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# No additional effect of different types of physical activity on 10-hour muscle protein synthesis in elderly men on a controlled energy- and protein-sufficient diet

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ABSTRACT

*Purpose:* The elderly lose skeletal muscle mass with age, which may be detrimental for function and quality of life. Both inactivity and heavy resistance exercise are known to have marked but opposite effects upon muscle mass. However, the potential effects of daily physical activity upon muscle protein synthesis (MPS) are less investigated. The aim of this study was to determine the effects of daily physical activities upon MPS in elderly individuals.
*Methods:* A total of 24 elderly men (70 ± 1 year) were recruited and randomly assigned: inactivity in form of bed-rest (IA), daily physical activities (DA), or heavy resistance exercise (RE). All groups undertook a normal eating routine containing carbohydrates (52 E%), fat (32 E%), and protein (16 E%). Ingestion of labeled milk protein ([1-$^{13}$C]leucine-labeled whey and caseinate) served to maintain tracer enrichment for determination of 10-hour myofibrillar protein fractional synthesis rates (FSR), and typical prerequisites for calculating FSR were fulfilled. Physical activities were monitored, and venous blood and muscle biopsies collected.
*Results:* Physical activity was highest in the DA compared to both the IA and RE groups. Nutrient ingestion increased insulin, leucine, and phenylalanine plasma concentrations in all groups. [1-$^{13}$C]leucine enrichment was stable throughout the 10-hour FSR period. Myofibrillar protein FSR were similar for IA, DA, and RE groups, 0.055 ± 0.003%/h, 0.058 ± 0.006%/h, and 0.065 ± 0.008%/h, respectively (means ± SE, $P = 0.44$).
*Conclusions:* In elderly males, inactivity, daily activities, and resistance exercise interventions result in equal 10-hour, whole day MPS during an energy- and protein-sufficient diet regimen.



## 1. Introduction

Maintenance of skeletal muscle mass with increasing age is crucial to remain functionally independent. Consequently, age-related loss of muscle mass is a challenge for elderly individuals and can lead to sarcopenia and limited functional abilities. Therefore, knowledge about ways to prevent sarcopenia is of great individual but also socio-economic importance (Holm et al., 2015).

It is well-documented that periods of heavy-load resistance exercise training are capable of increasing muscle mass, strength, and function (Aagaard et al., 2001; Holm et al., 2008; Kumar et al., 2009), as a result of accumulated states of positive net protein balance presumably driven by short-lived increments in muscle protein synthesis (MPS) (Chesley et al., 1992; Phillips et al., 1997; Kumar et al., 2009; Agergaard et al., 2013). Heavy-load resistance exercise is superior in measures of muscle mass accretion, strength improvements, and acute regulation of MPS as compared to moderate contraction intensities (Holm et al., 2008, 2010). However, 12 weeks of endurance-like training with 3–4 sessions per week of 20–45 min of cycling at 60–80% of heart rate reserve did in fact increase skeletal muscle size in young and elderly men (Harber et al., 2012). Therefore, both aerobic exercise and light-load resistance exercise are able to exert some stimulus on MPS acutely and on muscle mass and strength over time (Short et al., 2004; Sheffield-Moore et al., 2004; Holm et al., 2008; Harber et al., 2010; Durham et al., 2010; Harber et al., 2012; Bechshoeft et al., 2013; Di Donato et al., 2014).

The ability of even moderate contractile activity to enhance MPS has been proposed to be related to increased muscle perfusion, which can be utilized to exert a more powerful nutrient (amino acid)-derived stimulus to the muscle cells (Timmerman et al., 2012). This phenomenon suggests that less strenuous activities than heavy resistance exercise are sufficient to stimulate some muscle growth or maintenance of existing mass, at least as long as adequate amounts of protein are

*Abbreviations:* ANOVA, Analysis of variance; DEXA, Dual-energy X-ray absorptiometry; FSR, Fractional synthesis rate; LBM, Lean body mass; MPB, Muscle protein breakdown; MPS, Muscle protein synthesis; RM, Repetition maximum; RMR, Resting metabolic rate; SE, Standard error; TTR, Tracer to tracee ratio.

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available (Bechshoeft et al., 2013). For example, simple habitual daily activities such as walking and shopping, which may not be strenuous enough to be considered as exercise, may therefore serve as an important daily action and stimulus to maintain skeletal muscle mass in the elderly. However, the impact of such light-intensity activities in a daily living setting in community-dwelling elderly citizens is to a large extent unknown when it comes to regulation of skeletal muscle protein turnover.

The purpose of the present study was to investigate activities of daily living (bicycling, stair-climbing, and walking) under well-nourished conditions in elderly men, who, we hypothesized, would gain higher MPS and prolonged intramuscular protein synthesis signaling as compared to physical inactivity. Furthermore, as a positive control we included a group that performed a session of heavy resistance exercise, which, we hypothesized, would result in higher MPS as compared to both inactivity and activities of daily living.

## 2. Materials and methods

### 2.1. Subjects

A total of 24 elderly (70 ± 1 year) male subjects were recruited by advertisements in local newspapers. They were recruited with the criteria of being moderately active (i.e. not participating in systematic heavy resistance exercise or other high-intensity sports), non-smokers and with no family history of diabetes or other chronic diseases. They were allowed to take antihypertensive, cholesterol-lowering, and prophylaxis anticoagulation drugs. Before inclusion, the study design, purpose, and possible risks were explained to each subject. Subjects gave written consent to participate in the protocol, which adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Region Hovedstaden (H-4-2012-145). All included participants were assigned to one of three groups: inactivity (IA), daily physical activities (DA), or heavy resistance exercise (RE) by simple randomization.

### 2.2. General subject measurements

Lean body mass (LBM) was determined by dual-energy X-ray absorptiometry (DEXA, Lunar DPX-IQ, GE Healthcare, Chalfont St. Giles, United Kingdom), and leg muscle strength was determined by the one-repetition maximum (1RM) on leg extension and leg press machines for each subject. All participants had a blood sample screened for general health parameters related to liver and kidney function, hematology, inflammation, diabetes, and cholesterol. Subject characteristics are outlined in Table 1.

**Table 1**
Subject characteristics.

| | Inactivity (IA) | Daily activity (DA) | Resistance exercise (RE) |
|---|---|---|---|
| *N* | 8 | 8 | 8 |
| Age (y) | 70 ± 1 | 71 ± 2 | 69 ± 2 |
| Height (m) | 1.74 ± 0.02 | 1.80 ± 0.02 | 1.77 ± 0.03 |
| Weight (kg) | 77 ± 3.2 | 80 ± 1.9 | 81 ± 4.0 |
| Body mass index (kg/m$^2$) | 25.3 ± 0.9 | 24.6 ± 0.6 | 25.8 ± 0.9 |
| Lean body mass (kg) | 56.5 ± 1.9 | 57.2 ± 2.0 | 58.2 ± 2.7 |
| Fat mass (kg) | 17.1 ± 2.4 | 19.4 ± 1.6 | 20.7 ± 1.9 |
| Fat (%) | 22.7 ± 2.3 | 25.3 ± 1.9 | 26.0 ± 1.5 |
| 1 RM knee extension (kg) | 81 ± 6 | 79 ± 3 | 88 ± 8 |
| 1 RM leg press (kg) | 267 ± 10 | 235 ± 16 | 228 ± 16 |
| HbA1c (mmol/L) | 6.74 ± 0.18 | 6.45 ± 0.12 | 6.59 ± 0.15 |
| Glucose (mmol/L) | 4.84 ± 0.21 | 5.29 ± 0.17 | 4.95 ± 0.25 |
| Total cholesterol (mmol/L) | 5.73 ± 0.32 | 5.90 ± 0.49 | 5.66 ± 0.27 |
| HDL cholesterol (mmol/L) | 1.61 ± 0.18 | 1.48 ± 0.13 | 1.45 ± 0.11 |
| LDL cholesterol (mmol/L) | 3.45 ± 0.37 | 3.78 ± 0.46 | 3.65 ± 0.33 |
| Triglycerides (mmol/L) | 1.46 ± 0.34 | 1.43 ± 0.21 | 1.20 ± 0.13 |

Values are mean (±SE) and group data were analyzed by one-factor non-repeated measures ANOVA. No significant differences were found between the groups in any of the parameters.

### 2.3. Experimental protocol

All subjects were instructed to refrain from strenuous physical activities during the 3 days prior to the trial day. Alcohol consumption was prohibited for one week and caffeine for 1 day prior to the trial day. On the trial day all subjects arrived at the laboratory by taxi at 0730 after an overnight fast from 2100 the evening before. An antecubital venous catheter was inserted and a baseline blood sample was obtained just before serving the subjects breakfast. An activPAL (PAL Technologies Limited, Glasgow, United Kingdom) was attached to the upper thigh to register physical activities during the day from −1 h (0900) to 10 h (2000). The experiments were conducted as described below and as outlined in Fig. 1.

Blood samples were obtained from the antecubital venous catheter every 15 min from −1 h to 0 h, and thereafter once every hour until 10 h. Muscle biopsies were taken from the vastus lateralis muscle under local anesthesia (lidocaine, 1%) at 0 h and 10 h. Muscle biopsies were taken with 4-mm Bergström biopsy needles (Stille, Stockholm, Sweden) with manual suction. Fat and connective tissue were quickly removed from the specimens, which subsequently were washed free of blood with ice-cold saline. The specimens were swiftly cut and weighed in portions of 20 mg, frozen in liquid nitrogen and stored at −80 °C.

### 2.4. Study meals and tracer ingestion

The dietary food items were selected with help of a nutritional software program (Dankost 3000; Dansk Catering Center, Herlev, Denmark). The distribution of fat, carbohydrates and protein, which adhered to general nutritional recommendations (Sandström et al., 1996), and the energy intake are outlined in Table 2. The total energy requirement was adjusted to each subject's basal resting metabolic rate (RMR) as determined by the Cunningham equation using LBM from the DEXA scan and multiplied by an activity factor of 1.5 (Sandström et al., 1996) irrespective of group.

The subjects had their first meal at 0900. The second meal was served at 1300 and the third meal at 1700. The first meal consisted of dark bread, jam, orange juice, apple slices, and butter. The second and third meal consisted of mashed potatoes, carrots, tomato sauce, red pepper, apple juice, and olive oil. In addition, all meals consisted of a drink containing [1-$^{13}$C]leucine-labeled milk proteins dissolved in water. Labeled protein drinks were also served in between the regular meals, at 1100 and 1500. All meals had to be finished within 30 min, and all subjects had to ingest the labeled protein drink before the regular meal items. All subjects ingested all meals and protein drinks. Furthermore, the subjects were allowed to drink water throughout the day.

All participants received a total of 1.25 g/kg LBM of [1-$^{13}$C]leucine-labeled calcium caseinate (10% tracer to tracee (TTR)) dissolved in 1600 mL of water. This was given to the subjects in five boluses: 480 mL (~23 g calcium caseinate for a person with LBM of 60 kg) at 0900, 260 mL (~12 g) at 1100, 300 mL (~14 g) at 1300, 260 mL (~12 g) at 1500, and 300 mL (~14 g) at 1700. In addition to the caseinate, we added 6 g of 10% TTR enriched [1-$^{13}$C]leucine-labeled whey protein to the first bolus at 0900. Leucine content in the caseinate was 8.77 g/100 g protein and in whey it was 11.77 g/100 g protein (Reitelseder et al., 2011). Therefore, ingestion of caseinate during the study protocol provided a total of 0.11 g/kg LBM of leucine (i.e., for a person with 60 kg LBM, 6.6 g of leucine was provided in the five servings). The ingestion of 6 g whey with the first serving provided 0.7 g of leucine. Since whey protein is quickly digested and the constituent amino acids appear rather quickly in the blood, we used this approach as a 'prime' of the tracer. Hence, the [1-$^{13}$C]leucine tracer