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Meta-regression analysis of the effect of *trans* fatty acids on low-density lipoprotein cholesterol

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ABSTRACT

We conducted a meta-regression of controlled clinical trial data to investigate quantitatively the relationship between dietary intake of industrial *trans* fatty acids (iTFA) and increased low-density lipoprotein cholesterol (LDL-C). Previous regression analyses included insufficient data to determine the nature of the dose response in the low-dose region and have nonetheless assumed a linear relationship between iTFA intake and LDL-C levels. This work contributes to the previous work by 1) including additional studies examining low-dose intake (identified using an evidence mapping procedure); 2) investigating a range of curve shapes, including both linear and nonlinear models; and 3) using Bayesian meta-regression to combine results across trials. We found that, contrary to previous assumptions, the linear model does not acceptably fit the data, while the nonlinear, S-shaped Hill model fits the data well. Based on a conservative estimate of the degree of intra-individual variability in LDL-C (0.1 mmoL/L), as an estimate of a change in LDL-C that is *not* adverse, a change in iTFA intake of 2.2% of energy intake (%en) (corresponding to a *total* iTFA intake of 2.2–2.9%en) does not cause adverse effects on LDL-C. The iTFA intake associated with this change in LDL-C is substantially higher than the average iTFA intake (0.5%en).

1. Introduction

In November 2013, the U.S. Food and Drug Administration (FDA) published a tentative notice indicating their intention to remove the “generally recognized as safe” status for partially hydrogenated oils (PHO); this notice was finalized in June 2015 (FDA, 2013, 2015). This action means that use of any PHO in food manufacturing is now subject to FDA approval (FDA, 2013, 2015). The decision was based on the FDA's determination that there is a lack of consensus among scientific experts regarding the safety of *trans* fatty acids (TFA). There are two sources of TFA in the diet: those that are industrially produced (iTFA), primarily coming from PHO (Tarrago-Trani et al., 2006), and those that are naturally occurring in ruminant animal products (rTFA). Low levels of iTFA may also be found in nonhydrogenated refined oils (e.g., soybean oil), and in fully hydrogenated oils (concentrations below 2%), due to incomplete hydrogenation (FDA, 2013). The FDA referenced evidence suggesting that there is a significant dose-dependent linear relationship between iTFA intake from PHO and low-density lipoprotein cholesterol (LDL-C), a validated surrogate marker of coronary heart disease (CHD) risk (Institute of Medicine, 2002/2005).

The Institute of Medicine (2002/2005) report did not recommend setting an upper limit for total dietary TFA because increased risk would be expected to occur at low intake levels, which would require extreme changes in dietary intakes that could result in unintended health consequences. Therefore, the panel

Abbreviations: %en, percent energy; ΔLDL, change in low-density lipoprotein cholesterol; CHD, coronary heart disease; CI, credible interval; CVD, cardiovascular disease; FDA, Food and Drug Administration; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; iTFA, industrial *trans* fatty acids; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; MCMC, Markov chain Monte Carlo; MOA, mode of action; MUFA, monounsaturated fatty acids; PHO, partially hydrogenated oils; PUFA, polyunsaturated fatty acids; RCT, randomized controlled trial; rTFA, ruminant *trans* fatty acids; SFA, saturated fatty acids; TALC, iTFA-associated LDL change; TFA, *trans* fatty acids; WAIC, Watanabe-Akaike information criterion.

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recommended that individuals keep intake as low as possible while still consuming a nutritionally adequate diet.

Several regression studies have been conducted on the effects of iTFA on putative biomarkers of CHD, including lipid levels such as LDL-C, high-density lipoprotein cholesterol (HDL-C), and the ratio of low-density lipoprotein (LDL) to high-density lipoprotein (HDL) (Ascherio et al., 1999; Brouwer et al., 2010; Mensink et al., 2003; Mozaffarian and Clarke, 2009; Trumbo and Shimakawa, 2011; WHO, 2016).[2] However, each of these analyses assumed and applied a linear dose-response relationship and did not investigate the possibility of a nonlinear response. Brouwer et al. (2010) noted that logarithmic models were tested to determine whether the assumption of a linear dose response was appropriate, but they did not show any results. Moreover, these analyses measured the change in intake from a selected control group, but they did not account for the variability in actual intake among controls and resultant inter-study variance.

To further investigate the impact of iTFA, the current work evaluated the quantitative relationship between iTFA intake and LDL-C in controlled clinical trials. We focused on an early biomarker of CHD, rather than CHD, due to the complex and multi-factorial nature of CHD. We further chose to focus on LDL-C because the study was designed after the release of FDA's (2013) tentative notice (which focused largely on changes in LDL-C), and was largely completed prior to release of the final FDA (2015) notice. In addition, LDL-C is an accepted biomarker by regulatory agencies, while other lipid markers are not at this time validated for regulatory decision-making on health outcomes. It is noted, however, that the determination of appropriate lipoprotein biomarkers is an area of active scientific discussion, with lipids outcomes such as HDL-C and non-HDL-C under consideration as emerging markers. For example, in other recent reviews of the health effects of iTFAs, the primary outcome was on the ratios of total cholesterol(TC): LDL-C or LDL-C to HDL-C (high density lipoprotein cholesterol) (Brouwer et al., 2010, 2013; Gayet-Boyer et al., 2014). In addition, most of the prior regression studies evaluated one or more of the ratios TC:LDL-C or LDL-C to HDL-C (Ascherio et al., 1999; Brouwer et al., 2010; Mensink et al., 2003; Mozaffarian and Clarke, 2009; WHO , 2016), although it is of note that the regression conducted by FDA scientists (Trumbo and Shimakawa, 2011) focused on LDL-C. As addressed further in the discussion section, it is recognized that cardiovascular disease (CVD) is a complex disease, and likely best predicted based on multiple markers, although the specific best markers are an area of active scientific debate. However, due to the complexity of the modeling, an important first step is modeling one marker.

Although there is a large body of literature that assesses the relationship between iTFA intake and CHD or markers of CHD, data in the low-exposure region are limited (Liska et al., 2016). To address the question of the shape of the dose-response relationship between iTFA and changes in LDL-C, we used a two-pronged approach. First, we evaluated the mode of action (MOA) of the effect of iTFA on LDL-C (Reichard and Haber, 2016) in order to provide a biological basis for our understanding of the expected shape of the dose-response curve in the low-iTFA intake region. Second, we conducted a meta-regression analysis to assess the shape of the dose response, as described in this report.

[2] A meta-regression for the effect of rTFA on the putative cardiovascular risk markers (total cholesterol:HDL-C and LDL-C:HDL-C) was published in the latter stages of the preparation of this report (Gayet-Boyer et al., 2014). The authors reported that they found no relationship between rTFA intake at doses up to 4.19%en and any of the markers examined. In addition, no effect with rTFA dose was found in a multivariate regression analysis that included other dietary variables and subject baseline characteristics.

The analysis described in this paper builds on and enhances the previous analyses (Ascherio et al., 1999; Mensink et al., 2003; Mozaffarian and Clarke, 2009; Brouwer et al., 2010; Trumbo and Shimakawa, 2011; WHO , 2016) in several ways. First, an extensive literature search and evidence map was conducted to ensure that the relevant literature was captured (Liska et al., 2016), resulting in the inclusion of additional studies and information, particularly in the low-dose region of the dose response. Second, a meta-regression, rather than a simple regression, was used to analyze the data and improve the accuracy of the description of the dose-response relationship by incorporating measures of intra- and inter-study variance. Meta-regression is a weighted regression technique for combining the "dose" and response information across studies. The included studies are weighted to reflect the variance associated with each response measure; studies with less uncertainty (i.e., smaller variance) are given more weight. In this manner, meta-regression can reduce the skewing effects of random error and small sample sizes within individual studies (Collins et al., 1997; Sutton, 2000), producing greater accuracy in a combined estimate of the relationship between exposure and response. The meta-regression analysis evaluated the relationship between iTFA intake, measured as a percentage of energy intake (%en), and LDL-C, using blinded randomized controlled trials (RCT) with a cross-over study design that compared iTFA-based diets to diets with other lipid profiles.

Finally, the current work makes fewer assumptions regarding the shape of the dose-response curve (e.g., it was not assumed to be linear), a determination that can have significant risk assessment implications. Although meta-regression models typically assume a linear relationship between dose and response, there is nothing inherent to the method that says the model must be linear, and it is possible to use nonlinear dose-response models when biologically appropriate (Bagnardi et al., 2004; Rota et al., 2010).

Specifically, the current work explores both linear and nonlinear models in order to determine which is more consistent with the data. This was particularly important because our current understanding of the MOA of the effect of iTFA on LDL-C supports the plausibility of a nonlinear dose response (Reichard and Haber, 2016).

2. Methods

2.1. Search strategy

Liska et al. developed a systematic evidence map following the Institute of Medicine Standards for Systematic Reviews and using Medline and Scopus databases (through January 2014) to determine whether there are sufficient data to perform regression analysis on the effect of iTFA on LDL-C at 0–3%en (Liska et al., 2016). Additional search strategy details and results are presented in the manuscript by Liska et al. (2016). Of 123 RCTs identified, 102 reported iTFA intake. Of these 102 studies, 81 were determined to be potentially useful (based on the reporting of iTFA) and were reviewed in depth. We additionally conducted a backwards literature search on each of the previous regression analyses (Ascherio et al., 1999; Mensink et al., 2003; Mozaffarian and Clarke, 2009; Brouwer et al., 2010; Trumbo and Shimakawa, 2011) to ensure that no key studies were missed. Each study from the evidence map and literature search was reviewed to determine whether it met the following inclusion criteria: 1) it was a blinded RCT with a cross-over study design that utilized a controlled diet design (i.e., all food and beverages consumed during the treatment periods were provided to the participants), 2) it measured iTFA content as % en (or provided sufficient data for calculating iTFA as %en), 3) it compared iTFA only to other lipids (i.e., excluding carbohydrate-

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