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07 March 2014

Mical Honigfort
Center for Food Safety and Applied Nutrition (HFS-265)
Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD, 20740

RE: Comments on Docket No. FDA–2013–N–1317 -- Tentative Determination Regarding Partially Hydrogenated Oils; Request for Comments and Scientific Data and Information

Dear Ms. Honigfort,

The International Life Sciences Institute (ILSI), North American branch welcomes the opportunity to provide comments in response to Docket No. FDA-2013-N-1317, announcing the Food and Drug Administration's (FDA) tentative determination that partially hydrogenated oils (PHO) are no longer generally recognized as safe (GRAS) under any condition of use in food.

ILSI North America is a public, non-profit organization that actively collaborates with government and academia to identify and resolve scientific issues important to the health of the public. The organization carries out its mission by sponsoring relevant research programs, professional education programs and workshops, seminars and publications, as well as providing a neutral forum for government, academic, and industry scientists to discuss and resolve scientific issues of common concern for the well-being of the general public. ILSI North America's programs are supported primarily by its industry membership.

Attached you will find comments addressing questions requested by the FDA in the docket; specifically, we address the following:

1. Should FDA finalize its tentative determination that PHOs are no longer GRAS?
2. Are there data to support other possible approaches to addressing the use of PHOs in food, such as by setting a specification for *trans* fat levels in food?

The first section includes a report from an evidence mapping and dose-response modeling feasibility analysis that ILSI North America conducted with Biofortis Clinical Research following the publication of the tentative determination. This project is phase I of a two-phased scope of work aimed at better delineating the risk of PHO intake on risk for coronary heart disease. The details of this project are outlined in Section One of the comments and Appendix A, which immediately follows Section One, contains the report.

Section Two provides considerations for updating a replacement scenario modeling study, previously conducted by ILSI North America using 1999-2003 data. Given the changes in the food supply that have occurred since the 2003 FDA ruling on labeling, an update to this data would prove valuable in determining a path forward.

We appreciate the opportunity to provide comments to this docket and thank you for considering the scientific data that we have presented in these comments.

Sincerely,

Eric Hentges
Executive Director
ILSI North America

ILSI North America

Section One:
Evidence Mapping and Feasibility Analysis of Dose-Response Modeling

This section and the report that follows, address questions requested by the FDA in the docket; specifically, we are addressing the following:

1. Should FDA finalize its tentative determination that partially hydrogenated oils (PHO) are no longer Generally Recognized As Safe (GRAS)?
2. Are there data to support other possible approaches to addressing the use of PHOs in food, such as by setting a specification for *trans* fat levels in food?

Upon review of the 08 November 2013 notice, ILSI North America concluded that there are research questions that require further consideration, and alternative approaches to safety assessment potentially employed, before definitive action can comfortably be taken. In the 120 days since the notice, we have been able to address some of these (Appendix A), and in these comments we propose our phased research plan to complete this work. However, first we would like to make note of a published modeling study, conducted by ILSI North America using National Health and Nutrition Examination Survey data from 1999-2002.[1] While not the primary focus of these comments, we refer you to Appendix B (Considerations for Updating Replacement Scenario Modeling) for additional details on this project and a rationale for why we feel that this exercise should be updated to reflect changes that have taken place in the food supply since the FDA's labeling ruling in 2003.

For the purpose of these comments, we feel that these questions below are those for which answers will provide the critical data for determining the best regulatory course of action to ensure a safe food supply in a manner that is grounded in the best evidence available.

In a two-phased scope of work, we have addressed or plan to address the following:

1. Is the relationship between low levels of PHO intake and risk of coronary heart disease (CHD) progressive and linear?
2. Are there adequate data at low levels of exposure to a) use linear regression and b) to assume that risk begins at zero (as cited by Ascherio et al.[2] in the IOM report[3] and performed by Brouwer et al.[4])?
3. Is it feasible to identify models for a dose-response analysis? And, could this approach be used to investigate a threshold below which PHO intake does not adversely influence risk of CHD?

At the completion of this work, we aim to better delineate the risk of PHO intake on LDL-C and HDL-C at various exposures. We pragmatically approached this goal by separating the work into two phases, allowing for initial results (Phase I: Evidence Map) to be available to meet the 08 March 2014 deadline, and a perspective to be provided on the feasibility and/or necessity of carrying out Phase II (Dose-Response Modeling).

Phase I is an evidence map with a summary report of the totality of published human literature in English (intervention trials, cohorts and case studies) on PHO and cholesterol outcomes. The methodology and results of Phase I are provided in the attached detailed report (Appendix A). For summation purposes, some of the report findings are highlighted below:

- Despite a generous volume of literature assessing industrial *trans* fatty acid (TFA) exposure and health outcomes, limited data are available regarding low exposures. With current mean intakes of industrial TFA at ~0.5% energy, the impact of actual exposure on risk for CHD is critical in evaluating the safety of PHO in the food supply.
- There were 123 studies identified that used industrially produced TFA, with 102 reporting the amount of TFA consumed. Of these, 58 reported TFA intake as % total energy. Assessing amounts and sources of TFA, and adjusting for duplicate publications in these reports, resulted in 30 studies with non-hydrogenated oils or PHOs having an intervention group at <3% energy intake. This low number of studies is representative of the fact that the majority of the <3% energy interventions were control groups (not intervention arms), and that the source of TFA in studies with <1.5% energy were mainly non-hydrogenated oils.
- Only two studies were found in the PHO exposure range of >2.5 - ≤3% energy but these were not designed as dose response studies, but as comparisons to other oils.
- The TFA sources and dietary approaches across the studies were quite variable; therefore, combining the data from multiple studies was not warranted. Significant complexities exist in the available data that make cross-comparison difficult and therefore linear regression challenging: no dose-response data at 2.5 -<3% energy, use of different comparators (MUFA, PUFA and SFA), variable study duration, and confounding variables related to background diet and population disease state.

In summary, from the evidence mapping exercise, results indicate that:

- The quantity and comparability of data at low exposures of industrial TFA is not sufficient for linear regression to be used to predict a dose-response relationship at low exposure levels. Therefore, linear regression cannot be considered an appropriate approach to define the relationship of lipid biomarkers and exposure to PHO down to a level of zero in the diet.
- Given the quantity and type of data that are available, the use of dose-response models is a more appropriate approach to determine the safety of PHO with regard to LDL and HDL cholesterol.

Upon completion of the evidence map, we began to look at the data in terms of its utility for modeling. To do so, experts in the risk assessment field evaluated the data and concluded that given the quantity and type of data that are available, the use of dose-response models is a more appropriate approach to determine the relationship of PHO with regard to LDL and HDL cholesterol and this type of modeling would be necessary to determine a true safe level of PHO exposure. The following points provide the rationale for moving forward with Phase II of this project:

- The available data are extensive, and while problematic in several regards, particularly at low exposures, these data are sufficient for modeling purposes. Of note is that other federal agencies, such as the US Environmental Protection Agency routinely use models for projecting low dose effects, or safe doses, with similar, and even less data.
- A better understanding of several of the Modes of Action (MOAs) for CHD, or at least for a change in LDL due to PHO, is needed before determining whether a threshold for an increase in LDL-C is likely, or not, in the dose scale for industrially-produced TFA. Modeling available data would assist in this determination, and may provide the basis

for a projection of risk at all points in the dose scale, rather than default to a linear, or some other, empirical function.

- In the docket, it is suggested that TFA-mediated changes in lipid metabolism, pro-inflammatory effects, and endothelial dysfunction might lead to dose-dependent increases in CHD in humans. It is important to note that many other factors, such as other chemicals, microorganisms, changes in diet, exercise regimens, nutritional status and obesity can all impact CHD as well. Modeling the available data would assist in determining those factors most likely to be associated with various types of CHD. This evaluation will help with future research focusing on these factors as well.
- A brief look at the work of Brouwer et al. (2010) suggests a linear trend for industrial TFA, but the data for ruminant TFA are decidedly not linear, and in fact might be hormetic. An explanation for this difference can best be approached through an understanding of MOA, for which models are a useful supporting tool.

In Summary

Data from the evidence mapping suggest that that linear regression may not be the most appropriate method to determine the safety of PHO exposure. The second critical finding from this project is the determination that moving forward with dose-response modeling is not only feasible, but necessary, to determine a true safe level of PHO exposure. The available data are extremely complex, particularly at the low exposures where there is a significant degree of uncertainty. Given these findings, ILSI North America will proceed with Phase II, the dose-response assessment, to continue toward our aim of better understanding and more fully delineating the risk of PHO intake on LDL-C and HDL-C at various exposures.

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2. Ascherio A, Katan MB, Zock PL, Stampfer MJ, Willett WC. Trans fatty acids and coronary heart disease. *The New England journal of medicine.* Jun 24 1999;340(25):1994-1998.

3. Institute of Medicine (U.S.). Panel on Macronutrients., Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids.* Washington, D.C.: National Academies Press; 2005.

4. Brouwer IA, Wanders AJ, Katan MB. Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans--a quantitative review. *PloS one.* 2010;5(3):e9434.

APPENDIX A: Study Report

Evidence Mapping & Summary Tables

of the Industrial Trans Fatty Acids Literature

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Evidence Mapping and Study Tables of the Industrial Trans Fatty Acid Literature

Study Director: DeAnn Liska, PhD[1]
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Study Number: C-128

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1 Executive Summary

The objective of this study was to develop an evidence map to better characterize the existing literature regarding intervention trials on industrially-produced *trans* fatty acid (IP-TFA). In particular, this study focused on literature that assessed IP-TFA-containing oils as a substitute for other oils (e.g., *cis*-monounsaturated fatty acid [MUFA] or saturated fatty acid [SFA]) on changes in low-density lipoprotein (LDL)-cholesterol or high-density lipoprotein (HDL)-cholesterol. The main question addressed was: *Are there sufficient data to perform linear regression analysis on the effect of IP-TFA at 0 to 3% of energy intakes (%en) on LDL-cholesterol?*

A systematic evidence mapping process was implemented following IOM's Standards for Systematic Reviews and using Medline® (1946 to January 2014) and Scopus® databases. Database searches were conducted in January 2014 and for studies published in English language with human subjects that included oil-to-oil comparisons with IP-TFA and a measurement of LDL-cholesterol or HDL-cholesterol. One hundred twenty-three randomized controlled trials (RCTs) were identified by this strategy. Of these, 102 RCTs reported the amount of TFA, with 58 studies reporting TFA as %en and 41 containing at least one group in either intervention or control with ≤3%en TFA. After adjusting for duplicate publications of trials, 30 studies were finally identified with ≤3%en IP-TFA and an oil-to-oil comparison.

A total of 84 IP-TFA intervention arms were identified within the 30 studies, with the majority either ≤1%en (56% of the studies) or >3.0%en (35% of studies) from IP-TFA. The majority of the interventions at ≤1%en IP-TFA were control groups using a non-hydrogenated oil. Only 7 intervention groups were noted between 1.0 %en and ≤2.5%en IP-TFA, and no studies included an intervention in the >2.5 to 3.0 %en IP-TFA range. Further identification of the partically hydrogenated oil (PHO) interventions indicated the majority (30 interventions, 70%) were at >3%en intakes, with 5 at or below 0.5%en, and 8 interventions between 0.5%en and 3%en.

Many of the studies were not designed to assess a dose-response and contained only one intervention with IP-TFA most often as a control. Therefore, studies were reviewed for those that contained at least 3 interventions with different quantified levels of IP-TFA, in which 2 of

those utilized an IP-TFA at ≤3%en. Eleven studies met these criteria, however, the TFA sources and dietary approaches were quite variable. Therefore, combining these studies for the purpose of assessing linearity was not warranted.

It is surprising that no interventions were noted for either IP-TFA or, more specifically PHO, between the amounts of 2.5%en and 3%en intakes, and only 2 were found at >2%en and ≤2.5%en. In addition, many of the studies we identified were not designed to assess a dose-response for IP-TFA or PHO in the low intake ranges. Therefore, our overall findings indicate few dose-response data points exist at low levels of PHO exposure, with many of these confounded by mixtures of oils. Given the data that are available, a linear regression is not supported.

2 Background and Rationale

On November 8, 2013, the Food and Drug Administration (FDA) announced their tentative determination to revoke the Generally Recognized As Safe (GRAS) status of partially hydrogenated oils (PHO). This announcement (referred to as FDA PHO Notice in this report) was based on data relating to industrially produced *trans* fatty acids (IP-TFAs). The scientific basis comes from a series of expert panels, including the Institute of Medicine's 2002/2005 report on Dietary Recommended Intakes for fats (IOM 2005), as well as from a search of the literature since the 2003 publication of the FDA's final rule on the required declaration of TFAs on food and supplement labels.

The data that were considered by the IOM committee included studies published up through 2002. The committee did not establish an Adequate Intake (AI) or Recommended Daily Intake (RDI) for TFA because there is no known physiological need for TFA, although these fats can be used for energy production. In addition, no Upper Level (UL) was set due to a lack of clinical trial data and because the committee concluded "*any* incremental increase in TFA increases CHD risk."[1]

The IOM committee based their conclusion of any increase in TFA intake leading to CHD risk primarily on the report of Ascherio et al. (1999), which noted a linear relationship between increases in TFA intake and increases in the LDL-to-HDL-cholesterol ratio. The Ascherio study determined that an increase in TFA at 2%en would raise the LDL-to-HDL cholesterol ratio by 0.1.

In 2010, Brouwer et al. published a review of the literature and performed an updated and more expansive linear regression analysis to determine the relationship between TFA intake and the LDL-to-HDL-cholesterol ratio. Their results were similar to those of Ascherio et al. (1999), although these authors concluded an increase of 1%en from IP-TFA from replacement of cis-MUFAs would increase-the LDL-to HDL-cholesterol ratio by 0.055. It should be noted, however, that only a few data points for IP-TFA below 3%en were included in these assessments, with the majority of data occurring at >4%en.

Current mean intakes of IP-TFA are noted in the FDA document as around 1 g/person/day, or 0.5%en based on a 2000 kcal daily intake. In addition, the data included in the Ascherio et al. (1999) and Brouwer et al. (2010) reports do not show statistically significant changes in LDL-cholesterol until intakes of IP-TFA reach ~4%en. Moreover, statistically significant effects on HDL-cholesterol have not been demonstrated until 5%en to 6%en (Hunter, 2006). One of the issues with the conclusions of these reports is that so few data points have been included at intakes below 3%en in the analyses. Therefore, the conclusions on the impact of IP-TFA at lower level of intakes are extrapolated from much higher level intakes than current estimates for average daily consumption of IP-TFA.

This study was developed to evaluate how much data has been generated and published on the effect of IP-TFA consumption on LDL-cholesterol and/or HDL-cholesterol. Of particular

[1] CHD, Coronary Heart Disease

interest is whether enough data exists to document a linear relationship between LDL-cholesterol and IP-TFA ≤3%en. Findings are anticipated to be used with government agencies for identifying gaps in knowledge in setting recommendation levels for foods and food ingredients.

3 Objective

The objective of this study was to develop an evidence map to better characterize the existing literature regarding intervention trials on IP-TFA intake as a substitute for *cis*-MUFAs or saturated fatty acids (SFA) on changes in LDL-cholesterol or HDL-cholesterol. Specifically, this study was designed to address the question: *Are there sufficient data to perform linear regression analysis on the effect of IP-TFA on LDL-cholesterol at intakes between 0 and 3%en?*

4 Methods & Results

4.1 Definitions and Categorizations

Industrially-produced TFAs were categorized essentially as defined in the FDA PHO Notice:

- Non-hydrogenated refined oils
- Partially hydrogenated oils (PHOs
- Fully hydrogenated oils
- Naturally-occurring *trans* fats

4.2 Evidence Base Methodology

The methods for conducting the evidence base assessment essentially followed those outlined in the IOM's Standards for Systematic Reviews (Moher et al, 2009). The study results are reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (Moher et al., 2009). Key questions and search terms were developed and reviewed by the Biofortis scientists with guidance from the ILSI Lead. The ILSI sponsoring technical expert panel reviewed and helped refine the search terminology for expansive inclusion of all fats that may contain TFA, and assessed the literature search strategy. The ILSI panel was also consulted for comment upon the inclusion/exclusion criteria to help identify important unaccounted for variables, and appraise parameters for the review of evidence. During the review process we consulted the ILSI Lead regarding technical details (e.g., refinements of quality appraisal items and study eligibility criteria). The ILSI members did not participate in our research meetings, or review or synthesize the evidence except as described in the following sections.

4.3 Data Sources and Searches

Literature searches were conducted in Medline® (1946 to January 2014) and Scopus® databases. All studies published in English language with human subjects were screened to identify articles relevant to our inclusion/exclusion criteria. The search strategy employed the National Library of Medicine's (NIH) Medical Subject Headings (MeSH) keyword nomenclature developed for Medline®. The full search strategy is described in **Section 5**. The search strategy combined MeSH or search terms for TFAs and oils with MeSH or search terms for dyslipidemia and cholesterol-related terms (**Section 5.1**). Reference lists of selected reviews were also screened -- primarily those of Bouwer et al. (2010), Ascherio et al. (1999), and IOM (2005) -- for additional

publications. Unpublished studies, clinical trial databases and articles in the grey literature were not searched; therefore the strategy did not include assessment for unpublished data.

4.4 Study Selection and Eligibility Criteria

All abstracts identified through the literature search were screened based on eligibility criteria with a low threshold to exclude irrelevant abstracts, such as animal or *in-vitro* studies, as well as studies that did not investigate diet and disease associations. A web-based citation screening tool, Abstrakr™ (http://abstrackr.cebm.brown.edu/), was used to facilitate the abstract screening process. To assure quality and consistency of abstract review, the first 10% (N=250) of abstracts were reviewed independently by two investigators. Results were compared and indicated a >90% agreement (243/250; 97.2%); therefore, abstract review was continued with one reviewer only.

Articles that were excluded were documented with a rationale for exclusion based on prospective categorization for exclusions. Articles that were not excluded by the initial low threshold search were identified as definitely included, "yes," or marked "maybe." The "maybe" articles were reviewed by a second independent scientist from the study team. The ILSI expert panel was also consulted as secondary reviewers for "maybe" articles. Full-text articles were retrieved for studies that could not be resolved based on the title, keywords, and abstract search process.

Studies that identified industrially-produced fats and oils that might contain TFA and/or identified as having a PHO or clearly noted inclusion of an industrially-produced oil were selected for review against the inclusion/exclusion criteria. Inclusion/exclusion criteria were designed to identify studies that could lead to a dose response (multiple arms with IP-TFA) and/or a comparison against MUFA or SFA interventions. Inclusion criteria included clinical trials, cohorts, and case reports of an IP-TFA or TFA-containing oil against an IP-TFA or a predominantly MUFA or SFA intervention. Studies that compared a single intervention with a TFA containing oil to a carbohydrate replacement, a predominantly PUFA oil, or naturally occurring TFA only were excluded.

- Inclusion Criteria:
    - Human clinical trial, cohort, or case report
    - Published in English
    - Studies that evaluated dietary intake of an oil with TFA (e.g., PHO, vegetable oil, margarine) or isolated TFA
    - Included an outcome measurement for total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and/or high-density lipoprotein cholesterol (HDL-C)
    - Compared the TFA/industrially-produced oil to another oil or fat (e.g., cis-MUFA, SFA or other solid fat)

- Exclusion Criteria
    - Studies published in a language other than English
    - *In-vitro* studies, addresses, bibliographies, interviews, lectures, comments, dictionary entries, editorials, or guidelines

- Reviews, meta-analyses, systematic reviews
- No intervention or TFA containing oil compared to a non-oil treatment (e.g., carbohydrates)
- No measurement for TC, LDL-C, or HDL-C

Studies that provided fats intravenously as well as studies using fat as a vehicle for evaluating the effects of other components such as phytosterols, medium-chain triglycerides, or peptides were excluded. During the search, terms for natural TFAs (e.g., conjugated linoleic acid) were included; however, if the study did not include an oil-to-oil comparison as noted above, it was identified for a bibliography only output. Likewise, bibliographies were developed for studies comparing TFA to PUFA if they did not meet the criteria above, as well as cross-sectional studies, and meta-analyses and systematic reviews that were identified via the search strategy.

4.5 Data Extraction and Evidence Table Generation

Data were extracted using a standardized form that included study design, subject population characteristics, intervention and control oils on Evidence Map 1 (TFA Evidence Map May_2.2014_Final.xls). Each study was extracted by one investigator, and reviewed and confirmed by one other investigator. Any disagreements were resolved by discussion amongst the team members and review of the full-text articles. Duplicate studies were not resolved at this level of evidence review. IP-TFA interventions were categorized per identification as a PHO, a non-hydrogenated oil, or TFA general based on the amount of information directly identified in the study (**Section 5.2**).

Studies were then selected based on having at least one IP-TFA oil at ≤3%en and are reported in the tables below for those with a confirmed and identified PHO and those with a non-hydrogenated or unconfirmed PHO (**Section 6.2**). Duplicated publications of trials were identified from the search based on subject characteristics and interventions and are noted in the Tables with only one entry per dataset. Those studies identified as having more than one IP-TFA intervention ≤3%en, and therefore a candidate for being considered as a dose-response study, were further extracted in **Section 7**.

4.6 Results and Summary

123 studies were identified that used IP-TFA. In the screening and initial mapping, studies were categorized as containing a PHO or other TFA based on the coding shown in **Section 5.2**; using this approach, 51 studies were identified as having a PHO. During this process, it was noted that some studies appeared to include oils or components that would be considered a hydrogenated TFA, but did not directly indicate in the abstract or title as such. In addition, many of the studies used oils with low levels of TFA as controls or mixtures of oil types, which made categorization based on the defined main intervention group(s) difficult. Therefore, a further search of full-text articles was employed to include as many studies that potentially assessed a PHO intervention arm or an oil from a hydrogenation process, and the studies themselves were not categorized as a PHO or non-PHO study, but further assessed based on amount of IP-TFA. A total of 342 full-text articles were reviewed for categorization. As noted above, studies with ruminant TFA (r-TFA) were not included, although those that contained an IP-TFA comparison

group were included. The results of the search and selection process are provided in the PRISMA diagram (**Section 5.3**).

Bibliographies that were collected are noted below. The bibliographies were not reviewed past the abstract screening process, with the exception of the Review and Meta-analysis bibliography, which was reviewed against studies referenced in the FDA TFA Notices (notations in the bibliography. The Reviews and Meta-analysis bibliography contains many generally reviews and commentaries as well as systematic reviews and meta-analyses.

Bibliography A: Industrially-produced oils/ TFA compared to predominantly PUFA
(ILSI TFA v PUFA_2.2014.docx)
169 publications

Bibliography B: Studies on naturally occurring TFA
(ILSI TFA natural comparisons_2.2014.docx)
124 publications

Bibliography C: Industrially-produced oils/TFA Cross-sectional studies
(ILSI TFA Cross-sectional_2.2014.docx)
27 publications

Bibliography D: Reviews and Meta-analyses
(ILSI TFA Reviews.docx)
103 publications

Seven case-control and 8 cohort studies were identified and are summarized in **Section 8.**

A primary purpose of this evidence mapping project was to identify the studies that could be used to determine the relationship of PHO to LDL-C, therefore, quantification of the PHO intake was important. Of the 123 studies initially identified, 102 reported the amount of IP-TFA, whereas 21 did not. Most studies reported the amount of IP-TFA as %en (58 studies); however, a number of studies reported intake in grams per day (7 studies) or in other units (**Section 6.1**). Because the data included in the relevant reviews and by FDA used %en intakes, the studies reporting %en were selected for further assessment.

Studies were then reviewed for those that contained an intervention or control group with ≤3%en as IP-TFA. Of the 58 studies reporting TFA as %en, 41 contained at least one group in either intervention or control with ≤3%en IP-TFA. In addition, many studies were duplicates and/or sub-reports of other studies, and these were categorized together for evidence mapping purposes. Adjusting for duplicate publications resulted in 30 studies with ≤3%en IP-TFA. These studies are diagrammed in **Section 6.2**.

The individual intervention arms were then extracted to identify the types of IP-TFA in the studies and number of interventions with IP-TFA<3%en. A total of 84 intervention groups were extracted and are shown in **Section 6.3** (r-TFA or diets primarily including sources of r-TFA were not included). These interventions included non-hydrogenated as well as PHO sources, and also included mixtures of oils and dietary components. As shown in **Section 6.4,** the majority of

studies used either <1%en (56% of the interventions) or >3.0%en from IP-TFA (35% of interventions). Only 7 intervention groups were noted between 1.0%en and ≤2.5%en, and no interventions were included in the >2.5% to 3.0%en IP-TFA.

As can also be seen in the table in **Section 6.3**, a wide variety of oils, IP-TFA sources, and dietary approaches are represented in the different studies. Identifying those using margarines, shortening, or defined hydrogenated process (e.g., PHOs) yielded 43 interventions, with the majority (30 interventions, 70%) >3%en. Five interventions were identified at or below 0.5%en, and 8 interventions with PHO were found between 0.5%en and 3%en. Surprisingly, no interventions were noted for either IP-TFA or PHO specifically between the amounts of 2.5%en and 3%en intakes, and only 2 were found at >2%en and ≤2.5%en.

It is noteworthy that many of the studies were not designed to assess a dose-response for IP-TFA in the low intake ranges. Studies were reviewed for those that could be considered as a dose response study. A potential dose-response study was defined as a study that included investigation of at least 3 arms with different quantified levels of IP-TFA, with 2 of those 3 occurring at or below 3%en TFA. For this purpose, if a study included a 0%en TFA group, it was considered as a quantified level of IP-TFA. Sources of r-TFA were not included. Eleven studies met these criteria and **Section 9** shows data from 4 of these studies. As noted in these examples, the interventions are quite variable in the oils and background dietary approaches. Therefore, combining these studies for the purpose of assessing linearity was not warranted.

Our overall findings indicate few dose-response data points exist at low levels of PHO exposure, with many of these confounded by mixtures of oils. Given the data that are available, a linear regression is not supported.

4.7 References:

FDA Docket No. FDA-2013-N-1317. Tentative determination regarding partially hydrogenated oils; Request for comments and for scientific data and information. Fed. Reg. 2013; 78 (217): 67169-67175.

Institute of Medicine (U.S.). Panel on Macronutrients., Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids.* Washington, D.C.: National Academies Press; 2005.

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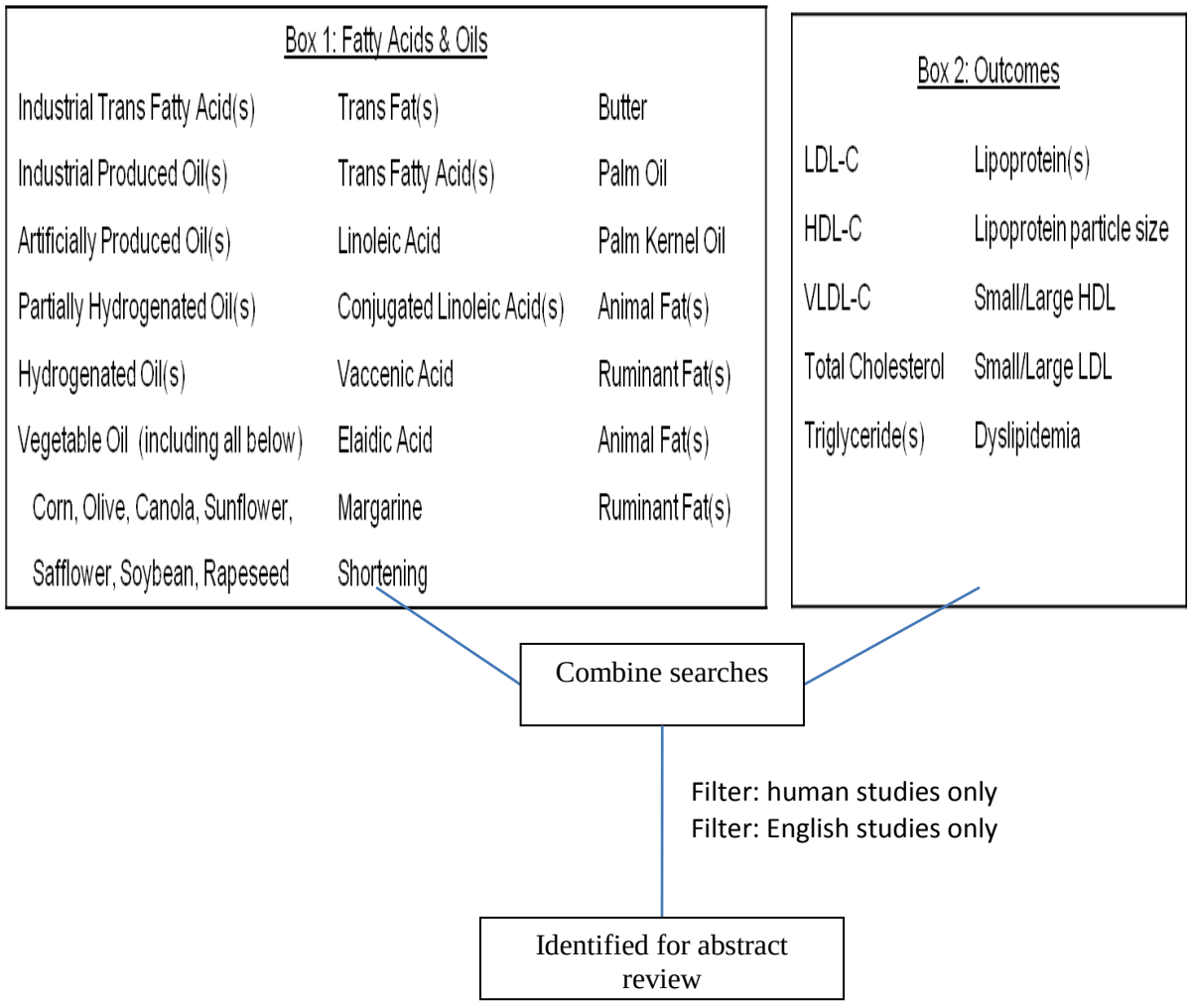
5 Search Strategy & Results

5.1 Search Strategy

- Database search: Medline & Scopus
- Search on Fatty acids & Oils terms (Box 1)
- Search on Outcomes terms (Box 2)
- Combine 1&2 to obtain output of articles with both Fatty acids & Outcomes
- Filters output for:
    - Human studies only
    - English language only



5.2 TFA Coding Categories

TFA Codings

- Partially Hydrogenated Oils (PHO)
    - Hydrogenated oil, Partially hydrogenated oil (soybean oil, sunflower oil)
    - Lightly hydrogenated soybean oil
    - PMS Blends 1 & 2 (poly/mono/sat fat veg oils partially hydrogenated
    - PHFO, partially hydrogenated fish oil,
    - Shortening
    - Margarine, Hard margarine, Squeeze margarine, Soft margarine, Tub margarine, Semiliquid margarine, Dietetic margarine, Corn oil margarine, TFA-margarine, TFA-enriched margarine, Trans-margarine, Low sat fat FA margarine
    - Soybean oil-based traditional stick margarine
    - Oil + margarine
    - Sunflower-enriched margarine
    - Low erucic rape seed oil margarine
    - Margarine with PUFA, Fish oil-enriched margarine
    - Palm margarine, High palm margarine
    - McGregor's margarine
    - SAFA margarine, high-SAFA margarine

- Industrial non-PHO
    - Corn oil
    - Canola oil/Rapeseed oil
    - Sunflower oil, Sunflower seed oil
    - Safflower oil
    - Soybean
    - Vegetable oil
    - High-oleic soybean oil, High-oleic acid with soybean oil
    - Gold'n canola
    - High oleic sunflower oil

- TFA general
    - Trans fats no specified, Trans fatty acids not specified
    - High TFA, Total TFA, Moderate TFA
    - Industrial TFA, TFA from industrially produced sources
    - Elaidic acid, Elaidic acid fat blends
    - Olive oil (type unknown)
    - Palm oil (type unknown)
    - Palm kernel oil (type unknown)
    - Coconut oil (type unknown)
    - Canola, corn, olive & rice bran oil
    - Olive oil + sunflower oil
    - High oleic, low trans diet
    - Canola + TFA
    - Trans MUFA, MUFA (with TFA amt documented)
    - Trans 18:1
    - High trans fat diet, TFA-enriched diet, High linoleic diet (with TFA amt documented)
    - High trans soybean oil
    - 50:50 palm and high oleic sunflower oil, Mix of palm & high oleic sunflower oil
    - Unhydrogenated soybean oil

For Comparison Oil Tags (purified fats, cold pressed, or no TFA documented)

- MUFA/SFA/PUFA
  - Linoleic, Oleate
  - Olive oil, palm, stearin (mix)
  - TFA-free canola
  - Mono cis blend, palm, oleic

- SFA
  - Saturated fat diet, High sat fat diet
  - SFA, Saturated fatty acids, Sat Fat
  - Stearate, stearic acid
  - Palmitic acid, Lauric, myristic
  - LMP, Lauric-Myristic-Palmitic acid mixture
  - Extra virgin palm oil, Extra virgin palm kernel oil
  - Extra virgin coconut oil

  - MUFA
    - MUFA, monounsaturated fatty acids
    - Monounsaturated fatty acid diet
    - High oleic fatty acid diet, oleic rich diet
    - Oleic fatty acids
    - Extra virgin olive oil, Virgin Olive oil
    - CMUFA, High cis MUFA

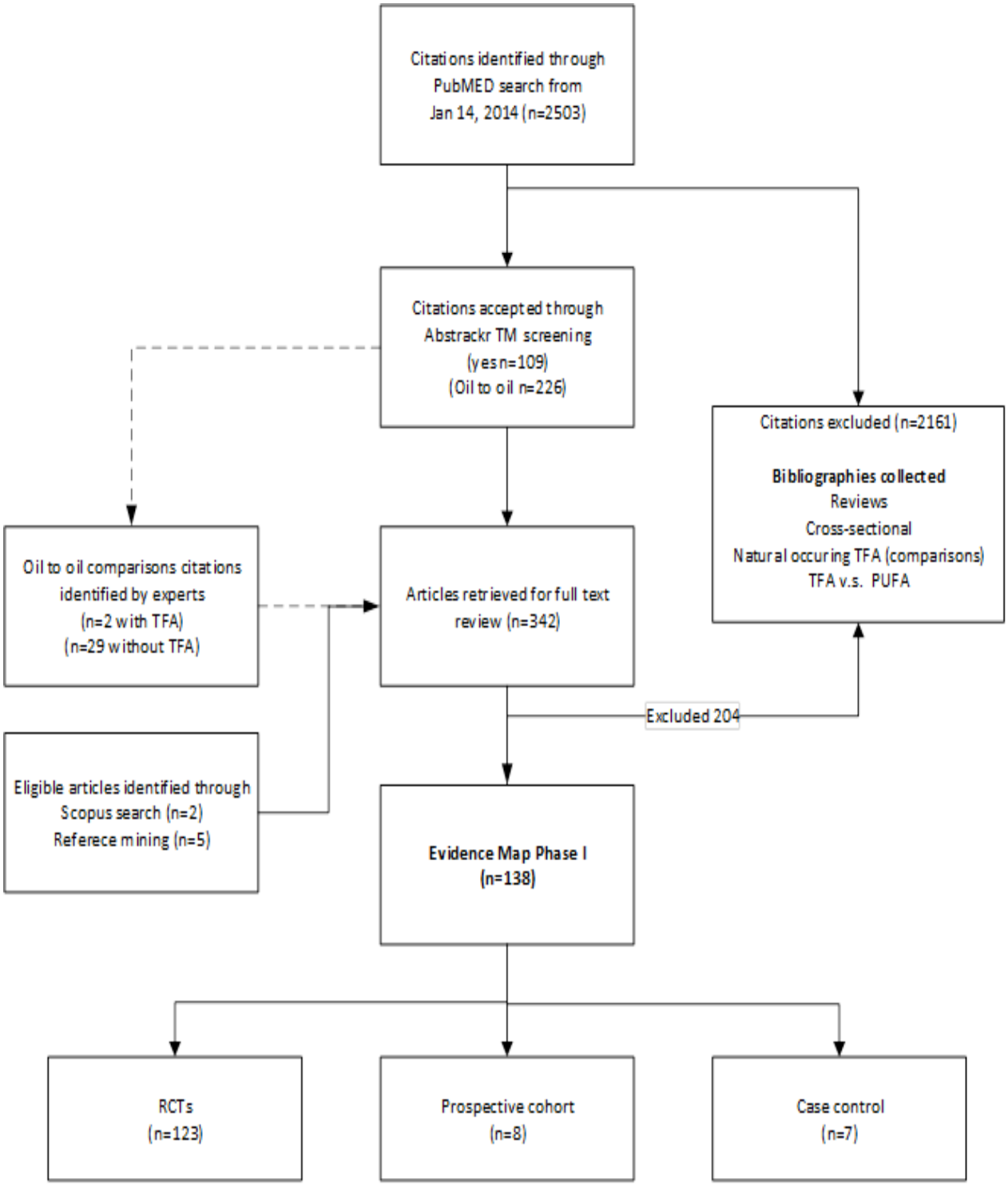
- PUFA
  - SDA, Stearidonic acid
  - N6 PUFA
  - N3 PUFA
  - Linolenic acid
  - Linoleic acid

- Naturally occurring TFA
  - TFA from natural, TFA from natural sources
  - Conjugated linoleic acid , CLA
  - rTFA, High rTFA, Moderate rTFA, Low rTFA butter
  - Butter & Hard cooking fat, Solid fat, Butter, Lard
  - Dairy or ruminant (e.g. lipids in milk/cheese), Dairy fat, Cow's milk,
  - Milk fat, Modified milk, Beef, Beef Tallow
  - Cocoa butter , Peanut
  - SAFA diet (mostly butter)
  - Vaccenic acid, Vaccenic acid enriched diet
  - Very low fat diet

- Mixture (unknown)
  - Butter & vegetable oil mix
  - Habitual butter, palm, canola
  - Habitual diet, Control diet, Baseline diet,
  - Butter, olive oil blend, Butter, sunflower oil blend

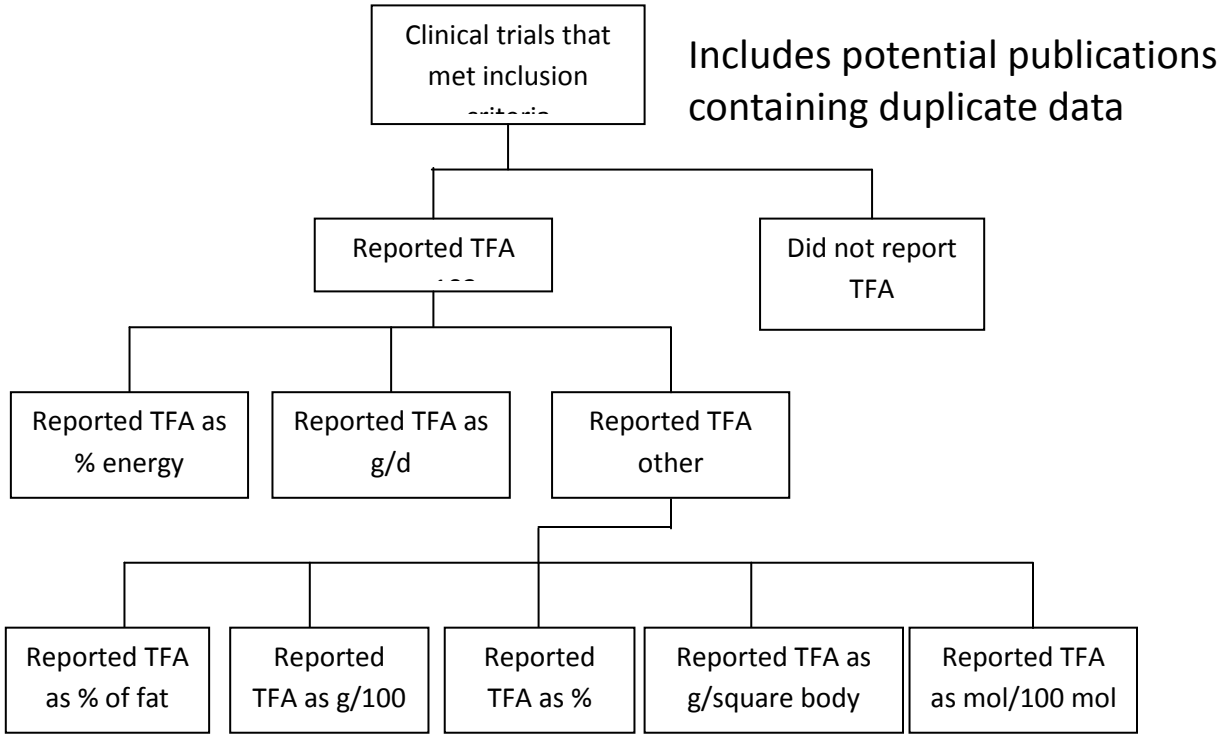
- Other
  - TFA + SFA, TFA + Stearic
  - No TFA

5.3 PRISMA Chart Results



6 Results for RCTs with Industrially-Produced Oils

6.1 Studies quantifying the amount of TFA.



6.2 Description of studies with at least one industrially-produced TFA intervention at <3%en.

Author, Year	Study Design	Subject Baseline Demographics	Background Diet	Test oil and TFA % en	Days on diet	Lipid Outcomes	Comments (if applicable)
Almendingen, 1995; Halvorsen, 1996	• R • C	• N = 31 (0% F) • Healthy • Mean Total-C: 5.35 mmol/L • Mean age: 38 y	22% total en from dairy, meat, fish, cereals; ≤0.5% en TFA	Butter • 0.9% en TFA PHFO marg (62% Norwegian capeline oil and 38% Peruvian anchovy oil) • 8.0% en TFA PHsoyO marg • 8.5% en 30% of refined soybean oil was added to each test fat to obtain equal content of linoleic and α-linolenic acids.	20	• LDL-C, HDL-C, Total-C, TG, LDL-C/HDL-C • Apo B, Apo A1 • Oxidized LDL • Lipid peroxidation	Primary lipid outcomes are reported in Almendingen, 1995 Ordinary raw materials for the food industry were used; PHOs were taken directly from the production line.
Aro, 1997	• P	• N = 80 (61% F) • Healthy • Mean Total-C: 4.75 mmol/L • Mean age: 29 y	High SFA diet (mainly dairy fat, meat fat, & coconut oil) lead-in for 35 d; mean = 0.8% en from TFA	Stearic acid rich marg (mix of fully hydrogenated sunflower oil, oleic acid rich sunflower oil, & linoleic acid) • 0.4% en TFA PHsunO marg (partially hydrogenated oleic acid rich sunflower oil + unaltered sunflower oil) • 8.7% en TFA	35	• <u>Cholesterol:</u> Total, VLDL, LDL, HDL, HDL_2, HDL_3, LDL/HDL • <u>TG:</u> Total, VLDL, LDL, HDL, HDL_2, HDL_3 • Apo B, Apo A1, Lp(a), CETP, PLTP	
Baer, 2004; Judd, 2002	• R <u>Stratified</u> BMI LDL-C • DB • C	• N = 50 (0% F) • Healthy • Mean Total-C: 4.768 mmol/L • Mean age: 42 y	All foods were provided (fat = 37% en); supervised weekday breakfast and dinner during each diet	Oleic acid • 0.1% en TFA CHO (replaced 8% en from fat with CHO) • 0.2% en TFA LMP • 0.2% en TFA Stearic acid • 0.3% en TFA TFA/stearic acid • 4.2% en TFA TFA	35	• LDL-C, HDL-C, HDL_2-C, HDL_3-C, Total-C, TG, Total-C/HDL-C • Apo B, Apo A1	**Possible study for dose response** TFA rich diets enriched with a spectrum of *trans* 18:1 positional isomers similar to that in the U.S. food supply Bear, et al. 2004 reported plasma biomarkers of inflammation from the same study

Author, Year	Study Design	Subject Baseline Demographics	Background Diet	Test oil and TFA % en	Days on diet	Lipid Outcomes	Comments (if applicable)
				• 8.3% en TFA			
Bendsen, 2011	• R • DB • P	• N = 49 (100% F) • Overweight, menopausal • Mean Total-C: NR • Mean age: 58.5 y	Intended to maintain body weight; specifics NR	50/50 palm oil & high oleic sunflower oil • 0.4% en TFA PHsoyO • 7% en TFA	112	• LDL-C, HDL-C, Total-C, TG, LDL-C/HDL-C, Total-C/HDL-C	
Christiansen, 1997	• R • C	• N = 16 (44% F) • Obese, NIDDM • Mean Total-C: 5.8 mmol/L • Mean age: 55 y	Isocaloric diets (fat = 30% en) based on Diabetes & Nutrition Study Group of the European Association for the Study of Diabetes	*Cis*-MUFA diet • 0% en TFA SFA diet (lean meat, butter, whole milk, cheese, and coconut products) • 2.5% en TFA (mainly rTFA) *Trans*-MUFA diet • 20% en TFA	42	• LDL-C, HDL-C, VLDL-C, Total-C, TG, LDL-C/HDL-C ratio, Total-C/HDL-C • Phospholipid, NEFAs, Apo B	
Cuchel, 1996; Lichtenstein, 1993	• C	• N = 14 (57% F) • Healthy • Mean Total-C: 5.77 mmol/L • Mean age: 63 y	Baseline diet: 0.77 % en TFA; study background diet = NCEP Step 2 guidelines (fat = 30% en)	Corn oil • 0.44% en *trans* 18:1 Corn oil marg • 4.16% en *trans* 18:1	32	• LDL-C, HDL-C, VLDL-C, Total-C, TG, LDL-C/HDL-C ratio • LDL particle score and lag time to oxidation • Apo B, Apo A1, Lp(a)	Lichtenstein, 1993 compared baseline diet vs. corn oil, baseline diet vs. margarine, and corn oil vs. margarine for lipid outcomes
Denke, 2000	• C	• N = 226 (48% F) (92 adults, 134 children) • Healthy • Adults Mean TC, 184 mg/dL, LDL-C 121 mg/dL; Children, mean TC 152 mg/dL. LDL-C 95 mg/dL • Mean age: adults 41 y; children 12y	Free-living population emphasizing low SFA diet with prescribed diets designed to maintain weights Isocaloric substitution of butter and margarine matched in fat content	Butter • 0.9% en rTFA Marg • 1.5% en TFA	35	• TC, LDL-C, HDL-C, TG • Apo B, Apo A1 • Weight	46 families included in study Only one PHO arm compared to naturally occurring TFA Margarine compared to butter lowered LDL-C 11% and 9% in adults and children respectively
de Roos, 2001a; de Roos,	• R • C	• N = 32 (66% F) • Healthy	Habitual energy intake estimated from FFQ;	**SFA diet**: marg 60% palm kernel fat and blend of vegetable oils and solid	28	• LDL-C, HDL-C, Total-C, TG, LDL-	de Roos, 2001b reported on endothelial function (FMD) from data obtained in the

Author, Year	Study Design	Subject Baseline Demographics	Background Diet	Test oil and TFA % en	Days on diet	Lipid Outcomes	Comments (if applicable)
2001b; de Roos, 2002; de Roos 2003		• Mean Total-C: 5.0 mmol/L • Mean age: 30 y	provided food to meet 90% of energy needs, remaining chosen from list of low-fat foods	vegetable fats • 0.3% en TFA **TFA diet:** Marg from PHsoyO and vegetable oil containing linoleic and oleic acid • 9.3% en TFA		C/HDL-C ratio	original study (de Roos, 2001a) de Roos, 2002a reported on serum paraoxonase activity from data obtained in the original study (de Roos, 2001a) de Roos 2003 essentially a summary of the other papers, however, it contained slightly different %en from TFA as well as results from 29 subjects instead of 32
Han 2012 (Portion publication of data in Lichtenstein, 2006 **)**	• R • C • DB	• N = 18 (61% F) • Healthy • Mean TC 5.96 mmol/L, LDL-C 3.81 mmol/L • Mean age: 63 y	Isocaloric controlled feeding trial Background for test diet 30%en as fat, test oils provided 2/3 of fat	SoyO • 0.61%en LoSFAsoyO • 0.64%en HiOleicSoyO • 0.33%en LoALAsoyO • 0.52%en PHsoyO • 2.45%en	35	• TC, LDL-0C, HDL-C, VLDL-C, TG • CRP • Immune & inflammatory markers • Plasma phospholipid profiles	**Possible study for dose response**
Han, 2002; Matthan, 2004	• R • DB • C	• N = 19 (58% F) • Generally healthy • Mean Total-C: 253 mg/dL (6.54 mmol/L) • Mean age: 64.7 y	30% en from fat; weight maintenance diet	Butter • 0.3% en TFA Soy oil • 0.6% en TFA Soy oil based stick marg • 6.7% en TFA	32	• LDL-C, HDL-C, VLDL-C, Total-C, TG, Total-C/HDL-C ratio • Biomarkers of inflammation	**Possible study for dose response** Matthan, 2004 reported data on lipoprotein kinetics from a subgroup of 8 women from the original study (Han, 2002).
Iggman, 2011	• R • C	• N = 20 (30% F) • Generally healthy • Mean Total-C: 6.7 mmol/L • Mean age: 50.9 y	Isocaloric (35% en fat), weight maintenance diets; all foods provided	Rapeseed oil diet • 0.8% en TFA Dairy fat diet • 0.9% en TFA	21	• LDL-C, HDL-C, VLDL-C, Total-C, TG, LDL-C/HDL-C ratio, Total-C/HDL-C ratio • Apo B, Apo A1, LP(a)	Goal of study was to replace SFA with MUFA; not to alter TFA levels in the interventions
Judd, 1998	• R Stratified	• N = 46 (50% F)	All foods provided (37%	PUFA rich marg • 2.4% en TFA	35	• LDL-C, HDL-C, HDL_2-C, HDL_3-C,	**Possible study for dose response**

Author, Year	Study Design	Subject Baseline Demographics	Background Diet	Test oil and TFA % en	Days on diet	Lipid Outcomes	Comments (if applicable)
	Sex LDL-C • DB • C	• Healthy, overweight • Mean Total-C: 5.12 mmol/L • Mean age: 46.8 y	en fat); supervised weekday breakfast and dinner during each diet	Butter • 2.7% en TFA TFA rich marg • 3.9% en TFA		Total-C, TG, Total-C/HDL-C ratio, LDL-C/HDL-C ratio • Apo B, Apo A1, Lp(a)	
Judd and Clevidence, 1993; Judd, 1994	• C	• N = 58 (50% F) • Healthy • Mean Total-C: 5.3 mmol/L • Mean age: 42.6 y	All foods provided (39-40% en fat); supervised weekday breakfast and dinner; each diet provided 6%en linoleic acid	Oleic acid • 0.7% en TFA SFA (mostly LMP & stearic acid) • 0.7 % en TFA Moderate TFA (hydrogenated fats used; specific types NR) • 3.8% en TFA High TFA (hydrogenated fats used; specific types NR) • 6.6% en TFA	42	• LDL-C, HDL-C, HDL_2-C, HDL_3-C, Total-C, TG, Total-C/HDL-C • Apo B, Apo A1	**Possible study for dose response** Oils specifically used in each intervention NR, but noted that oils used to prepare foods included coconut oil, high oleic sunflower oil, and high linoleic safflower oil; SFAs from meat and dairy products
Lichtenstein, 1999 Lichtenstein, 2001 Lichtenstein, 2003	• R • C	• N = 36 (50% F) • Healthy • Mean TC 245 mg/dL; LDL-C 167 mg/dL • Mean age: 63 y	Controlled isocaloric feeding trial Background for test diet 30%en as fat, test oils provided 2/3 of fat Test diet met NCEP Step 2 Criteria	Butter • 1.25%en TFA SoyO • 0.55%en TFA SoySemiMarg • 0.91%en TFA SoySoftMarg • 3.30%enTFA SoyShort • 4.15%2nTFA SoyStickMarg • 6.72%en TFA	35	• TC, LDL-C, HDL-C, VLDL-C, TG • Apo A1, Apo B, Lp(a), Apo A2 • TC-to-HDL-C ratio • HDL-C to Apo A1 ratio • LDL-C to Apo B ratio • CETP, PLTP • Glucose, Insulin, BP, CRP	**Possible study for dose response** Several same authors as Matthan, 2000 Same test oils as used by Matthan 2000 (described slightly differently, but TFA and form, source, and range of margarines consistent)
Matthan, 2000 Matthan, 2001	• R • C	• N = 14 (100% F) • Healthy • Mean TC 258.3 mg/dL, LDL-C 171.5 mg/dL • Mean age: 68 y	Baseline diet 39%en fat Background for test diet 30%en as fat, test oils provided 2/3 of fat	Baseline Diet • 1.69%en Butter • 1.25%en SoyO • 0.55%en SoySqu Marg • 0.91%en SoyTubMarg	35	• TC, LDL-C, HDL-C, VLDL-C, TG • Cholesterol synthesis using deuterium uptake • Lipoproteins • CETP, FFA, ASP, glucose, insulin	**Possible study for dose response** Several same authors as Lichtenstein, 1999; 14 of the subjects were the same as in Lichtenstein 1999 Same test oils as used by

Author, Year	Study Design	Subject Baseline Demographics	Background Diet	Test oil and TFA % en	Days on diet	Lipid Outcomes	Comments (if applicable)
			Test diet met NCEP Step 2 Criteria	• 3.30%en SoyStickMarg • 6.72%en			Lichtenstein 1999 (described slightly differently, but TFA and form, source, and range of margarines consistent)
Mensink and Katan, 1990	• R • C	• N = 59 (58% F) • Healthy • Mean Total-C: 4.75 mmol/L • Mean age: 25.5 y	3-d diet record to estimate daily energy needs to maintain body weight	**Oleic acid** (olive oil & marg from high oleic acid sunflower oil + palm & palm kernel oil) • 0.0% en TFA **SFA** (marg from lightly hydrogenated palm oil+palm kernel oil combined with high oleic acid sunflower oil) • 1.8% en TFA ***Trans*-C18:1** (partially hydrogenated high oleic acid sunflower oil) • 10.9% en TFA	21	• LDL-C, HDL-C, Total-C, TG • Apo B, Apo A1	**Possible study for dose response**
Mensink, 1992	• R • C	Exp III • N = 59 (59% F) • Healthy • Mean Total-C: 5.05 mmol/L • Mean age: 26 y	3-d diet record to estimate daily energy needs to maintain body weight	Exp III **Linoleic acid** (sunflower oil) • 0.1% en TFA **Stearic acid** (fully hydrogenated high oleic, linoleic sunflower oil) • 0.3% en TFA ***Trans*-C18:1** (hydrogenated high oleic acid sunflower oil) • 7.7% en TFA	21	• LDL-C, Lp(a)	**Possible study for dose response** Exp I not relevant; Exp II contained duplicate data from Mensink, 1990 described above.
Mensink, 2008	• R • DB • C	• N = 44 (75% F) • Healthy • Mean Total-C: men = 5.40 mmol/L; women = 5.31 mmol/L • Mean age: 41 y	40%en fat; ~15%en fat from test fats provided in margs, cookies, muffins, chocolate paste and chips	High palmitic acid; TFA "free" (semiliquid palm olein • 0.2% en TFA High oleic acid (semiliquid rapeseed oil) • 0.7 % en TFA	21	• LDL-C, HDL-C, VLDL-C, Total-C, TG, Total-C/HDL-C ratio	
Muller, 1998	• R • C	• N = 16 (100% F) • Healthy • Mean Total-C: 4.44 mmol/L • Mean age: 22 y	31-32%en fat; all foods provided on rotating 7 d menu	Vegetable oil marg (palm, soyabean, coconut, & partially hydrogenated palm oils & fractionated palm oil) • 1.1% en	14	• LDL-C, HDL-C, Total-C, TG, LDL-C/HDL-C ratio • Apo B, Apo A1, Lp(a)	

Author, Year	Study Design	Subject Baseline Demographics	Background Diet	Test oil and TFA % en	Days on diet	Lipid Outcomes	Comments (if applicable)
				PHFO marg (traditional hard marg with high PHFO + some soyabean & coconut oils) • 7.7% en TFA			
Mutanen and Aro, 1997	• P	• N = 80 (61% F) • Healthy • Mean Total-C: 4.75 mmol/L • Mean age: 29 y	5-week lead-in diet high in SFA, mainly dairy fat, meat fat, and coconut oil; 0.8% TFA	Stearic acid diet (mix of fully hydrogenated sunflower oil & high-oleic-acid & high-linoleic-acid sunflower oils • 0.4% en High TFA diet (mix of partially hydrogenated and unaltered high-oleic-acid sunflower oils) • 8.7% en TFA	35	• TG, Lp(a)	Lipid panel only measured at baseline and correlation analyses conducted with changes in markers of coagulation & fibrinolysis
Nestel, 1992	• R • C	• N = 27 (0% F) • Healthy • Mean Total-C: 221 mg/dL (5.72 mmol/L) • Mean age: 46.8 y	35%en fat; 15%en fat from dairy, meat, bread, & cereals & 20%en from test margarines	Habitual diet (butter, palm oil, canola oil) • <1% en TFA (exact amount NR) Palmitic acid diet (mostly palm oil) • <1% en TFA (exact amount NR) Oleic acid diet (canola oil) • 1.4% en TFA High elaidic acid diet (canola, linseed, and safflower oils + hardened canola/palmolein) • 5.7% en TFA	14 d habitual diet 21 d test oils diets	• LDL-C, VLDL-C, HDL-C, HDL_2-C, HDL_3-C, Total-C, TG, Total-C/HDL-C • Apo B, Apo A1 • Oxidized LDL	**Possible study for dose response**
Pedersen, 2005	• R • C	• N = 27 (100% F) • Healthy • Mean Total-C: • Mean age: 27 y	All foods provided; supervised weekday dinner; each diet provided Total fat = 30-31%en (fat from test margarines = 26%en)	PALM-diet (marg from palm oil, soybean oil, & rapeseed oil) • 0.1% en TFA PUFA-diet (marg from sunflower oil, rapeseed oil coconut oil, and palm oil) • 0.1% en TFA TRANS-diet (marg from PHsoyO, refined rapeseed oil, & refined soybean oil.) • 7.0% en TFA	17	• LDL-C, HDL-C, Total-C, TG, LDL-C/HDL-C • Apo B, Apo A1, Lp(a)	

Author, Year	Study Design	Subject Baseline Demographics	Background Diet	Test oil and TFA % en	Days on diet	Lipid Outcomes	Comments (if applicable)
Sanders, 2003	• R • SB • C	• N = 36 (0% F) • Healthy • Mean Total-C: 4.63 mmol/L • Mean age: 24.2 y	All foods provided; weight maintenance diet; SFA, PUFA, cholesterol & fiber held constant	High CHO diet (American Heart Association Step 1 diet) • 0.1% en TFA High oleic acid diet (marg containing oleic acid; specific oil NR) • 0.1% en High TFA diet (marg containing C18:1 isomers; specific oil NR • 9.6% en TFA	14	• LDL-C, HDL-C, HDL_2-C, HDL_3-C, Total-C, TG, LDL-C/HDL-C ratio • Apo B, Apo A1, Apo AII, Lp(a)	
Sundram, 2007	• R • C	• N = 30 (66% F) • Healthy • Mean TC 5.05 mmol/L, LDL-C 3.17 mmol/L • Mean age: 30 y	Controlled feeding study except for dinner and weekends Test diets delivered ~31%en fat with >70% from test fats	PHsoyO • 3.2%en PalmOlein • nd IE • nd	28	• TC, LDL-C, HDL-C, VLDL-C,TG • TC to HDL-C ratio • HDL-C to LDL-C ratio • Glucose, Insulin	Study included although 3.19%en TFA
Takeuchi, 2011	• R • C • DB	• N = 12 (75% F) • Healthy • No Hx dyslipidemia • Mean age: 22.8 y	TFA and control oil delivered in cookie. TFA added 0.6%en per day to Free-living subjects.	Rapeseed oil • 0.1%en PH Rapeseed Oil • 0.8%en	28	• TC, LDL-C, HDL-C, TG • LDL-C to HDL-C ratio • Lipoprotein • Glucose, Insulin, CRP, HbA1c • Serum & Erythrocyte TFA	Limited information on background diet Body weight during study not reported
Vega-Lopez, 2006	• R • C	• N = 15 (67% F) • Generally healthy • Mean Total-C: 253 mg/dL (6.54 mmol/L) • Mean age: 63.9 y	All foods provided; weight maintenance diet; experimental fats provided two-thirds of the total fat of the diet	Soybean oil • 0.55% en TFA Palm oil • 0.60% en TFA Canola oil • 0.98% en TFA PHsoyO • 4.15% en TFA	35	• LDL-C, HDL-C, HDL_2-C, HDL_3-C, Total-C, TG, Total-C/HDL-C ratio • Apo B, Apo A1, Apo AII, Lp(a), LDL-C/Apo B ratio, HDL-C/ApoA1 ratio • CETP, PLTP	

Author, Year	Study Design	Subject Baseline Demographics	Background Diet	Test oil and TFA % en	Days on diet	Lipid Outcomes	Comments (if applicable)
Vega-Lopez, 2009	• R • DB • C	• N = 30 (100% F) • Healthy • Mean Total-C: 5.94 mmol/L • Mean age: 64.2 y	All foods provided; weight maintenance diet; experimental fats provided two-thirds of the total fat of the diet	Corn oil • 0.3% en TFA PHsoyO • 4.3% en TFA	35	• LDL-C, VLDL-C, HDL-C, HDL_2-C, HDL_3-C, Total-C, TG, Total-C/HDL-C ratio • Apo B, Apo A1, Lp(a)	
Wanders, 2010	• R • C	• N = 63 (59% F) • Healthy • Mean Total-C: 4.54 mmol/L • Mean age: 30.9 y	Weight maintenance diets estimated from FFQ; diets intended to be identical except for 7% total en as CLA, industrial TFA or oleic acid provided as margarines and in yogurt drinks	Oleic acid marg (high oleic sunflower oil, palm oil, & palm kernel fat) • 0.2% en TFA TFA marg (partially hydrogenated vegetable oil, sunflower oil, high-oleic sunflower oil) • 7.3% en TFA CLA marg (CLA-rich oil, high-oleic sunflower oil, palm oil, & palm kernel fat • 9.1% en TFA (99% from CLA)	21	• LDL-C, VLDL-C, HDL-C, Total-C, TG, LDL-C/HDL-C ratio, Total-C/HDL-C ratio • Apo B, Apo B/LDL-C ratio	
Zock, 1994	• C • SB	• N = 59 (61%) • Healthy • Mean TC 5.06 mmol/L, • Mean age: 28.5 y	Controlled diet designed to match energy needs per subject 10%en provided by test oils	Syn MyristicMarg • 0.8%en Palm-R Marg • 0.2%en Oleic-R Marg • 0.3%en	21	• TC, LDL-C, HDL-C, TG • HDL-C to LDL-C ratio • Apo A1, Apo B	**Possible study for dose response**
Zock and Katan, 1992	• R • C	• N = 56 (54% F) • Healthy • Mean Total-C: 4.84 mmol/L • Mean age: 25 y	The planned nutrient content of the 3 diets was similar, except for 8% total en, provided by linoleic acid, stearic acid, or elaidic acid.	Linoleate-diet (marg high in linoleic acid + high-linoleic acid sunflower oil in study foods) • 0.1% en Stearate-diet (high-linoleic acid sunflower oil, high-oleic acid sunflower oil, unaltered high linoleic acid sunflower oil) • 0.3% en TFA	21	• LDL-C, HDL-C, Total-C, TG, HDL-C/LDL-C ratio, Total-C/HDL-C ratio • Apo B, Apo A1	**Possible study for dose response**

Author, Year	Study Design	Subject Baseline Demographics	Background Diet	Test oil and TFA % en	Days on diet	Lipid Outcomes	Comments (if applicable)
				Trans-diet (marg and shortening from hydrogenated high oleic acid sunflower oil + unaltered oleic acid rich oil) • 7.7% en TFA			

Abbreviations: Apo, apolipoprotein; **ASP**, acylation-stimulating protein; **BP**, blood pressure; **C**, crossover; **CETP**, cholesteryl ester transfer protein, **CHO**, carbohydrate; **CRP**, C-reactive protein; **DB**, double blind; **en**, energy; **F**, female; **FA**, fatty acid(s); **FFA**, free fatty acids; **FMD**, flow mediated dilation; **FFQ**, food frequency questionnaire; **HbA1c**, hemoglobin A1c; **HDL-C**, high-density lipoprotein cholesterol; **HiOleicSoyO**, high oleic soybean oil; **Hx**, history; **IE**, Interesterified fat; **LoALAsoyO**, ,low alpha linolenic acid soybean oil; **LDL-C**, low-density lipoprotein cholesterol; **LMP**, lauric, myristic, and palmitic acids; **LoSFAsoyO**, low saturated fatty acids soybean oil; **Lp(a)**, lipoprotein a; **N**, sample size; **NCEP**, National Cholesterol Education Program; **nd**, not detected; **NEFA**, non-esterified fatty acid(s); **NIDDM**, non-insulin dependent diabetes mellitus; **NR**, not reported; **P**, parallel; **Oleic-R Marg**, oleic acid rich margarine; **Palm-R Marg**, palmitic acid rich margarine; **PH**, partially hydrogenated; **PHFO**, partially hydrogenated fish oils; **PHsoyO**, partially hydrogenated soybean oil; **PLTP**, phospholipid transfer protein; **PHsunO**, partially hydrogenated sunflower oil; **PLTP**, phospholipid transfer protein; partially hydrogenated fish oil; **R**, randomized; **rTFA**, ruminant TFA; **SB**, single blind; **SFA**, saturated fatty acids; **SoyO**, soybean oil; **SoySemiMarg**, soybean oil-based semi-liquid margarine; **SoySoftMarg**, soybean oil-based soft margarine; **SoySquMarg**,soybean oil-based squeeze margarine; **SoyStickMarg**, soybean oil-based stick margarine **SoyShort**, soybean-oil-based shortening; **SoyTubMarg**, soybean oil-based tub margarine; **SynMyristic Marg**, synthetic myristic acid rich margarine; **TFA**, *trans* fatty acid(s); **TG**, triglyceride(s); **VLDL-C**, very-low-density lipoprotein cholesterol; **y**, year

6.3 Interventions in RCTs with Industrially-Produced Oils and TFAs.

Author, year	Industrial Oil or diet including industrial oils (no ruminant sources)	%TFA	Studies using margarine, SFA with TFA, or a defined PHO
Nestel 1992	Habitual diet	<1	
Nestel 1992	Palmitic acid diet	<1	
Sundram 2007	PamOlein	nd	
Sundram 2007	Interesterified	nd	
Christiansen 1997	cis-MUFA	0	
Mensink & Katan 1990	Oleic acid	0	
Baer 2004, Judd 2002	Oleic acid	0.1	
Mensink 1992	Linoleic acid	0.1	

Author, year	Industrial Oil or diet including industrial oils (no ruminant sources)	%TFA	Studies using margarine, SFA with TFA, or a defined PHO
Pedersen2005	PALM diet	0.1	
Pedersen2005	PUFA diet	0.1	
Sanders 2003	High CHO diet	0.1	
Sanders 2003	High oleic acid diet	0.1	
Zock & Katan 1992	Linoleate diet	0.1	
Takeuchi 2011	Rapeseed oil	0.1	
Baer 2004, Judd 2003	CHO with fat	0.2	
Baer 2004, Judd 2002	LMP	0.2	
Mensink 2008	High palmitic acid TFA free	0.2	
Wanders 2010	Oleic acid margarine	0.2	Yes
Zock 1994	Palm rich margarine	0.2	Yes
Baer 2004, Judd 2002	Stearic acid	0.3	
de Roos 2001a, 2001b, 2002, 2003	SFA diet - margarine 60% palm kernel fat and blend of vegetable oils and solid vegetable fats	0.3	Yes
Mensink 1992	Stearic acid diet - fully hydrogenated high oleic, linoleic sunflower oil	0.3	
Vega-Lopez 2009	Corn oil	0.3	
Zock & Katan 1992	Stearate diet	0.3	
Zock 1994	Oleic rich margarine	0.3	Yes
Han 2012, Lichtenstein 2006	High Oleic Soy oil	0.33	
Aro 1997	Stearic acid rich marg	0.4	Yes
Bendsen 2011	palm & high oleic sunflower oil	0.4	
Mutanen & Aro 1997	Stearic acid diet	0.4	
Cuchel 1996, Lichtenstein 1993	Corn oil	0.44	
Han 2012, Lichtenstein 2006	LoALA soy Oil	0.52	
Vega-Lopez 2006	Soybean oil	0.55	
Lichtenstein 1999, 2001, 2003	Soybean oil	0.55	

Author, year	Industrial Oil or diet including industrial oils (no ruminant sources)	%TFA	Studies using margarine, SFA with TFA, or a defined PHO
Matthan 2000, 2001	Soybean oil	0.55	
Han 2002, Matthan 2004	Soybean oil	0.6	
Vega-Lopez 2006	Palm oil	0.6	
Han 2012, Lichtenstein 2006	Soybean Oil	0.61	
Han 2012, Lichtenstein 2006	LoSFA soy O	0.64	
Judd & Clevindence 1993, Judd 1994	Oleic acid	0.7	
Judd & Clevindence 1993, Judd 1994	SFA diet (meat + dairy products)	0.7	
Mensink 2008	High oleic acid rapeseed oil	0.7	
Iggman 2011	Rapeseed oil	0.8	
Takeuchi 2011	PH rapeseed oil	0.8	Yes
Zock 1994	Syn Myristic margarine	0.8	Yes
Lichtenstein 1999, 2001, 2003	Soy Semiliquid margarine	0.91	Yes
Matthan 2000, 2001	Soy squeeze margarine	0.91	Yes
Vega-Lopez 2006	Canola oil	0.98	
Muller 1998	Veg oil mixture	1.1	
Nestel 1992	Oleic acid diet	1.4	
Denke 2000	Margarine	1.5	Yes
Matthan 2000, 2001	Baseline diet	1.69	
Mensink & Katan 1990	SFA diet - margarine from lightly hydrogenated palm oil+palm kernel oil combined with high oleic acid sunflower oil	1.8	Yes
Judd 1998	PUFA-rich margarine	2.4	Yes
Han 2012, Lichtenstein 2006	PHsoyOil	2.45	Yes
Sundram 2007	PHsoyO	3.2	Yes
Lichtenstein 1999, 2001, 2003	Soy soft margarine	3.3	Yes
Matthan 2000, 2001	Soy tub margarine	3.3	Yes
Judd & Clevindence 1993, Judd 1994	Moderate TFA (hydrogenated fats; specific types NR)	3.8	Yes

Author, year	Industrial Oil or diet including industrial oils (no ruminant sources)	%TFA	Studies using margarine, SFA with TFA, or a defined PHO
Judd 1998	TFA-rich margarine	3.9	Yes
Vega-Lopez 2006	PHsoyO	4.15	Yes
Lichtenstein 1999, 2001, 2003	Soy shortening	4.15	Yes
Cuchel 1996, Lichtenstein 1994	Corn oil margarine	4.16	Yes
Baer 2004, Judd 2002	TFA/ Stearic	4.2	Yes
Vega-Lopez 2009	PHsoyO	4.3	Yes
Nestel 1992	High elaidic acid diet	5.7	Yes
Judd & Clevindence 1993, Judd 1994	High TFA (hydrogenated fats; specific types NR)	6.6	Yes
Han 2002, Matthan 2004	Soybean oil-based stick margarine	6.7	Yes
Lichtenstein 1999, 2001, 2003	Soy stick margarine	6.72	Yes
Matthan 2000, 2001	Soy stick margarine	6.72	Yes
Bendsen 2011	PHsoyO	7	Yes
Pedersen2005	TRANS diet (margarine from PHsoyO, refined rapeseed oil, & refined soybean oil)	7	Yes
Wanders 2010	TFA margarine	7.3	Yes
Mensink 1992	Trans C18:1 diet - hydrogenated high oleic acid sunflower oil	7.7	Yes
Muller 1998	PHFO, soy, coconut mixture	7.7	Yes
Zock & Katan 1992	Trans diet (margarine and shortening from hydrogenated high oleic acid sunflower oil + unaltered oleic acid rich oil)	7.7	Yes
Almengdinden 1995, Halvorsen 1996	PHFO marg	8	Yes
Baer 2004, Judd 2002	TFA	8.3	Yes
Almengdinden 1995, Halvorsen 1996	PHsoyO marg	8.5	Yes
Aro 1997	PHsunO marg	8.7	Yes
Mutanen & Aro 1997	High TFA diet (partially hydrogenated and unaltered high-oleic-acid sunflower oils)	8.7	Yes
de Roos 2001a, 2001b, 2002, 2003	PHsoyO + non-hyd veg oil marg	9.3	Yes

Author, year	Industrial Oil or diet including industrial oils (no ruminant sources)	%TFA	Studies using margarine, SFA with TFA, or a defined PHO
Sanders 2003	High TFA diet (margarine containing C18:1 isomers; specific oil NR)	9.6	Yes
Mensink & Katan 1990	Trans-C18:1 diet (partially hydrogenated high oleic acid sunflower oil)	10.9	Yes
Christiansen 1997	Trans-MUFA	20	Yes

6.4 Number of interventions using an industrial oil and subgroup of PHO interventions

%en TFA Levels	# IP-TFA Interventions	# PHO Interventions
0 or < detectable	6	0
>0 and ≤0.5	24	5
>0.5 and ≤1.0	17	4
>1.0 and ≤1.5	3	1
>1.5 and ≤2.0	2	1
>2.0 and ≤2.5	2	2
>2.5 and ≤3.0	0	0
>3.0	30	30

6.5 Studies with at least one industrially-produced TFA intervention at <3%en

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7 Possible Studies to Use as Dose- Response

7.1 Lichtenstein 1999 (duplicate reporting in Lichtenstein 2001 and 2003)

This controlled cross-over feeding study included six test diets using a Latin Square design. All foods were provided and were administered in a double-blind fashion for 35 days per test condition. Diets were designed to deliver 30%en as total fat and provided per subject based on calculated caloric levels to maintain body weight. Ten percent of the fat was provided in the base diet, with 20% provided by the test ingredients. With the exception of butter, the test ingredients were all soybean oil-based. Eighteen postmenopausal women (mean age 67 ± 4 y; mean BMI 26.6 ± 2.4 kg/m^2) and 18 men (average age 60 ± 5 y, BMI 28.1 ± 3.4 kg/m^2). All subjects were generally healthy with LDL-C >130 mg/dL and not taking medications to manage blood lipids. Fasting blood lipids (14-hour fast) were obtained during screening and three times after day 28 of each diet. No other details are provided.

	Pre-trial	Soybean Oil	Semiliquid Margarine	Soft Margarine	Shortening	Stick Margarine	Butter
Trans Fat (%en)	NR	0.55	0.91	3.30	4.15	6.72	1.25
	Mean ± SD						
TC (mg/dL	245 ± 33	225 ± 32	226 ± 30	232 ± 28	235 ± 32	243 ± 37	251 ± 36
LDL-C (mg/dL)	167 ± 28	154 ± 28	155 ± 27	159 ± 26	164 ± 28	168 ± 30	177 ± 32
HDL-C (mg/dL)	48 ± 11	43 ± 9	43 ± 10	43 ± 9	43 ± 9	42 ± 9	45 ± 10

- Data are presented as Mean ± SD
- No statistics are provided in comparison to pre-trial values
- LDL-C following consumption of all diets were significantly different compared to butter ($p<0.05$ for all comparisons)
- LDL-C following consumption of soybean oil, semi-liquid margarine, and butter were significantly different vs. stick margarine ($p<0.05$ for all comparisons)
- LDL-C following consumption of butter and soybean oil were significantly different vs. shortening ($p<0.05$ for all comparisons)
- LDL-C following consumption of butter, stick margarine, and shortening were significantly different vs. soybean oil ($p<0.05$ for all comparisons)
- LDL-C following consumption of butter was significantly different than soft margarine ($p<0.05$ for all comparisons)
- LDL-C was not significantly different among the soybean oil, semi-liquid margarine, and soft margarine diets.
- HDL-C was not significantly different following consumption of any of the diets, with the exception of the comparison of butter vs. stick margarine ($p<0.05$)

7.2 Matthan 2000 (duplicate with Matthan 2001)

This randomized, crossover, isocaloric controlled feeding study included six test diets over 35 days per diet, with a 2 to 4 week washout between test periods. The study was designed to assess the impact of consumption of commonly available sources of dietary fats subjected to different degrees of hydrogenation on endogenous synthesis rate of free cholesterol using deuterium incorporation.

The 14 participants in this study were selected from the 18 subjects who participated in the Lichtenstein 1999 study. Subject characteristics included postmenopausal women (mean age = 68.1 ± 2.5 y and BMI = 26.5 ± 2.4 kg/m^2), generally healthy with LDL-C >130 mg/dL and not taking medications to manage blood lipids at baseline.

The background diet for the oil treatments was designed to follow the NCEP Step 2 guidelines (15%en protein, 55%en carbohydrate, 30%en fat, ≤7%en SFA, 10-15%en MUFA, ≤10%en PUFA, <85mg cholesterol/1000 kcal). The baseline diet did not follow Step 2 criteria and was assessed as 16.8%en protein, 44.6%en carbohydrates, 38.6%en fats, with 163.8 mg cholesterol/ 1000 kcal. Diets were designed per each subject for weight maintenance as calculated by caloric needs. Ten percent of the fat was provided in the base diet, with 20%en provided by the test ingredients. With the exception of butter, the test ingredients were all soybean oil-based.

TC, LDL-C, and HDL-C for the 14 subjects on the Matthan 2000 study after the dietary interventions. The Matthan 2001 study publishes data on 18 subjects, but essentially appears to be the same study.

	Baseline Diet	Soybean Oil + NCEP diet	Squeeze Margarine + NCEP diet	Tub Margarine + NCEP diet	Stick Margarine + NCEP diet	Butter + NCEP diet
Trans Fat (%en)	1.69	0.55	0.91	3.30	6.72	1.25
	Mean ± SD					
TC (mg/dL)	252 ± 31.9^{ab}	229 ± 28.3^{d}	232 ± 28.1^{cd}	239 ± 29.9^{bcd}	247 ± 33.5^{ab}	255.9 ± 33.3^{a}
LDL-C (mg/dL)	175.5 ± 30.8^{a}	156.5 ± 32.4^{b}	157.5 ± 22.4^{bc}	165.2 ± 29.3^{ab}	171.9 ± 30.3^{ac}	176.8 ± 28.7^{a}
HDL-C (mg/dL)	51.7 ± 12.0^{a}	46.5 ± 9.2^{bc}	46.5 ± 10.8^{bc}	47.2 ± 9.7^{bc}	45.3 ± 9.4^{c}	50.2 ± 9.6^{ab}

Values in the same row with different superscripts are significantly different $p<0.05$

7.3 Han 2012 (portion of the data from this study published as Lichtenstein 2006)

This was a double-blind, randomized isocaloric crossover controlled feeding study included six test diets over 35 days per diet with a minimum of 2 weeks between tests. The study was designed to test impact of different fatty acid compositions in the diet on immune function, and lipids were also obtained. The study included 11 postmenopausal women and 7 men average age 63.1 ± 2.3 y and BMI = 26.7 ± 0.9 kg/m^2), generally healthy with LDL-C between 3.37 and 4.14 mmol/L and not taking medications to manage blood lipids, nonsteroidal anti-inflammatory drugs or aspirin, or supplements known to affect immune function.

Diets were designed per each subject for weight maintenance as calculated by caloric needs. Diets provided 30%en as fat in which 20%en was contributed by the test ingredients. With the exception of butter, the test ingredients were all soybean oil-based. The background diets delivered 16.7

– 17.7%en protein, 52.1 – 54.6%en carbohydrate, 28.9 – 31.2%en fat, 4.91 – 7.25%en SFA, 6.19 – 18.90%en MUFA, 2.82 – 14.60%en PUFA, and 57-66 mg cholesterol/1000 kcal. Fiber was relatively consistent at 13.4 to 14.6 g/1000 kcal.

	Baseline	Soybean Oil	LoSFA-SO	HiOleic-SO	LoALA-SO	Hydrog-SO
Trans Fat (%en)	nd	0.61	0.64	0.33	0.52	2.53
	Mean ± SD					
TC (mmol/L)	5.75 ± 0.75	5.72 ± 0.91[b]	5.59 ± 0.96[b]	5.71 ± 0.87[b]	5.74 ± 0.81[b]	6.01 ± 0.84[a]
LDL-C (mmol/L)	3.86 ± 0.51	3.66 ± 0.67[b]	3.53 ± 0.77[b]	3.70 ± 0.66 [b]	3.71 ± 0.64[ab]	3.92 ± 0.70[a]
HDL-C (mmol/L)	1.28 ± 0.32	1.32 ± 0.32 [ab]	1.32 ± 0.35 [b]	1.36 ± 0.33 [a]	1.32 ± 0.33[b]	1.32 ± 0.32 [ab]

Values in the same row with different superscripts are significantly different $p<0.05$

7.4 Judd 2002 (duplicate with Baer 2004)

This study was referenced in Ascherio 1999 as an abstract published in 1998 with use on one datapoint corresponding to ~8%en TFA. The study was a randomized, controlled feeding, crossover design comparing 6 different diets with varying amounts of fats. The purpose of the study was to compare the cholesterol lowering by oleic acid rather than elevation by saturated fatty acids and TFA. Study diets were consumed for 5 weeks each. The feeding portions of the study were performed in two phases, with testing of three diets, followed by an 8-week break, and subsequent testing of the remaining 3 diets. Baseline blood lipids were taken in duplicate before initiation and rechecked for drift after the 8-week break and before the second set of diets were begun. Fifty-four subjects started and 50 completed the trial; only data from the 50 completers was included in the analyses. Subjects were men in generally good health and not on lipid lowering medications, with mean age 42 y and mean BMI 26.2 kg/m^2. Body weight was also controlled between the two sets of dietary intakes (mean difference 0.46 ± 0.32 kg). The comparison diets were kept constant with respect to protein (~25%en). All diets with the exception of the CHO (carbohydrate) diet delivered 45-46%en as carbohydrates; the CHO diet delivered higher at 54.5%en carbohydrates. Total fat was provided at 38-40%en for all diets but the CHO diet, which delivered 30.5%en as fat. The specific fatty acids provided were designed to compare:

- Oleic Diet: 8%en from fat provided as oleic acid
- LMP Diet: 8%en form fat provided as lauric, myristic, and palmitic
- STE Diet: 8%en from fat provided as stearic (STE) acid
- TFA Diet: 8%en from fat provided with a spectrum of 18:1 of trans positional isomers similar to that found in the U.S. food supply at the time of the study
- TFA/STE Diet: 4%en provided as TFA and 4%en provided as STE

	Pre-Trial-Phase 1	Pre-Trial Phase 2	CHO	Oleic Diet	TFA Diet	TFA/ STE Diet	STE Diet	LMP Diet
Trans Fat (%en)	NA	NA	0.2	0.1	8.3	4.2	0.3	0.2
	Mean ± SEM							

LDL-C (mmol/L)	3.082 ± 0.072	3.037 ±0.072	3.045 ± 0.083	2.948 ± 0.083	3.36 ± 0.08	3.320 ± 0.083	3.101 ± 0.083	3.209 ± 0.083
HDL-C (mmol/L)	1.160 ± 0.038	1.124 ± 0.043	1.195 ± 0.041	1.241 ± 0.041	1.159 ± 0.041	1.174 ± 0.041	1.156 ± 0.041	1.303 ± 0.041

- The TFA diet resulted in significantly different LDL-C measurements with the STE, LMP, Oleic, and CHO diets but not the TFA/STE diet
- The TFA/STE diet resulted in significantly different LDL-C measurements with the STE, Oleic, and CHO diets, but not with the LMP and TFA diets
- HDL-C was not significantly different among the TFA, TFA/STE, STE, and CHO diets, but was significantly different between the TFA & LMP and TFA & Oleic diets

7.5 Other Possible Dose Response Studies from Non-hydrogenated Oils

1. Han, 2002 (duplicate with Matthan, 2004)
2. Judd, 1998
3. Mensink and Katan, 1990
4. Mensink, 1992
5. Nestel, 1992
6. Zock, 1994
7. Zock and Katan, 1992

7.6 Comparison of Studies Included in Key Systematic Reviews/Meta-analyses

Studies with at least one treatment arm that included an IP-TFA at <3.0%en from Section 6.2

Author, Year	Brouwer et al, 2010	Ascherio et al, 1999	Mozaffarian & Clark, 2009	Mensink et al, 2008	Possible Dose-Response for low level intakes*
Almendingen, 1995; Halvorsen, 1996	X	Excluded	X	X	
Aro, 1997		X	X	X	
Baer, 2004; Judd, 2002	X	X			X
de Roos, 2001a; de Roos, 2001b; de Roos, 2002;de Roos 2003		X			
Han 2012 (Portion publication of data in Lichtenstein, 2006)	X				X
Han, 2002; Matthan, 2004	X				X
Judd, 1998			X	X	X
Judd and Clevidence, 1993; Judd, 1994	X	X	X	X	
Lichtenstein, 1999 Lichtenstein, 2001 Lichtenstein, 2003	X	X	X		X
Matthan, 2000 Matthan, 2001					X
Mensink and Katan, 1990	X	X	X	X	X
Mensink, 1992					X
Muller, 1998	X		X	X	
Nestel, 1992	X				X
Sundram, 2007	X		X		
Wanders, 2010	X				
Zock, 1994					X
Zock and Katan, 1992	X	X	X	X	X

* Studies were included if there were at least 3 treatment arms and at least 1 of the treatment arms provided a IP-TFA at <3.0%en

Review References

Brouwer et al. PLoS One. 2010; 5(3):e9434.
Ascherio et al. 1999. N Engl J med. 1999; 340:1994-1998.
Mozaffarian & Clarke. Eur J Clin Nutr. 2009; 63:S22-S33.
Mensink et al. Am J Clin Nutr. 2008; 87: 558-566.

8 Case Control and Cohort Tables

Case-Control Studies												
PMID	**Author**	**Title**	**Publ Year**	**Health status**	**Female %**	**Total N**	**Intervention (oil or TFA)**	**TFA %en reported**	**TFA %en**	**TFA (grams)**	**Comparator % en**	**LDL/HDL measurement**
23446892	Tokede OA, Petrone AB, Hanson NQ, Tsai MY, Weir NA, Glynn RJ, Gaziano JM, Djoussé L.	Plasma phospholipid trans fatty acids and risk of heart failure.	2013	healthy vs CVD/HF	0%	1576	TFA in diet	yes	in quintile	2% fat	2.1% fat	yes
15051840	Clifton PM, Keogh JB, Noakes M.	Trans fatty acids in adipose tissue and the food supply are associated with myocardial infarction.	2004	healthy vs heart disease	37%	383	TFA in adipose tissue	no		1.77g/100g	1.48g/100g	yes
8281700	Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC.	Trans-fatty acids intake and risk of myocardial infarction.	1994	healthy vs MI	22%	521	TFA intake	no		4.68g/d	3.8g/d	yes
8465781	Siguel EN. Lerman RH.	Trans-fatty acid pattern in patients with angiographically documented coronary artery disease.	1993	healthy vs CVD	26%	103	TFA in plasma	no		1.38% of fat	1.11% of fat	yes
9298578	Kohlmeier L, Simonsen N, van 't Veer P, Strain JJ, Martin-Moreno JM, Margolin B, Huttunen JK, Fernández-Crehuet Navajas J, et al.	Adipose tissue trans fatty acids and breast cancer in the European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer.	1997	healthy vs breast cancer	100%	698	TFA in adipose tissue	yes	1.30%		1.10%	no
9023464	Tavani A, Negri E, D'Avanzo B, La Vecchia C.	Margarine intake and risk of nonfatal acute myocardial infarction in Italian women.	1997	healthy and AMI diagnosed	1	1295	margarine	no				no
3405870	Tuyns AJ, Kaaks R, Haelterman M.	Colorectal cancer and the consumption of foods: a case-control study in Belgium.	1988	healthy vs colon/rectal cancers	nr	3669	margarine undefined; oil undefined	no				no

Prospective Cohort Studies											
PMID	**Author**	**Title**	**Pub Year**	**Sample size**	**Female %**	**Yrs Follow up**	**Dietary Assessment method**	**Exposure**	**% or quintile in range**	**LDL outcome**	**HDL outcome**
14525873	He K, Merchant A, Rimm EB, Rosner BA, Stampfer MJ, Willett WC, Ascherio A.	Dietary fat intake and risk of stroke in male US healthcare professionals: 14 year prospective cohort study.	2003	43732	0%	14	semi-quantitative food frequency questionnaire	trans unsaturated fat		no	no
9149659	Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J.	Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study.	1997	21930	0%	6.1 (5-8)	Diet questionnaire	TFA	1.3-6.2g	serum	serum
22135871	Sartika RA.	Effect of trans fatty acids intake on blood lipid profile of workers in East Kalimantan, Indonesia.	2011	388	3%	<1 yr	semi-quantitative food frequency question, Nutrisoft	TFA	0.48% total energy	serum	serum
8094827	Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH.	Intake of trans fatty acids and risk of coronary heart disease among women.	1993	85095	100%	8	dietary questionnaire	mean intake of TFA	2.4-5.7g/d	no	no
11117615	Zhang SM, Willett WC, HernÃ¡n MA, Olek MJ, Ascherio A.	Dietary fat in relation to risk of multiple sclerosis among two large cohorts of women.	2000	92422	100%	14	Food frequency questionnaire	TFA	1.3-3.2% en	no	no
11117615	Zhang SM, Willett WC, HernÃ¡n MA, Olek MJ, Ascherio A.	Dietary fat in relation to risk of multiple sclerosis among two large cohorts of women.	2000	95389	100%	4	Food frequency questionnaire	TFA	0.9-2.4% en	no	no
8688759	Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willett WC.	Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States.	1996	43757	0%	6	Food frequency questionnaire	TFA	0.8-1.6% en	no	no
9229205	Gillman MW, Cupples LA, Gagnon D, Millen BE, Ellison RC, Castelli WP.	Margarine intake and subsequent coronary heart disease in men.	1996	832	0%	18-21	24-hour dietary recall	margarine	0->5 tsp/day	serum	serum

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Section Two:
Considerations for Updating Replacement Scenario Modeling

This section addresses the following question requested by the FDA in the docket:

1. Should FDA finalize its tentative determination that PHOs are no longer GRAS?

Background

Using 1999-2002 NHANES data, the Committee conducted a modeling analysis of predicted changes in cardiovascular disease risk in response to application-appropriate substitutions for *trans* fatty acid containing oils.[1] From this modeling work we predicted that, when considering solid fat replacement scenarios for partially hydrogenated soybean oil, each of the functional replacements (ranging in TFA content from 0.7-3.9% fat), would decrease LDL-C, with the exception of the highly saturated (46% fat) palm oil based shortening. This modeling exercise supports the data that is presented throughout these comments in that, when compared to higher saturated fatty acid containing fats, low level of *trans* fatty acids in fats confer a less detrimental or even beneficial effect on blood lipids.

Since this study's completion, the literature has developed, providing for a more robust update of this exercise and would allow for modeling of low levels of *trans* fatty acids intake to be modeled. Studying low levels of *trans* fatty acid intake is incredibly important, given that current consumption is ~1.0g/d (0.55% total energy) of *trans* from PHO on average.[2] Since the FDA's 2003 ruling on *trans* fatty acid label declaration, intakes have been significantly reduced from when our study was performed. Further, our modeling work was unable to fully realize all potential replacement scenarios at the individual product level, given the complexities of lipid use in the food supply. We believe that the study could be repeated using current intake and product reformulation data and performed using an even more comprehensive and detailed replacement methodology. Such data would provide extremely valuable and practical scientific evidence by which decisions can be made regarding usage of PHO in the foods supply.

Supporting points for the consideration of using application-appropriate modeling:

The FDA's tentative determination applies to partially hydrogenated oils (PHOs), not to all industrial *trans* fatty acids. The scientific literature provides many examples to potentially support setting specifications for the level of *trans* fatty acids in foods, and for the overall diet. However, to do this will require careful consideration of *trans* fatty acids in the context of a fat or oil as consumed to avoid unintended consequences. The importance of considering the impact of PHOs in this context must not be overlooked given the following realities of how fats and oils are used as food ingredients:

- As food ingredients, fats and oils are the tools for making foods and are composed of a mixture of fatty acids. The individual fatty acids are *not* the tools for making foods.
- The functionality of a fat and/or oil is a major consideration for its ingredient use in various foods. Whether the functionality is oxidative stability as a frying medium, or melting profile as a shortening, physical properties of a fat or oil are critical considerations when making foods.
- PHO is a general term that, in reality, represents a vast array of fats with widely differing fatty acids profiles, and varying in levels of *trans* fatty acids.
- There are several uses of PHOs in the food supply, such as processing aids, that contribute little to no *trans* fat to the diet.

Within the field of nutrition, the prominent fatty acid of the fat or oil is often used as the surrogate marker for the assigned 'health' status of that fat or oil. This approach is understandable for nutrition policy development. The intervention studies providing support for nutrition policy take steps to minimize variables in experimental design; however, it is extremely difficult to isolate effects of a single fatty acid within an experimental diet, and even more challenging to isolate these effects in a population's overall diet. Given the realities outlined above, the following examples highlight limitations in this individual fatty acid approach to making a determination with regard to PHOs [Note: These examples will focus on the impact of fats and oils on LDL-C levels given the FDA's focus of the effect of *trans* fatty acids on biomarkers in the notice (78 FR 67169) and its acceptance by the agency as one of two validated surrogate endpoints for coronary heart disease]. It is worth mentioning that the purpose of these examples is to address the issue of PHO *safety*, and not to address dietary guidance. We acknowledge that an overall dietary pattern emphasizing lower saturated and *trans* fatty acid intakes generally supports a reduction in coronary heart disease risk.

Example 1: Butter vs. Margarine

Butter and margarine both provide solid fat functionality to food products. It is well known that PHOs are used to create margarine to mimic the functional properties of butter and that margarine has a different fatty acid composition than butter. A meta-analysis by Zock and Katan[3] examined the effects of butter versus margarine on LDL-C. **Their findings demonstrate that substitution of butter, with either stick or tub margarines, decreases LDL-C levels.** The nature of this decrease in LDL-C is positively correlated with percent energy, indicating a greater caloric displacement when margarine is consumed instead of butter. This study also demonstrates the range of fatty acid profiles that can occur in products with similar intended uses. With regard to the aggregate fatty acid composition per 100g of butter or margarine, the authors reported the following:

- *Butter:* 51% saturates, **3% *trans***, 2% polyunsaturates, 21% monounsaturates;
- *Stick margarine:* 21% saturates, **24% *trans***, 11% polyunsaturates, 22% monounsaturates;
- *Tub margarine:* 18% saturates, **8% *trans***, 30% polyunsaturates, 22% monounsaturates.

Since this 1997 meta-analysis, four additional clinical studies have compared the effect of butter versus margarine on LDL-C.[4-7] All four studies showed that the replacement of butter with margarine resulted in a significant decrease in LDL-C ($p \leq 0.05$, <0.05, <0.001 and <0.009, respectively). Given that each study used different types of margarines, an examination of the details from each study is warranted.

1) Judd et al.[4] compared butter with two margarines (M): TFA-M and PUFA-M. Butter intake resulted in significantly higher LDL-C than intake of either margarine, with the TFA-M resulting in significantly higher LDL-C than PUFA-M. Total cholesterol followed the same pattern and there were no effects on HDL for any diet group. The authors reported the following fatty acid compositions for the spreads:
   - *Butter:* 53.1% saturated, **3.14% *trans****, 5.12% polyunsaturated, 30.63% monounsaturated**;
   - *TFA-M:* 16.44% saturated, **17.35% *trans****, 26.57% polyunsaturated, 47.87% monounsaturated**;
   - *PUFA-M:* 20.55% saturated, **ND *trans****, 49.41% polyunsaturated, 20.5% monounsaturated**.

     *Expressed as *trans* 18:1 monoenes; **expressed as cis 18:1 oleic, ND-none detected

2) Lichtenstein et al[5] examined the replacement of butter with four types of *trans*-containing fats: three margarines (stick, soft and semiliquid) and shortening. Compared to butter, all *trans*-containing fats significantly decreased LDL-C. Total cholesterol was also lower with intake of shortening, as well as for soft and semiliquid margarines. No statistically significant HDL lowering occurred with the diet treatments, and butter intake resulted in the highest concentration. The authors reported the following fatty acid compositions for the test fats:
   - *Butter:* 61.7% saturated, **1.5% *trans***, 7.0% polyunsaturated*, 21.5% monounsaturated*;
   - *Stick margarine:* 27.0% saturated, **20.1% *trans***, 20.5% polyunsaturated*, 25.4% monounsaturated*;
   - *Shortening:* 29.3% saturated, **25.5% *trans***, 25.5% polyunsaturated*, 32.2% monounsaturated*;
   - *Soft margarine:* 25.2% saturated, **7.4% *trans***, 35.5% polyunsaturated*, 25.6% monounsaturated*;
   - *Semiliquid margarine:* 27.0% saturated, **<0.5% *trans***, 42.0% polyunsaturated*, 25.4% monounsaturated*.
     *Only isomers containing cis double bonds were included.

3) Denke et al.[6] compared intakes of butter versus margarine on LDL-C. Margarine intake resulted in a significant decrease in LDL-C in both adults and children ($p<0.001$). The fatty acid compositions for butter and margarine were not described, however mean energy intakes were reported as follows:
   - *Butter:* 16% saturated, **0.5% *trans***, 3% polyunsaturated, 14% monounsaturated;
   - *Margarine:* 9% saturated, **1.5% *trans***, 10% polyunsaturated, 14% monounsaturated.

4) Chisholm et al.[7] also compared the effect of consuming butter versus margarine on LDL-C and observed significantly lower concentrations following the margarine diet compared to the butter. The authors reported the following aggregate fatty acid composition per 100g of butter or margarine:
   - *Butter:* 50.6% saturated, **4.4% *trans*,** 1.9% polyunsaturated, 24.4% monounsaturated.
   - *Margarine:* 13.7% saturated, **12.7% *trans***, 22.7% polyunsaturated, 41% monounsaturated.

A 2003 meta-analysis by Mensink and colleagues[8] examined the effects of dietary fatty acids on total:HDL-C, serum lipids and apolipoproteins, demonstrating a spectrum of response when compared to the "average" US dietary fat. The analysis showed that butter had the greatest impact on raising total:HDL-C, while rapeseed oil had the greatest impact on lowering total:HDL-C. The hierarchy of fats producing a response, falling between butter and rapeseed oil were: shortening, margarine (stick), palm oil, chocolate fat, coconut fat, margarine (tub), palm kernel fat, mayonnaise and soybean oil. The 'line' between fats, indicating those that increased and those that lowered total:HDL-C, occurred between chocolate fat and coconut fat. **A critical consideration that these authors brought forth is the importance of focusing on fats and oils *as used for consumption* as food ingredients.** Example 2 illustrates another functional comparison that would be included in an updated modeling study.

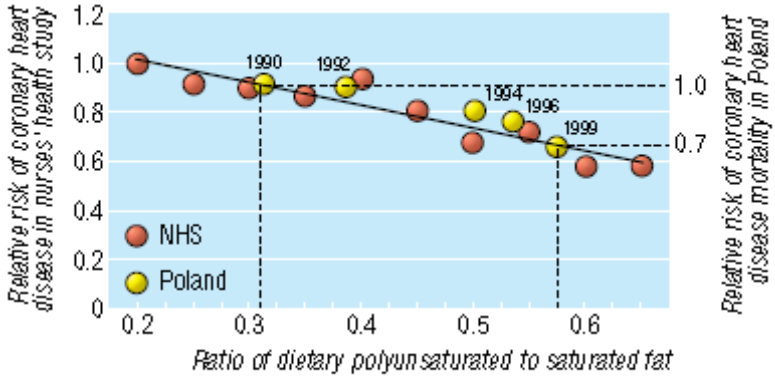
Example 2: High palmitic, trans 'free' vs. High oleic, 'low' trans

RP Mensink[9] tested equally functional semi-solid fats to determine their effect on various biomarkers related to cardiovascular disease and diabetes. The high palmitic, *trans* 'free' fat contained <2% *trans* fatty acids (1.0% *trans* fatty acids) and the high oleic, 'low' *trans* fat contained >2% *trans* fatty acids (4.5% *trans* fatty acids). In terms of percent energy (% en) from these experimental fats, the high palmitic, *trans* 'free' fat provided 0.2% en while the the high oleic, 'low' *trans* fat provided 0.7% en from *trans* fat. **The high palmitic, *trans* 'free' fat resulted in significantly greater LDL-C levels (p<0.001).** The author reported the following fatty acid compositions for the test fats:

- High palmitic, *trans* 'free': 43.5% saturated, **0.5% *trans***, 11.5% polyunsaturated, 44.0% monounsaturated;
- High oleic, 'low' *trans*: 22.0% saturated, **4.5% *trans***, 12.0% polyunsaturated, 61.5% monounsaturated.

The examples above explored the effects of *trans* fatty acid-containing fats versus highly saturated fats on LDL-C. As previously mentioned, PHOs represent a variety of fats, with varied fatty acid profiles. In many cases the PUFA content of PHO-containing fats is greater than for higher saturated fat alternatives. The removal of PHO from the food supply may decrease PUFA intake and increase saturated fat intake, depending on replacement scenarios. Increasing saturated fat in the diet while decreasing PUFA, could be of concern. Providing epidemiological support for this point are Zatonski and Willett's[10] data from Poland. The evidence presented may be less confounded than often seen with dietary epidemiological studies, as a result of its collection before and after significant agricultural policy changes caused shifts in food and food ingredient availability. These policies led to dramatic changes in consumption, reducing the intake of animal fats and increasing intake of vegetable-based fats. The authors expressed this change by reporting the polyunsaturated-to-saturated fatty acid ratio (P/S), shown in the figure below (Figure 1). An increased P/S was attributed for decreasing the relative risk (predicted, ~24% decrease) for coronary heart disease mortality, with predictions for smoking cessation (~5%) and increasing fruit and vegetable consumption (1-2%) much less impactful. The authors note that low levels of *trans* fatty acids were present in the diet, while stating that a conscious effort was underway to minimize their level in Polish margarines; however, estimated dietary intake of *trans* fatty acids from margarine was not reported. Nonetheless, the aforementioned meta-analysis by Zock and Katan, along with the studies of Judd, Lichtenstein, Denke and Chisolm each provide the clinical biomarker support for the observation reported by Zatonski and Willett.

Figure 1. Ratio of PUFA/Saturated Fat and Coronary Heart Disease Mortality in Poland[10]



Ratio of dietary polyunsaturated to saturated fat and mortality due to coronary heart disease in Poland (relative to rates in 1990), superimposed on the relation between the fat ratio and coronary risk observed in the nurses' health study. Changes in dietary polyunsaturated to saturated fat in Poland between 1990 and 1999 are predicted to result in a 24% drop in coronary mortality, which is similar to the observed decline

The evidence described above indicate that a level of *trans* fatty acids in food can be derived, from which there would be no significant impact on LDL-C levels, compared to the functional fats they would replace. This level could be based on either, the: 1) *trans* fatty acid level in fat or oil as consumed in a food, 2) *trans* fatty acid level in foods, 3) *trans* fatty acid level in the total diet, or 4) a combination of these three levels. Further, we present data that demonstrates replacement scenarios must receive careful consideration to avoid potential unintended consequences.

In Summary

These two substitution examples (butter vs. margarine and high palmitic/*trans* free vs. high oleic/low *trans*) present data that support a level of PHO containing *trans* fatty acids, particularly in fats and oils, which deliver comparable solid fat function and do not exhibit a detrimental effect on LDL-C, a validated surrogate endpoint for cardiovascular disease. When the data are examined for each application-appropriate substitution scenario, and fats are considered 'as consumed', it becomes possible to make informed decisions regarding the impact of fats in the food supply. Given that the function and use of fats in foods is complicated, we reiterate the importance of meticulously collecting all data available and consideration of each of the uses of PHOs in foods to model feasible replacement scenarios to understand if estimated nutritional improvements could be achieved.

Further, a consideration of the GRAS status of PHOs, which have a long history of use, requires a comprehensive review of the relevant science across the range of intended uses and intake levels. A focus on those studies where a low level was examined is necessary due to the current US intakes. It is important that the U.S. population be aware of the *trans* fatty acid content of the foods they consume and that they are encouraged to consume less through appropriate nutrition policy. However, given current functional substitutes, complete removal of PHOs from the food supply does not ensure safety, and may not be expected to result in the health benefits projected by the FDA.

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