Calorie restriction accelerates the catabolism of lean body mass during 2 wk of bed rest[1–3]

Gianni Biolo, Beniamino Ciocchi, Manuela Stulle, Alessandra Bosutti, Rocco Barazzoni, Michela Zanetti, Raffaella Antonione, Marion Lebenstedt, Petra Platen, Martina Heer, and Gianfranco Guarnieri

ABSTRACT

Background: Muscle inactivity and low energy intake commonly occur in persons with acute or chronic disease, in astronauts during space flight, and during aging.

Objective: We used a crossover design to investigate the effects of the interactions of inactivity and calorie restriction on whole-body composition and protein kinetic regulation in 9 healthy volunteers.

Design: Lean body mass (LBM) was measured by using dual-energy X-ray absorptiometry before and at the end of 14-d periods of bed rest (B) and controlled ambulation (A) in patients receiving eucaloric (E) or hypocaloric (H) ($\approx$80% of total energy expenditure) diets. Whole-body leucine kinetics were determined at the end of the 4 study periods by using a standard stable-isotope technique in the postabsorptive state and during a 3-h infusion of a 0.13 g · kg $LBM^{-1} \cdot h^{-1}$ amino acid mixture.

Results: In the postabsorptive state, we found a significant ($P = 0.04$) bed rest × hypocaloric diet interaction for the rate of leucine oxidation, an index of net protein catabolism (A+E: 0.23 ± 0.01; B+E: 25 ± 0.01; A+H: 0.23 ± 0.01; B+H: 0.28 ± 0.01 μmol · min^{-1} · kg LBM^{-1}). Bed rest significantly ($P < 0.01$) decreased amino acid–mediated stimulation of nonoxidative leucine disappearance, an index of protein synthesis (A+E: 35 ± 2%; B+E: 30 ± 2%; A+H: 41 ± 3%; B+H: 32 ± 2%). B+H decreased LBM by 1.10 ± 0.1 kg, which is significantly ($P < 0.01$) greater than the decrease seen with A+E, A+H, or B+E.

Conclusion: Calorie restriction enhanced the catabolic response to inactivity by combining greater protein catabolism in the postabsorptive state with an impaired postprandial anabolic utilization of free amino acids.   *Am J Clin Nutr* 2007;86:366–72.

KEY WORDS   Healthy volunteers, muscle inactivity, protein metabolism, hypocaloric diet, bed rest, leucine kinetics, lean body mass

INTRODUCTION

Disease states are associated with various degrees of muscle inactivity in some persons who are bedridden for a prolonged time. Physical activity also may be reduced during physiologic aging. Muscle unloading is the primary consequence of human exposure to a microgravity environment during space flight. The specific effects of inactivity on physiologic functions include decreased turnover of skeletal muscle proteins (1–3) and impaired dietary amino acid utilization (4), which lead to muscle dysfunction and atrophy (5, 6). Nonetheless, bedridden persons,

elderly persons, and astronauts often combine muscle inactivity with a reduced energy intake that is below their energy expenditure, and, thus, they lose not only skeletal muscle but also fat mass (7–9). Clinical evidence indicates that such a combination of physical inactivity and low energy intake may rapidly lead to protein-energy malnutrition, increased incidence of complications, and a poor clinical outcome (7). Anorexia is one of the most common consequences of disease (10), whereas potential causes of decreased food intake in space flight may include alterations in circadian rhythms, gastrointestinal function, and neuroendocrine mediators and cytokines, and greater exposure to radiation energy (11). Despite the fact that the combination of hyponutrition and reduced physical activity is so frequently observed, interactions between energy restriction and muscle unloading for regulation of lean body mass (LBM) and protein kinetics have been poorly investigated. In fact, the specific effects of inactivity and energy balance are difficult to separate from confounding variables that arise from disease, aging, or space flight.

Healthy volunteers assigned to prolonged bed rest provide an appropriate model in which to investigate the effects of muscle unloading on physiologic functions (12). We have hypothesized that a hypocaloric diet would be more catabolic in the bed rest state than in the ambulatory state. The combination of reduced physical activity and negative energy balance would accelerate the loss of LBM in healthy subjects through changes in whole-body protein kinetics, as assessed by using stable isotopes of amino acids. Subjects have been studied 4 times for 14-d periods over 2 y in bed rest or in ambulatory condition in combination with eucaloric or hypocaloric diets using a crossover experimental design. Energy intake was individually tailored to account for

TABLE 1
Nutrient intake during the ambulatory and bed rest periods with eucaloric and hypocaloric diets[1]

	Eucaloric diet		Hypocaloric diet		P[2]		
	Ambulatory	Bed rest	Ambulatory	Bed rest	Activity effect	Diet effect	Interaction
Total energy (kcal · d^{-1} · kg LBM^{-1})	45.4 ± 0.9[a,3]	35.6 ± 0.7[b]	37.0 ± 1.4[c]	30.4 ± 0.7[d]	< 0.01	< 0.01	0.08
Carbohydrate (kcal · d^{-1} · kg LBM^{-1})	26.0 ± 0.5[a]	19.3 ± 0.4[b]	22.6 ± 1.3[c]	15.9 ± 0.6[d]	< 0.01	< 0.01	0.96
Lipid (kcal · d^{-1} · kg LBM^{-1})	14.2 ± 0.3[a]	11.2 ± 0.2[b]	9.3 ± 0.2[c]	9.4 ± 0.2[c]	< 0.01	< 0.01	< 0.01

[1] LBM, lean body mass. Protein intake was constant in the 4 phases at the level of 1 g · d^{-1} · kg body wt^{-1}, or 5.1 kcal · d^{-1} · kg LBM^{-1}. Means in a row with different superscript letters are significantly different, $P < 0.01$ (Bonferroni's post hoc analysis).

[2] Data were analyzed with a 2-factor ANOVA (activity × diet interaction).

[3] $\bar{x}$ ± SD (all such values).

the decrease in energy requirement during bed rest and then decreased by ≈20% during the hypocaloric periods. This investigation was conducted within the frame of the Short-Term Bed Rest Study of Integrated Physiology (STBR-IP) set up by the German Aerospace Institute (DLR) and the European Space Agency. Results relative to the bed rest period in eucaloric conditions were previously reported (4).

SUBJECTS AND METHODS

Subjects

Nine healthy, male, sedentary subjects [mean (±SEM) age: 24 ± 1 y; body weight: 77 ± 2 kg; LBM: 61 ± 1 kg; fat mass: 16 ± 2 kg; body mass index (BMI; in kg/m^2): 23 ± 1] participated in the STBR-IP study at the Clinical Research Center of the German Aerospace Institute (Cologne, Germany).

Written informed consent was obtained from all subjects. The experimental protocol was in accordance with the local ethical standards on human experimentation and approval was obtained from the Ethics Committee of the Árztekammer Nordrhein (Düsseldorf, Germany).

Protocol

The study protocol was divided into 4 phases. During the first and second phases (July–August 2001 and February–March 2002, respectively), subjects received weight-maintaining, eucaloric diets in either bed rest or ambulatory conditions. During the third and fourth phases (July–August 2002 and February–March 2003, respectively), subjects received low-calorie diets—mainly fat intake reduction—containing ≈80% of total energy requirement in either bed rest or ambulatory conditions. Four subjects were studied in ambulatory conditions during the first and third phases and in bed rest conditions during the second and fourth phases. The other 5 subjects were studied in bed rest conditions during the first and third phases and in ambulatory conditions during the second and fourth phases. During the 14 d of bed rest, participants were exposed to 6° head-down-tilt bed rest for 24 h/d. Some activities (ie, food intake, using the toilet, showering, and weighing) were performed in near-horizontal position. During the ambulatory periods, the participants were in the normal upright position during the day, were allowed to walk around in the ward, and performed light muscular workload (including bicycle ergometry for 10 min 3 times/d).

Each 14-d examination period was preceded by 9 d of adaptation and followed by 3 d of recovery. During the adaptation and the recovery periods, all subjects received weight-maintaining, eucaloric diets in ambulatory conditions.

At the beginning of each examination period, resting energy expenditure (REE) was calculated for each individual according to the FAO/WHO equations (13). As expected, these equations overestimated the actual values of REE measured by indirect calorimetry at the end of each period. The energy content of diets was reduced during the bed rest and hypocaloric periods. During the eucaloric phases, participants received a specifically prepared diet containing 1.4 and 1.1 times their calculated REE during the ambulatory and the bed rest periods, respectively. During the hypocaloric phases, participants received a specifically prepared diet containing 1.1 and 0.9 times their resting metabolic rate during the ambulatory and the bed rest periods, respectively. Ten percent of total kcal was added to account for diet-induced thermogenesis (14). Total energy intake was ≈20% lower during the hypocaloric phases than during the correspondent eucaloric phases in bed rest or ambulatory conditions. Subjects received 1g protein · kg body wt^{-1} · d^{-1} in all study phases. Each day's protein intake was identical. Dietary fat content was planned to be ≈30% of the energy during the eucaloric periods. Fatty acid composition was provided as saturated and polyunsaturated fatty acids. During the hypocaloric periods, energy restriction was achieved by decreasing fat intake to a minimum of 60 g/d. The remaining energy was composed of carbohydrates. Total energy, carbohydrate, and lipid intakes during the 4 experimental phases are shown in **Table 1**. Daily intakes of water (50 mL/kg), sodium (2.5 mmol/kg), calcium (1000 mg) and vitamin D (400 IU) were monitored during the experimental periods. No caffeine, methylxanthine, or alcohol was allowed. Six meals were given daily—ie, 3 main meals (breakfast, lunch, and dinner) and 3 snacks. All foods were exactly weighed for each participant, and volunteers were asked to consume the complete meal. The body composition of all subjects was measured by DXA at the end of the adaptation period and at the beginning of the recovery period with the use of the Hologic QDR-2000 (Waltham, MA). The enhanced whole-body scans were analyzed for lean tissue mass.

On the morning of the last day of bed rest or ambulatory periods in eucaloric or hypocaloric conditions, after a 12-h overnight fast, a stable isotope infusion study was performed as described previously (4). Briefly, blood and breath samples were taken before the start of isotope infusion to determine baseline natural enrichments of [1-^{13}C]α-ketoisocaproic acid ([^{13}C]KIC) in arterialized plasma and of $^{13}CO_2$ in the expired air. Thereafter, a bolus injection (0.08 μmol/kg) of [^{13}C] sodium bicarbonate

TABLE 2
Body weight and composition before and at the end of the 4 experimental phases[1]

	Eucaloric diet		Hypocaloric diet		P^2		
	Ambulatory	Bed rest	Ambulatory	Bed rest	Activity effect	Diet effect	Interaction
Initial weight (kg)	77.1 ± 2.9^3	77.0 ± 3.0	80.3 ± 3.1	80.9 ± 3.0	0.47	< 0.01	0.72
Change (kg/14 d)	0.0 ± 0.3	-0.6 ± 0.2	-1.4 ± 0.3^4	-2.1 ± 0.2^4	< 0.01	< 0.01	0.68
Initial lean mass (kg)	61.1 ± 1.2	61.2 ± 1.3	61.7 ± 1.4	61.6 ± 1.6	0.88	0.53	0.56
Change (kg/14 d)	-0.1 ± 0.1^a	-0.3 ± 0.2^a	-0.3 ± 0.3^a	$-1.1 \pm 0.1^{b,4}$	< 0.01	0.04	0.03
Initial fat mass (kg)	16.0 ± 2.0	15.9 ± 1.9	18.6 ± 2.2	19.3 ± 1.8	0.57	< 0.01	0.56
Change (kg/14 d)	0.1 ± 0.3	-0.3 ± 0.2	-1.0 ± 0.3^4	-1.0 ± 0.3^4	0.35	0.01	0.24

[1] Means in a rows with different superscript letter are significantly different, $P < 0.01$ (Bonferroni's post hoc analysis).
[2] Data were analyzed with a 2-factor ANOVA (activity × diet interaction).
[3] $\bar{x} \pm$ SD (all such values).
[4] Changes were significantly different from zero, $P < 0.01$ (paired t test).

(Cambridge Isotope Laboratories, Andover, MA) was intravenously administered and was followed by a primed (5.4 μmol/kg) continuous (0.09 μmol $\cdot$ kg$^{-1} \cdot$ min^{-1}) infusion of L-[1-^{13}C]leucine ([^{13}C]leucine) (Cambridge Isotope Laboratories), which was continued for 6 h. After 160 min had been allowed for isotope equilibration, 3 blood and breath samples were obtained over 20 min to determine [^{13}C]KIC enrichment and to measure amino acid concentrations in arterialized plasma and $^{13}CO_2$ enrichment in the breath. Between 120 and 180 min, indirect calorimetry was performed to measure the rate of total carbon dioxide expiration by using a ventilated hood system (MBM-200; Deltatrac, Datex, Finland), which is an open-circuit computerized indirect calorimeter. A primed (0.13 g/kg LBM) constant (0.13 g $\cdot$ kg LBM$^{-1} \cdot$ h^{-1}) intravenous infusion of an amino acid solution (Freamine III 8.5%; Clintec, Milan, Italy) was initiated; the infusion was continued for 3 h. The amino acid concentrations reported by the manufacturer were 590, 770, 870, 450, 480, 340, 130, 560, 600, 810, 240, 500, 18, and 1190 mg/100 mL for isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, arginine, histidine, serine, cysteine, and glycine, respectively. The infusion rates of the unlabeled leucine under the ambulatory and bed rest conditions, respectively, were 1.46 $\pm$ 0.04 and 1.49 $\pm$ 0.03 μmol $\cdot$ min$^{-1} \cdot$ kg^{-1} during the eucaloric phase and 1.46 $\pm$ 0.04 and 1.46 $\pm$ 0.04 μmol $\cdot$ min$^{-1} \cdot$ kg^{-1} during the hypocaloric phase.

Amino acid concentrations in plasma were measured by using HPLC (4). Plasma [^{13}C]KIC and breath $^{13}CO_2$ isotopic enrichments were determined by using gas chromatography–mass spectrometry (5973 Mass Spectrometer; Agilent–HP, Albertville, MN) and isotope ratio–mass spectrometry (Delta S; Finnigan MAT, Bremen, Germany) as previously described (4, 15).

Estimates of whole-body leucine kinetics were made at isotopic steady state, which was attained at the end of each study period. Mean values of [^{13}C]KIC and $^{13}CO_2$ enrichments and of total carbon dioxide production in each study period were used for data calculation. The intracellular leucine rate of appearance [(Ra) an index of proteolysis], oxidation, and rate of nonoxidative disappearance [(Rd) an index of protein synthesis] were calculated as previously described (4, 15, 16). During amino acid infusion, the rate of net leucine deposition into body protein was calculated from the difference between nonoxidative leucine Rd (protein synthesis) and Ra (proteolysis) (4).

Statistical analysis

All data were expressed as means ± SEMs. The effects of bed rest in the eucaloric condition were assessed according to a crossover during study phases 1 and 2. The effects of bed rest in the hypocaloric condition were assessed according to a crossover during study phases 3 and 4. Results in the 4 different experimental conditions (ambulatory with eucaloric diet, bed rest with eucaloric diet, ambulatory with hypocaloric diet, and bed rest with hypocaloric diet) were analyzed by using a repeated-measures analysis of variance (ANOVA) with activity (ambulatory or bed rest) and diet (eucaloric or hypocaloric) as the 2 factors. Post hoc analysis was performed, when appropriate, by using a t test with Bonferroni's adjustment. Amino acid–mediated changes from the postabsorptive state in the ambulatory and bed rest conditions with eucaloric and hypocaloric diets were compared by using Student's paired t test. Statistical analysis was performed with SPSS software (version 12; SPSS Inc, Chicago, IL). P values ≤ 0.05 were taken as indicating significant differences.

RESULTS

REE relative to LBM did not differ significantly between the ambulatory and bed rest conditions during the eucaloric (25.7 ± 0.6 and 25.0 ± 0.6 kcal/d/kg LBM, respectively) and hypocaloric (24.8 ± 0.7 and 24.7 ± 1.1 kcal/d/kg LBM, respectively) diets ($P = 0.70$ for activity effect; $P = 0.57$ for diet effect; $P = 0.65$ for interaction). The respiratory quotient did not change significantly during the eucaloric periods in the ambulatory (0.87 ± 0.01) or bed rest (0.85 ± 0.01) conditions or during the hypocaloric periods in the ambulatory (0.85 ± 0.02) or bed rest (0.82 ± 0.02) conditions ($P = 0.17$ for activity effect; $P = 0.29$ for diet effect; $P = 0.54$ for interaction). Changes in body weight and in lean and fat masses during the ambulatory and bed rest periods with eucaloric and hypocaloric diets are shown in **Table 2**. Initial body weight and fat mass were greater during the hypocaloric periods (study phases 3 and 4) than during the eucaloric periods (study phases 1 and 2). During the eucaloric periods, in both the bed rest and ambulatory conditions, body weight and lean and fat masses did not change significantly. During the hypocaloric periods, body weight decreased significantly from baseline. Nonetheless, during the hypocaloric period in ambulatory conditions, changes in body weight were largely accounted for by decreases

TABLE 3
Plasma amino acid concentrations in the baseline postabsorptive state and the percentage increase from baseline after intravenous amino acid infusion

| | Eucaloric diet | | Hypocaloric diet | | P^1 | | |
	Ambulatory	Bed rest	Ambulatory	Bed rest	Activity effect	Diet effect	Interaction
Serine							
Baseline (μmol/L)	107 ± 5^2	111 ± 4	130 ± 7	139 ± 6	< 0.01	< 0.01	0.18
Increase (%)	111 ± 8	112 ± 6	126 ± 9	114 ± 7	0.28	0.01	0.19
Histidine							
Baseline (μmol/L)	96 ± 4	95 ± 4	105 ± 5	110 ± 6	0.48	0.06	0.24
Increase (%)	48 ± 2	54 ± 6	52 ± 4	52 ± 3	0.52	0.82	0.50
Glycine							
Baseline (μmol/L)	213 ± 7	220 ± 8	284 ± 11	272 ± 8	0.65	< 0.01	0.11
Increase (%)	163 ± 9	165 ± 12	175 ± 10	176 ± 9	0.80	0.22	0.94
Threonine							
Baseline (μmol/L)	142 ± 6	140 ± 5	165 ± 6	161 ± 4	0.49	< 0.01	0.83
Increase (%)	63 ± 6	65 ± 5	76 ± 4	72 ± 4	0.72	0.11	0.61
Arginine							
Baseline (μmol/L)	83 ± 4	76 ± 5	98 ± 5	94 ± 5	0.12	0.01	0.71
Increase	159 ± 8	177 ± 19	187 ± 20	172 ± 19	0.94	0.36	0.14
Alanine							
Baseline (μmol/L)	301 ± 21	264 ± 18	305 ± 25	274 ± 16	< 0.01	0.41	0.90
Increase (%)	42 ± 5	58 ± 5	66 ± 5	73 ± 4	0.03	< 0.01	0.41
Methionine							
Baseline (μmol/L)	24 ± 1	25 ± 1	27 ± 1	28 ± 1	0.36	0.02	0.61
Increase (%)	380 ± 20	361 ± 26	399 ± 20	390 ± 22	0.18	0.06	0.72
Valine							
Baseline (μmol/L)	226 ± 9	240 ± 10	260 ± 11	277 ± 8	0.02	< 0.01	0.92
Increase (%)	111 ± 4	115 ± 11	112 ± 5	114 ± 5	0.70	0.92	0.85
Phenylalanine							
Baseline (μmol/L)	65 ± 2	70 ± 2	73 ± 4	76 ± 4	0.03	0.10	0.53
Increase (%)	127 ± 5	118 ± 8	130 ± 5	123 ± 5	0.12	0.41	0.85
Isoleucine							
Baseline (μmol/L)	58 ± 3	64 ± 4	66 ± 5	70 ± 4	0.10	0.03	0.63
Increase (%)	268 ± 8	264 ± 26	305 ± 18	298 ± 18	0.76	0.09	0.97
Leucine							
Baseline (μmol/L)	140 ± 5	153 ± 6	163 ± 8	181 ± 7	< 0.01	< 0.01	0.60
Increase (%)	140 ± 5	135 ± 13	148 ± 8	137 ± 5	0.43	0.43	0.75
Lysine							
Baseline (μmol/L)	209 ± 16	204 ± 17	209 ± 22	222 ± 21	0.52	0.37	0.31
Increase (%)	75 ± 11	80 ± 10	105 ± 13	90 ± 13	0.72	0.08	0.53

[1] Data were analyzed with a 2-factor ANOVA (activity × diet interaction).
[2] $\bar{x} \pm$ SD (all such values).

in fat mass, whereas LBM did not change significantly. In contrast, during the hypocaloric period in bed rest, a decrease in fat mass was paralleled by a significant decrease in LBM.

Plasma concentrations of the infused amino acids in the baseline, postabsorptive state and the percentage increases after the infusions at the end of the ambulatory and bed rest periods in both the eucaloric and hypocaloric conditions are shown in **Table 3**. Calorie restriction increased serine, glycine, threonine, arginine, methionine, valine, isoleucine, and leucine concentrations. Bed rest increased plasma concentrations of serine, valine, leucine, and phenylalanine but decreased those of alanine. None of the activity × diet interactions was significant. Intravenous infusion of the amino acid mixture resulted in variable increments in plasma concentrations of the infused amino acids. Increments from the postabsorptive values varied according to the infusion rates and pool sizes of the individual amino acids. Increments in plasma concentrations of amino acids—except alanine and serine—did not differ significantly between the ambulatory and bed rest conditions during eucaloric or hypocaloric diets. There

were significant effects of calorie restriction in enhancing the infusion-mediated increases in serine and alanine concentrations and of bed rest in enhancing the increase in alanine.

Results of whole-body intracellular leucine kinetics in the postabsorptive state and during amino acid infusion at the end of the 4 study phases are shown in **Table 4**. In the baseline postabsorptive state, intracellular Ra (proteolysis) did not exhibit significant differences in the 4 study phases. In contrast, nonoxidative leucine Rd (protein synthesis) was significantly decreased by bed rest. The baseline postabsorptive values of leucine oxidation, an index of net protein catabolism (ie, difference between proteolysis and protein synthesis), did not differ significantly in the ambulatory conditions with the eucaloric or the hypocaloric diet. However, the activity effect and the activity × diet interaction were both significant. Baseline rates of leucine oxidation during bed rest with the hypocaloric diet were significantly greater than those during either the ambulatory or bed rest condition with the eucaloric diet. Amino acid infusion increased nonoxidative leucine Rd (protein synthesis) and leucine

TABLE 4
Whole-body intracellular protein kinetics in the baseline postabsorptive state and the percentage changes from baseline after intravenous amino acid infusion[1]

	Eucaloric diet		Hypocaloric diet		P^2		
	Ambulatory	Bed rest	Ambulatory	Bed rest	Activity effect	Diet effect	Interaction
Leucine Ra (proteolysis)							
Baseline (μmol · kg LBM^{-1} · min^{-1})	2.47 ± 0.05^3	2.36 ± 0.05	2.44 ± 0.12	2.34 ± 0.08	0.20	0.72	0.97
Change (%)	-15 ± 2	-20 ± 2	-12 ± 4	-18 ± 4	0.08	0.47	0.81
Leucine Rd (protein synthesis)							
Baseline (μmol · kg LBM^{-1} · min^{-1})	2.24 ± 0.05	2.11 ± 0.04	2.22 ± 0.12	2.05 ± 0.07	0.04	0.60	0.68
Change (%)	35 ± 2	30 ± 2	41 ± 3	32 ± 2	< 0.01	0.06	0.35
Leucine oxidation							
Baseline (μmol · kg LBM^{-1} · min^{-1})	$0.23 \pm 0.01^{a,b}$	0.25 ± 0.01^a	0.23 ± 0.01^a	0.28 ± 0.01^b	0.04	0.17	0.04
Change (%)	136 ± 12	155 ± 21	124 ± 16	139 ± 21	0.53	0.40	0.83

[1] Ra, rate of appearance; Rd, rate of disappearance. The rate of leucine oxidation in the basal postabsorptive state is a marker of net protein catabolism (ie, difference between proteolysis and protein synthesis). All amino acid–mediated changes from baseline are significantly different from zero, $P < 0.001$ (Student's t test). Means in a row with different superscript letters are significantly different, $P < 0.01$ (Bonferroni's post hoc analysis).
[2] Data were analyzed with a 2-factor ANOVA (activity × diet interaction).
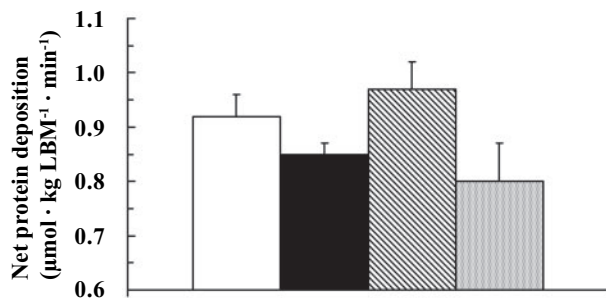[3] $\bar{x} \pm$ SD (all such values).

oxidation and decreased leucine Ra (proteolysis) in all conditions. Amino acid–mediated changes in leucine Ra (proteolysis) and leucine oxidation did not differ significantly during the 4 experimental conditions. In contrast, amino acid–mediated increases in nonoxidative leucine Rd (protein synthesis) were significantly blunted by bed rest, although no significant activity × diet interaction was found. The rates of net leucine deposition into body protein—ie, nonoxidative Rd (protein synthesis) minus Ra (proteolysis) during amino acid infusion were significantly lower in the bed rest than in the ambulatory condition; however, no significant activity × diet interaction was found (**Figure 1**).

DISCUSSION

We have assessed the interaction between 14 d of bed rest and calorie restriction on the regulation of LBM and whole-body protein kinetics in healthy young volunteers by using a crossover experimental design. Subjects were studied 4 times at the end of 14-d periods of any combination of normal physical activity or

FIGURE 1. Rates of net leucine deposition into body protein, ie, nonoxidative rate of disappearance (Rd) (protein synthesis) minus the rate of appearance (Ra) (proteolysis) during amino acid infusion in ambulatory and bed rest conditions with eucaloric and hypocaloric diets. □, ambulatory eucaloric diet; ■, bed rest eucaloric diet; ▨, ambulatory hypocaloric diet; ▨, bed rest hypocaloric diet. LBM, lean body mass. Data were analyzed by using ANOVA with activity × diet interaction. $P < 0.01$ for activity effect; $P = 0.94$ for diet effect; $P = 0.17$ for interaction.

strict bed rest with adequate energy intake or hypocaloric nutrition. Bed rest with hypocaloric nutrition led to the greatest wasting of LBM, as assessed by DXA. Whole-body kinetics of the stable isotope of leucine indicated that the mechanisms of such accelerated protein loss involved an increased net protein catabolism in the postabsorptive state combined with an impaired amino acid–mediated stimulation of protein synthesis in the fed state.

In the 4 study phases, energy intake was carefully tailored to the REE of individual subjects and to their level of physical activity. During bed rest in eucaloric conditions, energy intake was ≈21 ± 1% lower than that during the ambulatory period in eucaloric conditions. Achievement of an energy balance throughout the 2 eucaloric experimental periods was shown by the fact that the body weight and fat mass of subjects did not change significantly during either the bed rest or the ambulatory condition. During the 2 hypocaloric periods, energy intake was 18 ± 2% and 15 ± 2% lower than that during the corresponding eucaloric periods in the bed rest and ambulatory conditions. The periods of bed rest in the hypocaloric condition led to the greatest decrease in energy intake (ie, 34 ± 1% lower than that in the ambulatory eucaloric period). The negative energy balance of subjects was clearly shown by the fact that their fat mass significantly decreased by ≈8% in both the bed rest and ambulatory conditions. Energy balance did not differ significantly during the hypocaloric period between the ambulatory and the bed rest conditions. In fact, with an assumption of an energy density for fat and lean mass of 8192 and 800 kcal/kg, respectively (17), changes in body composition accounted for a negative energy balance of 8603 ± 2489/14 d and 9037 ± 1993 kcal/14 d ($P = 0.87$) during the hypocaloric period in the ambulatory and bed rest conditions, respectively. In addition, during the hypocaloric period in bed rest, the subjects lost ≈2% of their LBM. Such negative changes in lean body mass during the combination of hyponutrition and bed rest were significantly greater than those observed during hyponutrition in ambulatory conditions or during bed rest in eucaloric conditions. These results indicate that physical inactivity in conditions of negative energy balance may lead to a rapid loss of LBM and that such catabolic effects can be

prevented, at least in the short term, by a moderate level of physical activity.

We have shown that a blunted amino acid–induced stimulation of protein synthesis is the main catabolic mechanism associated with short-term inactivity in eucaloric conditions (4). This observation suggests that, during physical inactivity, a greater protein intake may counteract the postprandial defect in amino acid utilization (4). Such a hypothesis was confirmed in a study showing that supplementation with essential amino acids maintained muscle protein synthetic capacity and ameliorated muscle loss during 28 d of bed rest (18). Nonetheless, that study had a dietary caloric content that was sufficient to contribute to an increase in whole-body fat mass during bed rest, which suggests a positive energy balance throughout the experimental period. It remains to be shown whether such anticatabolic effects of increased amino acid availability in hypercaloric conditions during bed rest are also observed in eucaloric conditions or even during a hypocaloric diet. In our study, despite the fact that energy intake varied during the 4 experimental periods, daily protein intake remained constant at 1 g protein/kg body wt. In such controlled conditions, we have shown that bed rest–mediated impairment of protein anabolism in the fed state is quantitatively not different in the eucaloric and hypocaloric conditions.

During the 2 hypocaloric periods, energy restriction was achieved through decreases in carbohydrate and lipid intakes (*see* Subjects and Methods and Table 1). As expected (19), energy restriction in ambulatory conditions did not lead to significant alterations in whole-body protein kinetics. In contrast, we found that the combination of bed rest and calorie restriction led to a greater rate of leucine oxidation, as a marker of net protein catabolism, and to less nonoxidative Rd, as a marker of protein synthesis, in the postabsorptive state.

Physical inactivity is commonly associated with spontaneous or enforced reduction in the nutrient intake in persons with acute or chronic diseases (7, 10). Decreased energy intake is the major cause of negative energy balance in patients, because the frequent disease-mediated elevation in the resting metabolic rate is overridden by inactivity (7). The present study suggests that loss of LBM in bedridden persons is accelerated by inadequate energy intake and that this alteration may rapidly lead to severe malnutrition. Stress mediators, such as cortisol and cytokines, may further amplify the catabolic response of LBM to inactivity and hyponutrition (20, 21). Our results strongly indicate that nutrition of patients should be optimized by matching energy requirements with nutrient intake via either enteral or parenteral routes.

Studies conducted during human space-flight missions found that, despite nutritional advances, most astronauts were in negative energy balance. Anorexia in space can lead to food intakes ≈20–30% lower than preflight and postflight intakes (8, 11, 22), which can result in an average weight loss of ≈0.5 kg for each week spent in space (8). Potential causes for this weight loss may include alterations in circadian rhythms, gastrointestinal function, and neuroendocrine mediators and cytokines and space motion sickness, exposure to radiation energy, and busy work schedules (11). As space-flight missions increase in duration, nutritional deficiencies will become progressively more significant. Malnutrition may amplify the negative consequences of microgravity, including muscle atrophy, osteoporosis, and immune dysfunction (12). The present 2-wk bed rest study was designed to simulate the extent of energy restriction often observed in astronauts during space missions. Loss of body weight

has been comparable in our healthy volunteers and in most of the astronauts who underwent space missions of similar duration (8). Our kinetic results suggest that protein requirements may be increased in microgravity, whereas energy balance should be monitored and maintained throughout the space mission. Potential countermeasures to maintain energy balance during space flight could include medications or dietary interventions to improve appetite and nutrient intake.

Hypocaloric dieting is widely prescribed to overweight subjects to treat the metabolic syndrome and to prevent complications of obesity. In these subjects, loss of body fat is usually accompanied by a decrease in lean mass (23). In contrast, exercise training during calorie restriction may preserve lean mass while further reducing fat mass (24). By explaining the mechanisms of the interaction between calorie restriction and the level of physical activity on regulation of LBM, our study emphasizes the necessity of combating a sedentary lifestyle and of combining the prescription of exercise training with calorie restriction in the treatment of obesity.

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