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Daily myofibrillar protein synthesis rates in response to low and high frequency resistance exercise training in healthy, young men

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Running title: Resistance exercise frequency and muscle protein synthesis

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ABSTRACT

The impact of resistance exercise frequency on muscle protein synthesis rates remains unknown. The aim of this study was to compare daily myofibrillar protein synthesis rates over a seven-day period of low frequency versus high frequency resistance exercise training. Nine young men (21±2 y) completed a seven-day period of habitual physical activity (BASAL). This was followed by a seven-day exercise period of volume-matched, low frequency (10 x 10 repetitions at 70% 1RM, once per week; LF) or high frequency (2 x 10 repetitions at ~70% 1RM, five times per week; HF) resistance exercise training. Participants had one leg randomly allocated to LF and the other to HF. Skeletal muscle biopsies and daily saliva samples were collected to determine myofibrillar protein synthesis rates using $^{2}H_2O$, with intracellular signalling determined using Western blotting. Myofibrillar protein synthesis rates did not differ between LF (1.46±0.26 $\%\cdot d^{-1}$) and HF (1.48±0.33 $\%\cdot d^{-1}$) conditions over the seven-day exercise training period (P>0.05). There were no significant differences between LF and HF conditions over the first two days (1.45±0.41 vs 1.25±0.46 $\%\cdot d^{-1}$) or last five days (1.47±0.30 vs 1.50±0.41 $\%\cdot d^{-1}$) of the exercise training period (P>0.05). Daily myofibrillar protein synthesis rates were not different from BASAL at any time point during LF or HF (P>0.05). The phosphorylation status and total protein content of selected proteins implicated in skeletal muscle ribosomal biogenesis were not different between conditions (P>0.05). Under the conditions of the present study, resistance exercise training frequency did not modulate daily myofibrillar protein synthesis rates in young men.

Key words: Exercise frequency, muscle protein synthesis, skeletal muscle, deuterated water

INTRODUCTION

The muscle hypertrophic response to resistance exercise training can be modulated by manipulating variables such as absolute load, total exercise volume, proximity to failure and rest interval between exercise sets (Burd et al., 2010b; Mitchell et al., 2012; Schoenfeld et al., 2016). Less clear is the impact of resistance exercise training frequency (i.e., the number of times a muscle group is exercised over a given period of time) on muscle hypertrophy. Understanding the relative importance of exercise training frequency is necessary to optimize the skeletal muscle adaptive response to prolonged resistance exercise training.

Whilst some studies have shown muscle hypertrophy to be enhanced by a higher (i.e., two or more times per week) resistance exercise training frequency (Schoenfeld et al., 2015; Zaroni et al., 2018), most have shown no differences (Schoenfeld et al., 2018). However, most studies to date have examined the impact of resistance exercise training frequencies in the range of one-to-three times per week. It is possible that higher resistance exercise training frequencies (e.g., five times per week) are required to enhance muscle protein synthesis rates and subsequent muscle hypertrophy. The evidence currently available is equivocal, with one study (Zaroni et al., 2018) showing greater muscle hypertrophy with a relatively high (five times per week) resistance exercise training frequency whereas another study (Gomes et al., 2018) reported no differences. As such, the impact of high versus low resistance exercise training frequency on muscle hypertrophy remains unclear.

Muscle hypertrophy following prolonged resistance exercise training is the product of sustained elevations in muscle protein synthesis that exceed muscle protein breakdown. It has recently been posited that relatively high resistance exercise training frequency is required to maximize muscle hypertrophy by regularly stimulating the acute myofibrillar protein synthetic response to a single bout of resistance exercise (Dankel et al., 2017). Following an acute bout of resistance exercise, myofibrillar protein synthesis rates remain elevated for approximately twenty-four hours before returning to basal levels (Burd et al., 2011; Damas et al., 2016). Furthermore, a relatively low volume (~three sets) of resistance exercise appears to maximize post-exercise myofibrillar protein synthesis rates, at least in young men (Burd et al., 2010a; Kumar et al., 2012). On this basis, it has been speculated that more frequent, low-volume, resistance exercise could induce more frequent elevations in myofibrillar protein synthesis rates which in the long-term would lead to greater muscle hypertrophy (Dankel et al., 2017). Whilst plausible, this hypothesis has yet to be tested.

The purpose of the present study was to compare daily myofibrillar protein synthesis rates, measured using deuterated water (^2H_2O) under free-living conditions, in young men over a seven-day period while performing low (once per week; LF) versus high (five times per week; HF) frequency resistance exercise training. As muscle protein synthesis rates are facilitated by transcriptional capacity (Figueiredo & McCarthy, 2019), we also aimed to assess whether resistance exercise training frequency impacts the phosphorylation status and total protein content of selected proteins implicated in ribosomal biogenesis.

METHODS

Participants and ethical approval

Nine young men participated in the present study between February 2018 and August 2018. Participant characteristics are presented in **Table 1**. Prior to providing written consent, each volunteer was informed of the experimental procedures and potential risks. Participants were screened prior to inclusion and deemed healthy based on their responses to a general health questionnaire. Inclusion criteria included being male, aged 18-35 years, a BMI between 18.5-29.99 kg/m^2, being recreationally active and untrained (i.e., performing activities of daily living and recreation but no regular lower body resistance exercise in the last year), and being willing and able to comply with all procedures. Exclusion criteria included having a lidocaine allergy, hypertension (≥140/90 mmHg) or bleeding disorders, current participation in another study, being a current/recent smoker, vegetarian/vegan or a history of substance abuse and/or taking prescription or non-prescription medication or supplements that may influence normal metabolic responses. The study was approved by the National Research Ethics Service Committee West Midlands, Edgbaston, UK (Reference: 17/WM/0430) and conformed to standards for the use of human participants in research as outlined in the Declaration of Helsinki. The intervention was registered at clinicaltrials.gov prior to data collection (Identifier: NCT03275779).

Pretesting

During the initial screening visit, participants underwent maximal strength testing and a familiarization session. First, participants completed a 5 minute warm-up of self-paced cycling. Maximal leg strength was then determined for each leg on a plate loaded 45° leg press. This process was then repeated on a weight-stacked leg extension machine. Participants first performed a submaximal warm-up set of eight-to-ten repetitions and had their lifting form critiqued and corrected when necessary. This was followed by sets at progressively increasingly loads until only one valid repetition could be competed. The load for each set was chosen based on the participant's rating of perceived exertion following the previous set. A three-minute rest interval was provided between each set. Once completed, the corresponding load (~70% 1RM) to be used during the subsequent familiarization session and resistance exercise sessions was calculated.

To familiarize participants with the exercise volume to be completed during the experimental trials, and to minimize muscle damage associated with an unfamiliar bout of resistance exercise (Damas et al., 2016; Nosaka et al., 2001), participants completed five sets of bilateral leg press followed by five sets of bilateral leg extension at ~70% 1RM, with two minutes rest between each set. Total exercise volume completed during the familiarization (12121±2206 kg) was similar to that completed in total by both legs during the experimental resistance exercise sessions (11952±2700 kg). Pretesting and the first experimental trial (day 0) were separated by ≥ seven days.

Study overview

A study overview is presented in **Figure 1**. The study was designed to assess whether resistance exercise frequency impacts daily myofibrillar protein synthesis rates measured under free-living conditions. Participants arrived at ~08:00 in a fasted state on day 0 and had a muscle biopsy collected. All muscle biopsies were collected from the *vastus lateralis* using the Bergström needle with manual suction under local anaesthesia (1% lidocaine). Participants then completed a seven-day basal period (BASAL) where they were instructed to maintain habitual physical activity (i.e., activities of daily living and recreation without structured physical activity). Participants returned on day 7 and had a second muscle biopsy collected from the alternate leg. Following this, participants had each leg randomly allocated to one of low frequency (LF) or high frequency (HF) resistance exercise (see *Resistance exercise sessions* section below). A bout of LF and HF was completed on day 7. Approximately forty-eight hours later (day 9), participants returned and had one muscle biopsy collected from each leg. This was followed by the second bout of HF. Additional bouts of HF were completed on days 10, 11 and 12. Participants returned on day 14 (~48 hours after the final HF bout) and had the final muscle biopsies collected from each leg, signifying the end of the study. A pedometer was worn throughout and weighed food diaries were completed to assess daily step count and dietary intake, respectively, across the study.

^2H_2O dosing protocol

The ^2H_2O dosing protocol consisted of one dosing day and sixteen maintenance days (Shad et al., 2019). The ^2H_2O protocol was well tolerated with none of the participants reporting any adverse effects.

Dietary intake and physical activity

The evening prior to each experimental visit involving muscle biopsies, participants received the same standardized meal (~689 kcal, providing ~55 energy% (En%) carbohydrate, ~20 En% protein, and ~25 En% fat). A weighed four-day food diary was completed over the first seven-day period of habitual physical activity (BASAL) and over the second seven-day period of LF and HF resistance exercise to assess energy and macronutrient intake. Participants were required to include two week-days and both weekend days in their recordings. Dietary records were analysed using Dietplan software (Forestfield Software Ltd., v6.70.67). Participants were instructed to refrain from structured physical activity throughout the study other than the prescribed resistance exercise completed as part of the study. Participants were also provided with a hip-worn pedometer (Yamax Digi-Walker SW-200) to wear throughout the study to assess daily step count.

Resistance exercise sessions

Using a within-subject design, participants had one leg randomized to complete LF and the other to HF. Prior to all resistance exercise sessions, participants completed a five-minute warm-up of self-paced cycling at ~100 W. On day 7, a single bout of unilateral high volume LF was completed. This consisted of five sets of ten repetitions at ~70% 1RM on the 45° leg press machine followed by five sets of ten repetitions at ~70% 1RM on the weight-stacked leg extension machine. A single bout of unilateral low volume HF was also completed on day 7 using the opposite leg. This consisted of one set of ten repetitions at ~70% 1RM on the 45° leg press machine followed by one set of ten repetitions at ~70% 1RM on the weight-stacked leg extension machine. A further four bouts of unilateral low volume HF was completed on days 9, 10, 11 and 12. This design ensured that total exercise volume and the number of sets completed were matched between the LF and HF conditions. Total exercise volume was intentionally matched as exercise volume has been shown, at least when comparing low volumes of resistance exercise, to modulate the magnitude of the myofibrillar protein synthetic response to resistance exercise (Burd et al., 2010a). Two minutes of rest was allowed between all sets, and five minutes of rest was allowed between the bouts of LF and HF on day 7. Following all resistance exercise sessions, participants ingested 25 g of whey protein powder (Impact Whey Protein; Myprotein), containing 21 g of protein (equating to ~0.29 g/kg), dissolved in water.

Body water ^{2}H enrichment

Body water ^{2}H enrichment was analysed from daily saliva samples collected throughout the study as previously described (Holwerda et al., 2018; Shad et al., 2019).

Myofibrillar bound ^{2}H-alanine enrichment

^{2}H-alanine enrichment in the myofibrillar fraction of muscle biopsy samples was measured as previously described (Shad et al., 2019).

Western blotting

Western blot analyses were performed on the sarcoplasmic fraction obtained during myofibrillar protein extraction as previously described (McKendry et al., 2019). The following primary antibodies were used ((1:1000) in 2.5% bovine serum albumin (BSA)): total eukaryotic translation initiation factor 4E (eIF4E) (ab33766), phospho-eIF4E Ser209 (ab76256), total cyclin D1 (ab16663) and total upstream binding factor (UBF) (ab244287) all purchased from Abcam (Abcam, Cambridge, U.K). Imaging was undertaken using a G:Box

Chemi-XR5 (Syngene, Cambridge, UK) and bands were quantified using Image Studio Lite (Li-Cor, Lincoln, Nebraska, U.S).

Calculations

Myofibrillar protein fractional synthetic rate (FSR) was determined using the incorporation of ^{2}H-alanine into myofibrillar protein and the mean ^{2}H enrichment in body water between sequential biopsies, corrected by a factor of 3.7, as the surrogate precursor based upon ^{2}H labelling during *de novo* alanine synthesis (Belloto et al., 2007). The standard precursor-product method was used to calculate FSR:

$$FSR\ (\% \cdot day^{-1}) = \left(\frac{E_{m2} - E_{m1}}{E_{precursor} \times t}\right) \times 100$$

where E_{m1} and E_{m2} are the myofibrillar protein-bound ^{2}H-alanine enrichments between sequential muscle biopsies. $E_{precursor}$ represents the mean body water ^{2}H enrichment between sequential biopsies corrected by a factor of 3.7 based upon the ^{2}H labelling of alanine during *de novo* synthesis (Belloto et al., 2007). t represents the time between sequential biopsies in days.

Statistics

Based on the hypothesis that high frequency resistance exercise training would result in more frequent elevations in myofibrillar protein synthesis rates compared to low frequency resistance exercise training, and previous research (Holwerda et al., 2018; Wilkinson et al., 2014), an effect size of 1.1 was estimated. Sample size calculations showed that n=9 would be sufficient to detect a difference in daily myofibrillar protein synthesis rates between LF and HF conditions over the seven-day exercise training period using a two-tailed paired samples t test (80% power, α-level of 0.05, G*power). All statistical analyses were performed using SPSS 25.0 (SPSS, USA). Differences between the seven-day basal period and seven-day exercise period (i.e., BASAL vs. LF/HF) for daily step count and dietary intake were compared using paired sample t-tests. Differences between exercise conditions (LF vs. HF) for exercise variables (i.e., maximal strength and total exercise volume) were compared using a paired sample t-test. Body water ^{2}H enrichment was analysed using a one-factor repeated measures ANOVA with time as the within-subjects factor. Myofibrillar protein FSR over the seven-day resistance exercise training period was compared between LF and HF conditions using a paired samples t-test (n=9). All other comparisons over time and between conditions for myofibrillar protein FSR were analysed using two-factor repeated measures ANOVAs (condition x time) with condition (BASAL vs. LF vs. HF) and time (days 0-7, 7-9, 9-14 and 7-14) as within-subjects factors. Intracellular signalling was analysed using a two-factor repeated measures ANOVA (condition x time) with condition (BASAL vs. LF vs. HF) and time (days 7, 9 and 14) as within-subjects factors. A biopsy sample for one participant could not be collected on day 9, and thus myofibrillar protein FSR data for days 7-9 and 9-14 and all intracellular signalling data were analysed on n=8. When a significant main effect or interaction was found, t-tests with Bonferroni correction for multiple comparisons were performed. All data are presented as mean±SD.

RESULTS

Exercise variables

Maximal strength values at baseline were not different between the LF and HF conditions for the leg press (P=0.397) and leg extension (P=0.650) exercises (**Table 1**). By design, total exercise volume completed was not different between the LF (5933±1357 kg) and HF (6019±1347 kg) conditions (P=0.121).

Daily step count and dietary intake

Daily step count and dietary intake are presented in **Table 2.** Daily step count was not different between BASAL and the seven-day period of resistance exercise (P=0.167). The relative contribution of dietary fat to overall energy intake significantly decreased during the period of resistance exercise (P=0.041). There was also a trend for daily protein intake (P=0.061) and protein intake relative to body weight (P=0.089) to increase during the period of resistance exercise. All other dietary variables were unchanged across the study.

Body water ^{2}H enrichment

Figure 2A presents the mean body water ^{2}H enrichment. Following the loading phase on day -2 and a single maintenance day on day -1, body water ^{2}H enrichment reached 0.55±0.05% (day 0). Body water ^{2}H enrichment did not change significantly over the duration of the study, with an average body water ^{2}H enrichment of 0.58±0.08% during BASAL and 0.62±0.13% during the period of resistance exercise (P=0.107).

Myofibrillar protein synthesis

Daily myofibrillar protein synthesis rates were not different between LF (1.46±0.26 $\%\cdot d^{-1}$) and HF (1.48±0.33 $\%\cdot d^{-1}$) conditions over the entire seven-day exercise period (**Figure 2B**;

P=0.801). Moreover, there were no significant differences between LF and HF conditions over the first two days (days 7-9) (1.45±0.41 vs. 1.25±0.46 $\%\cdot d^{-1}$; **Figure 3**; P=0.342) or over the last five days (days 9-14) of the exercise period (1.47±0.30 vs. 1.50±0.41 $\%\cdot d^{-1}$; **Figure 3**; P=0.342). Daily myofibrillar protein synthesis rates were not different from BASAL at any time point during LF or HF (**Figures 2B and 3**; P=0.591).

Intracellular signalling

A main effect of time was observed for eIF4E total protein content (**Figure 4A**; P=0.029). Following correction for multiple comparisons, pairwise comparisons showed a tendency (P=0.056) for greater total protein content 48 hours (i.e., day 9) following the initial LF and HF resistance exercise bouts compared to day 7. A main effect of time was also observed for cyclin D1 total protein content (**Figure 4C**; P=0.046). However, following correction for multiple comparisons, pairwise comparisons showed no significant difference between time points. There were no significant changes over time (P=0.407) or differences between LF and HF conditions (P=0.345) for phosphorylation of eIF4E at Ser209 (**Figure 4B**). There were no significant changes over time (P=0.217) or differences between LF and HF conditions (P=0.891) for UBF total protein content (**Figure 4D**).

DISCUSSION

The present study is the first to determine the impact that resistance exercise training frequency may have on myofibrillar protein synthesis rates. The major finding was that daily myofibrillar protein synthesis rates did not differ between volume-matched low and high frequency resistance exercise training performed over a seven-day period in young men. In line with these findings, resistance exercise training frequency did not modulate the phosphorylation status and total protein content of selected proteins implicated in skeletal muscle ribosomal biogenesis.

Manipulation of resistance exercise training frequency (i.e., the number of times a muscle group is exercised over a given period of time) has been proposed as a key factor determining exercise training induced muscle hypertrophy (Dankel et al., 2017; Schoenfeld et al., 2018). This is based on the premise that high resistance exercise training frequency induces greater overall myofibrillar protein synthesis rates and thus results in a greater amount of time spent in a greater net positive protein balance (Dankel et al., 2017). In the present study, a unilateral exercise model was utilized where each participant had one leg assigned to complete resistance exercise training once per week (i.e., low frequency; LF) and the other leg to complete resistance exercise training five times per week (i.e., high frequency; HF). This experimental design ensured that factors known to influence day-to-day muscle protein synthesis rates (e.g., sleep (Saner et al., 2020), protein intake (Witard et al., 2014), dietary composition (van Vliet et al., 2017) and habitual physical activity (Shad et al., 2019)) were identical between conditions, thereby allowing the impact of different resistance exercise training frequency on myofibrillar protein synthesis rates to be assessed in isolation. In contrast to the aforementioned hypothesis, the findings of the present study demonstrate that under volume-matched conditions, a high resistance exercise training frequency did not result in greater daily myofibrillar protein synthesis rates. These findings lend support to the preponderance of evidence showing that resistance exercise training frequency has little impact on muscle hypertrophy (Barcelos et al., 2018; Schoenfeld et al., 2018).

The present data are in line with evidence showing no differences in muscle hypertrophy with a resistance exercise frequency of one versus five times per week (Gomes et al., 2018), but are inconsistent with findings showing greater muscle hypertrophy under similar conditions (Zaroni et al., 2018). It is important to note that the total exercise volume completed in the study by Zaroni et al. (2018) was significantly higher in the group with a resistance exercise training frequency of five times per week. In contrast, in the present study, total exercise volume was intentionally matched between the low and high frequency exercise training conditions, which likely explains the lack of agreement between findings. Indeed, a recent meta-analysis, published whilst the present study was being undertaken, suggests that resistance exercise training frequency does not significantly impact muscle hypertrophy when conducted under volume-matched conditions (Schoenfeld et al., 2018). Taken together, it would appear that resistance exercise training frequency *per se* (i.e., under volume matched conditions) does not impact daily myofibrillar protein synthesis rates or subsequent muscle hypertrophy in young individuals.

In contrast to most (Brook et al., 2016; Damas et al., 2016; Wilkinson et al., 2014), although not all (Davies et al., 2020) previous studies, resistance exercise training failed to induce a detectable increase in daily myofibrillar protein synthesis rates **(Figure 3)**. The volume of resistance exercise completed in the high volume, low frequency exercise bout would have been expected to increase daily myofibrillar protein synthesis rates, given that resistance exercise of a similar volume and relative intensity has previously been shown to increase muscle protein synthesis rates in young men (Wilkinson et al., 2014). As such, there appears to be no obvious explanation for the absence of a measurable increase in daily myofibrillar protein synthesis rates following resistance exercise training. A possible explanation is that the impact of resistance exercise training on myofibrillar protein synthesis was ‘diluted’ over the measurement period, as ^2H_2O measures myofibrillar protein synthesis rates continuously capturing all free-living activities including diet, sleep and inactivity. Whilst more representative of long-term muscle hypertrophy and remodelling (Damas et al., 2016), the free-living nature of the ^2H_2O measurement may have masked the well-established increase in myofibrillar protein synthesis in the hours following resistance exercise (Burd et al., 2010a; Kumar et al., 2012).

An alternative explanation could be related to familiarizing participants with resistance exercise prior to the study. During the screening visit, participants completed a high volume familiarization bout. Given that Damas and colleagues demonstrated that the 48-hour myofibrillar protein synthetic response following resistance exercise is no longer different from resting values once participants have been familiarized with resistance exercise, this may explain the undetectable increase in daily myofibrillar protein synthesis rates in the present study (Damas et al., 2016). A final possibility is that factors known to influence muscle protein synthesis rates (e.g., sleep (Saner et al., 2020) and energy balance (Areta et al., 2014)) could have differed during the basal period and the exercise period and thus could, in part, explain the lack of an exercise effect. It must be acknowledged that the inability to detect an increase in daily myofibrillar protein synthesis rates in response to resistance exercise training may also have precluded differences from being detected between low frequency and high frequency resistance exercise training.

As muscle protein synthesis is partly regulated by translational capacity (i.e., ribosomal biogenesis) (Figueiredo & McCarthy, 2019), a secondary aim was to assess whether resistance exercise training frequency impacts the phosphorylation status and total protein content of selected proteins implicated in skeletal muscle ribosomal biogenesis **(Figure 4)**. Transcription of ribosomal DNA (rDNA) requires the activation of eIF4E and cyclin D1 which can subsequently activate a number of transcription factors including UBF which forms part of the pre-initiation complex (Figueiredo & McCarthy, 2019). In line with previous findings (Figueiredo et al., 2016), there was a tendency (P=0.056) for total eIF4E protein content **(Figure 4A)** to increase 48 hours following the initial bouts of LF and HF resistance exercise training. Consistent with the finding that resistance exercise training frequency had no impact on daily myofibrillar protein synthesis rates, no differences were observed at any time point for any marker of skeletal ribosomal biogenesis between LF and HF resistance exercise training **(Figure 4)**. However, it should be acknowledged that skeletal muscle ribosomal biogenesis is activated at multiple time points following resistance exercise (Figueiredo et al., 2016) and thus it is possible that biopsy timing, primarily intended to assess myofibrillar protein synthesis rates, missed differences that may have occurred at earlier time points.

Although total exercise volume was intentionally matched to isolate the impact of resistance exercise training frequency *per se* on daily myofibrillar protein synthesis rates, it should be considered that higher resistance exercise training frequencies can be used effectively to increase overall exercise volume for a given muscle group (Barcelos et al., 2018). Indeed, under non-volume equated conditions, higher resistance exercise training frequencies have been associated with greater gains in muscle mass (Schoenfeld et al., 2018) and strength (Grgic et al., 2018). From a practical standpoint, high resistance exercise training frequency may be considered a useful means of achieving a given exercise training volume, particularly when time is a limiting factor.

It is also important to note that any change in muscle mass is ultimately determined by the overall protein balance between muscle protein synthesis and breakdown. Whilst the absence of a measure of muscle protein breakdown may be considered a limitation of the present investigation, the myofibrillar protein synthesis measurements made in the present study align well with the general finding that volume-matched resistance exercise training frequency has no impact on muscle hypertrophy (Schoenfeld et al., 2018). Finally, this study was conducted in individuals unaccustomed to regular lower limb resistance exercise, but it is possible that higher resistance exercise frequencies could be of greater benefit to more resistance-trained individuals as has been suggested previously (Dankel et al., 2017).

In conclusion, under the conditions of the present study, resistance exercise training frequency does not modulate daily myofibrillar protein synthesis rates or the phosphorylation status and total protein content of selected proteins implicated in skeletal muscle ribosomal biogenesis in young men. These findings suggest that for a given exercise volume, resistance exercise training frequency has little impact on skeletal muscle hypertrophy.

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AUTHOR CONTRIBUTIONS

B.J.S., J.L.T., A.M.H., L.J.C.v.L., and G.A.W. conception and design of research; B.J.S., J.M., Y.S.E., L.B., and G.A.W. performed experiments; B.J.S., J.M., and A.M.H. analysed samples; B.J.S., and G.A.W. prepared figures and drafted manuscript; B.J.S., J.L.T., J.M., A.M.H., Y.S.E., L.B., L.J.C.v.L., and G.A.W. edited and revised manuscript; B.J.S., J.L.T., J.M., A.M.H., Y.S.E., L.B., L.J.C.v.L., and G.A.W. approved final version of manuscript.

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CONFLICTS OF INTEREST

None of the authors have any conflicts of interest or financial disclosures to declare.

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Table 1. Participants' characteristics at baseline

Variable	Value
Age (y)	21.0 ± 2.3
Height (m)	1.79 ± 0.07
Body mass (kg)	72.4 ± 7.1
BMI ($kg \cdot m^{-2}$)	22.7 ± 2.6
LF leg press 1RM (kg)	104 ± 22
HF leg press 1RM (kg)	106 ± 22
LF leg extension 1RM (kg)	82 ± 11
HF leg extension 1RM (kg)	81 ± 12

Values are mean±SD. n=9. BMI, body mass index; 1RM, one repetition maximum; LF, low frequency; HF, high frequency.

Table 2. Daily step count and dietary intake during the seven-day period of habitual physical activity (BASAL) and seven-day period of low frequency (LF) and high frequency (HF) resistance exercise

Variable	BASAL	LF/HF	*P* Value
Daily step count	10000 ± 2420	11458 ± 1871	0.167
Energy intake (kcal·d^{-1})	2253 ± 316	2336 ± 208	0.477
Protein (g·kg^{-1}·d^{-1})	1.3 ± 0.4	1.5 ± 0.2	0.089
Protein intake (g·d^{-1})	93 ± 25	104 ± 15	0.061
Carbohydrate intake (g·d^{-1})	278 ± 53	280 ± 43	0.931
Fat intake (g·d^{-1})	82 ± 12	82 ± 8	0.906
Protein (En%)	16 ± 5	18 ± 2	0.402
Carbohydrate (En%)	51 ± 7	52 ± 4	0.602
Fat (En%)	32 ± 3	30 ± 4*	0.041

Values are mean±SD. n=9. *($P<0.05$) indicates a significant difference between BASAL and LF/HF conditions.

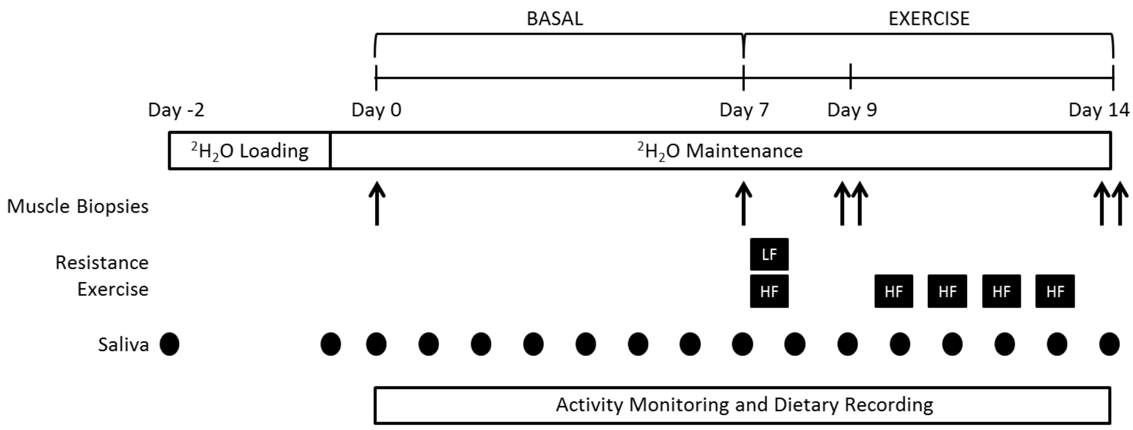
FIGURE LEGENDS

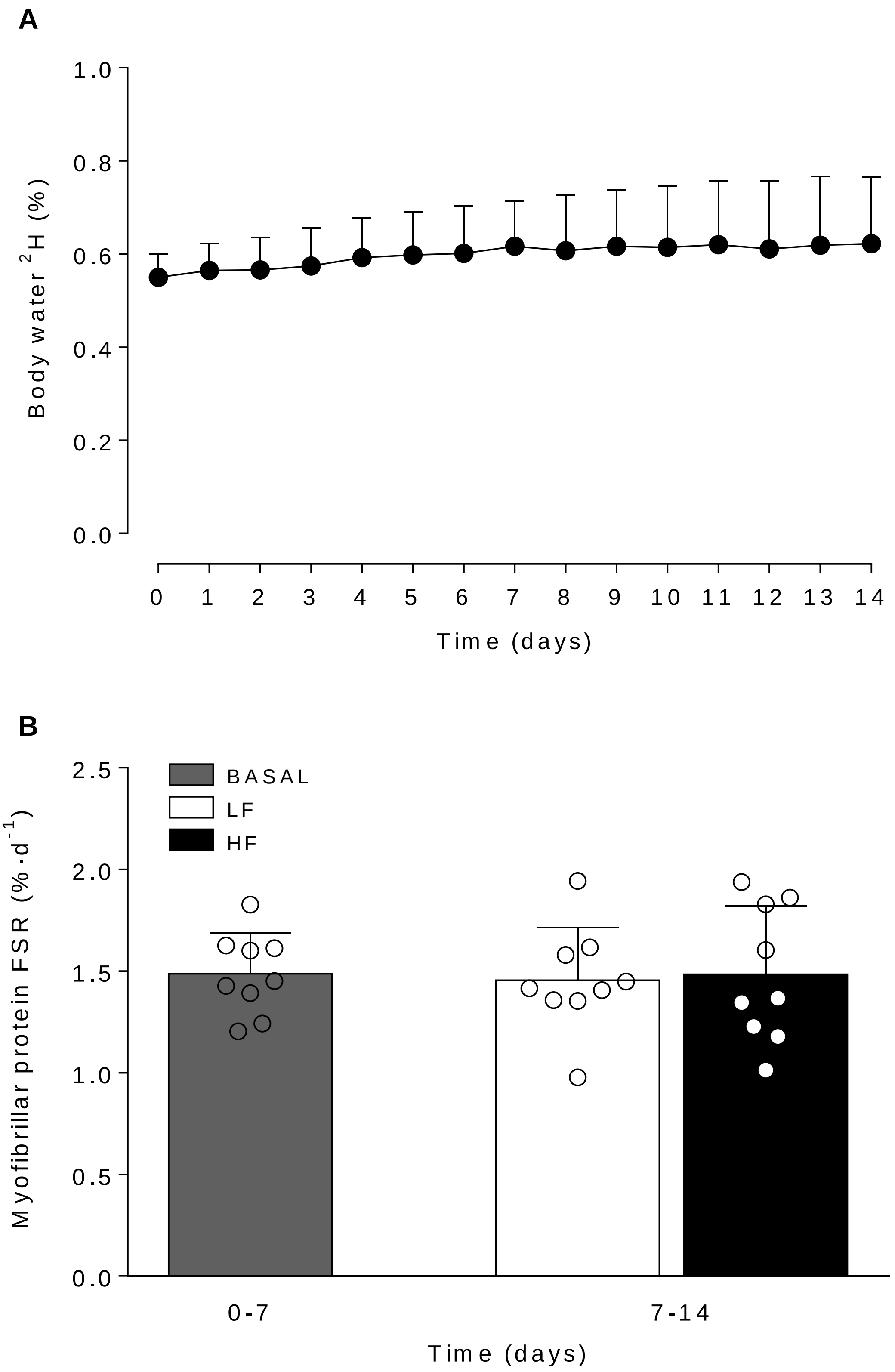
Figure 1. Study overview.

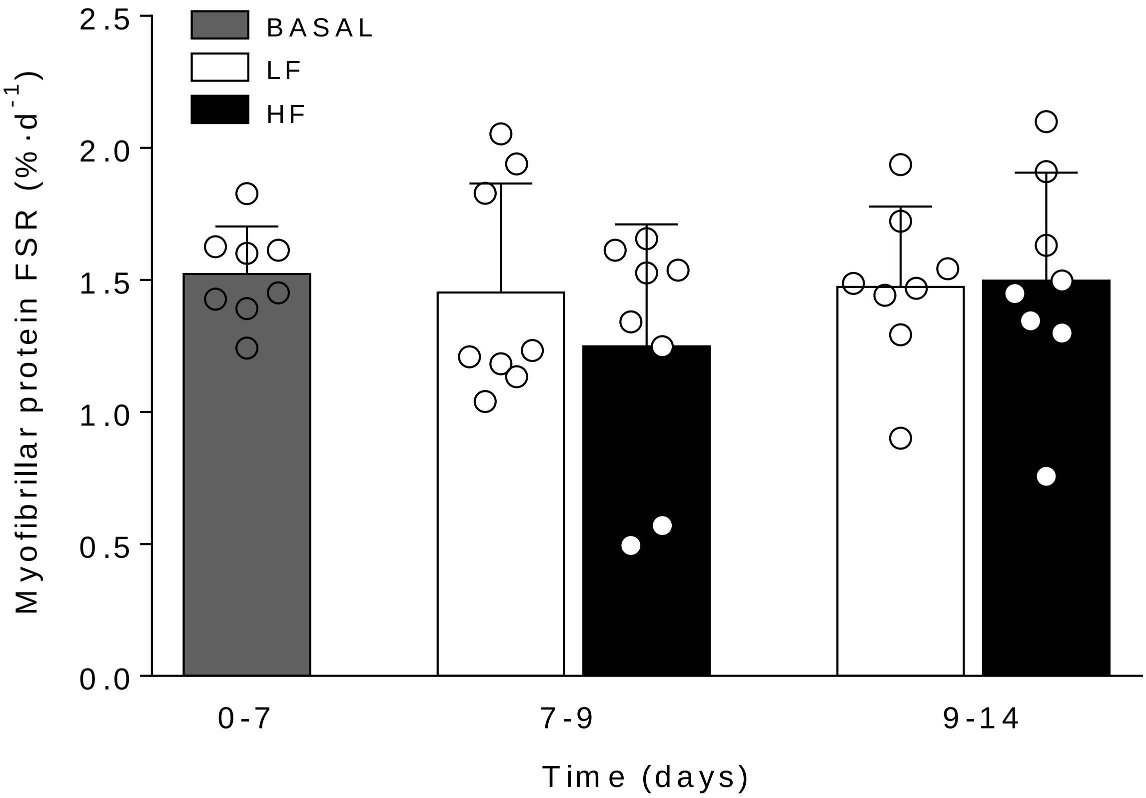
Figure 2. Body water ^{2}H enrichment and daily myofibrillar protein fractional synthesis rates (FSR) during a seven-day period of habitual physical activity (BASAL) and a seven-day period of low frequency (LF) and high frequency (HF) resistance exercise (n=9). Data are displayed as mean±SD with participants' individual FSR

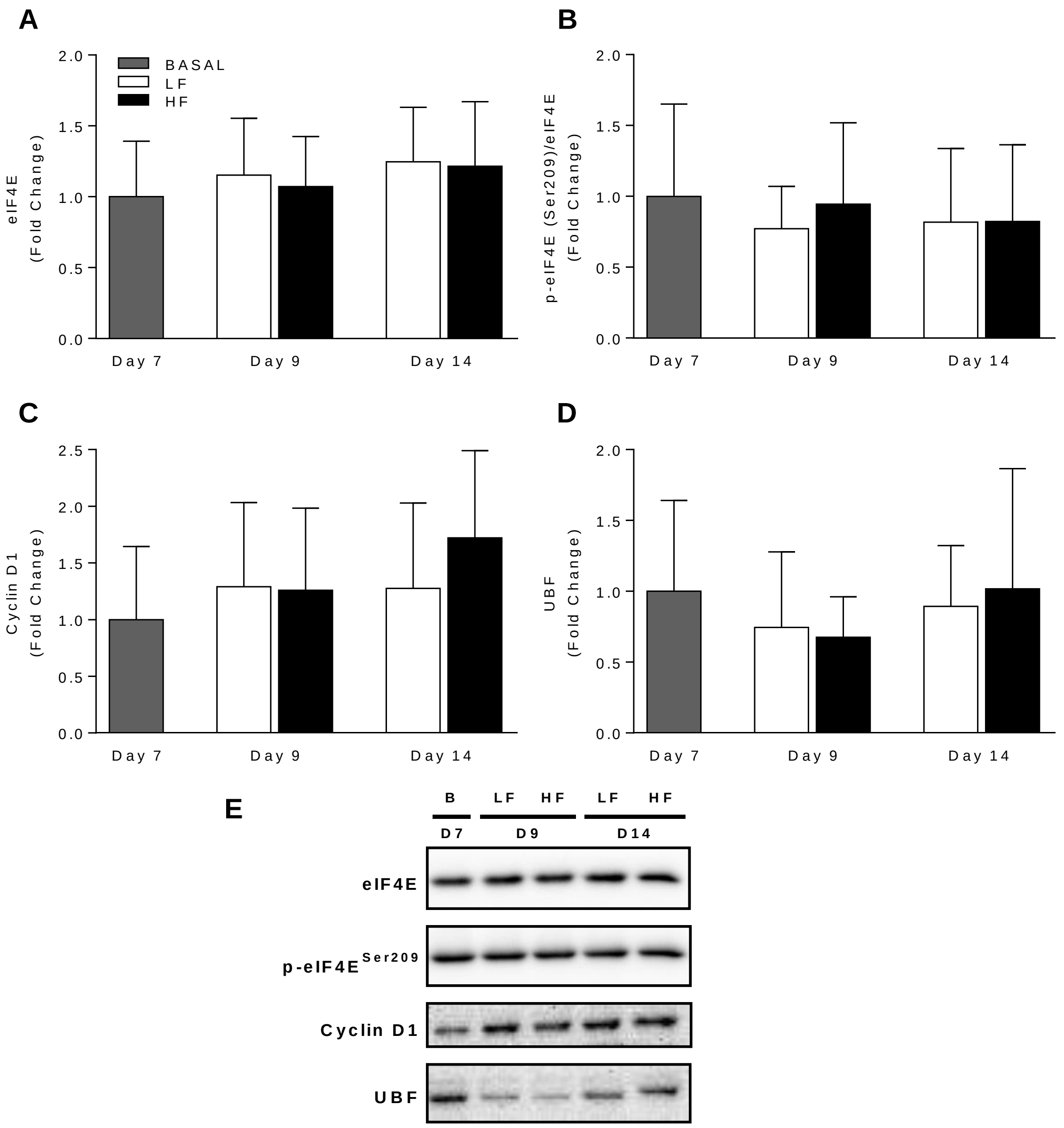
Figure 3. Daily myofibrillar protein fractional synthesis rates (FSR) during a seven-day period of habitual physical activity (BASAL) and a seven-day period of low frequency (LF) and high frequency (HF) resistance exercise (n=8). Data are displayed as mean±SD with participants' individual FSR

Figure 4. Impact of low frequency (LF) and high frequency (HF) resistance exercise on total protein content of eukaryotic translation initiation factor 4E (eIF4E; A), phosphorylation of eIF4E at Ser209 (B), total protein content of cyclin D1 (C) and total protein content of upstream binding factor (UBF; D) (n=8). Data are mean±SD.


BASAL
EXERCISE
Day -2
Day 0
Day 7
Day 9
Day 14
^2H_2O Loading
^2H_2O Maintenance
Muscle Biopsies
Resistance
Exercise
LF
HF
HF
HF
HF
HF
Saliva
Activity Monitoring and Dietary Recording


A
1.0
0.8
0.6
0.4
0.2
0.0
Body water 2H (%)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Time (days)
B
2.5
2.0
1.5
1.0
0.5
0.0
Myofibrillar protein FSR (%·d-1)
BASAL
LF
HF
0-7
7-14
Time (days)


BASAL
LF
HF
Myofibrillar protein FSR (%·d-1)
2.5
2.0
1.5
1.0
0.5
0.0
0-7
7-9
9-14
Time (days)


A
B
C
D
E
BASAL
LF
HF
eIF4E
(Fold Change)
p-eIF4E (Ser209)/eIF4E
(Fold Change)
Cyclin D1
(Fold Change)
UBF
(Fold Change)
0.0
0.5
1.0
1.5
2.0
2.5
Day 7
Day 9
Day 14
B
LF
HF
LF
HF
D7
D9
D14
eIF4E
p-eIF4E Ser209
Cyclin D1
UBF