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Building strength, endurance, and mobility using an astaxanthin formulation with functional training in elderly

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Abstract

Background Building both strength and endurance has been a challenge in exercise training in the elderly, but dietary supplements hold promise as agents for improving muscle adaptation. Here, we test a formulation of natural products (AX: astaxanthin, 12 mg and tocotrienol, 10 mg and zinc, 6 mg) with both anti-inflammatory and antioxidant properties in combination with exercise. We conducted a randomized, double-blind, placebo-controlled study of elderly subjects (65–82 years) on a daily oral dose with interval walking exercise on an incline treadmill.

Methods Forty-two subjects were fed AX or placebo for 4 months and trained 3 months (3×/week for 40–60 min) with increasing intervals of incline walking. Strength was measured as maximal voluntary force (MVC) in ankle dorsiflexion exercise, and tibialis anterior muscle size (cross-sectional area, CSA) was determined from magnetic resonance imaging.

Results Greater endurance (exercise time in incline walking, >50%) and distance in 6 min walk (>8%) accompanied training in both treatments. Increases in MVC by 14.4% ($\pm 6.2\%$, mean \pm SEM, $P < 0.02$, paired t -test), CSA by 2.7% ($\pm 1.0\%$, $P < 0.01$), and specific force by 11.6% (MVC/CSA, $\pm 6.0\%$, $P = 0.05$) were found with AX treatment, but no change was evident in these properties with placebo treatment (MVC, $2.9\% \pm 5.6\%$; CSA, $0.6\% \pm 1.2\%$; MVC/CSA, $2.4 \pm 5.7\%$; $P > 0.6$ for all).

Conclusions The AX formulation improved muscle strength and CSA in healthy elderly in addition to the elevation in endurance and walking distance found with exercise training alone. Thus, the AX formulation in combination with a functional training programme uniquely improved muscle strength, endurance, and mobility in the elderly.

Keywords Skeletal muscle; Interval training; Fatigue; Sarcopenia; Dynapenia

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Introduction

Age is characterized by a progressive loss of mobility, with muscle wasting (sarcopenia) and reduced endurance as key factors in this exercise intolerance. Exercise training has been the gold standard for slowing or reversing sarcopenia. Resistance training (RT) has been most effective at treating muscle wasting,¹ while endurance training has the greatest benefits to exercise intolerance.^{2,3} However, combining training

modes to treat both declines simultaneously typically yields a lower response than either mode applied separately.^{4,5}

Recent evidence indicates that dietary supplements may lead to improvements in both strength and endurance, which is a combination of adaptations that have not been possible with a traditional training programme alone.^{5,6} For example, anti-inflammatory supplements yield greater strength improvements when combined with training in elderly subjects,⁷ while antioxidant supplements lead to greater endurance

improvements.⁸ Astaxanthin is a natural product with both antioxidant and anti-inflammatory properties^{9–12} that has been found to increase strength and endurance. For example, 6 months of feeding in the healthy young resulted in ‘strength endurance’ gains of 55% as measured by the number of weighted knee bends completed by the end of the study.¹³ Muscle adaptations, such as these, in an elderly population would counter key muscle functional losses that are found with age.¹⁴ Additional benefit would come from combining a dietary treatment with functional training, which has the potential to not only reverse muscle wasting and improve exercise intolerance but also to increase mobility with a single intervention.

Astaxanthin accumulates in tissues of animals in the food chain, such as salmon, that feeds on the marine algae, *Haematococcus pluvialis*⁹ and is estimated to have been consumed at levels of 6 mg per day in populations with a salmon-based diet.¹⁵ Two factors supplied in the diet, vitamin E and zinc, also have antioxidant properties, but intake is often inadequate, especially in the elderly.¹⁶ Vitamin E has the additional benefit of enhancing the antioxidant effect of astaxanthin *in vitro*¹⁷ and zinc stabilizes SOD1 activity.¹⁸ Formulating these dietary supplements into a single treatment offers the possibility of combining or synergizing the beneficial effects of the individual natural compounds with antioxidant and anti-inflammatory properties.

Here, we test a dietary formulation of astaxanthin, vitamin E, and zinc in combination with exercise training as an approach to elevate mobility, endurance, and strength in the elderly.^{6,8} Previous intervention studies have focused on astaxanthin alone in young animals and humans, but there are few studies on the elderly.¹⁹ Evidence for the beneficial impact of AX on old muscle comes from a pilot study in mice, reported here, that found that AX accumulated in muscle with feeding and was associated with elevated muscle quality (specific force) after exercise training on a treadmill. The approach of this study was to build on this pilot study using a formulation of dietary factors with antioxidant and anti-inflammatory properties paired with exercise training. Our hypothesis was that this astaxanthin-based formulation in combination with a functionally based exercise training programme would activate improvements in both endurance and muscle strength, thereby providing a single approach to reverse loss of mobility and muscle properties in the elderly.

Methods

Pilot study

Twenty-nine-month-old male mice were treated with either 300 mg/(kg × day) astaxanthin ($n = 10$, Astareal, Inc. Moses Lake, WA, USA) or standard chow alone ($n = 9$). These mice were very old at the start of training given that 50%

survivorship generally occurs about 28 months of age for this strain²⁰ (vs. mean life expectancy of 78 years old for humans in the USA in 2013²¹). The AX dose was determined for mice by scaling²² from the level found to be effective in rat studies.²³ Exercise training occurred 3×/week on a 20° inclined treadmill at 10 m/min for 5 min at the start reaching 15 min in the final 4 weeks of training. *In vivo* muscle force of the gastrocnemius was measured as the maximum twitch and tetanic force during electric stimulations (200 Hz for 300 ms) at baseline and at 8 weeks in anaesthetised mice as described.²⁴ The quadriceps muscle was frozen at the end of training to determine the level of astaxanthin. The Institutional Animal Care and Use Committee of the University of Washington Animal approved this experimental protocol.

Human study

This randomized, double-blind, placebo-controlled study was conducted at the University of Washington Medical Center and the Fred Hutchison Cancer Research Center. Adults age 65–85 years old were recruited through public lectures, mailers, posted advertisements, and referrals from prior studies. To be included in the study, subjects had to be healthy and not under treatment for serious chronic conditions, ambulatory and able to perform activities of daily living without assistance, and able to speak and read English fluently. Exclusion criteria are listed in Table S1. A total of 365 subjects were phone screened; 58 subjects enrolled in the study and were randomly assigned to groups. Each subject had a physical examination, resting and exercise electrocardiogram, and blood testing to ensure that they were healthy and free from orthopaedic and neuromuscular problems.

Treatment and dosing

The dietary formulation consisted of astaxanthin (12 mg), tocotrienol (10 mg), and zinc (6 mg; Astamed, Bellevue, WA) and was ingested as two capsules per day. The astaxanthin dose was determined based on safety studies showing that a dose up to 45 mg per day for 4 weeks is well tolerated without serious side effects.¹⁹ Long-term intervention studies (4 mg/day for 52 weeks²⁵; 12 mg/day²⁶ and 16 mg/day for 12 weeks²⁷) reported no serious adverse side effects. The 12 mg daily dose resulted in significant improvement in cognitive measurements.²⁶ Two additional components were included in the formulation because they provide additional antioxidant benefits and are often ingested below the recommended intakes.¹⁶ Tocotrienols (vitamin E) reduce lipid peroxidation in combination with astaxanthin more than astaxanthin alone¹⁷ and quench reactive oxygen species in liposomes 40% better than the sum of the two antioxidants acting alone.²⁸ In addition, zinc stabilizes SOD1 activity.¹⁸ Thus, this formulation has more antioxidant protection than

the individual ingredients as well as supplementing nutrients that are often ingested below recommended levels.

Randomization and blinding

The subjects were assigned to the two treatment groups by an individual not associated with the study. A random number generator provided the assignments. Copies of the codes were held in separate locations that were not accessible to the investigators. Thus, the assignment of the individual subjects to the treatment groups was not known to the participants or to the investigators until the study was completed.

Figure 1 shows a chart of the subject flow through the study. Sixteen subjects dropped out after randomization: 8 for medical reasons unrelated to the treatment, 6 for protocol non-adherence, and 2 for personal reasons. Forty-three subjects ($n = 19$, placebo and $n = 23$, astaxanthin formulation) aged 65–82 years completed the study. The characteristics of the subjects that completed the study are shown in Table 1. All participants gave written informed consent consistent with the Declaration of Helsinki in a project approved by the University of Washington and Western Institutional Review Boards.

Exercise training

The 12 week training programme met 3× per week with a 10 min warm-up before and 5–10 min cool down period at the end of each session. Treadmill training involved walking at ~1.3 m/sec with periods at a high treadmill incline of 9–12% grade (interval training) separated by periods of low incline walking at 5–7% grade (recovery). Table S2 contains the time and incline grade (%) used at baseline, at the end of training, and the overall change with training. The training level was incremented based on the heart rate (HR) response (HR targets

Table 1 Subjects' characteristics

	Placebo	AX
Age, year	72.2 ± 5.2	69.1 ± 3.4
F, <i>n</i>	9	13
M, <i>n</i>	10	10
Height, cm	66.6 ± 3.4	66.4 ± 4.1
Weight, kg		
Pre	71.1 ± 14.8	73.8 ± 13.4
Post	71.3 ± 14.8	72.4 ± 13.9
BMI		
Pre	24.7 ± 3.1	26.3 ± 3.2
Post	25.4 ± 3.1	25.8 ± 3.2

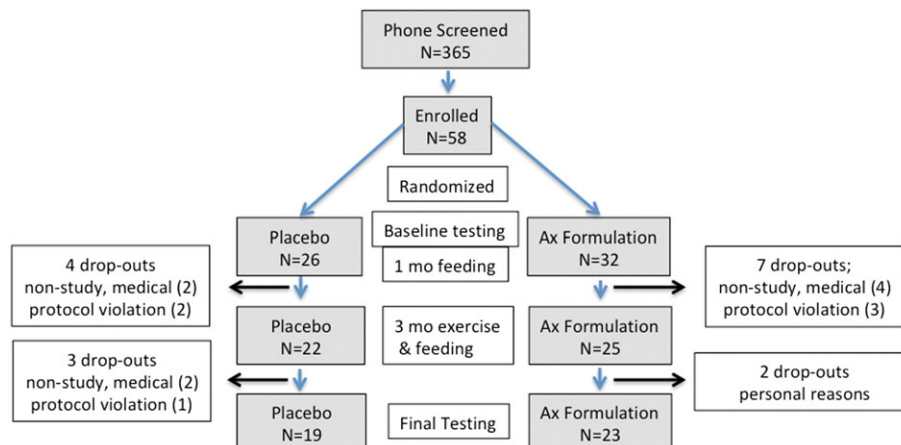
Value are means ± SD. BMI, body mass index.

listed in the succeeding text) as well as relative perceived exertion during the treadmill test. Training progressed in three steps: (i) familiarization with the treadmill protocol (weeks 1 and 2), which involved 8–10 intervals at the high incline for ~1 min each and recovery for 2 min, (ii) baseline interval training (weeks 3–7), which involved 1–1.5 min exercise in 8–10 intervals to achieve 70–80% HRmax with 2–3 min of recovery exercise between intervals, and (iii) ramping up (weeks 8–12), which involved 1.5–2 min exercise in 10–12 intervals to achieve 80–85% HRmax with 0.5–1.0 min of recovery exercise between intervals. All exercise training was overseen by an American College of Sports Medicine certified exercise physiologist at the Fred Hutchinson Cancer Research Center.

Magnetic resonance imaging

Tibialis anterior (TA) muscle cross-sectional area (CSA) was determined from magnetic resonance images (Bruker 4.7-T magnet with Biospin console; Bruker Corporation, Billerica, MA) acquired as axial plane T1-weighted, 2-D gradient-echo images collected with the following parameters: 500 ms repetition time, 2.5 ms echo time, 3 mm slice thickness,

Figure 1 The study flow diagram. Schema of subject progress through the phases of the parallel randomized study of the two treatment groups.



1 mm inter-slice interval, 192×192 matrix, and number of excitations = 2. Five slices of each right limb were analysed with NIH Image software (Image J, version 1.50 e) using manual planimetry²⁹ to determine the muscle CSA. The measurements by two independent investigators agreed to within 2.5% on average.

Single muscle test: isometric ankle dorsiflexion

The TA muscle strength and contractile properties were determined on the right leg using a custom-built isometric exercise apparatus, as previously described³⁰ (Figure S1A). The TA muscle is important in ankle dorsiflexion and represents a simple system connecting muscle mechanics and energetics to foot movement³⁰ and is a critical part of walking mobility. The subject performed a maximal voluntary contraction (MVC) using ankle dorsiflexion for ~5 sec by pulling on a strap that secured the foot to a force transducer platform. Three successive bouts were separated by 5 sec each (Figure S1B). A test, retest evaluation found no significant difference in MVC in two determinations separated by 30 days (-0.01 ± 0.04 N, mean \pm SEM, $n = 24$, paired t -test). The relative standard deviation for the difference scores was 21% (SD of difference/mean \times 100).

Statistical analysis

An unpaired Student's t -test was used to evaluate treatment vs. placebo in the pilot mouse study. To explore for measurements that change with treatment in the human study, a

paired, 2-tailed t -test (pre-training vs. post-training change) was used with significance assigned at $\alpha = 0.05$ ($P < 0.05$). No correction for multiple comparisons was used as is consistent with a proof of concept study.³¹ Data are reported as means \pm SEM in figures and text and \pm SD in the tables.

Results

Pilot study

The level of astaxanthin in muscle after the 8 week exercise programme was significantly elevated in the AX (236.7 ± 123.4 ng/g, $n = 4$) vs. the placebo (9.2 ± 9.2 ng/g, $n = 6$) treatment group. Specific force (maximum twitch force/muscle cross-sectional area) was significantly greater in AX vs. placebo-treated mice after training ($P < 0.004$, Table 2).

Human study

The subjects' physical characteristics are reported in Table 1. Figure 2A shows that the time in the interval stage (high % grade incline walking) was the predominant change with training (Table S2). The increased interval stage exercise time demonstrates that the subjects in both treatment groups could exercise longer (greater time) and at a higher intensity (higher % grade) after training. Walking distance in the 6 min walk also significantly improved by ~8% in both groups with training (Figure 2B, Table S3).

Figure 3 shows the relative change in muscle strength and size in the two groups with training (Table S4 presents the absolute changes). A significant change in human muscle strength, as measured by MVC ($\Delta 14.4 \pm \Delta 6.2\%$ mean \pm SEM, $P < 0.02$), is shown for the AX treatment group alone. The TA muscle CSA ($\Delta 2.7 \pm \Delta 1.0\%$) also only increased in the AX

Figure 2 Changes in endurance (training time) and mobility (walking distance) after 3 months of training in placebo (PL) and astaxanthin formulation (AX) treated elderly subjects. (A) Change in training time (min) in the recovery (REC) and interval (INT) exercise periods in the training session and (B) change in distance (m) in the 6 min walk test. Values are mean \pm SEM. * $P < 0.05$.

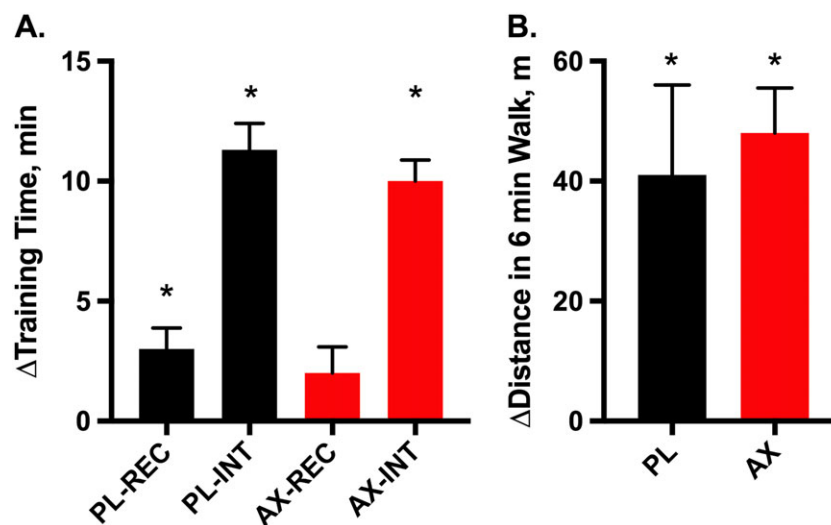
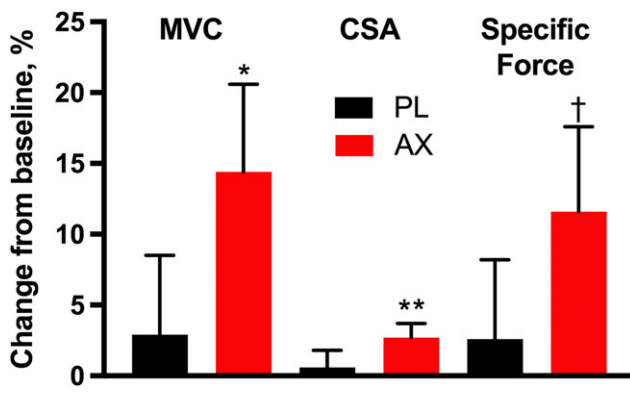


Figure 3 Changes in muscle properties after 3 months of training in AX formulation and placebo-treated elderly subjects. Changes in maximum voluntary contraction (MVC), muscle cross-sectional area (CSA), and specific force (MVC/CSA) are shown. Values are mean \pm SEM. * $P < 0.02$, ** $P < 0.01$, † $P = 0.053$ for paired 2-tailed *t*-test.



treatment group (both image analysers found CSA differences at $P < 0.01$). The ratio of these measures provides the muscle specific force (MVC/CSA), which trended to a higher value ($\Delta 11.6 \pm \Delta 6.1\%$, $P = 0.053$) in the AX treatment group alone. No significant change in muscle properties was found in the placebo treatment group (MVC, $\Delta 2.9\% \pm \Delta 5.6\%$; CSA, $\Delta 0.6\% \pm \Delta 1.2\%$; MVC/CSA, $\Delta 2.4 \pm \Delta 5.7\%$; $P > 0.6$ for all).

Discussion

The key findings from this study are that the astaxanthin formulation in combination with functionally based exercise training improved endurance and walking distance (Figure 2), as well as elevated muscle strength and size (sarcopenia) in elderly subjects (Figure 3). Thus, mobility,

endurance, and strength were all improved with a single training approach in elderly humans. This approach of pairing a dietary formulation with functionally based training holds promise as a single mode to reverse key deficits in muscle and mobility that limit the elderly.¹⁴

The pilot study involving exercise training of aged mice on an incline treadmill provided two results important for the human study. The first result was that AX accumulated in the muscle and was significantly above the levels found with placebo treatment at the end of exercise training. The second result was that this AX accumulation was associated with significantly greater specific force in the AX-treated vs. placebo-treated muscle after incline training (Table 2), which points to the biological activity of AX. The human study emulated this design with the addition of interval training that mixed level with incline treadmill walking to provide both endurance and strength training stimuli. Figure 2 shows that total walking time on the treadmill increased (endurance) due predominantly to more time in high incline (interval) walking vs. low incline (recovery) walking after 3 months of training. The similarity of the response of the two groups indicates that the antioxidant properties of the AX formulation did not inhibit the endurance training adaptation as has been found with other antioxidant treatments in exercise training interventions.³² Instead, both training groups increased walking distance in a 6 min test, thereby demonstrating both greater endurance (treadmill time in interval training) and mobility (walking distance) with this approach.

Accompanying these functional improvements were adaptations in both strength and muscle CSA in the group treated with the formulation of dietary supplements with antioxidant and anti-inflammatory properties (astaxanthin, vitamin E, and zinc) (Figure 3).^{9,11} The strength gain (14.4%) in the AX formulation group was similar to that found with RT in studies of the elderly (10–15%,^{1,7,33}) that used isokinetic or isometric methods to measure MVC. This strength gain was accompanied by an increase in muscle size (2.5%), which indicates that a greater muscle mass was a part of the gains in strength. Although this CSA gain is smaller than found in RT studies of the elderly (~10%,^{1,7,33,34}), the muscle size gain was one of a suite of changes that accompanied the training stimulus with the AX formulation. This muscle adaptive response has been found with anti-inflammatory treatments⁷ and is consistent with the anti-inflammatory properties reported for AX.^{10,11}

The difference in the strength (14.4%) vs. the CSA change (2.5%) points to an improved muscle quality with the AX formulation plus training, as measured by specific force (MVC/CSA; 11.5%, $P = 0.053$). Remarkably, this specific force gain was similar in size to the change found in many RT studies of the elderly (Figure S2). These results point to specific force as a key contributor to the strength increases with the AX formulation treatment in the human study and to the difference between placebo and AX treatment in the mice after exercise training (Table 2).

Table 2 Mouse muscle force, size, and specific force (MVC/mass) after 8 weeks of training with the astaxanthin formulation (AX) or placebo (PL) supplementation

	PL	AX	<i>P</i> (PL vs. AX)
Strength (max. force, mN)			
Pre	395 \pm 63	366 \pm 41	0.32
Post	351 \pm 73	406 \pm 74	0.12
Muscle size (mass, g)			
Post	0.14 \pm 0.01	0.13 \pm 0.02	0.49
Specific force (max. force/CSA, N/cm ²)			
Pre	4.6 \pm 1.0	4.7 \pm 0.9	0.73
Post	4.0 \pm 0.6	5.0 \pm 0.6	0.004

Values are mean \pm SD: Max. force: maximum twitch force elicited by electrical stimulation; CSA, cross sectional area; *P*: α level in a Student's *t*-test. MVC, maximal voluntary force.

Many factors have been implicated as contributors to a rise in specific force with training, including neuromuscular,³⁵ muscle architecture,³⁶ and cellular mechanisms.³⁷ Our testing approach and the observed changes with training and treatment minimize the impact of several of the factors and highlight one factor that could raise strength. For example, direct muscle electrical stimulation (mouse) and ballistic contractions of the TA muscle³⁸ are designed to recruit all fibres and thereby reduce the impact of muscle and fibre recruitment on strength measures. Also, the ankle dorsiflexion exercise protocol primarily activates the TA muscle with little contribution by other muscles³⁹ indicating a minimal impact of co-activation of antagonist muscles to the strength measure. A second factor, muscle architecture (fibre length and pennation angle), is reported to change strikingly with RT in old subjects³³ but, despite these changes, CSA (or related size measures) remains the predominant factor determining MVC.³⁶ The results of these RT studies suggest that other factors, in addition to muscle architecture, contribute to the 11.5% improvement in specific force found in this study.

Reversal of the denervation that comes with age is a potential mechanism for increasing specific force with AX formulation feeding. Such a reversal in neuromuscular function with RT was demonstrated by two studies: a 4% rise in MVC relative to an electrically stimulated maximum in healthy elderly³³ and improvement in skeletal muscle innervation reported in obese older adults.⁴⁰ The neuromuscular junction (NMJ) is the interface between the muscle and nervous system; it is known to be disrupted by oxidative stress, leading to degeneration and denervation with age.⁴¹ Reduction of oxidative stress has been shown to improve the NMJ and specific force in mouse studies.⁴² The AX formulation is reported to act as an antioxidant *in vitro* in rodent models and *in vivo* in humans.¹¹ This antioxidant property may reduce oxidative stress so that the biosynthetic signals raised by exercise training can activate rebuilding of degenerative NMJs with AX treatment. Rejuvenated NMJs could permit activation of more muscle fibres within the TA⁴³ and could account for the improved specific force of ankle dorsiflexion found in this study.

Limitations

Three improvements in the experimental design of future studies would provide insight into the mechanism of action of astaxanthin beyond this 'proof of concept' study. First, adding an untrained control group to the mouse study would reveal whether age-related, rather than training-related, changes were responsible for the lower specific force in the placebo group after the 8 week experiment. An age-related decline would point to a mechanism of action that elevates specific force with astaxanthin feeding and exercise training

in mice as was found in the human study. Second, this study provides the statistical information needed to power a future clinical trial to directly test AX formulation treatment vs. placebo for effectiveness in reversing the strength and muscle size declines that come with age. Third, a muscle biopsy would allow a direct test of the mechanism of action of the AX formulation using biomarkers of inflammation and oxidative stress. Despite the limitations of a 'proof of concept' study, the clear responses of the AX formulation group to exercise training provide a template for interventions designed to counter sarcopenia, exercise intolerance, and low mobility in elderly subjects.

Conclusion

Here, we show that functionally based exercise training combined with a formulation of natural anti-inflammatory and antioxidant compounds improved muscle strength and size in elderly subjects more than exercise training alone. This was done without sacrificing the improvements in walking distance and endurance that typically accompany endurance training. These results suggest that the potential for strength and endurance improvements in elderly muscle is realized when natural products that promote adaptation are combined with exercise training incorporating both resistance and aerobic components. The end result is an approach involving functional exercise and a dietary formulation that can improve endurance, strength, and function to remedy the deficits associated with sarcopenia that limit mobility in the elderly.

Author contributions

K.C., D.M., M.C., S.L., and Astavita, Inc. designed the research; S.L., A.A., M.C., K.C., and E.S. performed research; B.R. provided physician oversight; S.L., A.A., M.C., K.C., and D.M. analysed data; and K.C. and S.L. wrote the paper.

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K23DK099442); Department of Radiology and Office of the Provost of the University of Washington, and the Prevention Center at the Fred Hutchison Cancer Research Center. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Exclusion Criteria

Table S2. Treadmill time (min) and incline grade (%) during training at baseline (Pre), at the end (Post) and the change (Δ Post-Pre) with the study.

Table S3. Walking distance at baseline (Pre), at the end (Post) and the change (Δ Post-Pre) with the study in the placebo (PL) and astaxanthin formulation (AX) fed groups.

Table S4: Human TA muscle properties pre and post training and treatment in the placebo (PL) and astaxanthin formulation (AX) fed groups.

Figure S1. A) Apparatus for measuring muscle strength in ankle dorsiflexion, B) Example of a single maximum voluntary contraction at baseline and after three months of training in an AX formulation treated human elderly subject.

Figure S2. Specific force changes with resistance training (RT) and AX treatment with exercise training (red arrow). Note: “Tracy/M” represents the older male average and “Tracy/W” represents the older female data. Farup et al. (black bar) training data from young adults; Tracy, Ferrir, and Trappe et al. data points all represents elderly (>65 yr) subject training studies.

Conflict of interest

S.Z.L. declares she has no conflict of interest, A.S.A. declares he has no conflict of interest, M.D.C. declares he has no conflict of interest, K.K. declares he has no conflict of interest, E. G.S. declares he has no conflict of interest, B.R. declares he has no conflict of interest, D.J.M. declares he has no conflict of interest, and K.E.C. has received research funds and is a Scientific Advisor for Astavita, Inc.

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