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Oral nutritional supplement fortified with beta-alanine improves physical working capacity in older adults: A randomized, placebo-controlled study

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ABSTRACT

This study examined the effects of an oral nutritional supplement fortified with two different doses of beta-alanine on body composition, muscle function and physical capacity in older adults. Using a double-blind placebo controlled design, 60 men and women (age ± SD = 70.7 ± 6.2 yrs) were randomly assigned to one of three treatment groups: 1) oral nutritional supplement (ONS; n = 20) (8 oz; 230 kcal; 12 g PRO; 31 g CHO; 6 g FAT), 2) ONS plus 800 mg beta-alanine (ONS800; n = 19), and 3) ONS plus 1200 mg beta-alanine (ONS1200; n = 21). Treatments were consumed twice per day for 12 weeks. At pre- and post-supplementation period, participants performed a discontinuous, submaximal cycle ergometry test to determine physical working capacity at fatigue threshold (PWCFT). Fat mass, total body and arm lean soft tissue mass (ALSTM) were measured with DEXA while muscle strength was assessed with handgrip dynamometry (GRIP) and 30-s sit-to-stand (STS) was used to measure lower body functionality. Muscle quality (MQ) was calculated with GRIPmax and DEXA derived ALSTM [GRIP (kg)·ALSTM (kg)$^{-1}$]. Two-way analysis of variance was used to compare pre- to post-supplementation measures and group differences. There were 16 dropouts over the duration of the study. Final group sizes were ONS = 16 (m = 11, w = 5), ONS800 = 15 (m = 5, w = 10), and ONS1200 = 13 (m = 6, w = 7). No significant changes were observed for body composition or GRIP values pre to post. Significant increases in PWC_{FT} were seen in ONS1200 (13.6%) and ONS800 (17.8%) pre- to post-supplementation ($p < 0.05$). These changes were significantly greater ($p < 0.05$) than the changes in ONS (−6.3%). ONS1200 and ONS had significant increases in STS (22.2 and 10.7%, respectively). While ONS significantly increased in STS, no differences ($p > 0.05$) in change scores were found between ONS and ONS800. ONS fortified with beta-alanine may improve physical working capacity, muscle quality and function in older men and women. These findings could have importance in the perception of frailty, and the maintenance of health and independent living in older adults.

1. Introduction

Sarcopenia is an age-related condition characterized by a loss of skeletal muscle mass, with accompanying loss of strength, power, and the ability to resist fatigue (Brooks and Faulkner, 1994; Chandler and Hadley, 1996). These modifications may be the result of changing skeletal muscle morphology, including both a decrease in size and loss of type I and II muscle fibers (Doherty, 2003) and an increase in fat and connective tissue infiltration, which has been shown to decrease muscle quality (MQ) (Cadore et al., 2012; del Favero et al., 2012). Muscle quality has been shown to be associated with strength, power and anaerobic threshold in an older population (Cadore et al., 2012; Fukumoto et al.,

Abbreviations: PWC_{FT}, Physical Working Capacity at Fatigue Threshold; ONS, Oral Nutritional Supplement; MQ, Muscle Quality; DEXA, Dual-energy X-ray Absorptiometry; TLSTM, Total body lean soft tissue mass; ALSTM, Arm lean soft tissue mass; EMG, Electromyography.

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2012). Cadore et al. (2012) recently suggested that the age related decrease in MQ, due to the increase in intramuscular fat and connective tissue, may be associated with a decrease in the number of capillaries. This change in capillary density may disrupt the blood supply to adjacent muscle fibers and impair cardiorespiratory capacity resulting in the inability to resist fatigue at relatively low workloads due to anaerobic acidosis.

In addition to a decrease in MQ, several studies have shown a decrease in muscle carnosine levels in older adults (Baguet et al., 2012; Everaert et al., 2011; Stuerenburg and Kunze, 1999; Tallon et al., 2007). Carnosine is a dipeptide that works as an intramuscular hydrogen ion (H^+) buffer (Harris et al., 1990, 2006). The decrease in muscle carnosine levels may lead to a decrease in buffering capacity of the muscle, diminishing the ability to withstand the build-up of H^+ during anaerobic activities (Stout et al., 2008). With a decrease in MQ and carnosine content, daily activities like walking up stairs may result in muscle acidosis and lead to an inability to continue due to fatigue. The ability to resist fatigue may be important due to its potential relationship with balance, gait speed, and increased risk of falls, which has been related to an increased risk of injury and death (Alexander et al., 1997; Chandler and Hadley, 1996; del Favero et al., 2012; Kannus et al., 2005; Madureira et al., 2010; Stout et al., 2008).

There are a couple of factors that may cause this decrease in carnosine concentration with age. β-Alanine is the rate limiting amino acid for muscle carnosine synthesis which occurs naturally in the diet (e.g., chicken and fish) (Harris et al., 2006). The first possible cause for the decrease in muscle carnosine, therefore, may be due to a movement towards a more vegetarian diet, or a diet with a smaller amount of fresh meat as opposed to processed meat (Everaert et al., 2011; Kim, 2009). In support, one study of the elderly where it was known that subjects were continuing to eat a high meat (mainly chicken and fish) diet, muscle carnosine levels were unchanged when compared with a young population from the same country (Kim, 2009). The second cause of a decrease in muscle carnosine with aging is an atrophy of type II muscle fibers with area occupied decreasing (Tallon et al., 2007). Thus in a given volume or weight of muscle there will be less type II muscle even if the percentage of fibers has not changed, i.e. they just got thinner. Thus even if the carnosine content per kg of muscle does not change in either type I and II muscle fibers there will appear to be a reduction in the content of carnosine at the whole muscle level (Tallon et al., 2007).

Several studies have shown that muscle carnosine content increases significantly following β-alanine supplementation (Baguet et al., 2010; del Favero et al., 2012; Harris et al., 2006; Hill et al., 2007) and is directly related to an increase in exercise capacity. Two studies (del Favero et al., 2012; Stout et al., 2008) have shown a significant increase in exercise capacity in older adult populations following β-alanine supplementation. Recently, del Favero et al. (2012) demonstrated a significant increase (+85.4%) in muscle carnosine levels in an older adult population (65 ± 4 yrs) following 3.2 $g \cdot day^{-1}$ of β-alanine supplementation over an 84 day period. Following the supplementation period there were significant increases in time to exhaustion at a constant workload on a treadmill (36.5%) and in time to exhaustion during an incremental treadmill test (12.2%). In support, Stout et al. (2008) observed a 28.6% increase in physical working capacity at fatigue threshold (PWC_{FT}) following 12-weeks of β-alanine supplementation (2.4 $g \cdot day^{-1}$) in older (72.8 ± 11.1 years) men and women. Therefore, data showing β-alanine supplementation may improve PWC may have significant implications for the aging population.

The improvements in exercise capacity seen in older adults with β-alanine supplementation appear to be effective at moderate (3.2 $g \cdot day^{-1}$) and low (2.4 $g \cdot day^{-1}$) doses for more than 84 days, however, no study to our knowledge has investigated whether a very low dose (1.6 $g \cdot day^{-1}$) has similar efficacy in this population. The primary purpose of this study was to determine the effect of 12-weeks of β-alanine added to a commercially available oral nutrition supplement (ONS) on PWC_{FT}, MQ, muscle strength, and muscle function in an older adult population. This study was a preliminary examination testing the safety and efficacy of adding β-alanine to a commercially available product that people over the age of 60 commonly consume. We know of no studies to date that have examined the effects of β-alanine added to an ONS. A secondary purpose was to examine the efficacy of two lower dose levels (2.4 $g \cdot day^{-1}$ and 1.6 $g \cdot day^{-1}$) of β-alanine added to the ONS on the performance measures. Previous studies (Del Favero et al., 2012; Stout et al., 2008) have provided evidence that higher doses of β-alanine improved endurance capacity in a similar cohort of men and women; however, the higher dosing may produce paresthesia in some people. Therefore it is important to determine if a low dose of β-alanine can produce the positive effects on performance without paresthesia.

2. Methods

2.1. Participants

Sixty older men (n = 27) and women (n = 33) from Central Florida volunteered to participate in this double-blind, placebo-controlled study. A medical history questionnaire and interviews with participants prior to the testing revealed no one was supplementing with beta-alanine prior to the study period. No participants had major surgery within the previous 6 months, or a history of asthma, uncontrolled heart or pulmonary disease, uncontrolled hypertension, or were taking any medications that would interfere with exercise. All procedures were approved by the University Institutional Review Board. Following an explanation of all related risks and benefits associated with the experimental protocol, each participant gave his or her written informed consent to participate in the study.

2.2. Experimental design

Immediately following baseline testing, participants were randomly assigned to one of three treatment conditions. Each group supplemented with 8 liquid ounces (227 g) of a commercially available oral ONS containing 230 kcal, 12 g of protein, 31 g of carbohydrate, and 6 g of fat (Ensure High Protein, Abbott Nutrition). Group 1 ingested ONS only (n = 20); group 2 ingested ONS plus 800 mg β-alanine (ONS800; n = 19); and group 3 ingested ONS plus 1200 mg β-alanine (ONS1200; n = 21). Supplements were consumed twice per day for 12-weeks. The ONS was fortified with Carnosyn™, non-sustained release powder, β-alanine. The two dose levels of β-alanine used to fortify the ONS were examined to determine if they produce significant exercise improvements while attenuating the potential paresthesia observed in previous studies. The ONS was produced from the same batch and therefore the ingredient make-up was identical, with the exception of the addition of the β-alanine. At the outset of the study, participants were briefed on the number of calories in the ONS and dietary strategies to maintain an isocaloric diet in order to maintain weight throughout the study. Additionally, participants were asked to continue their normal daily exercise routine if they were exercising. Participants were asked to refrain from vigorous physical activity the day before testing. During each visit, participants were questioned to confirm compliance with the study protocol as directed. To measure compliance, participants returned empty bottles or bottle caps. Compliance for each group during the 12 week supplementation was: ONS =95%, ONS800 = 93%, and ONS1200 = 90%.

Participants were instructed to complete a three-day dietary recall at pre- and post-testing. Participants were instructed to write down everything consumed during two weekdays and one weekend day. These data were entered into a software program (Food Works 13, The Nutrition Company, Long Valley, NJ) which provided calculations for daily protein intake (g) and total calories (kcal).

Of the 60 participants that enrolled in the study, 44 completed follow-up testing. One participant was excluded for initiation of oral creatine supplementation following baseline testing, another for a sickness contracted late in the study, and the rest were voluntary dropouts due to personal reasons. Two of the dropouts from the ONS1200 group cited paresthesia as the reason for dropping out. Four other participants in the ONS1200 group noted paresthesia, but were willing to continue with the study. No dropouts from the ONS800 group noted paresthesia as the reason for leaving the study. One person from the ONS800 group did experience paresthesia, but was willing to continue with the study. Final group sizes were ONS = 16 (m = 11, w = 5), ONS800 = 15 (m = 5, w = 10), and ONS1200 = 13 (m = 6, w = 7). Characteristics of those participants who completed the study are presented in Table 1. In addition, Table 2 presents information detailing the medicines, supplements, and physical activity of those participants completing the study. Using the procedures described by Gravettier and Wallnau (1996) for estimating sample sizes for repeated measures designs, a minimum sample size of n = 12 was required for each group to reach a statistical power $(1-\beta)$ of 0.80 based on the findings of Stout et al. (2008).

Table 2
Medications, supplements, and physical activity of participants.

	Number/% of participants[a]
Medication	
High cholesterol	26/59%
Hypertension	17/39%
Acid reflux	10/23%
Heart issues	8/18%
Type II diabetes	5/11%
Osteoporosis/osteopenia	4/9%
Depression	4/9%
Thyroid	4/9%
Arthritis	3/7%
Taking no medication	8/18%
Supplements	
Vitamins	25/57%
Fish oil	18/41%
Calcium	16/36%
Aspirin	13/30%
Glucosamine–chondroitin	9/21%
Biotin	3/7%
Magnesium	2/5%
Zinc	2/5%
Taking no supplements	10/23%
Physical activity	
Structured physical activity[b]	30/68%
Physically active[c]	5/11%
Sedentary	9/21%

[a] Participants = only those completing the study (n = 44).
[b] At least twice·week^{-1}.
[c] Activities included gardening, housework, etc.

2.3. Measures

Dual-energy X-ray absorptiometry (DEXA) (GE Lunar Prodigy, Madison, WI) was performed to estimate fat mass, total body lean soft tissue mass (TLSTM) and arm lean soft tissue mass (ALSTM) pre and post supplement period. During both visits, participants performed a discontinuous, cycle ergometry test on an electronically-braked cycle ergometer to determine the physical working capacity at fatigue threshold (PWC_{FT}), a handgrip dynamometry test (GRIP) to assess muscle strength, and a 30-s sit-to-stand test (STS) to measure lower body functionality. To ensure safety from a hematological standpoint, a fasted blood draw was performed at the university health center at pre and post supplementation. Samples were analyzed at a commercial laboratory for complete blood counts and general blood chemistry.

2.4. Electromyography (EMG) measurements

A bipolar (4.6 cm center-to-center) surface electrode (Quinton Quick-Prep silver-silver chloride) arrangement was placed over the right vastus lateralis muscle, at approximately 60% of the distance from the lateral portion of the patella on a line with the greater trochanter. The reference electrode was placed over the lateral epicondyle of the distal femur. Inter-electrode impedance was kept below 5000 Ω with abrasion of the skin beneath the electrodes. The raw EMG signals were pre-amplified using a differential amplifier (MP150 BIOPAC Systems, Inc., Santa Barbara, CA), sampled at 1000 Hz, and stored on a personal computer (Dell Latitude E6530, Dell Inc., Round Rock, TX) for off-line analysis. The EMG signals were expressed as root mean square (rms) amplitude values (μV rms) by software (AcqKnowledge v4.2, BIOPAC Systems, Inc., Santa Barbara, CA).

2.5. Determination of PWC_{FT}

Determination of PWC_{FT} values was previously described by de Vries et al. (1987) for the vastus lateralis. The initial work rate was set at 30 W for each test. The participants pedaled at 50 revolutions per minute (rpm) for two-minute stages on an electronically-braked cycle ergometer (Lode, Excalibur Sport, Groningen, Netherlands). Toe clips were utilized for each participant. Following each stage of exercise, the EMG-rms amplitude values were saved on a personal computer and further analyzed with custom-written software (LabView, National Instruments Corporation, Austin, TX). If the exercise stage did not produce a statistically significant ($p < 0.05$), positive slope of the amplitudes (rms) across the two-minute work bout, the resistance was increased 10–20 W until a significant, positive amplitude (rms) slope was achieved or the participant reached 75% of their age-predicted maximal heart rate, or surpassed a rating of perceived exertion (RPE) of 13 ("Somewhat Hard") on the Borg scale. If a significant, positive amplitude (rms) slope was reached, one final stage was performed at 5–10 W less than the resistance of the stage that produced the significant, positive slope. The PWC_{FT} was estimated to be the mean resistance of the highest non-statistically significant positive slope and the lowest statistically significant positive slope. In the event the participant did not have a statistically significant, positive amplitude (rms) slope during any stage of their PWC_{FT}, a regression analysis was performed utilizing the amplitude (rms) slope for each two-minute stage against each corresponding workload (watts). The y-intercept (watts) produced in this analysis was then used as the PWC_{FT} (de Vries et al., 1982).

Test–retest reliability for the PWC_{FT} test was determined from 10 participants who were randomly selected from the ONS (control) group that were measured 40 days apart. The intraclass correlation coefficient (ICC) was 0.95 (SEM = 13.7 W). No significant difference

Table 1
Baseline participant characteristics of those completing study.

Treatment group	Age (yrs)	Height (cm)	Weight (kg)	BMI
ONS (n = 16, m = 11/w = 5)	70.6 ± 6.8	165.3 ± 16.3	77.8 ± 17.3	29.9 ± 7.2
ONS800 (n = 15, m = 5/w = 10)	70.8 ± 6.1	164.8 ± 10.2	73.7 ± 11.5	28.7 ± 6.0
ONS1200 (n = 13, m = 6/w = 7)	72.4 ± 6.4	169.9 ± 11.3	87.4 ± 25.8	27.1 ± 3.2

Values reported as mean ± standard deviation (SD).

($p > 0.05$) was noted between the mean PWC_{FT} values from trial 1 (69.5 ± 44.1 W) to trial 2 (68.5 ± 43.3 W). In addition, the ICC (0.95) and SEM (12.3 W) for estimating PWC_{FT} from plotting the slopes of EMG-rms versus time against each workload revealed no significant differences between the estimated mean PWC_{FT} values from trial 1 (46.7 ± 12.3W) to trial 2 (51.1 ± 11.1W). These ICC results were similar to de Vries et al. (1989) and Stout et al. (2008) who reported ICC values of 0.97 and 0.83, respectively in older adults.

2.6. Determination of strength measures

During the GRIP, participants were standing with the hand grip dynamometer (JAMAR, Sammons Preston Rolyan, Bolingbrook, IL) in their dominant hand. The dynamometer handle was adjusted so that the middle phalange of the third digit was comfortably perpendicular to the long axis of the handle. The arms were adducted with the dynamometer held at a 90° angle to their body. Participants were instructed to squeeze the handle as hard as they could for three to five seconds. Verbal encouragement was provided to illicit maximum effort. The participants performed three trials. GRIP values were recorded to the nearest whole number (kg). Average ($GRIP_{AVG}$) for the three trials and maximum ($GRIP_{MAX}$) values were recorded. Test–retest reliability for the GRIP test was determined using 16 participants from the control group measured 40 days apart. The ICC was 0.94 (SEM = 4.5 kg). There was no significant difference ($p > 0.05$) between the mean GRIP values from trial 1 (35.7 ± 3.1 kg) to trial 2 (34.9 ± 3.1 kg).

Muscle quality (MQ) was calculated as relative strength with $GRIP_{MAX}$ and DEXA derived ALSTM [GRIP (kg)·ALSTM $(kg)^{-1}$]. Test–retest reliability for the MQ was determined by using 16 participants from the control group measured 40 days apart. The ICC was 0.68 (SEM = 1.58 $kg \cdot kg^{-1}$). There was no significant difference ($p > 0.05$) between the mean MQ values from trial 1 (12.32 ± 2.06 $kg \cdot kg^{-1}$) to trial 2 (12.18 ± 1.87 $kg \cdot kg^{-1}$).

During the STS, participants sat in an armless chair with their arms crossed over their chest and upon first movement the timer started the stopwatch. Participants stood to a vertical position then sat down on the chair, repeating this process for 30 s. STS values were recorded to the nearest whole number, for those repetitions that the participant made it to a complete standing position. Test–retest reliability for the STS test was determined by using 16 participants from the control group measured 40 days apart. The ICC was 0.96 (SEM = 1.8). There was a significant difference ($p = 0.026$) between the mean STS values from trial 1 (17.5 ± 5.76) to trial 2 (18.6 ± 6.42).

2.7. Statistical analysis

A two-way [treatment (ONS vs. ONS800 vs. ONS1200) × time (pre vs. post)] Analysis of Variance (ANOVA) was used to analyze the data. If baseline values were different between the groups or as a follow-up to significant interaction, then an Analysis of Covariance (ANCOVA) was utilized to analyze differences between groups. If significant interactions were observed, then Tukey post hoc pair-wise comparisons were used to examine group differences. In addition, follow-up to a significant main effect included paired sample t-tests for each group over time. For effect size, the partial eta squared statistic was calculated and a value of 0.01, 0.06, and 0.14 were used to represent small, medium, and large effect sizes, respectively. An alpha level of $p < 0.05$ was used to determine statistical significance. Data were analyzed using SPSS v20 software (SPSS Inc., Chicago, IL).

3. Results

3.1. Body mass and dietary analysis

There were no significant changes in body weight, lean soft tissue, or fat mass across the time period for any group. There were no significant differences ($p > 0.05$) from pre- to post-supplement for protein intake or total calories consumed (Table 3). With no difference in protein intake it is believed that there were no changes in carnosine intake with the exception of those participants consuming ONS800 and ONS1200.

3.2. Blood analysis

The results of the pre- to post-supplementation blood analysis are presented in Table 4. Results revealed that 2 measures (glucose and mean corpuscular hemoglobin concentration) were outside the normal range at the beginning of the period for all three groups. Glucose stayed outside the normal range at post-supplement testing for all groups. With no measures moving outside the normal range, it appears β-alanine supplementation is safe from a hematological standpoint in older adults.

3.3. PWC_{FT}

There was a significant interaction ($p = 0.005$; $\eta^2 = 0.23$) for group × time for pre- to post-supplement. Groups ONS1200 and ONS800 exhibited significant ($p < 0.05$) improvements in their PWC_{FT}, while group ONS did not show an improvement in PWC_{FT}. Table 5 presents the mean and standard deviation values for the PWC_{FT} for each group as well as the percent change values from pre- to post supplementation testing. Of note, when the statistics are recomputed excluding those participants who experienced paresthesia, the statistical difference remained the same. Groups ONS800 and ONS12000 experienced significant increases in PWC_{FT}, and these two groups were not significantly different.

3.4. Strength measures

There were no significant differences in $GRIP_{MAX}$ or $GRIP_{AVG}$ for group × time for pre- to post-supplement. There was a significant group x time interaction ($p = 0.024$; $\eta^2 = 0.167$) for STS from pre- to post-supplement. Each group showed improvement with group ONS and ONS1200 showing significant improvement over time (Table 5). When post-supplement values were adjusted for the initial differences

Table 3
Dietary protein and caloric intake differences pre to post supplementation.

Treatment	Dietary protein content (g)			Total caloric intake (kcal)		
	Mean (g)			Mean (kcal)		
	Pre	Post	p[a]	Pre	Post	p[a]
ONS1200	78.72 ± 32.59	95.69 ± 22.31	0.171	1767.11 ± 730.31	2165.22 ± 436.71	0.777
ONS800	84.77 ± 25.87	87.39 ± 18.59	0.772	2103.4 ± 552.09	1974.6 ± 497.67	0.361
ONS	84.77 ± 25.87	79.36 ± 19.05	0.129	2204.08 ± 774.35	1983.34 ± 630.31	0.354

Values reported as mean ± standard deviation (SD).
[a] p value represents pre to post difference.

Table 4
Pre to post supplementation hematological results.

Variable	ONS1200 (n = 13)		ONS (n = 16)		ONS800 (n = 15)	
	Pre	Post	Pre	Post	Pre	Post
Sodium (mmol/L)	140.00 ± 3.74	140.00 ± 2.35	142.19 ± 3.15	140.88 ± 2.25	142.20 ± 2.78	141.87 ± 1.85
Potassium (mmol/L)	5.09 ± 0.46	4.99 ± 0.46	4.78 ± 0.37	5.13 ± 0.54[a]	5.05 ± 0.56	4.92 ± 0.36
Chloride (mmol/L)	104.77 ± 3.54	104.15 ± 2.97	104.94 ± 2.77	105.88 ± 1.71	104.93 ± 2.66	104.87 ± 2.29
Total CO_2 (mmol/L)	28.00 ± 1.83	25.54 ± 4.01[a]	28.19 ± 2.14	27.39 ± 1.93	27.33 ± 3.02	26.93 ± 2.58
Anion gap (mmol/L)	7.00 ± 2.49	9.45 ± 3.67	8.69 ± 2.24	7.63 ± 2.63	9.93 ± 3.31	9.93 ± 2.20
Glucose (mg/dL)	113.92 ± 32.00[a]	114.08 ± 31.23[b]	101.31 ± 11.91[c]	100.56 ± 10.97[b]	102.33 ± 10.17[c]	100.60 ± 14.94[b]
Urea nitrogen (mg/dL)	21.58 ± 8.04	22.69 ± 7.32	21.69 ± 5.44	22.75 ± 10.04	21.00 ± 5.36	22.20 ± 4.66
Creatinine (mg/dL)	1.04 ± 0.30	1.05 ± 0.22	1.14 ± 0.27	1.18 ± 0.33	0.88 ± 0.18	0.93 ± 0.16
BUN/creatinine ratio	20.92 ± 4.60	21.38 ± 3.86	19.38 ± 3.52	18.94 ± 3.80	24.53 ± 6.65	24.40 ± 5.05
Calcium (mg/dL)	9.54 ± 0.35	9.54 ± 0.44	9.40 ± 0.31	9.55 ± 0.26[a]	9.63 ± 0.38	9.71 ± 0.25
Total protein (g/dL)	7.05 ± 0.34	6.99 ± 0.37	6.84 ± 0.28	6.88 ± 0.27	7.17 ± 0.43	7.10 ± 0.32
Albumin (g/dL)	4.42 ± 0.24	4.08 ± 0.93	4.28 ± 0.18	4.26 ± 0.20	4.37 ± 0.24	4.34 ± 0.15
Globulin (g/dL)	2.63 ± 0.29	2.66 ± 0.25	2.57 ± 0.24	2.63 ± 0.27	2.80 ± 0.42	2.76 ± 0.33
A/G ratio	1.70 ± 0.22	1.65 ± 0.16	1.69 ± 0.19	1.64 ± 0.21	1.59 ± 0.25	1.59 ± 0.21
Total bilirubin (mg/dL)	0.58 ± 0.17	0.58 ± 0.26	0.58 ± 0.19	0.64 ± 0.23[a]	0.53 ± 0.16	0.68 ± 0.25[a]
Alkaline phosphatase (U/L)	57.31 ± 14.65	59.80 ± 13.18	64.44 ± 12.58	67.50 ± 16.30	64.40 ± 14.78	65.00 ± 13.79
AST (U/L)	22.92 ± 10.48	20.77 ± 4.44	22.06 ± 6.72	24.75 ± 14.32	25.33 ± 12.80	27.00 ± 21.60
ALT (U/L)	19.15 ± 9.48	17.69 ± 4.37	18.69 ± 7.84	19.69 ± 8.10	24.13 ± 23.48	26.93 ± 34.82
Cholesterol (mg/dL)	168.69 ± 27.70	167.62 ± 24.95	177.13 ± 40.65	168.19 ± 32.15	180.80 ± 19.89	174.27 ± 14.26
Triglycerides (mg/dL)	142.00 ± 48.70	140.85 ± 63.43	98.63 ± 45.21	101.00 ± 38.63	111.33 ± 31.46	123.20 ± 53.72
HDL cholesterol (mg/dL)	51.92 ± 14.91	53.46 ± 19.80	54.94 ± 14.50	54.00 ± 16.17	58.73 ± 10.62	55.20 ± 10.95
LDL cholesterol (mg/dl)	88.38 ± 31.67	85.77 ± 24.64	102.44 ± 35.59 9	3.94 ± 24.37	99.80 ± 19.38	94.53 ± 13.21
VLDL cholesterol (mg/dL)	28.08 ± 10.21	28.39 ± 12.81	19.75 ± 9.09	20.25 ± 7.68	22.27 ± 6.34	24.53 ± 10.83
Risk ratio (CHOL/HDL)	3.45 ± 0.97	3.23 ± 0.78	3.36 ± 0.93	3.28 ± 0.82	3.14 ± 0.52	3.26 ± 0.54
WBC ($\times 10^b$/μL)	6.09 ± 1.23	6.57 ± 2.66	5.79 ± 1.58	6.05 ± 1.87	5.91 ± 1.88	5.84 ± 1.80
RBC (M/μL)	4.40 ± 0.51	4.36 ± 0.53	4.75 ± 0.43	4.71 ± 0.49	4.67 ± 0.32	4.67 ± 0.36
Hemoglobin (g/dL)	13.53 ± 1.45	13.47 ± 1.02	14.41 ± 1.02 1	4.15 ± 1.12	14.31 ± 0.92	14.16 ± 1.10
Hematocrit (%)	42.36 ± 4.50	41.21 ± 4.39[a]	45.55 ± 2.93	44.06 ± 3.48[a]	45.75 ± 2.93	43.97 ± 3.11[a]
MCV (fL)	96.49 ± 4.30	94.82 ± 4.03[a]	96.23 ± 4.95	93.89 ± 3.80[a]	97.97 ± 3.35	94.35 ± 2.70[a]
MCH (pg)	30.82 ± 1.16	30.93 ± 1.42	30.45 ± 1.63	30.19 ± 1.66	30.65 ± 1.18	30.37 ± 1.06[a]
MCHC (g/dL)	31.97 ± 0.87[c]	32.65 ± 1.14[a]	31.64 ± 0.56[c]	32.13 ± 0.81[a]	31.27 ± 0.65[c]	32.21 ± 0.70[a]
Platelets ($\times 10^b$/μL)	251.31 ± 83.96	240.31 ± 60.67	245.06 ± 50.80	235.69 ± 57.22	247.47 ± 71.58	244.20 ± 80.33
RDW	13.48 ± 0.84	13.46 ± 1.14	13.29 ± 0.62	13.29 ± 0.70	13.31 ± 0.96	13.19 ± 0.89

[a] Statistically different from pre to post ($p < 0.05$).
[b] Out of standard at post measurement.
[c] Out of standard at pre measurement.

in pre-supplement values, the increase in STS value was significantly greater for ONS1200 versus ONS800 ($p = 0.021$). There was no difference between groups ONS and ONS800. There was a significant difference in group x time interaction ($p = 0.039$; $\eta^2 = 0.146$) for $MQ_{HANDGRIP}$ from pre- to post-supplement. Groups ONS and ONS800 did not show any improvement, but group ONS1200 showed a trend towards improvement ($p = 0.06$) of 7.4%. When post-supplement values are adjusted for initial differences, group ONS1200 showed a significantly greater increase than ONS800 and ONS (Fig. 1).

4. Discussion

The main findings in this study suggest that 12-weeks of a very low (800 mg twice daily) and low (1200 mg twice daily) amount of β-alanine added to an ONS may increase PWC_{FT}, as well as muscle function and quality measures in older adults. These results add support to previous research showing that regardless of age or gender, β-alanine supplementation, even at relatively low doses, may improve performance across a variety of physical tasks, including functionality and enhancing muscular endurance. Exercise capacity in the present study was measured using PWC_{FT}, which has been shown to be a valid measure of fitness in older adults (de Vries et al., 1989). The ONS800 group showed a significant improvement in performance of 17.8%, while the ONS1200 group showed a significant increase of 13.6% (Table 5) following supplementation, however, there was no significant difference between these two groups ($p = 0.547$). The effect size observed in this study for exercise capacity ($\eta^2 = 0.23$) is comparable to effect sizes (0.12–1.129) reported for several studies using similar total β-alanine dosing (109 to 189 g) (Hobson et al., 2012). Post hoc analysis of changes relative to body weight suggests little difference between groups ONS800 and ONS1200. This may be the reason why there was no significant difference between these two groups. Future studies should try and control for dose level and body weight when selecting dose levels. However, our results are about 10% lower than

Table 5
PWC_{FT} and sit-to-stand pre to post supplementation performance differences.

Treatment	PWC_{FT}			p[a]	STS			p[a]
	Pre (watts)	Post (watts)	% change		Pre	Post	% change	
ONS1200 (N = 13)	50.8 ± 22.6	58.8 ± 22.6	13.6	0.03	13.3 ± 3.6	17.1 ± 7.5	22.2	0.015
ONS800 (N = 15)	47.7 ± 17.4	57.3 ± 22.9	17.8	0.006	14.4 ± 2.5	14.9 ± 2.6	3.4	
ONS (N = 16)	59.4 ± 27.7	55.9 ± 31.2	−6.3		16.6 ± 5.8	18.6 ± 6.6	10.7	0.002

Values reported as mean ± standard deviation (SD).
[a] p value represents pre to post difference.

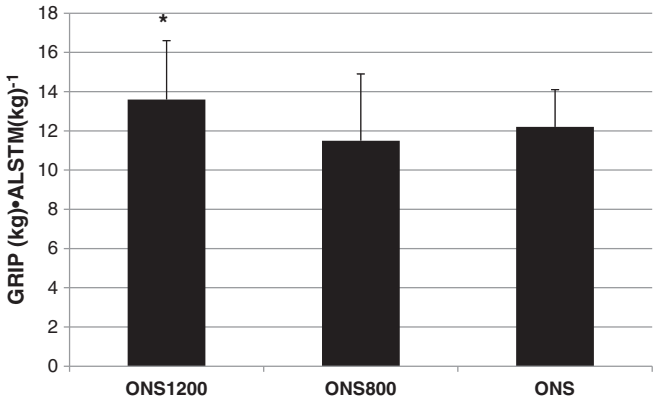

Fig. 1. Group differences in $MQ_{HANDGRIP}$ when pre-supplement group differences were accounted for. *ONS1200 had significantly greater increase than ONS and ONS800 ($p < 0.05$).

the significant improvement reported by Stout et al. (2008) in a similar cohort of older adults for the PWC_{FT} test (28.6%). A recent study by del Favero et al. (2012), measured physical capacity in two different ways, one by time to exhaustion on a treadmill at 3.5 mph with increasing elevation (TTE) and the other the limit of tolerance at a workload equivalent to 75% of the difference between ventilatory anaerobic threshold and VO_2peak (TLIM) on a treadmill. Their results following 12-weeks of β-alanine supplementation showed significant increases of 12.2% in TTE and 36.5% in TLIM which is similar to the percent increase in physical working capacity reported by Stout et al. (2008) (28.6%). All three studies examining β-alanine supplementation in older adults have shown significant improvements in working capacity.

The difference in the results of the PWC_{FT} seen in the present study and the results by Stout et al. (2008) may be due to the difference in the total dose of β-alanine consumed during the supplement period. The total dose of β-alanine consumed during the Stout et al. (2008) study was approximately 216 g. This represents approximately 20% more β-alanine than that consumed by the ONS1200 (~181 g) group and approximately 70% more β-alanine than the ONS800 group (~125 g). This is supported by research from Hill et al. (2007) that showed an improved performance in total cycling capacity with a larger total dose of β-alanine supplementation.

Recently del Favero et al. (2012) reported supplementing a total dose of 269 g of β-alanine which resulted in a mean increase of 85.4% in muscle carnosine levels in a similar cohort of older men and women. An increase in the muscle carnosine levels has been correlated with increases in exercise capacity in both the young and old (Artioli et al., 2010; del Favero et al., 2012; Hobson et al., 2012). One explanation for the increase in exercise capacity with an increase in muscle carnosine is the possible increase in muscle buffering capacity, which will help maintain muscle pH levels in exercising muscle (Hobson et al., 2012; Stout et al., 2008). This increase in buffering capacity within the muscle may explain the significant increase in PWC_{FT} observed in groups ONS800 and ONS1200 in the present study. In support, del Favero et al. (2012) demonstrated an increase in work capacity as measured on a treadmill, corresponded to a significant increase in muscle carnosine levels.

As seen in previous β-alanine supplement research with older adults (del Favero et al., 2012; Stout et al., 2008), the improvements in working capacity were seen without a training intervention. There are a number of implications in these findings. Research indicates that as many as 75% of adults over the age of 60 years do not meet physical activity recommendations as outlined by the United States Department of Health and Human Services in 2008 (Tucker et al., 2011). Without any intervention, this group of older adults will continue to see a decline in working capacity, muscle mass, and muscle function (Marcus et al., 2012). Supplementing with β-alanine and thus maintaining high muscle carnosine content may lead to improvements in working capacity and perhaps encourage older adults to maintain a more active lifestyle.

To our knowledge, there has been no research involving a training intervention along with β-alanine supplementation in older adults. In early work by de Vries et al. (1989), 10-weeks of aerobic training improved PWC_{FT} by 38.4 and 29.8% for older adults exercising at 85 and 70% of their pre-exercise PWC_{FT} levels, respectively. Future research needs to examine the impact of β-alanine supplementation and aerobic exercise training on working capacity in older adults.

In the present study, the GRIP (MAX and AVG) measures demonstrated no improvement following 12 weeks supplementing β-alanine. One explanation for this is the short duration of the activity, 3 to 5 s for the GRIP. It has been suggested increasing muscle carnosine content through β-alanine supplementation may not improve exercise performance lasting less than 60 s (Hobson et al., 2012). In support, del Favero et al. (2012) reported no significant improvement in the 30 s STS test following 12-weeks of β-alanine supplementation. In contrast, STS values in the current study were significantly improved in the ONS1200 group. The difference between our results and del Favero et al. (2012) is difficult to explain, but could be due to the reliability of the STS or difference in level of fitness between participants in each study. In the current study, baseline values for STS of the ONS1200 group averaged 13 compared to 16 in the β-alanine group in the del Favero et al. (2012) study. Interestingly, the ONS1200 group and the β-alanine group in the del Favero et al. (2012) study both averaged 17 STS post-testing. It is possible that if β-alanine supplementation increases muscle carnosine levels, thus improving intra-muscular buffering capacity, this may delay fatigue and improve the STS performance. Another explanation for the increase in STS performance could be the effect of carnosine on $Ca^{2\pm}$ ion sensitivity, leading to enhancements in force production and reduced fatigue (Sale et al., 2010). Utilizing in vitro techniques, Lamont and Miller (1992) demonstrated that the presence of carnosine reduced the amount of $Ca^{2\pm}$ required to produce half-maximum tension in skeletal muscle. Similarly, Dutka and Lamb (2004) showed, in vitro, increased $Ca^{2\pm}$ ion sensitivity of the contractile apparatus, concluding that carnosine enhances force production as a result of the sensitivity of the contractile apparatus to $Ca^{2\pm}$, without additional $Ca^{2\pm}$ release from the sarcoplasmic reticulum. While most studies have determined no benefit of β-alanine supplementation for exercise lasting less than 60 s in younger adults, our study suggests that β-alanine may be beneficial for older men and women during a maximal 30 second test. Future studies are needed to examine if β-alanine supplementation is beneficial for intense anaerobic exercise lasting less than 60 s.

A major limitation in this investigation was the fact that muscle carnosine levels were not measured. Previous research (Baguet et al., 2010; del Favero et al., 2012; Harris et al., 2006; Hill et al., 2007), however, using men and women (18–80 years) have shown that β-alanine supplementation (3.2–6.4 g/day) for 28 to 84 days can significantly increase muscle carnosine content by as much as 85%. In addition, a recent meta-analysis indicated that when ~179 g of β-alanine was consumed over a period of 3–13 weeks there is an expected 2.85% improvement in exercise performance (Hobson et al., 2012). In the current study, participants consumed ~125 to 181 g over an 84 day period which resulted in a mean increase of 13.6% to 17.8% for the PWC_{FT}. Therefore, while muscle carnosine levels were not directly measured in the present study, the results of previous investigations (Baguet et al., 2010; del Favero et al., 2012; Harris et al., 2006; Hill et al., 2007) suggest that it is likely that β-alanine supplementation resulted in an increase in muscle carnosine concentration which may have an impact on exercise performance. Future research is needed to examine the effect of low doses of β-alanine supplementation as used in the current study, on changes in muscle carnosine content and PWC_{FT}. A second limitation in this investigation was not performing a familiarization prior to testing. A familiarization trial was not administered prior to the PWC_{FT} as previous

research (de Vries et al., 1989; Stout et al., 2008) has shown the PWC_{FT} to be reliable without a familiarization trial. Finally, the rate of paresthesia was relatively high with this low dose ($2.4\ g \cdot day^{-1}$) of β-alanine. This was possibly due to utilizing a non-sustained release formulation of β-alanine.

5. Conclusions

The results of this study, showing a significant improvement in working capacity following 12-weeks of β-alanine supplementation, are in agreement with previous research (del Favero et al., 2012; Stout et al., 2008). The unique finding in this study was two different total doses of β-alanine (125 and 181 g) administered over 12-weeks led to significant improvements in PWC_{FT} in an older population. It is recommended that future research investigate the combined effects of β-alanine ingestion and exercise in an older adult population.

Conflict of interest

All authors except JR Stout declare no competing interests in this work. JR Stout has given presentations on behalf of Abbott Nutrition and received compensation.

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