9 ± 2.8*
Methionine	−8.6 ± 1.2	−0.6 ± 2.7	−4.8 ± 0.8
Ornithine	8.5 ± 2.6	12.4 ± 5.0	7.5 ± 1.8
Phenylalanine	−11.2 ± 1.8	13.9 ± 2.5	−5.5 ± 1.5*
Proline	−37.8 ± 4.3	11.5 ± 6.9	−7.2 ± 4.5*
Serine	11.9 ± 4.0	12.1 ± 4.4	11.9 ± 4.2
Taurine	68.8 ± 17.5	91.7 ± 33.8	61.9 ± 11.0
Threonine	−40.6 ± 14.5	−9.9 ± 15.8	−28.12 ± 7.5
Tryptophan	−0.3 ± 3.2	1.0 ± 2.7	1.2 ± 1.5
Tyrosine	−16.9 ± 2.4	1.4 ± 4.4	−7.3 ± 1.9
Valine	−30.7 ± 4.8	18.7 ± 6.0	−1.5 ± 4.2*
BCAA	−75.9 ± 10.3	41.3 ± 15.6	−12.6 ± 10.0*
EAA	−230.7 ± 32.5	19.5 ± 55.6	−112.8 ± 21.7*
NEAA	−142.1 ± 32.7	137.1 ± 73.6	−24.3 ± 16.8*
TAA	−372.8 ± 59.6	156.6 ± 118.0	−137.1 ± 27.1*

Values (nmol·100 ml leg volume^{-1}·min^{-1}) are reported as means ± SE for each period. Basal measurements are overall means for both protocols, because there were no significant differences between them by treatment. A positive balance indicates a net uptake of amino acids, and a negative balance indicates a net release of amino acids across the leg. *$P < 0.05$, EARLY vs. LATE.

ercise nutrient supplement. The mean leg fractional extraction of phenylalanine during the basal period was 9.8 ± 1.1%. When the oral postexercise supplement was given EARLY, mean leg fractional extraction of phenylalanine was increased to 17.0 ± 2.4%. However, when the supplement was administered 3 h after exercise, mean leg fractional extraction of phenylalanine was only 7.6 ± 1.4%.

Leg protein dynamics. Leg protein synthesis and net protein balance were both significantly affected by the timing of an oral postexercise nutrient supplement (Fig. 4). Leg protein synthesis was more than 3 times greater for EARLY compared with LATE. Leg protein breakdown, however, was not significantly different

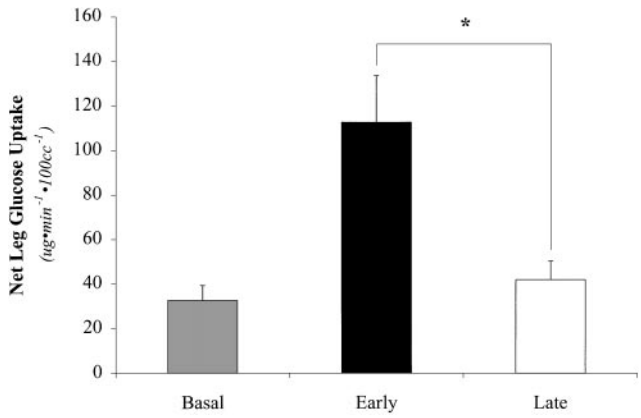

Fig. 3. Net leg glucose uptake for 10 subjects given an oral nutrient supplement either immediately after exercise (EARLY) or 3 h postexercise (LATE). *Significant difference ($P < 0.05$), EARLY vs. LATE.

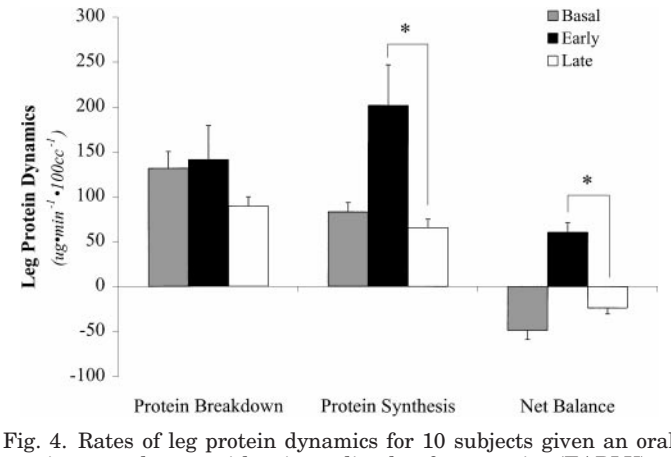

Fig. 4. Rates of leg protein dynamics for 10 subjects given an oral nutrient supplement either immediately after exercise (EARLY) or 3 h postexercise (LATE). *Significantly different ($P < 0.05$), EARLY vs. LATE.

between the two supplemental periods. Thus there was a net gain of protein in the leg for EARLY but a net loss of leg protein for LATE, with an absolute difference of 84.3 $\mu g \cdot min^{-1} \cdot 100\ mg^{-1}$ (+60.5 vs. −23.8 $\mu g \cdot min^{-1} \cdot 100\ ml^{-1}$, respectively).

Whole body protein dynamics. Whole body proteolysis, as estimated by endogenous leucine R_a, was not significantly affected by the timing of a postexercise nutrient supplement (2.52 ± 0.17 $mg \cdot kg^{-1} \cdot min^{-1}$ EARLY vs. 2.33 ± 0.15 $mg \cdot kg^{-1} \cdot min^{-1}$ LATE; Fig. 5). However, whole body protein synthesis was 12% greater (2.69 ± 0.13 vs. 2.40 ± 0.11 $mg \cdot kg^{-1} \cdot min^{-1}$) for EARLY vs. LATE. Thus there was a net whole body release of protein in the postabsorptive basal period, but there was a net uptake (protein balance was positive) when an oral nutrient supplement was given after exercise, regardless of timing. Net whole body protein deposition was significantly greater (0.17 ± 0.15 vs. 0.07 ± 0.07 $mg \cdot kg^{-1} \cdot min^{-1}$) when the oral postexercise supplement was given immediately after exercise vs. 3 h later.

Whole body glucose and glycerol kinetics. Whole body glucose utilization was 1.97 ± 0.07 $mg \cdot kg^{-1} \cdot min^{-1}$ during the basal period. Whole body glucose utilization was 44% greater when the oral postexercise supplement was given immediately after exercise vs. 3 h later (2.40 ± 0.11 $mg \cdot kg^{-1} \cdot min^{-1}$ EARLY vs. 1.67 ± 0.12 $mg \cdot kg^{-1} \cdot min^{-1}$ LATE). Whole body glycerol flux (7.82 ± 1.18 $\mu mol \cdot kg\ fat^{-1} \cdot min^{-1}$ EARLY vs. 6.16 ± 1.04 $\mu mol \cdot kg\ fat^{-1} \cdot min^{-1}$ LATE) was unaffected by the timing of the oral supplement postexercise.

Energy metabolism. There was no change in total EE, normalized to body weight, in EARLY vs. LATE (Table 5). In addition, the proportion of energy derived from protein, carbohydrate, or lipid was not different between the treatments. Whole body amino acid, carbohydrate, and fat oxidation were also unaffected by the timing of the oral postexercise supplement intake.

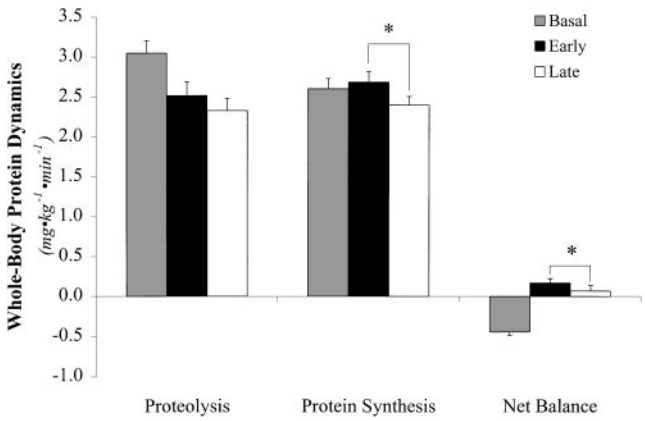

Fig. 5. Rates of whole body protein dynamics for 10 subjects given an oral nutrient supplement either immediately after exercise (EARLY) or 3 h postexercise (LATE). *Significantly different ($P < 0.05$), (EARLY) vs. (LATE).

Table 5. *Energy metabolism*

	Basal	EARLY	LATE
Total energy expenditure, $kcal \cdot kg^{-1} \cdot day^{-1}$	20.5 ± 1.0	22.6 ± 1.0	21.3 ± 1.2
Whole body amino acid oxidation, %	11.5 ± 0.7	13.8 ± 0.5	16.3 ± 0.8
Whole body carbohydrate oxidation, %	18.3 ± 2.6	4.7 ± 4.8	6.2 ± 2.7
Whole body fat oxidation, %	70.3 ± 3.0	81.5 ± 5.0	77.5 ± 2.7
Whole body amino acid oxidation, $mg \cdot kg^{-1} \cdot min^{-1}$	0.44 ± 0.04	0.60 ± 0.05	0.69 ± 0.06
Whole body carbohydrate oxidation, $mg \cdot kg^{-1} \cdot min^{-1}$	0.71 ± 0.12	0.21 ± 0.21	0.27 ± 0.13
Whole body fat oxidation, $mg \cdot kg^{-1} \cdot min^{-1}$	1.18 ± 0.06	1.55 ± 0.11	1.42 ± 0.08

Values are means ± SE for each period. Basal measurements are the overall means for both protocols, because there were no significant differences between them by treatment. *$P < 0.05$, EARLY vs. LATE.

DISCUSSION

Although several studies have focused on the timing of carbohydrate administration after exercise (21, 22, 43), little is known concerning the timing of postexercise nutrient supplementation and protein homeostasis. Therefore, the focus of the present study was to examine whether the timing of postexercise nutrient ingestion has an impact on whole body and leg protein homeostasis in healthy adults. Each subject in the present study was tested twice, with the nutrient supplement (10 g protein, 8 g carbohydrate, 3 g fat) being consumed either immediately (EARLY) or 3 h (LATE) after exercise. Whether taken immediately or 3 h after exercise, consumption of this supplement resulted in similar plasma glucose and insulin concentrations in the plasma for the 3 h after ingestion. Furthermore, concentrations of all EAA and NEAA, with the exception of glutamine, were similar during EARLY and LATE periods. Leg blood flow and all other hormones and metabolites measured, with the exception of cortisol, were also similar between the two postexercise periods. Although the substrate and hormonal milieu were similar whether the supplement was given immediately or 3 h after exercise, the leg uptake of glucose and amino acids was greater when the supplement was given immediately after exercise.

With the greater net uptake of amino acids and glucose during EARLY, more substrate and energy were available within the leg for protein synthesis. Although leg proteolysis was not significantly different between the two treatments, leg protein synthesis was increased more than threefold for EARLY vs. LATE. Hence, there was a net accretion of leg protein when nutrients were ingested immediately after exercise, which was in contrast to the net loss of leg protein when nutrients were given 3 h after exercise. These results confirm the conclusions of Okamura et al. (32). In that study, a 2-h intraportal infusion of glucose and amino acids was given to canines either immediately or

2 h after treadmill exercise. The earlier nutrient infusion increased leg protein synthesis by ~35% but did not alter leg proteolysis. In a longer-term study (10 wk), exercising rats were fed a mixed meal either immediately or 4 h after exercise (42). The rats that were fed immediately after exercise for 10 wk had increased total hindlimb muscle weight and decreased perirenal, epididymal, and mesenteric adipose tissue weight. These findings support the more acute studies, such as the present study and that of Okamura et al.

Whole body protein dynamics in the present study followed the same pattern as leg protein dynamics, with rates of proteolysis being similar but rates of protein synthesis and net protein deposition both being greater during EARLY vs. LATE. Previously, it has been demonstrated that gastrointestinal tract tissue protein homeostasis is in a net catabolic state, with protein breakdown increased by 40–50% during exercise (19, 46). Therefore, these tissues also could potentially benefit from an environment that would promote net protein synthesis. However, it is not possible in this study to speculate on changes in protein synthesis for nonexercising muscle tissues or specific nonskeletal muscle tissues (e.g., heart, kidney, or liver vs. intestinal tract).

The timing of carbohydrate administration after exercise has been studied extensively in recent years. Ivy et al. (22) demonstrated that a 25% glucose polymer supplement given immediately postexercise dramatically increased rates of glycogenesis, but the rate of glycogen storage was markedly decreased if ingestion was delayed for 2 h postexercise. The results from the present study confirm these previous conclusions, because leg glucose uptake and whole body glucose utilization were considerably greater when the nutrient supplement was given immediately postexercise. Glycogen in exercised muscle can be replenished within 24 h after exercise with the consumption of a high (500–600 g) carbohydrate diet (4, 8). Glycogen resynthesis is maximized at 5–8 mmol·kg wet weight of muscle^{-1}·h^{-1} by providing 0.7–1.5 g of glucose per kg body weight every 2 h for up to 6 h after exhaustive exercise (14, 21). Furthermore, if a high-carbohydrate diet is continued for 3 days, muscle glycogen content rises above preexercise levels (40). These observations have fostered the concept referred to as "carbohydrate loading".

Whereas exercise increases lipoprotein lipase activity in muscle but not in adipose tissue (39), plasma triacylglycerol concentrations are altered depending on the timing of sucrose consumption relative to exercise (43). The effects of when a postexercise nutrient supplement is ingested on lipid metabolism in rats also were reported by Suzuki et al. (42). In their study, exercising rats were given a meal either immediately or 4 h after exercise, and lipoprotein lipase activity in the soleus was 70% greater in the group given a meal immediately after exercise (42). Thus more energy after exercise would be directed away from fat stores and toward muscle stores, thereby potentially facilitating muscle protein and glycogen synthesis.

Studies examining the timing of postexercise nutrient supplementation suggest that repletion of leg nutrient stores is not determined by nutrient intake alone. Insulin action also appears to play an important role in controlling postexercise nutrient repletion. Insulin decreases circulating concentrations of glucose, amino acids, and lipids, promotes inward cellular transport of glucose and amino acids, enhances glycogen synthesis, stimulates adipose cells to synthesize and store fat, and promotes protein accretion (12). Although circulating insulin is reduced during exercise, muscle glucose transport (11, 18) and utilization (44) are still enhanced with exercise as a result of improved insulin sensitivity and responsiveness. Therefore, the role of exercise-stimulated insulin action may be critical to the observation that the rate of glycogen resynthesis is greater the earlier that carbohydrate intake is given after exercise. Although the exact time course of this enhanced insulin action has not been defined, Burstein et al. (7) demonstrated that a majority of this effect is lost after 60 h. The present study suggests that the peak stimulation in whole body glucose utilization and leg glucose uptake occurred within the 1st h after immediate postexercise consumption of the nutrient supplement (Fig. 6). These values steadily decreased to near basal over the subsequent 3 h. Interestingly, this response occurred during the first 3 h after exercise despite a lingering elevation in circulating cortisol, which is known to blunt the response of carbohydrate metabolism to insulin (36). When the same supplement was administered 3 h postexercise, there was no change in these measures of carbohydrate utilization compared with basal, suggesting that peak sensitivity to insulin-mediated carbohydrate metabolism occurs very soon after exercise.

Insulin also has been shown to be central in the regulation of protein dynamics (12). Numerous reports exist demonstrating that stimulation of amino acid transport, promotion of whole body and muscle protein synthesis, and inhibition of proteolysis occur when amino acid availability and insulin concentrations are increased (12). Very little is known, however, regarding how exercise modulates insulin's effects on protein metabolism. In the present study, whole body and leg protein synthesis were increased during the same postexercise period that insulin responsiveness to glucose metabolism appeared to be increased. Furthermore, although the changes were not as large as those noted for glucose utilization, protein synthesis also was greatest immediately after exercise and tended to decrease over the 3-h period (Fig. 7). Also similar to carbohydrate metabolism, there was no change in whole body or leg protein synthesis when the same supplement was provided 3 h after exercise. These data suggest that exercise's modulation of insulin action also may play a central role in the regulation of protein synthesis. This conclusion is supported by a previous report with canines in which net muscle protein balance became positive within 15 min after initiation of a postexercise glucose-amino acid infusion and contin-

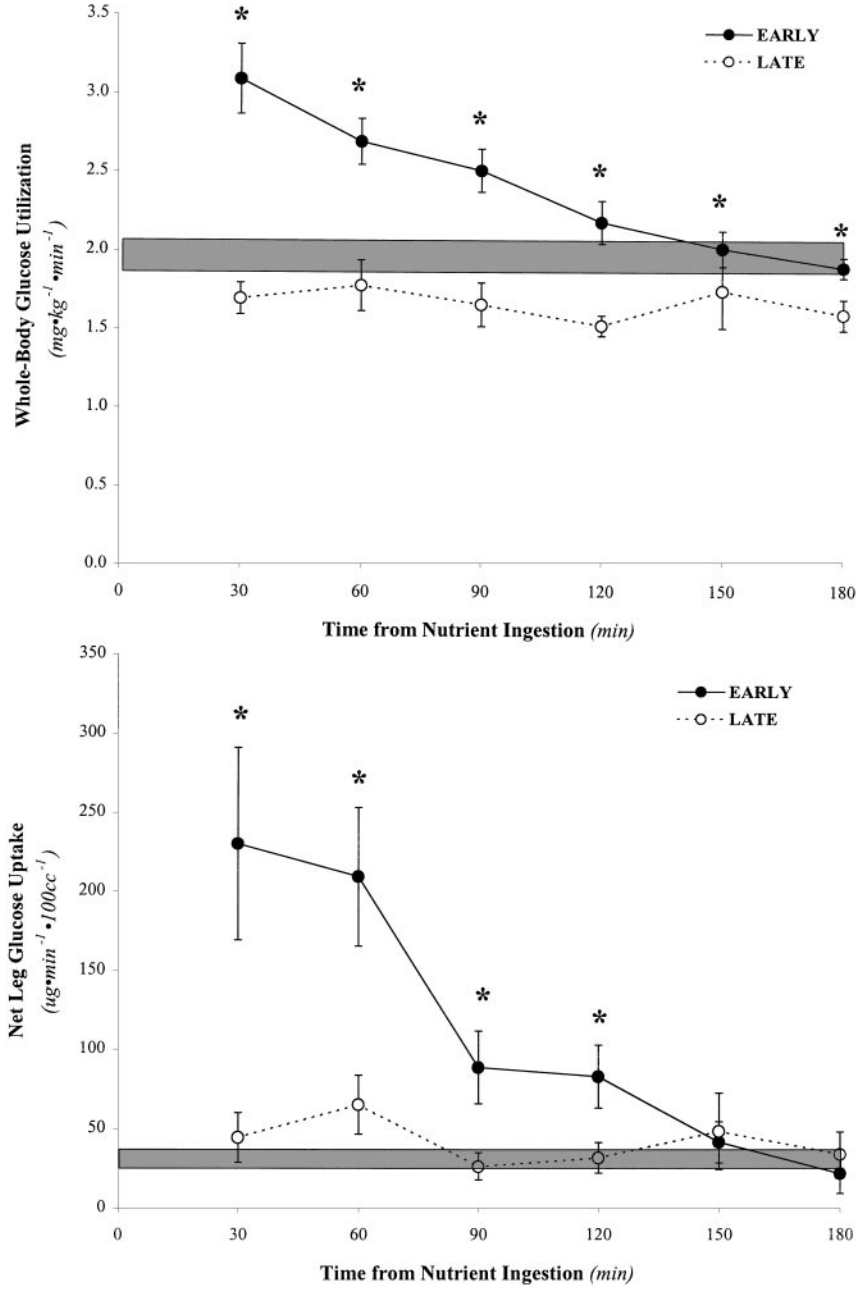

Fig. 6. Point-by-point timing of leg and whole body glucose uptakes for 10 subjects given an oral nutrient supplement either immediately after exercise (EARLY) or 3 h postexercise (LATE). *Significantly different ($P < 0.05$), EARLY vs. LATE. Shaded bar represents basal means ± SE.

ued as such for the entire 2-h infusion (32). In addition, when the nutrient supplementation was discontinued in the canines, net muscle protein balance became negative again.

Insulin also is important in the regulation of amino acid transport (12). Insulin-stimulated amino acid transport was elevated by muscular contraction even in the absence of an increase in insulin binding (48). Although transport was not directly measured in the present study, fractional extraction of phenylalanine by the leg was twofold greater for EARLY vs. LATE supplementation, suggesting that leg tissues were more effective at removing the amino acids presented to them immediately vs. 3 h after exercise. Taken together with the information on protein synthesis, these data support enhanced insulin sensitivity as a central component of the mechanism involved in how the timing of nutrient supplementation after exercise alters utilization of a nutrient load.

The importance of replenishing muscle glycogen content for subsequent moderate- to heavy-intensity exercise is also well established (3, 33, 41). Inadequate muscle glycogen results in fatigue and inability to train at high intensities. Therefore, sufficient stores of muscle glycogen are essential for optimum performance during intense, prolonged exercise. Although muscle glycogen was not tested in this study, it is reasonable to speculate that similar importance can be placed on the repair and synthesis of muscle protein after exercise.

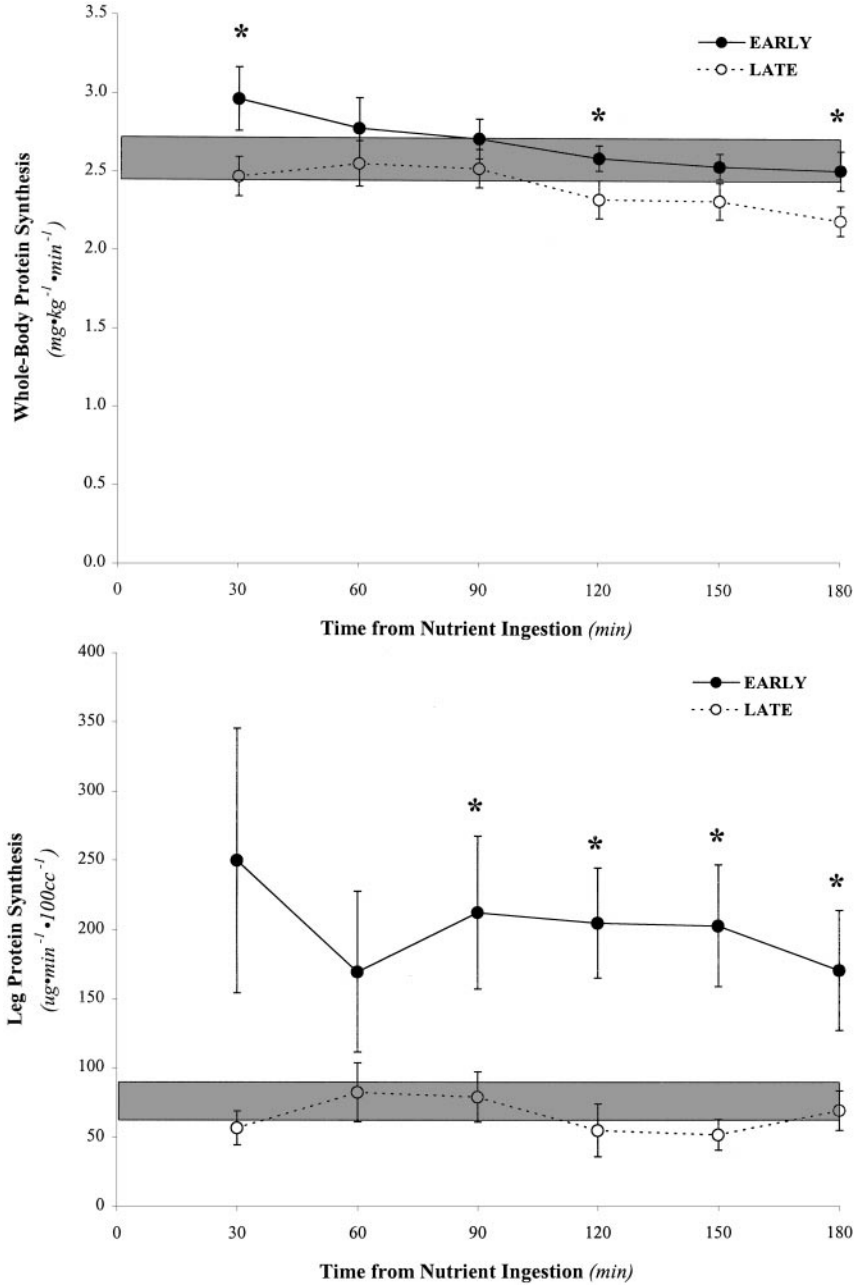

Fig. 7. Point-by-point timing of leg and whole body protein synthesis for 10 subjects given an oral nutrient supplement either immediately after exercise (EARLY) or 3 h postexercise (LATE). *Significantly different ($P < 0.05$), EARLY vs. LATE. Shaded bar, basal means ± SE.

Muscle glycogen resynthesis after prolonged exercise has been investigated with respect to amount, type, physical form, and timing of the ingested carbohydrate (45). Similar interactions with these variables and postexercise protein synthesis have not been well documented. Factors that can potentially affect nutrient utilization include age, gender, body composition, and physical training of the participants; the type, duration, and intensity of exercise performed; the amount and physical form of the nutrient ingested; and the protein and energy intake in the days before exercise. Extreme care was taken to maintain consistency in these variables between the two treatments in this study. However, it is possible that alterations in these variables from those used in the present study may result in different conclusions. For example, a more intense exercise may result in more prolonged catabolic hormone concentrations, thereby blunting insulin action. Furthermore, the ingestion of a supplement consisting of free amino acids, as opposed to the ingestion of an intact protein source, may produce significantly different results.

Therefore, our study clearly indicates that the timing of postexercise nutrient supplementation has a significant impact on whole body and leg protein homeostasis, similar to that observed for carbohydrate homeostasis. Thus whole body and leg protein synthesis, as well as net protein deposition, is enhanced when nutrients are consumed immediately after exercise as opposed to 3 h later. These data suggest that exercise's

modulation of insulin action may impact whole body and muscle protein accretion, as well as glucose deposition.

The aid of the personnel and use of the facilities at the General Clinical Research Center (RR-00095), Clinical Nutrition Research Unit (DK-26657), and Diabetes Research and Training Center (DK-20593) at Vanderbilt University is 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, and we are grateful for the expert technical and analytical assistance of Suzan Vaughan, Mu Zheng, Donna Schot, and Wanda Snead. Finally, we thank the subjects for their loyal compliance and participation.

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