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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

Marlou L. Dirks,¹  Joey S. J. Smeets,¹ Andrew M. Holwerda,¹ Imre W. K. Kouw,¹ Gabriel N. Marzuca-Nassar,¹ Annemie P. Gijssen,¹ Graham P. Holloway,² Lex B. Verdijk,¹ and Luc J. C. van Loon¹

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Dirks ML, Smeets JS, Holwerda AM, Kouw IW, Marzuca-Nassar GN, Gijssen AP, Holloway GP, Verdijk LB, van Loon LJ. Dietary feeding pattern does not modulate the loss of muscle mass or the decline in metabolic health during short-term bed rest. *Am J Physiol Endocrinol Metab* 316: E536–E545, 2019. First published January 15, 2019; doi:10.1152/ajpendo.00378.2018.—Short periods of bed rest lead to the loss of muscle mass and quality. It has been speculated that dietary feeding pattern may have an impact upon muscle protein synthesis rates and, therefore, modulate the loss of muscle mass and quality. We subjected 20 healthy men (age: 25 ± 1 yr, body mass index: 23.8 ± 0.8 kg/m²) to 1 wk of strict bed rest with intermittent (4 meals/day) or continuous (24 h/day) enteral tube feeding. Participants consumed deuterium oxide for 7 days before bed rest and throughout the 7-day bed rest period. Prior to and immediately after bed rest, lean body mass (dual energy X-ray absorptiometry), quadriceps cross-sectional area (CSA; CT), maximal oxygen uptake capacity ($\dot{V}O_{2peak}$), and whole body insulin sensitivity (hyperinsulinemic-euglycemic clamp) were assessed. Muscle biopsies were collected 7 days before, 1 day before, and immediately after bed rest to assess muscle tracer incorporation. Bed rest resulted in 0.3 ± 0.3 vs. 0.7 ± 0.4 kg lean tissue loss and a 1.1 ± 0.6 vs. $0.8 \pm 0.5\%$ decline in quadriceps CSA in the intermittent vs. continuous feeding group, respectively (both $P < 0.05$), with no differences between groups (both $P > 0.05$). Moreover, feeding pattern did not modulate the bed rest-induced decline in insulin sensitivity ($-46 \pm 3\%$ vs. $39 \pm 3\%$; $P < 0.001$) or $\dot{V}O_{2peak}$ (-2.5 ± 2.2 vs. $-8.6 \pm 2.2\%$; $P < 0.010$) (both $P > 0.05$). Myofibrillar protein synthesis rates during bed rest did not differ between the intermittent and continuous feeding group (1.33 ± 0.07 vs. $1.50 \pm 0.13\%$ /day, respectively; $P > 0.05$). In conclusion, dietary feeding pattern does not modulate the loss of muscle mass or the decline in metabolic health during 1 wk of bed rest in healthy men.

muscle atrophy; muscle disuse; enteral feeding; nutrition; tube feeding

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

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Table 1. *Participants' characteristics*

	Intermittent (n = 10)	Continuous (n = 10)
Age, yr	27 ± 1	24 ± 1
Body mass, kg	77.5 ± 5.1	77.3 ± 5.1
Height, m	1.81 ± 0.03	1.79 ± 0.03
BMI, kg/m ²	23.5 ± 1.3	24.0 ± 1.0
HbA _{1c} , %	5.2 ± 0.1	5.2 ± 0.2
RMR, MJ/day	7.6 ± 0.4	7.6 ± 0.3

BMI, body mass index; HbA_{1c}, glycated hemoglobin; RMR, resting metabolic rate.

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 ($\dot{V}O_{2peak}$, 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/m², a (family) history of deep vein thrombosis, Type 2 diabetes mellitus (determined by HbA_{1c} values >7.0%), and any back, knee, or shoulder complaints that could be problematic during the bed rest period. Additionally, participants who had been involved in progressive resistance-type exercise training during the 6 mo before the study were also excluded. All subjects were informed on the nature and risks of the experiment before written informed consent was obtained. During the screening visit, a fasting blood sample was taken to assess HbA_{1c}, and resting energy expenditure was measured with the use of a ventilated hood. The current study was part of a larger project investigating the impact

of short-term bed rest on muscle mass and metabolic health, registered on clinicaltrials.gov as NCT02521025 (<https://clinicaltrials.gov/>). The study was approved by the Medical Ethical Committee of Maastricht University Medical Centre⁺ (registration no. MEC 15-3-035), in accordance with the latest version of the Declaration of Helsinki.

Experimental outline. Following inclusion, participants visited the University for a deuterium oxide (D₂O) loading visit. On the subsequent day, on *test day 1*, a single muscle biopsy was taken from the musculus vastus lateralis. After this visit, a 7-day period of standardized nutrition was started. On *day 7* of this standardized diet (*test day 2*), a second muscle biopsy sample was obtained, and DXA and CT scans and a hyperinsulinemic-euglycemic clamp were performed. $\dot{V}O_{2peak}$ was assessed before the free-living period, and on the day following bed rest. On the same evening, participants arrived at the university for insertion of a nasogastric tube, and subsequently stayed overnight. The following morning at 0800, a 7-day period of strict bed rest was started. During this period, participants were tube-fed with an enteral food product in an intermittent (n = 10, intermittent: 4 boluses/day) or continuous (n = 10, continuous: 24 h per day at a constant rate) feeding pattern. After exactly 7 days, *test day 2* was repeated, and participants were allowed to go home.

One week of bed rest. Participants underwent a 7-day period of strict bed rest to mimic the effects of a standard hospitalization period. On the morning of *day 1*, at 0800, participants started the 7-day period of strict bed rest during which they were not allowed to leave the bed. During daytime, participants were allowed to use a pillow and slight elevation of the bed-back to be able to perform their daily activities. Washing and all sanitary activities were performed in bed. Participants were awakened at 0730, and lights were switched off at 2330 every day. Participants were continuously monitored by members of the research team.

Dietary intake. During the screening visit, resting energy expenditure was measured by indirect calorimetry using an open-circuit ventilated hood system [Omnicall, Maastricht University, Maastricht, The Netherlands (50)]. During the 7 days before bed rest, and during the bed rest period itself, dietary intake was fully controlled. During the pre-bed rest period, subjects received all food products from the research team. Energy requirements were estimated on the basis of indirect calorimetry data, 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 2. *Dietary intake*

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

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 (Nutrison 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 assessed 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 $\dot{V}O_{2\text{peak}}$ [described previously (20)]. Whole body insulin sensitivity was measured via a one-step hyperinsulinemic-euglycemic clamp, as described previously (20). In short, 20% glucose (Baxter B.V., Utrecht, The Netherlands) was coinfused with insulin (40 $\text{mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$; Novorapid, 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_2O 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 Gilson autoinjector, 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 $\mu\text{l}/\text{mg}$). The samples were spun at 2,500 g and 4°C for 5 min. The pellet was washed with 500 μl ddH_2O 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_4OH . 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 $^2\text{H}/^1\text{H}$ 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 (C_t) values of the target genes were normalized to C_t 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 $29 \pm 5\%$ 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 ± 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 ± 522 to 7430 ± 511 mm²) and $0.8 \pm 0.5\%$ (from $7,544 \pm 549$ to

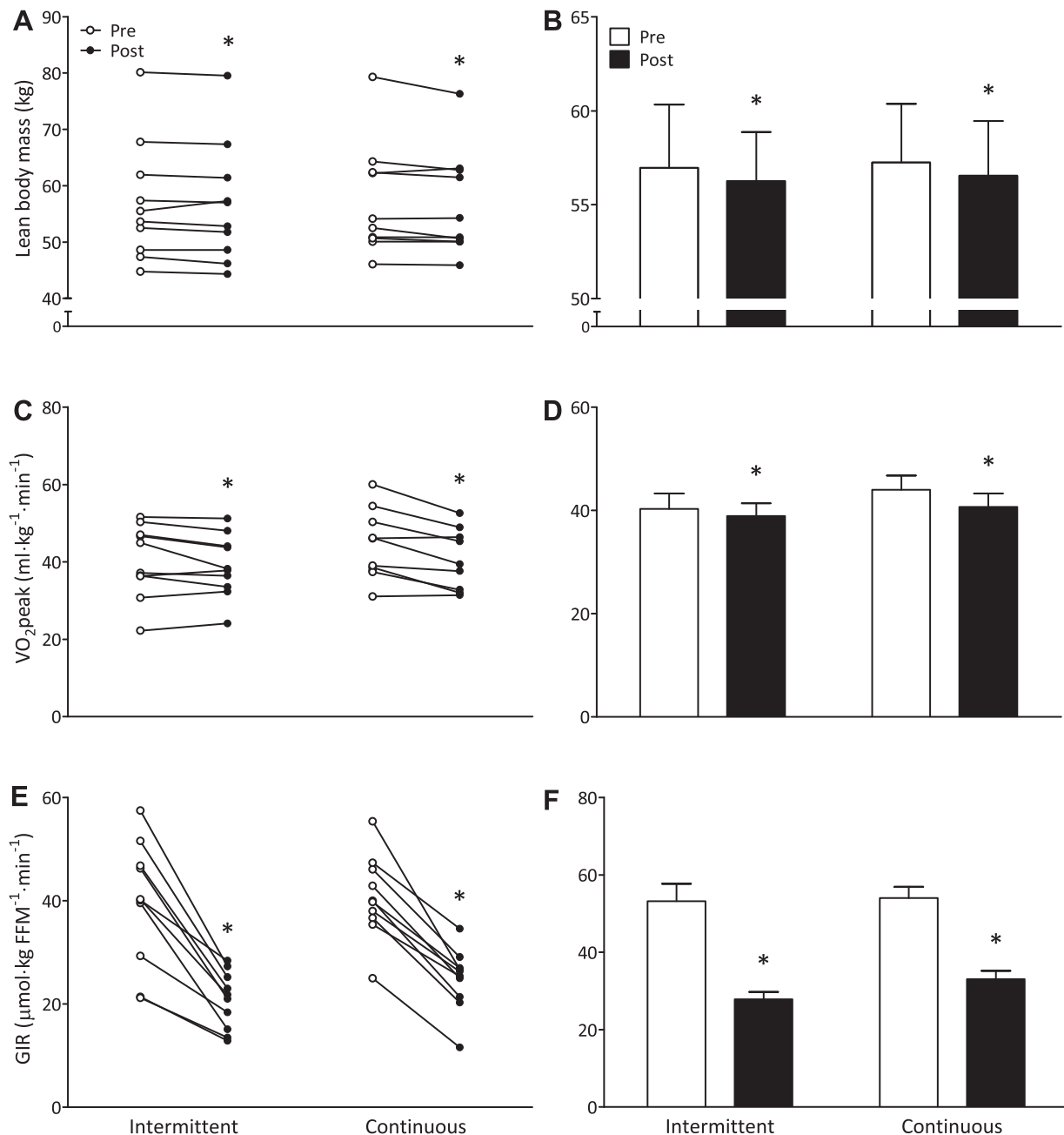
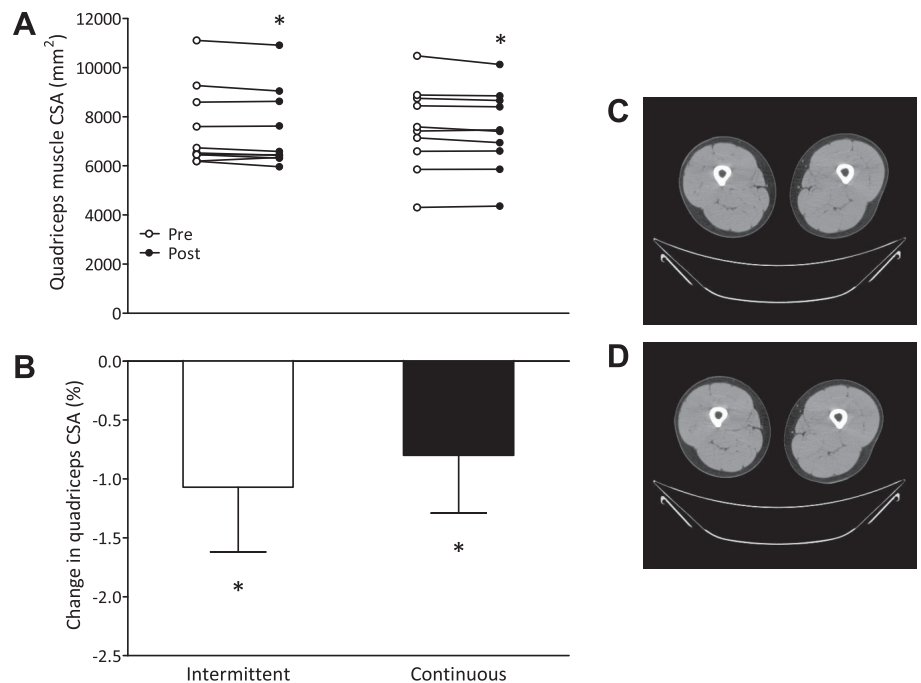


Fig. 1. Lean body mass (A and B), whole body oxygen uptake capacity (C and D), and whole body insulin sensitivity (E and F) at baseline and following 7 days of strict bed rest in healthy, young men, who were nasogastric tube-fed in an intermittent ($n = 10$) or continuous ($n = 10$) feeding pattern. 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.

Fig. 2. Individual participants' quadriceps cross-sectional area (CSA; A) and group mean changes in quadriceps CSA (B), 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. *Significantly different from pre-bed rest values ($P < 0.05$). Values are expressed as means \pm SE. (pre-bed rest) (C) and (post-bed rest) (D) display representative CT scans from a participant with an average decline in quadriceps CSA.



$7,469 \pm 522 \text{ mm}^2$) 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 $0.62 \pm 0.19 \text{ kg}$ 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 $-0.36 \pm 0.19 \text{ kg}$ 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. $\dot{V}O_{2\text{peak}}$ (Fig. 1, C and D) declined from 40.3 ± 3.0 to $38.9 \pm 2.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ following bed rest with intermittent feeding and from 44.8 ± 3.1 to $40.7 \pm 2.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ 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 $46 \pm 3\%$ following bed rest with intermittent and $39 \pm 3\%$ 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 P706SK, 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 $15.4 \pm 0.5 \text{ g/day}$ 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

	Intermittent ($n = 10$)		Continuous ($n = 10$)	
	Pre	Post	Pre	Post
Total mass, kg	77.7 ± 4.9	$77.3 \pm 5.0^*$	77.6 ± 5.3	$76.8 \pm 5.1^*$
Fat mass, kg	18.2 ± 2.1	18.3 ± 2.1	17.7 ± 2.3	17.6 ± 2.3
Fat percentage, %	22.9 ± 1.9	23.2 ± 1.9	22.3 ± 1.2	22.4 ± 1.3
Lean mass, kg	57.0 ± 3.4	$56.6 \pm 3.4^*$	57.2 ± 3.1	$56.5 \pm 2.9^*$
Trunk lean mass, kg	28.6 ± 1.8	$28.0 \pm 1.7^*$	28.0 ± 1.7	$27.6 \pm 1.6^*$
Leg lean mass, kg	9.5 ± 0.7	9.5 ± 0.6	9.5 ± 0.6	9.4 ± 0.5
Arm lean mass, kg	3.5 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	3.4 ± 0.2
BMD, g/cm^2	1.16 ± 0.03	$1.17 \pm 0.03^*$	1.16 ± 0.02	1.15 ± 0.02

Values (means \pm 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.

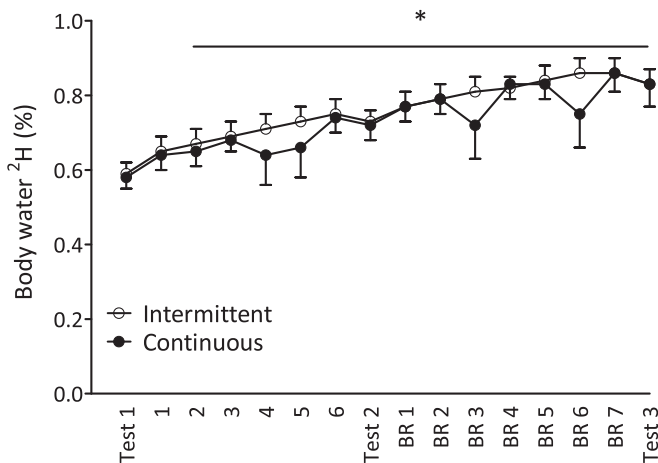


Fig. 3. Body water deuterium enrichments, measured the day after ingestion of 8×50 ml of 70% deuterium oxide (*test 1*) and every subsequent day, in healthy, young men under free-living (*test 1*-BR1) and bed rested (BR1-*test 3*) conditions. On all days, a 50-ml maintenance dose was provided. During bed rest, participants were nasogastric tube-fed in an intermittent or continuous feeding pattern. Values are expressed as means \pm 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 administration in an intermittent versus continuous manner, did not impact the bed rest-induced decline in muscle mass and metabolic 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 $0.9 \pm 0.4\%$ 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 $3.2 \pm 0.9\%$ decline in quadriceps CSA that we recently observed following 1 wk of bed rest in our laboratory (20). The apparent discrepancy may be attributed to the enteral feeding regimens as opposed to normal food consumption (13) and/or the composition 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 $\sim 5\%$ decline in maximal oxygen uptake capacity and a $\sim 40\%$ 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 modulate 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 participants. In contrast, the continuous enteral feeding group received 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 intermittent 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

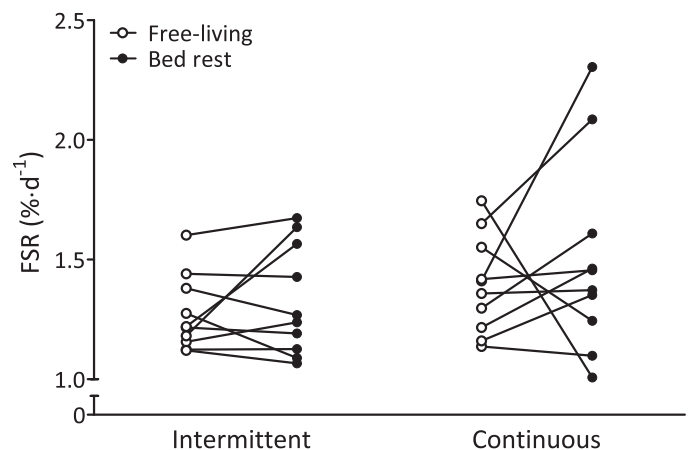


Fig. 4. Myofibrillar protein synthesis, expressed as fractional synthetic rate (FSR) per day, during free-living and bed-rested conditions in healthy, young men. Data are displayed as participants' individual FSR. During bed rest, food was administered via a nasogastric tube in either an intermittent ($n = 10$; 4 \times bolus/day) or continuous ($n = 10$, 24 h/day) pattern. A repeated-measures ANOVA revealed no significant effects.

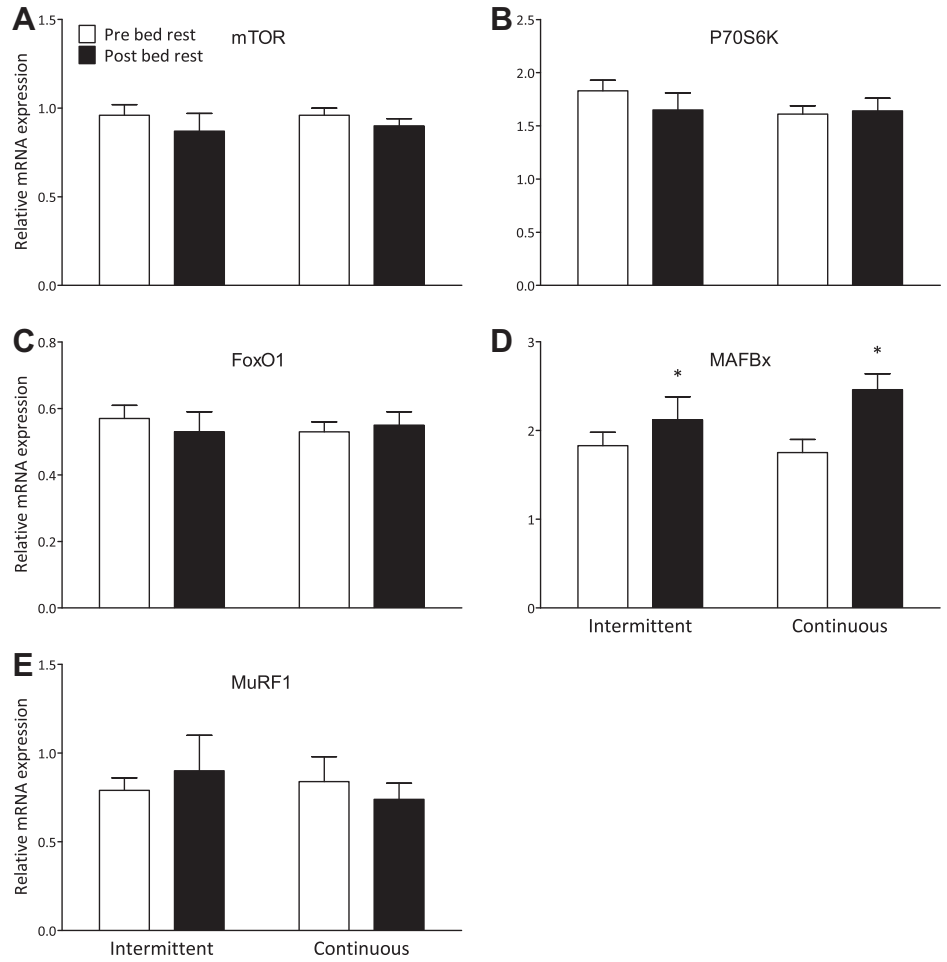


Fig. 5. Skeletal muscle mRNA expression of genes involved in the regulation of muscle protein synthesis [i.e., mTOR (A) and P70S6K (B)] and muscle protein breakdown [i.e., FoxO1 (C), MAFBx (D), and MuRF1 (E)]. Biopsies were taken between the free-living and the bed rested period (pre), and immediately following bed rest (post). *Significantly different from corresponding pre-bed rest values ($P < 0.01$).

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 rates over a more extended time frame (32). In the present study, muscle protein synthesis rates averaged $\sim 1.4 \pm 0.1\%$ /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. $1.50 \pm 0.13\%$ /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. $1.41 \pm 0.07\%$ /day dur-

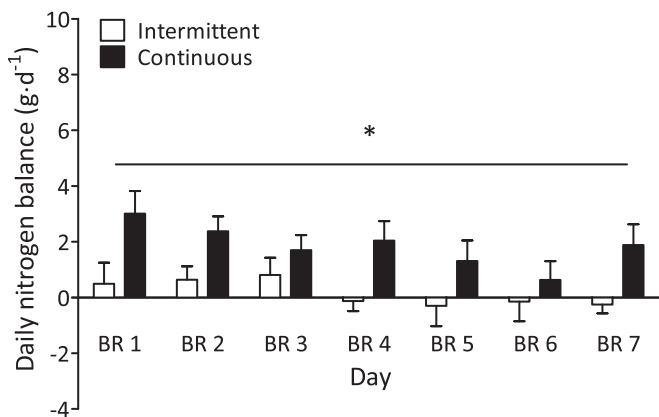


Fig. 6. Daily nitrogen balance during 7 days of strict bed rest. Participants were fed a standard enteral food product via a nasogastric tube, in either an intermittent ($n = 10$; 4 \times bolus per day) or continuous ($n = 10$, 24 h/day) pattern. *Significant time effect ($P < 0.001$). Values are expressed as means \pm SE.

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 seems to be in line with the observation that muscle protein synthesis rates are lower during caloric restriction (31, 41, 44). It 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

M.L.D. and L.J.v.L. conceived and designed research; M.L.D., J.S.S., I.W.K., G.N.M.-N., and G.P.H. performed experiments; M.L.D., A.M.H., and A.P.G. analyzed data; M.L.D., J.S.S., A.M.H., I.W.K., L.B.V., and L.J.v.L. interpreted results of experiments; M.L.D. prepared figures; M.L.D. drafted

manuscript; M.L.D. and L.J.v.L. edited and revised manuscript; M.L.D., J.S.S., A.M.H., I.W.K., G.N.M.-N., A.P.G., G.P.H., L.B.V., and L.J.v.L. approved final version of manuscript.

REFERENCES

- Abadi A, Glover EI, Isfort RJ, Raha S, Safdar A, Yasuda N, Kaczor JJ, Melov S, Hubbard A, Qu X, Phillips SM, Tarnopolsky M. Limb immobilization induces a coordinate down-regulation of mitochondrial and other metabolic pathways in men and women. *PLoS One* 4: e6518, 2009. doi:10.1371/journal.pone.0006518.
- Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, Jeacocke NA, Moore DR, Stellingwerff T, Phillips SM, Hawley JA, Coffey VG. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *J Physiol* 591: 2319–2331, 2013. doi:10.1113/jphysiol.2012.244897.
- Arnal MA, Mosoni L, Boirie Y, Houlier ML, Morin L, Verdier E, Ritz P, Antoine JM, Prugnaud J, Beaufrère B, Mirand PP. Protein feeding pattern does not affect protein retention in young women. *J Nutr* 130: 1700–1704, 2000. doi:10.1093/jn/130.7.1700.
- Arnal MA, Mosoni L, Boirie Y, Houlier ML, Morin L, Verdier E, Ritz P, Antoine JM, Prugnaud J, Beaufrère B, Mirand PP. Protein pulse feeding improves protein retention in elderly women. *Am J Clin Nutr* 69: 1202–1208, 1999. doi:10.1093/ajcn/69.6.1202.
- Bergouignan A, Rudwill F, Simon C, Blanc S. Physical inactivity as the culprit of metabolic inflexibility: evidence from bed-rest studies. *J Appl Physiol* (1985) 111: 1201–1210, 2011. doi:10.1152/jappphysiol.00698.2011.
- Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* 35: 609–616, 1975. doi:10.3109/00365517509095787.
- Biolo G, Ciochi B, Stulle M, Bosutti A, Barazzoni R, Zanetti M, Antonione R, Lebenstedt M, Platen P, Heer M, Guarnieri G. Calorie restriction accelerates the catabolism of lean body mass during 2 wk of bed rest. *Am J Clin Nutr* 86: 366–372, 2007. doi:10.1093/ajcn/86.2.366.
- Biolo G, Pišot R, Mazzucco S, Di Girolamo FG, Situlin R, Lazzar S, Grassi B, Reggiani C, Passaro A, Rittweger J, Gasparini M, Šimunič B, Narici M. Anabolic resistance assessed by oral stable isotope ingestion following bed rest in young and older adult volunteers: Relationships with changes in muscle mass. *Clin Nutr* 36: 1420–1426, 2017. doi:10.1016/j.clnu.2016.09.019.
- Bohé J, Low JF, Wolfe RR, Rennie MJ. Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol* 532: 575–579, 2001. doi:10.1111/j.1469-7793.2001.0575f.x.
- Burd NA, Cermak NM, Kouw IW, Gorissen SH, Gijsen AP, van Loon LJ. The use of doubly labeled milk protein to measure postprandial muscle protein synthesis rates in vivo in humans. *J Appl Physiol* (1985) 117: 1363–1370, 2014. doi:10.1152/jappphysiol.00411.2014.
- Burd NA, Gorissen SH, van Vliet S, Snijders T, van Loon LJ. Differences in postprandial protein handling after beef compared with milk ingestion during postexercise recovery: a randomized controlled trial. *Am J Clin Nutr* 102: 828–836, 2015. doi:10.3945/ajcn.114.103184.
- Campbell IT, Morton RP, Cole JA, Raine CH, Shapiro LM, Stell PM. A comparison of the effects of intermittent and continuous nasogastric feeding on the oxygen consumption and nitrogen balance of patients after major head and neck surgery. *Am J Clin Nutr* 38: 870–878, 1983. doi:10.1093/ajcn/38.6.870.
- Chen W, Codreanu I, Yang J, Li G, Servaes S, Zhuang H. Tube feeding increases the gastric-emptying rate determined by gastroesophageal scintigraphy. *Clin Nucl Med* 38: 962–965, 2013. doi:10.1097/RLU.0000000000000268.
- Churchward-Venne TA, Breen L, Di Donato DM, Hector AJ, Mitchell CJ, Moore DR, Stellingwerff T, Breuille D, Offord EA, Baker SK, Phillips SM. Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial. *Am J Clin Nutr* 99: 276–286, 2014. doi:10.3945/ajcn.113.068775.
- DeFronzo RA. Glucose intolerance and aging: evidence for tissue insensitivity to insulin. *Diabetes* 28: 1095–1101, 1979. doi:10.2337/diab.28.12.1095.
- Deutz NE, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, Cederholm T, Cruz-Jentoft A, Krznarić Z, Nair KS, Singer P, Teta D, Tipton K, Calder PC. Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. *Clin Nutr* 33: 929–936, 2014. doi:10.1016/j.clnu.2014.04.007.
- Di Girolamo FG, Situlin R, Fiotti N, Biolo G. Intermittent vs. continuous enteral feeding to prevent catabolism in acutely ill adult and pediatric patients. *Curr Opin Clin Nutr Metab Care* 20: 390–395, 2017. doi:10.1097/MCO.0000000000000397.
- Dirks ML, Hansen D, Van Assche A, Dendale P, Van Loon LJ. Neuromuscular electrical stimulation prevents muscle wasting in critically ill comatose patients. *Clin Sci (Lond)* 128: 357–365, 2015. doi:10.1042/CS20140447.
- Dirks ML, Wall BT, Snijders T, Ottenbros CL, Verdijk LB, van Loon LJ. Neuromuscular electrical stimulation prevents muscle disuse atrophy during leg immobilization in humans. *Acta Physiol (Oxf)* 210: 628–641, 2014. doi:10.1111/apha.12200.
- Dirks ML, Wall BT, van de Valk B, Holloway TM, Holloway GP, Chabowski A, Goossens GH, van Loon LJ. One week of bed rest leads to substantial muscle atrophy and induces whole-body insulin resistance in the absence of skeletal muscle lipid accumulation. *Diabetes* 65: 2862–2875, 2016. doi:10.2337/db15-1661.
- El-Kadi SW, Suryawan A, Gazzaneo MC, Srivastava N, Orellana RA, Nguyen HV, Lobley GE, Davis TA. Anabolic signaling and protein deposition are enhanced by intermittent compared with continuous feeding in skeletal muscle of neonates. *Am J Physiol Endocrinol Metab* 302: E674–E686, 2012. doi:10.1152/ajpendo.00516.2011.
- European Union. *Hospital Discharges and Length of Stay Statistics*. https://ec.europa.eu/eurostat/statistics-explained/index.php/Hospital_discharges_and_length_of_stay_statistics [Accessed 1 August 2018].
- Ferrando AA, Lane HW, Stuart CA, Davis-Street J, Wolfe RR. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Physiol Endocrinol Metab* 270: E627–E633, 1996. doi:10.1152/ajpendo.1996.270.4.E627.
- Ferrando AA, Stuart CA, Brunder DG, Hillman GR. Magnetic resonance imaging quantitation of changes in muscle volume during 7 days of strict bed rest. *Aviat Space Environ Med* 66: 976–981, 1995.
- Ferrando AA, Tipton KD, Bamman MM, Wolfe RR. Resistance exercise maintains skeletal muscle protein synthesis during bed rest. *J Appl Physiol* (1985) 82: 807–810, 1997. doi:10.1152/jappphysiol.1997.82.3.807.
- Gazzaneo MC, Suryawan A, Orellana RA, Torrazza RM, El-Kadi SW, Wilson FA, Kimball SR, Srivastava N, Nguyen HV, Fiorotto ML, Davis TA. Intermittent bolus feeding has a greater stimulatory effect on protein synthesis in skeletal muscle than continuous feeding in neonatal pigs. *J Nutr* 141: 2152–2158, 2011. doi:10.3945/jn.111.147520.
- Gorissen SH, Burd NA, Hamer HM, Gijsen AP, Groen BB, van Loon LJ. Carbohydrate coingestion delays dietary protein digestion and absorption but does not modulate postprandial muscle protein accretion. *J Clin Endocrinol Metab* 99: 2250–2258, 2014. doi:10.1210/jc.2013-3970.
- Gorissen SHM, Burd NA, Kramer IF, van Kranenburg J, Gijsen AP, Rooyackers O, van Loon LJC. Co-ingesting milk fat with micellar casein does not affect postprandial protein handling in healthy older men. *Clin Nutr* 36: 429–437, 2017. doi:10.1016/j.clnu.2015.12.011.
- Greenhaff PL, Karagounis LG, Peirce N, Simpson EJ, Hazell M, Layfield R, Wackerhage H, Smith K, Atherton P, Selby A, Rennie MJ. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab* 295: E595–E604, 2008. doi:10.1152/ajpendo.90411.2008.
- Groen BB, Horstman AM, Hamer HM, de Haan M, van Kranenburg J, Bierau J, Poeze M, Wodzig WK, Rasmussen BB, van Loon LJ. Increasing Insulin Availability Does Not Augment Postprandial Muscle Protein Synthesis Rates in Healthy Young and Older Men. *J Clin Endocrinol Metab* 101: 3978–3988, 2016. doi:10.1210/jc.2016-1436.
- Hector AJ, McGlory C, Damas F, Mazara N, Baker SK, Phillips SM. Pronounced energy restriction with elevated protein intake results in no change in proteolysis and reductions in skeletal muscle protein synthesis that are mitigated by resistance exercise. *FASEB J* 32: 265–275, 2018. doi:10.1096/fj.201700158RR.
- Holwerda AM, Paulussen KJM, Overkamp M, Smeets JSJ, Gijsen AP, Goossens JPB, Verdijk LB, van Loon LJC. Daily resistance-type exercise stimulates muscle protein synthesis in vivo in young men. *J Appl Physiol* (1985) 124: 66–75, 2018. doi:10.1152/jappphysiol.00610.2017.
- Hušek P. Amino acid derivatization and analysis in five minutes. *FEBS Lett* 280: 354–356, 1991. doi:10.1016/0014-5793(91)80330-6.

34. Jackman SR, Witard OC, Philp A, Wallis GA, Baar K, Tipton KD. Branched-Chain Amino Acid Ingestion Stimulates Muscle Myofibrillar Protein Synthesis following Resistance Exercise in Humans. *Front Physiol* 8: 390, 2017. doi:10.3389/fphys.2017.00390.
35. Kortebein P, Ferrando A, Lombeida J, Wolfe R, Evans WJ. Effect of 10 days of bed rest on skeletal muscle in healthy older adults. *JAMA* 297: 1772–1774, 2007. doi:10.1001/jama.297.16.1772-b.
36. Mamerow MM, Mettler JA, English KL, Casperson SL, Arentson-Lantz E, Sheffield-Moore M, Layman DK, Paddon-Jones D. Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults. *J Nutr* 144: 876–880, 2014. doi:10.3945/jn.113.185280.
37. Mazaherpour S, Khatony A, Abdi A, Pasdar Y, Najafi F. The Effect of Continuous Enteral Nutrition on Nutrition Indices, Compared to the Intermittent and Combination Enteral Nutrition in Traumatic Brain Injury Patients. *J Clin Diagn Res* 10: JC01–JC05, 2016. doi:10.7860/JCDR/2016/19271.8625.
38. Mitchell CJ, D'Souza RF, Mitchell SM, Figueiredo VC, Miller BF, Hamilton KL, Peelor FF III, Coronet M, Pileggi CA, Durainayagam B, Fanning AC, Poppitt SD, Cameron-Smith D. Impact of dairy protein during limb immobilization and recovery on muscle size and protein synthesis; a randomized controlled trial. *J Appl Physiol* (1985) 124: 717–728, 2018. doi:10.1152/jappphysiol.00803.2017.
39. Mitchell WK, Phillips BE, Williams JP, Rankin D, Lund JN, Smith K, Atherton PJ. A dose- rather than delivery profile-dependent mechanism regulates the “muscle-full” effect in response to oral essential amino acid intake in young men. *J Nutr* 145: 207–214, 2015. doi:10.3945/jn.114.199604.
40. Mitchell WK, Phillips BE, Williams JP, Rankin D, Lund JN, Wilkinson DJ, Smith K, Atherton PJ. The impact of delivery profile of essential amino acids upon skeletal muscle protein synthesis in older men: clinical efficacy of pulse vs. bolus supply. *Am J Physiol Endocrinol Metab* 309: E450–E457, 2015. doi:10.1152/ajpendo.00112.2015.
41. Murphy CH, Churchward-Venne TA, Mitchell CJ, Kolar NM, Kassis A, Karagounis LG, Burke LM, Hawley JA, Phillips SM. Hypoenergetic diet-induced reductions in myofibrillar protein synthesis are restored with resistance training and balanced daily protein ingestion in older men. *Am J Physiol Endocrinol Metab* 308: E734–E743, 2015. doi:10.1152/ajpendo.00550.2014.
42. Nair KS. Aging muscle. *Am J Clin Nutr* 81: 953–963, 2005. doi:10.1093/ajcn/81.5.953.
43. Oates BR, Glover EI, West DW, Fry JL, Tarnopolsky MA, Phillips SM. Low-volume resistance exercise attenuates the decline in strength and muscle mass associated with immobilization. *Muscle Nerve* 42: 539–546, 2010. doi:10.1002/mus.21721.
44. Oikawa SY, McGlory C, D'Souza LK, Morgan AK, Saddler NI, Baker SK, Parise G, Phillips SM. A randomized controlled trial of the impact of protein supplementation on leg lean mass and integrated muscle protein synthesis during inactivity and energy restriction in older persons. *Am J Clin Nutr* 108: 1060–1068, 2018. doi:10.1093/ajcn/nqy193.
45. Paddon-Jones D, Sheffield-Moore M, Urban RJ, Sanford AP, Aarsland A, Wolfe RR, Ferrando AA. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. *J Clin Endocrinol Metab* 89: 4351–4358, 2004. doi:10.1210/jc.2003-032159.
46. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300: 1140–1142, 2003. doi:10.1126/science.1082889.
47. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol Endocrinol Metab* 273: E99–E107, 1997. doi:10.1152/ajpendo.1997.273.1.E99.
48. Rennie MJ, Edwards RH, Halliday D, Matthews DE, Wolman SL, Millward DJ. Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting. *Clin Sci (Lond)* 63: 519–523, 1982. doi:10.1042/cs0630519.
49. Scheld K, Zittermann A, Heer M, Herzog B, Mika C, Drummer C, Stehle P. Nitrogen metabolism and bone metabolism markers in healthy adults during 16 weeks of bed rest. *Clin Chem* 47: 1688–1695, 2001.
50. Schoffelen PF, Westerterp KR, Saris WH, Ten Hoor F. A dual-respiration chamber system with automated calibration. *J Appl Physiol* (1985) 83: 2064–2072, 1997. doi:10.1152/jappphysiol.1997.83.6.2064.
51. Scrimgeour CM, Rollo MM, Mudambo SM, Handley LL, Prosser SJ. A simplified method for deuterium/hydrogen isotope ratio measurements on water samples of biological origin. *Biol Mass Spectrom* 22: 383–387, 1993. doi:10.1002/bms.1200220704.
52. Stein TP, Blanc S. Does protein supplementation prevent muscle disuse atrophy and loss of strength? *Crit Rev Food Sci Nutr* 51: 828–834, 2011. doi:10.1080/10408398.2010.482679.
53. Stein TP, Schluter MD, Leskiw MJ, Boden G. Attenuation of the protein wasting associated with bed rest by branched-chain amino acids. *Nutrition* 15: 656–660, 1999. doi:10.1016/S0899-9007(99)00120-3.
54. Stoll B, Puiman PJ, Cui L, Chang X, Benight NM, Bauchart-Thevret C, Hartmann B, Holst JJ, Burrin DG. Continuous parenteral and enteral nutrition induces metabolic dysfunction in neonatal pigs. *JPEN J Parenter Enteral Nutr* 36: 538–550, 2012. doi:10.1177/0148607112444756.
55. Strandberg S, Wretling ML, Wredmark T, Shalabi A. Reliability of computed tomography measurements in assessment of thigh muscle cross-sectional area and attenuation. *BMC Med Imaging* 10: 18, 2010. doi:10.1186/1471-2342-10-18.
56. Stuart CA, Shangraw RE, Prince MJ, Peters EJ, Wolfe RR. Bed-rest-induced insulin resistance occurs primarily in muscle. *Metabolism* 37: 802–806, 1988. doi:10.1016/0026-0495(88)90018-2.
57. Symons TB, Sheffield-Moore M, Chinkes DL, Ferrando AA, Paddon-Jones D. Artificial gravity maintains skeletal muscle protein synthesis during 21 days of simulated microgravity. *J Appl Physiol* (1985) 107: 34–38, 2009. doi:10.1152/jappphysiol.91137.2008.
58. Tanner RE, Brunker LB, Agergaard J, Barrows KM, Briggs RA, Kwon OS, Young LM, Hopkins PN, Volpi E, Marcus RL, LaStayo PC, Drummond MJ. Age-related differences in lean mass, protein synthesis and skeletal muscle markers of proteolysis after bed rest and exercise rehabilitation. *J Physiol* 593: 4259–4273, 2015. doi:10.1113/JP270699.
59. Urso ML, Scrimgeour AG, Chen YW, Thompson PD, Clarkson PM. Analysis of human skeletal muscle after 48 h immobilization reveals alterations in mRNA and protein for extracellular matrix components. *J Appl Physiol* (1985) 101: 1136–1148, 2006. doi:10.1152/jappphysiol.00180.2006.
60. Verdijk LB, Jonkers RA, Gleeson BG, Beelen M, Meijer K, Savelberg HH, Wodzig WK, Dendale P, van Loon LJ. Protein supplementation before and after exercise does not further augment skeletal muscle hypertrophy after resistance training in elderly men. *Am J Clin Nutr* 89: 608–616, 2009. doi:10.3945/ajcn.2008.26626.
61. Wall BT, Dirks ML, Snijders T, Senden JM, Dolmans J, van Loon LJ. Substantial skeletal muscle loss occurs during only 5 days of disuse. *Acta Physiol (Oxf)* 210: 600–611, 2014. doi:10.1111/apha.12190.
62. Wall BT, Dirks ML, Snijders T, van Dijk JW, Fritsch M, Verdijk LB, van Loon LJ. Short-term muscle disuse lowers myofibrillar protein synthesis rates and induces anabolic resistance to protein ingestion. *Am J Physiol Endocrinol Metab* 310: E137–E147, 2016. doi:10.1152/ajpendo.00227.2015.
63. Wall BT, Snijders T, Senden JM, Ottenbros CL, Gijsen AP, Verdijk LB, van Loon LJ. Disuse impairs the muscle protein synthetic response to protein ingestion in healthy men. *J Clin Endocrinol Metab* 98: 4872–4881, 2013. doi:10.1210/jc.2013-2098.
64. Wall BT, van Loon LJ. Nutritional strategies to attenuate muscle disuse atrophy. *Nutr Rev* 71: 195–208, 2013. doi:10.1111/nure.12019.
65. West DW, Burd NA, Coffey VG, Baker SK, Burke LM, Hawley JA, Moore DR, Stellingwerff T, Phillips SM. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *Am J Clin Nutr* 94: 795–803, 2011. doi:10.3945/ajcn.111.013722.
66. Wilkinson DJ, Franchi MV, Brook MS, Narici MV, Williams JP, Mitchell WK, Szewczyk NJ, Greenhaff PL, Atherton PJ, Smith K. A validation of the application of D(2)O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. *Am J Physiol Endocrinol Metab* 306: E571–E579, 2014. doi:10.1152/ajpendo.00650.2013.
67. Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr* 99: 86–95, 2014. doi:10.3945/ajcn.112.055517.
68. Yang Y, Breen L, Burd NA, Hector AJ, Churchward-Venne TA, Josse AR, Tarnopolsky MA, Phillips SM. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr* 108: 1780–1788, 2012. doi:10.1017/S0007114511007422.