Three weeks of caloric restriction alters protein metabolism in normal-weight, young men

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Friedlander, Anne L., Barry Braun, Margaret Pollack, Jay R. MacDonald, Charles S. Fulco, Steve R. Muza, Paul B. Rock, Gregory C. Henderson, Michael A. Horning, George A. Brooks, Andrew R. Hoffman, and Allen Cymerman. Three weeks of caloric restriction alters protein metabolism in normal-weight young men. Am J Physiol Endocrinol Metab 289: E446–E455, 2005. First published May 3, 2005; doi:10.1152/ajpendo.00001.2005.—The effects of prolonged caloric restriction (CR) on protein kinetics in lean subjects has not been investigated previously. The purpose of this study was to test the hypotheses that 21 days of CR in lean subjects would 1) result in significant losses of lean mass despite a suppression in leucine turnover and oxidation and 2) negatively impact exercise performance. Nine young, normal-weight men [23 ± 5 kg, 19.6 ± 5.7 kg, peak oxygen consumption (VO2 peak) 45.2 ± 7.3 mL·kg−1·min−1, mean ± SD] were underfed by 40% of the calories required to maintain body weight for 21 days and lost 3.8 ± 0.3 kg body wt and 2.0 ± 0.4 kg lean mass. Protein intake was kept at 1.2 g·kg−1·day−1. Leucine kinetics were measured using α-ketoisocaproic acid reciprocal pool model in the postabsorptive state during rest and 50 min of exercise (EX) at 50% of VO2 peak. Body composition, basal metabolic rate (BMR), and exercise performance were measured throughout the intervention. At rest, leucine flux (≈131 μmol·kg−1·h−1) and oxidation (R0.6 ≈ 19 μmol·kg−1·h−1) did not differ pre- and post-CR. During EX, leucine flux (129 ± 6 vs. 121 ± 6) and R0.6 (54 ± 6 vs. 46 ± 8) were lower after CR than they were pre-CR. Nitrogen balance was negative throughout the intervention (≈3.0 g N/day), and BMR declined from 1,898 ± 262 to 1,670 ± 203 kcal/day. Aerobic performance (VO2 peak, endurance cycling) was not impacted by CR, but arm flexion endurance decreased by 20%. In conclusion, 3 wk of caloric restriction reduced leucine flux and R0.6 during exercise in normal-weight young men. However, despite negative nitrogen balance and loss of lean mass, whole body exercise performance was well maintained in response to CR.

energy intake; energy expenditure; leucine flux; exercise; nitrogen balance; lean mass

Mountaineers, elite athletes competing in ultraendurance events, and deployed military personnel may be subjected to prolonged periods when caloric intake is limited yet energy expenditure is high. Reports on military field training suggest that energy deficits can be as high as 1,400 kcal/day (21). Under such conditions, loss of lean mass could be detrimental to physical performance, resulting in increased risk of injuries for the individual or group. Information on protein metabolism and physical performance under such conditions could elucidate the importance of adequate caloric intake and be used to design interventions to minimize protein losses, thus potentially reducing susceptibility to injury. To our knowledge, no studies have yet looked at the effects of prolonged negative energy balance on protein kinetics in combination with physical performance measures in young, fit adults.

Classical studies observing young, normal-weight men undergoing prolonged semistarvation indicate that the body preserves lean mass over time during caloric restriction (23). Initial rapid losses in body weight tend to slow as compensatory reductions in basal and voluntary energy expenditure occur (13, 14, 23). Whether the slowed rate of weight loss is accompanied by changes in protein turnover and oxidation in lean subjects is unknown. Studies of short-term fasting (<4 days) show increases in leucine flux and oxidation during both rest and exercise in nonobese subjects (20, 24, 30, 35), but few studies are available on the effects of longer duration fasting or energy restriction. Data from Yang et al. (41) demonstrated that 13 days of caloric restriction without a reduction in protein intake did not affect leucine flux in nonobese males at rest. Effects of energy restriction in obese subjects have been better characterized because of the important public health consequences of weight loss in that population. Fasting or hypocaloric diets of long duration (3–16 wk) suppress protein flux and oxidation in obese subjects, possibly as a protective mechanism to conserve lean mass during prolonged energy deficiency (10, 16, 22, 34, 39). Indeed, a greater portion of weight loss appears to come from adipose stores as opposed to lean-mass compartments when obese subjects are placed on hypocaloric diets (22, 34, 39). Whether lean, fit adults with substantially less body fat to buffer the negative energy balance and potentially different weight control mechanisms would respond similarly is unclear.

Short-term, severe caloric restriction has been shown to be detrimental to strength and aerobic performance in young men and women (1, 19). In contrast, modest energy restriction (~20% of intake) of up to 2 wk has been shown not to impair performance (42). Recent reviews on the topic of energy...
reduction and performance in athletes (8) and military personnel (29) suggest that the impact of energy deficit is varied and dependent on severity and duration of reduction and the type of exercise performed. Therefore, studies designed to test a variety of performance measures under conditions that reflect appropriate field stresses are needed.

The purpose of this study was to investigate the response of young men to prolonged negative energy balance. To this end, we measured protein metabolism, changes in body composition, and physical performance in normal-weight men underfed by 40% of normal caloric intake for 21 days. We hypothesized that 21 days of hypocaloric feeding would 1) cause a significant loss in lean mass despite a reduction in leucine turnover and oxidation, and 2) be associated with decrements in muscle and aerobic exercise performance.

METHODS

Subjects

Nine nonsmoking, normal-weight men between 18 and 35 yr old were recruited through advertisements and fliers placed in local newspapers and universities in and around the Palo Alto, CA, area. For inclusion, individuals were required to consume ≥2,700 kcal/day for maintenance of body weight, to have been weight stable for the previous 6 mo, to be in good health with no chronic illnesses, and to be participating in a regular exercise program. The protocol was approved by the Administrative Panels for the Protection of Human Subjects at Stanford University and the US Army Research Institute for Environmental Medicine. Each individual gave written informed consent before screening.

Screening

All studies were performed in the Clinical Studies Unit (CSU) at the Veterans Affairs (VA) Palo Alto Health Care System. Upon admission to the CSU, subjects completed a medical history questionnaire and underwent a physical examination, resting 12-lead electrocardiogram, and routine blood and urine analyses. Each subject then completed a continuous, progressive exercise test to volitional exhaustion on the cycle ergometer (SensorMedics 800, Yorba Linda, CA) for the determination of peak oxygen consumption (\(\dot{V}\)\(_{\text{O}2}\) peak) using an online TrueMax system (ParvoMedics Consentius Technologies, Sandy, UT). For participation, subjects were required to have a \(\dot{V}\)\(_{\text{O}2}\) peak of >40 ml·kg\(^{-1}\)·min\(^{-1}\) and to successfully complete a one-arm curl with ≥18.2 kg to ensure that they would be fit and strong enough to complete the fitness tests required by the study protocol. Habitual dietary intake and physical activity levels were assessed using a 3-day diet record and 7-day activity diaries, respectively.

Diet Stabilization Period

Before the intervention period, subjects underwent a 7-day dietary stabilization period. Basal energy expenditure was estimated using the Harris-Benedict equation, and energy need was calculated by multiplying the basal energy expenditure by an activity factor determined from their physical activity records (26). Subjects were weighed every other day during the stabilization period, and caloric intake was adjusted as needed to maintain body weight. Protein intake was set at 1.2 g·kg body wt\(^{-1}\)·day\(^{-1}\), with the mean macronutrient percentages for the stabilization diet consisting of 11% protein, 21% fat, and 68% carbohydrate. A protein intake of 1.2 g·kg body wt\(^{-1}\)·day\(^{-1}\) is 1.5 times the recommended dietary allowance (RDA) but representative of normal protein consumption rates for young, fit men. Subjects were given food for each day in an individual bag and were expected to finish all the food provided and eat nothing else. The food items consisted of whole foods (e.g., pasta with red sauce, bread, cheese, apples, etc.), which were adjusted in quantity according to subjects’ needs. The diets, during both the stabilization period and 3-wk intervention, provided 100% of the RDAs for nutrients. However, to ensure adequate intake of nutrients, a multivitamin and mineral supplement was provided daily. During the stabilization phase, subjects practiced all of the performance tests that would be performed during the testing phase. In addition, prior to the intervention, a second \(\dot{V}\)\(_{\text{O}2}\) peak test was performed and a fasting background blood sample was collected.

Intervention Diet

Upon the start of the intervention period, energy intake for each subject was cut by 40% (≈1,300 kcal). Protein content of the diet was held constant (1.2 g·kg body wt\(^{-1}\)·day\(^{-1}\)) to isolate the effects of negative energy balance on protein turnover without the confounding effects of varying protein intake. Thus energy intake was adjusted by adding or subtracting fat- and carbohydrate-containing foods. Carbohydrate intake was kept above ≥3 g·kg\(^{-1}\)·day\(^{-1}\) to minimize the effect of low carbohydrate intake on glycogen stores. By selecting only subjects who required ≥2,700 kcal/day to remain weight stable, we were able to meet all of the dietary criteria listed above.

Exercise and Dietary Controls

Subjects lived in the CSU for the full 21-day intervention period. All food was provided for them daily, and they were required to eat two meals in the unit every day (breakfast and dinner). Subjects were allowed to leave the CSU to attend classes, work, etc., during the day but were expected to return when not actively engaged in outside activities. Subjects were asked to continue with their normal activity patterns during the intervention period, and a daily schedule of activities was given to them based on their preintervention activity diaries.

Body Composition and Basal Metabolic Rate

Fasted body weight was measured each morning throughout the intervention period. Skinfold measurements were made on days 1– 3 during the stabilization period and on days 3, 10, and 21 of the intervention period and used to calculate lean body mass, fat mass, and percentage of fat. Basal metabolic rate (BMR) was determined by indirect calorimetry according to the method of Consolazio et al. (5) on days 1–4, 10–12, and 19–21. Briefly, expired air was collected in Douglas bags via one-way Hans Rudolf valves for 10 min (following a 5- to 10-min equilibration period) in the subjects’ rooms before rising, 10 h after their last meal, and after a minimum of 6 h of bed rest. Rooms were kept isothermic, dim, and quiet. Expired air was then analyzed for volume (Parkinson-Cowan dry gas meter) and O\(_2/\)CO\(_2\) content (ParvoMedics Metabolic Cart).

Nitrogen Balance

Twenty-four-hour urine collections were taken on days 1–7, 10–13, and 18–21. Collections began between 7 and 8 AM. At the end of the collection period, the volume was determined, and two 10-ml aliquots were frozen for future analysis of total nitrogen. Urinary creatinine was also analyzed using a routine auto-analyzer at the VA Clinical Laboratory as a measure of completeness of the urine collections. Total urinary nitrogen was measured using the Kjeldahl technique (Tecator 2300 Kjeltec Analyzer, Hoganas, Sweden). Compositions of the foods eaten by the subjects were homogenized and run through the same Kjeldahl machine for the determination of total nitrogen intake. Nitrogen balance (NBAL) was determined using the following equation:

\[
\text{nitrogen intake} = (\text{total urinary nitrogen} + \text{miscellaneous losses} + \text{fecal losses}).
\]
Miscellaneous losses were estimated conservatively at 5 mg/kg, and fecal losses were set at 2 g/day (3). For logistical reasons, urine samples were not collected on this group of subjects during the baseline weight stability period. However, baseline data collected on matched subjects \((n = 16)\) undergoing identical testing and dietary controls during the stabilization period are included in this study for comparison with the negative energy balance portion of the study. These data show that NBAL can be obtained in weight-stable subjects undergoing our standard protocol. It is likely that, because the protocols and subjects were similar between studies, the NBAL values were also comparable.

**Protein Turnover and Oxidation Studies**

Protein kinetics were assessed on days 1 and 18 of the 3-wk study intervention period. Subjects had been on the standardized diet and were weight stable at the time of testing on day 1. Subjects spent the night in the CSU before testing, were not allowed to exercise for the previous 24 h, and were allowed no food after 10 PM the night before the test. One-half of the subjects were tested in the morning following a pre-event meal \([\approx 676 \text{kcal}; 11\% \text{ protein}, 65\% \text{ carbohydrate (CHO)}, 24\% \text{ fat}]\) that was completed 2 h before the start of tracer cocktail infusion. The remaining subjects were tested in the afternoon following a standardized meal at 8 AM and the pre-event meal (as previously mentioned) at noon, 2 h before the start of infusion. Subjects maintained the same testing time for each of their trials. At the start of each trial, a catheter was placed in a hand vein for sampling of "arterialized" blood using the "heated-hand vein" technique, and an antecubital venous catheter was placed in the opposite arm for infusion of the stable isotope tracers. After the collection of background blood and expired air samples, a priming bolus of \([1-^{13}\text{C}]\)leucine \((0.75 \text{ mg/kg})\) and sodium bicarbonate \((\text{NaH}^{13}\text{CO}_3, 0.295 \text{ mg/kg})\) was given followed by a constant infusion of \([1-^{13}\text{C}]\)leucine at \(0.8 \text{ mg}\cdot \text{kg}^{-1}\cdot \text{h}^{-1}\) using a Gemini PC-1 infusion pump (IMed, San Diego, CA) for 90 min of rest and 50 min of exercise on the cycle ergometer. The exercise intensity was set at a workload that elicited 50% of baseline \(\dot{V}O_2\text{peak}\). Blood samples were taken at 0, 75, and 90 min of rest and 30, 40, and 50 min of exercise. All isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA), diluted in 0.9% sterile saline, and pharmaceutically tested for sterility and pyrogenicity (University of California San Francisco School of Pharmacy, San Francisco, CA).

At each of the blood sampling time points, respiratory gas exchange \((\dot{V}O_2, \dot{V}CO_2, \text{respiratory exchange ratio})\) was determined using the online metabolic system described previously, and a sample of expired air was collected in a 10-ml vacuum container to determine \(^{13}\text{CO}_2\) isotopic enrichment. The expired air samples were stored at room temperature until they were analyzed using isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH).

**Blood Sample Collection and Analysis**

Blood samples were immediately placed on ice, centrifuged for 10 min at 2,500 rpm, decanted, and frozen. Blood samples for the analysis of glucose and lactate concentrations were collected in 8% perchloric acid. Plasma glucose and lactate concentrations were determined by the VA Clinical Laboratories using a Beckman LX20 multichannel analyzer. Plasma free fatty acid (FFA) concentrations were collected in EDTA and measured on a spectrophotometer plate reader using a Wako enzymatic kit (Richmond, VA). Plasma for the analysis of insulin and cortisol was collected in chilled tubes containing EDTA and aprotinin (to prevent proteolysis of the peptide hormones) and stored at -80°C for future analysis using RIA kits (Diagnostic Systems Laboratories, Webster, TX).

Samples for the analysis of \(\alpha\)-ketosacaproic acid (KIC) isotopic enrichment (IE) and concentration were also collected in 8% perchloric acid. KIC IE and concentration were measured at UC Berkeley by using gas chromatography-mass spectrometry (GC-MS; GC model 6890 series and MS model 5973N, Agilent) of the trimethylsilyl-quinoxalinol derivative, using \(\alpha\)-ketovaleteral as the internal standard for concentration measurement. The method of Rocchiccioli et al. (32) was modified for use with perchloric acid extracts. After being spiked with \(\alpha\)-ketovaleteral, 1 ml of perchloric acid extract was mixed 1:1 with an o-phenylenediamine solution (5 mg/ml in 3 N HCL). The solution was heated for 60 min at 90°C and subsequently extracted with 4 ml of methylene chloride. The aqueous layer was discarded, and the remaining solution was dried under \(N_2\). Next, 100 \(\mu\)l of pyridine and 100 \(\mu\)l of \(N,O\)-bis(trimethylsilyl)trifluoroacetamide was added and the solution was vortexed and transferred to autosampler vials for GC-MS analysis.

For GC-MS analysis, an HP-1701 column was used, and the inlet temperature was set at 250°C, the initial oven temperature at 70°C, the source temperature at 250°C, and the quadrupole temperature at 106°C. Splitless injection was used, and the carrier gas was helium with a constant flow of 50 ml/min. Methane was used for chemical ionization, and selective ion monitoring was used to monitor ions \((m/e)\) of 261 for \(\alpha\)-ketovaleteral and 275 and 276 for \([^{13}\text{C}]\)- and \([^{12}\text{C}]\)KIC, respectively.

**Calculations**

Whole body leucine flux \((Q)\) was calculated using the IE of plasma \([^{13}\text{C}]\)KIC (reciprocal pool model) assuming steady-state enrichments (33). Plasma KIC IE is thought to be a better predictor of the intracellular \([^{13}\text{C}]\)leucine precursor pool than plasma \([^{13}\text{C}]\)leucine (18). The following equations were used:

\[
\text{leucine flux: } Q (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = \frac{|E_i/E_{iKIC} - 1|}{k} \text{ mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}
\]

where \(I = \text{l-}[^{13}\text{C}]\text{leucine infusion rate in } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} , E_i = \text{the l-}[^{13}\text{C}]\text{leucine infusate enrichment mol percent excess (MPE)} , \text{and } E_{iKIC} = \text{the KIC isotopic enrichment} \).  

\[
\text{leucine oxidation: } C (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = \frac{(E_{CO_2} \times V_{CO_2})}{(E_{iKIC} \times k)} ,
\]

where \(E_{CO_2}\) is the enrichment of \(^{13}\text{CO}_2\) in expired air in MPE, \(V_{CO_2}\) is the volume of an expired air in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \), and \(k\) is the bicarbonate correction factor. Although we did not experimentally derive \(k\) in this study, many previous investigations have done so in studies measuring leucine oxidation in similar populations under similar conditions. In all cases, the values for rest and exercise have ranged between 0.77 and 0.84 and between 0.9 and 1.06, respectively (16, 25, 27, 31, 40). In addition, evidence suggests that bicarbonate recovery is well maintained, even during severe conditions of starvation (36). Therefore, in the current investigation, \(k\) values of 0.8 for rest and 1.0 for exercise were used. Nonoxidative leucine disposal, an estimation of synthesis, was calculated as the difference between leucine flux and oxidation. Values for all kinetic variables were calculated during the last 15 min of rest and between min 30 and 50 of exercise.

**Physical Performance Test**

Four quantifiable exercise tests were selected to isolate different elements of physical performance (9). The performance tasks were as follows.

\(\dot{V}O_2\text{peak}\) Standard incremental progressive exercise bout to volitional exhaustion on a bicycle ergometer was used to assess peak, whole body aerobic exercise performance. \(\dot{V}O_2\text{peak}\) was determined during the baseline stabilization period and on days 2 and 20 of the intervention. The coefficient of variation (CV) for these subjects on this test was 3.6%.

**Submaximal test to exhaustion.** After the 50 min of cycling exercise at 50% of \(\dot{V}O_2\text{peak}\) performed during the protein turnover studies, isotope infusions were discontinued, and subjects rode to exhaustion at 70% of \(\dot{V}O_2\text{peak}\). For each test, the fan was set on low and directed at the subject’s bare torso and face. No music or videos were allowed...
during the test to exhaustion portion, encouragement was regulated throughout the test, and no clocks were visible. Exhaustion was defined as the inability to sustain a cadence greater than 50 rpm three times (e.g., if the cadence dropped below 50, subjects were given strong verbal encouragement to continue “a while” longer; on the third fall below 50, the test was discontinued).

“Shell loading” task. To determine the impact of energy deficit on heavy, weight-bearing work performance, an occupational task involving lifting and carrying was chosen. For this task, subjects lifted a metal cylinder (91 cm long, 38 cm diameter, 25 kg) from a 76-cm-high platform and then carried it 8 m and placed it on a 132-cm-high platform. Each subject had to accomplish 50 complete repetitions as quickly as possible. The performance outcome was time to complete all repetitions. In addition to practice tests, subjects performed this task during the baseline phase and on days 3, 11, and 21 of the intervention. The CV for the shell loading task in these subjects was 4.2%.

Elbow flexion weight-lifting task. This task was chosen to assess the impact of energy deficit on small-muscle performance during repetitive submaximal dynamic contractions to exhaustion. For this task, subjects used a dumbbell to perform full-range, one-arm elbow flexions (“curls”) to exhaustion while seated on a bench with their backs resting and stabilized against a 45° incline. Throughout the entire movement, the hand was positioned midway between full supination and pronation so that the thumb was facing up. All repetitions were performed with the left arm at the rate of 22 curls/min, paced by a traditional-style metronome. Subjects were not allowed to swing their arms at the bottom of the movement or drop their arms from the fully contracted position. The weight chosen had been previously selected via early familiarization sessions so that each subject could perform ~25–35 repetitions during the baseline phase. “Exhaustion” occurred when the subject could not maintain the repetition pace using only correct form. The weight of the dumbbell used varied among subjects but was constant for each subject throughout the study (~10% of baseline body wt). All subjects performed this task during the baseline phase and during the intervention on days 2 and 20. The arm curl CV during the baseline phase was 5.1%.

Statistics

One-way ANOVA with repeated measures was utilized for the analysis of protein kinetics, body composition, and physical performance comparisons. Post hoc analyses were performed where appropriate using Tukey’s least significant difference tests. Pearson correlation coefficients were used to determine associations between body composition and performance values. Statistical significance was accepted when \( P < 0.05 \). All values are presented as means ± SD.

RESULTS

Subject Characteristics, Diet, and Body Composition

All nine normal-weight, recreationally fit young men (age, 23 ± 5 yr; weight, 78.6 ± 5.7 kg; height, 177 ± 8 cm; body fat, 16 ± 4%; lean mass, 65.4 ± 6.1 kg; \( \text{VO}_2\text{peak}, 45.2 ± 7.3 \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) completed the intervention period. Post hoc analysis of the diet indicated that the goal of 40% caloric restriction (~1,300 kcal) was achieved (Table 1). Protein content of the diet remained relatively constant (between 1.1 and 1.2 g·kg\(^{-1} \cdot \text{day}^{-1} \)), whereas the quantity of CHO was cut by 36 and 46%, respectively. Subjects lost an average of 3.8 ± 0.3 kg (~5%) body wt over the 21-day intervention, with equal amounts coming from fat and fat-free tissue compartments (1.8 ± 0.4 and 2.0 ± 0.4, respectively; Fig. 1).

Basal Metabolic Rate and NBAL

Before caloric restriction, BMR was ~1,900 kcal/day and declined 15% over the intervention period reaching a maximum reduction of 230 kcal/day on days 19–21 (Fig. 2). No significant association was observed between quantity of weight or lean mass lost and decline in BMR among individual subjects. NBAL data for the seven subjects who were able to provide complete 24-h urine collections are presented in Fig. 3. NBAL was negative at each of the measurement periods during the caloric restriction intervention, although the values tended to improve by days 10–12 compared with the first week of collections (Fig. 3). Although no baseline urine samples for these subjects were collected (see METHODS), baseline NBAL values from matched subjects \( (n = 16) \) undergoing identical testing and dietary controls are provided for comparison in Fig. 3.

Protein Metabolism Studies

Calorimetry data and metabolite concentrations. All values presented in Table 2 were measured during the last 15 min of rest (3.5 h postmeal) and the last 30 min of exercise during the isotope trials on days 1 and 18 of the intervention. During both trials, subjects worked at the same absolute (~1.85 l/min) and relative (53% of \( \text{VO}_2\text{peak} \)) workloads during the exercise bout for 50 min. Respiratory exchange ratio was higher during exercise than rest for both trials and tended to be reduced.

Table 1. Mean daily caloric and macronutrient content of the diet

<table>
<thead>
<tr>
<th></th>
<th>kcal</th>
<th>CHO, g</th>
<th>Protein, g</th>
<th>Fat, g</th>
<th>%Change, kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (days −7 to −1)</td>
<td>3,245±187</td>
<td>555±42 (68%)</td>
<td>92±8 (11%)</td>
<td>73±6 (21%)</td>
<td></td>
</tr>
<tr>
<td>Hypocaloric (days 1 to 21)</td>
<td>1,950±260</td>
<td>299±34 (61%)</td>
<td>85±11 (18%)</td>
<td>46±6 (21%)</td>
<td>−40%</td>
</tr>
</tbody>
</table>

Values are means ± SD (%total energy). CHO, carbohydrate. Protein set between 1.1 and 1.2 g/kg.
following caloric restriction ($P = 0.06$, day 1 vs. day 18 for rest and exercise). Leucine concentration was higher during exercise than rest on day 1 ($P = 0.003$) but not on day 18.

There were no significant exercise or caloric restriction-induced differences in KIC concentration. Plasma glucose, lactate, and FFA all differed between rest and exercise but demonstrated no significant change in response to caloric restriction. In contrast, β-hydroxybutyrate was elevated at both rest and exercise following 18 days of underfeeding ($P < 0.002$) but did not respond to the exercise stimulus (Table 2).

**KIC isotopic enrichments and leucine kinetics.** Steady-state isotopic plateaus in KIC and $^{13}$CO$_2$ were reached in both trials during the exercise sampling periods (Fig. 4, A and B). Leucine flux did not differ between rest and exercise on day 1. However, 18 days of caloric restriction suppressed leucine flux during exercise relative to both rest on day 18 and exercise on day 1 (Fig. 5A). On days 1 and 18, leucine oxidation more than doubled between rest and exercise ($=19$ vs. 50 $\mu$mol·kg$^{-1}$·h$^{-1}$). After 18 days of caloric restriction, leucine oxidation was reduced during exercise relative to day 1 exercise values (Fig. 5B). Nonoxidative leucine disposal was at-

### Table 2. Gas exchange, metabolite, and hormone variables measured during isotope tests

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest Exercise Rest Exercise</td>
<td></td>
</tr>
<tr>
<td>$\text{VO}_2$, l/min</td>
<td>$0.27 \pm 0.06$ $1.87 \pm 0.28^\dagger$</td>
<td>$0.27 \pm 0.03$ $1.82 \pm 0.28^\dagger$</td>
</tr>
<tr>
<td>RER</td>
<td>$0.89 \pm 0.06$ $0.93 \pm 0.03^\dagger$</td>
<td>$0.85 \pm 0.06$ $0.90 \pm 0.03^\dagger$</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>$4.8 \pm 0.6$ $4.4 \pm 0.6^\dagger$</td>
<td>$4.6 \pm 0.6$ $4.3 \pm 0.3^\dagger$</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>$0.4 \pm 0.08$ $2.1 \pm 1.1$</td>
<td>$0.4 \pm 0.08$ $1.5 \pm 0.6^\dagger$</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>$0.32 \pm 0.11$ $0.61 \pm 0.26^\dagger$</td>
<td>$0.36 \pm 0.25$ $0.74 \pm 0.20^\dagger$</td>
</tr>
<tr>
<td>Leucine, $\mu$M</td>
<td>$78.3 \pm 8.7$ $85.0 \pm 6.2$</td>
<td>$77.6 \pm 12.6$ $76.5 \pm 7.3$</td>
</tr>
<tr>
<td>KIC, $\mu$M</td>
<td>$10.6 \pm 3.1$ $12.7 \pm 5.6$</td>
<td>$11.5 \pm 2.5$ $11.3 \pm 4.2$</td>
</tr>
<tr>
<td>β-OHB, mM</td>
<td>$0.78 \pm 0.57$ $0.90 \pm 0.60$</td>
<td>$1.66 \pm 0.60^<em>$ $1.83 \pm 1.18^</em>$</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>$105 \pm 82$ $51 \pm 25^\dagger$</td>
<td>$74 \pm 35$ $37 \pm 9^*^\dagger$</td>
</tr>
<tr>
<td>Cortisol, nM</td>
<td>$251 \pm 104$ $328 \pm 154^\dagger$</td>
<td>$226 \pm 52$ $303 \pm 33^\dagger$</td>
</tr>
</tbody>
</table>

Values are means ± SD. Exercise, last 30 min of a 50-minute exercise bout at 50% $\text{VO}_2$max. RER, respiratory exchange ratio; FFA, free fatty acid; KIC, α-ketoisocaproate; β-OHB, β-hydroxybutyrate. *Significantly different from day 1 at $P < 0.05$. †Significantly different from rest at $P < 0.05$.
tenuated with exercise but was not affected by the caloric restriction (data not shown).

**Hormone responses during the isotope trials.** Insulin concentration was lower during exercise compared with rest on both days 1 and 18, but the fall was greater during exercise on day 18 than on day 1 (Table 2). Exercise induced a significant increase in cortisol concentration during both trials, but there was no impact of dietary energy restriction on the cortisol response during rest or exercise (Table 2).

**Performance measurements.** Performance test results are presented in Table 3. \( \text{V} \dot{\text{O}}_2 \text{peak} \) did not change over the intervention period when expressed in liters per minute. However, it tended to increase when expressed relative to the declining body weight (ml·kg\(^{-1}·\text{min}^{-1}\)). Similarly, there was no significant effect of caloric restriction on submaximal endurance time on the cycle ergometer. Subjects experienced a mixed response to caloric restriction on the lift-and-carry vs. arm flexion test with a 12% improvement on the former, but a 20% decrement on the latter. There was a strong association between decrement in arm flexion performance and the quantity of weight lost during the intervention (\( r = 0.7, P > 0.05; \text{Fig. 6A} \)). When decrement in arm flexion performance was plotted as a function of percent change in body weight, the relationship was maintained with those losing more than 5% of their body weight, demonstrating the greatest reduction in performance (Fig. 6B). The relationship between change in arm flexion and fat-free mass was less robust (\( r = 0.5, \text{not significant (NS)} \)) and there was little association between improvements in lift-and-carry and change in either body weight or lean mass (\( r < 0.4, \text{NS} \)).

**DISCUSSION**

The primary finding of this investigation was that leucine flux and oxidation were decreased during exercise in response to 18 days of 40% energy restriction in young men. Despite reductions in leucine kinetics, NBAL did not significantly improve throughout the intervention, resulting in losses of fat-free mass representing 50% of the total body weight loss. Furthermore, significant reductions in body weight had modest effects on the endurance capacity of small muscle groups, but whole body exercise and aerobic capacity was not negatively impacted. These results are interpreted to mean that normal-weight young men adapt to 3 wk of moderate energy restriction
in a way that alters protein metabolism during exercise yet preserves work capacity.

**Protein Metabolism**

Leucine oxidation was reduced following caloric restriction. Although the reduction in carbohydrate availability during caloric restriction might be expected to create a greater dependence on protein as an energy source, gluconeogenic precursor, and to serve anaplerotic functions as TCA cycle intermediates, previous research suggests the opposite. An early study by Keys et al. (23) elucidated a curvilinear pattern of body weight and lean tissue loss (a rapid initial response followed by a slower rate of loss) in response to 6 mo of semistarvation in lean, healthy men. The pattern has since been replicated with NBAL measurements in less prolonged studies using obese subjects (13, 15). More recently, data from studies using stable isotopes to measure protein kinetics suggest a similar response. Fasting studies of up to 4 days have been performed in lean subjects and support the idea that leucine flux, proteinolysis, and oxidation are elevated in response to short-term energy deficiency (20, 24, 35). However, as the duration of intervention increases, there appears to be a reversal of the response. For example, healthy young men subjected to 13 days of 25% caloric restriction showed no change in leucine flux (41), but it is unclear whether the lack of response was a result of the milder energy deficit or whether subjects were already experiencing an adaptive response in protein metabolism.

Previously, weight loss interventions of greater than 2 wk investigating protein flux and oxidation have been conducted in obese subjects. Many of the studies suggest that the observed reduction in nitrogen excretion over time with weight loss may be accompanied by a suppression of leucine (or other amino acid) flux at rest (16, 22, 34). However, not all studies have demonstrated decreases in leucine flux (10) despite substantial weight loss, and none have observed a change in the rate of leucine oxidation at rest (10, 16). Our data collected after 18 days of caloric restriction also did not show suppression of leucine flux or oxidation at rest, but the underfeeding did reduce both flux and oxidation during exercise, suggesting that the adaptive response may only be evident under conditions of physical stress. Indeed, the only long-term study to our knowledge that included exercise demonstrated that the decrease in leucine flux observed during rest following 16 wk of weight loss was further suppressed by exercise in initially obese subjects (22). Because of differences in weight control mechanisms or the reduced buffering capacity of fat, some have hypothesized that lean and obese adults might differ in their response to underfeeding (13, 14), as observed in studies of laboratory rodents (12). Such a hypothesis is partially supported by the current investigation.

**Conservation of Mass with Substrate Availability**

The suppression of protein metabolism and gradual attenuation of negative NBAL observed in previous studies are hypothesized to be part of a systemic adaptation to conserve lean mass. One mechanism by which conservation may function is by promoting alternative (nonprotein) substrate availability. In the present investigation, alteration in substrate partitioning was reflected in the metabolic variables associated with the isotope trials (Table 2). Insulin was lower during exercise, and ketones were elevated during rest and exercise following the underfeeding intervention. Lower insulin concentrations spare blood glucose by reducing uptake by nonactive tissues, whereas higher ketone levels provide alternate fuel sources to normally carbohydrate-dependent tissues. The trends for lower respiratory exchange ratio values, lower lactate concentrations, and higher FFA following energy restriction also suggest a switch in substrate use toward lipid sources, thus reducing the need for CHO and protein as fuel. Suppression of protein oxidation in the presence of alternate fuel sources may be controlled through the downregulation of branched-chain 2-oxoacid dehydrogenase (BCOAD) enzyme activity. BCOAD catalyzes the rate-limiting, irreversible decarboxylation step leading branched-chain amino acids down the metabolic pathway to oxidation. As yet, no information is available on the effects of long-term caloric restriction on BCOAD, but regulation of BCOAD has been reported to be associated with acute exercise-induced increases and training-induced reductions in leucine oxidation (2, 27).

**Conservation of Mass with Energy Expenditure**

Minimizing weight and lean tissue losses may also be accomplished by decreasing energy expenditure, thus reducing total fuel requirements. Reductions in total energy expenditure resulting from decreases in both resting metabolic rate and spontaneous physical activity have been reported in response to reduced caloric intake (14, 34). Basal metabolic rate in the current investigation declined by an average of 165 kcal/day (maximum of 230 kcal/day) over the 21 days, representing a total of 3,480 kcal that did not need to be buffered by body stores. Assuming equal losses from lean and fat tissue (as occurred in this study), the reduction in BMR could have spared up to 0.27 kg each of lean and fat tissue (a 13% attenuated loss). BMR declines in our study were similar to others that reported decreases of 100 kcal/day in resting metabolic rate after 19 days of 20% caloric restriction in lean subjects, or 184 kcal/day following 4 wk of 50% reduction in obese subjects (14, 34). The same free-living, young, healthy men whose resting metabolic rate declined 100 kcal/day also experienced a reduction in daily physical activity equivalent to 196 kcal/day. In contrast, our subjects were encouraged to maintain their normal levels of physical activity. Subjectively, participants reported no reduction in their activity levels, but did report that they felt “less energetic” during exercise of long duration (such as soccer) towards the end of the caloric restriction period. Our calculations comparing total caloric deficit with changes in body composition, BMR, and “other activity” (assuming 10% of intake value for the thermic effect of food) suggest that outside energy expenditure did remain constant relative to baseline throughout the 21-day intervention period.

**Protein Kinetics vs. NBAL**

In this study, lean subjects suppressed protein turnover during exercise, but not rest. Constant resting protein flux and oxidation rates were reflected in a state of negative NBAL that did not significantly change over the course of the intervention. Others have shown in obese subjects that NBAL can return from a deficit of −4 to 0 g N/day after 3 wk of adaptation to a very low-calorie diet (500 kcal/day) when protein content of
the diet is high (1.5 g/kg) (15). Even obese women fasted for 3 wk have demonstrated a 40% improvement (≈ −10 to −6 g N/day) in NBAL (16). In our study, dietary restriction was less severe and protein intake was ≈1.2 g/kg, yet our results demonstrated only a nonsignificant trend toward improving NBAL (≈−3.3 to −2.7 g N/day). Therefore, lean subjects may possess less robust mechanisms to counter periods of negative energy balance than those with high body fat. Alternatively, the less severe caloric restriction with adequate protein in our study may have muted the initial fall in NBAL. Requiring our subjects to remain physically active throughout the protocol may also have prevented some of the later normalization of NBAL evident in previous studies. Finally, NBAL could have been underestimated (too negative) during the later stages of our investigation had greater conservation of nitrogen via fecal and miscellaneous losses occurred over time. However, in the two studies cited previously that observed a rebound in NBAL (15, 16), no adjustments to either estimated or measured miscellaneous/fecal losses over the course of caloric restriction were made.

Previous studies have demonstrated that leucine kinetics and NBAL measurements do not always correspond (15). In the present study, the leucine kinetics measurements provided a snapshot of protein metabolism in the fed, postabsorptive state, whereas NBAL data represents a 24-h period. Our data indicated a decrease in leucine flux and oxidation on day 18 during exercise only. Therefore, alterations in nitrogen excretion during exercise may have been masked in the NBAL measurements by 23 h of similar values in the resting fed or fasting conditions. However, because the bulk of amino acid oxidation occurs following meal consumption and/or during exercise, we anticipated our NBAL values would be more reflective of our protein turnover measurements than in other studies that tested subjects at rest following an overnight fast, when leucine oxidation and nitrogen excretion are lower.

Dietary Issues

The response of protein metabolism in the fed state is interesting because it demonstrates the chronic effects of underfeeding on meal distribution and accretion of protein. However, testing our subjects in the fed, postabsorptive state may have elevated the leucine kinetic values reported in our study. El-Kourey et al. (7) demonstrated that leucine oxidation was increased by up to 30% following a single meal containing 26.7 mg/kg leucine and did not return to baseline rates for up to 5 h. Our pre-event meal (676 kcal) with less leucine (18 mg/kg) was given 3.5 h prior to resting kinetic data collection time points, but leucine oxidation may still have been elevated relative to fasting conditions. However, the timing and the content of the meal were identical before both sets of tests, so the comparison between day 1 and day 18 would be unaffected. An additional consideration is that the metabolic accommodation to caloric restriction may have been attenuated by testing our subjects in the fed state. Garlick et al. (11) reported no change in leucine kinetics in obese subjects adapted to a 3-wk, very low-calorie diet (that included protein) when tested in the fed state. In contrast, a similar dietary intervention resulted in a 20 and 30% suppression in leucine turnover and oxidation when measured following a 12-h fast (15). Because we saw no difference at rest but did measure a 6–15% reduction in flux and oxidation during exercise, exercise may provide a stress that overrides the negating effect of feeding on those variables. Also relevant is that a meal containing leucine increases leucine oxidation (7), but feeding calories in CHO form during exercise actually reduces the reliance on leucine as a fuel source (6). Therefore, our mixed pre-event meal may have increased resting but decreased exercising rates of leucine oxidation.

For a given caloric deficit, NBAL is less negative when the protein content of the diet is higher (4, 15). Furthermore, it has been shown that in both the fed and fasted states, leucine oxidation during rest and exercise is altered by prior protein content in the diet (2, 37). To isolate the effects of caloric restriction on protein metabolism, we attempted to reduce caloric intake by ≈40% without altering dietary protein (1.2 g·kg⁻¹·day⁻¹). Our post hoc analysis of the diet indicated a slight decrease in protein content of the foods actually consumed (1.17 vs. 1.08 g·kg⁻¹·day⁻¹ of initial weight). However, when corrected for reduction in body weight, the g/kg remained essentially equivalent. Therefore, we are confident that suppression of leucine turnover and oxidation was an adaptive response to reduced caloric intake, not protein content of the diet.

Physical Performance

Data from athletes, such as wrestlers trying to make weight classifications, indicate that short-term, rapid weight loss can be detrimental to high-intensity sprint work (19, 28) and time to exhaustion (1). However, such severe weight loss programs often contain elements of dehydration and glycogen depletion and thus may not be applicable to more gradual weight loss situations. Much of the recent work on long-term underfeeding and performance has been conducted with military personnel undergoing field training, where food availability and/or appetite are limited. Under such conditions, mismatches between energy intake and expenditure of up to 1,400 kcal/day for 62 days have been reported, with accompanying weight loss reaching 16% of body weight and decrements in strength of 26% (21). However, the effects of less severe weight loss on performance is unclear. A recent review of the military performance literature by Montain and Young (29) illustrates that, although variation in length of interventions, quantity of weight lost, and types of performance measures have yielded mixed results, certain trends in the literature do exist. First, maximal aerobic capacity (l/min) seems to be sensitive to underfeeding, but the differences tend to disappear when values are corrected for the reduction in body weight (ml·kg⁻¹·min⁻¹). In contrast, our subjects showed no decline in V̇O₂ peak over the course of this study, nor did subjects from several other studies that achieved similar changes in body weight (29). Second, strength measures of larger muscle groups tend to decline with body weight losses of greater than 6%. Although we did not measure maximal strength of any large muscle groups, we did observe a strong relationship between percent of body weight lost and decrement in arm curl endurance. As is illustrated in Fig. 6B, arm curl endurance declined most severely in those subjects that lost more than 6% of their body weight. The fall in arm curl performance occurred between days 10 and 21, which supports the observation that initial protein losses with underfeeding come from the gut,
followed later by muscle (38). The observation that changes in arm endurance were more strongly correlated with changes in body weight than changes in lean mass may be an artifact of the precision of the weight-vs.-body fat (skin caliper) measurements. Finally, it appears that performance is well maintained on task-based tests such as obstacle courses or marksmanship (29). We actually observed a significant reduction in time to complete the lift-and-carry task in our subjects. Because the lift-and-carry task involves moving the subject’s body weight in addition to the 25-kg artillery shell, a loss of body weight could have facilitated completion of the task in a shorter time by reducing the total mass being moved. Alternatively, subjects could have improved their techniques throughout the course of the study (despite practicing the test prior to data collection). Overall, the changes in performance were small, thus supporting the concept that physical performance is well maintained in response to prolonged caloric deficiency.

Methodological Issues

Subjects served as their own controls in this investigation, but there was no separate control group. Such a design is adequate for the metabolic studies where workloads, sampling parameters, and measurements were controlled by the investigators. All samples were batched by subject, with pre- and postintervention samples run simultaneously. In contrast, the performance data could have been impacted by factors independent of the underfeeding protocol. For example, as mentioned previously, subjects may have demonstrated a learning effect on the lift-and-carry task by improving their techniques during the test. In addition, the possibility that a learning effect counteracted decrements in whole body submaximal endurance resulting from the caloric restriction cannot be eliminated. Tests to exhaustion have also been reported to have a wide range of reliability (2–27%). Therefore, changes in endurance performance could have been masked by a high CV of the test (17). However, efforts were made to minimize such an effect by standardizing testing conditions (room temperature, fan position, no music or video, no visible clocks), regulating verbal encouragement, and providing specific exhaustion criteria (see METHODS).

Although subjects lived in the CSU and were given all of their food for the duration of the study, they were out of our control for portions of most days. As a result, two subjects could not deliver complete 24-h urine collections and were deleted from the NBAL analysis. In addition, the dietary regimen could have been compromised while subjects were absent from the CSU. However, based on the average weight loss attained, the majority of the subjects appeared to have been compliant with the diet program.

In conclusion, three weeks of caloric restriction reduced leucine flux and oxidation during exercise, but not rest, in normal-weight young men. The suppression in leucine metabolism during exercise was not reflected in an attenuation of negative NBAL, thus resulting in a significant loss of lean mass. Despite changes in body composition, most measures of physical performance were well maintained throughout the intervention. These data are interpreted to mean that normal-weight individuals have less robust mechanisms to preserve lean mass than obese subjects but can still adapt to 3 wk of moderately severe caloric restriction in a way that minimizes decrements in physical performance.

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