Two Weeks of Reduced Activity Decreases Leg Lean Mass and Induces "Anabolic Resistance" of Myofibrillar Protein Synthesis in Healthy Elderly

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Background: Alterations in muscle protein metabolism underlie age-related muscle atrophy. During periods of muscle disuse, muscle protein synthesis is blunted, and muscle atrophy occurs in young and old. The impact of a short reduction in physical activity on muscle protein metabolism in older adults is unknown.

Purpose: The aim of this study was to investigate the impact of 14 days of reduced daily steps on fasted and fed-state rates of myofibrillar protein synthesis (MPS) to provide insight into the mechanisms for changes in muscle mass and markers of metabolic health.

Methods: Before and after 14 days of reduced daily step-count, 10 healthy older adults (age, 72 $\pm$ 1 y) underwent measures of insulin sensitivity, muscle strength, physical function, and body composition. Using a primed constant infusion of L-[*ring*-$^{13}C_6$]phenylalanine with serial muscle biopsies, basal, postabsorptive, and postprandial rates of MPS were determined before and after the 14-day intervention.

Results: Daily step-count was reduced by approximately 76% to 1413 $\pm$ 110 steps per day. Leg fat-free mass was reduced by approximately 3.9% ($P < .001$). Postabsorptive insulin resistance was increased by approximately 12%, and postprandial insulin sensitivity was reduced by approximately 43% after step reduction ($P < .005$). Concentrations of TNF-α and C-reactive protein were increased by approximately 12 and 25%, respectively, after step reduction ($P < .05$). Postprandial rates of MPS were reduced by approximately 26% after the intervention ($P = .028$), with no difference in postabsorptive rates.

Conclusion: The present study demonstrates that 14 days of reduced steps in older adults induces small but measurable reductions in muscle mass that appear to be underpinned by reductions in postprandial MPS and are accompanied by impairments in insulin sensitivity and systemic inflammatory markers and postprandial MPS. **(*J Clin Endocrinol Metab* 98: 2604–2612, 2013)**

Skeletal muscle is a vital organ for the maintenance of metabolic health and functional independence, especially in the elderly. The age-related decline in muscle mass, sarcopenia, and function, dynapenia (1), affect health and the general well-being of elderly individuals. Sarcopenic loss of muscle is rooted in part by an imbalance in muscle protein metabolism that, in otherwise healthy elderly, has been shown to be related to a reduced capacity

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Abbreviations: AUC, area under the curve; BM, body mass; CRP, C-reactive protein; DXA, dual-energy x-ray absorptiometry; EAA, essential amino acid; 4E-BP1, 4E binding protein 1; FFM, fat-free mass; FM, fat mass; FSR, fractional synthetic rate; HOMA-IR, homeostasis model of insulin resistance index; ISI, insulin sensitivity index; MPS, myofibrillar protein synthesis; mTOR, mammalian target of rapamycin; OGTT, oral glucose tolerance test; p70S6K, ribosomal protein S6 kinase; SPPB, short physical performance battery.

to synthesize skeletal muscle proteins in the postabsorptive (2, 3) and/or postprandial state (4–6). Although the mechanisms for this reduced synthetic capacity are likely multifactorial in nature, the level of contractile activity of skeletal muscle plays an important role in the sensitivity of elderly muscle to anabolic factors such as insulin (7) and dietary amino acids (8) and should be considered as a primary potential mechanism in sarcopenia.

Episodic periods of disuse, such as with limb immobilization or bed rest, have been well described and clearly accelerate the loss of muscle mass and strength in older adults (9–11) and may be a major contributing factor to the progression of sarcopenia (12). In addition to severe models of disuse, periods of reduced ambulatory activity, which occur with greater frequency in older adults due to illness or hospitalization, may have a detrimental effect on metabolic health. Recent studies demonstrate that healthy young adults who transition from relatively high to low ambulatory activity for 14 days (~80% reduction in step-count) display impairments in insulin sensitivity and lipid metabolism, increased visceral fat content (13, 14), a reduction in aerobic capacity, and loss of leg lean mass (15). Thus, whereas disuse atrophy with protracted bed rest or leg casting is relatively well described (9, 16), it is less clear how variations in habitual levels of ambulation, which superficially may appear far more benign than strict disuse, influence muscle protein metabolism and insulin sensitivity in older adults. In addition, older adults have an impaired ability to recover losses in muscle mass and strength after disuse compared with the young (9, 10); there is a clear need to improve our understanding of how physical inactivity, even acutely, influences the progression of sarcopenia (17). Therefore, we investigated the effect of 14 days of reduced ambulatory activity on changes in muscle strength, physical function, body composition, and insulin sensitivity in otherwise healthy elderly individuals. To identify potential mechanisms for the hypothesized decreases in lean mass, parallel measures of postabsorptive and postprandial myofibrillar protein synthesis (MPS) were performed.

Subjects and Methods

Participants

Ten older adults (5 men and 5 women), aged 66–75 years, were recruited to complete the study (Table 1). Participants were moderately active (all > 3500 steps/d), nonsmokers, nondiabetic (by fasting blood glucose, insulin, and glycosylated hemoglobin) and considered generally healthy (based on questionnaire responses). Participants were free from medication, with the exception of medications to control hypertension. The study was approved by the local Hamilton Health Sciences and McMaster University Research Ethics Board and conformed with current Canadian funding agency guidelines for use of human participants in research (18).

Table 1. Body Composition, Strength, and Function

Parameter	Preintervention	Postintervention	*P* Value
Age, y	72.3 ± 1.0	-	—
Weight, kg	81.8 ± 5.8	81.9 ± 5.7	.6
BMI, $kg \cdot m^{-2}$	29.0 ± 1.8	29.1 ± 1.8	.47
Daily step-count	5962 ± 695	1413 ± 110	<.001
PA ≥ 3.0 METs, $min \cdot d^{-1}$	32.1 ± 8.0	9.3 ± 2.4	<.001
Energy intake, $kcal \cdot d^{-1}$	1517 ± 133	1634 ± 185	.3
Energy expenditure, $kcal \cdot d^{-1}$	1921 ± 137	1694 ± 130	<.001
Total body fat, %	31.9 ± 2.9	32.7 ± 3.0	.024
Total FM, g	26 284 ± 2696	26 797 ± 3203	.18
Trunk FM, g	4506 ± 540	4834 ± 610	.053
Whole-body FFM, g	53 611 ± 4261	52 839 ± 417	.082
ALM, kg	22.18 ± 2.23	21.41 ± 2.15	<.001
Leg FFM, g	16 115 ± 1485	15 521 ± 1456	<.001
Leg SM, kg	11 152 ± 1028	10 742 ± 1007	<.001
Arm FFM, g	6070 ± 751	5884 ± 704	.078
Trunk FFM, g	28 006 ± 2108	27 696 ± 2002	.57
Isometric MVC, N · m	132 ± 17	134 ± 15	.69
SPPB score, total	10.6 ± 1.4	10.2 ± 2.2	.44

Abbreviations: BMI, body mass index; PA, physical activity; NS, not significant; SM, skeletal muscle mass; ALM, appendicular lean mass; MVC, maximal voluntary contraction (knee extensors); METs, metabolic equivalents. Values are presented as mean ± standard error of the mean; n = 10 (5 men, 5 women). Significance is set at $P \leq .05$.

General design

Before 14 consecutive days of reduced activity, participants underwent assessments of body composition, insulin sensitivity, maximal strength, and functional ability. During the 14 days of reduced activity, participants were instructed to reduce their daily step-count to ensure they completed no more than 1500 steps per day. All participants monitored and recorded their daily step-count to ensure they did not surpass 1500 steps per day.

Preliminary assessments

Daily step-count and energy intake

Upon consent, participants' habitual daily step-count and energy expenditure were determined over a 3-day period. Daily step-count was monitored using a portable pedometer (AccuSTEP 400; Accusplit, Livermore, California), and energy expenditure was measured by a SenseWear Pro energy expenditure armband device (BodyMedia, Pittsburgh, Pennsylvania). Habitual energy and macronutrient intakes were determined on the same 3 days of habitual activity by diet record and analyzed (Diet Analysis Plus 9.0; Cengage, Independence, Kentucky).

Preintervention and postintervention assessments

Assessments of function, metabolic health, and body composition were conducted within 1 week before the 14-day step-

reduction intervention and repeated 48 hours after the final day of the 14-day intervention (ie, d 16).

Metabolic health and body composition assessment

After a 10-hour overnight fast, an oral glucose tolerance test (OGTT) was performed as described elsewhere (19). Body composition was assessed by dual-energy x-ray absorptiometry (DXA) (QDR-4500A, software version 12.31; Hologic Inc., Bedford, Massachusetts). Fat mass of the abdominal region was determined from the scan region between lumbar vertebrae (L1–L4) as a surrogate for visceral adipose tissue (20). Lower limb skeletal muscle mass from DXA was calculated by utilizing the prediction equation developed and validated in the lower limbs by Shih et al (21). All DXA scans were performed by a trained technician and analyzed by an individual who was blinded to the overall study design.

Physical function and maximal leg strength

Functional abilities were assessed by the short physical performance battery (SPPB), described previously (22). Unilateral isometric knee extensor torque was measured using a dynamometer (Biodex system 3; Biodex Medical Systems, Shirley, New York) as described previously (23).

Experimental infusion trial

Before the 14-day step-reduction intervention, participants reported to the laboratory, and a catheter was inserted in an antecubital arm vein of each arm; one arm was wrapped in a 45°C heating blanket to "arterialize" blood for sampling and with stable isotope infusion into the opposite arm (Figure 1). After baseline blood sampling, a primed (2 $\mu mol \cdot kg^{-1}$) continuous (0.05 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) infusion of L-[*ring*-$^{13}C_6$]phenylalanine was initiated (Cambridge Isotopes, Andover, Massachusetts). After 150 minutes of infusion, a muscle biopsy was obtained using a Bergström needle (24), under local anesthesia from the vastus lateralis of the thigh from the same leg that performed unilateral strength tests $\leq$ 7 days previously. Muscle biopsies were rapidly frozen in liquid nitrogen for further analysis. After the muscle biopsy, participants ingested a drink with 25 g of egg white protein (80%; NOW Foods, Bloomington, Illinois) dissolved in 400 mL water. Drinks were enriched to approximately 5% with [*ring*-$^{13}C_6$]phenylalanine to minimize disturbances in isotopic steady state on consumption (25). Arterialized blood samples were processed as previously described (26). At 240 minutes, a second muscle biopsy was obtained from the same leg as the first biopsy. The infusion trial was repeated on the morning after the 14-day step-reduction intervention with biopsies from the contralateral leg to the preintervention infusion trial.

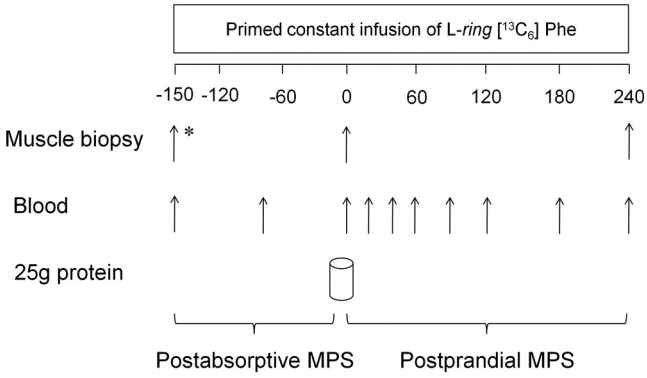

Figure 1. Schematic diagram of the preinfusion/postinfusion trial. Asterisk indicates muscle biopsy sample that was obtained during the postintervention infusion trial only. Preinfusion biopsy samples were taken from the same leg that performed the unilateral leg strength testing, and postinfusion biopsy samples were obtained from the contralateral leg.

Step-reduction intervention

The day after the first infusion trial, participants began 14 days of reduced ambulatory activity. Participants were instructed to remain as sedentary as possible, ensuring that no more than 1500 steps per day were completed. Participants were instructed to record their pedometer step-count at the end of each day before bed. Compliance with the intervention was monitored through a second step-count record provided by the armband accelerometer, the counts from which were inaccessible to the participants, and compared against pedometer values. The difference between pedometer-derived and armband accelerometer-derived step-counts over the 14-day intervention was consistently $< 10\%$.

Blood analyses

Plasma [$^{13}C_6$]phenylalanine enrichments were determined by gas chromatography-mass spectrometry as previously described (27). Blood essential amino acid (EAA) concentrations were analyzed by HPLC as previously described (27). Plasma glucose and insulin were measured as described previously (27). Commercially available ELISAs were used to determine concentrations of C-peptide (Cederlane Labs, Burlington, Ontario, Canada), IL-6 (Thermo-Scientific, Toronto, Ontario, Canada), TNF-α (Thermo-Scientific), and C-reactive protein (CRP; Cayman Chemical, Ann Arbor, Michigan) by following the manufacturers' instructions.

Muscle analyses

Analysis of [$^{13}C_6$]phenylalanine enrichment of muscle myofibrillar protein by gas chromatography combustion isotope ratio mass spectrometry was achieved as previously described (27). The cytosolic protein pool obtained during the isolation of muscle protein subfractions was used to determine im signaling via Western blot, as previously described (29). Protein phosphorylation was expressed relative to total protein content, and total protein was expressed relative to α-tubulin (for cytosolic protein targets). Molecular signaling proteins were determined with 9 subjects (5 male and 4 female), due to limited tissue availability for 1 subject. Primary antibodies obtained from Cell Signaling (Beverley, Massachusetts) were mammalian target of rapamycin (mTOR)^{Ser2448} (no. 2971), total mTOR (no. 2972), ribosomal protein S6 kinase (p70S6K)^{Thr389} (no. 9234), total p70S6K (no. 9202), 4E binding protein 1 (4E-BP1)$^{Thr37/46}$ (no. 2855), total 4E-BP1 (no. 9452), Akt^{Ser473} (no. 3787), total Akt (no. 8596), eEF2^{Thr56} (no. 2331), total eEF2 (no. 2332), and α-tubulin. A horseradish peroxidase-conjugated antirabbit secondary antibody (no. NA934VS) was obtained from GE Healthcare (Amersham Bioscience, Pittsburgh, Pennsylvania).

Calculations

Area under the curve (AUC)

Plasma glucose and insulin concentrations at 0, 10, 20, 30, 60, 90, and 120 minutes of the OGTT were used to determine AUC. Plasma insulin and amino acid concentrations at 0, 20, 40, 60, 90, and 240 minutes of the experimental infusion trial were also used to determine AUC.

Insulin sensitivity

Plasma glucose and insulin concentrations during the 120-minute OGTT were used to determine the whole-body insulin sensitivity index (ISI) (30). Postabsorptive insulin sensitivity was also estimated by the homeostasis model of insulin resistance index (HOMA-IR) (31).

Muscle protein synthesis

The fractional synthetic rate (FSR) of myofibrillar muscle proteins was calculated using the standard precursor-product method with the mean plasma phenylalanine enrichment as an estimate of tRNA labeling (32). Plasma protein enrichment was utilized as a proxy for baseline enrichment of muscle protein due to the use of "tracer naive" participants, as previously validated by our group (25).

Statistics

Preintervention to postintervention changes in body composition, insulin sensitivity, and physical function/strength were determined using a paired Student's *t* test. Changes in fasted- and fed-state im signaling and fraction-specific rates of muscle protein synthesis were analyzed using a 2-way repeated-measures ANOVA (time × feeding). Significance was set at $P \leq .05$. Data are presented as means ± standard error of the mean unless otherwise indicated. All analyses were performed using SPSS version 19 for Windows (SPSS, Inc., Chicago, Illinois).

Results

Step-count and energy expenditure

During the 14-day intervention, daily step-count was reduced by approximately 76% from habitual levels ($P < .001$; Table 1 and Figure 2), whereas daily physical activity ≥ 3.0 metabolic equivalents was reduced by approximately 72% ($P < .001$; Table 1). Daily energy expenditure was reduced by approximately 14% ($P < .001$; Table 1) and correlated with the reduction in daily step-count ($r = 0.66$; $P = .004$).

Body composition

Total body mass, body mass index, and total body fat mass (FM) were unchanged after 14 days of reduced ambulatory activity (Table 1). Total body fat percentage and trunk FM were increased by approximately 7.4% ($P = .053$) and approximately 2.6% ($P = .024$), respectively, after the intervention. There was a trend for reductions in total body fat-free mass (FFM) (~1.5%; $P = .082$) and arm FFM (~2.8%; $P = .078$) after the intervention. FFM and corrected skeletal muscle mass of the legs were reduced ($P < .001$) by approximately 3.9% after the intervention (Table 1).

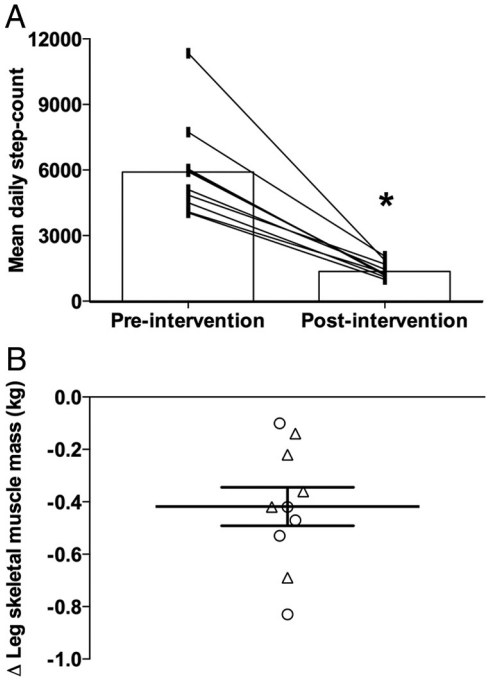

Figure 2. Mean preintervention and postintervention change in daily step-count (A) and leg skeletal muscle mass (B) in each male (circles) and female (triangles) participant. Asterisk indicates significantly lower compared with preintervention values ($P < .001$). Values represent individual and means (n = 10).

Physical function and strength

SPPB performance and maximal isometric torque were not affected by the 14 days of reduced ambulation (Table 1).

Dietary intake

There was no difference between preintervention and midintervention total daily energy (1517 ± 133 vs 1634 ± 185 kcal · d^{-1}, respectively), protein (0.8 ± 0.1 vs 0.8 ± 0.1 g · kg^{-1} body mass [BM], respectively), and carbohydrate intake (2.5 ± 0.3 vs 2.8 ± 4.3 g · kg^{-1} BM, respectively). Daily fat intake increased slightly during the step-reduction intervention compared with preintervention values (0.9 ± 0.1 g · kg^{-1} BM, from 0.7 ± 0.1; $P = .04$).

Blood metabolites

Fasting plasma glucose concentrations were not altered, whereas fasting plasma insulin concentration was significantly greater ($P < .05$) after step reduction (Table 2). Peak plasma glucose concentration at 30 minutes of OGTT was significantly greater after step reduction (8.6 ± 0.5 and 9.8 ± 0.5 mM, respectively; $P < .05$). AUCs for plasma glucose and insulin during OGTT were in-

Table 2. Measures of Metabolic Health and Systemic Inflammation

Parameter	Preintervention	Postintervention	*P* value
Glucose AUC, mmol · mL^{-1} · 120 min	454 ± 30	496 ± 28	<.01
Insulin AUC, μIU · mL^{-1} · 120 min	2308 ± 114	2575 ± 123	<.01
HOMA-IR	2.72 ± 0.27	3.08 ± 0.32	<.01
Matsuda ISI	0.71 ± 0.15	0.55 ± 0.08	.014
Postabsorptive TNF-α, pg · mL^{-1}	4.18 ± 0.35	4.67 ± 0.32	.047
Postabsorptive IL-6, pg · mL^{-1}	6.38 ± 0.70	7.11 ± 0.51	.11
Postabsorptive CRP, μg · mL^{-1}	0.96 ± 0.06	1.20 ± 0.13	.046
C-peptide AUC, pmol · L^{-1} · 120 min	2135 ± 28	2117 ± 40	.7

Abbreviation: NS, not significant. Values are expressed as means ± standard error of the mean; n = 10.

creased by approximately 9 and 12%, respectively, after step reduction ($P < .001$; Table 2). Postabsorptive insulin resistance (HOMA-IR) was increased by approximately 12% and postprandial insulin sensitivity (Matsuda ISI) was reduced by approximately 43% ($P < .05$; Table 2). In addition, AUC for plasma insulin in response to the egg protein beverage was also increased by approximately 7% after step reduction ($P < .05$; Figure 3, A and B).

Postabsorptive plasma concentrations of TNF-α and CRP were increased after the intervention by approximately 12 and 25%, respectively, compared with preintervention values ($P = .05$; Table 2). Circulating concentrations of IL-6 and C-peptide were not significantly elevated after step reduction (Table 2).

Amino acids

Plasma EAA concentrations were elevated 20 minutes after egg protein ingestion ($P < .05$) and remained elevated for 90 minutes before returning to postabsorptive values by 240 minutes. There was no difference before and after the 14-day step-reduction intervention in basal, postabsorptive, or postprandial plasma EAA concentrations (Figure 3, C and D).

Tracer enrichment

Plasma phenylalanine enrichment was stable throughout the protocol ($P < .01$; Figure 3E). Linear regression analysis indicated that the slopes of the plasma enrichment over time were not significantly different from zero ($P = .5$), indicating an isotopic steady state.

Intramuscular signaling proteins

Phosphorylation of p70S6K^{Thr389} increased robustly above postabsorptive values in the 4-hour postprandial state ($P < .01$), with no difference between the preintervention and postintervention response. Phosphorylation of Akt^{Thr473} was reduced below postabsorptive values in the 4-hour postprandial state ($P < .05$), with no difference between the preintervention and postintervention response. Before the 14-day step-reduction intervention, 4-hour postprandial phosphorylation of 4E-BP1$^{Thr37/46}$ was increased above postabsorptive values ($P = .04$), with no significant postprandial response apparent after the intervention. There was no feeding or time effect for mTOR^{Ser2448} or eEF2^{Thr56} phosphorylation. Total mTOR, eEF2, p70S6K, 4E-BP1, and Akt protein was not affected by the 14-day step-reduction intervention. Data are presented in Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) and representative blots in Supplemental Figure 1.

Myofibrillar protein synthesis

Rates of MPS increased significantly in the postprandial state at both time points ($P < .001$; Figure 4) but were attenuated by approximately 26% postintervention ($P = .028$; Figure 4). Basal, postabsorptive rates of MPS were not different from preintervention to postintervention.

Discussion

We demonstrate here for the first time that an approximately 76% reduction in daily step-count for as little as 14 days induced a significant loss of leg muscle in older men and women. Prolonged periods of disuse, due to hospitalization or bed rest, occur with greater frequency in adults > 65 years of age, and these bouts of inactivity have been hypothesized to contribute to the sarcopenic process (12, 17). Past investigations have demonstrated that 10–28 days of muscle disuse (via bed rest or leg casting) lead to decreases of approximately 4–10% in leg lean tissue mass, approximately 15% in strength, and approximately 30–50% reductions in postabsorptive and postprandial rates of MPS, with no apparent difference between young and older individuals (9, 16, 33, 34). Our current data provide a critically important extension to these studies in demonstrating that far less extreme forms of inactivity (ie, reduced stepping) have a detrimental effect on skeletal muscle mass as evidenced by a reduction of approximately 4% in leg lean mass (range, 1–9%; Figure 2) in otherwise healthy elderly individuals. This superfi-

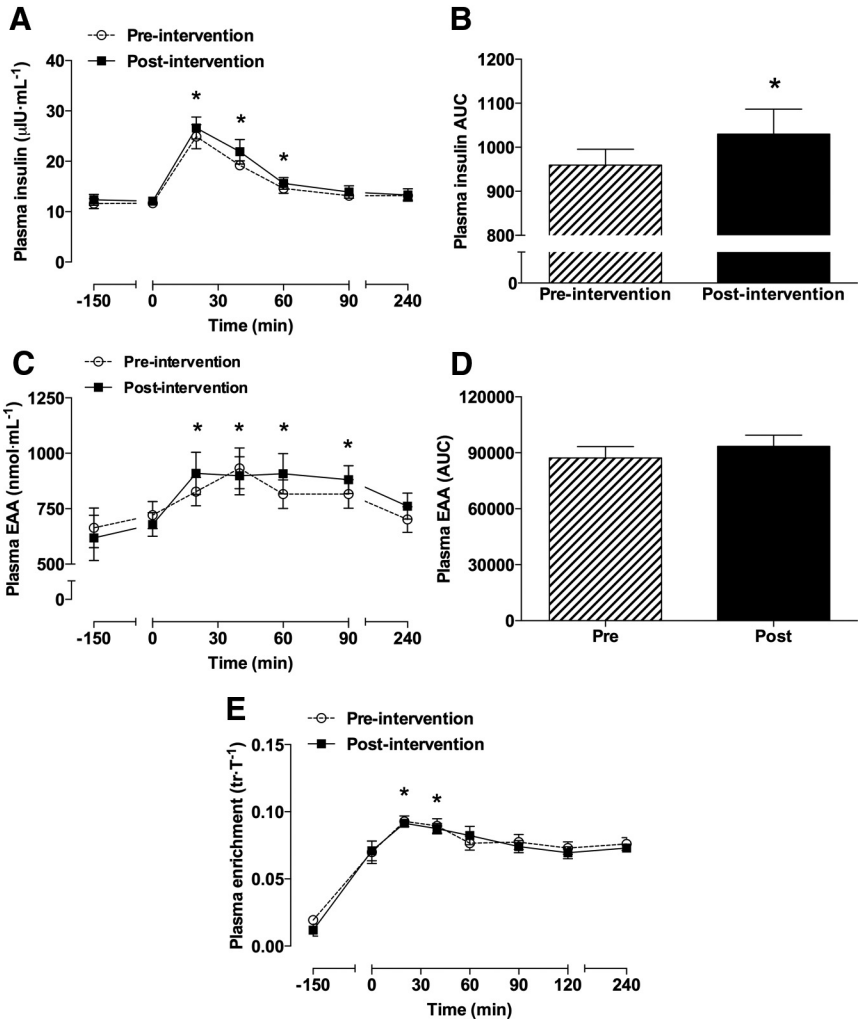

Figure 3. Mean preintervention and postintervention plasma insulin concentration (A), insulin AUC (B), EAA concentration (C), EAA AUC (D), and plasma enrichment (E) of [$^{13}C_6$]phenylalanine tracer, expressed as a percentage of the tracer-to-tracee ratio, during the infusion trial. At t = 0 minutes, 25 g of egg protein was consumed. Asterisk indicates significantly greater than basal, fasted values in both conditions or from preintervention values for AUC ($P < .05$). Values are expressed as means ± standard error of the mean (n = 10).

cially "benign" intervention of simply reducing daily steps demonstrates just how deleterious a period of inactivity, even without an overt pathology, can be for older persons. Our data are congruent with results in healthy young individuals who, after adopting a more sedentary lifestyle by reducing daily step-count by approximately 80% for 14 days, also displayed a reduction in leg lean tissue mass of approximately 4% (13, 15) during the same period. Because older adults do not recover, even with heavy resistance exercise, losses in muscle mass and strength after disuse compared with the young (9, 10), periods of disuse such as we have utilized here would be important to consider in the progression of sarcopenia (17).

Our findings demonstrate that reduced ambulation in elderly individuals is associated with a blunting of the anabolic response to a bolus of high-quality (complete EAA) dietary protein. Indeed, whereas the provision of 25 g of high-quality egg protein increased postprandial rates of MPS by approximately 123% before 14-day reduced ambulation, reduced habitual activity coincided with an approximately 26% reduction in postprandial rates of MPS. This "anabolic resistance" to the normal feeding-induced rise in MPS may explain in part the muscle atrophy associated with limiting habitual activity, especially in the absence of reduced MPS in the postabsorptive state, which is observed in bed rest (33). Despite the reduction in postprandial MPS, we were unable to detect a paralleled blunting in translational initiation signals. The absence of any robust blunting in anabolic signaling may relate to the timing of the postprandial biopsy (4 h after feeding), which was taken after the peak phosphorylation response (~1–2 h after feeding) (35). Nonetheless, a lack of congruence between the amplitude of signaling protein phosphorylation and that of protein turnover has also been documented (35, 36).

In addition to inducing muscle atrophy, a period of reduced ambulation also leads to impairments in insulin sensitivity in young men (13–15), with obvious consequences that would likely be similar, or possibly worse, in elderly people. As far as amino acid metabolism is concerned, it is difficult to estimate what a decline in insulin sensitivity, measured in response to carbohydrate, would mean because the impact of insulin on amino acid metabolism is complex (36). Blood flow has been shown to be an important aspect for delivery of amino acids to muscle, contributing to feeding-induced increases in MPS (37); thus, it is possible that impairments in insulin-mediated microvascular recruitment (38) may limit muscle availability of amino acids and induce fed-state anabolic resistance. Investigation into the influence of inactivity on microvascular flow/recruitment, particularly in response to nutrient provision, should be an important consideration for future studies. Also, and congruent with observations of increases in adiposity and in young men (13–15), we show that an increase in trunk adiposity occurred after 14 days of reduced ambulation and also occurs in older individu-

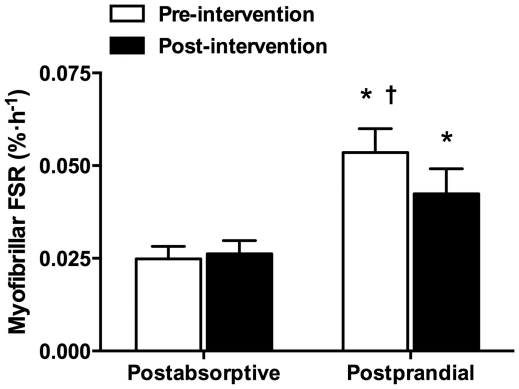

Figure 4. Mean preintervention and postintervention postabsorptive and postprandial FSR of myofibrillar muscle proteins. Asterisk indicates significant increase above fasting FSR within condition ($P < .001$). Dagger indicates significantly greater than postintervention ($P = .028$). Values are expressed as means ± standard error of the mean ($n = 10$).

als. Furthermore, fasting concentrations of insulin were increased (a postabsorptive marker of insulin resistance), whereas both OGTT and protein feeding-indexed insulin sensitivity were also reduced after reduced ambulation. It is important to note that insulin sensitivity was measured at the whole-body level and is not muscle-specific. Nonetheless, previous studies have used sophisticated insulin clamp techniques to demonstrate deleterious changes in insulin sensitivity after a near-identical model of inactivity in the young (15), and we are confident in the insulin sensitivity pattern of response observed herein. Previously, Guillet et al (39) demonstrated that obese young adults who displayed indicators of insulin resistance (eg, reduced insulin-mediated glucose disposal) had blunted postabsorptive and postprandial rates of MPS compared with healthy young adults. Thus, the modest increase in adiposity and decline in insulin sensitivity are parallel and may have contributed to the fed-state anabolic resistance and the muscle atrophy observed. Indeed, prior habitual physical activity can alleviate insulin resistance of MPS in elderly muscle (7), in part by inducing greater amino acid delivery. Nevertheless, changes in metabolic health with the cessation of physical activity precede the increase in adiposity as demonstrated by Knudsen et al (14), who reported a 37% reduction in insulin sensitivity after just 3 days of reduced ambulation combined with overfeeding in young adults. Thus, we speculate that a reduction in daily ambulation leads to rapid impairments in skeletal muscle insulin-mediated sensitivity of the vasculature in the elderly, which precedes overt body composition changes and the onset of metabolic disease seen with longer duration and more frequent periods of inactivity, which may contribute to the blunting of fed-state MPS.

Concomitant with impairments in insulin sensitivity, we observed a modest increase in circulating inflammatory markers TNF-α and CRP in older adults after 14 days of reduced ambulation that warrants discussion. Previously, in young adults, Krogh-Madsen et al (15) reported that circulating inflammatory markers were not altered by 14 days of reduced ambulation in younger individuals. The disparity between our current data in the old and that of the young may be due the pre-existence of, or propensity to develop, systemic inflammation in the old, associated with lower appendicular lean mass and greater adiposity (28). Our observations of a modest increase in inflammatory cytokines over the 14-day intervention may have contributed in part to the deterioration in insulin sensitivity, postprandial MPS, and the establishment of muscle atrophy, either indirectly by adversely affected skeletal muscle insulin sensitivity or directly by initiating catabolic signaling intermediates; clearly, further work is required to delineate these concepts.

In conclusion, we have demonstrated that 14 days of reduced ambulation in older adults results in a loss of leg lean mass and gains in trunk adiposity. The blunted postprandial rates of MPS may underpin the deleterious "shift" in body composition after inactivity in older adults and may be linked to impairments in insulin sensitivity and/or modest elevations in systemic inflammation. We suggest that habitual activity should be accounted for when evaluating the impact of nutrition on muscle protein metabolism in the elderly due to its ability to accelerate "biological age" and induce a relative "anabolic resistance" characteristic of overtly catabolic pathological states, such as bed rest. Ultimately, the extent of muscle atrophy and impaired rates of MPS after a single disuse event is comparable between young and old; however, we postulate that increasingly frequent periods of disuse, coupled with a decline in physical activity in older adults, may lead to losses in muscle mass from which older people do not fully recover (9). Importantly, these disuse periods need not be the result of pathological or traumatic catabolic events (12). Short periods of relative disuse, as we have utilized here, lead to modest increases in markers of inflammation, a gradual decay in insulin sensitivity, and blunting of fed-state MPS—all of which may transiently accelerate the trajectory of sarcopenia. From a healthcare perspective, our data highlight the importance of implementing ongoing nutritional and/or exercise support designed to mitigate or ablate the loss of muscle mass and maintain metabolic health, even during short and superficially benign periods of reduced activity in otherwise healthy elderly individuals.

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