Influences of carbohydrate plus amino acid supplementation on differing exercise intensity adaptations in older persons: skeletal muscle and endocrine responses

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Abstract Losses in physiological function in healthy ageing occur partly as a consequence of reduced protein intake and partly as a consequence of less than 30-min/day of moderate to vigorous physical activity. The current study aimed to compare the effects of two different intensities of resistance training in healthy older adults, whose habitual dietary intake was supplemented with carbohydrate and amino acid preparations. We hypothesised that although intensive exercise with appropriate carbohydrate and amino acid supplementation would result in the most profound impact on in vivo markers of healthy physiologic and endocrine functions in previously sedentary older individuals, the effectiveness of the less intense exercise prescription with supplementa-

tion would also result in beneficial adaptations over and above findings of previous studies on low intensity exercise alone. Twenty-nine older adults (out of 32) completed the study after being randomly assigned to low (SUP_LowR, i.e., ~40% 1RM; $n=16$) versus high resistance training (SUP_HighR, i.e., ~80% 1RM; $n=13$) for 12 weeks. A carbohydrate supplement was ingested immediately before and during every exercise session and an amino acid cocktail was ingested post-exercise. Neither intervention significantly impacted upon body composition assessed using: Body mass index, waist/hip ratio and bioelectric impedance. Muscle strength increased similarly in the two groups with the SUP_HighR protocol showing $46\pm 8\%$, $10.8\pm 4.4\%$ and $26.9\pm 4.9\%$ ($P<0.01$) improvements in 1-RM strength, unilateral and bilateral knee extension torque, respectively, compared with $39\pm 2\%$, $9.4\pm 3.7\%$ and $29.5\pm 8.2\%$ ($P<0.01$) increments in the same measures in the SUP_LowR group. Lean muscle thickness however, showed a greater benefit of the SUP_LowR protocol ($8.7\pm 3.9\%$ increase, $P<0.05$) compared with the SUP_HighR protocol, which elicited no significant change. In terms of functional abilities, only the standing-from-lying (SFL) test exhibited an improvement in rate in the SUP_HighR group (-11.4% , $P<0.05$). The SUP_LowR group, on the other hand, showed significant improvements in the get-up-and-go ($-8.7\pm 3.6\%$, $P<0.05$), the SFL (-4.7% change, $P=0.05$) and the 6-min walk ($7.2\pm 2.2\%$ increase in distance covered, $P<0.01$) tests. Following overnight

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fasting, serum levels of glucose changed significantly ($-13 \pm 4.7\%$ decrease, $P < 0.01$) in SUP_LowR. Serum levels of insulin ($-25 \pm 5.3\%$ decrease, $P = 0.05$), neuropeptide Y ($-24 \pm 15.3\%$ decrease, $P = 0.02$), and IGFBP-3 ($-11 \pm 6.6\%$ decrease, $P = 0.03$), changed significantly in SUP_HighR. Circulating levels of interleukin-6, tumour necrosis factor- α and insulin-like growth factor 1 did not alter significantly in either intervention group. These data suggest that whilst both interventions were beneficial in older persons, the end targets as well as metabolic and hormonal adaptations are different. The supplementation plus low exercise regimen tended to impact on muscle hypertrophy combined with increased habitual function. Supplementation plus high-intensity exercise regimen improved markers of strength, but not to a significantly greater extent than supplementation plus low intensity exercise.

Keywords Elderly · Endocrinology · Cytokines · Physiology · Resistance exercise intensity · Nutritional supplementation

Introduction

The evidence is that many of the biological changes, risks of, and occurrences in chronic diseases which have been attributed to ageing, are in fact caused by less than optimal nutritional intake, (Blumberg 1994; Vellas et al. 1997; Volkert et al. 1992), where adequate diet has been assessed anecdotally using a mini-nutritional assessment (which includes recent weight loss to amounts and types of foodstuff ingested; Guigoz 2006) or immunologically by determining CD4 lymphopenia (which has been associated with nutritional compromise in older persons; Rea et al. 1996; Kaiser and Morley 1994). These studies suggest that several macronutrient and proteins in particular are essential in the maintenance of a healthy lifestyle. Although skeletal muscle is composed of high protein content, it only contributes ~30% of whole body protein turnover in young people (Wagenmakers 1999) and declines to ~20% (or less) by 70 years of age (Chernoff 2004). In the face of reduced dietary protein intake, a loss in physiological function can therefore occur, with the body seeking to re-establish a steady metabolic state. Castaneda et al. (1995) found that elderly women who consumed

insufficient protein (56% recommended daily allowance; RDA) over a 9-week period lost ~9% lean muscle mass (sarcopenia), thus compromising their skeletal muscle functional capacity, as a direct consequence of diminished protein consumption. Furthermore, the marked hypertrophy of skeletal muscle after resistance training which is seen in the presence of protein supplementation in older persons (Esmarck et al. 2001) may involve increased stimulation of muscle protein synthesis (Tipton et al. 2007). Therefore, exercise alone may not be sufficient to increase muscle mass following exercise, particularly in undernourished or older people.

Studies trying to determine underlying mediators of muscle loss with age, specifically, the age-related decrement in myosin heavy chain (crucial in muscle contractile function) synthesis rate, suggested a correlation existed between this decline and circulating levels of insulin-like growth factor 1 (IGF-I), dehydroepiandrosterone, and testosterone in 24 participants aged 20–92 years (Balagopal et al. 1997). Taken together, these results may imply a decreased ability to synthesise or maintain this essential protein with age, which may underpin the decreases in muscle mass and muscle contractile function seen with the ageing process. Hence, attempts to prevent/delay age-related sarcopenia and thus to improve the quality of life in populations aged 60 and above should include assessments of/interventions in, both ‘less than optimal nutritional intake’ and ‘sub-nutrition’ in this group in the face of reduced physical activity. Indeed, the evidence for increased health benefits with protein supplementation only (Jensen and Hessel 1997), or protein plus carbohydrate (Gray-Donald et al. 1995) supplementation for instance, have been shown. Amino acids, even above the RDA (1–1.3 versus 0.8 kg/day) are potent and safe stimulators of muscle protein synthesis (Bohe et al. 2003) and hence muscle mass increases in the young, the elderly and the frail elderly (Jensen and Hessel 1997; Lauque et al. 2000), provided the person’s health status (Tobin and Spector 1986; Chevalier et al. 2003), as well as calcium intake (Lucas and Heiss 2005), are both adequate. However, due to impacts on satiety, ingestion of nutritionally balanced supplements has often been found to reduce the caloric intake of the rest of the food consumed in a day, by an amount equivalent to the calories supplied in the supplement (Fiatarone et al. 1994). Therefore, such dietary supplements in the elderly end up being dietary substitutes and do not

fulfil their desired goal. On the other hand, the ingestion of only essential amino acids (EAAs) has been found to be enough to stimulate muscle protein synthesis in this age group (Paddon-Jones et al. 2006; Volpi et al. 1999, 2003), without the negative impact on habitual food intake seen with bulkier protein preparations. In summary, following adequate health check-ups, it would appear that improved nutritional balance in older persons will have beneficial consequences in terms of skeletal muscle structural characteristics and hence sarcopenia.

Resistance exercise has also been demonstrated to be an effective intervention to combat age related sarcopenia (Frontera et al. 1988; Klitgaard et al. 1990). Indeed, provided the stimulus is sufficient and exceeds the rate of protein degradation, net muscle protein synthesis and hence positive balance (i.e., when synthesis outweighs breakdown) is increased after a bout of exercise (Short et al. 2004). Despite these compelling studies, any added benefit of combining exercise and supplementation regimens is yet to be fully assessed, particularly for older populations. Nevertheless, in support of the potential of combining these two interventions, a study by Levenhagen et al. (2002) showed that amino acid availability is more important than the availability of energy *per se* for post-exercise repair and synthesis of muscle proteins. Furthermore, studies also exist, which show that protein/amino acid supplements alone have little or no effect on muscle strength and mass without concomitant exercise interventions in frail elderly (Bonney et al. 2003; Fiatarone et al. 1994; Rosendahl et al. 2006) and otherwise undernourished sedentary individuals (Bonney et al. 2003; Fiatarone et al. 1994; Rosendahl et al. 2006).

The current study therefore aimed to determine the impact of nutrition and exercise in an otherwise healthy older population, and to assess whether combining nutritional supplementation (i.e., carbohydrate plus amino acids) with high vs. low resistance exercise, would elicit beneficial effects on several factors associated with physical wellbeing.

Methods

Participants

Thirty-six older adults volunteered to participate in the current study having responded to advertisements

posted locally. Four of these prospective volunteers were subsequently excluded from this study as they had a known history of either kidney, liver, cardiovascular, neurological, inflammatory or myopathic disease. The remainder of the participants gave written informed consent to take part in the study after they had obtained medical clearance to participate from their general practitioner. Thus, the current study included relatively healthy, community dwelling, and habitually active individuals, with no recent history of structured resistance training. The local Human Ethics Committee approved all experimental procedures.

Of the 32 healthy older adults who started the study, 29 completed the 12-week ‘combined exercise + supplementation’ intervention. As a result of adjustments owing to one participant’s own preference, as well as dropout rates, the study was completed with 13 ‘combined supplementation + low intensity resistance exercise’ (SUP_LowR) and 16 ‘combined supplementation + high intensity resistance exercise’ (SUP_HighR) training groups: the characteristics of the completing participants are presented in Table 1.

Muscle strength measurements

One repeated maximum measurement

During a familiarisation session, no more than 7-days prior to the 12-week intervention, participants’ 1 repetition maximum (1RM) was determined for all exercises employed in the training programme. Participants first performed a standardised warm-up on the leg press (6×50% perceived 1RM; 4×70% perceived 1RM with 3-min recovery). After warming up, the load was set at 90% of the estimated 1RM, and increased after each successful lift by 5 kg until failure. Each participant was given six lifting attempts in order to achieve their 1RM and a maximum of two attempts to lift the chosen weight, once it had been established. The greatest amount of weight lifted successfully was recorded to determine the training load. Between successive attempts, 3-min rest periods were allowed. A repetition was valid if the participant used correct form and was able to complete the entire lift in a controlled manner without assistance. Participants 1RM for each exercise was reviewed every 2-weeks during training; if 1RM had increased, the training load was adjusted accordingly. Additionally,

Table 1 Completing participants' baseline characteristics

Group	Gender (N)	Age (years)	Activity (min/week)	Height (m)	Weight (Kg)
SUP_LowR	Male (9)	71.8±3.7	360.0±167.4	1.77±0.08	82.2±11.5
	Female (9)	72.6±5.9	319.6±202.4	1.61±0.07	70.8±14.9
	Both (18)	71.8±4.8	339.8±184.9	1.69±0.08	76.5±13.2
SUP_HighR	Male (7)	68.9±5.2	277.5±89.3	1.80±0.07	85.6±13.9
	Female (6)	67.2±5.0	227.5±145.0	1.58±0.08	65.7±13.1
	Both (13)	68.1±5.1	252.5±117.2	1.69±0.08	75.5±13.5

Means ± SD

if any participants felt that in-between 1RM assessments the training load was not providing adequate resistance, the load was increased so they were always lifting at the desired percentage of their maximum. Participants were familiarised with the resistance exercise training protocol on a subsequent visit to the laboratory.

Isometric knee extensors muscle strength measurements

Participants were familiarised with the experimental procedures on a separate occasion no more than 7 days prior to the baseline test sessions. In one single testing session, quadriceps isometric strength measures were taken on the right leg and isokinetic measures on the left leg, using a Cybex Dynamometer (Cybex Norm, Cybex International Inc., NY, USA). The centre of rotation of the lever arm of the dynamometer was aligned with the axis of rotation of the knee. Participants were positioned with the hip joint at 85° (supine = 0°). In order to minimise any extraneous movement of the hip joint or the trunk, participants were strapped over the shoulders, pelvis and thighs. Settings of chair height and positioning relative to the dynamometer were adjusted individually with all settings recorded and replicated at the post-intervention testing phase. Gravity corrections were then made following the manufacturers' own procedure, having adjusted the attachment of the lever arm cuff relative to the length of the participant's shank. Previous work from within our laboratory has utilised similar methods of measurement, finding them to be both valid and reliable (Pearson and Onambele 2005, 2006).

Maximal unilateral isometric torque Maximal isometric knee extension torque was measured with the knee at 70° angle (full knee extension = 0°) on the

right leg of all participants. After a series of warm-up trials consisting of ten isokinetic contractions at 60°s⁻¹ at 50-75% maximal effort, participants were instructed to rapidly exert maximal isometric force against the Cybex lever arm over a 3-4 s period. Participants were given both verbal and visual encouragement/feedback throughout their effort. Joint torque data were displayed on the screen of a Macintosh G4 computer (Apple Computer, Cupertino, CA, USA), which was interfaced with an A/D system (Acknowledge, Biopac Systems, Santa Barbara, CA, USA) with a sample frequency of 500 Hz. Isometric contractions were held for ~2 s at the plateau with a 90 s rest period between contractions. Peak torque was averaged over a 500 ms period at the plateau phase. The mean peak torque of three extensions was used as the measure of strength in each participant.

Maximal Bilateral Isometric Torque Maximal bilateral isometric knee extension torque was measured with the two knees at 70° angle (full knee extension = 0°) pushing against a customised lever arm. Similar precautions to record time of day were taken. After a series of warm-up trials consisting of three isokinetic contractions at 60°s⁻¹ at 50% maximal effort, participants were instructed to rapidly exert maximal isometric force against the Cybex lever arm over a 3-4 s period. Participants were given both verbal and visual encouragement/feedback throughout their effort. Joint torque data were acquired and processed as during the unilateral efforts, with the only difference that here the best of the three efforts was used as the measure of strength for each participant.

Mid-thigh lean tissue thickness

Real-time B mode ultrasonography with a 7.5 MHz linear array probe (AU5, Esaote, Genoa, Italy) was

used to study mid-thigh muscle thickness in the area of the vastus intermedius (VI) and the vastus lateralis (VL) muscles. Muscle thickness was taken at rest with the knee at 70° angle. Scans were acquired in the mid-sagittal plane, at approximately 50% length of the VL muscle as measured from the origin at the linea aspera and lateral femur to insertion at the tibial tuberosity via patella tendon on the anterior surface. Medio-lateral width of the VL was determined over the skin surface and the position of one-half of the width was used as the measurement site. The ultrasound probe was coated with water-soluble transmission gel to provide acoustic contact and was held in place without depressing the dermal surface by the experimenter. Three separate recordings of mid-thigh muscle thickness were made with 90 s rest between each recording. Ultrasound images were acquired using a digital recorder and frames exported to capture software (iMovie HD6, Apple Computer Inc, USA). The VL thickness was measured as the distance from the top of the peripheral muscle aponeurosis to the deep aponeurosis. VI thickness was from the deep aponeuroses to the surface of the femur. The three points of interest were measured at three standardised points on each ultrasound frame to obtain an average tissue thickness using ImageJ analysis software (ImageJ 1.37, Maryland, USA). Total muscle thickness was computed as the sum of VI + VL muscle thickness measured.

Body composition

Waist/hip ratio

A measuring tape was used to measure the circumference of the hips at the widest part of the buttocks, and the waist at the smaller circumference of the individual's natural waist (just above the navel). The waist/hip ratio was used as the individual's score of central adiposity.

Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA; BODYSTAT, Isle of Man, British Isles) was used to estimate body composition based on the difference in electric conductive properties of various tissues. The BODYSTAT applied 500 μ Amps at a single frequency of 50 kHz through self adhesive electrodes placed on the

right hand and foot, of a participant lying flat on their back with their arms away from the trunk, thighs not touching and ankles at least 20 cm apart for at least 5 min prior to any measures being recorded. Although BIA has acknowledged limitations when applied to non-standard or elderly populations due to the built-in equation utilised to calculate body composition, it is important to state that the BIA in this study was used to assess within participant adaptations as a consequence of the intervention and, as such, carries more validity. What is more, to lend greater external validity to data from this instrument, raw values were corrected for the inherent 15% overestimation in body fat content (when comparing pilot participants BIA versus dual energy X-ray absorptiometry (DEXA) outputs in our laboratories—others have drawn similar conclusions regarding the differences in body fat content values depending on the methodology used (Jorgensen et al. 1996; Lintsi et al. 2004) and have tended to consider DEXA readings as gold standard since it yields values similar to those obtained from the hydrostatic weighing method (Prior et al. 1997).

Body mass index

Body mass index (BMI) was defined as the individual's body mass (Kg) divided by the height in metre squared.

Functional ability measures

The tests of functional ability were all performed on 1 day at the outset and completion of the study and were similar to a battery of functional tests performed by participants in previous research (Chandler et al. 1998; Skelton et al. 1995).

Get up and go

Three cones were placed 1 m apart on the floor in front of a rigid chair of adjustable height. The participant's knee-to-floor height (i.e., the distance from the knee joint axis to the floor) was recorded prior to testing to determine the appropriate chair height for each test. Once knee height was determined the chair height was set at 100%, 80% and 60% of each individual knee-to-floor height for the tests. The test started with the participant seated on the chair (at the appropriate height), with feet flat on the floor and

arms folded across the chest. They were then asked to rise unaided as quickly as possible, walk around the furthest cone (3 m away) and back to the initial seated position on the chair. The elapsed time between the rising from the chair to sitting back down was recorded. The quickest of three trials was used as the participant's score.

Standing from lying

Participants were asked to lie flat on the floor on a gym mat and on their preferred side with their arm on the floor outstretched and their head resting on the outstretched arm. Participants were then instructed to rise as quickly as possible using their preferred technique. The time elapsed between the instruction to 'Go' and the participant standing upright and steadily with both feet firmly on the ground, was recorded. The fastest of three trials was utilised as their score.

Six-minute walk

A 10-m course was set with cones 1 m apart in a straight line.

Participants were instructed to walk around this course using their fastest, non-running, walking pace, with the aim of completing as many revolutions of the circuit as possible in 6 min. The score was calculated as the total distance covered in the allocated time.

Metabolic and endocrine profiling

At the onset and end of the intervention and following an overnight fasting period, participants reported to the laboratory. A 21-gauge 1-inch ultra thin wall needle (Terumo Medical Corporation, New Jersey, USA) was inserted into the antecubital vein of the forearm. Using a vacutainer assembly and serum separator tubes (Monovette, Sarstedt, Numbrecht, Germany), 10 mL blood samples were collected. Blood glucose was analysed immediately using a single drop of freshly sampled blood using the AccuChek Advantage System (Roche Diagnostics Ltd, Lewes, UK. Sensitivity of <10 mg/dL (i.e., minimum detectable concentration); Intra-assay variability of 2% (i.e., coefficient of variation). The remainder of the sample was centrifuged at 2–5°C for 5 min at 4,000 rpm, with the supernatant being

removed and stored in eppendorfs at –70°Celsius for later analyses. Insulin (Biosource, Nivelles, Belgium. Sensitivity of 0.15 µl U/ml; Intra-assay variability of 4.2%). IGF-I (Biocode-Hycl, Liege, Belgium. Sensitivity of 4.9 ng/ml; intra-assay variability of 8.0%). Tumor necrosis factor-alpha (TNF-α; Diaclone, Besancon Cedex, France. Sensitivity <8 pg/ml; intra-assay variability of 3.3%). Neuropeptide Y (NPY; Phoenix Europe GmbH, Karlsruhe, Germany. Sensitivity of 0.13 ng/ml; intra-assay variability <5%). IGFBP-3 (Biocode-Hycl, Liege, Belgium. Sensitivity of 10.5 ng/ml; intra-assay variability of 6.5%). Finally, interleukin-6 (IL-6; Diaclone, Besancon Cedex, France. Sensitivity <0.8 pg/ml; intra-assay variability of 3.3%) were analysed using standard enzyme-linked immuno-sorbent assay procedures.

Training programme

The training programme was 12 weeks in duration and consisted of one supervised gym-based class and two home-based sessions per week in LowR. In HighR, the programme was for two supervised gym-based classes and one home-based session per week. All exercise sessions were 1 h in duration.

Briefly, the supervised exercise classes consisted of a warm-up (stretching, aerobic and coordination work), resistance exercises (using therabands for all major muscle groups, leg press, leg extension, ankle rotator and glute conditioner (Technogym, Gambettola, Italy), with a progression from eight to 11 reps in two to four sets at 40% or 80% 1RM), and a cool-down (i.e., stretches, pilates, tai chi). The unsupervised home-based exercises were similar in design to the supervised classes with the exception that all the resistance work was carried out using therabands, and a 20-min brisk walk was also included. An exercise booklet illustrated, using photographic and/or cartoons, all the exercises in detail. Home-based exercise was not to be performed the day preceding or following the supervised class exercise.

Nutritional supplementation

All participants received the supplement and all were administered the same dosage, regardless of the exercise intensity they had been allocated to carry out. Administration of the combined treatments is illustrated below in Fig. 1.

Fig. 1 Illustration of the weekly schedule for training and supplementation during the study programme. **a** Scheduling of exercise sessions. **b** Timing of supplement ingestion during the exercise sessions (both gym- and home-based)

A)	Day of week	1	2	3	4	5	6	7
	SUP_LowR	Home-Based		Gym-Based			Home-Based	
	SUP_HighR	Gym-Based		Home-Based		Gym-Based		

B)	ACTIVITY	15mins Pre-Exercise	60 mins Exercise Session	15mins Post-Exercise
	INGESTION	½ of Carbohydrate solution	½ of Carbohydrate solution	Amino-Acid Solution

Carbohydrate supplementation

This was provided in the form of a 500 mL (containing 26 g of carbohydrates), orally administered isotonic Lucozade drink (kindly donated by GlaxoSmithKline, Basildon, Essex, England). Participants were instructed to ingest 250 mL of the drink upon arrival for each training session prior to participating in any exercise, and to consume the rest of the beverage during the exercise session. For home exercise sessions, participants were given a personal stock to keep at home.

Protein supplementation

Supplementation of protein came in the form of an orally administered mixed amino acid supplement (Bodyfortress, Holland and Barret, Warwickshire, UK). Participants were instructed to ingest the supplement within 15 min post-training mixed with water. Each participant ingested a two tablespoon dose of supplement containing 22 g of essential amino acids. As with the carbohydrate supplement, for the home-based exercise sessions participants received a personal stock of the amino acid supplement and were given clear instructions regarding the dosage and mixture of the formula. Demonstrations of the correct dose measurement were given at all face-to-face sessions.

Statistical analyses

T tests were carried out to compare data at baseline. Two-way factorial analysis of variance were carried out with group as one factor (two levels: SUP_HighR vs. SUP_LowR) and phase as the second factor (two

levels: baseline vs. post-intervention) to determine any main effects of the group or interventions. Analysis of covariance (ANCOVAs) were used where the two groups were heterogeneous at baseline. Data are expressed as mean $\pm$ SE unless otherwise stated. Significance was set at $P \leq 0.05$.

Results

The two intervention groups did not differ in age, habitual physical activity levels, height or weight at the onset of the current programme. Post-intervention, both groups showed slight tendencies towards increased body mass (SUP_HighR = $\sim 2.6\%$ vs. SUP_LowR group = $\sim 0.9\%$), though this effect was not significant.

Body composition changes

As a consequence of unaltered body weight, no significant differences in BMI were observed in the two groups after the 12-week interventions (26.5 ± 0.9 to 26.7 ± 1 in SUP_LowR and 26.4 ± 1.3 to 26.5 ± 1.2 in SUP_HighR). What is more, there was no change in waist/hip ratio in either the SUP_LowR population (0.91 ± 0.02 to 0.87 ± 0.02) or the SUP_HighR population (0.91 ± 0.03 to 0.89 ± 0.03). In terms of body fat percentage, neither SUP_LowR ($28.3 \pm 2.0\%$ to $27.5 \pm 2.0\%$), nor SUP_HighR populations ($26.0 \pm 2.4\%$ to $27.2 \pm 2.5\%$) showed any changes.

Lower limbs muscle strength changes

1-RM Prior to training, there were no significant differences in mean 1RM strength of the leg press,

leg extension or gluteus maximus conditioner between groups. Ankle rotation strength was different between the groups at baseline ($P<0.05$, see Table 2). At 12 weeks, the average strength increase for each exercise group was significant ($P<0.05$; Table 2). The average 12-week increase in strength was $39\pm2\%$ (i.e., 20.3 ± 4.2 Kg) for SUP_LowR and $46\pm8\%$ (i.e., 20.3 ± 5.0 Kg) for SUP_HighR. The group effect was present even after accounting for the baseline difference in ankle rotator measures (using an ANCOVA test).

Isometric Unilateral and Bilateral torque- Prior to training there was no significant difference in either the mean isometric unilateral (MVCuni-ext) or bilateral knee extensor strength (MVCbilat-ext) between the two intervention groups. Table 2 shows the mean changes in torque after the 12-week interventions in each group. Briefly, unilateral isometric torque increased significantly following both interventions. Indeed the SUP_LowR group exhibited an increase in MVCext of $9.4\pm3.7\%$ (124.8 ± 8.6 Nm to 135.3 ± 9.3 Nm; $P=0.04$). Similarly, SUP_HighR showed an increase in MVCuni-ext of $10.8\pm4.4\%$ (121.6 ± 13.8 Nm to 137.6 ± 18.8 Nm; $P=0.04$). The 12-week interventions also resulted in similar and significant increases in bilateral torque in both SUP_HighR ($26.9\pm4.9\%$ $P\leq0.05$) and SUP_LowR ($29.5\pm8.2\%$ $P\leq0.05$) groups.

Mid-thigh lean tissue thickness

Total muscle thickness (VL + VI) was significantly increased in the SUP_LowR participants by $8.7\pm3.9\%$ (33.6 ± 1.7 mm to 36.1 ± 1.4 mm; $P<0.05$). SUP_HighR exhibited no change in total muscle thickness (36.1 ± 2.2 mm to 35 ± 2.2 mm; NS).

Functional measures

Get up and go At baseline, there was no significant difference in reaction time (as determined by the get-up-and-go (GUG) test) between the two groups. GUG time significantly improved by $8.7\pm3.6\%$ (5.7 ± 0.52 s to 5.1 ± 0.2 s) in the SUP_LowR population. However, SUP_HighR did not change their performance in this test (5.1 ± 0.4 s to 5.1 ± 0.4 s).

Standing from lying Functional power determined by SFL time showed significant improvements in rate for both SUP_HighR (3.33 ± 0.41 to 2.67 ± 0.29 , i.e., -11.4% change, $P<0.05$) and SUP_LowR (3.26 ± 0.32 to 3.00 ± 0.23 , i.e., -4.7% change, $P=0.05$) populations.

Six-minute walk The distance covered during the 6-min walk test was increased by $7.2\pm2.2\%$ (27 ± 1 m to 28.7 ± 0.7 m) in the SUP_LowR population ($P<0.003$). The SUP_HighR participants exhibited a non-significant change in distance covered (26.3 ± 1.8 m to 26.8 ± 1.7 m).

Metabolic and endocrine characteristics changes

Summary of the findings in terms of endocrine changes (i.e., post-intervention values normalised for baseline values) are presented below and summarised in Fig. 2.

Metabolic profile—plasma levels of glucose and insulin

At baseline, there was a significant difference ($P<0.01$) in the mean plasma glucose levels between intervention groups. The 12-week intervention

Table 2 Change in 1RM load lifted (Kg), and maximum isometric torque (Nm) from pre- to post 12-week intervention

	SUP_LowR			SUP_HighR		
	Pre	Post	Δ (%)	Pre	Post	Δ (%)
Leg press (Kg)	85.2 ± 8.1	121.1 ± 7.7	42	86.1 ± 6.3	124.4 ± 6.8	44
Leg extensor (Kg)	31.4 ± 2.3	42.1 ± 2.3	34	26.3 ± 2.4	40.1 ± 3.2	52
Ankle rotator (Kg)	37.2 ± 3^a	53.5 ± 2.8	44	29.8 ± 1.8	50.1 ± 2.6	68
Glute conditioner (Kg)	48.5 ± 4.8	66.8 ± 3.5	38	50.5 ± 1.5	59.4 ± 2.4	18
Unilateral MVC (Nm)	124.8 ± 8.6	135.3 ± 9.3	9.4	121.6 ± 13.8	137.6 ± 18.8	10.8
Bilateral MVC (Nm)	136.5 ± 13.8	166.8 ± 13.8	29.5	149.5 ± 19.5	188.7 ± 24.5	26.9

^a Indicates significantly lower baseline value in SUP_HighR compared to SUP_LowR ($P<0.05$). Values are Mean $\pm$ S.E

Fig. 2 Summary of endocrine characteristics changes with the two levels of exercise (high versus low intensity) in the presence of nutritional supplementation. The asterisk indicates where post-intervention data were significantly different to baseline values, with $P \leq 0.05$

resulted in no change in plasma glucose in the SUP_HighR group (4.77 ± 0.16 mmol/L to 4.79 ± 0.18 mmol/L). Interestingly, the SUP_LowR group exhibited significantly lower post-intervention plasma glucose of $-13 \pm 4.7\%$ (5.45 ± 0.18 mmol/L to 4.71 ± 0.18 mmol/L). Even after accounting for the baseline differences (using an ANCOVA test), the difference in intervention-induced changes in glucose levels was still significant ($P=0.031$).

Prior to training, there was no significant difference in the mean plasma insulin levels between the two intervention groups. The 12-week intervention resulted in a trend for decreased plasma insulin level in SUP_LowR ($-17 \pm 6.7\%$, i.e., 8.8 ± 1.2 μ U/ml to 7.2 ± 0.9 μ U/ml; $P=0.08$). SUP_HighR group, on the other hand, exhibited a significant $-25 \pm 5.3\%$ decline (i.e., 11.68 ± 0.42 μ U/ml to 8.79 ± 0.83 μ U/ml; $P=0.05$) in plasma insulin levels.

Hormonal profiles—plasma levels of NPY

Prior to training, there was no significant difference in the mean plasma NPY levels between the two intervention groups. The 12-week interventions resulted in a significant decrease in plasma NPY levels for SUP_HighR ($-24 \pm 16.3\%$ change (i.e., 24.7 ± 4.26 ng/ml to 16.1 ± 1.90 ng/ml), $P=0.016$) but no change in SUP_LowR groups ($+16 \pm 20.9\%$ change (i.e., 19.0 ± 1.72 ng/ml to 19.3 ± 1.8 ng/ml).

Plasma levels of IGF-I and IGFBP-3

Serum levels of IGF-I did not differ significantly between the two groups at baseline. Post intervention

values did not change significantly in either group (318.3 ± 39.9 ng/ml at baseline to 282.0 ± 26.5 ng/ml post training in the SUP_LowR group, and 309.9 ± 35.1 ng/ml at baseline to 322.8 ± 25.1 ng/ml post training in the SUP_HighR group).

Serum levels of IGFBP-3 were similar in the two groups at baseline. Post intervention, in the SUP_HighR group, values changed significantly from $3,716.6 \pm 507.5$ ng/ml at baseline to $3,171.1 \pm 370.2$ ng/ml post training, a -11.0% ($P=0.03$) decrease with the intervention. However, in the SUP_LowR group, values did not change significantly with the intervention whilst increasing from $4,252.6 \pm 314.0$ ng/ml at baseline to $4,506.1 \pm 273.7$ ng/ml post training, a 6.0% but non-significant change. The difference in changes seen in the two groups was significant ($P=0.049$).

Plasma levels of TNF-α and IL-6

Serum levels of TNF-α did not differ significantly between the two groups at baseline. Post intervention values did not change significantly in either group (29.3 ± 8.2 pg/ml at baseline to 31.4 ± 7.1 pg/ml post training in the SUP_LowR group and 35.4 ± 13.7 pg/ml at baseline to 27.6 ± 11.8 pg/ml post training in the SUP_HighR group).

Similar to the observations on TNF-α, serum levels of IL-6 did not differ significantly between the two groups at baseline, nor did the interventions have any significant effects on the amount of circulating IL-6; values changed from 3.4 ± 0.6 pg/ml at baseline to 2.6 ± 0.3 pg/ml post training in the SUP_LowR group, and 2.31 ± 0.32 pg/ml at baseline

to 2.08 ± 0.26 pg/ml post training in the SUP_HighR group.

Discussion

Protocol design

The current study proposed to systematically quantify the changes associated with two alternative lifestyle changes in an otherwise healthy older population, comparing (a) a high intensity protocol which combined nutritional supplementation (i.e., carbohydrate plus amino acids) with high intensity resistance exercise, twice supervised and once self-directed each week, and (b) a lower intensity protocol which combined nutritional supplementation (also carbohydrate plus amino acids) with low intensity resistance exercise, once supervised and twice self-directed each week. Notably here, we used amino acids rather than proteins to (a) diminish the likelihood of any deleterious impact on amount of habitual food intake (Fiatarone Singh et al. 2000), but also (b) as amino acids have been shown to be more effective for required end results (e.g., increased net muscle protein synthesis) than protein preparations in this age group (Fiatarone Singh et al. 2000; Paddon-Jones et al. 2005). Crucial also to the study design, was the issue of timing of supplementation. In a recent study of older persons aged 70–80 years (Esmarck et al. 2001), it was demonstrated that the timing of a carbohydrate + protein supplement ingestion affected the degree of response to the treatment in that increments in the cross-sectional area (CSA) and strength of the quadriceps femoris, as well as the mean fibre area in this muscle, were greater where ingestion was immediately after exercise. Arguably, the timing of the protein supplement is key; hence, the current study required for participants to adhere to a 15-min post-exercise window.

Summary of findings in view of the study hypotheses

The principal tenet of the current study was that supplementing lifestyle with low or high resistance exercise protocols in the presence of nutritional supplements would elicit beneficial metabolic, hormonal and functional benefits. Further, while the high intensity regimen may elicit the greater response, a

lower regimen exercise protocol, if successful would be more likely to succeed in terms of compliance, if rolled out on a larger scale and may therefore ultimately elicit the greater population-wide benefit. We also aimed to discuss our findings in the context of recent publications on the benefits of exercise-only and/or combined exercise-supplementation therapies in older persons. Our findings show that both protocols elicited significant increases in strength over the 12-week period, with no significant differences in the protocols employed. Furthermore, functional ability tests showed a significant advantage in those participants following the SUP_LowR protocol. Linked to these observations, to our surprise, lean tissue data in fact showed a greater benefit following the SUP_LowR protocol. Metabolic (a combination of glucose and insulin effects), hormonal (a snapshot of NPY, IGF-I and IGF-BP-3), and cytokine (as shown through IL-6 and TNF- α trends) measures did not tend to favour either protocol.

Muscle size and strength changes

Cuthbertson et al. (2005) have previously demonstrated an anabolic signalling deficit to EAA ingestion in older adults and stated that a deficit in protein synthesis in the basal state is unlikely. These authors attributed the loss of muscle mass with age to decreased sensitivity and responsiveness of muscle protein synthesis to EAAs. It was also proposed that this lack of responsiveness may be associated with decrements in the expression and activation of components of anabolic signalling pathways, and hence, this may be a major contributor to the failure of muscle maintenance in older adults. Previous studies in older adults have shown that provision of dietary supplements has not been effective in improving lean body mass (Campbell et al. 1995; Welle and Thornton 1998). What our data seem to suggest is that different adaptations may occur when combining exercise (intensity) and EAA ingestion. We propose that in the older age group (a) in the presence of relatively low exercise levels, muscle strengthening is significant and there is no impairment in protein synthesis and functional ability is improved, (b) if exercise intensity is high the observed pronounced muscle strengthening appears to favour other, non-hypertrophic (at least in the first few weeks) as yet unidentified factors (thought likely neural related;

Onambélé et al. 2008; Onambele et al. 2006). Taking into consideration both our current data in the SUP_HighR group and a previous observations made by Borsheim et al. (2008) who found that 16-weeks of EAA supplementation (total 22 g per/day—with no exercise), increased IRM in the order of 22% in older adults (but did not significantly increase muscle mass), it seems evident that further studies are required to discriminate between the effects of exercise versus those of protein supplementation in combined therapies.

Molecular mechanisms underpinning adaptation

A key intracellular pathway coordinating signals in the regulation of protein synthesis is the mammalian target of rapamycin or mTOR (Bodine et al. 2001), the signalling of which to its downstream effectors, ribosomal S6 kinase 1 (S6K1) and 4E binding protein 1 (4E-BP1), plays a key regulatory role in the regulation of translation initiation, an initial step in protein synthesis (Wang et al. 2006). Following resistance exercise in animals and humans, components of the mTOR pathway are rapidly up-regulated (Bolster et al. 2003). Whilst the acute muscle protein synthesis (MPS) response after resistance exercise and EAA ingestion is similar between the young and the old, the response is in fact delayed in ageing (Dreyer et al. 2008). It is thought that reduced specificity in older muscle signalling may be the reason for the delay relative to younger persons' MPS signalling. Others have proposed 'potential mechanisms through which mechanical signals and IGF-I-derived signals may be mediated through overlapping pathways (Tidball 2005). While signalling was not assessed in our study, the fact that there was no change in circulating IGF-I but a significant decrease (−11.0%) in IGFBP-3 in SUP_HighR suggests that other mechanisms may be evoking the small but significant hypertrophic response in SUP_LowR group only. Critically, we must not forget that hormonal measures were made at the beginning and end of the trial and therefore do not report early adaptations which may or may not have occurred. Importantly, the pathways accounting for improved strength and functional adaptations in the absence of extensively altered size in the SUP_HighR group also warrants further investigation. While no major changes were evident in IGF or IGFBP-3, there were also no significant

changes in TNF- α /IL-6 either, a further argument to support the need for further studies to begin to elucidate mechanisms.

Body composition and metabolism at the whole body as well as endocrine level

In a study of the effects of a protein + carbohydrate supplement cocktail with heavy resistance training, it was demonstrated that the supplemented group displayed significantly increased weight, skinfold thickness and subcutaneous mid-thigh adiposity (Meredith et al. 1992). By contrast, our results show no effect on BMI, waist/hip ratio or bioelectric impedance measures.

In previous studies, both increased carbohydrate and increased protein supplementation have individually demonstrated positive effects on insulin sensitivity (Baba et al. 1999; Solerte et al. 2004). Similarly exercise, and in particular cardiovascular training (Tonino 1989), has been linked to improved insulin sensitivity. Therefore, the potential for a positive outcome in terms of insulin sensitivity through the combined interventions was investigated. Our data were encouraging in that both levels of exercise were associated with decreased but normal insulin levels (significantly so for SUP_HighR) and these were coupled with fasting glucose effects which also had a potential to indicate increased health (no change in glucose for SUP_HighR, significant decrement for SUP_LowR). Some have suggested the loss of muscle mass commonly associated with the ageing process is a relative resistance to insulin stimulated amino acid uptake and stimulation of muscle protein synthesis (Volpi et al. 2000). Insulin itself is a known regulator of protein metabolism. However, the manner by which insulin promotes anabolism in human skeletal muscle is unresolved. Available data show that insulin activates several proteins (e.g., phosphatidylinositol-3 kinase), that cause downstream phosphorylation of factors known to play key roles in regulating protein and glycogen synthesis (Kimball and Jefferson 1994). Moreover, insulin has been shown to attenuate ubiquitin proteolysis (Roberts et al. 2003), which is believed to be responsible for the degradation of the bulk of muscle proteins (Ventadour and Attaix 2006). Following resistance-type exercise insulin appears to have little effect on protein synthesis. This may be due to a reduction of amino acids in the intracellular pool,

if insulin does indeed reduce muscle protein breakdown. In addition, post-exercise insulin concentrations elevated independently of amino acid availability, through carbohydrate ingestion alone, have been found to reduce proteolysis and promote muscle protein accretion (Borsheim et al. 2004). In the present study, beneficial effects on metabolic profiles were evident as a consequence of the exercise plus supplement administration and these benefits, although subtly different were evident in both groups, regardless of intensity.

Functional abilities changes with exercise training

The energy required to build new tissues appears to increase with age (Shizgal et al. 1992). Yet the direct impact of supplementation-only interventions on habitual functional ability tends to vary from limited (Gray-Donald et al. 1995) to non-existent (Payette et al. 2002). In fact, prior to the current study, any validation of the use of EAAs (supplements in combination with resistance training) in improving functional abilities in older adults was scarce and tended to be limited to frail older persons (Fiatarone et al. 1994), rather than the comparatively healthy group in the current study. In fact, where it existed in healthy populations, earlier evidence was as contradictory as in frail persons, with some studies showing no additional benefit (Rosendahl et al. 2006) and others highlighting clear benefits (Bonney et al. 2003) of supplementation on improvements in functional abilities. Further than this, our data would suggest that increased background physical activity even of a relatively low level, works well in combination with supplementation to improve markers of increased independence in old age, such as get-up-and-go, standing-from-lying and 6-min walk tasks.

Conclusion

The current data suggest that both levels of lifestyle changes are beneficial to older person, with the low level exercise plus supplement having profound impact not only on muscle mass, but also on strength, functional ability and metabolism. The mechanisms underpinning the differential responses remain to be defined. Nevertheless, we have identified a protocol

(SUP_LowR), which over a 12-week period appears to have beneficial effects on all participants, was palatable (in terms of compliance) and may therefore form the basis for a larger roll-out trial.

References

- Baba NH, Sawaya S, Torbay N, Habbal Z, Azar S, Hashim SA (1999) High protein vs high carbohydrate hypoenergetic diet for the treatment of obese hyperinsulinemic subjects. *Int J Obes Relat Metab Disord* 23(11):1202–1206
- Balogopal P, Rooyackers OE, Adey DB, Ades PA, Nair KS (1997) Effects of aging on in vivo synthesis of skeletal muscle myosin heavy chain and sarcoplasmic protein in humans. *Am J Physiol* 273(4 Pt 1):E790–E800
- Blumberg J (1994) Nutrient requirements of the healthy elderly—should there be specific RDAs? *Nutr Rev* 52(8 Pt 2):S15–S18
- Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R et al (2001) Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* 3(11):1014–1019
- Bohe J, Low A, Wolfe RR, Rennie MJ (2003) Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. *J Physiol* 552(Pt 1):315–324
- Bolster DR, Kubica N, Crozier SJ, Williamson DL, Farrell PA, Kimball SR et al (2003) Immediate response of mammalian target of rapamycin (mTOR)-mediated signalling following acute resistance exercise in rat skeletal muscle. *J Physiol* 553(Pt 1):213–220
- Bonnefoy M, Cornu C, Normand S, Boutitie F, Bugnard F, Rahmani A et al (2003) The effects of exercise and protein-energy supplements on body composition and muscle function in frail elderly individuals: a long-term controlled randomised study. *Br J Nutr* 89(5):731–739
- Borsheim E, Aarsland A, Wolfe RR (2004) Effect of an amino acid, protein, and carbohydrate mixture on net muscle protein balance after resistance exercise. *Int J Sport Nutr Exerc Metab* 14(3):255–271
- Borsheim E, Bui QU, Tissier S, Kobayashi H, Ferrando AA, Wolfe RR (2008) Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. *Clin Nutr* 27(2):189–195
- Campbell WW, Crim MC, Young VR, Joseph LJ, Evans WJ (1995) Effects of resistance training and dietary protein intake on protein metabolism in older adults. *Am J Physiol* 268(6 Pt 1):E1143–E1153
- Campbell WW, Trappe TA, Wolfe RR, Evans WJ (2001) The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. *J Gerontol A Biol Sci Med Sci* 56(6):M373–M380
- Castaneda C, Charnley JM, Evans WJ, Crim MC (1995) Elderly women accommodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. *Am J Clin Nutr* 62(1):30–39
- Chandler JM, Duncan PW, Kochersberger G, Studenski S (1998) Is lower extremity strength gain associated with

- improvement in physical performance and disability in frail, community-dwelling elders? *Arch Phys Med Rehabil* 79(1):24–30
- Chernoff R (2004) Protein and older adults. *J Am Coll Nutr* 23 (6 Suppl):627S–630S
- Chevalier S, Gougeon R, Nayar K, Morais JA (2003) Frailty amplifies the effects of aging on protein metabolism: role of protein intake. *Am J Clin Nutr* 78:422–429
- Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P et al (2005) Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* 19(3):422–424
- Dreyer HC, Drummond MJ, Pennings B, Fujita S, Glynn EL, Chinkes DL et al (2008) Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. *Am J Physiol Endocrinol Metab* 294(2):E392–E400
- Esmarck B, Andersen JL, Olsen S, Richter EA, Mizuno M, Kjaer M (2001) Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *J Physiol* 535(Pt 1):301–311
- Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME et al (1994) Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* 330(25):1769–1775
- Fiatarone Singh MA, Bernstein MA, Ryan AD, O'Neill EF, Clements KM, Evans WJ (2000) The effect of oral nutritional supplements on habitual dietary quality and quantity in frail elders. *J Nutr Health Aging* 4(1):5–12
- Frontera WR, Meredith CN, O'Reilly KP, Knuttgen HG, Evans WJ (1988) Strength conditioning in older men: skeletal muscle hypertrophy and improved function. *J Appl Physiol* 64(3):1038–1044
- Guigoz Y (2006) The Mini Nutritional Assessment (MNA) review of the literature—what does it tell us? *J Nutr Health Aging* 10(6):466–485, Discussion 485–7
- Gray-Donald K, Payette H, Boutier V (1995) Randomized clinical trial of nutritional supplementation shows little effect on functional status among free-living frail elderly. *J Nutr* 125(12):2965–2971
- Jensen MB, Hessel I (1997) Dietary supplementation at home improves the regain of lean body mass after surgery. *Nutrition* 13(5):422–430
- Jorgensen JO, Vahl N, Hansen TB, Thuesen L, Hagen C, Christiansen JS (1996) Growth hormone versus placebo treatment for one year in growth hormone deficient adults: increase in exercise capacity and normalization of body composition. *Clin Endocrinol (Oxf)* 45(6):681–688
- Kaiser FE, Morley JE (1994) Idiopathic CD4⁺ T lymphopenia in older persons. *J Am Geriatr Soc* 42(12):1291–1294
- Kimball SR, Jefferson LS (1994) Mechanisms of translational control in liver and skeletal muscle. *Biochimie* 76(8):729–736
- Klitgaard H, Mantoni M, Schiaffino S, Ausoni S, Gorza L, Laurent-Winter C et al (1990) Function, morphology and protein expression of ageing skeletal muscle: a cross-sectional study of elderly men with different training backgrounds. *Acta Physiol Scand* 140(1):41–54
- Lauque S, Arnaud-Battandier F, Mansourian R, Guigoz Y, Paintin M, Nourhashemi F et al (2000) Protein-energy oral supplementation in malnourished nursing home residents. A controlled trial. *Age Ageing* 29(1):51–56
- Levenhagen DK, Carr C, Carlson MG, Maron DJ, Borel MJ, Flakoll PJ (2002) Post-exercise protein intake enhances whole body and leg protein accretion in humans. *Med Sci Sports Exerc* 34(5):828–837
- Lintsi M, Kaarma H, Kull I (2004) Comparison of hand-to-hand bioimpedance and anthropometry equations versus dual-energy X-ray absorptiometry for the assessment of body fat percentage in 17–18-year-old conscripts. *Clin Physiol Funct Imaging* 24(2):85–90
- Lucas M, Heiss CJ (2005) Protein needs of older adults engaged in resistance training: a review. *J Aging Phys Act* 13(2):223–236
- Meredith CN, Frontera WR, O'Reilly KP, Evans WJ (1992) Body composition in elderly men: effect of dietary modification during strength training. *J Am Geriatr Soc* 40(2):155–162
- Onambélé GL, Maganaris CN, Mian OS, Tam E, Rejc E, McEwan IM et al (2008) Inertial vs. isotonic training in old age: neuromuscular and balance effects. *J Biomechanics* 41(15):3133–3138
- Onambele GN, Bruce SA, Woledge RC (2006) Oestrogen status in relation to the early training responses in human thumb adductor muscles. *Acta Physiol (Oxf)* 188(1):41–52
- Paddon-Jones D, Sheffield-Moore M, Aarsland A, Wolfe RR, Ferrando AA (2005) Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion. *Am J Physiol Endocrinol Metab* 288(4):E761–E767
- Paddon-Jones D, Sheffield-Moore M, Katsanos CS, Zhang XJ, Wolfe RR (2006) Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. *Exp Gerontol* 41(2):215–219
- Payette H, Boutier V, Coulombe C, Gray-Donald K (2002) Benefits of nutritional supplementation in free-living, frail, undernourished elderly people: a prospective randomized community trial. *J Am Diet Assoc* 102(8):1088–1095
- Pearson SJ, Onambele GN (2005) Acute changes in knee-extensors torque, fiber pennation, and tendon characteristics. *Chronobiol Int* 22(6):1013–1027
- Pearson SJ, Onambele GN (2006) Influence of time of day on tendon compliance and estimations of voluntary activation levels. *Muscle Nerve* 33(6):792–800
- Prior BM, Cureton KJ, Modlesky CM, Evans EM, Sloniger MA, Saunders M et al (1997) In vivo validation of whole body composition estimates from dual-energy X-ray absorptiometry. *J Appl Physiol* 83(2):623–630
- Rea IM, Alexander HD, Crockard AD, Morris TC (1996) CD4 lymphopenia in very elderly people. *Lancet* 347 (8997):328–329
- Roberts RG, Redfern CP, Goodship TH (2003) Effect of insulin upon protein degradation in cultured human myocytes. *Eur J Clin Invest* 33(10):861–867
- Rosendahl E, Lindelof N, Littbrand H, Yifter-Lindgren E, Lundin-Olsson L, Haglin L et al (2006) High-intensity functional exercise program and protein-enriched energy supplement for older persons dependent in activities of daily living: a randomised controlled trial. *Aust J Physiother* 52(2):105–113
- Shizgal HM, Martin MF, Gimmon Z (1992) The effect of age on the caloric requirement of malnourished individuals. *Am J Clin Nutr* 55(4):783–789

- Short KR, Vittone JL, Bigelow ML, Proctor DN, Nair KS (2004) Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am J Physiol Endocrinol Metab* 286(1):E92–E101
- Skelton DA, Young A, Greig CA, Malbut KE (1995) Effects of resistance training on strength, power, and selected functional abilities of women aged 75 and older. *J Am Geriatr Soc* 43(10):1081–1087
- Solerte SB, Gazzaruso C, Schifino N, Locatelli E, Destro T, Ceresini G et al (2004) Metabolic effects of orally administered amino acid mixture in elderly subjects with poorly controlled type 2 diabetes mellitus. *Am J Cardiol* 93(8A):23A–29A
- Tidball JG (2005) Mechanical signal transduction in skeletal muscle growth and adaptation. *J Appl Physiol* 98(5):1900–1908
- Tipton KD, Elliott TA, Cree MG, Aarsland AA, Sanford AP, Wolfe RR (2007) Stimulation of net muscle protein synthesis by whey rotein ingestion before and after exercise. *Am J Physiol Endocrinol Metab* 292:E71–E76
- Tobin J, Spector D (1986) Dietary protein has no effect on future creatinine clearance. *Gerontologist* 26:59A
- Tonino RP (1989) Effect of physical training on the insulin resistance of aging. *Am J Physiol* 256(3 Pt 1):E352–E356
- Vellas BJ, Hunt WC, Romero LJ, Koehler KM, Baumgartner RN, Garry PJ (1997) Changes in nutritional status and patterns of morbidity among free-living elderly persons: a 10-year longitudinal study. *Nutrition* 13(6):515–519
- Ventadour S, Attaix D (2006) Mechanisms of skeletal muscle atrophy. *Curr Opin Rheumatol* 18(6):631–635
- Volkert D, Kruse W, Oster P, Schlierf G (1992) Malnutrition in geriatric patients: diagnostic and prognostic significance of nutritional parameters. *Ann Nutr Metab* 36(2):97–112
- Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR (2003) Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* 78(2):250–258
- Volpi E, Mittendorfer B, Rasmussen BB, Wolfe RR (2000) The response of muscle protein anabolism to combined hyper-aminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab* 85(12):4481–4490
- Volpi E, Mittendorfer B, Wolf SE, Wolfe RR (1999) Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol* 277(3 Pt 1):E513–E520
- Wagenmakers AJ (1999) Tracers to investigate protein and amino acid metabolism in human subjects. *Proc Nutr Soc* 58(4):987–1000
- Wang Y, Barbaro MF, Baraban SC (2006) A role for the mTOR pathway in surface expression of AMPA receptors. *Neurosci Lett* 401(1–2):35–39
- Welle S, Thornton CA (1998) High-protein meals do not enhance myofibrillar synthesis after resistance exercise in 62- to 75-yr-old men and women. *Am J Physiol* 274(4 Pt 1):E677–E683