Calorie restriction accelerates the catabolism of lean body mass during 2 wk of bed rest

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) (<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 · 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.

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 hypnutation 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

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2 Supported by grants from the Italian Space Agency and by grant COFIN 2001 from the Italian Ministry of Education, University, and Research. The general experimental design was supported by grants from the European Space Agency and from the German Space Agency.
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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/m²): 23 ± 1] partici-
pated 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 Ärztekammer 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, eu-
caloric 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 6h 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 adap-
tation 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 pre-
pared 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 specifi-
cally 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 correspon-
dent 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 polyun-
saturated 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 exper-
imental 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 com-
plete 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 over-
night fast, a stable isotope infusion study was performed as de-
scribed previously (4). Briefly, blood and breath samples were
taken before the start of isotope infusion to determine baseline
natural enrichments of [1-13C]α-ketoisocaproic acid ([13C]KIC)
in arterIALIZED plasma and of [13C]CO₂ in the expired air. Thereafter,
a bolus injection (0.08 μmol/kg) of [13C] sodium bicarbonate

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient intake during the ambulatory and bed rest periods with eucaloric and hypocaloric diets</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total energy (kcal · d⁻¹ · kg LBM⁻¹)</td>
</tr>
<tr>
<td>Carbohydrate (kcal · d⁻¹ · kg LBM⁻¹)</td>
</tr>
<tr>
<td>Lipid (kcal · d⁻¹ · kg LBM⁻¹)</td>
</tr>
</tbody>
</table>

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 ± SD (all such values).
and to measure amino acid concentrations in arterialized plasma pic steady state, which was attained at the end of each study. Finnigan MAT, Bremen, Germany) as previously described spectrometry (5973 Mass Spectrometer; Agilent–HP, Al-}
ments were determined by using gas chromatography–mass (protein synthesis) and Ra (proteolysis) (4).

tative disappearance [(Rd) an index of protein synthesis] were calculated from the difference between nonoxidative leucine Rd (protein synthesis) and Ra (proteolysis) (4).

**TABLE 2**

<table>
<thead>
<tr>
<th>Eucaloric diet</th>
<th>Hypocaloric diet</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambulatory</td>
<td>Bed rest</td>
</tr>
<tr>
<td>Initial weight (kg)</td>
<td>77.1 ± 2.9²</td>
<td>77.0 ± 3.0</td>
</tr>
<tr>
<td>Change (kg/14 d)</td>
<td>0.0 ± 0.3</td>
<td>−0.6 ± 0.2</td>
</tr>
<tr>
<td>Initial lean mass (kg)</td>
<td>61.1 ± 1.2</td>
<td>61.2 ± 1.3</td>
</tr>
<tr>
<td>Change (kg/14 d)</td>
<td>−0.1 ± 0.1²</td>
<td>−0.3 ± 0.2²</td>
</tr>
<tr>
<td>Initial fat mass (kg)</td>
<td>16.0 ± 2.0</td>
<td>15.9 ± 1.9</td>
</tr>
<tr>
<td>Change (kg/14 d)</td>
<td>0.1 ± 0.3</td>
<td>−0.3 ± 0.2</td>
</tr>
</tbody>
</table>

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 ± SD (all such values).
⁴ Changes were significantly different from zero, P < 0.01 (paired t test).

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 (ambula-
tory 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 peri-
ods, body weight decreased significantly from baseline. Nonetheless, during the hypocaloric period in ambulatory conditions, changes in body weight were largely accounted for by decreases

(Cambridge Isotope Laboratories, Andover, MA) was intrave-

ously administered and was followed by a primed (5.4 μmol/ kg) continuous (0.09 μmol·kg⁻¹·min⁻¹) infusion of L-[1-13C]leucine ([13C]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 [13C]KIC enrichment and to measure amino acid concentrations in arterialized plasma and 13CO₂ enrichment in the breath. Between 120 and 180 min, indirect calorimetry was performed to measure the rate of total carbon dioxide production 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·kg LBM⁻¹·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, 840, 1000, 2000, 3000, 4000, 5000, and 6000 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 ± 0.04 and 1.49 ± 0.03 μmol·min⁻¹·kg⁻¹ during the eucaloric phase and 1.46 ± 0.04 and 1.46 ± 0.04 μmol·min⁻¹·kg⁻¹ during the hypocaloric phase.

Amino acid concentrations in plasma were measured by using HPLC (4). Plasma [13C]KIC and breath 13CO₂ isotopic enrichments were determined by using gas chromatography–mass spectrometry (5973 Mass Spectrometer; Agilent–HP, Alber-ville, MN) and isotopic ratio–mass spectrometry (Delta S; Finnigan MAT, Bremen, Germany) as previously described (4, 15).

Estimates of whole-body leucine kinetics were made at iso-
topic steady state, which was attained at the end of each study period. Mean values of [13C]KIC and 13CO₂ 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 differences between nonoxidative leucine Rd (protein synthesis) and Ra (proteolysis) (4).
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 during amino acid infusion 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 \( \times \) 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 \( \times \) 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 3

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Eucaloric diet</th>
<th>Hypocaloric diet</th>
<th>Activity effect</th>
<th>Diet effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambulatory</td>
<td>Bed rest</td>
<td>Ambulatory</td>
<td>Bed rest</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>107 ± 5(^2)</td>
<td>111 ± 4</td>
<td>130 ± 7</td>
<td>139 ± 6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>111 ± 8</td>
<td>112 ± 6</td>
<td>126 ± 9</td>
<td>114 ± 7</td>
<td>0.28</td>
</tr>
<tr>
<td>Histidine</td>
<td>96 ± 4</td>
<td>95 ± 4</td>
<td>105 ± 5</td>
<td>110 ± 6</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>48 ± 2</td>
<td>54 ± 6</td>
<td>52 ± 4</td>
<td>52 ± 3</td>
<td>0.52</td>
</tr>
<tr>
<td>Glycine</td>
<td>213 ± 7</td>
<td>220 ± 8</td>
<td>284 ± 11</td>
<td>272 ± 8</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>163 ± 9</td>
<td>165 ± 12</td>
<td>175 ± 10</td>
<td>176 ± 9</td>
<td>0.80</td>
</tr>
<tr>
<td>Threonine</td>
<td>142 ± 6</td>
<td>140 ± 5</td>
<td>165 ± 6</td>
<td>161 ± 4</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>63 ± 6</td>
<td>65 ± 5</td>
<td>76 ± 4</td>
<td>72 ± 4</td>
<td>0.72</td>
</tr>
<tr>
<td>Arginine</td>
<td>83 ± 4</td>
<td>76 ± 5</td>
<td>98 ± 5</td>
<td>94 ± 5</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>159 ± 8</td>
<td>177 ± 19</td>
<td>187 ± 20</td>
<td>172 ± 19</td>
<td>0.94</td>
</tr>
<tr>
<td>Alanine</td>
<td>301 ± 21</td>
<td>264 ± 18</td>
<td>305 ± 25</td>
<td>274 ± 16</td>
<td>&lt; 0.01</td>
</tr>
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<td></td>
<td>42 ± 5</td>
<td>58 ± 5</td>
<td>66 ± 5</td>
<td>73 ± 4</td>
<td>0.03</td>
</tr>
<tr>
<td>Methionine</td>
<td>24 ± 1</td>
<td>25 ± 1</td>
<td>27 ± 1</td>
<td>28 ± 1</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>380 ± 20</td>
<td>361 ± 26</td>
<td>399 ± 20</td>
<td>390 ± 22</td>
<td>0.18</td>
</tr>
<tr>
<td>Valine</td>
<td>226 ± 9</td>
<td>240 ± 10</td>
<td>260 ± 11</td>
<td>277 ± 8</td>
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</tr>
<tr>
<td></td>
<td>111 ± 4</td>
<td>115 ± 11</td>
<td>112 ± 5</td>
<td>114 ± 5</td>
<td>0.70</td>
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<tr>
<td>Phenylalanine</td>
<td>65 ± 2</td>
<td>70 ± 2</td>
<td>73 ± 4</td>
<td>76 ± 4</td>
<td>0.03</td>
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<tr>
<td></td>
<td>127 ± 5</td>
<td>118 ± 8</td>
<td>130 ± 5</td>
<td>123 ± 5</td>
<td>0.12</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>58 ± 3</td>
<td>64 ± 4</td>
<td>66 ± 5</td>
<td>70 ± 4</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>268 ± 8</td>
<td>264 ± 26</td>
<td>305 ± 18</td>
<td>298 ± 18</td>
<td>0.76</td>
</tr>
<tr>
<td>Leucine</td>
<td>140 ± 5</td>
<td>153 ± 6</td>
<td>163 ± 8</td>
<td>181 ± 7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>140 ± 5</td>
<td>135 ± 13</td>
<td>148 ± 8</td>
<td>137 ± 5</td>
<td>0.43</td>
</tr>
<tr>
<td>Lysine</td>
<td>209 ± 16</td>
<td>204 ± 17</td>
<td>209 ± 22</td>
<td>222 ± 21</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>75 ± 11</td>
<td>80 ± 10</td>
<td>105 ± 13</td>
<td>90 ± 13</td>
<td>0.72</td>
</tr>
</tbody>
</table>

\(^1\) Data were analyzed with a 2-factor ANOVA (activity \( \times \) diet interaction).  
\(^2\) Mean ± 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 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 \(\approx 21 \pm 1\%\) 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 \(18 \pm 2\%\) and \(15 \pm 2\%\) 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, \(34 \pm 1\%\) 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 \(\approx 8\%\) 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 \(\approx 2\%\) 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

### Table 4

Whole-body intracellular protein kinetics in the baseline postabsorptive state and the percentage changes from baseline after intravenous amino acid infusion.

<table>
<thead>
<tr>
<th></th>
<th>Eucaloric diet</th>
<th>Hypocaloric diet</th>
<th>(P^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambulatory</td>
<td>Bed rest</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bed rest</td>
<td>Activity effect</td>
<td>Diet effect</td>
</tr>
<tr>
<td>Leucine Ra (proteolysis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (µmol·kg LBM(^{-1})·min(^{-1}))</td>
<td>2.47 ± 0.05(^a)</td>
<td>2.36 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Change (%)</td>
<td>−15 ± 2</td>
<td>−20 ± 2</td>
<td>0.20</td>
</tr>
<tr>
<td>Leucine Rd (protein synthesis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (µmol·kg LBM(^{-1})·min(^{-1}))</td>
<td>2.24 ± 0.05</td>
<td>2.11 ± 0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Change (%)</td>
<td>35 ± 2</td>
<td>30 ± 2</td>
<td>0.06</td>
</tr>
<tr>
<td>Leucine oxidation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (µmol·kg LBM(^{-1})·min(^{-1}))</td>
<td>0.23 ± 0.01(^ab)</td>
<td>0.25 ± 0.01(^a)</td>
<td>0.04</td>
</tr>
<tr>
<td>Change (%)</td>
<td>136 ± 12</td>
<td>155 ± 21</td>
<td>0.17</td>
</tr>
</tbody>
</table>

\(^{1}\) 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).

\(^{a}\) ± SD (all such values).

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**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.
achieved through decreases in carbohydrate and lipid intakes in eucaloric and hypocaloric conditions. 

We have shown that bed rest–mediated impairment of protein anabolism in the fed state is quantitatively not different in the 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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