Nutrition 30 (2014) 1404–1408

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Erythrocyte saturated fatty acids and systemic inflammation in adults

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ARTICLE INFO

Article history:
Received 7 August 2013
Accepted 15 April 2014

Keywords:
C-reactive protein
Interleukin-6
White blood count
Palmitic acid
Stearic acid
Erythrocyte phospholipid
Saturated fat

ABSTRACT

Objective: The role of saturated fatty acids (SFAs) in chronic disease remains controversial; inflammation is one pathway by which SFAs influence the risk for chronic disease. The aim of this study was to investigate the associations between red blood cell (RBC) phospholipid SFAs and systemic inflammation.
Methods: As part of a randomized controlled trial, we measured RBC phospholipid FA composition in 55 generally healthy adults twice at 3-mo intervals. We estimated associations of RBC total SFAs and two major SFA subtypes, palmitic and stearic acids, with C-reactive protein (CRP), interleukin (IL)-6, white blood count (WBC), and a composite inflammation measure using generalized estimating equations in multivariable FA substitution models.
Results: Mean (±SD) SFA level across both visits was 45% ± 3% of the total RBC FAs, mainly palmitic (21% ± 1%) and stearic (17% ± 3%) acids. In models adjusted for age, sex, race, smoking, body mass index, statin use, aspirin use, transunsaturated FAs, and ω-3 FAs, SFAs were significantly associated with IL-6 (20% increase per 1 SD increment; 95% confidence interval [CI], 0.03%–43%; $P = 0.05$) and the composite inflammation measure ($P = 0.05$) and marginally associated with CRP (34% increase; 95% CI, −1% to 81%; $P = 0.06$), but not associated with WBC. Stearic acid was positively associated with CRP (35% increase; 95% CI, 2%–79%; $P = 0.04$). Palmitic acid was marginally associated with the composite inflammation measure ($P = 0.06$) and, upon additional ω-6 FA adjustment, significantly associated with IL-6 (15% increase; 95% CI, 0.4%–27%; $P = 0.006$).
Conclusions: RBC SFAs, which represent longer-term dietary intake, are positively associated with inflammation. In particular, palmitic acid was associated with IL-6, and stearic acid was associated with CRP after multivariable adjustment.

Introduction

The role of saturated fatty acids (SFAs) in chronic diseases such as coronary heart disease (CHD) and cancer remains an area of ongoing controversy. For example, a 2010 meta-analysis of 16 prospective cohorts found that dietary SFA intake is not significantly associated with risk for CHD [1]. A systematic review based on the Bradford Hill guidelines also concluded that insufficient evidence relates SFA intake with CHD [2]. By contrast, a pooled analysis of 11 cohorts [3] and a meta-analysis of eight

This study was funded by the National Institutes of Health Harvard Catalyst Grant 1UL1RR025758-02 and conducted with support from Harvard Catalyst|The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award 8UL1TR000170-05 and financial contributions from Harvard University and its affiliated academic health care centers). The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, or the National Institutes of Health. All authors contributed to the conception and design of this study, data interpretation, and manuscript revision. LM conducted the data analysis and drafted the manuscript. KJM and AZN obtained funding, acquired data, and provided supervision. We acquired data of this study from a clinical trial on docosahexaenoic acid (DHA) and periodontitis, which received a donation of DHA and placebo capsules from Martek Corporation. Martek provided no other resources or funds and has no role in the conduct or analysis of this study. Martek had no role whatsoever in the current manuscript. There are no other competing interests to disclose.

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http://dx.doi.org/10.1016/j.nut.2014.04.020

randomized controlled trials [4] suggested that isocaloric replacement of SFAs with polyunsaturated FAs reduces coronary events and deaths.

Similarly, a meta-analysis found that higher SFA intake was associated with lower risk for breast cancer in 19 cohort studies but higher risk in 8 case–control studies, although neither relation was significant [5]. Similar controversies exist in ovarian [6–9], prostate [10–12], and pancreatic cancers [13–16]. In reconciling these discrepancies, the accuracy of dietary assessment instruments, particularly food frequency questionnaires, has been critiqued for contributing to the contradictory research results [17,18].

One pathway by which SFAs have been proposed to influence the risk for chronic disease is inflammation. A recent systematic review suggested that the current evidence regarding the association of SFA with inflammation remains inconclusive; however, it pointed to a potential positive relationship between SFA and C-reactive protein (CRP) [19]. In the Whitehall study of older men, plasma lipid SFA level was positively correlated with plasma CRP and fibrinogen levels [20]. Additionally, among healthy men fed controlled diets, consumption of combined lauric, myristic, and palmitic acids increased plasma CRP, fibrinogen, and interleukin (IL)-6 concentrations [21]. Surprisingly, the consumption of stearic acid alone, which appears to be non-hypercholesterolemic [22–24], also appears proinflammatory [21].

To date, few studies have related measured levels of SFAs to systemic inflammation. To evaluate the potential effect of SFAs and their two most prevalent subtypes (palmitic and stearic acids) on systemic inflammation, we assessed the associations of systemic CRP and IL-6 concentrations and white blood count (WBC) with red blood cell (RBC) phospholipid SFAs on two occasions in a cohort of generally healthy adults with periodontitis. Periodontitis is a common, chronic inflammatory disease with a global prevalence exceeding 10% in 2010 [25]; studying healthy adults with periodontitis allows us to include individuals with a broad range of inflammation levels. RBC SFAs are objective physiological measurements that reflect dietary intake within the past 3 mo and thus allow for more direct assessment of the SFA physiological role in inflammation than dietary survey methods [26].

Methods

Participants

This study analyzed data from a randomized control trial conducted at Beth Israel Deaconess Medical Center (BIDMC) in Boston, Massachusetts, that examined the effect of supplementation of docosahexaenoic acid, a marine-source ω-3 fatty acid, on adults aged ≥40 y with moderate periodontitis. Relevant exclusion criteria included pregnancy, diabetes, rheumatic diseases, cirrhosis, cancer, hemorrhagic stroke, gastrointestinal bleeding, poorly controlled hypertension, conditions requiring antibiotic prophylaxis, systemic steroids, immunomodulatory therapies, warfarin, clopidogrel, end-stage renal disease, severe caries or periodontitis, recent antibiotic or periodontal therapy or ω-3 FA use, and regular use of nonsteroidal anti-inflammatory drugs other than aspirin. A total of 560 potential participants responded to advertisements and underwent a prescreening telephone interview, and those eligible by history also underwent a screening appointment at the BIDMC Clinical Research Center within 2 wk. From June 2009 to December 2011, 55 eligible participants underwent randomization in a parallel, double-blinded design to receive either 2 g/d DHA or corn/soy oil placebo for 3 mo (Supplementary Fig. 1). All participants also received 81 mg aspirin, which prolongs the anti-inflammatory effect of DHA in vitro [27,28]. We provided participants with standard oral hygiene instructions but asked them not to undergo periodontal treatment, alter diet, or change physical activity for the duration of the study. Each participant received $100 remuneration. The study was approved by the Committee on Clinical Investigations, and all participants gave written informed consent.

Laboratory measurements

Blood specimens were collected after an overnight fast at baseline and 3-mo follow-up. Complete blood count and high-sensitivity CRP concentration were both assessed at Laboratory Corporation of America (Raritan, NJ, USA), using a Sysmex X-series analyzer (Sysmex Corporation, Kobe, Japan) and a particle-enhanced turbidimetric assay on a COBAS INTEGRA system (Roche Diagnostics, Mannheim, Germany), respectively. RBC FAs were quantified in the Department of Nutrition at Harvard School of Public Health (Boston, MA, USA), using gas-liquid chromatography on a fused silica capillary *cis–trans* column SP2560 (Supelco, Bellefonte, PA, USA) with peak retentions analyzed in Agilent Technologies ChemStation A.08.03 software. Individual RBC FA was reported as a proportion of total phospholipid FAs. Serum IL-6 concentration was measured at the Harvard Catalyst Central Laboratory (Boston, MA, USA) using access chemiluminescent immunoassay (Beckman Coulter, Fullerton, CA, USA). The intra-assay coefficients of variation for CRP, IL-6, palmitic and stearic acids were 1.3%, 1.7% to 4.6%, 5%, and 19%, respectively.

Statistical analysis

We analyzed data using the Statistical Analysis Software (version 9.3, 2011, SAS Institute Inc, Cary, NC, USA). Baseline participant characteristics across tertiles of SFA were compared using simple linear regression for trends in continuous variables or Fisher's exact test for categorical variables. RBC FA data generally followed a normal distribution, which we rescaled in units of their SDs before analysis. CRP, IL-6, and WBC data were right-skewed and log-transformed. We also created a composite measure of inflammation by averaging the standardized levels of log-transformed CRP, IL-6, and WBC.

We used generalized estimating equations (PROC GENMOD) to evaluate the association between inflammation measures and SFAs accounting for repeated measures within individual. We first adjusted for age and sex, and then additionally controlled for race, smoking (current, former, never), body mass index (BMI), statin use, aspirin use (two individuals at baseline and all at follow-up), and levels of transunsaturated FA (TFA) and ω-3 FA.

Because individual FA levels represent proportions of RBC FAs and hence sum to 1, adjustment for TFA and ω-3 FA (but not monounsaturated fatty acid [MUFA] or ω-6 FA) represents a substitution model, in which the estimate for SFA represents the effect of substituting SFAs for an equivalent amount of MUFAs and ω-6 FAs, which we considered physiologically neutral. In models where MUFA and ω-6 FA appeared to show heterogeneous associations with inflammation, we additionally adjusted for ω-6 FA. We further applied these models to examine the individual effects of palmitic and stearic acids, the two most prevalent SFA subtypes, adjusting for the modest levels of other SFAs. To maximize model convergence, we specified 1) a Poisson distribution and a log link in CRP and IL-6 models and 2) a Gaussian distribution and an identity link in models of WBC and composite inflammation. We tested for linearity with fractional polynomials and tests of heterogeneity across quartiles of SFA, palmitic acid, and stearic acid.

Results

Table 1 shows the baseline characteristics of the study participants. Except for moderate periodontitis, participants were generally healthy. Among all 55 participants across both visits, the mean (±SD) SFA level was 45% (±3%) of the total RBC FAs, which were mainly palmitic acid (21% ± 1%) and stearic acid (17% ± 3%). As expected, CRP and IL-6 concentrations were strongly correlated (Spearman $r = 0.54$; $P < 0.001$), and each was marginally correlated with WBC (CRP: $r = 0.16$, $P = 0.13$; IL-6: $r = 0.19$, $P = 0.08$).

In age- and sex-adjusted models, SFA was positively, albeit not significantly, associated with inflammation. Further adjustment for confounders strengthened this association. In multivariable models, SFA was significantly associated with IL-6 ($P = 0.05$) and the composite inflammation measure ($P = 0.05$) and marginally associated with CRP ($P = 0.06$), but not with WBC (Table 2). Among all covariates, BMI was the main confounder and most significantly associated with CRP ($P < 0.001$), conferring a 13% (95% confidence interval [CI], 7%–19%) increase in CRP concentration per 1 unit BMI increment; it was also significantly associated with the composite inflammation measure ($P = 0.03$) and marginally associated with IL-6 ($P = 0.10$). We did not find TFA or ω-3 FA to associate significantly with any inflammatory