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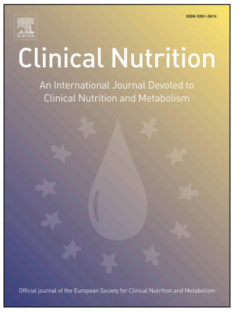

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Enriching a protein drink with leucine augments muscle protein synthesis after resistance exercise in young and older men

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Running title: Leucine enhances MPS responses to exercise in ageing

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ABSTRACT

Maximizing anabolic responses to feeding and exercise is crucial for muscle maintenance and adaptation to exercise training. We hypothesized that enriching a protein drink with leucine would improve anabolic responses to resistance exercise (RE: 6×8 knee-extension repetitions at 75% of 1-RM) in both young and older adults. Groups (n=9) of young (24±6 y, BMI 23±2 $kg.m^{-2}$) and older men (70±5 y, BMI 25±2 $kg.m^{-2}$) were randomized to either: (i) RE followed by Slim-Fast Optima (SFO 10 g PRO; 24 g CHO) with 4.2 g of leucine (LEU) or, (ii) RE+SFO with 4.2 g of alanine (ALA; isonitrogenous control). Muscle biopsies were taken before, immediately after, and 1, 2 and 4 h after RE and feeding. Muscle protein synthesis (MPS) was measured by incorporation of [1, 2-$^{13}C_2$] leucine into myofibrillar proteins and the phosphorylation of p70S6K1 by immunoblotting. In young men, both area under the curve (AUC; FSR 0-4 h $P<0.05$) and peak FSR (0.11 *vs.* 0.08%.$h.^{-1}$; $P<0.05$) were greater in the SFO+LEU than in the SFO+ALA group, after RE. Similarly, in older men, AUC analysis revealed that post-exercise anabolic responses were greater in the SFO+LEU than SFO+ALA group, after RE (AUC; FSR 0-4 h $P<0.05$). Irrespective of age, increases in p70S6K1 phosphorylation were evident in response to both SFO+LEU and SFO+ALA, although greater with leucine supplementation than alanine (fold-change 2.2 *vs.* 3.2; P<0.05), specifically in the older men. We conclude that addition of Leucine to a sub-maximal PRO bolus improves anabolic responses to RE in young and older men.

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INTRODUCTION

Ingestion of protein at rest (1) or after resistance exercise (RE) (2) stimulates muscle protein synthesis (MPS) through anabolic signaling (mechanistic target of rapamycin (mTOR) signaling pathway) in both young and elderly muscle (3,4). However, synthetic responses after acute resistance exercise in fasted (4) and postprandial states (5) and in response to feeding alone (6–9) have been shown to be blunted in older age. Since basal muscle protein turnover in the post-absorptive condition in healthy old people is found to be similar to rates in young muscle (6,9) these blunted responses of elderly muscle seem to be key factors in the aetiology of their gradual age related muscle loss and would thus be a target for intervention to prevent or slow the progression of sarcopenia.

Post-exercise ingestion of nutrients (protein and essential amino acids (EAA) with or without CHO) has been shown to elevate MPS above that measured following RE alone in both young (10–12) and elderly individuals (3,13) primarily due to EAA (14–16) and particularly leucine (17,18) (at least in young individuals). Although leucine, and other EAA (19) have been shown to stimulate MPS in humans acutely over 90 min, it is unlikely that this anabolic effect would be sustainable without provision of other EAA which would become limiting for MPS; clearly this is not a viable long term strategy to promote MPS and muscle growth, but it may provide a route by which MPS can be maximised when protein intake is low or insufficient to maximally stimulate MPS i.e. less than 20g in any meal. Furthermore, recent studies have shown, that the attenuated muscle protein synthetic and anabolic signalling responses to food intake in the elderly, can be compensated by increasing the leucine concentration of a meal in resting state (8,20). However, Dickinson and colleagues demonstrated that MPS following RE was maximally stimulated with 20 g EAA (containing 1.85 g Leu), and further supplementation with Leucine to 3.5 g could not further stimulate MPS (21). We have also recently demonstrated in elderly women that a low dose leucine enriched EAA mix (3g EAA, 1.2g leucine), was as effective as 20 g Whey protein in extending the stimulation of MPS following RE (22), suggesting there is a ceiling beyond which adding leucine is ineffectual. In contrast however, Yang et al showed a clear dose response of MPS to RE with increasing amounts of whey protein (up to 40 g, equivalent to approx. 1g of leucine for every 10 g of Whey) (13), indicating there was no maximum response. Despite this spurious data, it seems that the ingestion of leucine enriched EAA/ protein supplements following RE may provide an effective strategy to improve post-exercise MPS in the elderly, without the need for ingesting overly large amounts of protein.

Therefore, the goal of the present study was to assess the impact of leucine and sub-maximal protein ingestion using a meal replacement strategy i.e. Slimfast Optima, after an acute bout of resistance exercise on muscle protein synthesis (MPS) and anabolic signalling, particularly activation of mTOR signalling pathway, in young and older muscle. We hypothesised that enriching a sub-maximal protein feed, i.e. 10g with leucine shortly after a bout of RE would enhance anabolic responses in elderly men.

METHODS

Subject Recruitment and screening

The study was approved by the University of Nottingham Ethics Committee and complied with the Declaration of Helsinki. Written informed consent was obtained from the volunteers following explanation of the study protocol and procedures and any associated risks. Groups of 27 young and 27 older men were recruited for the exercise ± nutritional intervention studies (Subject Characteristics, see Table 1). All our recruits were physically independent and healthy. Screening procedures included a clinical history, physical examination, electrocardiogram, by a qualified physician. In addition a full blood count, coagulation profile, fasting blood glucose, and markers of liver, kidney, and thyroid function were assessed. Subjects were excluded if they had a history of metabolic disease (e.g. diabetes, thyroid disorders, obesity, anaemia, cancer) and any of the following cardiac, pulmonary, liver, kidney, vascular (including clotting) disorders, and poorly controlled hypertension; also excluded were those who showed evidence of alcohol abuse, palpable muscle wasting, corticosteroid use or the inability to discontinue aspirin therapy. Older subjects with mild controlled hypertension (<140/90 mm Hg) were admitted to the study, but refrained from taking medication on the study day.

For subjects passing screening procedures, we measured the maximal strength of the dominant leg on a leg extension machine (ISO leg extension, Leisure Lines (GB) Ltd) and they underwent a familiarization protocol of the exercise regime. Body composition, i.e. lean body mass, was assessed by dual-energy X-ray absorptiometry (DXA; GE Lunar Prodigy II, GE Healthcare).

Study design and optimization of feeding timing

Preliminary studies were undertaken to: (i) determine the time-course of the rise in blood AA after consumption of a can of SlimFast Optima, and particularly the timing of the peak AA concentration; (ii) determination of the time-course of the rise of leucine concentration in the blood after consuming gelatine capsules containing 4.2 g of leucine; (iii) adjusting the timing of ingestion of the leucine capsule in relation to the SlimFast Optima, to ensure the peak AA concentration coincided, thereby determining the post-exercise feeding schedule. This approach was chosen in order to synchronize the appearance of peak AA, which would be determined by digestion, gut transport and splanchnic metabolism – rather than exercise conditions – hence we did this simply under resting conditions in a small number of subjects.

These were performed on 3 young volunteers, who took part in all three studies. In each case an 18 g cannula was inserted into an antecubital vein of the postabsorptive volunteer and a blood sample was taken before subjects ingested either, (i) a full can (325ml) of SlimFast Optima; or (ii) 4.2 g of leucine alone; finally 4.2 g leucine was given followed by SlimFast Optima 30 min later (estimated from the difference in peak AA concentrations from i and ii) to confirm coincident appearance in the blood. Blood was sampled over 2.5 h at 20 min intervals into Lithium-Heparin tubes and plasma separated immediately and analyzed for AA (Figure 2) using an ion-exchange AA analyser (Biochrom 30, Biochrom Ltd, Cambridge).

For the principal studies, three groups each (n=9) of young and old were randomly assigned to: (i) RE + 325 ml SlimFast Optima (SFO) with 4.2 g of Leucine (LEU) (SFO+LEU), or (ii) RE+SFO with 4.2 g of alanine (ALA) (SFO+ALA) as control. All subjects performed 6 sets of 8 repetitions of an isotonic, full cycle unilateral leg extension and flexion exercise at 75% of 1 RM. Each subject received a full can (325 ml) of SlimFast Optima (10 g PRO + 24 g CHO; protein and AA composition: 8g casein, 2g whey, 0.05g soy, 0.95 g leucine, 0.30 g alanine, 0.36 g isoleucine and 0.76 g valine) and 4.2 g of leucine or alanine (the latter as an isonitrogenous control for the leucine) capsules. We purposely decided to give SFO containing a sub-maximal dose of protein i.e. 10g, (~4.5 g of EAA) to our subjects in both the leucine and alanine groups following the resistance exercise to demonstrate the efficacy of adding leucine; also since Moore *et al.*, have recently shown in healthy young men that ingestion of 20 g intact protein (or about 8.6 g EAAs as in the SFO+LEU group) is sufficient to maximally stimulate MPS (1); we gave a sub-maximal dose in order to observe an increase in response to added leucine or alanine.

Acute study Protocol

Subjects reported to the laboratory after an overnight fast, having refrained from any intense exercise for at least 72 h. At ~ 0900 h, subjects had catheters (18G) inserted in the antecubital veins of both arms, one for tracer infusion and one for venous blood sampling. A primed, continuous infusion (0.7 $mg.kg^{-1}$, 1 $mg.kg.h^{-1}$) of leucine tracer (99 Atoms % of [1, 2-$^{13}C_2$], Cambridge Isotopes Limited, Cambridge, MA, USA) was then initiated (at 0 h) immediately after the first biopsy and continued for 7 h. After taking biopsies at rest at 0 and 2.5 h in the post-absorptive pre-exercise state, the subjects performed 6 sets of unilateral leg extensions at a moderate contraction velocity (1-2 s concentric, 1-2 s eccentric) and 75%of 1-RM, with three min rest in between sets. After RE, each subject took first 4.2 g of alanine or leucine capsules and then SFO 30 min later (on the basis of feeding optimization studies described below, to ensure peak appearance coincided). Subjects in the rest group first took 4.2 g of leucine capsules and then SFO at 30 min following their 2nd muscle biopsy. Muscle biopsies were taken under sterile conditions from the m. vastus lateralis under local anaesthesia (1% lignocaine) using our standard conchotome technique. The muscle tissue was washed in ice cold saline to remove excess blood, and dissected free of visible fat and connective tissue, then snap frozen in liquid nitrogen and stored at -80°C prior to analysis. After the study, cannulae were removed; the subjects were fed and assessed for 30 min before being escorted home. The protocol scheme is shown in figure 1.

Muscle preparation for MPS analysis

Muscle tissue (~ 25 mg) was snipped with scissors in ice cold homogenization buffer (50 mM Tris HCl (pH 7.4), 1 mM EGTA, 1 mM EDTA, 10 mM β-glycerophosphate; all Sigma-Aldrich, Poole, UK) including protease inhibitors (Roche, West Sussex, UK). The homogenate was centrifuged at 3,000 *g* for 20 min to precipitate the myofibrillar fraction, the supernatant removed for western analyses, and the pellet was then solubilized with 0.3 M NaOH and centrifuged at 3,000 *g* for 20 min to pellet the insoluble collagen fraction. The solubilized myofibrillar protein was precipitated with ice cold 1M PCA, washed twice with 70% ethanol, to ensure free amino acids were removed, and collected by centrifugation. The Myofibrillar protein bound amino acids were subsequently released by acid hydrolysis in Dowex H^+ resin slurry (0.05M HCl) at 110°C overnight. The amino acids were then derivatized as their n-acetyl-N-propyl esters (23). The enrichment of [1, 2-$^{13}C_2$] leucine incorporated into protein was then measured by gas chromatography- combustion-isotope ratio mass spectrometry (Delta plus XP, Thermofisher Scientific, Hemel Hempstead,UK) using our standard techniques (24). The fractional synthetic rate (FSR) of the myofibrillar fraction was calculated from the incorporation of [1,2 $^{13}C_2$] leucine, using venous plasma KIC labelling between muscle biopsies to represent the immediate precursor for protein synthesis as previously described (17,18); using the standard precursor-product method: fractional protein synthesis (k_s, $\%\cdot h^{-1}$) = $\Delta E_m/E_p \times 1/t \times 100$, where ΔE_m is the change in protein labelling between two biopsy samples, E_p is the mean value over time of venous α-KIC, and t is the time between biopsies in hours.

Immunoblotting

Phosphorylated protein concentrations of p70 ribosomal S6 kinase^{Thr389} (p70S6K1) was determined using our standard methods as previously described (24). After homogenising the muscle tissue the sarcoplasmic protein fraction was separated from the myofibrillar fraction by centrifugation at 3,000 × g. Proteins were solubilised in Laemmli buffer prior to separation by electrophoresis at 200 V. h^{-1}, then transferred to 100 % methanol permeabilized 0.2 mm PVDF membranes at 100 V over 45 minutes. Membranes were blocked in 5% BSA solution for 60 min before overnight exposure at 4°C to p70S6K1^{Thr389} primary antibody (Abcam) diluted 1:2000. The next morning membranes were incubated with anti-rabbit IgG secondary at 1:2000 for 1 h before quantification using a Chemidoc XRS system (Bio-Rad Laboratories, Inc. Hercules, CA).

Statistical analysis

All data are shown as means ± standard error of mean (SEM). Area under the curve for MPS and p70S6K1 data was analysed as above baseline. Statistical Analyses were made using GraphPad Prism (Graph Pad software, version 5.0, La Jolla, CA, USA). Two-way ANOVA with Bonferroni post hoc test and Student's t-test were used to identify statistical differences as a result of age and treatment. Significance was accepted as $P < 0.05$.

RESULTS

Plasma amino acid concentrations

The results clearly show higher plasma essential amino acid concentrations after SFO in all groups following the resistance exercise, which was further significantly enhanced with the addition of leucine in both groups and the time course of this rise was similar in both young and older group. Thus we achieved the aim of increasing the availability of leucine, as a prerequisite to testing the hypothesis that it would improve the metabolic responses of MPS and cell anabolic signalling after resistance exercise.

Myofibrillar protein synthesis (MPS) and p70S6K1 phosphorylation

On examination of the responses of MPS (Fig 4): 1) in young men, SFO+LEU stimulated (*P*<0.05) MPS more than SFO+ALA (AUC; 0.15±0.01 vs. 0.12±0.01 $\%.4h.^{-1}$ FSR 0-4 h *P*<0.05) and peak FSR at 2h (0.11±0.008 *vs.* 0.08±0.008 $\%.h.^{-1}$; *P*<0.05); 2) in older men, SFO+LEU stimulated MPS more than SFO+ALA (AUC: O 0.14±0.01 vs. 0.11±0.01 $\%.4h.^{-1}$, *P*<0.05); 3) in older men, MPS following SFO+LEU didn't return to baseline at 4 h as seen in other groups therefore the net positive balance (effect of feeding over ex alone) was probably even greater as it lasted beyond the 4h. SFO supplemented with leucine enhanced p70S6K1 phosphorylation in the older (*P*<0.05) but not younger men. Under exercised conditions, there were no age-related differences when comparing overall anabolic responses (i.e. net MPS over the 4 h measurement period) in response to SFO+LEU or SFO+ALA.

DISCUSSION

This study has provided novel information, that it is possible to further enhance MPS by giving leucine enriched suboptimal protein supplementation immediately after exercise. Specifically, ingestion of 325 ml of CHO + PRO drink containing 5.2 g of leucine in total (i.e. ~1g from protein plus 4.2 g in capsules) immediately after an acute bout of RE at 75% 1RM markedly enhanced MPS and p70S6K1 responses of the older men such that their rates were similar to those of the young. We purposely provided SFO containing ~4.5 g of EAA to our subjects in both leucine and alanine groups following the resistance exercise as it was recently shown that ingestion of 20 g intact protein (~8.6 g EAA) was sufficient to stimulate MPS maximally (1). Thus we expected therefore, that addition of free-leucine to 10 g whole protein would have an additive effect on MPS.

Indeed, several studies have highlighted the importance of combining RE and AA supplementation to maximize the MPS response and shown that consuming essential amino acids (25) or leucine-enriched EAA after RE augments the contraction induced increase in MPS (26). For example, Dreyer *et al.* recently showed that leucine-enriched EAA+CHO ingestion following an acute bout of RE enhanced mTOR signaling and MPS in young human subjects when compared to those following exercise without nutrition (26). More recently supplementation of 6.25g of whey protein with either Leu (2.25g) or an EAA mix with no added leucine have been shown to stimulate MPS following RE (14). However, only young men were studied. Thus, to our knowledge, this is the first study reporting a comparison of the time-course of changes in MPS and p70S6K1 responses after RE and the provision of leucine in both young and old men to a suboptimal dose of protein.

Data surrounding leucine supplements have yielded contrasting results. Recently, Katsanos *et al.* demonstrated that ingestion of 6.7 g of an EAA mix containing 41% leucine (1.7 g over a 3.5 h period) stimulated MPS rates in the elderly to a greater extent than an EAA mixture with only 26% leucine, producing similar synthetic responses to those seen in young muscle (8). Similarly Rieu *et al.* showed that co-ingestion of leucine with protein, carbohydrate and fat administered as small meals (50ml every 20 min, a total of 3g Leu) over a 5 h period improved MPS in elderly men in the rested state (20). This supports our present findings and indicates that leucine should represent a high proportion of dietary protein intake and post-exercise supplementation to maximally stimulate MPS. Although it should also be noted that supplementation of a small dose of whey (6.25g) with an EAA mix containing no additional Leu yielded an improvement in MPS similar to a whey plus leucine (2.25 g) only group (14). Which supports previous findings of ours suggesting that EAA other than leucine i.e. phenylalanine valine and threonine are also capable of promoting MPS acutely and anabolic signalling when administered as a large bolus (19,27), suggesting the recently proposed “leucine trigger” hypothesis (28) needs to be revised.

On the other hand, the present data is in contrast with recently published study by Koopman (29), who showed that co ingestion of leucine with carbohydrate and protein (4.7 g leucine vs.17.6 g leucine over a 6h period) following physical activity did not further elevate MPS in elderly men, despite whole body protein balance being 2.8% greater ($p<0.05$) in the higher leucine group. The apparent discrepancy is likely explained by the fact that in the present study, post-exercise MPS responses following the RE and nutritional supplementation were measured at regular intervals (at 1, 2 and 4 h) during the post-exercise period, where MPS rates showed a faster rise and peaked over the 1-2 h post exercise before showing a downwards towards trend at 2-4 h. However, in Koopmans study, MPS was measured only at 6h post exercise, thereby missing this peak of MPS rise, perhaps giving the reported indistinguishable MPS responses. This highlights the on/off nature of MPS, and thus importance of temporal data gathering over short periods in determining cause and effect related to interventional strategies (24,30,31). It seems to us that there is a clear dose response of MPS to protein, EAA or Leu ingestion (6,13,32), and that although the duration of the stimulation is extended by prior exercise, there is a maximal response to providing additional amino acid substrate, of around 10g of EAA, 20g Whey or 3g of leucine. There are a number of studies that demonstrate, in both the fed only (8,32) and fed plus exercised condition (21,29), that providing additional leucine has no further impact upon MPS; an exception to this being the study of Yang et al, who although they show a maximal ie saturable MPS response to whey protein feeding alone i.e. MPS is the same at 20 and 40g, MPS continues to significantly increase following RE with increasing doses of whey in elderly men (13).

Regarding signalling proteins, it has been shown that the leucine supplementation in resting conditions as well as following resistance exercise enhance MPS via activating insulin-dependent and as well as insulin-independent mTOR pathway signalling proteins (3,18,33).

Correspondingly, we saw quantitatively similar increases in p70S6K1 phosphorylation, a robust proxy for mTORc1 anabolic signalling (4,18), which were maximal 2 h post-exercise + nutritional supplementation in all groups, however it was significantly enhanced (P<0.05) in old SFO+LEU group. This enhanced response of p70S6K1 in old SFO+LEU group could explain their greater increase in myofibrillar protein synthesis, when compared to the isonitrogenous alanine control. Finally, it should be pointed out that cell signals do not always match with MPS such that tying cause and effect is limited (24). Moreover, signaling responses are complex and involve many signals outside of those we have looked at and which could be important in regulating the heightened response in MPS we see when providing a leucine-enriched meal supplement, e.g. the leucine senor Sestrin 2 (34). Future work should hone in on such mechanisms.

Despite demonstrating a blunted response of myofibrillar protein synthesis to exercise in the elderly in postabsorptive state (4), we saw no differences between MPS responses to feeding plus RE between the young and elderly subjects. This lack of an obvious "blunted" response has been observed previously at low levels of protein or EAA feeding (6) and may represent an analytical limitation of the technique in detecting small differences between the groups. Despite this, in the present study, we observed an enhanced MPS response in old SFO+LEU group, identical to those seen in young, and interestingly MPS was still elevated at 4h after the exercise, thus highlighting the potential of combining RE with leucine enriched supplementation to maximise the anabolic responses. It would be a key next step to combine the anabolic influence of RE and ingestion of a amino acid source enriched with leucine over longer periods i.e. in order to determine if longer term supplements can increase clinically important aspects of muscle mass and muscle function in older individuals. Indeed, initial studies are in support of this notion, with one study showing that leucine enriched supplements show improvements indices of muscle mass/function, supporting this notion (35). Perhaps our study highlights potential mechanisms underlying this.

In conclusion, this study shows that it is possible to enhance MPS and p70S6K1 responses in young and older men by giving leucine enriched sub-optimal protein supplement immediately after exercise.

AUTHORS' CONTRIBUTIONS

P.A.: analysis, interpretation, critical revision, final approval; A.S.: analysis, critical revision, final approval; V.K.: study design, recruitment and screening of subjects, conduction of acute studies, interpretation, drafting, final approval; D.R.: analysis, critical revision, final approval; W.H.: clinical support; J.W.: clinical support; N.H: study design; K.S.: study design, analysis, interpretation, critical revision and final approval;

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Table 1 Subjects' characteristics (mean±SEM)

	Young men (n =27)	Older men (n=27)
Age (y)	24±6	70±5*
Weight (kg)	75±10	76±10
Height (m)	1.79±0.05	1.74±0.05
BMI (kg.m^2)	23±2	25±2
Lean Mass (kg)	59±7	54±4
Fat mass (kg)	13±5	19±8
% Body fat	17±6	25±9
1 repetition maximum (RM) (N)	683±171	392±111*
Blood glucose (mM) overnight fasted	4.6±0.5	5.0±0.4

* Significant difference between groups P<0.05

Figure 1

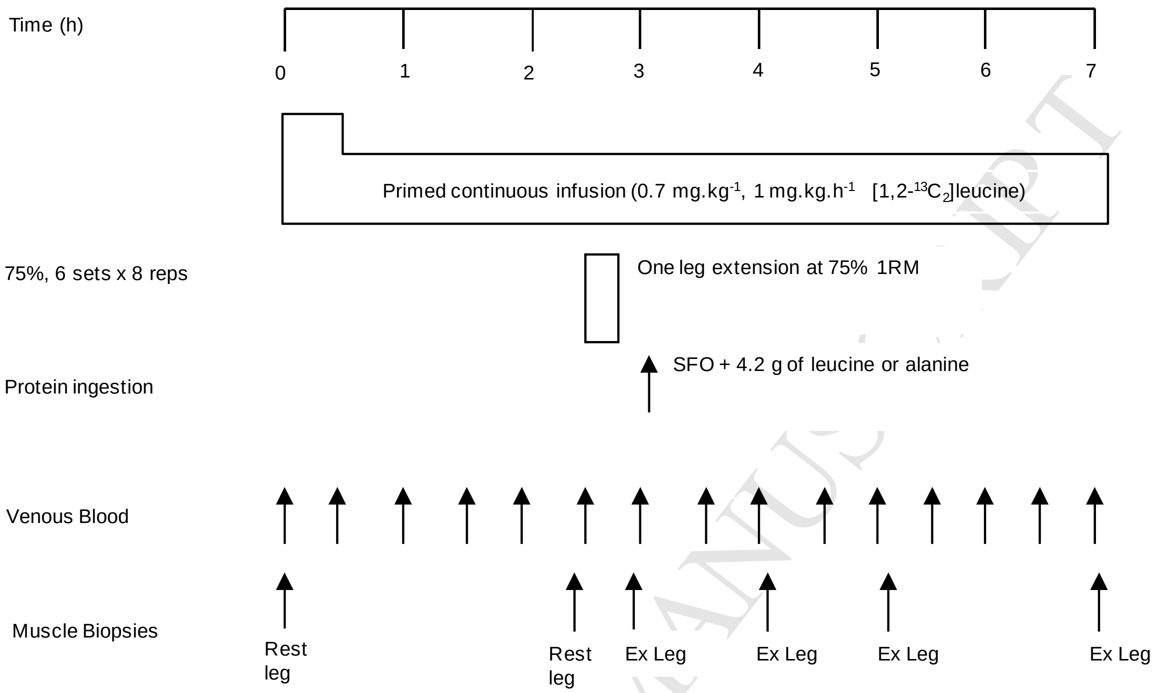
Time (h)
0
1
2
3
4
5
6
7
Primed continuous infusion (0.7 mg.kg-1, 1 mg.kg.h-1 [1,2-13C2]leucine)
75%, 6 sets x 8 reps
One leg extension at 75% 1RM
Protein ingestion
SFO + 4.2 g of leucine or alanine
Venous Blood
Muscle Biopsies
Rest leg
Rest leg
Ex Leg
Ex Leg
Ex Leg
Ex Leg

Figure 2

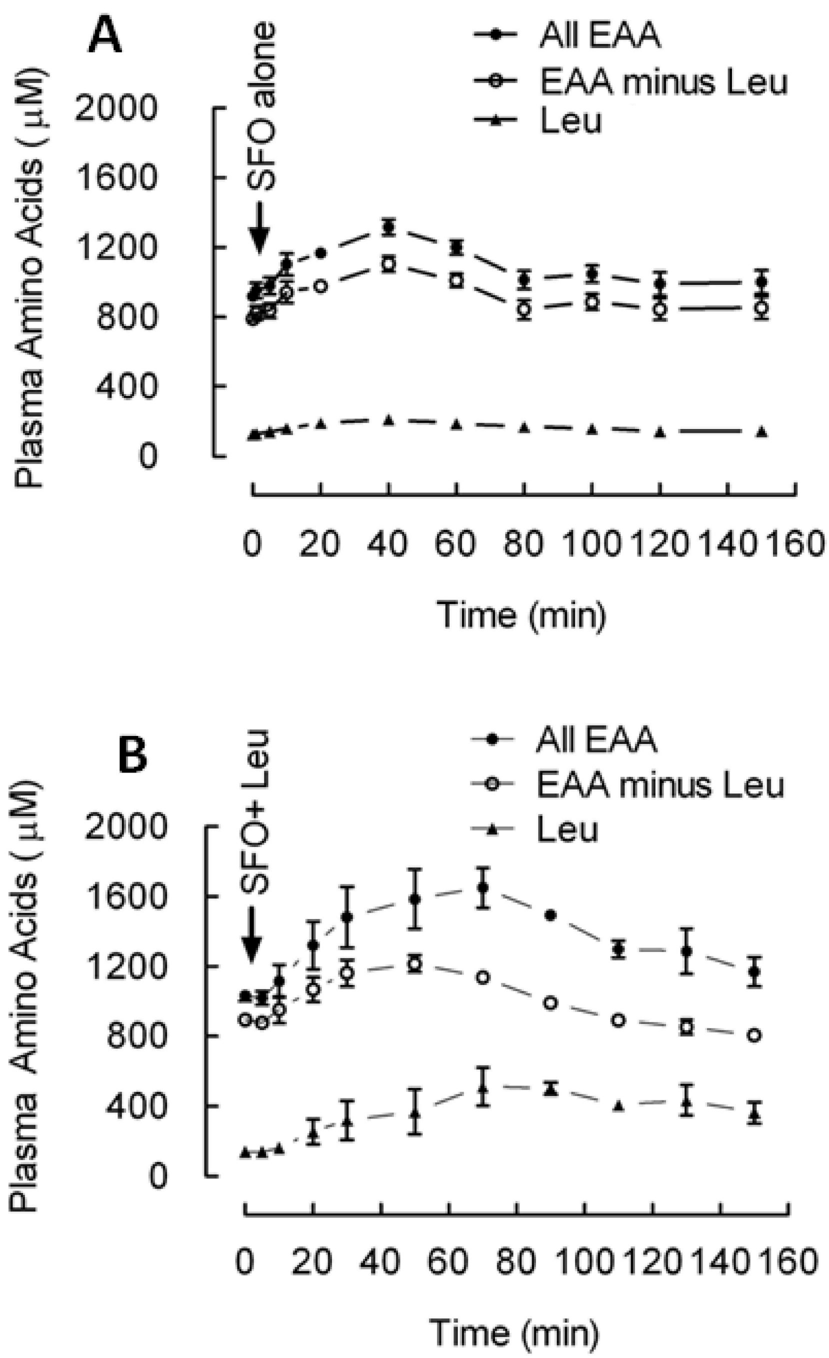
A
SFO alone
All EAA
EAA minus Leu
Leu
Plasma Amino Acids (µM)
2000
1600
1200
800
400
0
0 20 40 60 80 100 120 140 160
Time (min)
B
SFO+ Leu
All EAA
EAA minus Leu
Leu
Plasma Amino Acids (µM)
2000
1600
1200
800
400
0
0 20 40 60 80 100 120 140 160
Time (min)

Figure 3

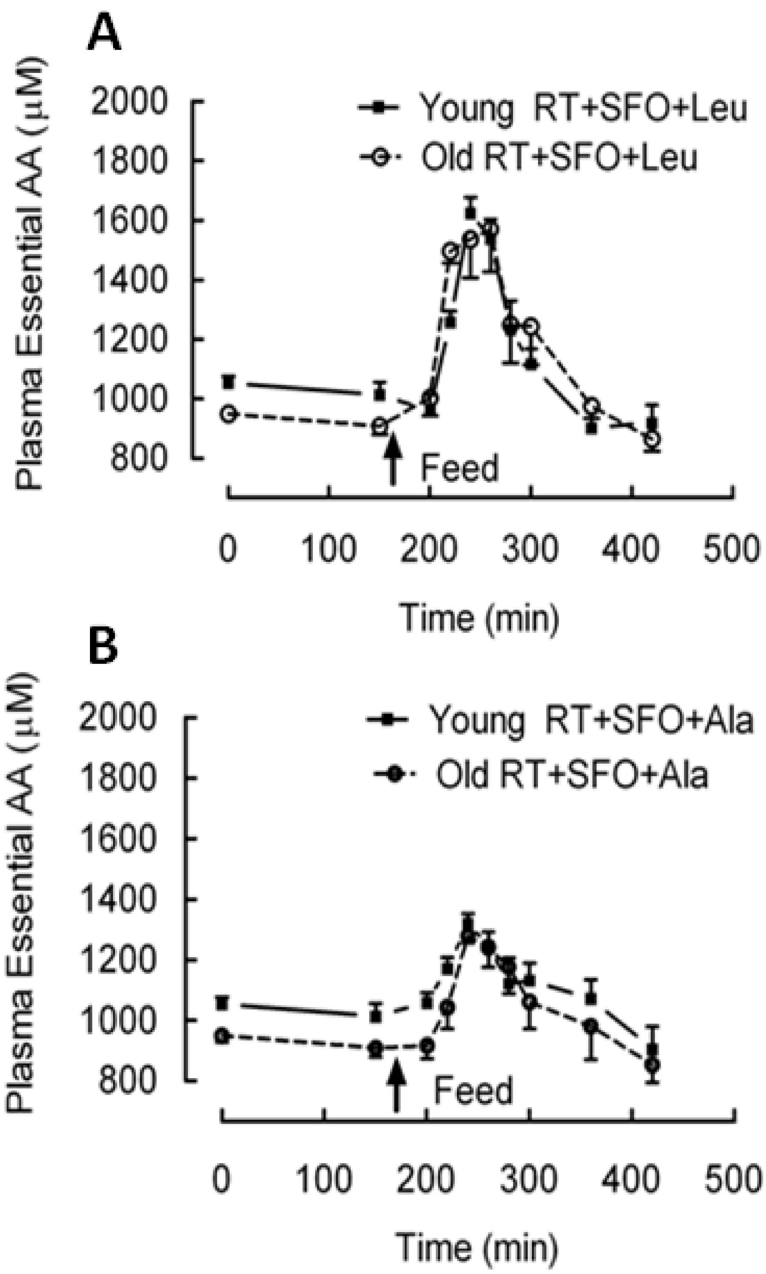
A
Plasma Essential AA (µM)
2000
1800
1600
1400
1200
1000
800
Young RT+SFO+Leu
Old RT+SFO+Leu
Feed
0
100
200
300
400
500
Time (min)
B
Plasma Essential AA (µM)
2000
1800
1600
1400
1200
1000
800
Young RT+SFO+Ala
Old RT+SFO+Ala
Feed
0
100
200
300
400
500
Time (min)

Figure 4

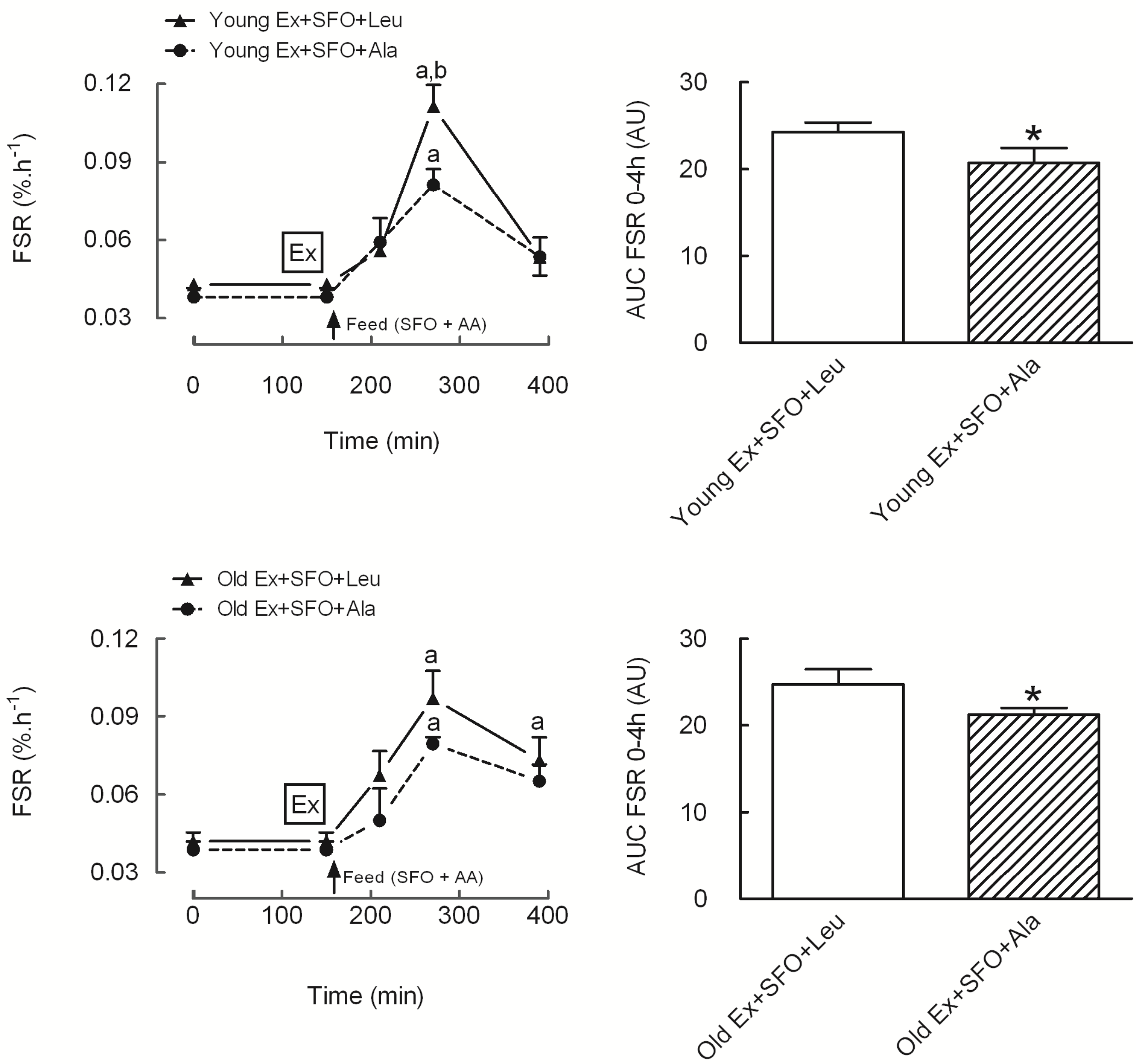
Young Ex+SFO+Leu
Young Ex+SFO+Ala
FSR (%.h-1)
0.12
0.09
0.06
0.03
a,b
a
Ex
Feed (SFO + AA)
0
100
200
300
400
Time (min)
AUC FSR 0-4h (AU)
30
20
10
0
*
Young Ex+SFO+Leu
Young Ex+SFO+Ala
Old Ex+SFO+Leu
Old Ex+SFO+Ala
FSR (%.h-1)
0.12
0.09
0.06
0.03
a
a
a
Ex
Feed (SFO + AA)
0
100
200
300
400
Time (min)
AUC FSR 0-4h (AU)
30
20
10
0
*
Old Ex+SFO+Leu
Old Ex+SFO+Ala

Figure 5

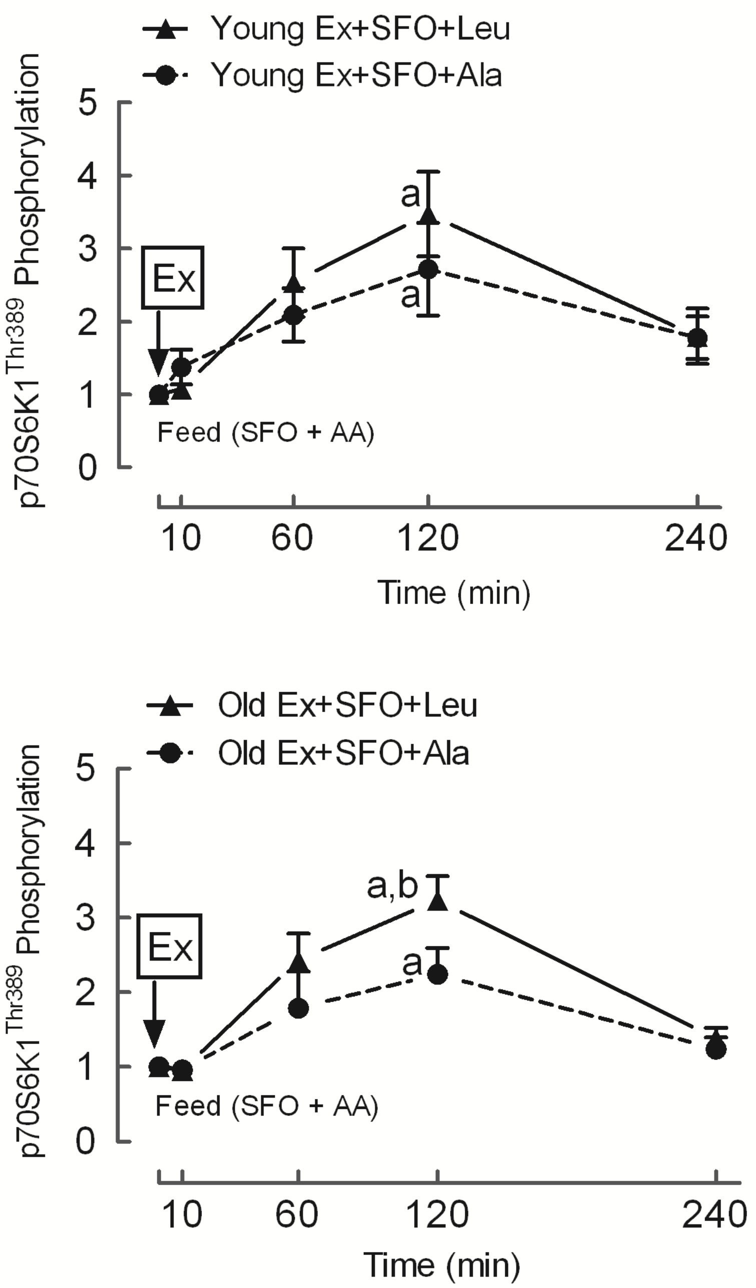
Young Ex+SFO+Leu
Young Ex+SFO+Ala
p70S6K1 Thr389 Phosphorylation
Ex
Feed (SFO + AA)
a
a
Time (min)
Old Ex+SFO+Leu
Old Ex+SFO+Ala
p70S6K1 Thr389 Phosphorylation
Ex
Feed (SFO + AA)
a,b
a
Time (min)

FIGURE LEGENDS

Figure 1. Study protocol for the measurement of myofibrillar protein synthesis and muscle anabolic signalling phosphorylation to unilateral leg extension exercise at 75% 1RM followed by the ingestion of Slim-Fast Optima (SFO) with 4.2 g leucine or alanine in post-absorptive young and older men (n=9). NB 9 older men were studied at rest consuming SFO and 4.2g leucine without exercise.

Figure 2. Concentrations of essential amino acids (total or with leucine subtracted) or leucine in plasma after drinking 325 ml of SlimFast Optima with (A) or without (B) 4.2g of leucine taken in a gelatine capsule 30 min before the SlimFast Optima. Values are means±SEM for n = 3. In some cases the error bars are within the symbols.

Figure 3. Plasma essential amino acid concentrations after 6×8 repetitions unilateral leg extension exercise at 75% 1RM in older and young men (RT) after SlimFast Optima supplemented with leucine (RT+SFO+Leu) (A) or alanine (RT+SFO+Ala) (B).

Figure 4. Responses of myofibrillar protein synthesis to resistance exercise in older men with or without SlimFast Optima plus leucine or alanine (control) and in young men after resistance exercise with SlimFast Optima +leucine or alanine.

Figure 5. Responses of p70S6K11 phosphorylation to resistance exercise in older men with or without SlimFast Optima plus leucine or alanine (control) and in young men after resistance exercise with SlimFast Optima +leucine or alanine.