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Effects of rumenic acid rich conjugated linoleic acid supplementation on cognitive function and handgrip performance in older men and women

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ABSTRACT

The purpose of this study was to investigate the effects of 8 weeks at 6 g per day of RAR CLA versus placebo on cognitive function and handgrip performance in older men and women. Sixty-five (43 women, 22 men) participants (mean ± SD; age = 72.4 ± 5.9 yrs; BMI = 26.6 ± 4.2 $kg \cdot m^{-2}$) were randomly assigned to a RAR CLA (n = 30: 10 men, 20 women) or placebo (PLA; high oleic sunflower oil; n = 35: 12 men, 23 women) group in double-blind fashion and consumed 6 $g \cdot d^{-1}$ of their allocated supplement for 8 weeks. Before (Visit 1) and after supplementation (Visit 2), subjects completed the Serial Sevens Subtraction Test (S_7), Trail Making Test Part A (TM_A) and Part B (TM_B), and Rey's Auditory Verbal Learning Test (RAVLT) to measure cognitive function. The RAVLT included 5, 15-item auditory word recalls (R_{1-5}), an interference word recall (R_B), a 6th word recall (R_6), and a 15-item visual word recognition trial (R_R). For handgrip performance, subjects completed maximal voluntary isometric handgrip strength (MVIC) testing before ($MVIC_{PRE}$) and after ($MVIC_{POST}$) a handgrip fatigue test at 50% $MVIC_{PRE}$. Hand joint discomfort was measured during $MVIC_{PRE}$, $MVIC_{POST}$, and the handgrip fatigue test. There were no treatment differences ($p > 0.05$) for handgrip strength, handgrip fatigue, or cognitive function as measured by the Trail Making Test and Serial Seven's Subtraction Test in men or women. However, RAR CLA supplementation improved cognitive function as indicated by the RAVLT R_5 in men. A qualitative examination of the mean change scores suggested that, compared to PLA, RAR CLA supplementation was associated with a small improvement in joint discomfort in both men and women. Longer-term studies are needed to more fully understand the potential impact of RAR CLA on cognitive function and hand joint discomfort in older adults, particularly in those with lower cognitive function.

1. Introduction

Conjugated linoleic acids (CLA) are naturally occurring geometric and positional isomers of linoleic acid, or octadecadienoic acid, produced naturally by microbes in the rumen of ruminant animals (Blankson et al., 2000; Jenkins et al., 2014b; Ritzenthaler et al., 2001). CLA contains two double bonds separated by a single bond in a *cis*, *trans* configuration that commonly occurs between the 8- and 13-carbon positions (Gaullier et al., 2002). The two most common isomers of CLA are *trans*-10, *cis*-12 and *cis*-9, *trans*-11 (Gaullier et al., 2002). However, while most commercial synthetic CLA supplements contain an approximately equal amount of the *trans*-10, *cis*-12 and *cis*-9, *trans*-11 isomers (i.e., 50:50 blend) (Bhattacharya et al., 2006; Penedo et al., 2013), the latter represents approximately 90–95% of the total CLA in rumenic food (i.e., dairy) products (Elgersma et al., 2006; Nunes and Torres, 2010). Consequently, the *cis*-9, *trans*-11 isomer is commonly referred to as rumenic acid (RA).

Rumenic acid has shown promise as an anti-inflammatory dietary supplement in humans (Penedo et al., 2013; Sofi et al., 2010; Turpeinen et al., 2008). For example, Penedo et al. (2013) reported that 8 weeks of RA-enriched butter improved inflammatory markers in young, healthy men and women. Turpeinen et al. (2008) reported that 8 weeks of RA supplementation reduced the allergic responses mediated by inflammation in young men and women with birch pollen allergy. Sofi et al. (2010) showed that 10 weeks of dietary supplementation with cheese naturally rich in RA (e.g., pecorino) reduced inflammation in middle-aged men and women. Therefore, although there are limited applied studies in humans, existing evidence suggests that RA may have anti-inflammatory effects.

The mechanism of action for the anti-inflammatory effects of RA may be due to its actions as an agonist of peroxisome proliferator-activated receptor-γ (PPARγ) (Turpeinen et al., 2008; Yu et al., 2002). PPARγ is a ligand-activated transcription factor that regulates gene transcription. PPARγ is expressed in most tissues of the body and has

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important metabolic and inflammatory effects. Jaudszus et al. (2005) demonstrated that RA reduced inflammatory responses in human epithelial cells via activation of PPARγ. Similarly, Yu et al. (2002) showed that RA activated PPARγ and served as an antioxidant in mouse macrophage cells. Therefore, it has been suggested that RA may have therapeutic value in the management of conditions characterized by chronic inflammation such as atherosclerosis, asthma, inflammatory bowel disease, obesity, and aging (Bassaganya-Riera et al., 2004; Bhattacharya et al., 2006; Penedo et al., 2013; Turpeinen et al., 2008; Yu et al., 2002).

Aging is associated with neurodegeneration, which describes a progressive deterioration and/or loss of neurons (Chen et al., 2012). Activation of PPARγ may reduce the risk of neurodegeneration. For example, in rodents, PPARγ activation has been shown to reduce cerebral ischemia/reperfusion injury (Collino et al., 2006) and vascular aging (Modrick et al., 2012) by inhibiting oxidative stress and inflammation. Gama et al. (2015) showed that dietary RA-enriched butter was associated with improved memory in rats. Thus, RA may be neuroprotective by way of PPARγ-mediated anti-inflammatory, antioxidant, and vascular protection effects (Ulrich-Lai and Ryan, 2013). Indeed, Yaffe et al. (2008) demonstrated that older adults with a specific PPARγ polymorphism (e.g., Pro12Ala) had a decreased risk for age-related cognitive decline. Therefore, from an applied perspective, PPARγ activation by RA supplementation may improve cognitive function in older adults.

Aging is also characterized by greater oxidative stress in tissues such as cartilage (Choi et al., 2013; Loeser, 2009). Aryaeian et al. (2009) studied the effects of a 50:50 isomeric blend of *cis*-9, *trans*-11 (i.e., RA) and *trans*-10, *cis*-12 CLA on symptoms of rheumatoid arthritis. The authors (Aryaeian et al., 2009) observed a decrease in disease activity, pain, and stiffness with CLA supplementation and hypothesized that these decreases were due to reduced inflammation. Therefore, RA may have protective effects against age-related joint dysfunction and/or discomfort through anti-inflammatory and antioxidant effects (Chen et al., 2012; Collino et al., 2006; Fahmi et al., 2011; Kapadia et al., 2008; Loeser, 2009; Matsui et al., 2007; Penedo et al., 2013; Turpeinen et al., 2008).

Although many studies have examined PPARγ's cellular effects (Collino et al., 2006; Garcia-Bueno et al., 2007; Garcia-Bueno et al., 2005; Jiang et al., 1998; Kelly et al., 1998; Modrick et al., 2012; Ricote et al., 1998; Ulrich-Lai and Ryan, 2013; Wang et al., 2014; Yaffe et al., 2008) and demonstrated RA's activity as a PPARγ agonist (Bassaganya-Riera et al., 2004; Jaudszus et al., 2005; Yu et al., 2002), few studies have examined the effects of RA on applied, functional outcomes in humans (Penedo et al., 2013; Sofi et al., 2010; Tricon et al., 2006; Turpeinen et al., 2008). In theory, if RA works through the aforementioned cellular mechanisms, then it may reduce the cognitive decline and joint dysfunction and/or discomfort that occur with age. In addition, because of the possible influence of gender on age-related declines in physical function and cognition (Barrett-Connor and Kritz-Silverstein, 1999; Cooper et al., 2011; Goodpaster et al., 2006; Gur and Gur, 2002; Morishita et al., 2013), RA may have gender-dependent effects in older adults. However, no previous studies have investigated the effects of RA-rich conjugated linoleic acid (RAR CLA) on age-related decreases in cognitive function or handgrip performance in men and women. Therefore, the purpose of this study was to investigate the effects of 8 weeks at 6 g per day of RAR CLA versus placebo on cognitive function and handgrip performance in older men and women. We hypothesized that RAR CLA would improve cognitive function and handgrip performance in older adults.

2. Methods

2.1. Experimental design

This was a prospective, randomized, double-blind, placebo-controlled, parallel design clinical trial. There were three visits to the laboratory: Visits 0, 1, and 2 (Fig. 1). During Visit 0, the participants were familiarized with the testing procedures and received 3-day dietary food logs. Three to seven days later, the participants returned for Visit 1 and completed pre-supplementation testing, which consisted of a series of cognitive tests and measures of handgrip performance and discomfort. Following testing, participants were randomly assigned to either the supplement (RAR CLA; $n = 30$: 10 men, 20 women) or placebo (PLA; $n = 35$: 12 men, 23 women) group and began 8 weeks of supplementation. Forty-eight hours after Visit 1, the participants were called to provide a joint discomfort rating over the phone. At regular 2 week intervals after Visit 1, participants were called to verify supplement compliance, collect information regarding the occurrence of adverse events, and ask about changes in dietary intake or supplement and medication usage. Following 8 weeks of supplementation, the participants returned to the laboratory for Visit 2 for post-supplementation testing, which was a replication of Visit 1. Forty-eight hours after Visit 2, the participants were called to provide a hand joint discomfort rating over the phone. The participants recorded all food and drink consumed for 2 week days and 1 weekend day between Visit 0 and Visit 1 and during the week prior to Visit 2 in the 3-day dietary food logs provided at Visit 0. The participants continued to take their supplement (RAR CLA or PLA) until Visit 2 when they returned all unused product (RAR CLA or PLA).

2.2. Participants

Seventy-five (53 women, 22 men) participants were enrolled, but only the data of 65 (43 women, 22 men) participants (mean ± standard deviation (SD); age = 72.4 ± 5.9 yrs.; height = 168.8 ± 8.5 yrs.; weight = 76.1 ± 14.4 kg; BMI = 26.6 ± 4.2 $kg \cdot m^{-2}$) were analyzed for this study (Fig. 1). Therefore, all the participants were protocol evaluable as opposed to intent to treat. The participants in this study were between 65 and 85 years of age inclusive, had a body mass index ≤35 $kg \cdot m^{-2}$, had not participated in any other clinical trials for 30 days prior to study enrollment, consumed <500 $mg \cdot day^{-1}$ of aspirin, and were not taking any of the following "sartans" or "glitazones": Losartan (Cozaar), Candesartan (Atacand), Valsartan (Diovan), Irbesartan (Aprovel, Karvea, and Avapro), Telmisartan (Micardis), Eprosartan (Teveten), Olmesartan (Benicar), Azilsartan (Edarbi, Edarbyclor), Fimasartan (Kanarb), Candesartan (Atacand), Rosiglitazone (Avandia), and/or Pioglitazone (Actos). In addition, all participants had stopped eating ≥3 servings of fish per week and taking any anti-inflammatory dietary supplements such as quercetin, curcumin, resveratrol, and/or other flavonoids for at least 1 month prior to the study. Participants were also instructed not to consume any amount of non-steroidal anti-inflammatory drugs or acetaminophen on the days of Visits 1 or 2. All participants were recruited via flyers, newspaper advertisements, and/or word of mouth in the local community from August through September 2016. This study was approved by the university's Institutional Review Board for the protection of human subjects, and all subjects completed a health history questionnaire and informed consent document prior to any testing.

2.3. Sample size calculation and participant randomization

Sample size was estimated a priori based on Turpeinen et al. (2008) who examined the effects of RA on functional outcomes in adults, in addition to a priori sample size calculation using G*Power 3.1 (Faul et al., 2009). Turpeinen et al. (2008) observed improved allergy symptoms in subjects with allergies utilizing a sample size of 40 (28 women, 12 men). In addition, sample size calculations (Faul et al., 2009) indicated that a total sample size of 22 subjects would be needed to observe an effect of 0.2 (Yurko-Mauro et al., 2010) with a power of 0.8 and a correlation of 0.8 between repeated measures using a mixed factorial ANOVA with 2 groups and pre- and post-measurements.

A randomization code that assigned subject enrollment numbers 1–40 and 41–80 to the RAR CLA or PLA group was generated using a free,