Effects of Essential Amino Acid Supplementation on Muscular Adaptations to 3 Weeks of Combined Unilateral Glenohumeral & Radiohumeral Joints Immobilisation

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Abstract

Background: Short-term immobilisation results in a decrease in muscle size and strength. Ingestion of essential amino-acids (EAAs) stimulates net protein synthesis and supplementation is shown to improve lean body mass, strength and physical function, even without exercise. This study set out to determine whether EAA supplementation would attenuate immobilisation-induced changes in muscle characteristics.

Methods: In n=16 healthy, habitually active participants from a mixed gender (n=10 females, 5 males) population aged 21 ± 3.1 years, the non-dominant arm was immobilised in a sling for 9 waking hours a day over 3 continuous weeks. Participants were randomly assigned to one of two groups: EAA or placebo treatment. The nutritional supplement treatment was consumed throughout the immobilisation period. Measures of muscle thickness (via b-mode ultrasonography), upper and lower arm girth (anthropometry), isotonic torque (dynamometry), muscle activation (electromyography), and serum interleukin-6 (ELISA) were taken immediately before immobilisation (PRE) and immediately on removal of the sling (POST).

Results: The decrease relative to baseline in arm girths was significantly greater with the placebo (-1.75% and -1.48%) than with the EAA supplement (-0.31% and -0.55%) in both the upper (p=0.01) and lower (p=0.045) arm. The direction of change in biceps sub-cutaneous adipose thickness differed significantly between the placebo and EAA supplements (-3.3% and 19.6%, respectively, p=0.03). Torque normalised by muscle thickness in the biceps decreased less in the EAA compared with the placebo group (-6% vs. -20%, P<0.05). Similarly, normalised torque in the triceps also changed differently in the EAA compared with the placebo group (+15% vs. -23%, P<0.05).

Conclusion: We conclude that EAA supplementation impacts positively on the immobilisation-induced changes in the structural and functional characteristic of the remaining muscle. Our findings are relevant to both sporting (e.g. off-season detraining modulation) as well as clinical (e.g. injury/illness induced short-term immobilisation/bed rest) populations.

Keywords

Essential amino acids; Immobilisation; Muscle size; Muscle strength; Sub-cutaneous adiposity

Abbreviations: EAA: Essential Amino Acid; EAAs: Essential Amino Acids; CSA: Cross-Sectional Area; IL-6: Interleukin-6; MVC: Maximum Voluntary Contraction; EMG: Electromyography

Introduction

Skeletal muscle has adaptive potential, meaning it is capable of altering its structure in response to environmental changes. In other words, the human muscular system shows great plasticity in response to different levels of physical activity. Prolonged reductions in muscle activity and mechanical loading, such as those experienced during limb suspension [1] bed-rest [2] and immobilisation/hypo-activity [3] result in numerous physiological adaptations in skeletal muscle structure and function. It has consistently been demonstrated that long periods of hypo-activity result in skeletal muscle atrophy [4,5] and a decrease in maximal voluntary strength [3,6-10]. However, discrepancies exist between the reductions in muscle strength and in muscle size seen with hypo-activity models [3,11-13]. These observations would tend to suggest that part of the observed reduction in Maximal Voluntary Contraction (MVC) may be due to reduced drive from the central nervous system to the muscle.

Reductions in muscle mass with hypo-activity may be the result of a decrease in protein synthesis, an increase in protein breakdown or a combination of the two [14]. On the topic of potential for increased protein breakdown, it is noted that periods of immobilisation have been associated with increased fatty tissue deposition [15]. Adipose tissue produces and secretes inflammatory cytokines, for example IL-6 [16] and the expression and plasma levels of such cytokines increase with increased adiposity [17,18]. The elevation of “pro-inflammatory” cytokines such as IL-6 is generally viewed to be potentially deleterious with regard to skeletal muscle. For example, it has been observed that IL-6 either directly or indirectly mediates catabolic effects on skeletal muscle [19]. It should be noted that whilst some IL-6 signalling is also important to initiate the cascade to signal protein synthesis [20] findings from Haddad et al. suggest that down-regulation of growth factor-mediated intracellular signalling may be a mechanism contributing to the development of muscle atrophy induced by significantly elevated IL-6 [21]. On the topic of potential for decreased protein synthesis, it is noted that Ferrando et al. reported a loss of lean muscle mass, accompanied with a 14% decrease in protein synthesis and no change in protein breakdown in response to 14 days simulated microgravity [22]. Gibson et al. noted a marked fall in muscle protein synthesis in response to 7 weeks leg immobilisation [23]. More recently a shorter period of immobilisation (21 days) provided very little evidence of any increases in mRNA for catabolic enzymes or in enzyme activity during this period [24]. In contrast to this there is some suggestion in the literature that such increases in catabolic potential do occur, and for this event to happen very quickly

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(48 hours) after immobilisation [25]. Nonetheless, the weight of the evidence would suggest that it is unlikely that protein breakdown is a key modulator in the process of muscle atrophy occurring during immobilisation in humans [26,27].

There is clear evidence that physical activity (and resistance exercise in particular) provides an anabolic stimulus during hypoactivity [14,28,29]. Moderate-resistance exercise (5 sets × 8 reps to volitional fatigue) every other day during 14 days bed-rest prevented a decline in muscle protein synthesis, whereas bed-rest alone elicited a 46% decrease in muscle protein synthesis [29]. Isometric leg press training (3 sets × 30 reps) during 20 days of bed-rest resulted in no changes in physiological CSA, whereas in the bed-rest control group physiological CSA decreased by 7.8% [28]. A number of nutritional interventions exist that have proven anabolic effects. For instance, when supplemented with either whole proteins [30] or simply essential amino acids (EAAs) [31], the benefits of exercise appears additive. It may not, however, always be practical to prescribe exercise to counteract the atrophy brought about by hypo-activity. This would tend to be linked to the presence of counter-indications for exercise such as pain or immobilisation in a cast. In these cases, supplementing the diet with potential/recognised hypertrophic nutrients may be an intervention of choice for preventing the loss of muscle mass/function seen with hypo-activity. Promising results have been observed in research utilising an increased protein intake (from 0.6 to 1.0 g·kg⁻¹·day⁻¹) to ameliorate the catabolic responses to 1 week of bed rest [32].

Unlike protein supplementation, EAA supplementation does not affect satiety, and further, does not alter the metabolic effects of subsequent meals [33]. This thus means that EAAs are likely to be the preferred nutritional supplement. Bolus oral ingestion of EAAs produces a rapid, several-fold increase in plasma amino acid levels [34] and has been shown to stimulate net protein synthesis to a greater extent than a mixed meal or a solution containing nonessential amino acids [35]. Studies have in fact shown that providing a nutritional supplement enriched with EAAs could improve lean body mass, strength and physical function, even without exercise [36].

It is evident from previous research that immobilisation decreases lean body mass and that EAA supplementation improves lean body mass in several models from increased physical activity [37] to bed rest [34]. Whilst these findings are interesting, they cannot be extended to all models of hypoactivity. Indeed previous studies show that each model of hypo-activity impacts on muscle structure and function differently [38] hence decreasing our ability to extrapolate the effects of one treatment between models of hypo-activity. To our knowledge, research is lacking in immobilisation studies in humans in which EAA supplementation is given as an intervention.

The aim of the present study was therefore to determine the role that EAA supplementation may play in attenuating atrophy induced through a model that would emulate relatively short-term decreased local mobility/activity in humans. The differential effect of immobilisation on in vivo muscle (size, strength), sub-cutaneous adipose tissue (thickness), neural (EMG activity) and cytokine (circulating Interleukin-6) factors, in the presence or absence of EAA supplementation, was systematically monitored. It was hypothesised that EAA supplementation during combined shoulder and arm immobilisation would attenuate the deleterious changes in muscle, fat and cytokine in vivo characteristics associated with hypo-activity models.

Materials and Methods

Participants

Eighteen healthy volunteers were recruited from the local university campus; via word of mouth and posters. All participants gave written informed consent to take part in the study. Prior to commencement of the study, the local Ethics Committee of Manchester Metropolitan University approved all procedures and experimental protocols. Exclusion criteria included any conditions requiring the use of medications likely to affect muscle function or musculoskeletal health, and any current or history of kidney/liver disease. Before taking part in the study, participants completed a questionnaire to ascertain health and habitual physical exercise levels. The questionnaire confirmed that all participants were recreationally active and free from recent upper limb injury. Participants were randomly assigned to one of two groups, though two subsequently withdrew during the course of the study for personal reasons. Hence, the present report describes data from the remaining sixteen participants: essential amino acid group [EAA: n = 9 (3 male, 6 female), 20 ± 1.5 yrs, 63.8 ± 8.1 kg, 169.6 ± 8.7 cm] and placebo group [Placebo: n = 7 (2 male, 5 female), 23 ± 3.9 yrs, 66.9 ± 9.5 kg, 167.0 ± 7.2 cm].

Study design

The study used a randomised, double-blind, placebo-controlled design. Following familiarisation with testing procedures at least one week prior to testing, participants attended the laboratory between 8:00 and 10:00 AM after an overnight fast. The non-dominant arm of the participants was placed in a sling immediately after baseline testing and the correct procedure for sling wear was explained to them. Essentially the aim was to minimise any movement medio-laterally at the elbow and shoulder (Figure 1) whilst requiring participants to not contract the upper musculature (including the hands) during the hours of immobilisation. Participants were required to wear the sling for approximately 9 waking hours per day, over a 3-week period. Removal of the sling was only permitted when necessary (e.g. taking a bath/showering, driving, going to bed at night). Measures of muscle and subcutaneous fat thickness, upper and lower arm girth, serum cytokine, isometric torque and electromyographic activation were taken immediately before immobilisation (PRE; i.e. on day 1 of immobilisation) and immediately on removal of the sling (POST; i.e. within 2 days of remobilisation). Details of all the procedures are outlined below.

During the 3 weeks immobilisation participants in the EAA group ingested 2×30 mL per day of a commercially available essential amino acid drink (BodyFortress, Holland & Barrett, UK), whereas participants in the placebo group ingested a placebo drink. Each 30mL dose of the Liquid Amino nutritional supplement is described by
manufacturer as containing 22g of protein i.e.: 1121 mg L-Alanine; 1223 mg L-Arginine; 655 mg L-Aspartic Acid; 1456 mg L-Glutamic Acid; 3394 mg Glycine; 131 mg L-Histidine; 218 mg L-Hydroxylysine; 1791 mg L-Hydroxyproline; 175 mg L-Isoleucine; 378 mg L-Leucine; 480 mg L-Lysine; 131 mg L-Methionine; 233 mg L-Phenylalanine; 1995 mg L-Proline; 495 mg L-Serine; 277 mg L-Threonine; 87 mg L-Tyrosine; and 320 mg L-Valine. The placebo mixture consisted of a homemade blend of water, wild cherry flavouring, honey, salt and a red food colorant, designed to emulate the appearance and to a degree the taste of the EAA supplement. At day 1 of immobilisation, participants were given clear instructions regarding the dosage of the formula. Participants ingested the twice-daily dose of EAA or placebo at 8h00 and 18h00. During the 3-week immobilisation period, participants were instructed to maintain their normal diet and refrain from unaccustomed strenuous activity. To monitor this, participants completed a daily food and activity diary and wore a pedometer to record the number of steps taken each day.

**Muscle and sub-cutaneous adipose thickness measures**

To avoid fluid shifts that might induce interstitial and/or intracellular changes all images were recorded after approximately 20 minutes of seated rest [39]. B-mode ultrasonography (AU5, Esaote, Genoa, Italy), using a 7.5-MHz linear phased-array probe (image depth: 37.1-92.8 mm), applied in the sagittal plane to obtain images of the muscles and sub-cutaneous adipose tissue of the upper arm. Images were recorded using an analogue capture card (Pinnicale DV500, Adobe, Maidenhead, UK) and stored for later analysis. Care was taken to apply minimal pressure onto the tissue area of interest during scanning in order to avoid any image distortion. This method has been used many times previously both by others and ourselves, with great reliability [40-42].

Images were obtained with the participant in an upright seated position, their arms hanging by the sides in the anatomical position. In the upper arm the proximal and distal insertions of the biceps and triceps brachii were identified, marked on the skin and the mid-point was identified. Upper arm ultrasonography images were collected in the sagittal plane, mid-limb length, and at the level of the mid-acromio-radial. Muscle thickness was measured as the distance from the top of the superficial muscle aponeurosis to the bone in the biceps and triceps brachii (Figure 2). Sub-cutaneous adipose thickness was measured as the distance from the bottom of the epidermis to the top of the superficial muscle aponeurosis in the biceps and the triceps brachii (Figure 2). These distances were measured at three standardised points on each ultrasound frame to obtain average muscle and sub-cutaneous adipose thicknesses using ImageJ analysis software (Image) 1.37, Maryland, USA).

**Arm girths**

Participants were asked to assume a relaxed standing position with arms hanging by the sides, palms facing the sides of the hips. Upper arm girth (relaxed) was measured using a measuring tape at the level of the mid-acromio-radial: with the arm abducted slightly to allow the tape to be passed around. Forearm girth was measured at a fixed point 1/4 of the way (from the proximal end) along the length of the radiale-styloion: with the arm slightly flexed at the shoulder and the elbow extended.

**Isometric dynamometry**

Maximal isometric torque was measured using a Cybex dynamometer (Cybex, New York, USA). Participants were positioned as per the manufacturers’ recommendations. Briefly, they were fastened to the dynamometer in a supine position with the trunk and lower limbs firmly strapped to minimise any extraneous movement. The axis of rotation of the dynamometer was aligned with the anatomical axis of rotation of the elbow joint (lateral epicondyle) and the upper arm secured using padded strapping, mid acromial-radial. The participant’s functional range of motion was measured and safety stops were set both electronically and manually, to prevent hyper-extension/flexion. A gravity correction was made for limb weight on torque measurements. All measurements were preceded by a warm-up period consisting of 2 mins of isokinetic contractions increasing in intensity, followed, after 2 mins rest, by one isometric contraction at a 90° elbow joint angle. Following a further 2 mins rest, two repetitions of isometric contractions, 60 sec apart, were performed at a 90° elbow joint angle. During all MVC attempts participants were instructed to rapidly exert maximal torque against the Cybex lever arm over a 3-4 s period, first in the direction of flexion and, 5 sec after return to baseline, in the direction of elbow extension. Participants were encouraged to exert maximal torque through a combination of verbal and visual feedback. Torque and angle were displayed on the screen of a computer (Macintosh G4; Apple Computer, Cupertino, CA), which was interfaced with an A/D system (Acknowledge, Biopac Systems, Santa Barbara, CA) with a sample frequency of 200 Hz. Peak torque was averaged over a 500 ms period (i.e. 250 ms either side of the instantaneous peak). The highest of the repeated efforts was used as the participant’s measure of MVC.

**Electromyographic measurement**

EMG was used to assess muscle activation patterns. The skin was prepared by shaving, abrading and cleaning with an alcohol-wipe to minimise resistance below 5kΩ. Self-adhesive Ag-Cl (Medicotest, Rugmarken, Denmark) electrodes were then placed in pairs on the
midsagittal plane of the biceps and the triceps brachii muscles, with reference electrodes placed on the lateral and medial epicondyle of the humerus. These data were obtained using the same system that acquired the torque data (as described above). Raw EMG data were recorded at 2000 Hz, with a band pass filter set at 10-500 Hz, with a notch set at 50 Hz.

Interleukin-6 (IL-6)

After a 10-12-hour overnight fasting period, before any of the other laboratory measures, blood samples were taken from the antecubital vein of the forearm by a hospital-trained phlebotomist, using a 21 ml gauge needle (S-Monovette, Sarstedt, Germany). Subsequently, 5-10 mL of venous blood was permitted to clot on ice for up to 1 hour. Samples were then centrifuged (Hermle Z 380, Huddersfield) at 5°C at 7500 rpm for 15 minutes to separate the serum from the blood cells. Aliquots (~1000 μl each) of the resulting sera were stored at -2°C for later analysis. IL-6 (R&D systems, Abingdon, UK) in the sera samples was quantified using standard quantikine high sensitivity (i.e. up to 10 pg/mL of IL-6) enzyme linked immunosorbant assay (ELISA). Optical density was read at 490 nm with a lambda correction at 650 nm. Intra-assay (CV = 7.4%) and inter-assay (CV = 7.8%) precisions, were all within acceptable boundaries. The minimum detectable dose (MDD) ranged from 0.016 to 0.110 pg/mL (average = 0.039 pg/mL).

Statistics

Data were analysed using IBM SPSS v19 (IBM Inc, USA). The Shapiro-wilk test revealed some of the data to be non-parametric (triceps fat thickness, triceps muscle thickness change, biceps torque, biceps torque change, EMG and serum IL-6) and in those cases, the data sets were log transformed. Once transformed the data was checked for normality. Parametric PRE to POST difference data were analysed using paired sample t-tests. If non-parametric, the differences between PRE to POST were analysed using the Wilcoxon signed-rank test. Between group differences in relative change data were analysed either using unpaired t-tests (if parametric), or Mann-Whitney U test (if non-parametric). All data are presented as mean ± standard deviation (SD). Statistical significance was set with alpha at ≤0.05.

Results

Measurements reliability

The assessment of the intra and inter day repeatability of measurements was conducted in a sub-sample of five participants. Those randomly chosen participants were asked to attend a separate repeatability testing date for between day measures; no less than 7 days prior to their pre-immobilisation testing session. The repeatability of unilateral isometric strength, EMG, muscle and fat thickness, as well as anthropometry were measured at the same time-of day to avoid any difference owing to diurnal effects [43]. Within-day coefficients of variation (CV in %) of 4.9%, 4.9%, 3.6%, 8.9%, 0.6% and 4.4%, and between-day CVs of 8.6%, 8.6%, 4.0%, 7.4%, 1.7% and 6.4% were yielded for unilateral elbow flexion torque at 90°, unilateral elbow extension torque at 90°, mid-upper arm biceps muscle thickness, mid-upper arm triceps muscle thickness, mid-upper arm biceps fat thickness and mid-upper arm triceps fat thickness, respectively.

Daily physical functioning and nutritional intake

No significant change was observed in habitual physical activity (p>0.05) or in calorific intake (p>0.05) during the course of immobilisation. This effect was true for both the EAA and the placebo groups. Dietary analysis revealed no significant difference in protein intake over the three week period between the EAA and placebo groups (Figure 3).

Muscle and sub-cutaneous adipose thicknesses

Muscle and sub-cutaneous adipose thickness values are displayed in Table 1. Comparisons of percentage change in biceps and triceps muscle thicknesses revealed no significant differences between the EAA and placebo groups (p>0.05 in both cases). Comparison of percentage change in biceps sub-cutaneous adipose thickness revealed a significant difference between the EAA and placebo groups (Student’s t-test; p<0.03). Comparison of percentage change in triceps sub-cutaneous adipose thickness revealed no significant difference between the EAA and placebo groups (Student’s t-test; p>0.05).

Arm girth

Pair-wise (Students t-test) comparisons revealed that upper and lower arm girths decreased significantly post-immobilisation in the placebo group (30.6 ± 3.6 cm to 30.1 ± 3.6 cm (p<0.01) and 25.9 ± 1.9 cm to 25.4 ± 1.8 cm (p<0.01), respectively) but not the EAA group (29.3 ± 3.3 cm to 29.2 ± 3.2 (p>0.05) and 25.0 ± 1.8 to 24.9 ± 1.8 (p>0.05), respectively). Percentage change in arm girth

![Figure 3: Protein intake as a function of study phase and group. A) Habitual dietary content of protein. There was no difference in the food diary reported diet of the two study populations. B) Total protein intake with amino acid supplementation accounted for. † denotes significant group differences in protein intake.](http://dx.doi.org/10.4172/2324-9080.1000116)
was significantly greater in the placebo group than the EAA group in both the upper and lower arm (Student’s t-test; p=0.01, p=0.045, respectively) (Figure 4).

Isometric dynamometry

Elbow flexion torque values are shown in Table 2a. Pair-wise (Students t-test) comparisons revealed elbow flexion torque decrease from pre- to post-immobilisation only in the EAA group (p=0.04).

Elbow extension torque values are shown in Table 2b. Despite the apparent trend for relative decrease in elbow flexion and extension torque values, to be greater in the placebo group than EAA group, this effect was not significant (p>0.05).

Normalising elbow flexion torque by biceps muscle thickness, this ratio decreased by 6% in the EAA group (0.81 Nm/mm (PRE) to 0.76 Nm/mm (POST)) and 20% in the placebo group (0.80 Nm/mm (PRE) to 0.64 Nm/mm (POST)). Normalising elbow extension torque by triceps muscle thickness, this ratio increased by 15% in the EAA group (0.60 Nm/mm (PRE) to 0.69 Nm/mm (POST)) and decreased by 23% in the placebo group (0.65 Nm/mm (PRE) to 0.50 Nm/mm (POST)).

Agonist and antagonist EMG activity

EMG data were non-parametric. Values for agonist EMG activity and antagonist co-contraction ratios are shown in Table 2a and 2b respectively. Statistical analysis revealed no within or between group differences in agonist EMG activity or antagonist co-contraction ratios in either flexion or extension isometric contractions.

Circulating IL-6 levels

Changes in IL-6 are shown in Figure 5. A pair-wise (Students t-test) comparison revealed no significant effect of supplement group on the degree of percentage change in IL-6 values from pre- to post-immobilisation (p>0.05). Interestingly, the graphical representation suggests a slight trend towards increased IL-6 levels pre- to post-immobilisation (p>0.05). † Significant difference in % change between essential amino acid and placebo groups. Percentage changes from pre- to post-immobilisation are also reported. * Significantly different change from pre- to post-immobilisation (p<0.05). † Significant difference in % change between essential amino acid and placebo groups.

Table 1: Differences in muscle and sub-cutaneous adipose thickness measurements pre- and post-immobilisation (mm ± SD) of the biceps and the triceps brachii. Values are reported for the essential amino acid and placebo groups. Percentage changes from pre- to post-immobilisation are also reported (% change ± SD). * Significantly different change from pre- to post-immobilisation (p<0.05). † Significant difference in % change between essential amino acid and placebo groups.

Table 2a: Differences in elbow flexion torque (Nm ± SD), agonist activation (mV ± SD) and co-activation ratio (% ± SD) pre- and post-immobilisation. Values are reported for the essential amino acid and placebo groups. Percentage change in mean torque and agonist EMG activity from pre- to post-immobilisation are also reported. * Significantly different change from pre- to post-immobilisation (p<0.05). † Significant difference in % change between essential amino acid and placebo groups.

Table 2b: Differences in elbow extension torque (Nm ± SD), agonist activation (mV ± SD) and co-activation ratio (% ± SD) pre- and post-immobilisation. Values are reported for the essential amino acid and placebo groups. Percentage change in mean torque and agonist EMG activity from pre- to post-immobilisation are also reported. * Significantly different change from pre- to post-immobilisation (p<0.05). † Significant difference in % change between essential amino acid and placebo groups.

Association studies

Bivariate correlations between relative change values for all reported measures are displayed in Table 3. The change in bicep sub-cutaneous adipose thickness correlated with the change in triceps brachii muscle thickness (r=0.57, p=0.01). The relative change in upper arm girth correlated with percentage changes in lower arm girth (r=0.70, p=0.001), elbow extension torque (r=0.52, p=0.03), and IL-6 levels (r=0.56, p=0.02). Percentage changes in extension torque and IL-6 levels were also strongly correlated (r=0.80, p<0.001).
The decrease in lower arm girth (1.1% = 0.05%/day) and volume (11.6% = 0.4%/day) with arm immobilisation supports previous findings of a decrease in elbow flexor muscles and a decrease in muscle CSA in the upper arm in the placebo group and with a notable trend towards a greater decrease in triceps brachii muscle thickness, in the current study suggest that changes in muscle CSA are lower than those reported in previous studies [8,10]. This apparent discrepancy with the rest of the literature, whereby we have observed relatively small degrees of atrophy during limb immobilisation where others have observed (though not always) a substantial atrophic response could be due to the following: a) the method of measuring the change in muscle CSA.

In the present study we used arm girth and sagittal plane ultrasound scans as markers of muscle size, whereas previous studies have used MRI as a direct measure; b) the method of immobilisation and resultant immobilisation compliance: the immobilisation in the present study was for 9 waking hours a day with a self-administered sling, compared to continuous immobilisation in other studies which used casts for the full immobilisation period. Nonetheless, differences in changes in arm girth from pre- to post-immobilisation between the EAA and placebo groups suggest that EAA supplementation may attenuate changes in muscle size. This supports promising results observed in 1 week of bed rest that showed increased protein intake (from 0.6 to 1.0 g·kg⁻¹·day⁻¹) to modulate the catabolic response [32].

The significant difference in limb CSA is not reflected entirely in our ultrasound data, since the current study found no change in biceps muscle thickness but a decrease in triceps brachii muscle thickness post-immobilisation. The fact that there is a decrease in skeletal muscle tissue with immobilisation, even though relatively moderate in its duration/restriction of movement, was in fact not surprising [8]. However, we had in fact expected the muscle held in the shortened position (i.e. the biceps), to be impacted on more, than the muscle held in the lengthened position (i.e. the triceps brachii). Muscle stretch is a modulator of sarcomeres being added in series and as such acts as a signal for protein synthesis. Previous research suggests that when the muscle is immobilised in the lengthened position sarcomeres are added in series, and when the muscle is immobilised in the shortened position sarcomeres are lost [46,47].

Our findings are in contrast with those of Yue et al. [10] who...
investigated the effect of 4 weeks elbow joint immobilisation with a fibre glass cast and reported decreases in elbow flexor CSA (11.2% = 0.4%/day) and volume (11.6% = 0.4%/day). One probable cause of the discrepancy between ours and Yue et al. [10] study could be the method of quantifying muscle size. Whereas those authors utilised CSA using MRI scans of the whole muscle, in the present study, we on the other hand, used single plane scan of the biceps at mid-limb length (which does not correspond to mid muscle length). These differences are key, in relation 1) the fact that the biceps is a fusiform muscle and thus any positional change along its length would result in a difference in size. Since it is recognised that the greatest degree of muscle hypertrophy for a muscle is at the belly [48] it is not surprising that in the present study the degree of change reported is reduced relative to that of those previous authors. 2) the reliability of the single sagittal scans (e.g. CV = 2.2% in our hands) versus that of CSA (error for repeated measures = <1%) [10] could also be a factor on the ability to capture the real size alteration events in the muscle. It should be noted nonetheless, that a large number of studies have previously found decreases in thigh (0.8-1.2%/day) [3,13] and calf (0.3-0.6%/day) [49,50] muscle CSA with lower limb immobilisation using a variety of techniques including computed tomography [3,49] ultrasonography [13] and limb circumference measures [50]. Observations are that the degree of muscle atrophy is greater in the lower limbs than in the upper limbs. This may be explained by the weight-bearing nature and greater musculature in the lower limbs. It may be the case, that the threshold of muscle synthesis is such that it takes little decrease in loading of lower limbs to demonstrate a change in protein metabolism in favour of decreased protein synthesis [51].

Contrary to our expectation, no main effect was shown for supplement group on muscle thickness of the biceps or of triceps brachii. This does not support a study that showed a decrease in the catabolic response to 1 week of bed-rest with increased protein intake [32]. Stuart et al. investigated the effects of 7 days bed-rest on two groups consuming iso-caloric diets containing either 0.6 or 1.0 g·kg⁻¹·day⁻¹ of protein and found that the decrease in protein synthesis seen with low dietary protein, was prevented by higher dietary protein [32]. However, they did not measure muscle size and the changes found in protein synthesis may not have resulted in phenotypical changes in muscle size. Comparisons between the study of Stuart et al. and the current study are therefore difficult, particularly as their treatment manipulated whole protein content of iso-caloric diets, whereas the current study supplemented the habitual diet with EAs.

The amount of supplement provided in the present study may not have been sufficient to induce an attenuation of muscle thickness decrease. An alternative explanation for the small effectiveness of EAA supplementation in this study can be through the "muscle full" hypothesis [52], in which there is an upper limit of amino acid delivery before muscle cells can no longer use them as a substrate for muscle protein synthesis, instead diverting them toward oxidation [32,53]. We found that the mean percentage change in biceps subcutaneous adipose thickness was significantly different in the placebo group than in the EAA group, whereby placebo demonstrated an increase in sub-cutaneous adipose thickness from pre- to post-immobilisation, and the EAA group exhibited a slight decrease in sub-cutaneous adipose thickness. This is in agreement with previous studies [15]. EAA supplementation therefore appeared to attenuate the increase in biceps subcutaneous adipose thickness. Studies have shown that providing a nutritional supplement enriched with EAs could improve lean body mass even without exercise [36]. In this case, the results may suggest that the EAA supplement is working towards maintaining lean mass by reducing the increase in fat mass. Indeed levels of circulating insulin help modulate the long-term level of fat stored in the body in any particular environment [54,55]. EAA ingestion has been shown to significantly increase insulin levels [34] and therefore, this may provide an explanation as to why the EAA supplement attenuated the increase in subcutaneous adipose thickness in the current study.

As mentioned in the introduction, previous research suggests that immobilisation results in a greater decrease in muscle strength than muscle size. Miles et al. investigated the effects of 9 days cast immobilisation suspended from the neck by a sling on the muscles acting on the wrist [8]. They reported a decrease in muscle CSA (4.1%) and reductions in isometric (29.3 to 32.5%), concentric (8.9% to 21.7%) and eccentric strength (12.5 to 18.5%) [8]. A study using casting to immobilise the elbow joint for four weeks found decrements in MVC and a decrease in the maximum load that could be lifted [10]. Again here, the reduction in MVC (35%) was greater than the observed decrease in muscle size (11%) [10]. Similarly, in the present study we generally found a greater percentage loss in muscle torque (elbow flexion: -11.5 to -19.8%; elbow extension: -1.0 to -27.8%) than in muscle thickness (biceps: -0.4% to -5.1%; triceps brachii: -5.3% to -11.7%). This therefore further supports the idea of neural factors being involved in the mal-adaptations to immobilisation.

Elbow flexion torque significantly decreased in the EAA supplement group. Looking at the percentage change in mean values it appears that elbow flexion torque decreases in both supplement groups with immobilisation and that this percentage decrease appears greater in the placebo group, although not significantly so. Similarly, in elbow extension the percentage change in muscle torque appears to decrease more in the placebo group than the EAA group. Gross ratios of ‘isometric strength to muscle thickness’ revealed greater decrements in the placebo group compared to EAA group. For the biceps muscle this ratio decreased by 6% in the EAA group and 20% in the placebo group. For elbow extension torque by triceps muscle thickness, the ratio in fact increased by 15% in the EAA group whereas it decreased by 23% in the placebo group. This is interesting as it appears that despite a decrease in triceps muscle thickness in the EAA group, the intrinsic quality of the remaining muscle was in fact improved. This may be due to the triceps brachii being immobilised in a lengthened position, allowing sarcomeres to be added in series [46,47] with additional availability of EAs.

Data collected for agonist and antagonist EMG activity highlighted no differences in agonist or antagonist co-activation from pre- to post-immobilisation. Previous research on EMG responses to immobilisation point to large decreases in EMG amplitude measurements during flexion in both the agonist and antagonist muscle [10,56]. Vaughan examined the EMG characteristics during extension and found significant decreases occurred in agonist peak EMG amplitude and antagonist peak EMG amplitude [56]. Care should be taken when drawing conclusions from the EMG findings of the above as well as the present study. Indeed, a) the dimensional changes in the muscle could mean that a different population of motor units is likely being recorded from [57], b) the reliability of EMG assessment in the present study (CV = -11% within day, and
~20% between days) as in previously published studies [58,59] is not very high, and this is a general limitation of studies utilising longitudinal EMG monitoring.

We found no significant changes in IL-6 from pre- to post-immobilisation in either EAA or placebo group. However, there appeared to be a non-significant trend towards increased IL-6 levels post-immobilisation in both groups. Research in humans demonstrates an increase in cytokine release in response to periods of bed-rest [60-62]. The muscle investigated in the current study is small (upper arm contains ~5.1% of body mass) and as such any endocrine effects would be limited when compared to bed-rest in which more of the musculature is sedentary [60-62]. As mentioned in the introduction, there is a link between increased fatty tissue deposition [15], and increased expression and plasma levels of cytokines, such as IL-6 [17,18]. In the present study, it would appear that any observed changes in subcutaneous adipose tissue was not great enough to impact on circulating IL-6 levels.

Correlation analyses revealed that those participants who experienced a greater change in triceps brachii muscle thickness also had a greater change in triceps brachii sub-cutaneous adipose thickness. This suggests that any adaptations in the triceps brachii with immobilisation occur in both the muscle and the sub-cutaneous adipose content. Unsurprisingly, greater changes in upper arm girth were associated with greater changes in lower arm girth, denoting that both the directly immobilised, as well as anatomically proximate musculature, exhibit deleterious responses to increased local hypo-activity. Changes in upper arm girth were associated with elbow extension torque. The changes to the musculature therefore, appear to be not only quantitative but also qualitative. Similarly, the fact that relative changes in IL-6 levels were associated with changes in upper arm girth and elbow extension torque, suggests that increased circulating IL-6 may only be a small reflection of greater changes at a cellular level.

Conclusion

We observed a trend for a positive effect of EAA supplementation on upper and lower arm girth post-immobilisation. This was accompanied by a trend towards a positive effect of EAA supplementation on elbow flexor (biceps) and extensor (triceps brachii) torque. The multi-component nature of the supplement makes it difficult to identify which specific amino-acid or combination thereof, may have been responsible for the observed changes. Based on the literature, the likelihood is that leucine was the main effector (for a recent review, read Breen and Phillips [63]). We suggest that our results warrant future research in arm immobilisation of similar duration. In particular, we propose that the model used in this current study could have relevance to a sporting population in which short-term immobilisation may be prescribed (e.g. treatment for minor injury). We would propose the use of single genders populations, as well as multiple sites being monitored along the length of the muscle. In this manner, the overall impact of EAA supplementation (e.g. on elbow flexors/ extensors) may be further clarified.

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