NUTRITION RESEARCH 36 (2016) 989–994

Available online at www.sciencedirect.com

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Red blood cell oleic acid levels reflect olive oil intake while omega-3 levels reflect fish intake and the use of omega-3 acid ethyl esters: The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico–Heart Failure trial

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ARTICLE INFO

Article history:
Received 3 February 2016
Revised 22 June 2016
Accepted 23 June 2016

Keywords:
Omega-3 fatty acids
Cardiovascular disease
Heart failure
Omega-3 index
Biomarker
Olive oil
Fish

ABSTRACT

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico–Heart Failure (GISSI-HF) study reported benefits of n-3 fatty acid (FA) treatment on cardiovascular (CV) events, but the effects of treatment on a putative CV disease risk factor, the red blood cell (RBC) n-3 FA level (the omega-3 index), have not been examined in this context. We hypothesized that treatment with prescription omega-3 acid ethyl esters (O3AEE) would increase the omega-3 index to the proposed cardioprotective value of 8%. RBCs were collected from a subset of patients participating in the GISSI-HF study (n = 461 out of 6975 randomized), at baseline and after 3 months of treatment with either an olive oil placebo or O3AEE (1 g/d). RBC FA levels were expressed as a percentage of total FA. Patients also reported their typical olive oil and fish intakes. RBC oleic acid levels were directly correlated with reported frequency of olive oil consumption, and the omega-3 index was correlated with reported fish intake (*P* for trends <0.001 for both). After treatment, the omega-3 index increased from 4.8 ± 1.7% to 6.7 ± 1.9% but was unchanged in the placebo group (4.7 ± 1.7 to 4.8 ± 1.5%) (*P* < .0001 for changes between groups). At 3 months, more patients reached the proposed target omega-3 index level of 8%-12% in the treated vs placebo group (22.6% vs. 1.3%, *P* < .0001), however, what omega-3 index levels were ultimately achieved after four years in this trial are unknown.

Abbreviations: ANOVA, analysis of variance; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPAn-3, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GISSI-HF, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico - Heart Failure; O3AEE, omega-3 acid ethyl esters; OA, oleic acid; Q, quartile; RBC, red blood cell; RBC EPA + DHA, the omega-3 index.

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[1] A complete list of centers participating in the GISSI-HF substudy and their investigators was published in the European Journal of Heart Failure 2010;12:338–347.

http://dx.doi.org/10.1016/j.nutres.2016.06.012
0271-5317/

1. Introduction

There are multiple controversies surrounding the effects of saturated, mono-unsaturated and omega-6 polyunsaturated fatty acids (FAs) on coronary heart disease risk [1-4], and even around the omega-3 FA, which for many years have enjoyed virtually universal support as being cardioprotective [5,6]. In the Chowdhury et al meta-analysis [1], both intakes and blood levels of the long-chain, marine-derived omega-3 FAs (eicosapentaenoic [EPA], docosahexaenoic [DHA], and docosapentaenoic acids [DPAn-3]) were inversely related to risk, but randomized trials have, at least in recent years, not supported this relationship.

FA intakes and blood levels vary around the world, presumably driven by differences in dietary patterns. Omega-3 FA levels are typically low in most western countries where fish consumption is low, and high in cultures like Japan or Korea where consumption is high [7]. Among the European Mediterranean countries, Spain has the highest fish intake [8], but their red blood cell (RBC) omega-3 levels, although high [8] relative to the United States [9] and Germany [10] (ie, 7.1% versus 5.6% and 4.7%, respectively), have not been compared across Europe. The other FA family most commonly associated with Mediterranean diets, particularly in Italy, is oleic acid (OA), which is a major component of olive oil. Since OA levels are determined by both endogenous synthesis and exogenous consumption, its levels in the blood have not been considered to be good markers of dietary OA [11], but cross-cultural analyses using the same laboratory methods are few, and the possibility that chronically elevated intakes might be reflected in membrane OA levels has not been tested.

The primary hypotheses tested here were (1) treatment with omega-3 acid ethyl esters (O3AEE) will increase the RBC EPA + DHA level (ie, the Omega-3 Index) to the proposed cardioprotective level of 8% or greater, (2) the Omega-3 Index at baseline is directly associated with reported fish intake, and 3) RBC OA levels at baseline are directly associated with reported olive oil intake. These hypotheses were tested by measuring the RBC FA composition at baseline and after 3 months in a sub-cohort of Italian patients who participated in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico–Heart Failure (GISSI-HF) study and who were treated with 1 g/d of O3AEE (or placebo).

2. Methods and materials

2.1. Subjects

The GISSI-HF trial was a randomized, double-blind, placebo-controlled, multicenter study that enrolled 6975 patients with clinical evidence of chronic, stable HF, as previously described [12]. The trial investigated the effect of taking 1 g/day of O3AEE providing about 850 to 882 mg of EPA and DHA combined (in the average ratio of 1:1.2) or an olive oil placebo for about 4 years. In a prospectively planned biomarker substudy, blood samples were collected at randomization and 3 months later from a subset of 461 patients recruited in 51 clinical centers [13]. A brief food questionnaire was administered that included questions on the frequency of consumption of fish and olive oil [14]. The study was approved by local ethics committees, and informed consent was obtained from all patients before the study started. The trial was registered at Clinicaltrials.gov (NCT00336336).

2.2. Laboratory methods

Blood samples were collected in EDTA tubes in the fasting state. The tubes were centrifuged, an aliquot of the packed RBC fraction was transferred to a cryovial and placed in a –70°C freezer, all within 30 minutes of collection. The samples remained at temperature until thawed at room temperature for analysis. RBC FA composition was analyzed according to the HS-Omega-3 Index® methodology as modified from Harris et al [10]. FA methyl esters were generated from erythrocytes by transesterification with boron trifluoride and analyzed by gas chromatography. FAs were identified by comparison with a standard mixture of fatty acids characteristic of RBCs. Omega-3 index results are given as EPA plus DHA expressed as a percentage of 20 total FAs (Table) after calibration-curve derived response factor correction was applied to each fatty acid [15]. The average coefficient of variation for FAs of <1% prevalence was 11.3%; for those between 1% and 5% prevalence, 3.4%; and for those with >5% prevalence, 1.6%.

2.3. Statistical analyses

Categorical variables are presented as proportions and continuous variables as means (±SDs) or medians (Q1-Q3). The effect of the study drugs (O3AEE vs placebo) on 3-month changes in circulating FA in RBC was tested by analysis of variance (ANOVA). Baseline associations between reported olive oil intake and RBC OA levels, and between fish intake and RBC EPA + DHA, were evaluated by ANOVA after checking normality distribution assumptions and log-transforming any data that were not normally distributed. Although multiple hypothesis tests were carried out, a nominal 2-sided significance level of 0.05 was used, with no formal adjustment for multiple testing given the exploratory nature of the present investigation. All analyses were performed with SAS software, version 9.3 (SAS Institute, Cary, NC).

3. Results

The mean (SD) age of the 461 patients comprising the GISSI-HF subcohort was 67.1 (11.5) years, 77% were male, the mean (SD) body mass index was 26.8 (4.6) and omega-3 index was 4.7% (1.7%). The effects of treatment with O3AEE (1 g/d) or placebo for 3 months on RBC FA composition are shown in Table. O3AEE increased the omega-3 index by 2 percentage points (a 42% increase), and levels of DPAn-3 by 30%. Treatment also lowered levels of all long chain omega-6 FAs downstream of linoleic acid including gamma-linolenic (–20%), docosapentaenoic n-6 (–17%), docosatetraenoic (–14%), and dihomo-gamma linolenic (–13%) acids (all $P < .0001$). The major long-chain omega-6 fatty acid, arachidonic acid, decreased by