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Longitudinal hypertrophic and transcriptional responses to high-load eccentric-concentric vs concentric training in males

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High-load eccentric training reputedly produces greater muscle hypertrophy than concentric training, possibly due to greater loading and/or inflammation. We quantified the temporal impact of combined maximal concentric-eccentric training vs maximal concentric training on muscle cross-sectional area (CSA), volume, and targeted mRNA expression (93 transcripts). Eight recreationally active males (24 ± 5 years, BMI 23.5 ± 2.5 kg/m^2) performed 3 x 30 maximal eccentric isokinetic knee extensions and 2 x 30 maximal concentric knee extensions in dominant limb (ECC + CON) and 5 x 30 maximal concentric contractions (CON) in the non-dominant limb for 12 weeks (all 90°/s, 3x/wk). Quadriceps muscle CSA and volume were measured at baseline, 28 days (d), and 84 d in both limbs (3T MRI). Resting vastus lateralis biopsies were obtained from both limbs at baseline, 24 hours (h), 7, 28, and 84 d for mRNA abundance measurements (RT-PCR microfluidic cards). Work output was greater throughout training in ECC + CON vs CON (20.8 ± 9.7%, $P < .001$). Muscle CSA increased from baseline in both limbs at 28 d (CON 4.3 ± 2.6%, ECC + CON 4.0 ± 1.9%, both $P < .001$) and 84d (CON 3.9 ± 2.3%, ECC + CON 4.0 ± 3.1%, both $P < .001$), and muscle volume and isometric strength at 84 d (CON 44.8 ± 40.0%, $P < .001$; ECC + CON 36.9 ± 40.0%, $P < .01$), but no between-limb differences existed in any parameter. Ingenuity Pathway Analysis identified several cellular functions associated with regulation of muscle mass and metabolism as altered by both modalities at 24 h and 7 d, but particularly with ECC + CON. However, mRNA responses waned thereafter, regardless of modality. Initial muscle mRNA responses to training did not reflect chronic training-induced hypertrophy. Moreover, ECC + CON did not produce greater hypertrophy than CON, despite greater loading throughout and a differential mRNA response during the initial training week.

KEYWORDS
concentric training, eccentric training, muscle hypertrophy, muscle transcriptional response

Mallinson and Taylor shared joint first authorship.

1 | INTRODUCTION

High-load eccentric exercise training has been reported to increase muscle hypertrophy, type IIx muscle fiber cross-sectional area (CSA) and strength gains over 10 to 12 weeks when compared to concentric exercise training,[1,2] which has been at least partially attributed to the greater work done during eccentric contractions.[3] However, these reports are equivocal, reflected by reports of no differences in muscle hypertrophy or strength gains when comparing high-load concentric and eccentric exercise training modalities.[4]

High-load eccentric exercise is also known to cause short-term muscle force loss and delayed onset muscle soreness (DOMS) compared to high-load concentric training,[5] both of which diminish with repeated bouts of exercise.[6] Muscle inflammation is commonly reported to accompany high-load eccentric exercise,[5] though is not commonly measured. This has resulted in combined high-load concentric and eccentric training regimens being adopted,[7,8] aimed at improving tolerability and volunteer compliance over high-load eccentric exercise training alone, and also because it is normally difficult to isolate eccentric contractions in a training setting. As far as we are aware no study has examined the temporal response to these two different training modalities. Furthermore, studies comparing muscle adaptations to concentric versus eccentric training have used different training protocols, varying in training loads and number of repetitions, methods of assessment of architectural and functional gains, and have involved both trained and untrained volunteers.[1,3] It is therefore difficult to draw conclusions on the comparative efficacy of the two modalities at inducing muscle training related adaptations, which we aimed to address in this study by adopting a within-subject bilateral exercise study design controlled for contraction number and speed of contractions.

The mechanisms that underpin purported greater muscle adaptation to eccentric exercise training are not well understood. Proposals such as greater neural activation and differing recruitment strategies, greater work done, and increased post-exercise muscle protein synthesis (MPS) have not been definitive and knowledge gaps exist.[1,3,9] Focus has also been directed toward high-load eccentric exercise-induced muscle inflammation being a mechanistic driver of hypertrophy. Application of gene transcript profiling in a study showed that 300 concentric and 300 eccentric contractions (in the form of rising from and controlled lowering to a seated position), induced greater muscle inflammatory mRNA responses after 4-8 h compared with concentric contractions alone.[10] Furthermore, these inflammatory-related mRNA responses were maintained 6h following a second bout of eccentric exercise.[11] It has also been suggested that there is a potentiating effect of inflammation on muscle satellite cell proliferation.[12] In keeping with this, an increase in the serum concentration of the pro-inflammatory cytokine interleukin 6 (IL-6) was accompanied by increased expression of myogenic regulatory factors (MRFs) between 24 to 96 h following maximal electrically evoked eccentric contractions in a rodent model.[13] IL-6 has also been demonstrated to stimulate satellite cell-mediated hypertrophy in isolated cells,[14] and local non-steroidal anti-inflammatory drug (NSAID) infusion following acute maximal eccentric exercise in healthy volunteers has been shown to decrease muscle satellite cell proliferation.[12] McKay et al[15] also showed an increase in satellite cell number 24 h following maximal eccentric exercise, which was associated with an increase in muscle IL-6 mRNA expression and serum IL-6 concentration. However, these studies examined acute responses to acute exercise intervention. In a chronic setting, high-load concentric-eccentric elbow flexor exercise for 6 weeks (5x/wk) combined with daily NSAID ingestion had no differential effect on muscle hypertrophy and strength compared to placebo group.[16] Conversely, 6 weeks of NSAID administration alongside progressive resistance training in older volunteers increased muscle volume and strength above placebo.[17] Importantly, as far as we are aware, no time-course data are available regarding muscle molecular adaptations to combined high-load concentric and eccentric exercise training compared to concentric exercise alone.

We hypothesized that in healthy, young male volunteers who were recreationally active, 12 weeks of unilateral combined maximal isokinetic concentric and eccentric resistance training would produce greater gains in muscle CSA, muscle volume, and isometric strength compared to maximal isokinetic concentric exercise training performed by the contralateral limb. We also hypothesized that these greater architectural and functional gains in the limb performing combined concentric and eccentric training would be preceded by greater exercise-induced changes in the expression of genes involved in inflammation, myogenesis and other cellular functions thought to regulate muscle mass and fuel metabolism.

2 | MATERIALS AND METHODS

2.1 | Participants

Eight healthy, non-smoking, non-vegetarian males (24 ± 5 years, BMI 23.5 ± 2.5 kg/m^2) were recruited to the study. Participants had to have participated in sports such as squash, football, and basketball on at least 2-3 days per week for a minimum of 2 years so were not unaccustomed to regular exercise. The study was approved by the University of Nottingham Medical School Ethics Committee (Ethics Reference No: I 07 2011) in accordance with the Declaration of Helsinki. Prior to taking part, all participants provided informed, written consent, underwent a routine medical

screening and completed a general health questionnaire. Participants were asked to refrain from taking anabolic supplements, that is, protein and creatine, for the duration of the study, but were allowed to continue with their regular training regimens apart from lower limb resistance training. Based upon documented changes in muscle CSA at 10 weeks of a maximal isokinetic resistance training protocol in healthy, recreationally active male volunteers[18] and assuming (a) an effect size of 0.7, (b) a significance level of 0.05 (α), and (c) 95% power, the required sample size was calculated (using G*Power, v3.1.2) to be n = 6. We recruited n = 8 volunteers in total for the current study.

2.2 | Exercise training protocol

One of the main objectives of the study was to determine whether a greater degree of muscle inflammation caused by high-load eccentric exercise training was associated with greater muscle mass and strength gains. We therefore wished to model a training protocol of sufficient intensity to induce inflammation in the quadriceps, but was also tolerable for the duration of the study. An exercise protocol consisting of a bout of 5 sets of 30 maximal isokinetic concentric knee extensions has been shown by our laboratory to be well-tolerated and effective at increasing muscle mass and strength.[19] However, preliminary protocol development work for this study revealed that five sets of 30 maximal eccentric isokinetic contractions of the quadriceps caused considerable post-exercise discomfort and muscle force loss in volunteers, and to an extent that this would likely deter volunteers from complying with a 12-week training program. Hence, we formulated a combined concentric-eccentric protocol that was likely to induce muscle inflammation while remaining tolerable for the duration of the study. Volunteers trained 3 times per week for 12 weeks and were not in an overnight fasted state for their training sessions (Figure 1). During each session, participants performed 3 bouts of 30 maximal isokinetic eccentric contractions of the knee extensors with their dominant leg, interspersed with two bouts of 30 maximal isokinetic concentric extensions (ECC + CON) with the same leg. Additionally, volunteers performed 5 bouts of 30 maximal isokinetic concentric contractions of the knee extensors with the non-dominant leg (CON).

Contractions in both legs were performed on an isokinetic dynamometer (HUMAC Norm, CSMi Solutions). For this, the participant was seated securely in the chair of the dynamometer with the knee flexed at 90°, the lever arm attached to the subject's ankle and the actuator axis aligned to the lateral femoral epicondyle. Contractions were performed at an angular velocity of 90°/s with 3 minutes rest between bouts, which ensures extensive quadriceps muscle fiber recruitment.[20] Volunteers were required to exert maximal voluntary force during each isokinetic knee extension (CON) or flexion (ECC) maneuver, and the ergometer lever arm was returned passively to the leg start position between contractions, thereby ensuring minimal muscle loading. Volunteers received real-time visual feedback on their performance, and verbal encouragement throughout each training session to expedite maximum effort being achieved. All volunteers completed 150 contractions per leg per session and 5400 contractions per leg over the 12-week training period. As expected, during each bout of 30 maximal contractions peak torque per contraction decreased substantially in each CON bout with only a partial recovery between bouts, but peak torque per contraction remained approximately the same within and over each bout of ECC exercise.

A within-subject bilateral internally controlled design was used as (a) such a model minimizes the variability of responses to training[21] and (b) it is known that hypertrophic adaptations

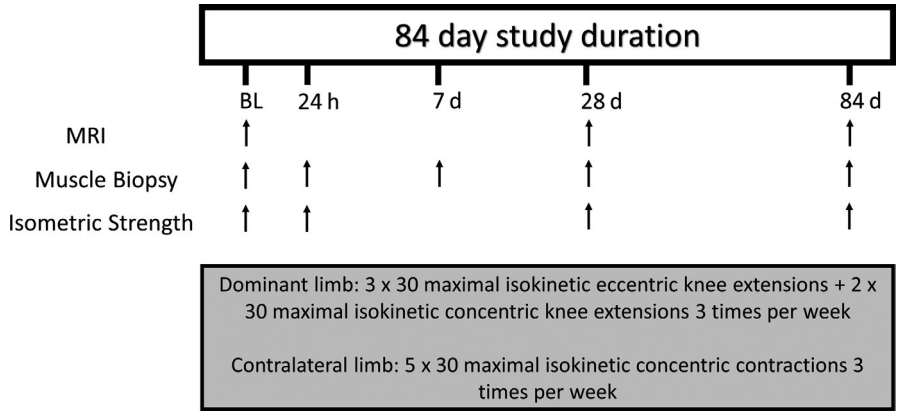

FIGURE 1 Study protocol. Participants performed 3 x 30 maximal isokinetic eccentric and 2 x 30 maximal isokinetic concentric knee extensions 3 times per week for 12 wk in the dominant limb and 5 x 30 maximal isokinetic concentric contractions 3 times per week for 12 wk in the contralateral limb. Muscle biopsies were taken at baseline (BL), 24 hours (24 h), 7 days (7 d), 28 days (28 d) and 84 days (84 d). Isometric strength was measured at BL, 24 h, 28 d and 84 d. MRI scans of the upper limb of both legs were performed at BL, 28 and 84 d

are a result of localized responses to exercise.[22] A potential concern with a within-subject design is the possibility of a cross-education effect. However, such a design has been used in several previous resistance exercise training studies.[9,23,24] Further, since the main targets and outputs of the present study were related to (a) hypertrophy and (b) localized muscular adaptations in mRNA expression measured in muscle samples, we foresaw cross-education having little to no impact.

2.3 | Total work output during exercise training

Total work output (both positive and negative) per week of training by each limb was calculated as the work done during the 450 maximal contractions performed each week. Total work output for each limb over the 12-week exercise training protocol was calculated as the sum of work done from all exercise sessions by each limb.

2.4 | Muscle strength

Isometric strength was determined (best of three attempts) in the knee extensor muscles, with the knee flexed at 90°, during a static maximal voluntary contraction using an isometric knee extension unit (Medical Physics, University of Nottingham, UK) at baseline (BL), and 24 h, 28 days (28 d) and 84 days (84 d; Figure 1). All measurements were made at the same time of day, at least 72 h after the last training session, and the legs were tested in a random order.

2.5 | Quadriceps muscle cross-sectional area and volume

Quadriceps muscle cross-sectional area (CSA) and volume measurements were performed at the BL, 28 d, and 84 d time points (Figure 1). For this, axial plane scans of the thigh were taken using a 3 Tesla MRI scanner (GE 3T 750 Discovery). A T1- Fast Spin Echo protocol was used (repetition time 600 ms, echo time 15 ms, number of excitation 2, field of view 22 mm, slice thickness 10 mm, no gap between slices). Participants were asked to lie supine on the MRI bed. Axial plane scans along the entire length of the quadriceps muscle were obtained to determine anatomical cross-sectional area (ACSA). The ACSA shown represents the changes in the peak value ($ACSA_{peak}$) at the middle of the femur length. The number of axial scans (48) taken in each leg of every volunteer was the same for the BL, 28 d, and 84 d time points. Using these axial scans, the contours of the whole quadriceps muscle group of each MRI scan were digitized using Osirix image analysis software (Pixmeo, Geneva, Switzerland). Subsequently, quadriceps muscle volume was calculated as follows[4]:

$$\text{Volume Quads } \left(\text{cm}^3\right) = \sum \text{ACSA}_{\text{peak}} \times \text{slice thickness}$$

2.6 | Muscle sample collection

Vastus Lateralis muscle biopsies were obtained in the resting state at BL, 24 h, and a minimum of 72 h from the previous exercise session at 7, 28, and 84 d (Figure 1). Volunteers attended the laboratory in a fasted state, having abstained from alcohol and strenuous exercise for 48 h prior. Muscle samples were obtained from both legs using the Bergstrom percutaneous needle biopsy technique,[25] and samples were immediately snap-frozen and stored in liquid nitrogen for further analysis.

2.7 | Muscle mRNA expression

We have previously demonstrated that serial needle muscle biopsy sampling, where repeated biopsies are taken 2.5 cm distal to each other, does not elicit confounding changes in the muscle transcriptome when analyzed by microarray technologies.[26] RNA was extracted from ~30 mg snap-frozen muscle from all but four biopsy samples (ie, 76 biopsies in total due to tissue availability) as previously described.[27] First strand cDNA was synthesized from 1 μg of total RNA, using Superscript III reverse transcriptase (Invitrogen Ltd) and random primers (Promega) and stored at −80°C until analysis. TaqMan low-density arrays were performed using an ABI PRISM 7900HT sequence detection system, and data analyzed using SDS 2.1 software (Applied Biosystems). Data were further analyzed using RQ Manager software (Applied Biosystems), where the threshold level was normalized across all plates before Ct values were calculated for each gene target and sample. Relative quantification of mRNAs of interest was measured using the $2^{-\Delta\Delta Ct}$ method with hydroxymethylbilane synthase (HMBS) as the endogenous control, with the mean of the baseline sample used as the calibrator. Mean Ct values for HMBS were no different between CON and ECC + CON (data not shown). A total of 93 transcripts were targeted for analysis in the present study (Appendix A). As limited muscle gene array data exist pertaining to chronic eccentric training, muscle transcripts identified as being altered following acute eccentric exercise in array studies involving healthy male volunteers were selected for inclusion in the present study,[1,10] along with muscle transcripts seen to be differentially expressed in healthy male volunteers over the course of 10 weeks of concentric resistance training.[26] Based on these studies, approximately 50 genes were selected that were linked to cell growth and regulation, DNA damage, stress responses,

energy metabolism, inflammation, extracellular matrix, and muscle differentiation and signaling. Additionally, mRNA expression of myogenic transcription/regulatory factors such as myoblast determination protein 1 (MyoD), myogenin (MYOG), myostatin (MSTN), myogenic factor 5 (MYF5) were selected for inclusion. Further genes of interest known to be differentially regulated in muscle inflammatory states and linked to muscle mass regulation were also included,[28,29] namely AKT serine/threonine kinase 1 (AKT1), forkhead box protein O 1 (FOXO1), protein tyrosine kinase 2 (PTK2), pyruvate dehydrogenase kinase 4 (PDK4), DNA (cytosine-5)-methyltransferase (Met1A), cathepsin-L, muscle-specific ligases atrophy F-box protein (MAFbx), muscle RING-finger protein-1 (MuRF1) and proteasomal alpha subunit 1 (PSMA1).

2.8 | Pathway analysis

To associate a biological function to the identified probe sets, Ct values were uploaded to Ingenuity Pathway Analysis (IPA) software (Redwood City, CA, USA) for pathway analysis of gene expression data. Ingenuity Pathway Analysis is a commercially available online software application that allows for the integration, analysis, and comprehension of data from gene expression, miRNA, and single nucleotide polymorphism (SNP) microarrays, as well as proteomics, metabolomics, and RNA sequencing (RNAseq) experiments. The outcome of IPA analysis, especially for the upstream regulator and cellular function analysis could appear biased because the input was the result of an array analysis of 93 pre-selected genes, as opposed to an unbiased analysis of gene expression on a global level. For example, since several target inflammatory genes were selected for measurement, inflammatory pathways were more likely to be flagged in the IPA analysis. However, the overall outcome of each IPA analysis (eg, upstream regular analysis, cellular function, activation status) was predicted by calculating a regulation Z-score and an overlap p-value, which were based on the number of regulated target genes' function, size change, and direction of expression, and their degree of agreement with the IPA curated database of published literature. Furthermore, irrespective of the genes selected the same battery of targeted genes were compared longitudinally and between CON and CON + ECC trained limbs.

2.9 | Statistics

All values are presented as mean ± SD unless otherwise stated, with significance accepted at the $P < .05$ level. Normality was tested using a Shapiro-Wilks test. Where data were not normally distributed, values were log transformed and parametric statistical tests used. Comparison of mean values between ECC + CON and CON legs was performed using a repeated measures two-way analysis of variance (ANOVA) (time and exercise regimen as independent factors) with Tukey's or Sidak's post hoc test analysis where appropriate (GraphPad Prism 7.03).

To identify the statistical difference in total work output between ECC + CON and CON legs over the entire exercise protocol, a paired sample, two-tailed t test was used (GraphPad Prism 7.03).

For muscle mRNA cellular function analysis, the Benjamini-Hochberg method for multiple hypothesis correction was employed to account for the possibility of false-positive results (type I errors). This is a default function within the IPA platform.

3 | RESULTS

3.1 | Baseline comparisons

The mean age of the participants was 24 ± 5 years, and mean BMI was 23.5 ± 2.5 kg/m^2. There was no difference in isometric strength, muscle CSA or muscle volume when comparing ECC + CON and CON legs at baseline (Table 1).

3.2 | Muscle function during training

Total work output per week during training was greater in ECC + CON compared to CON (Figure 2A), such that there was a treatment effect ($P < .001$), and cumulative work done over the 12-week protocol was greater in ECC + CON than CON (20.8 ± 9.7%, $P < .001$; Figure 2B).

Isometric strength of the knee extensors increased over time ($P < .05$), such that it was increased above baseline at 84d (44.8 ± 40.0% in CON, $P < .001$; 36.9 ± 40.0%. in ECC + CON, $P < .01$). However, there was no difference between groups in the change in isometric strength from baseline at any time point.

TABLE 1 Isometric strength and mid-thigh quadriceps muscle cross-sectional area (CSA) and quadriceps muscle volume at baseline are shown for a leg trained using a concentric exercise protocol (CON) and the contralateral leg trained using a combined concentric and eccentric exercise protocol (ECC + CON)

	CON	ECC + CON
Isometric Strength (kg)	51.6 ± 18.6	56.5 ± 21.6
Quadriceps CSA (cm^2)	88.9 ± 12.3	89.3 ± 13.2
Quadriceps Volume (cm^3)	2520.8 ± 388.5	2483.4 ± 408.5

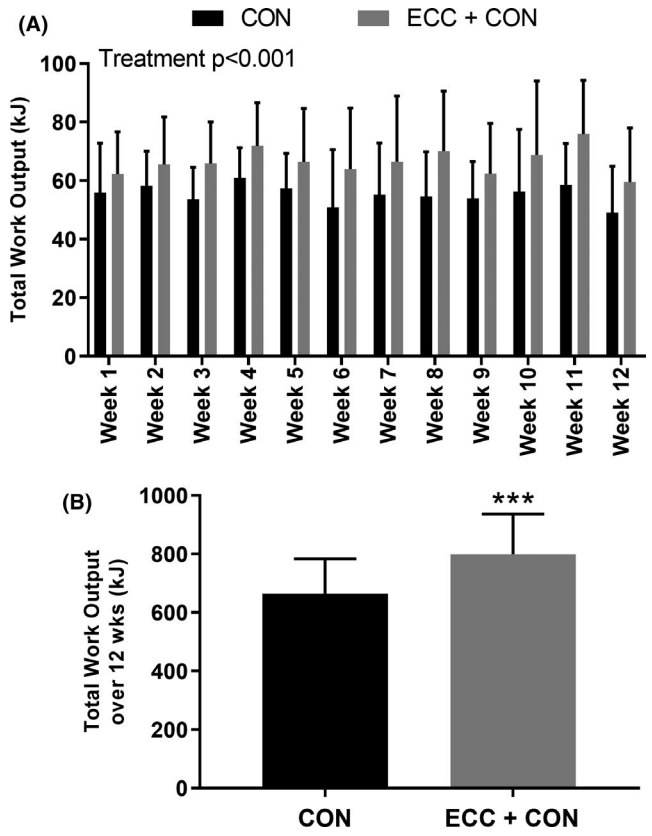

FIGURE 2 (A) Total work output (kJ) per week, (B) total work output (kJ) over 12 wk in concentric (CON) and combined concentric + eccentric (ECC + CON) trained legs. Values expressed as mean ± SD. B, ***$P < .001$ compared to CON

3.3 | Muscle cross-sectional area and volume

Quadriceps muscle CSA was increased above baseline in both legs by 28d (CON $P < .001$, ECC + CON $P < .001$) and 84d (CON $P < .001$, ECC + CON $P < .001$; Figure 3A). There was no increase in muscle CSA in either the CON leg or the ECC + CON leg from 28 d to 84 d. No difference was seen in change in muscle CSA with exercise training at any time point when comparing CON and ECC + CON legs.

Thigh muscle volume was also increased above baseline in both legs at 28 d (CON $P < .001$, ECC + CON $P < .001$) and 84 d (CON $P < .001$, ECC + CON $P < .001$; Figure 3B). Similar to muscle CSA, there was no increase in muscle volume in either the CON leg or the ECC + CON leg from 28 to 84 d. No difference was seen in change in muscle volume with exercise training between CON and ECC + CON legs at any time point.

3.4 | Muscle mRNA expression

Heat maps were generated (Figure 4; GraphPad Prism version 7.03) to depict muscle mRNA expression relative to baseline (log_2) in both CON and ECC + CON legs at each time point

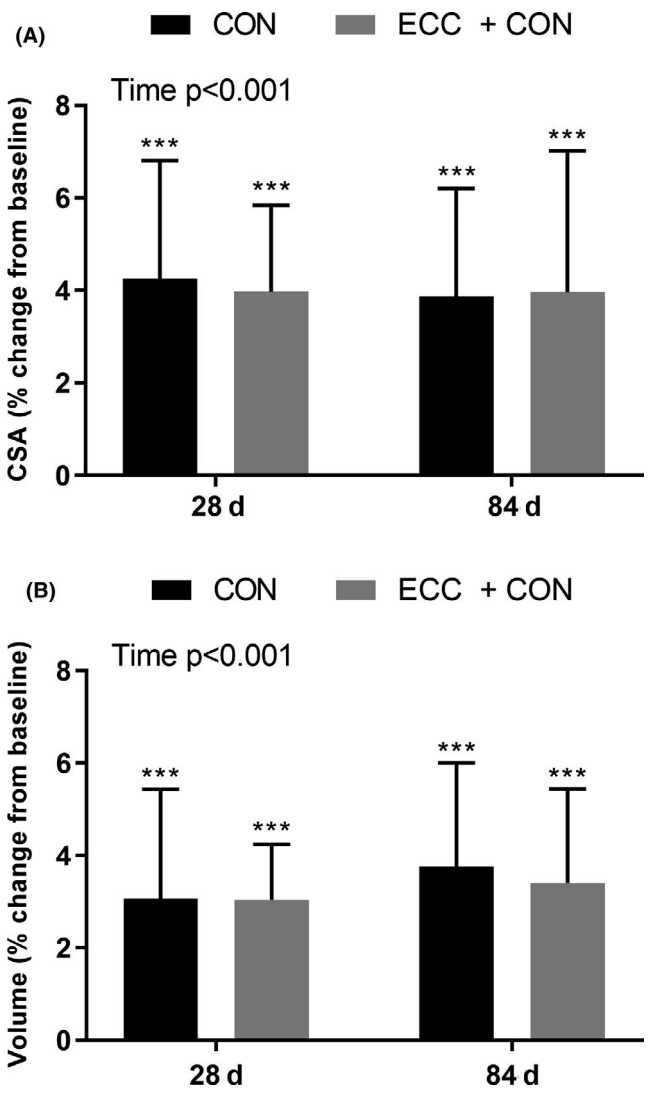

FIGURE 3 (A) Percentage change from baseline in mid-thigh quadriceps muscle cross-sectional area (%), and (B) quadriceps muscle volume (%) in concentric (CON) and combined concentric + eccentric (ECC + CON) trained legs at 28 days (28 d) and 84 days (84 d) of training. Values expressed as mean ± SD. ***$P < .001$ compared to baseline

for each volunteer. Figure 4 illustrates the change in mRNA abundance from mean baseline abundance in both CON and ECC + CON legs at 24 h (Figure 4A), 7d (Figure 4B), 28d (Figure 4C), and 84 d (Figure 4D). A temporal response to resistance training was evident, such that generally there was an apparent increase in mRNA abundance above baseline in both legs at 24 h (Figure 4A) and 7 d (Figure 4B), but this response had waned by 28 d (Figure 4C) and 84 d (Figure 4D). There was a noticeable pattern of divergence of response between legs, such that there was a greater increase in abundance of muscle mRNA transcripts in the ECC + CON leg compared to the CON leg at 24 h (Figure 4A) and 7 d (Figure 4B). This divergence in response between legs had also waned by 28 d (Figure 5A) and 84 d (Figure 5B).

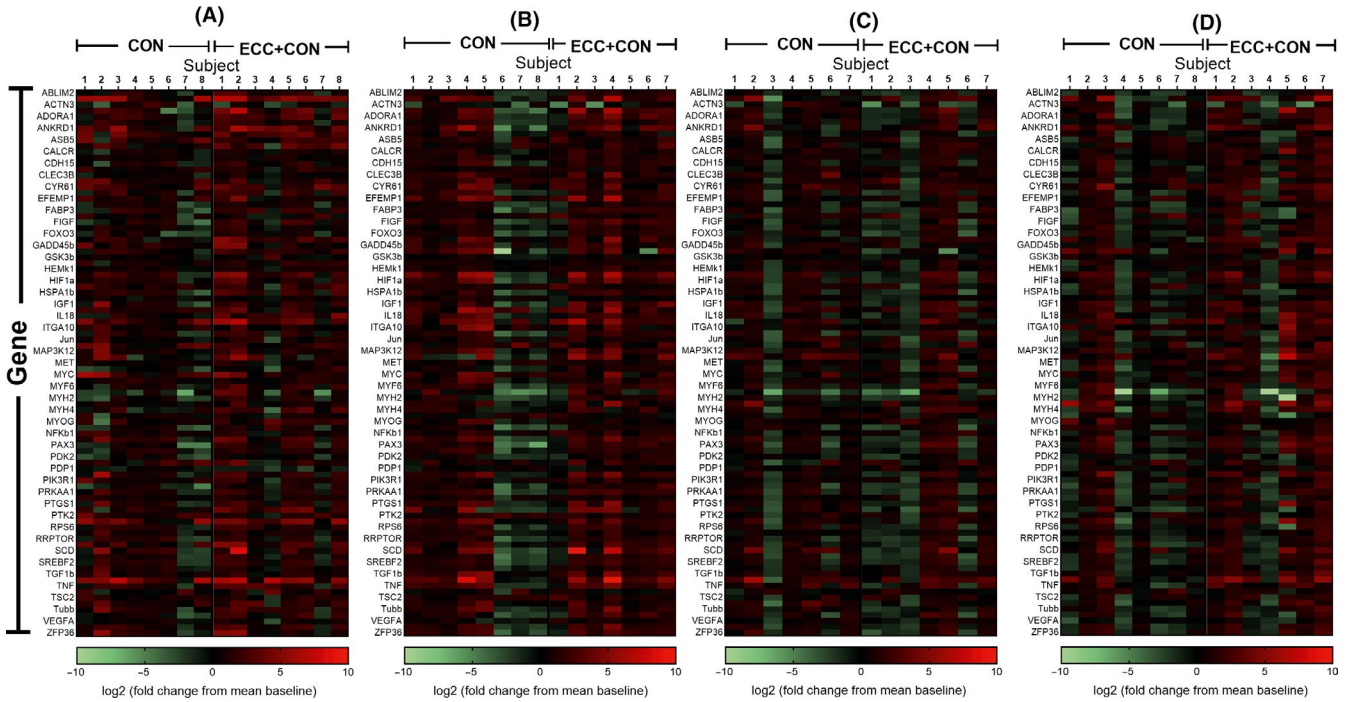

FIGURE 4 Data reflecting change in vastus lateralis mRNA abundance at 24 h (A), 7 d (B), 28 d (C) and 84 d (D) in concentric (CON) and combined concentric + eccentric (ECC + CON) trained legs for each participant (1 to 8). Change is depicted relative to mean baseline in the form of colors (heat map). Values are log2 of fold change from mean baseline. Individual gene abbreviations shown on y-axis (see Appendix A for definitions). Red indicates a greater abundance and green indicates less abundance relative to baseline

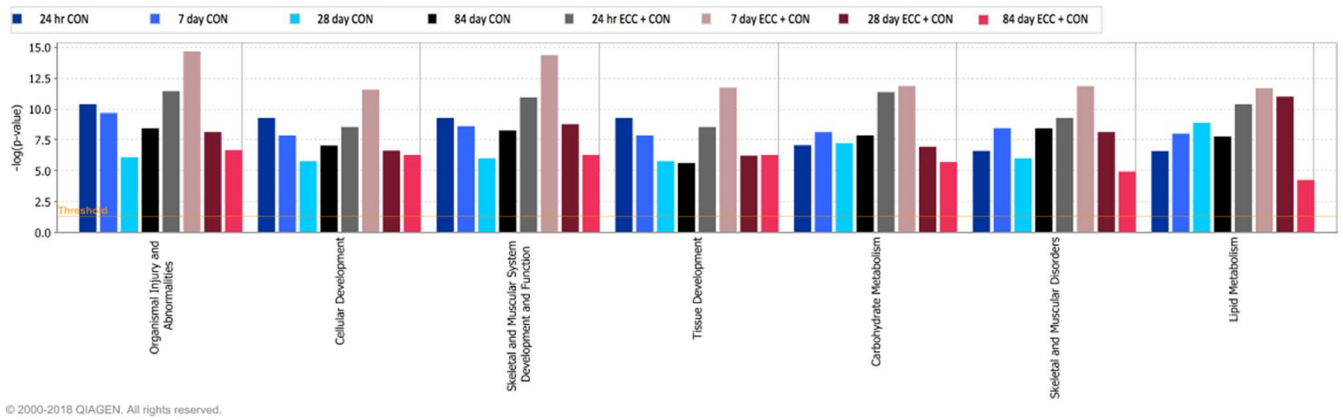

FIGURE 5 Cellular functions identified by IPA as being altered from baseline (n = 8) in vastus lateralis muscle in concentric (CON) and combined concentric + eccentric (ECC + CON) trained legs at 24 h, 7 d, 28 d, and 84 d based on mRNA expression data generated using the low-density microarray cards. The x-axis displays cellular functions most affected by training, whereas the y-axis displays the –log of the *P*-value. The –log of the *P*-value was calculated by Fisher's exact test right-tailed ($P < .05$). The threshold line corresponds to a *P*-value of .05

3.5 | Pathway analysis

Cellular functions most altered from baseline in the CON leg and ECC + CON leg at 24 h, 7, 28, and 84 d are depicted in Figure 5. The x-axis displays cellular functions most affected, while the y-axis displays the -log of the *P*-values. The *P*-value associated with each cellular function is a measurement of the likelihood that the association between a set of focus transcripts and a given function is due to random chance. The -log of *P*-value was calculated using Fisher's exact test (right-tailed). Overall, IPA detected altered cellular functions [–log (*P*-value) >5.0; Figure 5] associated with organismal injuries and abnormalities, cellular development, skeletal and muscular development and function, tissue development, carbohydrate metabolism, skeletal and muscular disorders, and lipid metabolism. Figure 5 also illustrates that in the main the largest magnitude of response to exercise training occurred at 24 h and 7 d in both limbs, which was invariably greatest in the ECC + CON trained leg and particularly at 7 d.

Figures 6 and 7 are included by way of example to illuminate precisely how any cellular function identified as being altered in Figure 5, in this case skeletal and muscular system development and function, was influenced by training, and to also highlight the clear differences in response between limbs at 24 h and 7 d. Each figure depicts the most differentially regulated mRNAs from baseline (outer ring), and the cellular events predicted to result from these collective mRNA changes in CON and ECC + CON legs at 24 h and 7 d. Similar schematics could be generated for all of the cellular functions highlighted in Figure 5, but in the interest of space this is impracticable. In the case of skeletal and muscular system development and function at 24 h (Figure 6), 16 mRNAs were identified as being altered in abundance relative to baseline in the CON leg (Figure 6A). The most upregulated mRNAs from baseline were tenascin C (TNC) and IL-6, while myostatin (MSTN), paired box gene 3 (PAX3) and peroxisome proliferator-activated receptor gamma coactivator 1 α (PPARGC1A) were the most downregulated transcripts. Based on the collective changes in mRNA abundance from baseline, IPA predicted among a number of things activation of migration of muscle cells, and inhibition of muscle formation and quantity of muscle with highest confidence at 24 h. In the ECC + CON leg at 24 h, 18 mRNAs were identified as being altered in abundance relative to baseline (Figure 6B). In contrast to the CON leg however, all mRNAs were increased in abundance. In common with the CON leg, IPA predicted activation of migration of muscle cells with high confidence. Proliferation of myoblasts and muscle cells, and formation of muscle were also predicted to be activated with high confidence. This latter prediction was in direct contrast to the CON leg, where formation of muscle was predicted to be inhibited with high confidence. In addition to this, inhibition of muscle function was predicted in the ECC + CON leg at 24 h, which was not predicted for the CON leg.

Figure 7 relates to skeletal and muscular system development and function at 7 d. Fourteen mRNAs were differentially regulated from baseline in the CON leg (Figure 7A), which were in the main the same transcripts that were changed in abundance at 24 h. Not surprisingly therefore, similar to 24 h, IPA predicted activation of migration of muscle cells, and inhibition of muscle formation with highest confidence at 7 d. Twenty-four mRNAs were found to be differentially expressed from baseline at 7 d in the ECC + CON leg, and similar to 24 h all were increased in expression (Figure 7B). Also, in keeping with 24 h, IPA predicted activation of migration of muscle cells, proliferation of myoblasts and muscle cells, formation of muscle, and inhibition of muscle function

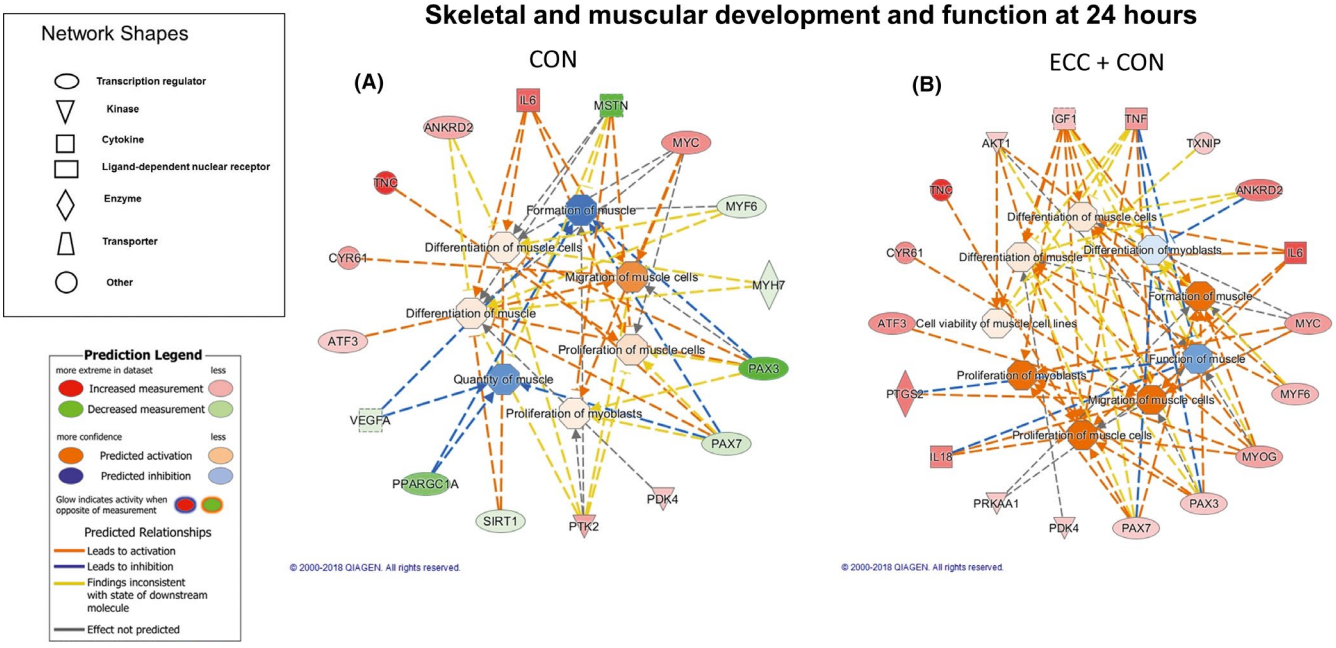

FIGURE 6 Differentially regulated muscle mRNAs (outer ring) associated with skeletal and muscular system development and function in (A) concentric (CON) and (B) combined eccentric + concentric (ECC + CON) trained legs at 24 h of training compared to baseline, and the cellular events predicted by Ingenuity Pathway Analysis to results from the collective changes in mRNA abundance. IL-6, interleukin 6; MSTN, myostatin; MYC, MYC proto-oncogene; MYF6, myogenic factor 6; MYH7, myosin heavy chain 7; PAX3, paired box 3; PAX7, paired box 7; PDK4, pyruvate dehydrogenase kinase 4; PTK2, protein tyrosine kinase 2; SIRT1, sirtuin 1; PPARGC1A, peroxisome proliferator-activated receptor α; VEGFA, vascular endothelial growth factor A; ATF3, activating transcription factor 3; CYR61, cysteine-rich angiogenic inducer 61; TNC, tenascin C; ANKRD2, ankyrin repeat domain 2; AKT1, AKT serine/threonine kinase 1; PRKAA1, protein kinase AMP-activated catalytic subunit α 1; IGF1, insulin growth factor 1; TNF, tumor necrosis factor; TXNIP, thioredoxin-interacting protein; IL-18, interleukin 18; PTGS2, prostaglandin-endoperoxide synthase 2

with highest confidence at 7 d in the ECC + CON leg. In addition to this, differences between legs in the cellular events predicted at 24 h were still evident at 7 d, that is, formation of muscle was predicted to be inhibited in CON but activated in ECC + CON, while the predicted inhibition of muscle function in ECC + CON was not seen in CON.

4 | DISCUSSION

The present findings revealed that the greater amount of work done during 84 d of unilateral combined concentric and eccentric maximal voluntary isokinetic exercise training in young, trained males did not result in differential temporal responses or gains in muscle CSA, muscle volume or strength when compared with the contralateral leg which performed only maximal voluntary concentric exercise training. Of importance, mRNA abundance associated with a number of cellular functions was altered from baseline in both limbs throughout the course of training, with changes in abundance being most marked at 24 h and 7 d in the limb that performed combined concentric and eccentric training. However, the early changes in mRNA abundance had waned in both limbs by 28 and 84 d of training. We conclude that initial mRNA abundance responses to maximal load exercise training are unlikely to reflect chronic training-induced muscle hypertrophy, suggesting some caution when extrapolating chronic muscle adaptive responses from acute exercise intervention studies. Moreover, neither the greater amount of work done over the course of 84 d of high-load combined concentric and eccentric training, nor marked differences in mRNA responses during the early phase of training, impacted on muscle CSA, volume or strength gains compared to concentric training alone.

We detected a ~4% increase in muscle CSA in both limbs. This magnitude of change is similar to other studies comparing hypertrophic responses to 10-12 weeks of maximal concentric resistance training versus maximal eccentric resistance training.[4,24] However, some studies have shown that eccentric training produces greater gains in muscle mass than concentric training at low-moderate intensities[30] and high intensities.[1] A meta-analysis by Roig et al[3] considered a number of studies that utilized a variety of exercise protocols, to compare muscle mass and strength gains following concentric versus eccentric training. The authors reported that eccentric training performed at high intensities may have resulted in greater muscle mass gains than concentric training. Of note however, in those studies where workloads were

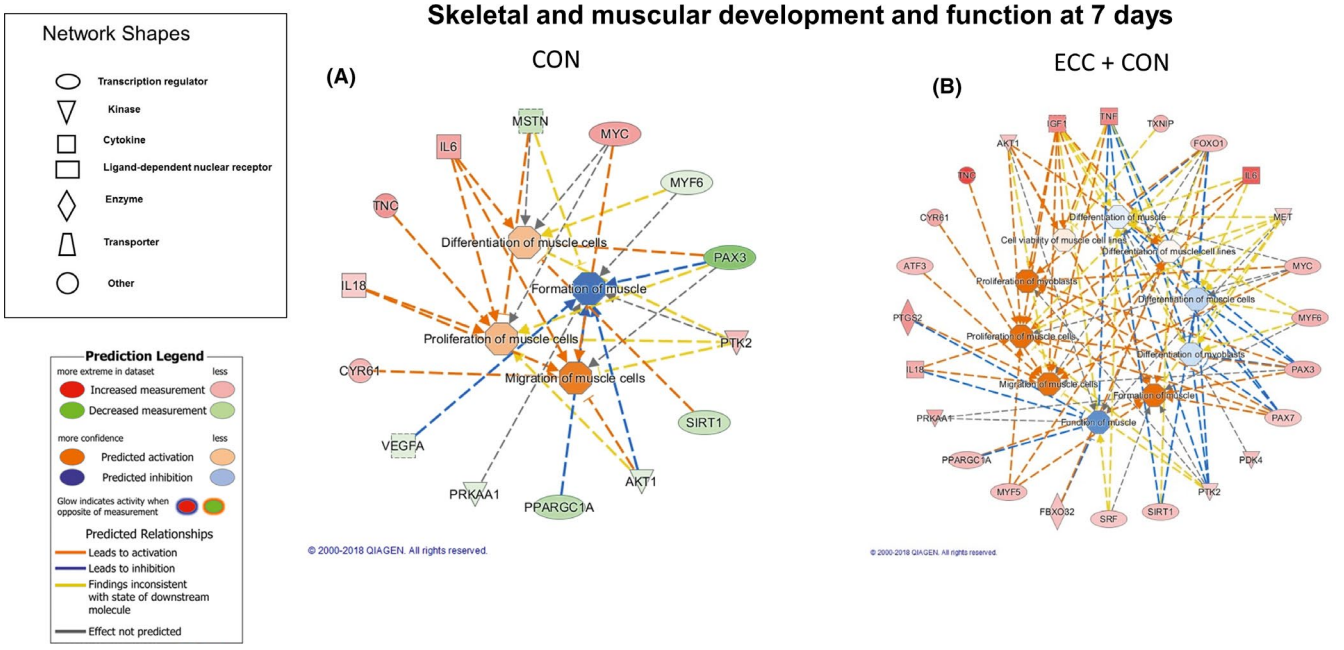

FIGURE 7 Differentially regulated muscle mRNAs (outer ring) associated with skeletal and muscular system development and function in (A) concentric (CON) and (B) combined eccentric + concentric (ECC + CON) trained legs at 7 d of training compared to baseline, and cellular events predicted by Ingenuity Pathway Analysis to results from the collective changes in mRNA abundance. AKT1, AKT serine/threonine kinase 1; ATF3, activating transcription factor 3; CYR61, cysteine-rich angiogenic inducer 61; FBXO32, F-box protein 32; FOXO1, forkhead box protein O 1; IGF1, insulin growth factor 1; IL-6, interleukin 6; IL-18, interleukin 18; MSTN, myostatin; MYC, MYC proto-oncogene; MYF5, myogenic factor 5; MYF6, myogenic factor 6; PAX3, paired box 3; PAX7, paired box 7; PDK4, pyruvate dehydrogenase kinase 4; PPARGC1A, peroxisome proliferator-activated receptor α; PRKAA1, protein kinase AMP-activated catalytic subunit α 1; PTGS2, prostaglandin-endoperoxide synthase 2; PTK2, protein tyrosine kinase 2; SIRT1, sirtuin 1; SRF, serum response factor; TNC, tenascin C; TNF, tumor necrosis factor; TXNIP, thioredoxin-interacting protein; VEGFA, vascular endothelial growth factor A

matched between concentric and eccentric training protocols, no difference was noted in muscle mass gains between the two modalities. The authors concluded therefore that the greater muscle forces generated during eccentric training was responsible for the differential gains in muscle mass. Moore et al[21] tested this proposition by having young male volunteers perform lengthening contractions of the biceps brachii using an isokinetic dynamometer with one arm and shortening contractions in the contralateral arm two times per week for nine weeks, but with work output matched between arms. In keeping with the conclusion of Roig et al,[3] a similar increase in muscle CSA was observed in each limb at the end of the training period. Conversely, more recent studies have reported when workloads were unmatched between concentric and eccentric training at 80% of 1-RM (1 repetition-maximum, using modified leg press), still no differences in gains in vastus lateralis muscle volume,[4] or vastus lateralis muscle thickness[31] were demonstrable over 8-10 weeks of training. Here we show that despite the ECC + CON leg performing more work per week (Figure 2A) and across the whole training period (Figure 2B), no differential temporal gains in muscle CSA (Figure 3A), volume (Figure 3B) or isometric strength over the CON leg were observed. This observation is supported by Cadore et al[32] who reported no difference in the increase in vastus lateralis muscle thickness, determined using ultrasound, over the course of 6-weeks isokinetic eccentric exercise training of the quadriceps compared to another group of volunteers who performed isokinetic concentric exercise, despite work output being ~40% greater over the course of training in the eccentric exercise trained group. Indeed, several studies have reported no differences in muscle mass adaptation when comparing maximal eccentric vs maximal concentric exercise training,[4,18] and recent studies have shown similar hypertrophic responses following concentric training versus eccentric training at 80% of 1-RM when workloads were not matched between the two training modalities.

Many studies to date have focused on acute muscle responses to eccentric exercise to provide insight on chronic muscle adaptation. However, based on the collective results of this study, it would be misleading to conclude that early mRNA responses to eccentric exercise training will reflect chronic training-induced muscle hypertrophy. This is illustrated by Figure 5 where, with perhaps the exception of lipid metabolism, the heightened effect of combined eccentric and concentric exercise on a number of cellular functions altered by exercise was found to be transitory. Moreover, Figures 6 and 7 clearly demonstrate mRNA responses in the first week of training were very different when comparing exercise modalities, with wholesale differences in mRNA abundance changes from baseline and cellular predictions evident between limbs. It remains unclear why the magnitude of mRNA responses declined from 28 d onwards in the ECC + CON limb despite training workloads remaining different between limbs.

Acute exercise intervention studies have reported greater muscle damage and inflammatory responses to be associated with high-load eccentric exercise, which has been proposed to stimulate myogenesis and cellular processes involved in muscle growth. For example, utilizing gene transcript profiling Chen et al[10] reported a greater muscle inflammatory response 4-8 h following 300 eccentric contractions of one leg when compared with 300 concentric contractions in the contralateral leg. Similarly, Kostek et al[23] identified 51 differentially expressed genes associated with protein synthesis, stress response/early growth, and sarcolemmal structure when comparing concentrically and eccentrically exercised limbs (in the form of rising from and controlled lowering to a seated position) over 24 h following exercise, albeit in n = 3 volunteers. In keeping with this, local NSAID infusion following acute maximal eccentric exercise in healthy volunteers was reported to decrease muscle satellite cell proliferation.[12] On the other hand, Deane et al[33] found the muscle transcriptional response to unilateral concentric and eccentric resistance exercise in young volunteers (using a modified leg press) was highly similar following 5 h of recovery, which may have been attributable to the volunteers being exercise naïve. The point being made here is that studies to date have invariably only documented the acute muscle responses to eccentric and concentric exercise. In the context of this point, although "organismal injury and abnormalities" was identified by IPA as a cellular function significantly altered by training in the present study, which in keeping with the literature was most altered in the ECC + CON limb at 24 h and 7 d of training compared to the CON limb (Figure 5), the magnitude of this insult had waned in both limbs by 28 d of training, and to an extent that the response between limbs was similar at 28 and 84 d (Figure 5). In short, the heightened effect of eccentric exercise on the muscle injury was short-lived and may therefore explain why eccentric training did not produce greater gains in muscle CSA and volume in the present study.

As an aside, studies investigating the impact of resistance exercise–induced inflammation per se on myogenesis and other processes linked to regulation of muscle mass appear to be equivocal. Using robust experimental designs involving local and systemic NSAID administration, it has been reported that the presence of inflammatory mediators in the first eight days of post-eccentric exercise recovery stimulated satellite cell proliferation (greater Pax7 + cells per myofiber) Mikkelsen et al.[12] Importantly, few studies have investigated the impact of chronic resistance exercise-induced inflammation on muscle adaptation. Trappe et al[17] reported 6-weeks of progressive resistance exercise training produced a ~25%-50% greater increase in muscle volume in elderly volunteers consuming either acetaminophen or ibuprofen during the

training period. The authors suggested that NSAID ingestion, by blocking inflammation, positively impacted on muscle hypertrophy. This may indeed be the case, given a number of cellular functions linked to muscle injury and growth were found to be significantly altered throughout the 12 weeks of exercise training in the present study, irrespective of whether concentric or eccentric exercise was performed (Figure 5). However, we must contextualize such a conclusion, given our participants were young, healthy males. Aging is associated with low-grade inflammatory state.[34] Hence, the muscle hypertrophy noted by Trappe et al[17] in elderly volunteers in response to a combination NSAID ingestion and progressive resistance training might have been due to an inhibition of this low-grade inflammation, rather than solely of inflammation induced by resistance training.

We examined the temporal effects of concentric versus combined concentric and eccentric chronic training on muscle CSA, muscle volume and strength. We observed that muscle CSA (Figure 3A) and volume (Figure 3B) increased by ~4% by 28 d with both training modalities, but perhaps surprisingly was unchanged thereafter despite work done during training being maintained. In keeping with this observation, DeFreitas et al[35] demonstrated the magnitude of increase in muscle CSA from baseline was greatest in the initial 4 weeks (6.7%) of high-intensity resistance training of the quadriceps compared to the increase recorded over 8 weeks (9.6%). Similarly, Brook et al[36] demonstrated a 2.66 ± 0.09 cm increase in vastus lateralis thickness from 0–3 weeks of leg resistance exercise training, which was unchanged (2.72 ± 0.09 cm) after 6 weeks despite a progressive increase in the training workload in this time. In keeping with this observation, by quantifying chronic changes in MPS using deuterated water tracer incorporation, the authors were also able to report an increase in quadriceps MPS over the initial 3 weeks of training, which then waned from 3-6 weeks. Hence, hypertrophic remodeling was most active during the early stages of training. This is in line with evidence indicating that chronic resistance training blunts the MPS response to acute resistance training.[37] Therefore, it seems that the MPS response to resistance training in the present study reached a plateau in both CON and ECC + CON legs between 28 and 84 d, which supports the concept that muscle adaptations to resistance exercise do not occur in a linear fashion.

Clearly, it is important to identify why greater force generation during eccentric training did not translate to greater gains in muscle CSA and volume in the present study. One possible explanation is that work produced by the muscle did not equate to that measured at the joint during the isokinetic maneuvers employed in the present study,[38] which could be viewed as a methodological limitation. An alternative explanation is that the MPS response to resistance exercise reaches an upper ceiling at submaximal exercise intensities. Kumar et al[24] reported that the MPS response to resistance exercise was dose-dependent and plateaued at an intensity of 75%-80% of 1-RM, suggesting that MPS was maximized in both CON and ECC + CON legs in the present study. Alternatively, it is known that the energy cost of eccentric exercise is considerably less than concentric exercise, despite muscle mechanical force generation being greater.[39] This being the case, it is entirely feasible that increases in muscle metabolic stressors known to be linked to muscle protein synthetic and hypertrophic responses, such as the cellular energy charge, the cellular redox state, and muscle lactate and pH,[40] would be less during eccentric compared with concentric exercise. However, is worthy of note that the plateauing of the muscle hypertrophic response to training in both limbs after 28 d in the present study coincided with the waning of muscle mRNA responses in both limbs. Additionally, there was no progression in the training workload after 28 d of training (Figure 2A), suggesting a connection between events. Indeed, as volunteers performed maximal intensity isokinetic contractions throughout training, one possibility is that an over training response occurred at the onset of training in both limbs that blunted subsequent adaptations.

One potential limitation of a within-subject bilateral study design is the possibility of a cross-education effect. However, this invariably applies to muscle functional adaptations.[41] Given primary end-point measurements of the present study were muscle hypertrophy and localized muscle mRNA responses to the two different exercise modalities (rather than functional gains), we foresaw cross-education having little to no impact. Indeed, since both limbs performed maximal intensity contractions, and the ECC + CON limb performed 20% more work throughout training when compared with CON (due to the biomechanical properties of an eccentric contraction; see Figure 2), it is difficult to rationalize how a cross-over effect could have impacted on these primary end-point measurements. This conclusion is corroborated by muscle CSA and volume gains being no different throughout training, and muscle mRNA expression changes from baseline being similar at 28 and 84 d of training when comparing limbs. Moreover, strength gains were different between ECC + CON and CON limbs, which also supports our stance.

To conclude, this study found that while combined maximal, isokinetic concentric and eccentric training produced greater work output during a 12-week training regimen compared to maximal, isokinetic concentric training, this did not translate to more rapid or greater increases in quadriceps muscle CSA, volume or strength. Furthermore, changes in muscle mRNA abundance associated with a number of cellular functions were observed with both training modalities, but was most marked at 24 h and 7 d in the limb that performed combined concentric and eccentric training. However, the early changes in mRNA abundance waned in both limbs by 28 d of training, and it is unclear why this occurred in the

face of training workloads being sustained and different between limbs. Thus, neither the greater amount of mechanical work done during combined eccentric and concentric training, nor marked differences in mRNA responses during the early phase of training, was associated with differential gains in muscle CSA, volume or strength compared to concentric training alone. This may be explained by a non-linear relationship existing between mechanical force generation and muscle protein synthetic responses to exercise and/or the blunted metabolic stress of eccentric exercise diminishing hypertrophic stimulation.

5 | PERSPECTIVE

Whether high-load eccentric exercise training induces greater muscle hypertrophy and strength gains than concentric exercise training alone, possibly due to greater muscle loading and/or inflammation, is controversial. In this study, 12 weeks of combined maximal concentric-eccentric training did not produce greater hypertrophy or strength gains compared to maximal concentric training alone, despite ~20% more work being done in the former. TaqMan low-density gene arrays revealed changes in several cellular functions associated with muscle mass regulation/metabolism during the first week of training with both exercise modalities, but particularly during concentric-eccentric training. However, these mRNA responses waned thereafter regardless of exercise modality. Initial mRNA responses to maximal load exercise training did not reflect chronic training-induced muscle hypertrophy, suggesting some caution when extrapolating chronic adaptive responses from acute studies. Moreover, neither greater loading nor differential mRNA responses with combined concentric-eccentric training produced greater hypertrophy or strength gains than concentric training alone.

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CONFLICT OF INTERESTS

None.

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REFERENCES

1. Higbie EJ, Cureton KJ, Warren GL 3rd, Prior BM. Effects of concentric and eccentric training on muscle strength, cross-sectional area, and neural activation. *J Appl Physiol*. 1996;81(5):2173-2181.
2. Vikne H, Refsnes PE, Ekmark M, Medbo JI, Gundersen V, Gundersen K. Muscular performance after concentric and eccentric exercise in trained men. *Med Sci Sports Exerc*. 2006;38(10):1770-1781.
3. Roig M, O'Brien K, Kirk G, et al. The effects of eccentric versus concentric resistance training on muscle strength and mass in healthy adults: a systematic review with meta-analysis. *Br J Sports Med*. 2009;43(8):556-568.
4. Franchi MV, Atherton PJ, Reeves ND, et al. Architectural, functional and molecular responses to concentric and eccentric loading in human skeletal muscle. *Acta Physiol (Oxf)*. 2014;210(3):642-654.
5. Paulsen G, Mikkelsen UR, Raastad T, Peake JM. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev*. 2012;18:42-97.
6. McHugh MP. Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scand J Med Sci Sports*. 2003;13(2):88-97.
7. Hather BM, Tesch PA, Buchanan P, Dudley GA. Influence of eccentric actions on skeletal muscle adaptations to resistance training. *Acta Physiol Scand*. 1991;143(2):177-185.
8. Dudley GA, Tesch PA, Miller BJ, Buchanan P. Importance of eccentric actions in performance adaptations to resistance training. *Aviat Space Environ Med*. 1991;62(6):543-550.
9. Moore DR, Phillips SM, Babraj JA, Smith K, Rennie MJ. Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions. *Am J Physiol Endocrinol Metab*. 2005;288(6):E1153-E1159.
10. Chen YW, Hubal MJ, Hoffman EP, Thompson PD, Clarkson PM. Molecular responses of human muscle to eccentric exercise. *J Appl Physiol*. 2003;95(6):2485-2494.
11. Hubal MJ, Chen TC, Thompson PD, Clarkson PM. Inflammatory gene changes associated with the repeated-bout effect. *Am J Physiol Regul Integr Comp Physiol*. 2008;294(5):R1628-R1637.
12. Mikkelsen UR, Langberg H, Helmark IC, et al. Local NSAID infusion inhibits satellite cell proliferation in human skeletal muscle after eccentric exercise. *J Appl Physiol*. 2009;107(5):1600-1611.
13. Armand AS, Launay T, Gaspera BD, Charbonnier F, Gallien CL, Chanoine C. Effects of eccentric treadmill running on mouse soleus: degeneration/regeneration studied with Myf-5 and MyoD probes. *Acta Physiol Scand*. 2003;179(1):75-84.
14. Serrano AL, Baeza-Raja B, Perdiguero E, Jardi M, Munoz-Canoves P. Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. *Cell Metab*. 2008;7(1):33-44.
15. McKay BR, De Lisio M, Johnston AP, et al. Association of interleukin-6 signalling with the muscle stem cell response following muscle-lengthening contractions in humans. *PLoS One*. 2009;4(6):e6027.
16. Krentz JR, Quest B, Farthing JP, Quest DW, Chilibeck PD. The effects of ibuprofen on muscle hypertrophy, strength, and soreness during resistance training. *Appl Physiol Nutr Metab*. 2008;33(3):470-475.
17. Trappe TA, Carroll CC, Dickinson JM, et al. Influence of acetaminophen and ibuprofen on skeletal muscle adaptations to resistance exercise in older adults. *Am J Physiol Regul Integr Comp Physiol*. 2011;300(3):R655-R662.

18. Blazevich AJ, Cannavan D, Coleman DR, Horne S. Influence of concentric and eccentric resistance training on architectural adaptation in human quadriceps muscles. *J Appl Physiol*. 2007;103(5):1565-1575.
19. Jones SW, Hill RJ, Krasney PA, O'Conner B, Peirce N, Greenhaff PL. Disuse atrophy and exercise rehabilitation in humans profoundly affects the expression of genes associated with the regulation of skeletal muscle mass. *FASEB J*. 2004;18(9):1025-1027.
20. Tesch PA, Dudley GA, Duvoisin MR, Hather BM, Harris RT. Force and EMG signal patterns during repeated bouts of concentric or eccentric muscle actions. *Acta Physiol Scand*. 1990;138(3):263-271.
21. Moore DR, Young M, Phillips SM. Similar increases in muscle size and strength in young men after training with maximal shortening or lengthening contractions when matched for total work. *Eur J Appl Physiol*. 2012;112(4):1587-1592.
22. West DW, Burd NA, Tang JE, et al. Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. *J Appl Physiol*. 2010;108(1):60-67.
23. Kostek MC, Chen YW, Cuthbertson DJ, et al. Gene expression responses over 24 h to lengthening and shortening contractions in human muscle: major changes in CSRP3, MUSTN1, SIX1, and FBXO32. *Physiol Genomics*. 2007;31(1):42-52.
24. Kumar V, Selby A, Rankin D, et al. Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol*. 2009;587(1):211-217.
25. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest*. 1975;35(7):609-616.
26. Murton AJ, Billeter R, Stephens FB, et al. Transient transcriptional events in human skeletal muscle at the outset of concentric resistance exercise training. *J Appl Physiol*. 2014;116(1):113-125.
27. Constantin D, Constantin-Teodosiu D, Layfield R, Tsintzas K, Bennett AJ, Greenhaff PL. PPARdelta agonism induces a change in fuel metabolism and activation of an atrophy programme, but does not impair mitochondrial function in rat skeletal muscle. *J Physiol*. 2007;583(Pt 1):381-390.
28. Crossland H, Constantin-Teodosiu D, Gardiner SM, Constantin D, Greenhaff PL. A potential role for Akt/FOXO signalling in both protein loss and the impairment of muscle carbohydrate oxidation during sepsis in rodent skeletal muscle. *J Physiol*. 2008;586(22):5589-5600.
29. Mallinson JE, Constantin-Teodosiu D, Sidaway J, Westwood FR, Greenhaff PL. Blunted Akt/FOXO signalling and activation of genes controlling atrophy and fuel use in statin myopathy. *J Physiol*. 2009;587(1):219-230.
30. Hortobagyi T, Barrier J, Beard D, et al. Greater initial adaptations to submaximal muscle lengthening than maximal shortening. *J Appl Physiol*. 1996;81(4):1677-1682.
31. Franchi MV, Ruoss S, Valdivieso P, et al. Regional regulation of focal adhesion kinase after concentric and eccentric loading is related to remodelling of human skeletal muscle. *Acta Physiol*. 2018;223(3):e13056.
32. Cadore EL, Gonzalez-Izal M, Pallares JG, et al. Muscle conduction velocity, strength, neural activity, and morphological changes after eccentric and concentric training. *Scand J Med Sci Sports*. 2014;24(5):e343-e352.
33. Deane CS, Ames RM, Phillips BE, et al. The acute transcriptional response to resistance exercise: impact of age and contraction mode. *Aging*. 2019;11(7):2111-2126.
34. Schaap LA, Pluijm SM, Deeg DJ, et al. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. *J Gerontol Ser A Biol Sci Med Sci*. 2009;64(11):1183-1189.
35. DeFreitas JM, Beck TW, Stock MS, Dillon MA, Kasishke PR 2nd. An examination of the time course of training-induced skeletal muscle hypertrophy. *Eur J Appl Physiol*. 2011;111(11):2785-2790.
36. Brook MS, Wilkinson DJ, Mitchell WK, et al. Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling. *FASEB J*. 2015;29(11):4485-4496.
37. Phillips SM, Parise G, Roy BD, Tipton KD, Wolfe RR, Tamopolsky MA. Resistance-training-induced adaptations in skeletal muscle protein turnover in the fed state. *Can J Physiol Pharmacol*. 2002;80(11):1045-1053.
38. Herzog W. The relation between the resultant moments at a joint and the moment measured by an isokinetic dynamometer. *J Biomech*. 1988;21(1):5-12.
39. Hoppeler H. Eccentric exercise: physiology and application in sport and rehabilitation. In: *Science RRiSaE*. Oxon, UK: Routledge Taylor and Francis Group; 2014.
40. Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab*. 2013;17(2):162-184.
41. Hortobagyi T, Lambert NJ, Hill JP. Greater cross education following training with muscle lengthening than shortening. *Med Sci Sports Exerc*. 1997;29(1):107-112.

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APPENDIX A

List of genes used on low-density RT-PCR array microfluidic cards (Applied Biosystems Inc, Foster City, CA, USA).

Symbol	Gene Name
ABLIM2	Actin Binding LIM Protein Family Member 2
ACTC1	Actin, alpha, cardiac muscle 1
ACTN3	Alpha-actinin-3
ACVRC1	Activin A receptor 1
ADORA1	Adenosine A1 receptor
AKT1	Serine-threonine protein kinase
ANKRD1	Ankyrin repeat domain 1
ANKRD2	Ankyrin repeat domain 2
ASB5	Ankyrin repeat and SOCS box containing 5
ATF3	Activating transcription factor 3
CALCR	Calcitonin receptor
CD34	CD34
CDH15	Cadherin 15
CEBPB	CCAAT/enhancer binding protein (C/EBP), beta
CLEC3B	C-type lectin domain family 3, member B
CTSL1	Cathepsin-L
CYR61	Cysteine-rich angiogenic inducer 61
DNAJB4	DnaJ (Hsp40) homolog, subfamily B, member 4
EFEMP1	EGF containing fibulin-like extracellular matrix protein 1
EIF2B	Eukaryotic translation initiation factor 2B, subunit 2 beta
FABP3	Fatty acid binding protein 3, muscle and heart
FBXO32	F-box protein 32
FIGF	Cellular oncogene c-fos
FOXO1	Forkhead transcription factor 1A
FOXO3	Forkhead transcription factor 3
GADD45a	Growth arrest and DNA damage-inducible protein alpha
GADD45b	Growth arrest and DNA damage-inducible protein
GEM	GTP-binding protein overexpressed in muscle
GSK3b	Glycogen synthase kinase 3 beta
HDAC9	Histone deacetylase
HEMk1	HemK methyltransferase family member 1
HGF	Hepatocyte growth factor
HIF1a	Hypoxia inducible factor 1, alpha subunit
HSPA1a	Heat shock protein (70 kDa) 1A
HSPA1b	Heat shock protein (70 kDa) 1B
IFRD1	Interferon-related developmental regulator 1
IGF1	Insulin-like growth factor 1
IGJ	JCHAIN
IL-18	Interleukin-18
IL-6	Interleukin-6
ITGA10	Integrin, alpha 10
JKAMP	Mitogen-activated protein kinase 8
Jun	c-jun
MAFF	MAF bZIP transcription factor F
MAP3K12	Mitogen-activated protein kinase kinase kinase 12
MDF1	MAD (yeast Mitosis arrest deficient) related
MET	MET proto-oncogene
MSTN	Myostatin
MYC	MYC proto-oncogene
MYF5	Myogenic factor 5
MYF6	Myogenic factor 6
MYH1	Myosin heavy chain 1
MYH2	Myosin heavy chain 2
MYH3	Myosin heavy chain 3
MYH4	Myosin heavy chain 4
MYH7	Myosin heavy chain 7
MYOG	Myogenin
NEDD4	Neural precursor cell expressed, developmentally downregulated 4-like
NFKb1	Nuclear factor of kappa light polypeptide gene enhancer in B cells 1
NFKb2	Nuclear factor of kappa light polypeptide gene enhancer in B cells 2
PAX3	Paired box 3
PAX7	Paired box 7
PDK2	Pyruvate dehydrogenase kinase, isoenzyme 2
PDK4	Pyruvate dehydrogenase kinase, isoenzyme 4
PDP1	Pyruvate dehydrogenase phosphatase catalytic subunit 1
PFK1	Phosphofructokinase 1
PIK3R1	Phosphatidylinositol 3-kinase, regulatory 1 (p85 alpha)
PPARGC1A	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
PRKAA1	Protein kinase AMP-activated catalytic subunit α 1
PSMA1	Proteasome subunit alpha 1
PTGS1	Cyclooxygenase 1
PTGS2	Cyclooxygenase 2
PTK2	Focal adhesion kinase
Rabb15	Member RAS oncogene family
RPS6	Ribosomal protein S6
RPS6KA	Ribosomal protein S6 kinase
RRPTOR	Regulatory-associated protein of MTOR, complex 1

(Cotninues)

(Cotninues)

APPENDIX A Continued

Symbol	Gene Name
RRAD	Ras-related Glycolysis Inhibitor And Calcium Channel Regulator
SCD	Stearoyl-Coenzyme A desaturase 1
SIRT1	Sirtuin 1
SREBF2	Sterol Regulatory Element Binding Protein
SRF	Serum Response Factor
TGF1b	Transforming growth factor, beta 1
TNC	Tenascin C
TNF	Tumor necrosis factor alpha
TRIM63	Tripartite motif containing 63
TSC2	Tuberous sclerosis 2
Tubb2A	Tubulin beta 2A
Tubb	Tubulin beta
TXNIP	Thioredoxin-interacting protein
VEGFA	Vascular endothelial growth factor alpha
YWZHA	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase
ZFP36	Zinc finger protein 36, C3H type