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ORIGINAL ARTICLE

Effects of divergent resistance exercise contraction mode and dietary supplementation type on anabolic signalling, muscle protein synthesis and muscle hypertrophy

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Abstract Greater force produced with eccentric (ECC) compared to concentric (CONC) contractions, may comprise a stronger driver of muscle growth, which may be further augmented by protein supplementation. We investigated the effect of differentiated contraction mode with either whey protein hydrolysate and carbohydrate (WPH + CHO) or isocaloric carbohydrate (CHO) supplementation on regulation of anabolic signalling, muscle protein synthesis (MPS) and muscle hypertrophy. Twenty-four human participants performed unilateral isolated maximal ECC versus CONC contractions during exercise habituation, single-bout exercise and 12 weeks of training combined with WPH + CHO or CHO supplements. In the exercise-habituated state, p-mTOR, p-p70S6K, p-rpS6 increased by approximately 42, 206 and 213 %, respectively, at 1 h post-exercise, with resistance exercise per se; whereas, the phosphorylation was exclusively maintained with ECC at 3 and 5 h post-exercise. This acute anabolic signalling response did not differ between the isocaloric supplement types, neither did protein fractional synthesis rate differ between interventions. Twelve weeks of ECC as well as CONC resistance training augmented hypertrophy with WPH + CHO group compared to the CHO group (7.3 ± 1.0 versus 3.4 ± 0.8 %), independently of exercise contraction type. Training did not produce major changes in basal levels of Akt-mTOR pathway components. In conclusion, maximal ECC contraction mode may constitute a superior driver of acute anabolic signalling that may not be mirrored in the muscle protein synthesis rate. Furthermore, with prolonged high-volume resistance training, contraction mode seems less influential on the magnitude of muscle hypertrophy, whereas protein and carbohydrate supplementation augments muscle hypertrophy as compared to isocaloric carbohydrate supplementation.

Keywords Whey protein · Hypertrophy signalling · Eccentric/concentric exercise

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Introduction

Resistance exercise and protein supplementation constitute separate ostensibly potent stimulators of muscle protein synthesis (MPS). Accordingly, resistance exercise in the protein-fed state augments MPS during the immediate post-exercise recovery (Phillips et al. 1997; Rasmussen and Phillips 2003) with protein high in branched chain amino acids (BCAA) and especially leucine being particularly capable of augmenting the positive effect of resistance exercise on MPS (Churchward-Venne et al. 2012;

Pennings et al. 2011). It has been proposed that increased MPS after resistance exercise is reliant on upstream activation of the mammalian target of rapamycin (mTOR)-complex 1 (mTORC1), including activation of its targets 4E binding protein 1 (4EBP1) and the 70-kDa ribosomal protein S6 kinase (p70S6K) (Fujita et al. 2007). mTOR itself is believed to constitute a nodal point integrating several types of stimuli, including hormones (Menon et al. 2014), essential amino acids (Dibble and Manning 2013; Dickinson et al. 2011; Jewell and Guan 2013; Kakigi et al. 2014), and/or mechanotransduction (Hornberger 2011; O'Neil et al. 2009; Spangenburg et al. 2008).

With regard to resistance exercise, muscle contraction mode performed during resistance exercise constitutes a factor that may differentially stimulate MPS and mTOR signalling. Accordingly, based on the ability to develop higher force during ECC exercise as compared to CONC (Roig et al. 2009), isolated eccentric resistance exercise (ECC) has been suggested to provide a stronger stimulus of MPS than isolated concentric resistance (CONC). However, very little information exists on this issue. In one elegant study by Eliasson and co-workers (2006), maximal eccentric, but not maximal concentric or submaximal eccentric contractions lead to an augmented mTOR pathway signalling (Eliasson et al. 2006). This study was performed on fasted individuals, however, and did not include measures of MPS or investigation on the effects of prolonged training responses. Other studies have reported, that when work-matched isolated ECC versus CONC resistance exercise is performed in the protein-fed state, ECC contractions produce a more rapid rise in myofibrillar MPS during the early, but not later hours, as compared to CONC contractions (Cuthbertson et al. 2006; Moore et al. 2005). Accordingly, some information exists that favours the view that ECC contractions constitute a stronger initiator of MPS and perhaps muscle hypertrophy. Yet, comparison of short-term effects between maximal ECC and CONC contractions (i.e. taking advantage of the different force-potential inherent of the two exercise modalities) in the fed state has not been conducted. Furthermore, previous studies have not included both investigation on both the acute responses to single-bout ECC versus CONC exercise and accumulated training responses with prolonged ECC versus CONC training.

With regard to supplementation, nutrients such as glucose and protein that cause an insulin response (Gannon and Nuttall 2010) can directly activate mTOR through PI3K-Akt signalling (Nave et al. 1999) and/or indirectly activate mTOR by inhibiting the tuberous sclerosis 2 (TSC2) (Latres et al. 2005; Proud 2014). The essential amino acid, leucine, has received special attention, since it has been demonstrated to augment MPS synthesis independently of all other amino acids in rats (Anthony et al. 2000a, b; Crozier et al. 2005) and to constitute an important stimulator of the mTOR pathway (Churchward-Venne et al. 2012; Moberg et al. 2014). It has been shown that leucine is capable of increasing MPS in humans even when the background total protein intake is low (Churchward-Venne et al. 2014, 2012). Accordingly, nutrients that stimulate insulin secretion through, e.g. increased plasma glucose or essential amino acid levels (Gannon and Nuttall 2010), may take partly different paths and/or exert different impact on activation on mTOR signalling and perhaps protein synthesis. Commercially available ergogenic supplements are often based on either carbohydrate (CHO), or a combination of CHO and protein. However, short- and long-term effects of such divergent available supplementation types on resistance exercise-induced muscle accretion, are neither well defined nor comprised in one and same study.

To investigate these aspects, in a human study, we employed a comparative paired design. Accordingly, we investigated the acute exercise effects as well as the accumulated training effects on muscle hypertrophy, to isolated unilateral maximal ECC versus CONC contractions with either whey protein hydrolysate + carbohydrate (WPH + CHO) or isocaloric carbohydrate (CHO) supplementation.

Measurements were conducted before and after: (a) a short period of preliminary ECC versus CONC resistance exercise habituation; (b) single-bout ECC versus CONC resistance exercise, and; (c) 12 weeks of ECC versus CONC resistance training.

We hypothesized (1) that in the exercise-habituated state, single-bout ECC contractions would augment MPS and mTOR signalling compared to CONC; (2) that WPH + CHO would augment MPS and mTOR signalling compared isocaloric CHO supplement; (3) that ECC and WPH + CHO combined would comprise a stronger stimulus than CONC and CHO to increase muscle hypertrophy and post-training basal levels of proteins involved in the mTOR signalling pathway after 12 weeks of intervention.

Materials and methods

Participants

Twenty-four young healthy recreationally active men (height, 1.82 ± 0.015 m; weight, 78.1 ± 1.8 kg; age, 23.9 ± 0.8 years; fat %, 16 ± 0.9 %) volunteered to participate in the study. All participants were informed about the purpose and the risks related to the study and gave written, informed consent to participate. The study was approved by The Central Denmark Region Committees on Health Research Ethics (j. no. M-20110003) and performed in accordance with the Declaration of Helsinki. Exclusion criteria were: (1) participation in systematic resistance or

high-intensity training (more than one session per 14 days) for lower extremity muscles 6 months prior to inclusion in the study; (2) history of lower extremity musculoskeletal injuries; and (3) vegan diet or use of dietary supplements or prescription medicine that would potentially affect muscle size.

Study design

The entire study comprised three parts: (1) a short period of exercise habituation; (2) a single-bout resistance exercise trial to investigate the acute phase exercise responses, and; (3) a 12-week training period to investigate accumulated resistance exercise responses. Following inclusion, participants were randomly allocated into either the WPH + CHO group ($n = 12$) or the isocaloric CHO group ($n = 12$). The ECC exercise leg was randomly chosen to be either the dominant (preferred kicking leg) or the nondominant leg to exclude any potential pre-training difference between the two legs. The study was conducted in a double blinded fashion in relation to dietary supplementation, so that the participants, the exercise supervisors and the staff during biopsy sampling were blinded with regard to supplement group. All later analyses were conducted by an investigator blinded with regard to interventions (supplementation and contraction type). Twenty-four participants completed the habituation and acute trials, five participants dropped out before the training period. Three additional participants were recruited to the training period only, i.e. 22 participants completed the training period. All participants performed eccentric work (ECC) with one leg and concentric work (CONC) with the other leg. Eccentric exercise was randomly ascribed to either the dominant (preferred kicking leg) or the nondominant leg to exclude any potential pre-training differences between the legs. In addition, the effects of a WPH + CHO supplementation versus isocaloric CHO supplementation were investigated in the single-bout trial and the training period. The participants were randomly divided into one of the two supplement groups and supplements were provided in a double blinded fashion. This within-participant design with regard to contraction mode was used to minimize the potential differences in the training response that are inherent with group designs because of differences in initial training, nutritional and hormonal status. Accordingly, the following four interventions were compared: (I) ECC with WPH + CHO; (II) ECC with CHO; (III) CONC with WPH + CHO, and; (IV) CONC and CHO. Throughout the study period, the participants were instructed to maintain their normal habitual physical activity level and dietary intake. Three days prior to the start of the study, the participants were asked to refrain from physical exercise. One basal muscle biopsy was then sampled from a randomly chosen leg. Three days after the muscle biopsy, an exercise-habituation period was commenced consisting of three habituation sessions conducted during the subsequent 7 days (shown in Fig. 1a). The participants completed ECC and CONC exercise on alternate legs using the same protocol as for to the single-bout exercise trial. Three days after the end of the exercise-habituation period, muscle biopsies were sampled from both legs after an overnight fast and 30 min of rest in the supine position. A dual-energy X-ray absorptiometry (DEXA) scan (QDR-2000, Hologic Discovery; Hologic, Bedford, MA, USA) was performed prior to the single-bout study day to determine the lean body mass (LBM) of the participants. Three days after sampling of the post-exercise-habituation biopsies, the participants completed the acute single-bout exercise trial with a stable isotope infusion (see Fig. 1b). Following the single-bout trial, the participants commenced a 12-week training period. A final (post-training) biopsy was sampled from ECC and CONC trained legs 3–6 days after the last exercise session of the training period.

Exercise protocol

All three exercise-habituation sessions and the single-bout exercise session were conducted in an isokinetic dynamometer (Humac Norm, CSMi Medical Solutions, Stoughton, MA, USA) guided and assisted by instructors. Participants were seated in the isokinetic dynamometer with 90° hip flexion and restraining straps holding back the torso and the passive leg. The transverse axis of the participant's knee was aligned with the axis of the dynamometer. One leg was placed behind a stabilization bar while the other leg was attached to the dynamometer arm to perform either ECC or CONC contractions. An exercise session consisted of 6 sets of 10 maximal ECC contractions for one leg and 6 sets of 10 maximal CONC repetitions for the other leg. The angular velocity of the dynamometer was 30°/s, and sets were interspaced by 1 min of recovery. All sets were completed using the same leg before switching to the other leg. Participants were verbally encouraged to perform maximal voluntary contractions throughout the training. The three habituation sessions were performed on separate days interspaced by at least 48 h.

Single-bout exercise protocol

The acute single-bout protocol is shown in Fig. 1b. The participants were instructed to abstain from physical activity 3 days prior to the single-bout trial. On the morning of the trial day, the participants arrived at the laboratory at 8.00 am after an overnight fast (10 h). They were then rested in supine position for 30 min. A catheter was then inserted into an antecubital vein of each forearm; one used for tracer

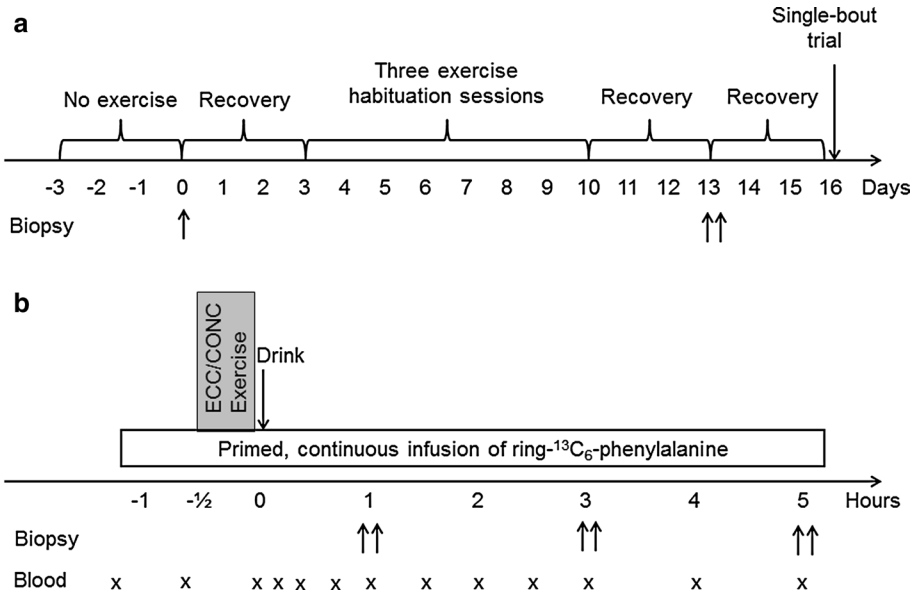

Fig. 1 Overview of exercise habituation and single-bout exercise protocols. **a** The exercise-habituation protocol period included 3 exercise sessions during a 7-day period, with at least 48 h interspacing each exercise session. Biopsies were sampled from one leg prior to exercise-habituation and from both the ECC and the CONC leg following habituation. Participants were instructed to refrain from exercise 3 days prior to all biopsies. Biopsies were followed by 3 days of recovery. Exercise was conducted as 6 sets of 10 maximal isolated eccentric or concentric contractions of the knee extensor muscles. 1 min of recovery interspaced sets. **b** The single-bout exercise protocol included an single exercise bout identical to the exercise bouts performed during the exercise habituation. Immediately after exercise, a protein (WPH + CHO) or a placebo (CHO) supplement was ingested (0 h). Blood samples and muscle biopsies were sampled at selected time points during the post-exercise recovery to allow analysis of protein phosphorylation and measurements of the myofibrillar fractional synthesis rate (FSR) by tracer (ring-$^{13}C_6$-phenylalanine) technique

infusion and the other used for collection of blood samples. A background blood sample was obtained and a primed [8 μmol/kg lean body mass (LBM)], constant (8 μmol/kg LBM/h) infusion of L-[ring-$^{13}C_6$]phenylalanine (Cambridge Isotope Laboratories, Andover, MA, USA) was initiated with a target tracer-to-tracee ratio of 12.5 % in arterial blood. Tracer solutions were made by the local dispensary under sterile conditions and tested to be free of bacteria and pyrogens before use. Participants rested in the supine position for 30 min after tracer infusion was initiated. A blood sample was collected and an exercise session identical to the exercise-habituation sessions was then conducted. Immediately after termination of exercise, a blood sample was collected and the participants ingested the assigned supplement (described below). The supplement was consumed within ~1 min and complete supplement ingestion was designated as time zero (0 min). The participants then rested during the subsequent 5 h of post-exercise recovery and only ingestion of water ad libitum was allowed. Blood samples were collected at time points: 0, 10, 20, 45, 60, 90, 120, 150, 180, 240 and 300 min for determination of $^{13}C_6$-phenylalanine enrichments. Blood samples for measurement of serum glucose and insulin concentrations were collected at: pre, 0, 1, and 3 h. Muscle biopsies were obtained from both legs at time points 1, 3 and 5 h after drink ingestion, providing two postprandial intervals for fractional synthesis rate (FSR) measures; 1–3, and 3–5 h. To control for potential confounding effects of circadian rhythm, all participants strictly adhered to the absolute daily time points and time resolution of the protocol.

Training protocol

Each participant completed 2–3 exercise sessions per week over a 12-week period to a total of 33 training sessions. All exercise sessions were initiated with 5 min light bicycling warm-up. Eccentric load was aimed at 120 % relative to concentric loading, with a training supervisor assisting to allow isolated exercise modality of the two legs. The load difference is parallel to the approximate strength difference between slow ECC and CONC contractions during isokinetic strength testing (Aagaard et al. 2000). Both the ECC and CONC leg training programmes consisted of isotonic knee extensions [repetition loading equal to repetition maximum (RM)] with the following set × repetitions; 6 × 10–15 RM (sessions 1–4), 8 × 10–15 RM (sessions 5–10), 10 × 10–15 RM (sessions 11–20), 12 × 6–10 RM (sessions 21–28), and 8 × 6–10 RM (sessions 29–33) (Technogym-Selection line, Technogym, Italy). Participants were instructed to perform each repetition in a controlled

manner (2 s tempo) during both the CONC and the ECC phase of the exercise. Two minutes of recovery were interspaced between sets and all training sessions were closely supervised and monitored by qualified training instructors to ensure proper execution and loading.

Supplementation

On the single-bout trial day, the participants received a 500-mL beverage immediately after completion of the exercise session. Participants in the WPH + CHO group ingested a beverage consisting of 0.30 g WPH + 0.30 g CHO/kg lean body mass, while participants of the CHO group ingested an isocaloric solution consisting of 0.60 g CHO/kg lean body mass. In the specific participants randomized into the two groups, this ended up with a WHP and CHO content of: (mean ± SEM) WHP-group, 18.2 ± 0.4 g whey protein hydrolysate and 18.2 ± 0.4 g carbohydrate and CHO group, 35.9 ± 0.9 g carbohydrate, with no difference in calories provided to the two supplement groups. The supplements were diluted in artificially flavoured water. All WPH + CHO-beverages were added L-[ring-$^{13}C_6$]-phenylalanine to reach a tracer-to-tracee ratio of 12.5 %. The BCAA content of the whey protein supplement (produced by Arla Foods Ingredients P/S, Viby J., Denmark) was 27.7 % (leucine 14.2 %, isoleucine 6.6 %, valine 6.9 %), which is considered high compared to standard milk-based whey protein sources (Hulmi et al. 2010). During the 12-week training period following the single-bout trial, the participants in both groups received a fixed amount of the respective supplements on all training days. Each drink consisted of an 8 % solution (663 kJ); the WPH + CHO drink consisted of 19.5 g whey protein hydrolysate (produced by Arla Foods Ingredients Group P/S, Viby J., Denmark) + 19.5 g of carbohydrate (both equal to 4 % solution) and the isocaloric placebo drink consisted of 39 g of carbohydrate. Half of the solution was ingested before each training session and the other half was ingested immediately after. The participants were instructed not to eat or drink anything calorie-containing for 90 min prior to and 60 min after the exercise sessions throughout the 12-week training period.

Muscle biopsies

Muscle biopsies were obtained from separate incisions in the middle section of the m. vastus lateralis of the quadriceps femoris using a Bergström needle with manual suction. A 5- to 7-mm incision was made using local anaesthesia (Lidokain: Amgros, Copenhagen, Denmark) and sterile conditions. It was attempted to sample all biopsies at similar depth of the muscle and incision holes were at least 3 cm apart. The muscle tissue was immediately quickly dissected free of visible fat and connective tissue. The muscle specimens assigned for tracer incorporation analysis were thoroughly wiped clean from blood on gauze wetted in ice-cold saline. All muscle samples were snap frozen in liquid nitrogen following preparation and stored at −80 °C until further analysis.

Serum insulin and glucose analyses

Venous blood samples were collected in 14-mL tubes, cooled for 1 h and centrifuged (3,000*g* at 4 °C for 10 min), and the serum phase was then stored at −80 °C until further analysis. From serum samples insulin and glucose concentrations were measured: insulin by using time-resolved fluoroimmunoassays (TF-IFMA; AutoDELFIA, PerkinElmer, Turku, Finland) and glucose by measuring duplicates on an YSI 2700 Select (YSI life sciences, Yellow springs, OH, USA).

Tracer sample preparation

From serum samples free ring-$^{13}C_6$-phenylalanine enrichment was measured. Briefly, 50 μL serum was acidified with 1 mL 50 % acidic acid before poured over 1 mL slurry of resin (Dowex AG50 W-X8 resin, BioRad, Copenhagen, Denmark). After multiple washes with demineralised water to obtain neutral pH, the amino acids were eluted by adding two times 1 mL 2 M NH_4OH. The NH_4OH was evaporated under a stream of nitrogen at 70° before amino acids were derivatized. The muscle free ring-$^{13}C_6$-phenylalanine enrichment was isolated from a ~15 mg wet weight muscle specimen by repeating the following homogenisation procedure three times: 2 × 15 s at speed 4 (FastPrep 120A-230; Thermo Savant, Holbrook, NY, USA) in 1.0 mL 2 % perchloric acid (PCA), centrifugation at 3,060*g* for 10 min at 4 °C, and saving the supernatant. The 3 mL of pooled supernatant was poured over acidified resin columns and amino acids purified as explained for serum samples. The serum and muscle free amino acids were derivatized with heptafluorobutyric anhydride (HBFA) using ethyl acetate as solvent. Briefly, purified amino acids from serum and muscle specimen samples were added 50 μL of 4 M HCl in methanol and left at 85 °C for 60 min. Hereafter centrifuged 5 min at 1,810*g* and subsequently dried down under a stream of nitrogen without heat. To the dried sample, 50 μL HFBA (Sigma-Aldrich, Steinheim, Switzerland) was added and left at 85 °C for 45 min, centrifuged 5 min at 1,820*g*, and subsequently dried down under a stream of nitrogen without heat. The sample was then resuspended in 50 μL of ethyl acetate and analysed within a couple of days. The myofibrillar protein-bound ring-$^{13}C_6$-phenylalanine abundances were determined from separate muscle specimens. 15–20 mg wet weight muscle specimens were

homogenized (FastPrep 120A-230; Thermo Savant, Holbrook, NY, USA) for 4 × 15 s, speed 4, in 1.0 mL buffer (NaCl 0.15 M, TritonX-100 0.5 %, EDTA 2 mM, EGTA 2 mM, sucrose 0.25 M, Tris 0.02 M, pH 7.4), left 5 h at 4 °C, and centrifuged at 800*g* for 20 min at 4 °C. The pellet, containing the structural proteins, was homogenized once more in 1 mL buffer as above, left for 1 h, and centrifuged at 800*g* for 20 min at 4 °C. The supernatant was discarded and to the pellet added 1.5 mL buffer (KCl 0.7 M and pyrophosphate 0.1 M), and left overnight at 4 °C. The day after, the samples were centrifuged (1,600*g*, 20 min, 4 °C). The supernatant containing the myofibrillar proteins was added 2.3× buffer volume ice-cold ethanol (99 %), vortexed, left for 2 h at 4 °C, and hereafter centrifuged (1,600*g*, 20 min, 4 °C) and the supernatant discarded. The pellet was washed once with 1 mL of 70 % ethanol before hydrolysed at 110 °C in 6 M HCl overnight. The constituent amino acids were purified over resin columns as described above and amino acids were derivatised as the *N*-acetyl-propyl (NAP) derivative.

Tracer analyses

The ^{13}C-enrichments of the HFBA-derivatives of phenylalanine were determined in both serum and muscle free pools using tandem mass spectrometry using a Thermo Scientific, TSQ Quantum GC–MS/MS (San Jose, CA, USA) under electron ionization mode. The derivatives were separated on a Varian CP-Sil 8 CB capillary column (30 m, id 0.32 mm, coating 0.25 um) using PTV mode injection. The enrichments were measured by MS/MS by monitoring the fragments of the parent ions of *m/z* [161–172] in the neutral loss mode [−31]. The protein-bound ring-$^{13}C_6$-phenylalanine abundances in the myofibrillar fraction were determined on a GC-combustion-isotope ratio MS (Delta Plus XL 6890, Thermo Finnigan, Bremen, Germany). Prior to combustion, the NAP-derivatised phenylalanine was isolated in the GC on an Agilent J&W capillary column (CP-Sil 19 CB 60 m, id 0.25 mm, coating 0.25 um). During combustion, the phenylalanine was converted to CO_2 and the presence of ^{13}C was measured as the $^{13}CO_2$ abundance in the sample compared to a known reference gas, reported as the delta-value in ‰, which was subsequently converted to an actual isotope ratio.

Western blotting

The Western blotting procedure has been described in detail previously (Moller et al. 2013). In short, the frozen muscle biopsies (approximately 30 mg) were minced between nitrogen-cooled pistons and homogenized in an ice-cold buffer (Moller et al. 2013). Samples were rotated for 15 min at 4 °C and insoluble materials were centrifuged off at 14,000×*g* for 20 min at 4 °C and protein concentration of the supernatant was determined using a Bradford assay (Bio-Rad, California, USA). Aliquots of protein were resolved by SDS-PAGE on precast StainFree 4–15 % gels (Bio-Rad) and electroblotted onto PVDF membranes (Bio-Rad). Control for equal loading was performed using the stain-free technology (Gurtler et al. 2013). Membranes were incubated overnight with primary antibodies. Membranes were initially incubated in phosphor-specific antibodies. Subsequently, the antibodies were stripped off (Moller et al. 2013), and the membranes were reblocked and re-incubated in antibodies against epitopes on sites not modified by phosphorylations. Antibodies against mTOR (Cat. no. 2972), phospho-specific mTOR (Ser^{2448}) (Cat. no. 2971), Akt (pan) (Cat. no. 4691), phospho-specific Akt (Ser^{473}) (Cat. no. 9271), eIF-4E (Cat. no. 9742), phospho-specific eIF-4E (Ser^{209}) (Cat. no. 9741), 4E-BP1 (Cat. no. 9644), non-phospho-specific 4E-BP1 (Thr^{46}) (Cat. no. 4923), p70 S6 kinase (Cat. no. 9202), phospho-specific p70 S6 kinase (Thr^{389}) (Cat. no. 9205), phospho-specific ribosomal protein S6 (Ser 235/236) (Cat. no. 4858), phospho-specific ACC (Ser79) (Cat. no. 3661) were purchased from Cell Signalling Technology (Danvers, MA, USA). Horseradish peroxidase-conjugated goat anti-rabbit antibody (Invitrogen) was used as secondary antibody. Proteins were visualized by chemiluminiscence (Thermo Scientific, MA, USA) and quantified with ChemiDoc™ MP imaging system (Bio-Rad). Precision Plus Protein All Blue standards was used as markers of molecular weight (Bio-Rad).

Muscle cross-sectional area

The cross-sectional area (CSA) of both thigh muscles on each participant was measured by magnetic resonance imaging (MRI) before and after the 12-week training period and has previously been described in detail by Farup et al. (2013). In brief, MRI measurements of thigh muscle were performed with a 1.5-T MRI scanner (Philips Achieva, Best, the Netherlands). A minimum of 48–72 h was interspaced between the last training session and MRI scanning to minimize the risk of fluid shifts and participants were seated in a resting position for 30–45 min before entering the scanner. The MRI scans were performed on both legs. Offline analyses were performed using Osirix, 4.1.1 (Osirix Foundation, Geneva, Switzerland). A T1-weighted, fast spin echo sequence with the following parameters was used: scan matrix = 576 × 576, field of view = 46 × 46 cm, number of slices = 50, slice thickness = 7 mm, slice gap = 3 mm, repetition time = 2 s, echo train length = 18, number of signal averages = 2, TR = 500 ms, TE = 6.2 ms, and pixel size = 0.8 × 0.8 mm. From the frontal and transverse scans, the femur length was calculated and the knee extensor muscle CSA (mm. vastus

lateralis, vastus medialis, vastus intermedius, and rectus femoris) was manually outlined at half of the femur length.

Calculations

FSR calculations The myofibrillar protein fractional synthesis rate (myoFSR) was calculated by using the standard precursor–product model and is reported in percent per hour: FSR = $\Delta E_{\text{product}} \times E_{\text{precursor}}{}^{-1} \times \Delta\text{time}^{-1} \times$ atom dilution factor $\times$ 100 %, where E_{product} is the change in tracer enrichment in two tissue samples taken with a time interval of Δtime in hours, during which period the precursor pool enrichment was constant and equal to $E_{\text{precursor}}$. We used the muscle free ^{13}C-enrichments as estimates for the precursor pool to calculate the myoFSR. To convert the ^{13}C-enrichments measured as CO_2 to phenylalanine amino acids atomic percent excess (ape) enrichments, we multiplied with an atom dilution factor of 14/6 (given by the ratio between the number of unlabeled carbons divided by number of labelled carbons in the NAP-derivatised phenylalanine). We corrected the precursor enrichments with the fluctuations measured by the more frequently sampled serum tracer enrichments by multiplying the average ratio of the muscle free and serum free tracer enrichments at the adjacent biopsy time points used to calculate each FSR measurement with the weighted average of the serum tracer enrichment between the neighbouring biopsy time points.

Western blot Phosphoprotein data were expressed as a ratio of phosphorylated/expressed protein measured on the same membrane (probing, stripping, and reprobing) for each target, except for phospho-S6 and phospho-ACC, which were normalized to the total amount of protein loaded, from the specific sample. The amount of expressed protein measured after the 12-week training period was normalized to the total amount of protein loaded, from the specific sample. The total amount of protein loaded was measured using the stain-free technology (Gurtler et al. 2013).

Statistical analysis

All protein data were log transformed before statistical analyses to achieve normal distribution.

The effects of exercise habituation (pre-habituation basal vs. post-habituation basal) on protein phosphorylation were tested with paired *t* tests. Comparison of the effect of contraction mode (ECC versus CONC) during exercise-habituation on protein phosphorylation was made with paired *t* test.

Difference in eccentric versus concentric workload in the acute study exercise bout was assessed with a paired *t* test. Workload differences between supplement groups in the acute study exercise bout were tested with unpaired *t* tests.

Venous serum phenylalanine $^{13}C_6$-enrichments, glucose and insulin levels for the two supplement groups (WPH + CHO vs. CHO) were compared by a mixed-effect two-way ANOVA, with repeated measures for time.

The effects of time (habituated basal vs. 1, 3 and 5 h post-exercise), group (WPH + CHO versus CHO) and contraction mode (ECC versus CONC) and their interactions on dependent variables (myoFSR, muscle free ^{13}C-enrichment, protein phosphorylation levels) were assessed using a mixed-effect three-way ANOVA with repeated measures for time. The within-participant design (repeated measures on the same participant within contraction mode and time) was accounted for in the model by using participant id and participant id $\times$ time as random effects. Linear pairwise comparisons were used post hoc to compare differences within and between individual conditions. The level of significance was set at $p \leq 0.05$. All statistical analyses were performed using Stata (Stata v 12.1, StataCorp LP, College Station, Texas, USA).

All phosphorylation changes are shown relative to the basal level of phosphorylation as geometric means $\pm$ back-transformed standard error of the mean. Data for serum and muscle free ring-13C6-phenylalanine enrichments were subtracted the venous serum background $^{13}C_6$-phenylalanine abundance and represented as the mean tracer-to-tracee ratios $\pm$ standard error of the mean. Glucose, insulin, myofibrillar FSR, and CSA data are represented as mean $\pm$ standard error of the mean. Graphs were designed in SigmaPlot (SigmaPlot v 11.0, Sysstat Software, Inc. San Jose, California, USA) or in GraphPad Prism (Version 5.0d, San Diego, California, USA).

Results

Effects of exercise habituation

The exercise-habituation protocol is shown in Fig. 1. The effect of exercise habituation on p-mTOR and its downstream signalling proteins are shown in Fig. 2. Representative Western blots are shown in Fig. 3. Exercise habituation did not lead to any phosphorylation changes in ACC, Akt, mTOR or eIF4E. Non-phospho-4EBP1 decreased by 18 $\pm$ 6 and 23 $\pm$ 9 % following ECC and CONC habituation exercise, respectively ($p < 0.05$). A decrease in the level of non-phosphorylated 4EBP1 is equivalent to an increased 4EBP1 phosphorylation. Exercise habituation caused an increase in the phosphorylation level of protein p70S6K by 54 $\pm$ 26 % ($p = 0.05$) compared to pre-habituation level following ECC. p-rpS6 increased by 49 $\pm$ 21 % ($p < 0.05$) compared to pre-habituation level following ECC habituation and exhibited a tendency to increase following CONC habituation (39 $\pm$ 21 %, $p = 0.07$). None of the proteins exhibited differences in phosphorylation levels between ECC and CONC contraction mode.

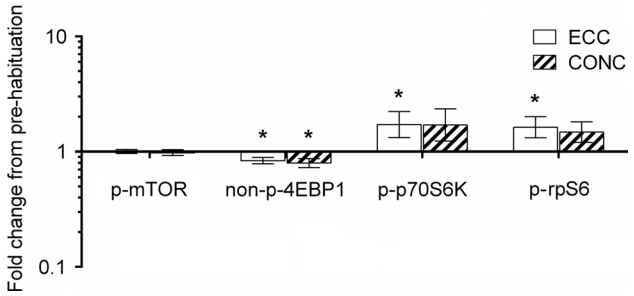

Fig. 2 Effect of exercise habituation on major mTOR signalling pathway proteins. *Open and hatched bars* represent concentric and eccentric work modalities, respectively. Contraction mode-specific (ECC versus CONC) effects of exercise habituation on levels of p-mTOR; non-p-4EBP1; p-p70S6K; p-rpS6 are shown as geometric means ± back-transformed SE, expressed as fold changes from individual pre-habituation basal level. *Denotes differences from pre-habituation basal level ($p < 0.05$). Examples of representative Western blots are shown for phosphor-specific mTOR pathway proteins for contraction mode-specific effects of exercise habituation in Fig. 3

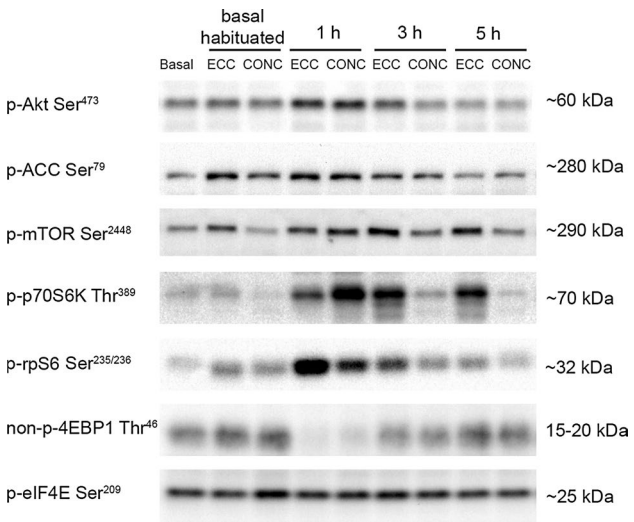

Fig. 3 Representative Western blots are shown for phospho-specific Akt-mTOR pathway proteins for contraction mode-specific effects of exercise habituation, and single-bout exercise

Single-bout trial

During the acute trial the total work performed by the ECC leg was on average 27 % higher than the total work performed by the CONC leg: 13,740 ± 462 and 10,855 ± 361 joule ($p < 0.001$), respectively. There were no differences between supplement groups (WPH + CHO vs. CHO) in the amount of concentric and eccentric work performed.

The serum $^{13}C_6$-phenylalanine enrichment for the supplement groups is shown in Fig. 4. No differences between groups were observed. A main effect of time ($p < 0.001$) was found. In accordance, at 120, 240 and 300 min the

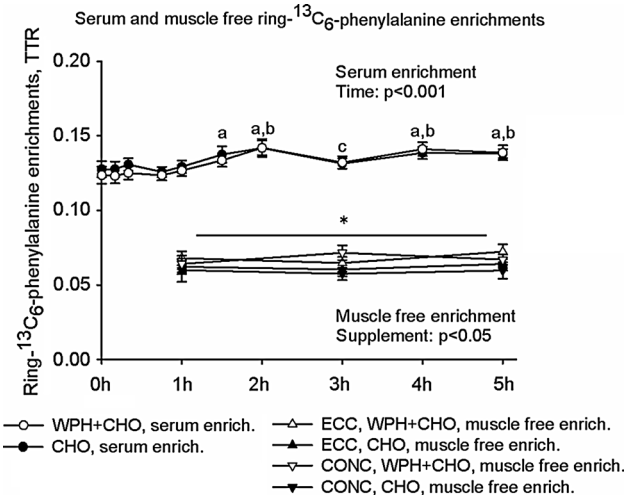

Fig. 4 Changes over time in serum and muscle free ring-$^{13}C_6$-phenylalanine tracer-to-tracee ratio (TTR) enrichments are shown for WPH + CHO and CHO, and calculated as: $^{13}C_6$-phenylalanine abundance subtracted the venous serum background $^{13}C_6$-phenylalanine abundance. Significant results of ANOVA analysis are shown in the figure. For the serum enrichment curve, *a* denotes an increased level of $^{13}C_6$-phenylalanine compared to 0-, 10 and 45 min; *b* denotes an increased level of $^{13}C_6$-phenylalanine compared to 20 min, and *c* denotes a decreased level of $^{13}C_6$-phenylalanine compared to 120 min ($p < 0.05$). Muscle free $^{13}C_6$-phenylalanine TTR enrichment at time 1, 3 and 5 h post-exercise, are shown for CHO, ECC; WPH + CHO, ECC; CHO, CONC; and WPH + CHO, CONC groups. *Denotes a difference in muscle free enrichment between supplement groups ($p < 0.05$)

level of $^{13}C_6$-phenylalanine was higher than at 0, 10, 20 and 45 min; at 90 min the level was elevated compared to 0, 10 and 45 min; and at 180 min the level was lower than at 120 min (all $p < 0.05$).

For the amount of free phenylalanine tracer in the muscle (i.e. precursor pool, shown in Fig. 4), no interactions between time (1, 3 and 5 h), group (WPH + CHO vs. CHO) and contraction mode (eccentric vs. concentric) were found. A main effect of supplement was found ($p < 0.05$) such that WPH + CHO muscle free $^{13}C_6$-enrichment levels were slightly elevated compared to CHO (6.7 ± 0.2 versus. 5.9 ± 0.3 %, $p < 0.05$).

Serum glucose and insulin levels before and during the recovery from single-bout exercise are shown in Fig. 5a, b, respectively. A supplement × time interaction was found ($p < 0.05$) for serum glucose which increased equally in both WPH + CHO and CHO groups by 15 ± 3 % at 0 min; however, at 1 h post-exercise the glucose concentrations exhibited a group difference ($p < 0.05$) in which the WPH + CHO and the CHO group were 90 ± 4 versus 108 ± 10 % compared to pre-level, respectively (Fig. 5a). Insulin concentrations were equal in the two supplement groups at all time points measured (Fig. 5b). A main effect of time ($p < 0.001$) was observed for the insulin levels; at

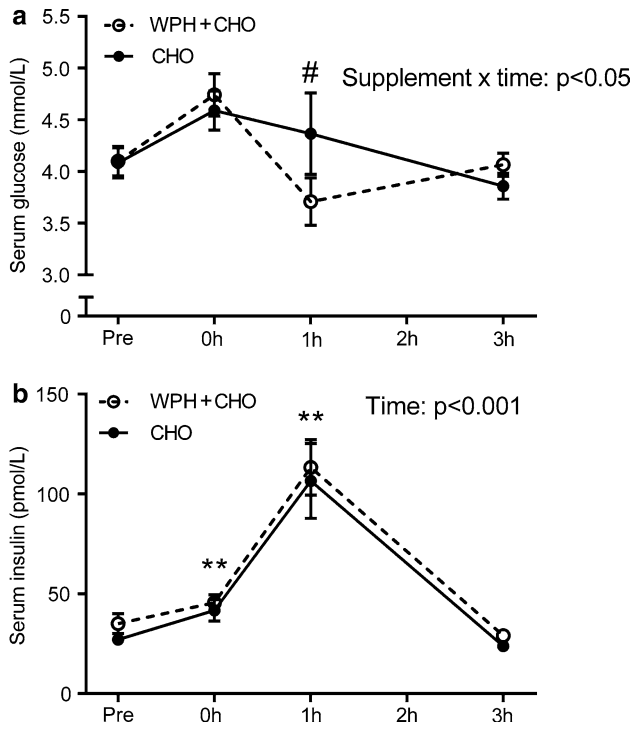

Fig. 5 Responses to single-bout resistance exercise of **a** serum glucose and **b** serum insulin concentrations, before and during the recovery phase after the single-bout exercise, are shown as mean ± SE for the WPH + CHO and CHO groups. #Denotes a difference between the two supplement groups ($p < 0.05$). **Denotes a difference from the pre-single-bout exercise concentration ($p < 0.001$)

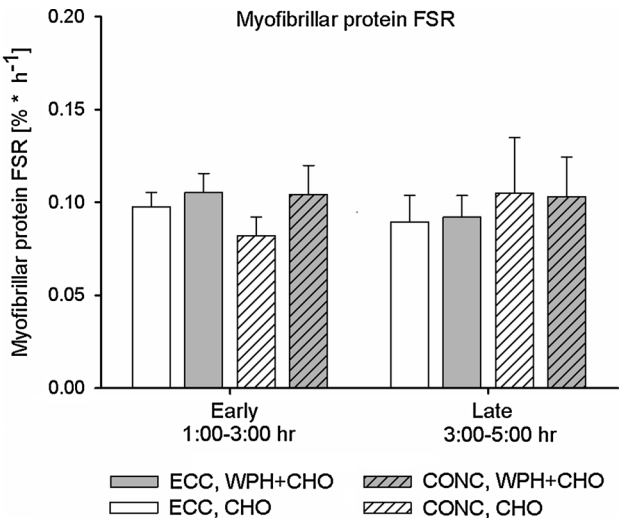

Fig. 6 The myofibrillar protein FSR for WPH + CHO and CHO supplement groups ± ECC versus CONC resistance exercise contraction mode exercise, are shown as mean ± SE during time intervals 1–3 h and 3–5 h, respectively

0 min the insulin level was 50 ± 11 % higher than pre-level ($p < 0.001$) and at 1 h the insulin level was 305 ± 54 % higher than at 0 min ($p < 0.001$) followed by a return to pre-level at 3 h.

Results of the myofibrillar protein FSR measurements are shown Fig. 6. No interactions or main effects were found for myofibrillar protein FSR at any conditions (time, supplement and exercise mode).

Phosphorylation changes of Akt-mTOR pathway proteins during recovery from single-bout ECC and CONC exercise are shown in Fig. 7 and representative blots are shown in Fig. 3. All single-bout phosphorylation results were compared to the basal habituated level hereafter referred to as the basal level.

For p-Akt (Fig. 7a), a main effect of time was observed; at 1 h post-exercise p-Akt was increased by 34 ± 7 % ($p < 0.001$), followed by decreases at 3 h (31 ± 6 %, $p < 0.001$) and at 5 h (34 ± 6 %, $p < 0.001$), compared to basal level. No interactions between interventions were evident.

For p-ACC levels (Fig. 7b) a supplement × time interaction ($p < 0.05$) and an exercise mode × time interaction ($p < 0.001$) was found. At 1 h post-exercise the p-ACC levels differed between the ECC and the CONC groups; they decreased and increased by 9 ± 9 % and 27 ± 8 % ($p < 0.05$), compared to basal level, respectively. At 3 h post-exercise; the p-ACC levels were different between ECC and CONC groups; they decreased by 39 ± 11 % and increased by 8 ± 8 % ($p < 0.05$), compared to basal level, respectively. At 3 h post-exercise, the p-ACC levels were also different between the WPH + CHO and the CHO groups; they decreased by 13 ± 9 and 35 ± 11 % ($p < 0.05$), compared to basal level, respectively. At 5 h post-exercise the ECC and the CONC p-ACC levels were different between groups; they changed by −43 ± 11 versus 5 ± 8 % ($p < 0.001$), compared to basal level, respectively. Only the WPH + CHO and the ECC interventions increased the p-ACC level above basal level at 5 h.

For the p-mTOR levels, shown in Fig. 7c, an exercise mode × time interaction was observed ($p < 0.001$). The p-mTOR levels were equally raised for all interventions at 1 h by 53 ± 4 % ($p < 0.05$) compared to basal habituation level. At 3 and 5 h post-exercise the p-mTOR levels were different between the ECC and CONC groups; they increased by 39 ± 5 % versus 13 ± 5 % ($p < 0.001$) at 3 h, and 31 ± 5 versus 10 ± 5 % ($p < 0.001$) at 5 h, compared to basal level, respectively. All interventions increased the p-mTOR level above basal level at 3 and 5 h post-exercise ($p < 0.05$).

The phosphorylation levels of p70S6K (Fig. 7d), showed an exercise mode × time interaction ($p < 0.001$). The p-p70S6K were equally raised for all interventions at 1 h post-exercise by 686 ± 24 % ($p < 0.05$) compared to basal level. At 3 and 5 h post-exercise the p-p70S6K levels were different between the ECC and the CONC groups; they were elevated by 410 ± 33 versus 66 ± 37 % ($p < 0.001$)

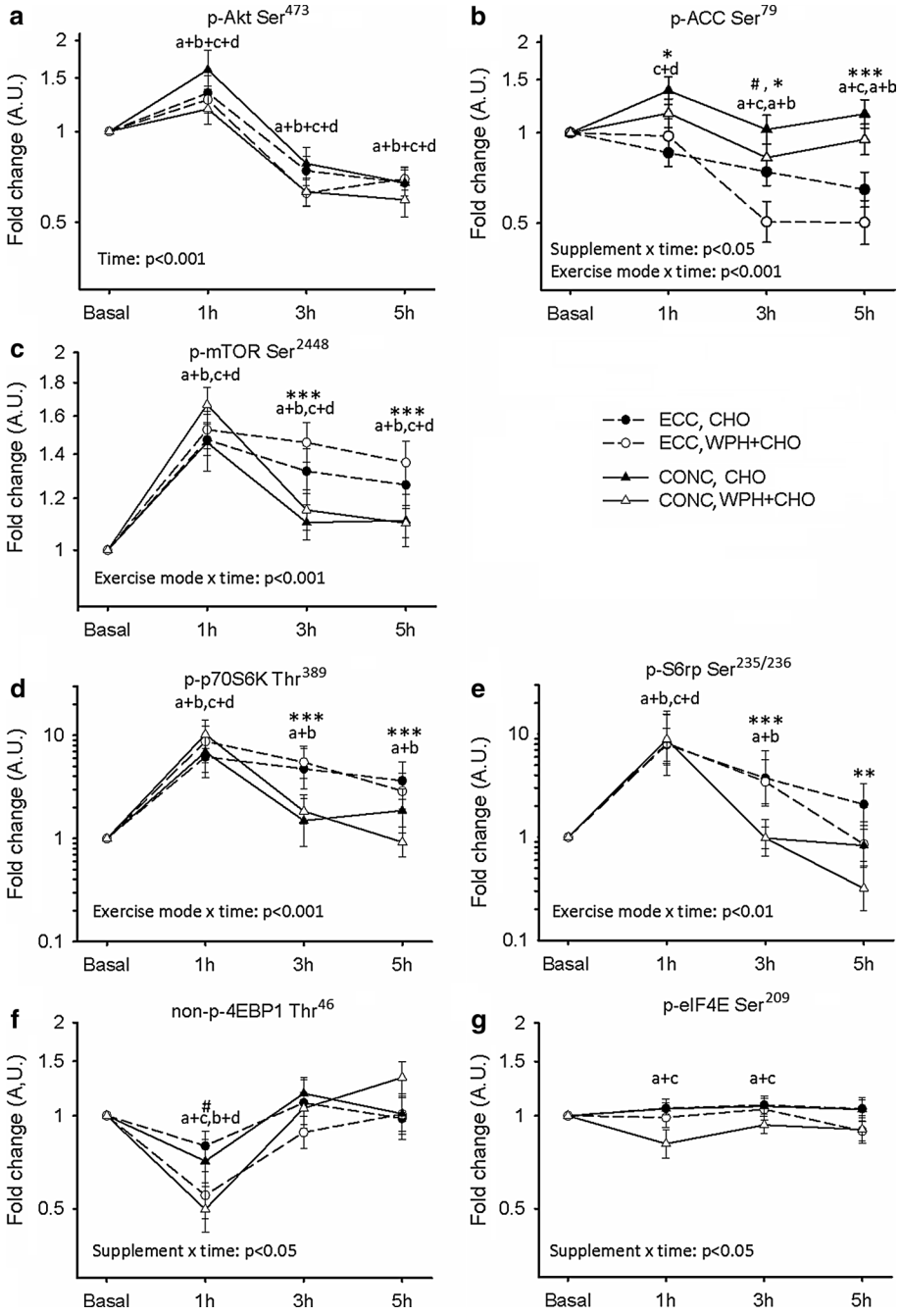

Fig. 7 Phosphorylation changes of **a** Akt kinase (p-Akt), **b** Acetyl-CoA carboxylase (p-ACC), **c** mammalian target of rapamycin (p-mTOR), **d** p70S6 kinase (p-p70S6K), **e** ribosomal protein S6 (p-rpS6), **f** (non-phospho) eukaryotic initiation factor 4E binding protein (4E-BP1), and **g** eukaryotic translation initiation factor 4E (p-eIF4E). Phosphorylation changes are shown relative to the basal level of phosphorylation as geometric means ± back-transformed SE after 1, 3 and 5 h of post-exercise recovery from single-bout ECC or CONC exercise ± WPH + CHO or CHO for. Overall significant ANOVA results are shown in the *lower left corner* of each graph. #Denotes differences between supplement groups (WPH + CHO vs. CHO, $p < 0.05$). Differences between exercise contraction modes (ECC versus CONC) are denoted by *($p < 0.05$), **($p < 0.01$) or ***($p < 0.001$), respectively. *a, b, c*, and *d* denotes differences from basal habituation level for groups ECC, WPH + CHO; ECC,CHO; CONC, WPH + CHO; and CONC + CHO, respectively ($p < 0.05$). +Denotes that groups are collapsed and different from basal habituation level ($p < 0.05$). Examples of representative Western blots are shown for phosphor-specific mTOR pathway proteins in Fig. 3

at 3 h, and 224 ± 32 versus 31 ± 35 % ($p < 0.001$) at 5 h, compared to basal level, respectively. At 3 and 5 h the p-p70S6k level of the ECC group was elevated compared to the basal level ($p < 0.05$).

rpS6 phosphorylation levels (Fig. 7e) showed an exercise mode × time interaction ($p < 0.01$). The p-rpS6 levels were equally increased for all interventions at 1 h post-exercise by 744 ± 32 % ($p < 0.05$) compared to the basal level. The p-rpS6 levels were different between the ECC and the CONC groups; they were changed by 258 ± 47 versus −2 ± 26 % ($p < 0.0001$) at 3 h, and 28 ± 43 versus −50 ± 38 % ($p < 0.001$) at 5 h, compared to basal level, respectively. At 3 h the p-rpS6 level of the ECC group was raised compared to the basal level ($p < 0.05$).

For the non-phosphorylation level of 4EBP1, shown in Fig. 7f, a supplement x time interaction ($p < 0.05$) was found. The non-p-4EBP1 levels were different between the WPH + CHO and the CHO groups; they decreased by 48 ± 13 versus 24 ± 10 % ($p < 0.05$) at 1 h, compared to basal level, respectively. The decreases of the non-p-4EBP1 levels at 1 h were significantly different from the basal level for all interventions.

For p-eIF4E a supplement × time interaction ($p < 0.05$) was found. There were no between-supplement group differences at any time points, but within the WPH + CHO group the p-eIF4E level was changed by −8 ± 6 % at 1 h and by 11 ± 4 % at 3 h post-exercise, whereas the p-eIF4E level remained constant for the CHO group.

Effects of 12-week resistance training

The relative increases of the mid-level CSA following 12-week training have previously been reported by Farup et al. (2013). In brief, no differences in quadriceps CSA were observed between the supplement groups when the training period was initiated. When the training period was completed, the quadriceps mid-level CSA of the ECC and CONC leg, in the WPH + CHO group were increased by 8.3 ± 1.3 and 6.2 ± 1.4 % ($p < 0.001$), respectively, with no difference between contraction modes. In the CHO group, the ECC and CONC leg mid-level CSA increased by 2.7 ± 1.1 % ($p < 0.01$) and 4.0 ± 1.0 % ($p < 0.001$), respectively, with no difference between contraction modes. A supplement × time interaction was observed at the mid-level CSA ($p < 0.01$) with a greater increase in the WPH + CHO group compared to the CHO group (7.3 ± 1.0 vs. 3.4 ± 0.8 %) independent of contraction type. The absolute numbers for CSA at the quadriceps mid-level pre- and post-training are shown in Table 1.

Changes in protein expression of Akt-mTOR signalling intermediates in response to 12 weeks of intervention are shown in Fig. 8. Twelve weeks of ECC and CONC resistance exercise with either WPH + CHO or CHO supplementation did not lead to any changes in the amount of Akt (pan, all isoforms), mTOR and p70S6K. For 4EBP1 there was an exercise mode × time interaction ($p < 0.01$), with a greater decrease following concentric compared to eccentric (25 ± 6 versus 9 ± 5 %, $p < 0.001$) training. For eIF4E protein a main effect of time was observed with a decrease of 13 ± 4 % ($p < 0.001$).

Table 1 Effect of 12 weeks of unilateral isolated ECC and CONC resistance training with WPH + CHO or CHO supplementation on absolute measures of quadriceps mid-level CSA

	WPH + CHO		CHO	
	ECC	CONC	ECC	CONC
Quadriceps mid-level CSA (cm^2)				
Pre-training	76.6 ± 2.7	77.8 ± 2.6	80.9 ± 2.6	79.4 ± 2.5
Post-training	82.9 ± 2.7*	82.5 ± 2.6*	83.1 ± 2.6	82.5 ± 2.5

The absolute pre-training quadriceps mid-level CSA has been published previously by Farup et al. (2013), where also the relative changes following 12 weeks resistance training are shown

Values are mean ± SE

* Supplement time interaction ($p < 0.01$), only the WPH + CHO group had a larger post-training CSA

Discussion

The current comparative study aimed to investigate the acute exercise effects as well as the accumulated training effects of divergent resistance exercise contraction modes and divergent dietary supplementation types, combined. Our main findings comprise: (1) that mTOR and its downstream pathway proteins display sensitivity to acute resistance exercise in a manner that is augmented by ECC contraction mode, but which did not differ between different isocaloric dietary supplementation types; (2) that regulatory differences at the level of mTOR signalling, does not translate into comparable differences at the level of myoFSR, and; (3) that prolonged high-volume ECC and CONC resistance training produce similar increases in muscle hypertrophy, which is augmented by whey protein supplementation.

Effect of exercise habituation on the anabolic signalling

We have previously found that stressors related to unaccustomed exercise may potentially confound interpretation of acute exercise-induced transcription and protein phosphorylation (Stefanetti et al. 2014; Vissing et al. 2005, 2013). To diminish such effects the participants first completed an initial exercise-habituation period. Resistance exercise habituation per se did produce an increase in 4EBP1 phosphorylation and ECC specifically produced minor increases in p-p70S6K and p-rpS6, which support that appropriate familiarization procedures in human exercise study designs may aid valid interpretation of results. Accordingly, this would increase the ability to distinguish between true exercise-induced responses and effects of exercise-unrelated stressors.

Acute responses to divergent single-bout resistance exercise and dietary supplementation

Leucine has previously been found to cause a substantially augmented insulin response when ingested with glucose as compared to ingestion of glucose alone (Gannon and Nuttall 2010; van Loon et al. 2000). Thus, the virtually identical insulin responses between supplement types observed in the present study were most likely caused by the high leucine content of the WPH + CHO supplement. It therefore makes sense that p-Akt Ser^{473} was observed to exhibit identical increases with WPH + CHO and isocaloric CHO. Since insulin and p-Akt levels did not differ between contraction modes, insulin-induced signalling to mTOR therefore cannot immediately be ascribed responsibility for the

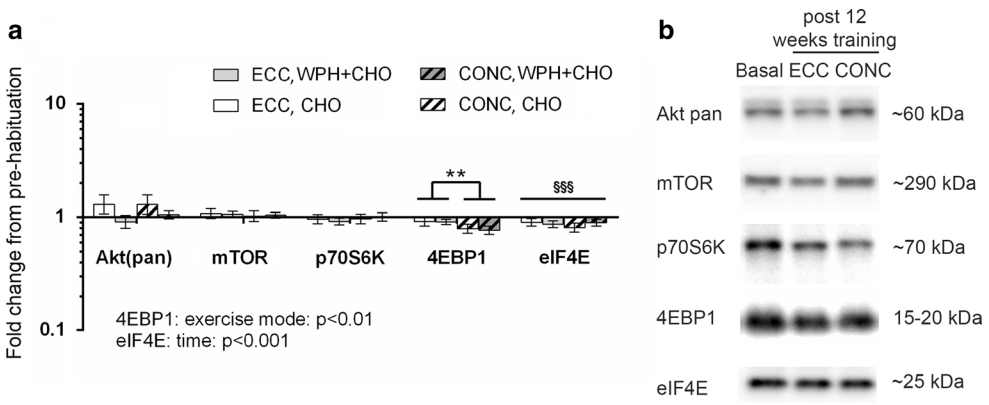

Fig. 8 Effect of 12 weeks of ECC and CONC training on the amount of **a** Akt-mTOR pathway proteins. *Grey* and *white bars* represent WPH + CHO and CHO supplement groups, respectively. *Open and hatched bars* represent eccentric and concentric work modalities, respectively. Contraction mode-specific (ECC versus CONC) and supplement-specific (WPH + CHO versus CHO) effects of 12-week resistance training (pre-habituation basal versus post-training level) on total amount of: Akt(pan); mTOR; p70S6K; 4EBP1; and eIF4E are shown as geometric means ± back-transformed SE, expressed as fold changes from individual pre-habituation basal level. Overall significant ANOVA results are shown in the lower left corner of the graph. **Denotes an effect of exercise mode ($p < 0.01$). §§§Denotes a difference from basal pre-habituation level ($p < 0.001$). **b** Representative Western blots are shown for mTOR pathway proteins pre and post 12 weeks of ECC and CONC training

differentiated mTOR and downstream signalling that we observe.

mTOR has previously been reported to be exhibit sensitivity to mechanical stress as well as certain amino acids. With regard to mechanical stress, studies by Hornberger and co-workers strongly support the contention that activation of mTOR can occur independently of Akt via phospholipase D (PLD)-generated phosphatic acid (PA) (Hornberger 2011). PLD is localized in the Z-bands in skeletal muscle, which constitute a critical site of mechanical force transmission (Friden and Lieber 2001). PLD is inactive in the resting muscle, but repetitive mechanical deformations during contractions of muscle fibres (ex vivo) induce activation of PLD and thereby an increase in PA, which activate the mTOR pathway (Hornberger and Chien 2006; O'Neil et al. 2009). Other proposed mechanotransducing mechanisms to activate mTOR comprise, e.g. focal adhesion kinase (Li et al. 2013) and α7 integrins (Lueders et al. 2011). With regard to amino acids branched chain amino acids (BCAA), and especially the BCAA leucine, are believed to interact with intracellular sensors that activate mTOR and allow its further stimulation by growth factors (Dibble and Manning 2013; Dickinson et al. 2011).

In the current study, phosphorylation of mTOR Ser^{2448} was upregulated with both contraction modes, but exhibited a much more persistent upregulation with ECC compared to CONC contraction mode. An exact similar pattern was observed for phosphorylation of the mTOR downstream substrate p70S6K and its substrate rpS6, responsible for allowing translation processes to occur. Overall, these findings support the hypothesis that higher force/mechanical stress produced during maximal voluntary ECC compared to maximal voluntary CONC contractions, is the determinant for the magnitude and preservation of acute resistance exercise-induced mTOR signalling (Eliasson et al. 2006). In the study by Eliasson et al. (2006), protein signalling was tracked during fasted post-exercise recovery and did not include analysis of MPS. Our results add to this by suggesting that in the fed state there is a relatively fast responding component of resistance exercise-induced mTOR phosphorylation, which do not differ between exercise contraction modes. This is subsequently followed by a slower more persistent upregulation in mTOR phosphorylation response, elicited by intrinsic mechanisms inherent of ECC contractions specifically. In accordance, the fast response could be related to insulin-mediated Akt signalling, as peak insulin levels and peak p-Akt at 1 h of recovery corresponds well with the initial parallel increase of p-mTOR at 1 h. The effect of mechanotransduction may then be more long-lived, with the level of force transmission from ECC superseding that of CONC.

One alternative explanation could be that the faster rate of decline in p-mTOR, p-p70S6K, and p-rpS6 at 3 and 5 h into recovery with CONC compared to ECC, rely on increased ATP turnover during CONC compared to ECC (Abbott et al. 1952). In accordance, under circumstances of decreased energy levels, AMP-activated protein kinase (AMPK) is believed to respond by switching off ATP-consuming pathways, such as MPS, in favour of pathways for ATP regeneration, such as muscle fat oxidation through phosphorylation action on acetyl-CoA carboxylase (ACC) (Dean et al. 2000). AMPK activates the TSC complex (Dibble and Manning 2013), and inhibits regulatory-associated protein of mTOR (raptor), thereby causing decreased

mTORC1 activity (Gwinn et al. 2008). While the time points of biopsy sampling of our protocol were not ideal to measure AMPK activation [i.e. AMPK is very transiently activated during the early minutes after exercise (Gehlert et al. 2012)], the p-ACC response we observe provides an indication of prior AMPK activity (Park et al. 2002). This may indicate that AMPK activation influenced the decline in mTOR and downstream signalling following CONC exercise as compared to ECC exercise.

As for 4EBP1, the repressive action of 4EBP1 on translation processes is known to be released by a resistance exercise-induced increase in its phosphorylation (Atherton et al. 2005). In the current study, this was evaluated as non-p-4EBP1, which exhibited a downregulation with resistance exercise per se that was accentuated by WPH + CHO compared to CHO. Accordingly, this part of mTORC1-related signalling also reacted in a manner to favour initiation of protein synthesis. Yet, in contrast to the mTOR–p70S6K–rpS6 axis, which reacted in a contraction mode-dependent manner, 4EBP1 reacted in a supplement type-dependent manner, although to a quite modest extent. This indicates that other upstream mediators exert control on 4EBP1 and favour the notion that different anabolic signalling proteins possess different sensitivity to amino acid supplements.

With regard to our outcome measure of protein synthesis, for practical reasons, we did not include an assessment of basal fasting and resting level of MPS. However, with reference to the absolute levels observed during post-exercise recovery as well as the literature (Cuthbertson et al. 2006; Mitchell et al. 2014), we can reasonably assume that the myofibrillar protein synthesis rates are markedly increased above resting levels during the hours after single-bout exercise and supplementation. Yet, contrary to our hypothesis, myoFSR was not observed to differ by either contraction mode (within-participant design) or by either type of dietary supplement (inter-participant design). Although immediately surprising, this lack of difference in myoFSR by contraction models actually in support of other recent reports, showing that different contraction modes neither produce divergent effects on acute changes in muscle protein synthesis rate (Cuthbertson et al. 2006) nor accumulated changes in muscle hypertrophy (Farup et al. 2013; Moore et al. 2012). Oppositely, one study by Moore et al. (2005) did show a difference in the acute MPS (Moore et al. 2005). Accordingly, the temporal changes in exercise-induced MPS rate appear rather variable in the literature despite provision of protein nutrition (Cuthbertson et al. 2006; Moore et al. 2005). In this respect, exercise intensity (Holm et al. 2010; Wilkinson et al. 2008) and total work (Burd et al. 2010a, b, 2012) seems to comprise two independent factors with impact on the pattern of the acute MPS response as well as on the longitudinal muscle hypertrophy response (Mitchell et al. 2012). Thus, the diversities in exercise protocols between otherwise seemingly comparable studies may be responsible for the temporal diversities in the response patterns (Cuthbertson et al. 2006; Moore et al. 2005). Based on such diverse results it can be questioned to which extent protein signalling can actually be expected to correlate well with myofibrillar FSR and/or muscle hypertrophy and render it speculative if signalling measured at discrete time points actually constitute reliable proxies of myofibrillar FSR. Given our sample size (i.e. $n = 24$ in a paired design), the statistical power of our study is quite robust by comparison to most human exercise studies. However, one explanation for the identical MPS responses we observe may be that our choice of dietary compositions (i.e. both high in CHO) influences the balance between MPS and breakdown in a manner to equalize net myofibrillar FSR. Another explanation may be that the early peak in signalling responses constitutes the stronger determinant for protein synthesis, which overrules the consequences of contraction mode-specific changes in signalling at later time points. Finally, our employment of prior exercise habituation may have exerted influence on protein signalling and myofibrillar FSR. However, this is all speculative and the link between measures of signalling and muscle protein turnover is an issue that calls for further attention.

Accumulated training responses to divergent resistance exercise and dietary supplementation

Somewhat contrary to the acute changes in anabolic signalling, prolonged intervention produced increases in muscle hypertrophy that were similar between exercise contraction modes. This may partly relate to the fact that with high volumes of resistance exercise work, differences between contraction modes can fade out (Wernbom et al. 2007). Therefore, we cannot exclude that lesser volumes of work would have produced more substantial muscle hypertrophy with one contraction mode over the other. On the other hand, we previously reported, that whey protein supplementation can augment muscle as well as interconnected tendon hypertrophy irrespective of resistance exercise contraction mode [these results are elaborated in much more detail in Farup et al. (2013)]. This observation that whey protein can augment muscle hypertrophy is in line with some (Andersen et al. 2005; Hartman et al. 2007), but not all studies (Hulmi et al. 2009) and may relate to the composition of amino acids (i.e. our supplement was rich in leucine) or strategy of intake. In the current study, the participants fasted right before and after each exercise session and received the half of the supplement immediately before and immediately after exercise session, respectively. Finally, we also investigated if total protein levels of Akt-mTOR pathway proteins changed in response to prolonged intervention. Virtually

no changes were observed to occur with differentiated contraction mode and supplement type. Thus, the function of these anabolic signalling proteins in promoting muscle protein synthesis seems to rely primarily on acute phosphorylation changes.

Conclusion

In conclusion, with regard to acute effects of intervention, we observed ECC resistance exercise to be a stronger driver of acute anabolic signalling than CONC resistance exercise, in a manner that was not altered by divergent isocaloric supplementation types. Yet, MPS did not differ between exercise and/or dietary interventions. Furthermore, with prolonged training, divergent contraction modes produced similar magnitude of muscle hypertrophy, with whey protein but not isocaloric CHO augmenting muscle hypertrophy.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard All participants were informed about the purpose and the risks related to the study and gave written, informed consent to participate. The study was approved by The Central Denmark Region Committees on Health Research Ethics (j. no. M-20110003) and performed in accordance with the Declaration of Helsinki.

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