Dietary feeding pattern does not modulate the loss of muscle mass or the decline in metabolic health during short-term bed rest

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RESEARCH ARTICLE

Dietary feeding pattern does not modulate the loss of muscle mass or the decline in metabolic health during short-term bed rest

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INTRODUCTION

Periods of bed rest are often required for the recovery from illness or injury. Despite the necessity of such periods of disuse for recovery, bed rest leads to substantial changes in body composition, characterized by a decrease in skeletal muscle mass of 0.5–0.6% per day (64), and an overall decline in metabolic health (5). The impact of bed rest on muscle mass and quality is already evident after as little as 5–7 days of bed rest (20, 24, 56, 58). This is of important clinical relevance, as the current overall average duration of hospitalization for all ages and reasons for hospital admission is 7 days (22). However, the reason for the bed rest-induced decline in muscle mass and muscle quality remains to be elucidated.

Both physical activity and food intake are key anabolic stimuli, which are required to maintain skeletal muscle tissue mass and quality. Muscle contractions, as well as food intake (i.e., ingestion of protein meals), strongly increase muscle protein synthesis rates and improve net muscle protein balance (47, 48). Hospitalization is characterized by a strong decline or even absence of physical activity due to restricted bed rest. Furthermore, in many patients food intake is reduced, often as a result of surgical stress, anxiety, nausea, lack of appetite, and/or gastrointestinal disorders. Maintaining energy balance and habitual protein consumption have been shown to be requirements to attenuate muscle loss during a period of bed rest or limb immobilization (7, 52). In many conditions, this is performed by nutritional supplementation or even enteral (tube) feeding.

Previous work has shown that ingestion of 20 g of a high-quality protein maximizes muscle protein synthesis rates during a 4-h postprandial period (67, 68). This has led to the formation of guidelines advocating consumption of 20 g protein with each main meal (16). Because of the stimulation of muscle protein synthesis following ingestion of each meal, an intermittent feeding strategy has been suggested to be preferred over more continuous feeding. Furthermore, the hormonal response to continuous feeding may be suboptimal to fully suppress postprandial muscle protein breakdown (29). However, whether intermittent feeding leads to an attenuated decline in skeletal muscle mass and/or quality when compared with continuous feeding is far from evident. Animal work has suggested that continuous feeding leads to lower rates of muscle protein synthesis (21, 26) and a more rapid decline in insulin sensitivity (54). However, work in humans is inconclusive (12, 37), and the impact of dietary feeding pattern on bed rest-induced muscle atrophy remains to be assessed. We hypothesized that continuous enteral feeding would lead to greater loss of muscle mass and quality when compared with
intermittent enteral feeding during 1 wk of bed rest in healthy volunteers fed in energy balance.

To test this hypothesis, we subjected 20 young, healthy men to one week of bed rest while being tube-fed in energy balance using either a continuous (24 h) or an intermittent (4 boluses daily) enteral feeding protocol. Muscle mass [CT, dual-energy X-ray absorptiometry (DXA)] and metabolic health (VO_{2peak}, whole-body insulin sensitivity via hyperinsulinemic-euglycemic clamp) were assessed before and after 1 wk of bed rest. Muscle protein synthesis rates were assessed for 1 wk before bed rest and during 1 wk of bed rest using deuterated water administration and muscle biopsy sampling. This is the first study to compare the impact of continuous versus intermittent enteral feeding on changes in muscle mass and quality during 1 wk of bed rest in vivo in humans.

METHODS

Participants. Twenty healthy, young men (age 25 ± 1 yr) were included in the present study. Participants’ characteristics are presented in Table 1. Prior to inclusion, participants completed a general health questionnaire and visited the university for a routine medical screening to ensure their eligibility to take part. Exclusion criteria included a body mass index below 18.5 or above 30 kg/m^2, a (family) history of deep vein thrombosis, Type 2 diabetes mellitus (determined from the research team. Energy requirements were estimated on the basis of indirect calorimetry, multiplied by an activity factor of 1.60 (free-living) and 1.35 (bed rest). Energy intake was adjusted if participants reported to be hungry or felt overfed for more than 1 day. In those situations, food provision was adjusted by decreasing or increasing the activity factor by 0.1. Macronutrient composition of the diet was identical between free-living and bed rest periods (Table 2). During bed rest, food administration in both groups was performed via a nasogastric tube (Flocare PUR tube Enlock, Ch8, 110 cm; Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands). Correct positioning of the tube in the stomach was assessed by means

Table 1. Participants’ characteristics

<table>
<thead>
<tr>
<th></th>
<th>Intermittent (n = 10)</th>
<th>Continuous (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>27 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>77.5 ± 5.1</td>
<td>77.3 ± 5.1</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.81 ± 0.03</td>
<td>1.79 ± 0.03</td>
</tr>
<tr>
<td>BMI, kg/m^2</td>
<td>23.5 ± 1.3</td>
<td>24.0 ± 1.0</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>RMR, MJ/day</td>
<td>7.6 ± 0.4</td>
<td>7.6 ± 0.3</td>
</tr>
</tbody>
</table>

BMI, body mass index; HbA1c, glycated hemoglobin; RMR, resting metabolic rate.

Table 2. Dietary intake

<table>
<thead>
<tr>
<th></th>
<th>Free-Living (n = 10)</th>
<th>Bed Rest</th>
<th>Continuous (n = 10)</th>
<th>Bed Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, MJ/day</td>
<td>11.3 ± 0.7</td>
<td>9.8 ± 0.4*</td>
<td>10.8 ± 0.3</td>
<td>10.1 ± 0.4*</td>
</tr>
<tr>
<td>Protein, g/kg body wt^{-1}day^{-1}</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1*</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.1*</td>
</tr>
<tr>
<td>Protein, g/day</td>
<td>108 ± 7</td>
<td>94 ± 4*</td>
<td>107 ± 5</td>
<td>96 ± 3*</td>
</tr>
<tr>
<td>Carbohydrates, g/day</td>
<td>323 ± 19</td>
<td>276 ± 12*</td>
<td>302 ± 8</td>
<td>282 ± 10*</td>
</tr>
<tr>
<td>Fat, g/day</td>
<td>100 ± 6</td>
<td>89 ± 4*</td>
<td>95 ± 4</td>
<td>91 ± 3*</td>
</tr>
<tr>
<td>Fibers, g/day</td>
<td>32 ± 2</td>
<td>35 ± 2*</td>
<td>31 ± 1</td>
<td>36 ± 1*</td>
</tr>
<tr>
<td>Protein, En%</td>
<td>16 ± 0</td>
<td>16</td>
<td>17 ± 0</td>
<td>16</td>
</tr>
<tr>
<td>Carbohydrate, En%</td>
<td>48 ± 1</td>
<td>47</td>
<td>47 ± 1</td>
<td>47</td>
</tr>
<tr>
<td>Fat, En%</td>
<td>33 ± 1</td>
<td>34</td>
<td>33 ± 0</td>
<td>34</td>
</tr>
<tr>
<td>Fibers, En%</td>
<td>2 ± 0</td>
<td>3*</td>
<td>2 ± 0</td>
<td>3*</td>
</tr>
</tbody>
</table>

Values (means ± SE) represent parameters of dietary intake from n = 20 healthy, male volunteers during 7 days of free-living and 7 days of strict bed rest. During bed rest, participants were fed a standard enteral food product in an intermittent (4 meals/day) or continuous (24 h/day) manner. BW, body weight; En%, energy percentage; MJ, Mega Joule. *Significantly different (P < 0.05) from corresponding free-living values.
of a pH check directly following insertion and on every morning during the bed rest period. A standard enteral food product (Nutrition Multi Fiber, Nutricia Advanced Medical Nutrition) was given, composed of 47 energy percent (en%) carbohydrates, 34 en% fat, 16 en% protein (blend of casein, whey, soy, and pea), and 3 en% fibers. Participants in the intermittent feeding group received the same product provided in four daily boluses. These boluses were administered at a rate of 25 ml/min (providing ~28 g protein per bolus) at 0800 (30% of total daily food intake), 1300 (30%), 1800 (30%), and 2300 (10%, representing a smaller pre-sleep meal), with the first meal administered on the morning of the first day of bed rest. Participants in the continuous feeding were fed in a continuous manner, using a Flocare Infinity enteral feeding pump (Nutricia Advanced Medical Nutrition) at a constant speed (i.e., ~100 ml/h) based on daily energy requirements. Continuous feeding started at 0000 on the evening before bed rest and ended at 0000 on the evening of day 7 to ensure fasting conditions on test day 3. Nasogastric tubes were removed at 0000 on the evening of day 7 in both groups.

**Body composition.** During test days 2 and 3 (one day before and immediately after bed rest, respectively, at 0900, anatomical cross-sectional area (CSA) of the quadriceps muscle was measured via a single-slice CT scan (Philips Brilliance 64, Philips Medical Systems, Best, The Netherlands), as described previously (20). Briefly, a 3-mm-thick axial image was made at 15 cm above the patella, with participants in supine position, while their legs were extended and their feet were secured. On test day 2, the exact scanning position was marked on the skin with semipermanent ink for replication on test day 3. CT scans were analyzed for quadriceps muscle CSA by manual tracing using ImageJ software [version 1.50c, National Institutes of Health, Bethesda, MD (55)]. On the same days, a DXA-scan (Hologic, Discovery A, Waltham, MA) was made at 1400 to assess body composition. The system’s software package Apex version 4.0.2 (en-CORE 2005, version 9.15.00 Hologic, Marlborough, MA) was used to determine whole body and regional lean mass, fat mass, and bone mineral content.

**Metabolic health.** Prior to the free-living period and on the day following bed rest, maximal oxygen uptake capacity was measured as $V_{\text{O}_2 \text{peak}}$ [described previously (20)]. Whole body insulin sensitivity was measured via a one-step hyperinsulinenemic-euglycemic clamp, as described previously (20). In short, 20% glucose (Baxter B.V., Utrecht, The Netherlands) was co-infused with [2H$_2$]oligoargin (4.1 mU·m$^{-2}$·min$^{-1}$; Novarapid, Novo Nordisk Farma, Alphen aan den Rijn, The Netherlands) during a 2.5-h clamp, which was started at 0930. Arterialized blood glucose concentrations were measured every 5 min, and the glucose infusion rate was altered to maintain euglycemia at 5.0 mmol/l.

**Deuterium oxide loading and body water enrichments.** To increase body water deuterium oxide ($D_2O$, or $^2H$) enrichments, participants attended the university for a $D_2O$ loading day. During that day, participants consumed 8 × 50 ml oral doses of 70% $D_2O$ (Cambridge Isotope Laboratories, Tewksbury, MA) with 1.5 h in between doses. To maintain body water enrichments throughout the study period, participants consumed one daily 50-ml oral dose every morning of the study period. Daily saliva samples were collected using a cotton swab at 1800 on every study day, to determine body water enrichment. Samples were frozen in liquid nitrogen and stored at ~80°C. Body water $^2H$-alanine enrichments were measured as described elsewhere (32). In short, samples were centrifuged at 10,000 g to remove debris and subsequently diluted 70-fold with ddH$_2$O to achieve deuterium enrichments within the detection limits of the GC-C-IRMS. Samples were prepared for analysis using the protocol by Scrimgeour et al. (51). This involved placing small plastic cups holding 4 mg of catalyst (5% platinum on alumina, 325 mesh, Sigma-Aldrich, St. Louis, MO) inside 3 ml glass vials, after which 300 µl of diluted saliva sample was added, and vials were sealed using rubber septa and a screw cap. Air in each vial was evacuated and replaced by hydrogen gas simultaneously, after which vials were left at 21°C for 24 h for deuterium equilibration to occur between the hydrogen gas and the saliva samples. The deuterium enrichment of the hydrogen gas was then measured in duplicate on a GC-C-IRMS (Micromass Optima IRMS fitted with a Multiprep and Glosa Pyrolytic Graphite, Micromass UK Limited, Manchester, UK). Standard regression curves were applied from a series of known standard enrichment values against the measured values to assess the linearity of the mass spectrometer and to account for deuterium loss during equilibration.

**Myofibrillar protein synthesis.** On test days 1, 2, and 3, a single muscle biopsy sample was collected from musculus vastus lateralis at 0815. After local anesthesia was induced, a percutaneous needle biopsy was taken ~15 cm above the patella using the Bergström technique (6). The collected muscle tissue was freed from any visible blood and nonmuscle tissue and rapidly frozen in liquid nitrogen. Muscle samples were subsequently stored at ~80°C until further analyses. Myofibrillar protein-enriched fractions were extracted from ~60 mg of wet muscle tissue by hand-homogenizing on ice using a pestle in a standard extraction buffer (10 µl/mg). The samples were spun at 2,500 g and 4°C for 5 min. The pellet was washed with 500 µl ddH$_2$O and centrifuged at 2,500 g and 4°C for 10 min. The myofibrillar protein was solubilized by adding 1 ml of 0.3 M NaOH and heating at 50°C for 30 min with vortex mixing every 10 min. Samples were centrifuged at 9,500 g and 4°C for 5 min, the supernatant containing the myofibrillar proteins was collected, and the collagen pellet was discarded. Myofibrillar proteins were precipitated by the addition of 1 ml of 1 M PCA and spun at 700 g and 4°C for 10 min. The myofibrillar protein was washed twice with 70% ethanol and hydrolyzed overnight in 2 ml of 6 M HCl at 110°C. The free amino acids from the hydrolyzed myofibrillar protein pellet were dried under a continuous nitrogen stream while being heated at 120°C. The free amino acids were then dissolved in 25% acetic acid solution, passed over cation exchange AG 50W-X8 resin columns (mesh size: 100–200, ionic form: hydrogen; Bio-Rad Laboratories, Hercules, CA), and eluted with 2 M NH$_4$OH. Thereafter, the eluate was dried, and the purified amino acids were derivatized to their N(O,S)-ethoxycarbonyl ethyl esters (33). The derivatized samples were measured using a gas chromatography-isotope ratio mass spectrometer (GC-IRMS; Thermo Fisher Scientific, MAT 253; Bremen, Germany) equipped with a pyrolysis oven and a 60 m DB-17MS column (no. 122-4762; Agilent, Wilmington, DE) and 5 m precolumn. Ion masses 2 and 3 were monitored to determine the $^2H$/H ratios of muscle protein-bound alanine. A series of known standards was applied to assess linearity of the mass spectrometer and to control for the loss of tracer.

**Skeletal muscle gene expression.** A second part of the obtained muscle sample (~15 mg) was used to measure mRNA expression of target genes, as described in detail elsewhere (61). Briefly, total RNA was isolated from frozen muscle tissue and spectrophotometrically quantified. Next, after RNA purity was determined and cDNA synthesis was performed, TaqMan PCR was carried out using 18S as a housekeeping gene. We have previously demonstrated that 18S expression does not change with muscle disuse (63). TaqMan probe sets were obtained from Applied Biosystems (Foster City, CA) for the following genes of interest: Atrogen-1/muscle atrophy F-box (MAFbx), Forkhead box protein O1 (FoxO1), mammalian target of rapamycin (mTOR), muscle RING-finger protein-1 (MuRF1), and ribosomal protein 70-kDa S6 kinase (p70S6K). Cycle threshold (Ct) values of the target genes were normalized to Ct values of 18S, and final results were calculated as relative expression against the standard curve.

**Nitrogen balance.** On every day of the bed rest period, 24-h urine collection was performed starting from the second voiding of the day until the first voiding on the day after. Urine was collected into containers with 10 ml of 4 M HCl. After the total daily urine production was measured, aliquots of urine were snap-frozen in liquid nitrogen and stored at ~80°C. The Dumas combustion method was used to determine nitrogen using the vario MAX cube CN (Elementar Analysensysteme, Germany), as described before (60).
Statistics. The two-tailed sample size calculation ($\alpha = 0.05$, power $= 0.8$) was based on an expected 29% decline in insulin sensitivity following 1 wk of bed rest with intermittent feeding (20), and an expected 25% worsening thereof (i.e., $-36 \pm 5\%$) in the continuous feeding group (54). This resulted in a required sample size of $n = 10$ participants per group. Baseline differences between groups were assessed using an independent samples $t$-test. Changes over time were analyzed using a repeated-measures ANOVA with time (free-living vs. bed rest or pre- vs. post-bed rest) as within-subjects factor and group (intermittent vs. continuous) as between-subjects factor. In the case of a significant interaction, a Bonferroni post hoc test was applied to locate individual differences. Statistical data analysis was performed using SPSS version 24.0 (IBM, Armonk, NY). Statistical significance was set at $P < 0.05$. All data are expressed as means $\pm SE$.

RESULTS

Body composition. The two experimental groups did not differ in any of the participants’ characteristics (Table 1) before the start of the study (all $P > 0.05$). After 1 wk of bed rest, whole body lean mass had decreased by $525 \pm 219$ g ($P < 0.05$, Fig. 1, A and B). Quadriceps cross-sectional area (CSA; Fig. 2, A and B) had declined by $1.1 \pm 0.6\%$ (from $7513 \pm 522$ to $7430 \pm 511$ mm$^2$) and $0.8 \pm 0.5\%$ (from $7,544 \pm 549$ to $7,444 \pm 549$).

![Fig. 1. Lean body mass (A and B), whole body oxygen uptake capacity (C and D), and whole body insulin sensitivity (E and F) at baseline and following 7 days of strict bed rest in healthy, young men, who were nasogastric tube-fed in an intermittent (n = 10) or continuous (n = 10) feeding pattern. A, C, and E show individual data, whereas panels B, D, and F display group means. GIR, glucose infusion rate. *Significantly different from pre-bed rest values ($P < 0.05$). Values are expressed as means $\pm SE$.](https://journals.physiology.org/journal/ajpendo/)

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7,469 ± 522 mm²) in the intermittent and continuous feeding groups, respectively (P < 0.05). No differences were observed between groups (interaction effect, P > 0.05). Bed rest led to an average 0.62 ± 0.19 kg decline in total body mass (P < 0.01; Table 3), which was predominantly attributed to a loss of trunk lean mass (−0.52 ± 0.12 and −0.36 ± 0.19 kg in the intermittent and continuous feeding group, respectively; P < 0.01), which did not differ between groups (P > 0.05). Because of the maintenance of energy balance during bed rest, no changes in whole body fat mass were observed (interaction effect, P > 0.05).

Maximal oxygen uptake capacity and whole body insulin sensitivity. VO2peak (Fig. 1, C and D) declined from 40.3 ± 3.0 to 38.9 ± 2.5 ml·kg⁻¹·min⁻¹ following bed rest with intermittent feeding and from 44.8 ± 3.1 to 40.7 ± 2.6 ml·kg⁻¹·min⁻¹ following bed rest with continuous feeding (time effect P < 0.001), with no differences between groups (interaction effect, P > 0.05). Glucose infusion rate (Fig. 1, E and F), representing whole body insulin sensitivity, declined by 46 ± 3% following bed rest with intermittent and 39 ± 3% following bed rest with continuous feeding (time effect P < 0.001), with no differences between groups (interaction effect, P > 0.05).

Cumulative muscle protein synthesis. Analyses of daily saliva samples revealed a gradual increase in body water enrichments (Fig. 3; time effect P < 0.001), with no differences between groups. Cumulative myofibrillar protein fractional synthetic rates (FSRs; Fig. 4) were not different between groups during the free-living period. Moreover, no significant differences between free-living and bed rest (time effect, P > 0.05) or between groups during bed rest (interaction effect P > 0.05, treatment effect P > 0.05) were found.

Skeletal muscle gene expression. Skeletal muscle mRNA expression of genes involved in muscle mass regulation, are depicted in Fig. 5. For mTOR and P70S6K, both key players in the regulation of muscle protein synthesis, no significant effects were found (interaction effect, all P > 0.05). FoxO1 and MuRF1 mRNA expression also were not influenced by bed rest or dietary feeding pattern (interaction effect, both P > 0.05). MAFbx mRNA expression (Fig. 5D) showed a time effect (P < 0.01) but no interaction effect (P > 0.05), demonstrating increased expression following bed rest in both feeding strategies. Skeletal muscle mRNA expression of the housekeeping gene 18S was not affected by bed rest or dietary feeding pattern (interaction and time effect both P > 0.05).

Nitrogen balance. Dietary nitrogen intake during bed rest, derived from dietary protein intake, was on average 15.0 ± 0.6 and 15.4 ± 0.5 g/day in the intermittent and continuous feeding groups, respectively, with no differences over time or between groups (both P > 0.05). Urinary nitrogen loss showed a time effect (P < 0.05), such that urinary nitrogen loss was greater on day 7 than on day 1. From these data, 24-h nitrogen balance was calculated (Fig. 6). We show that 7 days of bed rest, irrespective of dietary feeding pattern (interaction effect, P > 0.05), leads to a decline in whole body nitrogen balance.

Table 3. Body composition before and after 7 days of strict bed rest in participants fed either intermittently (4 boluses per day) or in a continuous manner

<table>
<thead>
<tr>
<th></th>
<th>Intermittent (n = 10)</th>
<th>Continuous (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Total mass, kg</td>
<td>77.7 ± 4.9</td>
<td>77.3 ± 5.0*</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>18.2 ± 2.1</td>
<td>18.3 ± 2.1</td>
</tr>
<tr>
<td>Fat percentage, %</td>
<td>22.9 ± 1.9</td>
<td>23.2 ± 1.9</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>57.0 ± 3.4</td>
<td>56.6 ± 3.4*</td>
</tr>
<tr>
<td>Trunk lean mass, kg</td>
<td>28.6 ± 1.8</td>
<td>28.0 ± 1.7*</td>
</tr>
<tr>
<td>Leg lean mass, kg</td>
<td>9.5 ± 0.7</td>
<td>9.5 ± 0.6</td>
</tr>
<tr>
<td>Arm lean mass, kg</td>
<td>3.5 ± 0.2</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>BMD, g/cm²</td>
<td>1.16 ± 0.03</td>
<td>1.17 ± 0.03*</td>
</tr>
</tbody>
</table>

Values (means ± SE) represent parameters of body composition from n = 20 healthy, male volunteers before (pre) and after (post) 7 days of strict bed rest, as measured by dual-energy X-ray absorptiometry. BMD, bone mineral density. *Significantly different (P < 0.05) from corresponding Pre values.
1.25 g·kg body wt

normal foods). Daily protein intake in the present study was

position of the standard enteral feeds (which are typically higher

opposed to normal food consumption (13) and/or the compo-

ancy may be attributed to the enteral feeding regimens as

1 wk of bed rest in our laboratory (20). The apparent discrep-

decline in quadriceps CSA that we recently observed following

basis of the 1.4

quadriceps CSA was less than what we had expected on the

rest, participants were nasogastric tube-fed in an intermittent or continuous

feeding pattern. Values are expressed as means ± SE. *Significantly different

from test 1 (P < 0.001).

(time effect, P < 0.05). However, a significant treatment effect

(P < 0.05) indicated that at all time points, the continuous

feeding group was in a more positive nitrogen balance.

**DISCUSSION**

In the current study, we observed that 1 wk of strict bed rest

reduced muscle mass, lowered oxygen uptake capacity, and

impaired insulin sensitivity in healthy volunteers fed in energy

balance. Dietary feeding pattern, i.e., enteral food administra-

tion in an intermittent versus continuous manner, did not

impact the bed rest-induced decline in muscle mass and met-

abolic health. Moreover, measures of muscle protein synthesis

rates and markers of muscle protein breakdown were not

influenced by the pattern of food administration.

In line with previous work in our laboratory (20), as well as

others (7, 23, 24, 52, 56), we show the impact of 1 wk of bed

rest on muscle mass and metabolic health. The average

525 ± 219 g loss of lean tissue and 0.9 ± 0.4% decline in

quadriceps CSA was less than what we had expected on the

basis of the 1.4 ± 0.2 kg lean tissue loss and 3.2 ± 0.9% decline in quadriceps CSA that we recently observed following 1 wk of bed rest in our laboratory (20). The apparent discrep-

ancy may be attributed to the enteral feeding regimens as

opposed to normal food consumption (13) and/or the compo-

sition of the standard enteral feeds (which are typically higher in protein and/or branched chain amino acids content than normal foods). Daily protein intake in the present study was 1.25 g·kg body wt⁻¹·day⁻¹ (Table 2) compared with 0.98 g·kg-body wt⁻¹·day⁻¹ in our previous study (20). Furthermore, the enteral feeding product had a branched-chain amino acid content (22 g/100 g protein) that is even higher than milk or beef (11). The anabolic properties of the branched chain amino acids (14, 34) may have contributed to the lesser muscle loss (45, 52) in the present study when compared with our previous work. The observed muscle atrophy was accompanied by a substantial ~5% decline in maximal oxygen uptake capacity and a ~40% decrease in whole body insulin sensitivity (Fig. 1).

To put this in perspective, such a decline in muscle mass and metabolic health is similar to what is generally observed over many years of aging (15, 42, 46). Clearly, it is of important clinical relevance to gain more insight in the mechanisms underlying disuse-induced atrophy and insulin resistance, to develop interventions that can attenuate a decline in muscle mass and health during short episodes of muscle disuse.

We hypothesized that dietary feeding pattern would modu-
late the rate of muscle atrophy, as well as the bed rest-induced impairments in oxygen uptake capacity and insulin sensitivity. Therefore, we provided 20 healthy subjects with nasogastric feeding tubes to allow continuous and intermittent feeding with exactly the same clinical enteral feeding product. To mimic the ingestion of various meals, we administered the enteral feed in an intermittent pattern, providing four daily boluses mimicking three main meals and a pre-bed snack, to half of the partici-

pants. In contrast, the continuous enteral feeding group re-

ceived the same amount of food continuously (24/7). Previous work has suggested that dietary feeding pattern forms an important factor driving postprandial muscle protein synthesis. Specifically, ingestion of a single meal-like bolus of 20 g protein is required to significantly increase muscle protein synthesis rates and inhibit protein breakdown, thereby resulting in net muscle protein accretion (10, 30, 62, 67, 68). On the basis of these findings, it has been suggested that each main meal should contain ample protein to allow such a postprandial anabolic response and that a dietary intake pattern containing less protein in each meal would be suboptimal in maintaining muscle mass. In support, some studies (2, 4, 12, 21, 26, 65), but certainly not all (3, 36, 37, 39, 40), have shown a more positive impact of bolus feeding on muscle protein synthesis and/or muscle protein retention when compared with more frequent feeding of smaller quantities of food. Subjects in the intermit-
tent enteral feeding group were administered four daily boluses containing 28 ± 1 g protein, 83 ± 4 g carbohydrate, and 27 ± 1 g fat. This amount of high-quality protein would provide sufficient amino acids to stimulate muscle protein synthesis, inhibit muscle protein breakdown, and, as such, stimulate postprandial muscle protein accretion. Although a

![Fig. 3. Body water deuterium enrichments, measured the day after ingestion of 8 × 50 ml of 70% deuterium oxide (test 1) and every subsequent day, in healthy, young men under free-living (test 1-BR1) and bed rested (BR1-test 3) conditions. On all days, a 50-ml maintenance dose was provided. During bed rest, participants were nasogastric tube-fed in an intermittent or continuous feeding pattern. Values are expressed as means ± SE. *Significantly different from test 1 (P < 0.001).](image-url)

![Fig. 4. Myofibrillar protein synthesis, expressed as fractional synthetic rate (FSR) per day, during free-living and bed-rested conditions in healthy, young men. Data are displayed as participants’ individual FSR. During bed rest, food was administered via a nasogastric tube in either an intermittent (n = 10; 4× bolus/day) or continuous (n = 10, 24 h/day) pattern. A repeated-measures ANOVA revealed no significant effects.](image-url)
minor delay in protein digestion may occur when other macronutrients are coingested with protein (27, 28), this does not modulate total plasma amino acid availability or postprandial muscle protein synthesis rates (27, 28). As such, the repeated stimulation of muscle protein synthesis with the intermittent mixed-meal feeding pattern should theoretically lead to an attenuated decline in skeletal muscle mass and metabolic health when compared with a situation where participants are fed in a continuous manner. In contrast to our hypothesis, we observed no differences in the decline in muscle mass, oxygen uptake capacity, or insulin sensitivity following 1 wk of bed rest combined with continuous versus intermittent feeding (Fig. 2 and Table 3). Therefore, we conclude that feeding pattern does not modulate the decline in muscle mass and health during short periods of bed rest in healthy volunteers when fed in energy balance.

To assess whether potential differences in muscle mass loss during continuous versus intermittent feeding could be (partly) explained by differences in daily muscle protein synthesis rates, we applied the deuterated water method as a means to assess muscle protein synthesis over a more extended time frame (32). In the present study, muscle protein synthesis rates averaged ~1.4 ± 0.1%/day. These findings are in agreement with previous studies from our laboratory (32), as well as others (38, 66), applying the deuterated water method. In line with the absence of measurable differences in muscle mass loss between the intermittent and continuous feeding regimen, no differences were observed in daily protein synthesis rates between groups (1.33 ± 0.07 vs. 1.50 ± 0.13%/day with intermittent and continuous feeding, respectively; Fig. 4). To our surprise, we also did not observe significant differences in daily protein synthesis rates assessed in the week before bed rest and the week during bedrest, independent of the feeding regimen applied during bed rest (1.33 ± 0.04 vs. 1.41 ± 0.07%/day dur-
ing free-living and bed rest, respectively). This is surprising as lower postabsorptive (23, 25, 57) and postprandial (8, 45) muscle protein synthesis rates have been reported in young individuals following 1–4 wk of bed rest. In contrast, our data seem to be more in line with recent work showing that a shorter period (i.e., 5 days) of bed rest does not affect muscle protein synthesis rates in healthy young volunteers. Nonetheless, the amount of leg muscle mass lost in the present study (i.e., less than 50 g) may have been insufficient to allow the detection of significant declines in daily protein synthesis rates using the deuterated water method (58). More work is required in applying deuterated water as a means to assess the impact of changes in muscle protein synthesis rates as a key factor in explaining net muscle loss during (short) periods of disuse.

Consequently, the observed muscle atrophy (Figs. 1 and 2) may be largely caused by an increase in muscle protein breakdown rates. Although data are quite limited, all available direct (57) and indirect (23) measurements of muscle protein breakdown rates suggest no changes in postabsorptive muscle protein breakdown rates following several weeks of muscle disuse. However, we (19, 61, 62) and others (1, 59) have demonstrated a rapid but transient increase in molecular proxies for muscle protein breakdown during the first few days following the onset of muscle disuse. In line, we observed an increase in MAFBx expression following bedrest in both treatment groups (Fig. 5). Although it remains unclear whether muscle protein breakdown rates are increased following short-term disuse, and if so, whether this is attributed to increased postabsorptive and/or postprandial muscle protein breakdown rates, our data seem to support previous suggestions that muscle protein breakdown is increased following the onset of disuse (1, 19, 59, 61, 62). It has been suggested that continuous enteral feeding may have a greater impact on muscle protein breakdown due to the continuous insulin-mediated suppression of proteolysis (29), whereas intermittent feeding has a greater impact on protein synthesis due to the repeated hyperinsulinemia and hyperaminoacidemia (9). Although we did not assess muscle proteolysis, mRNA expression of key proteins involved in the regulation of muscle protein breakdown did not show differences between feeding strategies. Consequently, our data do not support that large differences in muscle protein breakdown rates exist between continuous versus intermittent enteral feeding (Fig. 5).

Although muscle protein synthesis rates (using deuterated water) and markers of muscle protein breakdown do not seem to support this (Figs. 4 and 5), our observations of nitrogen balance seem to indicate that continuous feeding leads to greater whole body nitrogen retention when compared with intermittent feeding (Fig. 6). This is in agreement with some (37), but not all (12), work in patients, and could suggest that continuous feeding may lead to better preservation of whole body protein during more prolonged bed rest. Although a positive nitrogen balance during bed rest has been shown before in some (23, 53) but not all (35, 49) studies, it seems to be at odds with the decline in lean mass that was observed in the present study (Figs. 1 and 2). Because of the nature of the whole body nitrogen balance method, it is impossible to determine the tissue(s) responsible for the greater nitrogen retention, which likely include splanchnic tissues, other organs, as well as the impact on the microbiota. However, as we failed to see any preservation of muscle mass or metabolic health with continuous versus intermittent feeding, we assume that the observed greater nitrogen retention following continuous versus intermittent feeding is not per se reflective of skeletal muscle tissue.

This is the first study to assess the impact of continuous versus intermittent enteral feeding during bed rest in healthy men fed in energy balance. Under these conditions, the enteral feeding pattern had no impact on the decline in muscle mass, oxygen uptake capacity, and insulin sensitivity. These data are important for clinical practice, as the proposed benefits of intermittent over continuous enteral feeding strategies are currently a topic of intense debate (17). Bed-rested individuals under conditions of reduced energy intake tend to lose more muscle mass than those fed in energy balance (7). This could be speculated that dietary feeding pattern has a more potent effect under conditions of an energy and/or protein deficit. Therefore, similar approaches should be applied to assess the impact of different feeding strategies on muscle health. However, under conditions where appropriate energy and protein are provided to support muscle mass maintenance, enteral feeding pattern does not modulate the decline in muscle mass or metabolic health during a short period of bedrest. Of course, besides appropriate nutrition, some level of physical activity and/or muscle contraction will always be required to allow preservation of skeletal muscle mass and metabolic health during a period of disuse (18, 19, 43). As such, strategies need to be developed to define the minimal amount of physical activity required to maintain muscle mass and metabolic function under conditions where malnutrition is no longer evident.

In conclusion, dietary feeding pattern does not modulate the decline in skeletal muscle mass, oxidative capacity, or insulin sensitivity during 1 wk of bed rest in healthy men fed in energy balance.

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AUTHOR CONTRIBUTIONS


E543 DIETARY FEEDING PATTERN DURING BED REST
Dietary Feeding Pattern During Bed Rest


