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ORIGINAL ARTICLE

Fish oil administration combined with resistance exercise training improves strength, resting metabolic rate, and inflammation in older adults

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Abstract

Background While fish oil (FO) has attracted great attention due to their health-enhancing properties, its potential to enhance benefits from resistance exercise training (RET) has not been fully elucidated yet.

Aims The aim of this study was to investigate effects of FO administration during 12 weeks of programmed RET on muscular strength, resting metabolic rate (RMR), and systemic inflammation in healthy older adults.

Methods Twenty-eight healthy older adults were randomly assigned to three experimental groups: sedentary control (CON), resistance exercise training (RET), or RET combined with FO (RET-FO). A one-repetition (1RM) of maximum muscle strength, RMR, substrate oxidation, and blood inflammatory biomarkers were assessed before and after the intervention. Statistical significance was set at $p \leq 0.05$.

Results 1RM muscle strength was significantly increased in RET and RET-FO while substantially decreased in CON. RMR greatly increased in RET and RET-FO with no change in CON. RET-FO exhibited significantly increased fatty acid oxidation, but no change was found in CON and RET. Systemic interleukin 6 (IL-6) and C-reactive protein (CRP) were significantly decreased from baseline in RET-FO while no change was observed in CON and RET.

Conclusion Our data indicate chronic RET reversed aging-induced loss of muscle strength and improved RMR, while FO administration combined with RET appears to enhance fat metabolism and mildly reduce some indicators of systemic inflammation.

Keywords Resistance exercise training · Fish oil · Resting metabolic rate · Fat metabolism · Inflammation

Introduction

With age, humans unavoidably experience gradual loss of muscle mass (i.e., sarcopenia), muscular strength, metabolic rate, and respiratory function [1]. This aging-related disorder may render older adults at greater risk of premature physical impairment, resulting in increased incidence of falls [2–4], physical disability [5, 6], and mortality [7, 8]. The major consequences of sarcopenia include the loss of his or her functional independence associated with activities of daily living, which would impair the quality of life, leading to health inequalities in older adults. Recent evidence implicates chronic inflammation as a potential pathophysiologic factor to promote the sarcopenic process [9, 10] via up-regulation of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and IL-6, which contribute to muscle loss [11–13]. As skeletal muscle is responsible for approximately 20% of the whole-body resting metabolic rate (RMR) [14], anti-sarcopenic strategies are needed to combat aging-induced reduction in muscle strength and RMR.

It has been generally acknowledged that resistance exercise training (RET) enhances muscle hypertrophy and physical function, strength, and power as well as reducing morbidity in various age groups [15]. RET effectively combats muscle wasting in older populations by enhancing multiple signaling intermediators (i.e., Akt-mTOR) to promote adaptive responses to the mechanical loading in specific muscle groups [16]. The activity of these mediators stimulates

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anabolic responses through increased translational events and attenuates catabolic processes by attenuating primary mediators of ubiquitination (proteasomal degradation) [17]. Repeated mechanical loading down-regulates TNF-α signaling in skeletal muscle (mRNA and protein) in older individuals, enhancing protein synthesis in aged skeletal muscle, which points to favorable effects of RET in senescence [18]. Evidence indicated that chronic RET results in decrements in TNF-α which links with increased muscle mass in the elderly [19]. Since TNF-α levels inversely correlate with protein synthesis, RET-mediated TNF-α decrements may at least partly explain muscle growth.

Omega-3 polyunsaturated fatty acids (*n*–3 FAs) have attracted great attention for their health-enhancing properties. *n*–3, mainly composed of eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), possess anti-inflammatory and anti-catabolic properties [20], thus widely used to improve inflammatory diseases [21]. Further, *n*–3 contain strong anabolic [22] and lipolytic [23] properties, attenuating activities of inflammation and oxidative stress mediators secreted from adipose tissue. Further, previous research reported that chronic FO enriched with *n*–3 FA administration improved muscle mass of lower extremity, hand grip strength, and 1-repetition maximum (1RM) muscle strength in healthy elderly populations [24]. However, the efficacy of a long-term *n*–3 administration independently or in combination with RET on combating alterations in maximal strength of skeletal muscle, RMR, and inflammation remains to be established. Therefore, the primary objective of the present study was to investigate the efficacy of chronic FO administration during 12 weeks of programmed RET on 1RM maximum muscle strength, RMR, and systemic inflammatory biomarkers in healthy older adults. We hypothesized that 12 weeks of programmed RET would (1) increase muscular strength, (2) improve RMR, and (3) attenuate inflammation in healthy older adults; further, the benefits from RET would be enhanced by FO administration.

Materials and methods

Participants

The study protocol was approved by the New Mexico State University Institutional Review Board for Human Subjects (no. 17616). Twenty-eight healthy older adults (10 males, 18 females; 66.75 ± 5.49 year) were included in the study. They were considered eligible to be enrolled in this study if they (1) were healthy without any physical or mental disorders; (2) were nonsmoker; (3) have not consumed omega-3 supplements; (4) did not take anti-inflammatory drugs; (5) did not engage in resistance exercise training; and (6) did not drink excessive alcohol (no more than five drinks per week). Prior to participation, all participants completed their health history and physical activity questionnaire. After the screening procedures, all participants were provided written informed consent prior to being admitted to the study.

Experimental design

A longitudinal design was used to investigate changes in all outcome measurements from pre- to post-intervention. Participants visited the laboratory for pre-intervention assessments including 1RM muscle strength, RMR, and systemic inflammatory biomarkers. Upon completion of all assessments, they were randomly assigned to one of three groups; (1) control (CON, $n = 8$), (2) resistance training (RET, $n = 10$) or (3) RET with FO supplementation (RET-FO, $n = 10$) for 12 weeks of intervention. After the 12-week intervention period, they reported to the lab again for post-intervention assessments. All participants were instructed to maintain their regular diets and normal daily activity throughout the intervention period. They were also asked to avoid any strenuous exercise or physical activity for 48 h prior to their lab visit.

Resistance exercise training

The RET and RET-FO groups performed programed resistance exercise training twice per week for 12 weeks. Each participant in the exercise group was asked to perform two sets of 10 repetitions or until failure (whichever came first) for 5 muscle groups in the upper and lower body (lat pulldown, seated row, biceps curl, leg press, calf rise). They performed each training session under close supervision by the research team to ensure the appropriate form and technique during the RET sessions and to minimize the potential risk of injury. Exercise intensity was set at 50% of their 1 RM for the initial week. Then, training load was elevated to 70% of 1 RM on the second week and progressively increased (+ 5% weekly if they were able to complete the given workload) to promote the maximal adaptive hypertrophic response. If they failed to complete the 10 repetitions for the given workload, the same workload was given on the following session. Each exercise session was initiated with a warm-up with stretching and one set of low intensity exercise (30% of 1 RM, 10 repetitions). Each training session was recorded in a logbook to quantify load increments.

Fish oil supplement

The FO supplement consisted of a proprietary combination of eicosapentaenoic acid (EPA; 0.7 g) and docosahexaenoic acid (DHA; 0.24 g). The supplement groups consumed 3 capsules of FO, one capsule for each meal, which provided 2.1 g EPA and 0.72 g DHA per day. The RT and CON

groups received placebo capsules, which were identical in appearance (safflower oil; 3 capsules/day). This dose has been approved by the Food and Drug Administration (FDA) to effectively reduce triglyceride concentrations in those with high triglyceride levels [25]. Also, a previous study reported that whole-body protein synthesis is substantially increased with similar doses in rodent animals (relative to their body weight) [26], while greater doses are not effective on protein metabolism or may even impair protein synthesis [27, 28]. Participants consumed the supplement pills daily and returned the empty and/or remaining pills to ensure their compliance. The tablet counts revealed > 90% compliance in each group. They were instructed to duplicate the similar food and beverage during the 24 h period prior to each test session.

Maximum muscular strength assessment

While actual 1-RM assessment is the most reliable and thus widely used test to evaluate the maximal strength of local muscle, this method may not be appropriate for evaluating 1-RM of individuals with no previous RET experience due to a higher risk of injury. Indeed, Braith et al. reported that actual 1-RM assessment of skeletal muscle may be complicated in untrained individuals [29]. Further, given that the participants in the present study are older adults with no previous RET experience, it would be more appropriate to apply the multiple repetition strength test to minimize any risk of injury of the participants during the test. It has been reported that this estimation method presents high validity to evaluate strength of upper and lower body musculature [30].

To assess estimated 1-RM of upper and lower body muscle strength, all participants conducted a series of five exercises in order of lat pull-down, leg-press, seated row, calf raise, and biceps curl. Since most participants are initially naive to the 1-RM test, they were instructed on proper technique and execution for correct body mechanics and breathing during the test. All participants underwent two sessions of familiarization for each exercise equipment prior to the testing to become acquainted with the investigators. During the familiarization sessions, participants lifted low intensity with correct position and breathing technique to complete 10 repetitions for each exercise.

Upon completion of the familiarization period, the multiple repetition of strength test was conducted using previously described methods [31]. On the test day, the testing procedures were explained to the participants. For the consistent test assessment, each participant performed their multiple strength test at the same time with the same testers. After a 5 min warm up, participants lifted the same weight for the familiarization session, then the resistance gradually increased until they were able to complete fewer than 10 repetitions. Based on the weight and number of repetitions, 1-RM was estimated using Brzycki 1-RM prediction equation, which has been used in calculating estimated 1-RM from the multiple repetition test [32].

Resting metabolic rate assessment

Resting metabolic rate (RMR) was measured to evaluate an estimated daily basal metabolic rate using the dilution function on the TrueOne metabolic analyzer (TrueOne 2400, Parvo Medics, Sandy, UT). All equipment was calibrated prior to each test. Participants were instructed to refrain from any strenuous physical activity and alcohol and caffeine intake at least 24 h prior to the test as well as to maintain the same diet a day before each lab visit to minimize variation of the test results between pre- and post-intervention. All participants visited the lab at the same time frame, early morning around 6:00 to 8:00 a.m. following an overnight fast (> 10 h). Upon arrival to the lab, participants sat on a chair in a quiet condition for 5 min prior to the test. Then, they were asked to lie on a bed in a supine position in a quiet and thermo-neutral environment and refrain from sleeping or moving for the 30 min RMR assessment period. The canopy was placed over the head, shoulders and upper chest of the participant to collect their gas (i.e., oxygen consumed by and carbon dioxide produced from their body). The first 5 min data were discarded and the remaining 25 min-data were used for the RMR measurement as per manufacture's guideline. Resting oxygen consumption (VO_2) and Carbon dioxide production (VCO_2) were collected to calculate respiratory exchange ratio (RER) as VCO_2/VO_2. Resting metabolic rate (RMR) was calculated using non-protein RER tables and carbohydrate and fat oxidation was calculated by using the following equations [33].

$$\text{CHO oxidation (g)} = \left(4.585 \text{ x } VCO_2\right) - \left(3.226 \text{ x } VO_2\right),$$

$$\text{Fat oxidation (g)} = \left(1.695 \text{ x } VO_2\right) - \left(1.701 \text{ x } VCO_2\right),$$

$$\begin{aligned}\text{RMR (Kcal)} = {} & VO_2(\text{L/min}) \\ & \text{x RER cal equivalent (Kcal/L)} \\ & \text{x Time (min).}\end{aligned}$$

Blood collection and analysis of systemic biomarkers

Blood samples of each subject (10 mL) were collected from the participant' forearm vein via a 23-gauge needle and vacutainer tubes containing EDTA at pre- and

post-interventions. The collected samples were immediately centrifuged to obtain plasma from the collected samples and stored in multiple aliquots at – 80 °C for analyses of relative biomarkers. IL-6, TNF-α, and CRP were analyzed using commercially available ELISA kits from Raybiotek (Atlanta, GA). All samples were analyzed in duplicate.

Statistical analysis

Statistical analysis was performed using a SPSS software (SPSS version 25, IBM). All data are presented as the mean ± standard deviations (SD). After normality assurance, 3 (experimental condition) × 2 (time) repeated measure ANOVA was used with baseline as a covariate to determine if changes in the dependent variables over the 12 week experimental period were attributable to sex or group and whether a sex-by-group interaction was detected. When the significant main effects and interactions were noted, pairwise comparisons using Bonferroni adjustments were used to identify where the differences occurred. Cohen's *d* effect size were evaluated using the equation: *d* = (mean difference between pre- and post-intervention)/(pooled standard deviation). Using Cohen's conventions, values of 0.2, 0.5, 0.8 were considered small, medium, and large effects, respectively. Cohen's *d* values ≥ 0.5 were interpreted as a practical/functional impact of intervention.

Table 1 Descriptive characteristics of subjects at baseline

	CON	RET	RET-FO
Age (years)	66.5 ± 5.0	66.6 ± 7.3	67.1 ± 4.4
Height (cm)	167.2 ± 10.24	167.9 ± 5.7	171.6 ± 9.3
Weight (kg)	68.9 ± 15.8	66.5 ± 11.5	70.8 ± 13.5
Body mass index (kg/m^2)	24.3 ± 3.4	23.5 ± 3.6	24.0 ± 3.2

Values are mean ± SD

CON control; *RET* resistance training; *RET-FO* resistance exercise training + fish oil supplementation

Results

Descriptive data are presented in Table 1. There was no difference in age and anthropometric characteristics between groups at baseline. Exercise training groups (RET, RET-FO) exhibited 90% compliance with similar progression of the exercise intensity over the 12 week experimental period. In addition, the workload is not significantly different between RET and RET-FO during the entire intervention period.

Maximum muscle strength

The data for 1-RM are presented in Table 2. No significant difference was detected in all estimated 1-RM assessments between groups at pre-intervention. The lat-pulldown 1-RM strength greatly increased in both RET (+ 11.7%, $p = 0.013$, $d = 0.27$) and RET-FO (+ 20.5%, $p = 0.001$, $d = 0.49$) from baseline, while it significantly decreased in CON (− 4.4%, $p < 0.001$, $d = 0.14$). Lat-pulldown 1-RM strength was substantially higher in RET ($p = 0.012$) and RET-FO ($p < 0.001$) than CON at post-intervention. The seated row 1-RM strength significantly increased in RET (+ 24.4%, $p = 0.001$, $d = 0.69$) and RET-FO (+ 45.6%, $p < 0.001$, $d = 0.92$) from baseline, while no change was found in CON. Seated row 1-RM strength was significantly higher in RET ($p = 0.005$) and RET-FO ($p < 0.001$) than CON at post-intervention. The biceps curl 1-RM strength substantially increased in RET (+ 22.8%, $p = 0.004$, $d = 0.79$) and RET-FO (+ 35.3%, $p < 0.001$, $d = 0.85$)) from pre- to post-intervention, but decreased in CON (-7.5%, $p = 0.003$, $d = 0.36$). 1-RM of biceps curl strength at post-intervention was greatly higher in RET ($p = 0.002$) and RET-FO ($p < 0.001$) compared to CON.

Table 2 Estimated maximal muscular strength pre- and post-intervention

	CON		RET		RET-FO	
	Pre	Post	Pre	Post	Pre	Post
Lat-pulldown (kg)	48.0 ± 15.5	45.9 ± 15.4*	52.9 ± 23.9	59.1 ± 22.0*$^{!}$	48.3 ± 17.4	58.2 ± 22.5*$^{!}$
Seated row (kg)	45.6 ± 14.9	44.2 ± 14.5	48.0 ± 19.2	59.7 ± 14.2*$^{!}$	44.5 ± 17.3	64.8 ± 25.8*$^{!}$
Biceps curl (kg)	16.1 ± 3.1	14.9 ± 3.5*	16.2 ± 5.1	19.9 ± 4.2*$^{!}$	15.6 ± 5.5	21.1 ± 7.3*$^{!}$
Leg press (kg)	158.9 ± 42.6	154.4 ± 44.2*	180.8 ± 47.3	223.6 ± 43.6*	159.8 ± 43.6	247.5 ± 65.5*$^{!\#}$
Calf rise (kg)	55.6 ± 14.8	52.4 ± 15.2*	57.3 ± 18.5	75.4 ± 16.2*$^{!}$	59.4 ± 18.1	86.1 ± 22.5*$^{!}$

CON control; *RET* resistance exercise training; *RET-FO* resistance exercise training + fish oil supplementation.

Values are mean ± SD

*$p \leq 0.05$, significantly different from pre-intervention

$^{!}p \leq 0.05$, significantly different from CON post-intervention

$^{\#}p \leq 0.05$, significantly different from RET post-intervention

The leg press 1-RM strength was markedly increased in RET (+23.7%, $p=0.002$, $d=0.94$) and RET-FO (+54.9%, $p<0.001$, $d=1.58$) from baseline, while CON exhibited significantly lower strength (-2.8%, $p=0.028$, $d=0.1$). RET and RET-FO exhibited significantly higher leg-press 1-RM strength than CON at post-intervention ($p=0.006$, $p<0.001$). In addition, RET-FO showed remarkably greater leg-press strength than RET ($p=0.007$) post-intervention. Similarly, the calf rise 1-RM strength greatly increased in RET (+31.6%, $p=0.002$, $d=1.04$) and RET-FO (+45%, $p<0.001$, $d=1.31$) from baseline, but CON showed a significant decrease (-5.8%, $p<0.001$, $d=0.21$). RET and RET-FO showed remarkably higher calf rise 1-RM strength than CON at post-intervention ($p<0.001$).

Resting metabolic rate

The data for resting metabolic rate (RMR) are presented in Table 3. There was no detectable difference of RMR between groups at baseline. Significant increase in VO_2 after 12-weeks of intervention in both RET (+6.2%, $p=0.01$, $d=0.38$) and RET-FO (+9.8%, $p<0.001$, $d=0.74$) occurred, while no notable change was found in CON. Similarly, VCO_2 was greatly increased in RET (+5.1%, $p=0.009$, $d=0.36$) and RET-FO (+4.8%, $p=0.029$, $d=0.35$) with no difference in CON after 12 weeks of intervention. The increase in VO_2 induced markable change in RMR of RET (+6%, $p=0.008$, $d=0.38$) and RET-FO (+8.5%, $p>0.001$, $d=0.64$) from baseline. RET-FO presented a significant increase in fatty acid (FA) oxidation (+27.2%, $p>0.001$, $d=1.41$) and decrease in carbohydrate oxidation (− 45.1%, $p=0.002$, $d=1.07$) after 12 weeks of intervention, while unchanged in CON and RET.

Inflammation

Figure 1 describes values of inflammatory biomarkers in each experimental group at baseline and after the 12 week intervention. There was no difference in IL-6 TNF-α, and CRP between groups at pre-intervention. IL-6 significantly

Table 3 Resting metabolic rate pre- and post-intervention

	CON		RET		RET-FO	
	Pre	Post	Pre	Post	Pre	Post
VO_2 (mL/min)	207.7 ± 30.7	205.1 ± 31.8	209.9 ± 34.8	222.8 ± 33.8*!	209.0 ± 27.3	229.5 ± 28.2*!
VCO_2 (mL/min)	158.7 ± 25.0	156.3 ± 24.4	160.1 ± 22.6	168.3 ± 22.5*!	161.8 ± 20.4	169.5 ± 23.3*!
RER	0.76 ± 0.03	0.76 ± 0.02	0.77 ± 0.03	0.76 ± 0.02	0.78 ± 0.04	0.74 ± 0.03*!#
Fat oxidation (mg/min)	82.1 ± 14.1	81.8 ± 15.7	83.5 ± 23.0	91.3 ± 20.5	79.1 ± 17.9	100.6 ± 12*!#
Carbohydrate oxidation (mg/min)	57.5 ± 29.1	54.8 ± 23.3	56.8 ± 25.2	53.0 ± 18.3	67.4 ± 31.1	37.0 ± 25.5*!#
RMR	1414 ± 213	1409 ± 245	1429 ± 230	1515 ± 229*	1432 ± 183	1554 ± 196*

Values are mean ± SD

CON control; *RET* resistance exercise training; *RET-FO* resistance exercise training + fish oil supplementation. *VO_2* resting oxygen consumption; *VCO_2* resting carbon dioxide production; *RER* respiratory exchange ratio; *RMR* resting metabolic rate

*$p \leq 0.05$, significantly different from pre-intervention

!$p \leq 0.05$, significantly different from CON post-intervention

#$p \leq 0.05$, significantly different from RET post-intervention

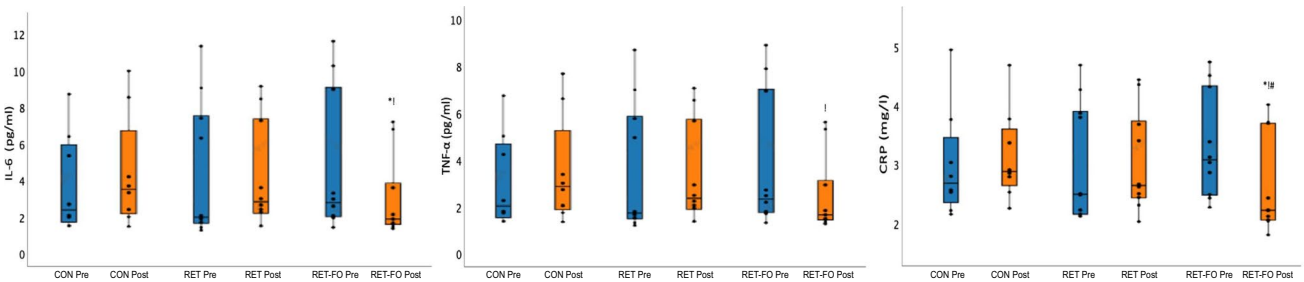

Fig. 1 Systemic Inflammation pre- and post-intervention. *IL-6* Interleukin-6; *TNF-α* Tumor necrosis factor-alpha; *CRP* C-reactive protein; *CON* control; *RET* resistance exercise. Training; *RET-FO* resistance exercise training + fish oil supplementation. Values are mean ± SD. *$p \leq 0.05$, significantly different from pre-intervention. !$p \leq 0.05$, significantly different from CON post-intervention. #$p \leq 0.05$, significantly different from RET post-intervention

decreased over time only in RET-FO (-41.2%, $p = 0.019$, $d = 0.53$), with the post-intervention value being substantially lower than CON ($p = 0.005$). TNF-α tended to increase in CON from baseline (+ 15%, $p = 0.072$, $d = 0.42$) and was significantly lower in RET-FO (-20%) compared to CON at post-intervention ($p = 0.019$). CRP substantially reduced from pre-intervention (− 22%, $p = 0.02$, $d = 0.79$) and was lower than CON ($p < 0.001$) and RET ($p = 0.003$) at post-intervention.

Discussion

The purpose of this present study was to determine the effects of chronic FO administration combined with RET on 1RM muscular strength, RMR, and systemic inflammation on healthy older adults. The key findings of this present study were that 12 weeks of programmed RET reversed aging-induced 1-RM muscle strength decline and FO administration augmented some aspects of muscle strength improvement (i.e., leg press). In addition, 12 weeks of FO consumption combined with RET enhanced RMR and FA oxidation while improving systemic inflammation in healthy older adults.

Muscle strength

The programed RET in the present study appeared to prevent or reverse aging-induced decline in muscle strength in healthy older adults. The fact that RET counteracts aging-induced loss of muscular strength is in accordance with previous studies in the healthy and frail elderly humans [34–37].

The data in the present study indicate FO administration enhanced RET-induced skeletal muscle strength gain in older adults. Particularly, FO supplementation combined with RET appeared to reverse aging-induced decline in leg muscle strength. In agreement with our findings, Rodacki and colleagues report 90 days of FO supplementation (2 g/day) during RET significantly enhanced peak torque and rate of torque development of leg muscles with greater improvement than RET only in elderly women [38]. Preserving skeletal muscle mass and strength is crucial for older adults to maintain their independent life due to its association with activities of daily living (ADL). In addition, Smith et al. reported FO enriched with n-3 FAs administration (1.86 g EPA and 1.5 g DHA) for 6 months markedly increased handgrip strength and 1RM muscle strength in healthy older male and females [24]. On the other hand, another research study reported no effect of n-3 FA supplementation (660 mg EPA and 440 mg DHA per day) on muscle strength change and physical performance in older adults [39]. The authors indicated the results were attributable to low sample size and low dosage of n-3 FAs, which was not sufficient to detect supplementation-induced changes in the outcomes. In our study, participants consumed higher dosage of n-3 FAs than their study (2.1 g EPA and 0.72 g DHA per day), which would be sufficient to enhance RET-induced muscle strength gain.

Resting metabolic rate and substrate oxidation

Our data indicated that the 12 weeks of RET substantially increased RMR (~ 86 kcal/day, + 6%) and this increment was much greater when combined with FO consumption (~ 122 kcal/day, + 8.5%). While RET has been recommended to maintain strength and physical function related to ADL in older adult, effects of RET on RMR and substrate metabolism has not been fully established. Findings from previous studies conducted to determine the efficacy of RET on RMR are inconsistent. Previous research reported no beneficial effects of RET on RMR in individuals engaged in weight loss intervention [40, 41]. Further, Geliebter and colleagues reported a significant decrease in RMR after 8-weeks of diet intervention, which was not prevented by RET [42]. In contrast, Lemmer and colleagues observed that 24-weeks of RET significantly increased RMR by 7% in both young and older adults [43], which agreed with Pratley and colleagues who found a substantial increment of RMR (+ 7.7%) after 16 weeks of heavy RET in healthy older adults [44] and Aristizabal and colleagues who reported improved RMR by 17% in young adults after 9 month of RET [45]. The positive effect of chronic RET on RMR was further supported by our data, which also indicated that chronic RET greatly increased RMR in healthy old adults. The participants in our study were healthy adults that maintained their normal diet with no caloric restriction. While chronic RET may not prevent or improve RMR in those with energy restriction, chronic RET with normal diet appeared to enhance RMR in healthy older adults.

While FO has attracted great attention due to their health-enhancing benefits (e.g., cardiovascular) [46], whether FO administration enhances RMR and substrate metabolism (e.g., FA oxidation) in older adults is yet to be determined. Previous investigations reported conflicting results regarding the effect of FO supplementation in RMR and FA oxidation in healthy humans. While some previous studies found no beneficial effect of FO administration [47, 48], Gerling et al. reported notable increase in RMR (+ 5.3%) after 12 weeks of $n-3$ supplementation in healthy young males [49]. In agreement with their findings, Logan and Spriet observed 12 weeks of FO supplementation (3 g/d) substantially increased RMR by 14% with significant FA oxidation by 19% in healthy older women, indicating FO administration as a potential interventional strategy to enhance metabolic capacity in older adults. The results from the present study

indicated that chronic FO administration enhanced RET-induced benefits of RMR. In addition, combined with RET, FO supplementation significantly improved fat metabolism (+ 27.2%) while RET alone did not affect FA oxidation.

It could be hypothesized that FO enriched with *n*–3 FAs may enhance fat metabolism by activating peroxisome proliferator-activated receptor alpha, (PPAR-α), which serves as a regulator of lipid homeostasis. EPA and DHA, major components of *n*–3 FAs, activate PPAR-α agonists and subsequently activates specific mediators for fat metabolism [50]. PPAR-α up-regulates specific transcript factors encoding for mitochondrial enzymes, which enhances ß-oxidation of FAs as well as proliferation of peroxisome [51]. Research reported a remarkable increase medium-chain acyl-CoA dehydrogenase (ß-oxidation enzyme) and acyl-CoA oxidase, the rate-limiting enzyme in the peroxisomal ß-oxidation in liver of mice fed FO for 8 weeks [52]. Their findings were supported by Fiamoncini and colleagues who also reported that 8 weeks of FO intake substantially enhanced FA ß-oxidation and thus reduced live fat accumulation in mice, indicating anti-lipogenic capacity of FO administration [53]. In agreement with their findings, the present study indicated a significant increase in resting metabolic rate and FA oxidation in RET-FO at post-intervention compared to baseline. Previous studies designed to determine beneficial effects of RET on fat oxidation reported inconsistent results. Kirk and colleagues found 6 months of RT significantly increased fat oxidation by decreasing sleep and resting RER by 1.7% and 1.5%, respectively, in overweight young adults [54]. However, Aristizabal et al. found that despite a significant increase in RMR, no detectable change in fat oxidation in healthy adults after 9 months of RET were found [45]. Therefore, it could be speculated that FO administration combined with RET would provide sufficient stimulus for older adults to maintain energy balance and prevent fat gain, which would help to better manage their body composition during aging.

Inflammation

We assessed blood IL-6, TNF-α, and CRP as biomarkers of systemic inflammation. Our data indicate 12-weeks of FO supplementation combined with RET lowered IL-6 and CRP while blunting the increase in TNF-α during aging. It has been generally known that inflammation is closely associated with aging-related diseases such as cardiovascular diseases [55], metabolic disorders [56], and osteoarthritis [57]. In addition, chronic low-grade inflammation during aging accelerates aging-induced muscle wasting followed by the loss of strength and physical function (i.e., sarcopenia) as an up-regulation of inflammatory cytokines impairs muscle anabolic signaling [58]. Thus, lowering chronic low-grade inflammation is crucial for older adults to improve their muscle mass and strength, maintain physical function and independence, and thus enhance quality of life.

Previous research designed to determine the anti-inflammatory effect of chronic RET reported inconsistent results. Greiwe and colleague found 3 months of RET significantly reduced TNF-α mRNA expression in frail elderly individuals [18]. However, Ogawa et al. presented that 12 weeks of RET resulted in no notable changes in systemic IL-6 and TNF-α in elderly women [19]. Our data in the present study also indicate that chronic RET fails to produce an anti-inflammatory benefit in healthy older adults. On the other hand, when FO was administered with RET, there was a substantial reduction in IL-6 from baseline. Further, RET-FO exhibited significantly lower systemic IL-6 and TNF-α values compared to CON post intervention. These findings indicate chronic FO administration with RET promoted anti-inflammatory effect in older adults. The anti-inflammatory impact of combined treatment may improve muscle mass followed by an increase in muscular strength. Indeed, we found the RET-FO group exhibited significant improvement of leg muscle strength from pre-intervention with the post-intervention value being substantially greater than CON and RET. In agreement with our findings, Smith and colleagues found that 6 months of n-3 FAs increased hand-grip strength and 1-RM muscle strength of upper and lower body [24]. Further, a number of research support pro-anabolic [59, 60] and anti-inflammatory [61, 62] impacts of *n*–3 FAs. Future research may need to be conducted to elucidate the suggested speculation.

While the present study provided meaningful findings, we should note that the present study has some limitations. First, we did not control diet and daily activity levels of participants, which may produce some variations in the results. Also, we did not assess body composition of participants which could well support the proposed speculations of the result.

Conclusion

In conclusion, our findings suggest that chronic programmed RET can reverse age-induced loss of muscle strength and improve RMR. Combining RET with FO supplementation appeared to enhance some RET-induced benefits in healthy older adults such as lower body strength. Further, FO administration combined with RET increased whole body fat oxidation and lowered some indicators of systemic inflammation such as IL-6 and CRP. While the effects observed on these circulating markers were mild, possible anti-inflammatory effects by RET plus FO supplementation and their biological significance in older adults may be worth exploring further. Overall, the present study provided some meaningful

implication for future clinical investigations. Future research may need to be conducted to determine clinical values of FO intervention strategies to improve aspects of physiological and physical function in older populations.

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Author contributions SL conceived and designed research and conducted experiments. SL and DD analyzed data. SL wrote manuscript. DD and AK reviewed and edited manuscript. All authors read and approved the manuscript.

Declarations

Conflict of interest The authors declare no conflicts of interest associated with this manuscript.

Ethical approval This study was approved by the New Mexico State University Institutional Review Board for Human Subjects. All the research procedures has been conducted in accordance with the Declaration of Helsinki and its later amendments.

Informed consent Informed consent was obtained from all participants prior to being admitted to the study.

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