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A multi-ingredient nutritional supplement enhances exercise training-related reductions in markers of systemic inflammation in healthy older men

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ABSTRACT

We evaluated whether twice daily consumption of a multi-ingredient nutritional supplement (SUPP) would reduce systemic inflammatory markers following 6wk of supplementation alone (Phase 1), and the subsequent addition of 12wk exercise training (Phase 2) in healthy older men, in comparison to a carbohydrate-based control (CON). Tumour necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) concentrations were progressively reduced (P -time<0.05) in the SUPP group. No change in TNF- α or IL-6 concentrations was observed in the CON group.

KEYWORDS: whey protein, creatine, calcium, vitamin D, omega-3 polyunsaturated fatty acids, resistance exercise, high-intensity interval training

INTRODUCTION

Age-associated low-grade systemic inflammation (termed "inflammaging") has been linked to declines in physical function (Marzetti et al. 2014), skeletal muscle insulin resistance (Buffiere et al. 2015), the occurrence of multiple morbidities (Stepanova et al. 2015), as well as reduced overall survival (Brown et al. 2016). Exercise training (Singh and Newman 2011), and certain isolated nutritional supplements such as whey protein (de Aguilar-Nascimento et al. 2011; Sugawara et al. 2012), creatine (Santos et al. 2004; Bassit et al. 2008), vitamin D/calcium (Bjorkman et al. 2009; Waterhouse et al. 2015), and omega-3 polyunsaturated fatty acids (n-3 PUFA) (Zhao et al. 2009; Tartibian et al. 2011), have previously been shown to reduce circulating concentrations of pro-inflammatory cytokines. Recently we demonstrated that a broad spectrum nutritional supplement (which included these aforementioned ingredients: creatine, whey, vitamin D, calcium, and n-3 PUFA) improved strength and lean tissue mass in a group of

healthy older men versus a nutrition control (Bell et al. 2017). Furthermore, the addition of multimodal exercise training enhanced improvements in strength and glycemic control in the same participants relative to the control group.

The objectives of this study were to determine whether the same protein-based multi-ingredient nutritional supplement, which has previously been shown to improve various aspects of health in older men in the absence of exercise (Bell et al. 2017), would reduce markers of systemic inflammation in a group of sedentary older men following 6 weeks of twice daily consumption. Additionally, we aimed to determine whether the same supplement would enhance exercise training-related improvements following 12 weeks of a combined resistance exercise training (RET) and high intensity interval training (HIIT) program. We hypothesized that markers of systemic inflammation would decrease following supplementation alone, and that further reductions would occur with the addition of exercise training.

METHODS

Experimental design. Details of the study protocol, testing procedures, and eligibility criteria have previously been described (Bell et al. 2017). Briefly, 49 healthy men ≥ 65 years old were randomly assigned to receive either a multi-ingredient nutritional supplement (SUPP; $n = 25$) or a control (CON; $n = 24$) drink for 20 weeks (weeks 0-19 inclusive). After 6 weeks of consuming their study beverages at home (Phase 1: SUPP/CON), subjects completed a 12-week supervised exercise training program at McMaster University while continuing to consume their assigned beverages (Phase 2: SUPP + EX and CON + EX). Plasma concentrations of tumour necrosis factor alpha (TNF- α), interleukin-6 (IL-6), and C-reactive protein (CRP) were evaluated at weeks -1 (baseline), 6, and 19 (post-exercise training). During week 19, blood samples were

collected at least 72 h after the last exercise training session. This trial was approved by the Hamilton Integrated Research Ethics Board and complied with the guidelines set out in the Tri-Council policy statement on ethical conduct for research involving humans (http://www.pre.ethics.gc.ca/pdf/eng/tcps2/TCPS_2_FINAL_Web.pdf). All participants were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. This trial was registered at ClinicalTrials.gov (NCT02281331).

Nutritional supplements. Participants in the SUPP group consumed a multi-ingredient beverage containing: 30 g whey protein, 2.5 g creatine, 400 mg calcium, 500 IU vitamin D, and 1500 mg n-3 PUFA (which delivered 700 mg eicosapentaenoic acid [EPA] and 445 mg docosahexaenoic acid [DHA]) twice daily (116 kcal/drink). Participants in the CON group consumed a control beverage containing 22 g of carbohydrate twice daily (56 kcal/drink). The exact composition of the supplement and control drinks has been previously outlined (Bell et al. 2017). Subjects consumed their first daily beverage within the hour after breakfast, and the second 1h prior to bed. The control beverages were matched in volume and flavour to the active blend. All study beverages were prepared and labeled in a blinded manner by Infinit Nutrition (Windsor, ON), and both subjects and researchers were blind to individual group assignments. Participants were instructed not to alter their habitual dietary or physical activity habits for the duration of the study.

Exercise training. From weeks 7 to 18 inclusive, subjects engaged in a 12-week progressive exercise training program at the Physical Activity Centre of Excellence (PACE) at McMaster University. In brief, subjects completed three supervised exercise sessions per week: whole body RET twice per week (Mondays and Fridays) and HIIT on a cycle ergometer once per week (Wednesdays). At every RET session, participants completed two upper body (chest press and

horizontal row on Mondays; lateral pulldown and shoulder press on Fridays) and two lower body exercises (leg press and leg extension on both Mondays and Fridays). Training was performed at 80% 1RM (6-8 repetitions) for three sets, with the last set completed until volitional fatigue.

During their HIIT sessions, subjects completed 10 x 60 s intervals cycling against a workload predetermined to elicit ~90% maximal HR, while maintaining a cadence of ≥ 90 rpm. Intervals were interspersed with 60 s of low-intensity cycling where subjects pedaled at a self-selected pace against 25 W.

Biochemical analysis. Fasting plasma TNF- α and IL-6 concentrations were measured using a Bio-Plex system (Bio-Rad Laboratories; Hercules, CA), and plasma CRP concentrations were measured using an Express Plus Autoanalyzer (Chiron Diagnostics Co; Walpole, MA) and a commercially available high-sensitivity CRP latex kit (Pulse Scientific; Burlington, ON). Intra-assay coefficients of variation for TNF- α , IL-6, and CRP were 4.5%, 6.0%, and 3.5%, respectively.

Statistical analysis. Statistical analysis was completed using SPSS (IBM SPSS Statistics for Windows, version 23.0; IBM Corp., Armonk, NY). We conducted an intention-to-treat analysis using a linear mixed model with an unstructured covariance matrix, group and time as fixed factors, and subject as a random factor. Trunk fat mass (assessed using dual-energy X-ray absorptiometry; DXA) was included as a covariate due to the high degree of correlation between visceral adiposity and systemic inflammation (Shoelson et al. 2007). Following significant group-by-time interactions, significant between (SUPP or CON) and within (weeks -1, 6, or 19) group differences were identified using post hoc tests with a Bonferroni correction for multiple comparisons. Based on recommendations for human clinical trials with missing data (Elobeid et al. 2009), all participants (completers as well as participants who withdrew prior to week 6 or

week 19 testing) were included in the final analyses, and missing values were not replaced.

Statistical significance was accepted as $P < 0.05$.

RESULTS

At baseline, there were no differences in inflammatory markers between groups. We observed significant group-by-time interactions for CRP ($P < 0.001$), TNF- α ($P < 0.001$), and IL-6 ($P < 0.001$). In both the SUPP and CON groups, plasma CRP concentrations did not change in Phase 1; although significant reductions were observed in both groups following Phase 2, the magnitude of this change was numerically larger in the SUPP group (SUPP: -10% and CON: -1%; $P < 0.05$; **Figure 1**). In the SUPP group, mean plasma TNF- α and IL-6 concentrations each decreased ~1-3% during Phase 1 and a further ~11-12% in response to Phase 2. In the CON group, plasma TNF- α and IL-6 concentrations did not improve over the course of the study. Plasma CRP, TNF- α , and IL-6 concentrations were all significantly lower in the SUPP group compared to the CON group at week 19.

We observed a main effect of time for trunk fat mass ($P < 0.033$) such that no change was detected during Phase 1, however following Phase 2 mean trunk fat mass was reduced from 17.4 to 16.7 kg across all participants (SUPP: -8% and CON: 0%; $P < 0.01$).

DISCUSSION

Six weeks of twice daily consumption of a protein-based multi-ingredient nutritional supplement resulted in modest yet significant reductions in certain circulating markers of systemic inflammation in a group of older men. These reductions were enhanced with the addition of 12 weeks of RET + HIIT.

Low-grade systemic inflammation is associated with a variety of negative health consequences in older age including reduced physical function (Stenholm et al. 2010; Marzetti et al. 2014), diminished aerobic capacity (Buffiere et al. 2015), cardiovascular disease (Liu and Li 2015), and insulin resistance (Buffiere et al. 2015). Accordingly, in the present study our subjects ingested a multi-ingredient supplement, in the absence of exercise, containing supplement components aimed at improving muscular strength, body composition, and cardiometabolic health, as well as reducing circulating concentrations of pro-inflammatory cytokines. Low-grade sterile inflammation due to increased oxidative stress (Singh and Newman 2011) and/or central (particularly visceral) adiposity (Ryan 2000; Singh and Newman 2011) has been suggested as a mechanism by which cardiometabolic disease develops in older age, and is therefore a worthwhile target for intervention. Previous studies have investigated the independent ability of whey protein (de Aguilar-Nascimento et al. 2011; Sugawara et al. 2012), creatine (Santos et al. 2004; Bassit et al. 2008), vitamin D (Bjorkman et al. 2009; Waterhouse et al. 2015), and n-3 PUFA (Zhao et al. 2009; Tartibian et al. 2011) to reduce various markers of systemic inflammation in older adults and, by extension, improve other health outcomes. A recent review (Ticinesi et al. 2016) concluded that sufficient evidence existed only in support of n-3 PUFA supplementation to reduce inflammation in older adults. However, the authors postulated that nutrition interventions which combine whey protein, vitamin D, and n-3 PUFA may be especially effective since the anti-inflammatory activity of one isolated nutrient could be influenced by the intake of another. The multi-ingredient nature of the supplement used in the present study prevents us from determining which ingredient(s) was (were) responsible for the anti-inflammatory effects that we observed. However, as Ticinesi et al. (2016) suggest, the interaction between the various ingredients in the supplement (whose combined effects may be

either additive or synergistic) may have been important for the observed reduction in systemic inflammation. However, this is speculative and should be confirmed in future studies.

During 12 weeks of exercise training, twice daily consumption of the nutritional supplement enhanced reductions in circulating concentrations of pro-inflammatory cytokines in the SUPP group only. This observation parallels (and may help to explain) the superior improvement in glycemic control in the SUPP group compared to the CON group that we described in our previously published study (Bell et al. 2017). Both TNF- α and IL-6 interfere with insulin signaling in skeletal muscle primarily via inhibition of phosphoinositide-3 (PI3) kinase (Kim et al. 2004; Plomgaard et al. 2005) and subsequent GLUT4 translocation to the plasma membrane. Decreased circulating concentrations of TNF- α and IL-6 may therefore have permitted greater GLUT4 vesicle trafficking to the sarcolemma, and enhanced insulin-stimulated glucose uptake following an oral glucose challenge in the SUPP group. It may be that exercise training is itself an anti-inflammatory stimulus; however, support for the anti-inflammatory effect of exercise alone is somewhat equivocal (Beavers et al. 2010). Indeed, we did not observe any exercise training-related improvement in circulating concentrations of TNF- α or IL-6 in the CON group.

Differential reductions in visceral adipose tissue content may have also contributed to the greater relative improvement in systemic inflammation (and indeed, glycemic control) in the SUPP versus CON group over the course of exercise training. Following 12 weeks of RET + HIIT participants lost 0.7 kg of trunk fat mass, which may be indicative of decreased visceral adiposity. Visceral adipose tissue content is an independent risk factor for glucose intolerance (Despres et al. 1989) and insulin resistance (Cefalu et al. 1995) possibly as a result of heightened pro-inflammatory cytokine production in visceral compared to subcutaneous adipose tissue

depots (Singh and Newman 2011). Furthermore, in overweight and obese individuals visceral adipose tissue can become infiltrated with macrophages which also secrete inflammatory cytokines (Weisberg et al. 2003). The observed decrease in trunk fat mass during exercise training in the present study may have been predominantly driven by the -8% change in the SUPP group, with the 0% change in the CON group constituting a relatively small contribution. Admittedly, we did not observe a statistically significant difference in the amount of trunk fat mass lost between the two groups: our relatively small sample size may have reduced the ability to detect between-group differences, which is a limitation of this study. Nonetheless, it remains possible that preferential reductions in trunk fat mass (which is a proxy of visceral adipose tissue) during exercise training with multi-ingredient nutritional supplementation may have underpinned improvements in both systemic inflammation as well as glucose handling; however, this hypothesis remains to be confirmed in future with larger trials.

In the present study, we have demonstrated that consumption of a multi-ingredient nutritional supplement, with and without exercise training, stimulated greater improvements in systemic inflammation compared to a carbohydrate-based control drink in a group of healthy older men. Improvements in muscular strength, body composition, and insulin sensitivity may prevent or delay health degeneration in older individuals, and this may be mediated by reductions in systemic inflammation. However, the role that inflammation plays in the development of age-related physiological decline remains to be further elucidated.

CONFLICTS OF INTEREST

SMP reports receipt of competitive grant support, travel expenses, and honoraria for speaking received from the US National Dairy Council. No other conflicts of interest, financial or otherwise, are reported by any of the authors.

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FIGURE CAPTION

Figure 1. Plasma IL-6 (A), TNF- α (B) and CRP (C) concentrations throughout the study. Boxes (SUPP: grey; CON: white) represent interquartile ranges, with the horizontal lines indicating the medians. Whiskers represent the maximal and minimal values, and crosses indicate the means. Dissimilar letters denote changes over time within a given treatment group (SUPP or CON). *indicates a significant difference from the CON group at that time.

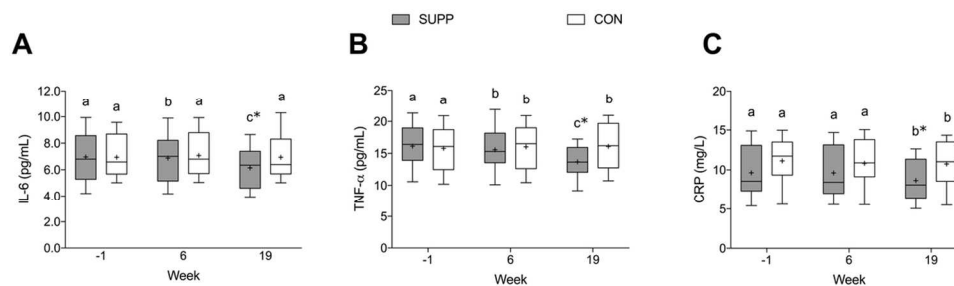


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