Supporting Document 1

Updated systematic review examining the effect of fatty acids on serum lipids

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Executive Summary

In 2003, a meta-analysis of 60 trials was published estimating under iso-caloric conditions the effects of the dietary fatty-acid composition (a mixture of saturated fatty acids (SFA)), *cis*-monounsaturated fatty acids (MUFA) and *cis*-polyunsaturated fatty acids (PUFA) on the serum lipoprotein profile. MUFA was mainly restricted to oleic acid and PUFA to linoleic acid plus α-linolenic acid. It was concluded that the most optimal lipoprotein profile as related to the risk of coronary heart disease was achieved when SFA in the diet was replaced by MUFA and PUFA. Since then, several new studies have been published and an update of this earlier meta-analysis was indicated.

In the end the total data set now comprised 74 well-controlled dietary studies from 15 different countries providing 177 data points (e.g. diets). Intake of *trans* fatty acids in all these studies was below 2% of energy. Results indicated that the cholesterol-raising effect of a mixture of SFA was about twice as strong as the cholesterol-lowering effect of PUFA. The effects of MUFA on serum total cholesterol were comparable to those of carbohydrates. For LDL-cholesterol, MUFA had a statistically significant LDL cholesterol-lowering effect relative to carbohydrates. All three classes of fatty acids increased HDL-cholesterol relative to carbohydrates, although the effects of the c*is*-unsaturated fatty acids were less than those of a mixture of SFA. Replacement of carbohydrates by any class of fatty acids decreased fasting triacylglycerol concentrations. The effect was larger for PUFA than for MUFA and a mixture of SFA. The total to HDL cholesterol ratio did not change if a mixture of SFA replaced carbohydrates. The ratio decreased, however, if carbohydrates or a mixture of SFA were replaced by MUFA and even more if replaced by PUFA.

In 37 studies including 91 data points, the intakes of oleic acid, linoleic acid and α-linolenic acid were reported. Effects of oleic acid and linoleic acid were very similar to those for respectively MUFA and PUFA. For total cholesterol, LDL-cholesterol and HDL-cholesterol, coefficients for α-linolenic acid differed slightly from those of linoleic acid, but confidence intervals overlapped. For triacylglycerol and the total to HDL-cholesterol ratio, coefficients were very similar.

Abbreviations and Terms

Carb	Carbohydrates
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
TFA	*Trans* fatty acids
Carb → SFA	when 1% energy from carbohydrates is replaced with an equal amount of energy from saturated fatty acids
Carb → MUFA	when 1% energy from carbohydrates is replaced with an equal amount of energy from *cis*-monounsaturated fatty acids
Carb → PUFA	when 1% energy from carbohydrates is replaced with an equal amount of energy from *cis*-polyunsaturated fatty acids
%En	percent of energy
Δ	change in (delta)

Contents

1. Introduction

In 2003, a meta-analysis of 60 trials was published estimating the effects of the amount and fatty-acid composition of the diet on the serum lipoprotein profile [Mensink et al., 2003]. At that time, it was concluded, that the most optimal lipoprotein profile as related to the risk of coronary heart disease was achieved when *trans* and saturated fatty acids (TFA and SFA, respectively) in the diet were replaced by monounsaturated and polyunsaturated fatty acids (MUFA and PUFA, respectively). In that publication, MUFA was mainly restricted to oleic acid and PUFA to linoleic acid plus α-linolenic acid. Since then, several new studies have been published and an update of this earlier meta-analysis was indicated.

2. Methods

This report describes a subset of data and analyses as part of a larger project being carried out at the same time in support of updating WHO guidance on TFA and SFA on risk and risk factors of cardiovascular disease [Brouwer 2016, Mensink 2016]. The larger project also examined the effect of TFA and studies in which diets were specifically enriched in one of the individual SFA. Consequently, many details in this report reflect the larger WHO-project. This will be specifically noted where it occurs. At request of the Food Standards Australia New Zealand (FSANZ), two additional analyses were performed. First, oleic acid, linoleic acid and α-linolenic acid were included into the statistical model instead of MUFA and PUFA. Secondly, subgroup analyses were performed to compare the results of industry vs. non-industry funded studies. In addition, further results relating to MUFA and PUFA from the subgroup analyses are presented.

2.1 Criteria for selecting studies

2.1.1 Study characteristics

Study design

The study design used had to eliminate the effect of aspecific drifts of the outcome variables with time. This could be achieved by feeding the different diets side-by-side (parallel design) or by giving the diets to the volunteers in random order (cross-over or Latin square design). "Before-and-after" (sequential) designs were therefore excluded. Dietary periods had to be at least 13 days, because time is otherwise too short for serum lipids to reach a new steady-state situation [Brussaard et al. 1982, Keys et al. 1957].

Diets and interventions

Only studies with a thorough daily control of food intake were selected. Protein and alcohol intake had to be constant. Fatty acids had to be exchanged for other fatty acids or for carbohydrates. Possible effects of protein and alcohol on the serum lipids could therefore not be estimated. Daily cholesterol intake between diets within a study had to be comparable (<100 mg difference). Only diets with a reported TFA intake of 2 En% or less were included. If TFA intake was not reported, it was assumed to be less than 2 En%. Other concomitant interventions (e.g. weight loss) were not allowed. Diets that focused on (hydrogenated) very long chain (n-3) *cis*-PUFA (fish oils) or arachidonic acid were excluded. Therefore, differences in the intakes of *cis*-PUFA between the diets can be considered to equal the PUFA with eighteen carbon atoms (linoleic acid plus α-linolenic acid). Diets focusing on one specific SFA were also excluded. As estimates for the effects of the various fatty acids on serum lipids were based on within-study comparisons (see Statistical Methods), studies that could only provide one data point due to these selection criteria were also excluded.

Participants

Only studies were considered with apparently healthy adult subjects, who did not suffer from gross disturbances of lipid metabolism or from diabetes.

Outcome measures

Studies had to report one or more of serum / plasma total cholesterol, LDL-cholesterol, HDL-cholesterol or triacylglycerol concentrations.

2.2 Data collection and analysis

2.2.1 Identification of studies

Search strategy and selection of studies

This meta-analysis is an update of the results of an earlier published meta-analysis that examined the effects of a range of fatty acids on different serum lipid and lipoprotein parameters, including the relationships of interest to the current report [Mensink et al. 2003]. At that time, controlled dietary trials were identified - published between January 1970 and December 1998 - as an original article in English through a computer-assisted literature search. We also scanned reference lists and hand-searched journals. In total 60 studies were identified that met our inclusion criteria.

In 2009, a computer-assisted literature search was performed for articles published between January 1999 and December 2008 and the total data set now comprised 83 studies. Finally, on January 12, 2014 a computer-assisted literature search was performed in the PubMed database for

articles published between January 2009 and December 2013. Search terms can be found in **Annex 1**. After screening, an additional 8 articles were identified.

Selection of studies

A study was excluded if it was evident from the title or abstract that the study did not meet the inclusion criteria (e.g. the study addressed the effects of fish oils only, was not adequately controlled, was not an intervention study). Full texts of the remaining citations were reviewed for inclusion.

2.2.2 Data extraction and management

For studies meeting the inclusion criteria, data were extracted using standard data extraction forms. Data were then transferred in duplicate to EXCEL. Typing errors were corrected and the data were analysed for consistency (e.g. sum of fatty acids, sum of percent energy provided by the macronutrients). No attempt was made to contact authors to obtain additional data.

2.2.3 Assessment of risk of bias in included studies

Considering the stringent selection criteria, including control of food consumed by subjects, all studies were considered to be of good quality. As reported, there was little variation among the studies in the characteristics that are commonly used to assess the risk of bias.

2.2.4 Calculations

Plasma values for total and HDL cholesterol were multiplied by 1.030 and those for triacylglycerols by 1.029 to convert them to serum values [Laboratory Methods Committee of the Lipid Research Clinics 1977]. LDL-cholesterol concentrations were calculated using the Friedewald equation [Friedewald et al. 1972]. For the sake of uniformity, the total to HDL-cholesterol ratio and LDL-cholesterol concentration for all studies were also calculated, even if reported by the authors themselves.

Dietary fat contains on average 96 percent by weight as fatty acids; the other 4 percent are glycerol and other lipids [Greenfield and Southgate, 2001]. For publications in which the intakes of the various fatty acid classes had been normalized so as to add up to 100 percent of total fat, we converted intakes back into true fatty acid intakes by multiplying them by 0.96.

2.2.5 Statistical analysis

As dependent variables absolute lipid concentrations or the total to HDL-cholesterol on each diet were used. A dummy variable for each study was introduced into the model to ensure that only within-study diet-induced differences were analyzed. The estimate for that dummy variable can be envisaged as the mean estimated serum lipid parameter ("the intrinsic level"), when the participants from that study consumed a standardized fat-free diet. It varies between studies, due to differences in study population (e.g. genetic makeup, age, and body mass index), but also by for example the fiber, protein or cholesterol content of the background diet, which was constant within studies, but differed between studies.

Each data point consisted of the fatty acid composition of a particular diet (the independent variables) and the mean serum lipid concentration or ratio (the dependent variable) of a group of subjects, as obtained at the end of a dietary period. For parallel-designs, levels were adjusted for differences between the intervention groups at baseline. The regression coefficients estimated in this way are the predicted change in the mean serum lipid concentration or a ratio when carbohydrate intake decreases by one percent of energy and that of a particular fatty acid increases by the same amount of energy.

Effects on a particular outcome of all fatty acids within a certain category - a mixture of SFA, MUFA or PUFA - were estimated. Diets, in which the fatty acid composition of a particular class of fatty acids diverged markedly from that in normal mixed diets, were excluded as specified in the request from FSANZ. For example, diets specifically enriched in stearic acid were excluded. Including these data points would have resulted in less reliable estimates of the effects of a normal mixture of SFA, because the individual SFA have different effects on the serum lipid profile [Mensink et al., 2003]. In this model, effects were expressed as compared to those of an iso-caloric amount of carbohydrates. In a second model, effects were expressed relative to those of an iso-caloric amount of SFA. In this model, carbohydrates, MUFA and PUFA were included as independent variables.

In a third model, the effects of SFA, oleic acid, linoleic acid and α-linolenic acid as compared with carbohydrates were examined.

The validity of the regression models was examined in several ways. First of all, normality of the residuals was checked. If the residual was not normally distributed, the most extreme value(s) were excluded. This approach did not change conclusions, but resulted in narrower confidence intervals of the estimates. Also, the influence of each separate observation on the estimated regression coefficients was assessed using the Cook's distance. Observations with a Cook's distance >0.4 were excluded in the final analysis. Finally, visual inspection of plots did not suggest a relationship between residuals and the independent variables. This suggests that the differences between observed and predicted values (i.e. the residuals) did not depend on the absolute intake of a

particular (class of) fatty acid(s). Each data point was weighed for the number of participants. All statistical analyses were carried out SPSS version 23.

2.2.6 Subgroup and sensitivity analyses

To examine the robustness of the results, the impact of various parameters that differed between studies on the outcomes were examined into more detail. For this, analyses with SFA, MUFA and PUFA as dependent variables were repeated by

- baseline total, LDL and HDL cholesterol levels (defined as above or below the fraction-specific medians in the dataset)
- excluding studies that used liquid formula diets,
- comparing results of studies published before and in 1993 and later - as at that time the detrimental effects of TFA on serum lipids became known
- comparing results studies not funded by industrial parties vs. those of studies funded by at least 1 industrial party.

3. Results

3.1 Search results

The initial search for articles published between January 2009 and December 2013 returned 629 potentially eligible articles. After removing citations based on title or abstract, the full texts of 66 articles were assessed for inclusion, of which 8 were included. Together with the 83 articles from previous searches, in the end 91 dietary trials were included. Seven of these studies could not be used for the final calculations, because they yielded only one data point, as the intake of TFA in the other diets exceeded 2 En% and were therefore excluded. As specified in the request from FSANZ, another 10 studies had to be excluded, because they yielded only one or no data point, when diets were excluded that were specifically enriched in one of the individual SFA. The flow of records through screening, exclusion and inclusion of studies is shown in **Figure 1.** Full references and characteristics of the 74 studies included are presented in **Annex 2** and **Annex 3**.

3.2 Characteristics of included studies

The 74 trials used to examine the effects of the classes of fatty acids on serum lipids and lipoproteins yielded 177 diet data points and included 2172 volunteers, 65% (n=1412) men and 34% (n=746) women (**Annex 3**). For two studies with in total 14 subjects, the number of men and women was not specified. Thirty-eight studies were carried out in men only, two studies in women only, and 34 studies in men and women. The diets were fed for 13 to 91 days. Sixty-three studies used a cross-over design and eleven studies a parallel design. Forty-two studies were from the U.S.A.; seven from the Netherlands; six from Canada, three from Denmark or the United Kingdom; two from Israel, Germany or Spain; and one each from Finland, Italy, Malaysia, Norway, New Zealand, Austria and Sweden. Eleven diets from five studies consisted of liquid formula diets. Sixty-two trials reported the

mean age of their participants, which varied between 21 and 72 years (mean 39 years). For 56 studies, mean BMI values were reported, which ranged between 20.3 to 28.6 kg/m^2 (mean 24.3 kg/m^2). For serum total cholesterol (56 studies), mean pre-study levels ranged between 3.8 and 6.7 mmol/L (mean 5.1 mmol/L), for LDL-cholesterol (48 studies) between 2.3 and 4.8 mmol/L (mean 3.4 mmol/L), for HDL-cholesterol (47 studies) between 0.9 and 1.8 mmol/L (mean 1.2 mmol/L) and for triacylglycerol (51 studies) between 0.7 and 2.2 mmol/L (mean 1.2 mmol/L).

The number of diet data points included in the calculations varied from 159 for the total to HDL-cholesterol ratio (66 studies) to 177 (74 studies) for total cholesterol. Mean intake of fat in these 177 diets was 34.0 percent of total daily energy (En%: range 4.5-53.0 En%), of SFA 9.8 En% (1.6 to 24.4 En%), of MUFA 13.6 En% (1.6 to 39.8 En%), and of PUFA 8.4 En% (0.4 to 28.8 En%) **(Figure 2)**.

3.3 Effect estimates

The regression equations indicated that cholesterol-raising effect of a mixture of SFA was about twice as strong as the cholesterol-lowering effect of PUFA (**Table 1**). The effects of MUFA on serum total cholesterol were comparable to those of an iso-caloric amount of carbohydrates. For LDL-cholesterol, however, MUFA had a statistically significant LDL cholesterol-lowering effect relative to carbohydrates.

All three classes of fatty acids increased HDL-cholesterol relative to carbohydrates, although the effects of the c*is*-unsaturated fatty acids were less than those of a mixture of SFA. Effects on fasting serum triacylglycerol concentrations were opposite to those on HDL-cholesterol. Replacement of carbohydrates by any class of fatty acids decreased fasting triacylglycerol concentrations. The effect was larger for PUFA than for MUFA and a mixture of SFA.

The total to HDL cholesterol ratio did not change if a mixture of SFA was replaced by carbohydrates. The ratio decreased, however, if carbohydrates or a mixture of SFA were replaced by MUFA and even more if replaced by PUFA.

As explained in the statistical method section, the regression coefficients in Table 1 represent the predicted change in the mean serum lipid or apolipoprotein concentration or a ratio when carbohydrate intake decreases by one percent of energy and that of a particular fatty acid increases by the same amount. Likewise, effects of total carbohydrate intake, MUFA intake and of PUFA intake can be expressed relative to those of a mixture of SFA. Regression coefficients will change, as another point of reference is used (SFA instead of carbohydrates). Theoretically, the coefficient for Carb $\rightarrow$ SFA (Table 1) should be exactly opposite to the coefficient for SFA $\rightarrow$ Carb (**Table 2**). Minor differences may exist, as the sum of the energy intakes of the macronutrients did not always add up to exactly 100%. For example, the regression coefficient for the exchange of Carb $\rightarrow$ SFA for LDL-cholesterol is 0.036 mmol/L. When calculations were repeated using SFA as point of reference (Table 2), the regression coefficient for SFA $\rightarrow$ Carb was slightly different (-0.033 mmol/L). From Table 1, it

can further be calculated that for LDL-cholesterol the difference in the coefficients of PUFA and SFA is -0.022 – 0.045 = -0.067 mmol/L. This difference can be interpreted, as the expected change in LDL-cholesterol when one percent of energy in the diet from SFA is replaced isocalorically by PUFA. Indeed, Table 2 shows that the coefficient for LDL-cholesterol for the exchange SFA $\rightarrow$ PUFA equals -0.064 mmol/L.

In 37 studies including 91 data points, the intakes of oleic acid, linoleic acid and α-linolenic acid were reported. In these studies, oleic acid contributed on average ~94% to total *cis*-MUFA, linoleic acid ~90% to total *cis*-PUFA intake and α-linolenic acid ~10% to total *cis*-PUFA intake (Figure 2). **Table 3** shows that coefficients for oleic acid and linoleic acid were very similar to those for respectively MUFA and PUFA, as reported in Table 1. In particular for total cholesterol, LDL-cholesterol and HDL-cholesterol, coefficients for α-linolenic acid differed slightly from those of linoleic acid, but confidence intervals overlapped. For triaclyglycerol and the total to HDL-cholesterol ratio, coefficients were very similar.

3.4 Subgroup analyses and sensitivity analyses

3.4.1 Baseline levels

As explained (see 2.2.5), the estimate for the dummy variable in the regression model can be envisaged as the mean estimated serum lipid level, when the participants from that study consumed a standardized fat-free diet. This estimate is a constant within studies, but differs between studies. In other words, it can be considered as a proxy for baseline lipid concentrations.

To examine if baseline levels were related to responses, subgroup analyses were performed. For this, the group was split into a low and high baseline groups based on the median level as estimated for each parameter based on the model presented in Table 1. The median level when subjects consumed a standardized fat-free diet was for total cholesterol 4.45 mmol/L, for LDL-cholesterol 2.89 mmol/L, for HDL-cholesterol 0.97 mmol/L, for triacylglycerol 1.48 mmol/L, and for the total to HDL-cholesterol ratio 4.36.

Effect estimates

Results are presented in **Annex 4**. The direction and statistical significance of the estimates did not depend on baseline levels. Effects, however, were in general more pronounced at higher baseline levels.

3.4.2 Liquid formula diets

Eleven diets from five studies consisted of liquid formula diets. To examine the impact of these diets on the outcomes, analyses were repeated by excluding these studies.

Effect estimates

Results, as shown in **Annex 5**, do not suggest that removing studies that employed liquid formula diets substantially changed the results.

3.4.3 Year of publication

In 1990, the detrimental effects of TFA on the serum lipoprotein profile were published for the first time. This may have resulted in an increasing awareness to better analyse and report the intake of TFA of the study diets. Thirty-four studies were published before 1993 and 40 studies in 1993 or later.

Effect estimates

Results are presented in **Annex 6**. The direction and statistical significance of the estimates did not depend on the year of publication. Also, the magnitude estimates were in good agreement, although effects of PUFA on serum total and LDL-cholesterol were higher for studies published in 1993 or later.

3.3.4 Source of funding

Of the 74 studies, 8 studies did not report any information on the source of funding. Of the other 66 studies, 34 reported only non-industrial parties as source of funding, while 32 studies reported at least 1 industrial party as source of funding.

Effect estimates

The results, as presented in **Annex 7**, the source of funding was not related to the direction and statistical significance of the estimates. However, for total and LDL cholesterol and for the total to HD-cholesterol ratio, effects of PUFA were more pronounced for studies with at least 1 industrial party as source of funding. It should be noted that in kind contributions of, for example margarines/oils/foods, were not defined as funded by industry.

References

Brouwer, IA. Effect of trans-fatty acid intake on blood lipids and lipoproteins: a systematic review and meta-regression analysis. Geneva: World Health Organization; 2016

http://www.who.int/nutrition/publications/nutrientrequirements/tfa_systematic_review/en/

Brussaard JH, Katan MB, Groot PH, Havekes LM, Hautvast JG. Serum lipoproteins of healthy persons fed a low-fat diet or a polyunsaturated fat diet for three months. A comparison of two cholesterol-lowering diets. Atherosclerosis. 1982;42:205–219

Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502

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Keys A, Anderson JT, Grande F. Prediction of serum-cholesterol responses of man to changes in fats in the diet. Lancet. 1957;273:959–966

Laboratory Methods Committee of the Lipid Research Clinics. Cholesterol and triglyceride concentrations in serum/plasma pairs. Clin Chem. 1977;23:60–63

Mensink, RP. Effects of saturated fatty acids on serum lipids and lipoproteins: a systematic review and regression analysis. Geneva: World Health Organization; 2016.

http://www.who.int/nutrition/publications/nutrientrequirements/sfa_systematic_review/en/

Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003;77:1146–1155

Tables and annexes

Table 1: Estimated multiple regression equations for the mean changes in serum lipids and lipoproteins when one percent of energy in the diet from carbohydrates in the diet is replaced isocalorically by saturated fatty acids (Carb → SFA), by *cis*-monounsaturated fatty acids (Carb → MUFA) or by *cis*-polyunsaturated fatty acids (Carb → PUFA)

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
ΔTotal cholesterol	mmol/L	0.045	-0.004	-0.022	177/74/2172
95% CI		0.038 to 0.051	-0.010 to 0.001	-0.028 to -0.016	
P-value		<0.001	0.097	<0.001	
ΔLDL-cholesterol	mmol/L	0.036	-0.009	-0.022	165/69/2026
95% CI		0.030 to 0.043	-0.014 to -0.003	-0.028 to -0.015	
P-value		<0.001	0.002	<0.001	
ΔHDL-cholesterol	mmol/L	0.011	0.008	0.006	163/68/2017
95% CI		0.010 to 0.013	0.007 to 0.010	0.004 to 0.008	
P-value		<0.001	<0.001	<0.001	
ΔTriacylglycerol	mmol/L	-0.012	-0.015	-0.021	172/72/2156
95% CI		-0.015 to -0.008	-0.018 to -0.011	-0.025 to -0.017	
P-value		<0.001	<0.001	<0.001	
ΔTotal to HDL-cholesterol		-0.002	-0.029	-0.036	159/66/1990
95% CI		-0.009 to 0.005	-0.035 to -0.023	-0.043 to -0.029	
P-value		0.553	<0.001	<0.001	

The 95 percent confidence intervals (CI) refer to the regression coefficients on the preceding line.
No: Number of diets/number of studies/number of subjects.

Table 2: Estimated multiple regression equations for the mean changes in serum lipids and lipoproteins when one percent of energy in the diet from saturated fatty acids (SFA) is replaced isocalorically by carbohydrates (SFA → Carb), by *cis*-monounsaturated fatty acids (SFA → MUFA) or by *cis*-polyunsaturated fatty acids (SFA → PUFA)

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		SFA → Carb	SFA → MUFA	SFA → PUFA	
ΔTotal cholesterol	mmol/L	-0.041	-0.046	-0.064	177/74/2172
95% CI		-0.047 to -0.035	-0.051 to -0.040	-0.070 to -0.058	
P-value		<0.001	<0.001	<0.001	
ΔLDL-cholesterol	mmol/L	-0.033	-0.042	-0.055	165/69/2026
95% CI		-0.039 to -0.027	-0.047 to -0.037	-0.061 to -0.050	
P-value		<0.001	<0.001	<0.001	
ΔHDL-cholesterol	mmol/L	-0.010	-0.002	-0.005	163/68/2017
95% CI		-0.012 to -0.008	-0.004 to 0.000	-0.006 to -0.003	
P-value		<0.001	0.014	<0.001	
ΔTriacylglycerol	mmol/L	0.011	-0.004	-0.010	172/72/2156
95% CI		0.007 to 0.014	-0.007 to -0.001	-0.014 to -0.007	
P-value		<0.001	0.022	<0.001	
ΔTotal to HDL-cholesterol		0.001	-0.027	-0.034	159/66/1990
95% CI		-0.006 to 0.007	-0.033 to -0.022	-0.040 to -0.028	
P-value		0.842	<0.001	<0.001	

The 95 percent confidence intervals (CI) refer to the regression coefficients on the preceding line.
No: Number of diets/number of studies/number of subjects.

Table 3: Estimated multiple regression equations for the mean changes in serum lipids and lipoproteins when 1% of energy in the diet from carbohydrates is replaced isocalorically by saturated fatty acid (SFA), oleic acid (OA), linoleic acid (LA) or α-linolenic acid (ALA)

Lipid or lipoprotein	Unit	Change per percent of energy replaced				No
		Carb → SFA	Carb → OA	SFA → PUFA	Carb → ALA	
ΔTotal cholesterol	mmol/L	0.039	-0.013	-0.028	-0.049	91/37/1125
95% CI		0.026 to 0.051	-0.023 to -0.002	-0.038 to -0.017	-0.077 to -0.022	
P-value		<0.001	0.017	<0.001	0.001	
ΔLDL-cholesterol	mmol/L	0.036	-0.014	-0.023	-0.039	87/35/1041
95% CI		0.024 to 0.047	-0.024 to -0.005	-0.033 to -0.014	-0.063 to -0.014	
P-value		<0.001	0.003	<0.001	0.003	
ΔHDL-cholesterol	mmol/L	0.010	0.009	0.005	0.000	87/35/1041
95% CI		0.008 to 0.013	0.007 to 0.011	0.003 to 0.008	-0.006 to 0.006	
P-value		<0.001	<0.001	<0.001	0.996	
ΔTriacylglycerol	mmol/L	-0.012	-0.015	-0.021	-0.023	91/37/1125
95% CI		-0.019 to -0.006	-0.021 to -0.010	-0.027 to -0.016	-0.037 to -0.008	
P-value		<0.001	<0.001	<0.001	0.003	

Lipid or lipoprotein	Unit	Change per percent of energy replaced				No
		Carb → SFA	Carb → OA	SFA → PUFA	Carb → ALA	
ΔTotal to HDL-cholesterol ratio		-0.001	-0.034	-0.034	-0.032	85/34/1021
95% CI		-0.011 to 0.009	-0.043 to -0.026	-0.043 to -0.025	-0.054 to -0.010	
P-value		0.834	<0.001	<0.001	0.005	

The 95 percent confidence intervals (CI) refer to the regression coefficients on the preceding line.

No: Number of diets/number of studies/number of subjects.

Figure 1: Flow diagram showing the study selection procedure of human intervention studies for the meta-analysis to examine the relationship between dietary fatty acid intake with serum lipids and lipoproteins

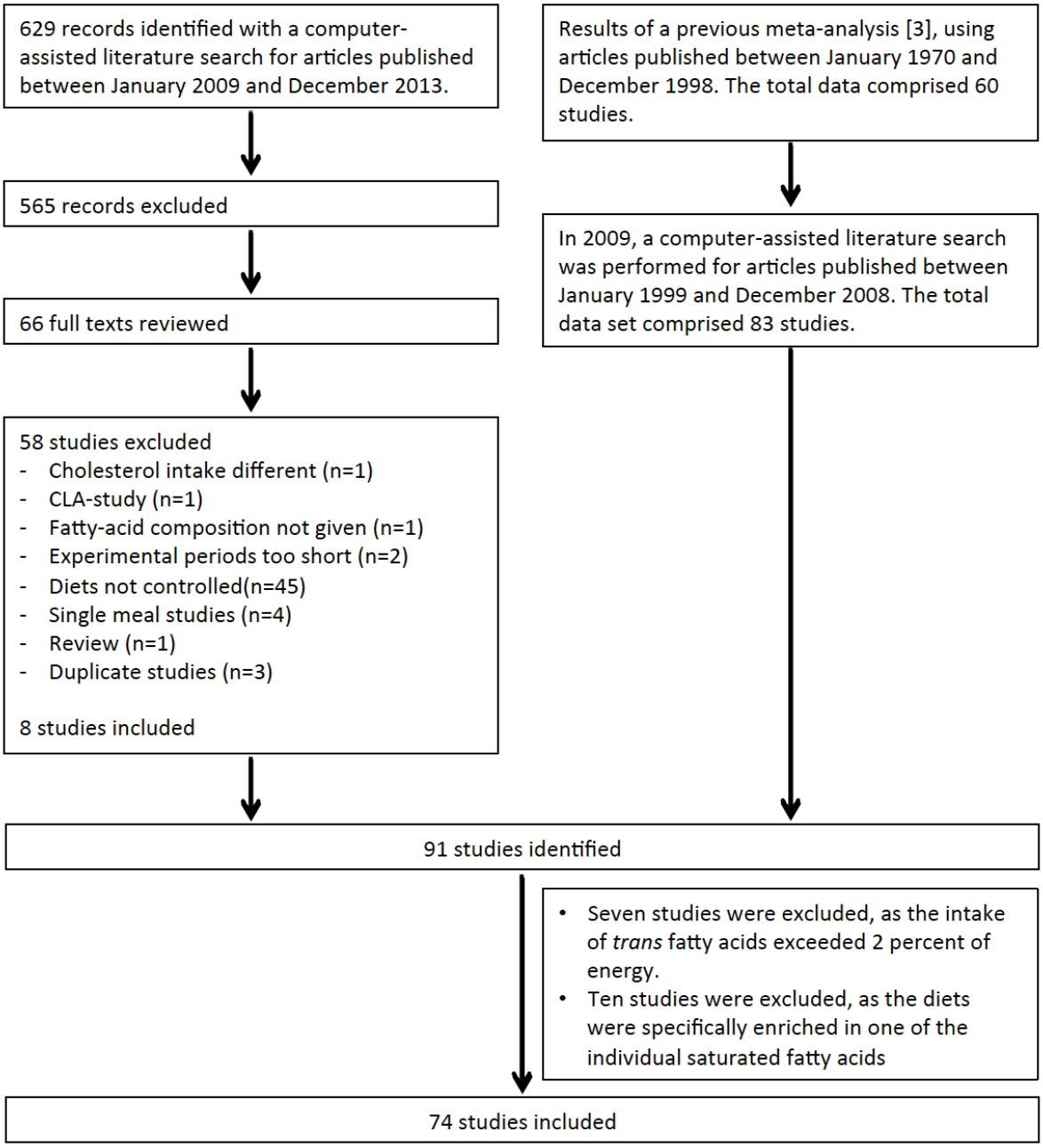
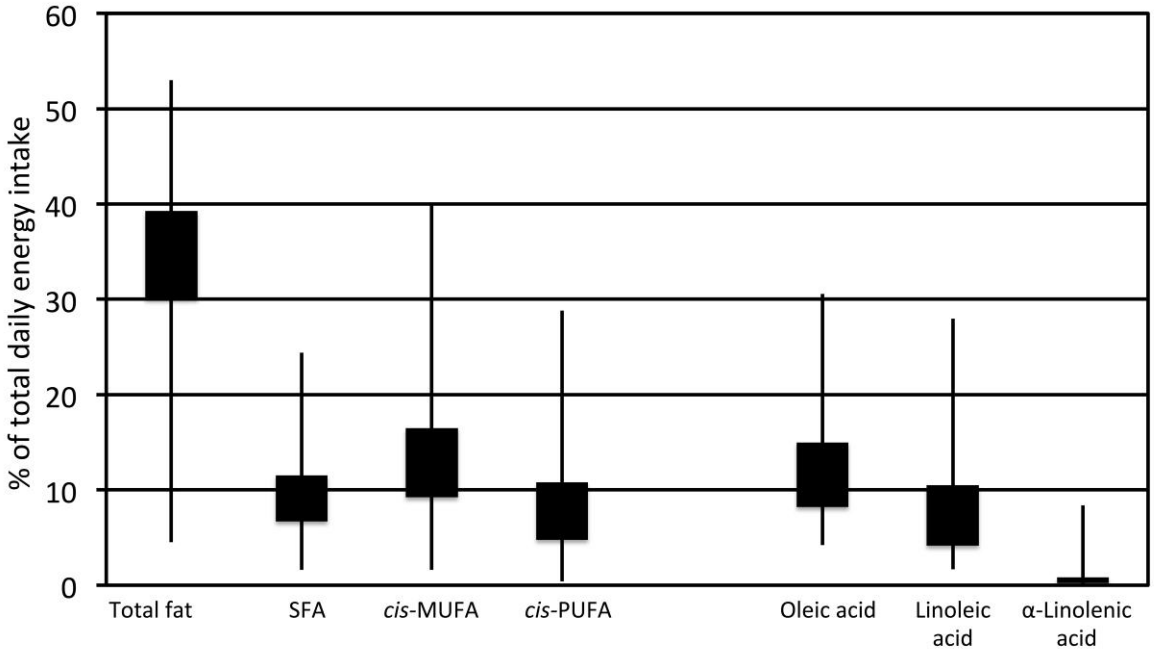


Figure 2: Intakes of total fat and the various fatty acids. The solid rectangles indicate the 25th percentile and 75th percentile, and the lines the minimum and maximum intakes



Annex 1: Search strategy

PubMed

(((((((("comparative study"[Publication Type]) OR "randomized controlled trial"[Publication Type]) OR "controlled clinical trial"[Publication Type]))

AND

((((("cholesterol/blood"[MeSH Terms]) OR "cholesterol, ldl/blood"[MeSH Terms]) OR "lipids/blood"[MeSH Terms]) OR "lipoproteins/blood")))

AND

"humans"[MeSH Terms]))

AND

((dietary fat*[MeSH Terms]) OR (((((palmitic acid*[MeSH Terms]) OR stearic acid*[MeSH Terms]) OR myristic acid*[MeSH Terms])) OR lauric acid*[MeSH Terms]))

Annex 2: List of studies included (listed in the order shown in Annex 3)

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Annex 3: Characteristics of the studies included

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Mensink and Katan, 1987 Mensink et al., 1989 The Netherlands	Randomized parallel design with two interventions Experimental period: 35 days	1. 2.	6.7 9.8	9.3 24.0	5.2 5.1		• Initial: 57, final: 48 • Reason for loss: influenza (n=3), change in smoking habits (n=2), weight loss (n=4)	• Diet 1: 12 men, 12 women • Diet 2: 12 men, 12 women • Mean age: 27 years	• The Commission of the European Communities
Mattson and Grundy, 1985 USA	Randomized crossover design with three interventions	1. 2. 3.	19.1 3.3 4.3	15.4 28.2 5.6	3.9 6.9 28.1		• Initial: 12, final: 12 • No dropouts reported	• 12 men • Mean age: 59 years	• Veterans Administration • National Institutes of Health • Moss Heart Foundation

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 28 days								
Grundy, 1986 USA	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	3.8 6.4	26.9 6.4	7.7 6.4		• Initial: 7, final: 7 • No dropouts reported	• Sex not reported • Mean age: 58 years	• Veterans Administration • National Institutes of Health • Southwestern Medical Foundation • Mead Johnson and Company • Moss Heart Foundation
Brussaard et al., 1980 The Netherlands	Randomized parallel design with four interventions	1. 2. 3.	8.0 10.0 11.0	10.0 8.0 8.0	3.0 11.0 19.0		• Initial: 60, final: 60 • No dropouts reported	• 37 men and 23 women • Diet 1: 16 subjects • Diet 2: 15 subjects • Diet 3: 15 subjects	• The Netherlands Heart Foundation

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 35 days	4.	18.0	16.0	3.0			• Diet 4: 14 subjects • Sex distribution not reported. • Age: 18-28 years	
Brussaard et al., 1982 The Netherlands	Randomized parallel design with two interventions Experimental period: 91 days	1. 2.	9.0 7.0	10.0 8.0	11.0 4.0		• Initial: 35, final: 35 • No dropouts reported	• Diet 1: 11 men and 6 women • Diet 2: 12 men and 6 women • Age: 19-30 years	• The Netherlands Heart Foundation
Mensink and Katan, 1989 The Netherlands	Randomized parallel design with two interventions	1. 2.	12.9 12.6	15.1 10.8	7.9 12.7		• Initial: 60, final: 58 • No reason for loss reported	• Diet 1: 14 men and 15 women • Diet 2: 13 men and 16 women • Mean age: 25 years	• Netherlands Nutrition Foundation • The Netherlands Heart Foundation • The Netherlands Ministry of Health

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 35 days								
Harris et al., 1983 USA	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	14.4 6.4	16.4 10.8	7.2 21.6		• Initial: 7, final: 7 • No dropouts reported	• Sex not reported • Mean age: 40 years	• National Heart, Lung, and Blood Institute • Clinical Research Center Grant
Becker et al., 1983 USA	Randomized crossover design with two interventions	1. 2.	2.7 4.0	29.2 15.1	6.5 17.5		• Initial: 12, final: 12 • No dropouts reported	• 12 men • Mean age: 32 years	• Clinical Research Center Program • National Institutes of Health • Corn Products

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 28 days								
Bonanome and Grundy, 1988 USA	Randomized crossover design with two interventions Experimental period: 21 days	1. 2.	19.6 3.1	14.9 30.6	3.7 4.7		• Initial: 11, final: 11 • No dropouts reported	• 11 men • Mean age: 72 years	• Not reported
Grundy et al., 1986 USA	Randomized crossover design with two interventions Experimental period: 60 days	1. 2.	9.6 9.6	12.5 9.6	16.3 9.6		• Initial: 9, final: 9 • No dropouts reported	• 9 men • Mean age: 63 years	• Veterans Administration / National Institutes of Health • Southwestern Medical Foundation • Moss Heart Foundation

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Katan et al., 1988 The Netherlands	Randomized crossover design with two interventions Experimental period: 21 days	1. 2.	23.4 11.6	14.1 11.7	5.2 20.9	1.9 0.5	• Initial: 54, final: 47 • Reason for loss: illness, weight loss, poor compliance	• 24 men and 23 women • Mean age: 44 years	• The Netherlands Heart Foundation
Grande et al., 1972 USA	Randomized crossover design with four interventions Experimental period: 28 days	1. 2. 3. 4.	2.3 3.3 5.2 8.7	1.6 6.5 16.9 7.1	0.6 2.7 6.7 13.3		• Initial: 48, final: 38 • Reason for loss: transport to another institution, illness, poor eating habits	• 38 men • Mean age: 56 years	• Public Health Service Research Grants
Anderson et al., 1976	Randomized crossover design	1. 2.	19.6 4.8	8.4 5.1	5.2 22.7		• Initial: 12, final: 12 • No dropouts reported	• 12 men • Mean age: 21 years	• Public Health Service Research Grants

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
USA	with two interventions Experimental period: 14 days								
Anderson et al., 1976 USA	Randomized crossover design with two interventions Experimental period: 14 days	1. 2.	19.4 4.8	8.4 5.1	5.1 22.9		• Initial: 12, final: 12 • No dropouts reported	• 12 men • Mean age: 21 years	• Public Health Service Research Grants
Grundy et al., 1988 USA	Randomized crossover design with two interventions	1. 2.	6.7 6.7	25.9 6.7	5.8 5.8		• Initial: 10, final: 10 • No dropouts reported	• 10 men • Mean age: 64 years	• Veterans Administration • National Institutes of Health • Moss Heart Foundation

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 42 days								
Reiser et al., 1985 USA	Randomized crossover design with two interventions Experimental period: 35 days	1. 2.	9.4 1.6	10.4 2.2	0.4 16.2		• Initial: 19, final: 19 • No dropouts reported	• 19 men • Mean age: 26 years	• National Heart and Blood Vessel Research • National Heart, Lung, and Blood Institute • National Institutes of Health • Clinical Research USDHS Grant • Lipid Research Clinics • National Live Stock and Meat Board • The Texas Cattle Feeders Association • The Standard Meat Co of Fort Worth
Laine et al., 1982 USA	Randomized crossover design	1. 2.	8.6 2.6	7.7 4.6	1.8 11.1		• Initial: 24, final: 24 • No dropouts reported	• 13 men and 11 women • Mean age: 25 years	• American Soy Bean Association • General Clinical Research Centers Program

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	with three interventions Experimental period: 20 days	3.	3.0	4.2	11.1				• National Institutes of Health
Lewis et al., 1981 United Kingdom	Randomized crossover design with three interventions Experimental period: 35 days	1. 2. 3.	9.6 9.4 13.4	9.2 9.2 13.2	7.2 7.3 11.7		• Initial: 12, final: 12 • No dropouts reported	• 12 men • Mean age: 45 years	• Not reported
McDonald et al., 1989 Canada	Randomized crossover design with two interventions	1. 2.	5.1 6.8	20.2 7.4	10.3 21.6		• Initial: 8, final: 8 • No dropouts reported	• 8 men • Age: 19-32 years	• Canola Council of Canada

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 18 days								
Mensink and Katan, 1990 The Netherlands	Randomized crossover design with two interventions Experimental period: 21 days	1. 2.	9.3 19.4	23.7 13.6	4.4 3.0	0.0 0.7	• Initial: 59, final: 59 • No dropouts reported	• 25 men and 34 women • Mean age: 26 years	• The Netherlands Nutrition Foundation • The Netherlands Ministry of Welfare, Public Health, and Cultural Affairs • The Commission of the European Communities
Valsta et al., 1992 Finland	Randomized crossover design with two interventions Experimental period: 25 days	1. 2.	12.4 12.7	16.2 10.2	7.6 13.3		• Initial: 59, final: 59 • No dropouts reported	• 29 men and 30 women • Mean age: 30 years	• Food Research Foundation • The Ministry of Agriculture and Forestry • The Yrjö Jahnsson Foundation • The Academy of Finland • The Finnish Cultural Foundation

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Wahrburg et al., 1992 Germany	Randomized crossover design with two interventions Experimental period: 23 days	1. 2.	10.2 10.1	16.0 9.9	4.1 10.3		• Initial: 40, final: 38 • Reason for loss: illness (n=1), genetic anomaly of lipid metabolism (n=1)	• 21 men and 17 women • Mean age: 24 years	• The Commission of the European Communities
Wardlaw and Snook, 1990 USA	Randomized crossover design with two interventions Experimental period: 35 days	1. 2.	6.7 7.7	26.9 13.4	5.8 18.2		• Initial: 22, final: 20 • Reason for loss: not reported	• 20 men • Mean age: 35 years	• SVO Enterprises
Ginsberg et al., 1990	Randomized parallel design	1. 2.	9.0 8.8	10.6 17.2	10.0 10.1		• Initial: 39, final: 36 • Reason for loss: allergy (n=1), poor compliance (n=2)	• Diet 1: 12 men • Diet 2: 12 men • Diet 3: 12 men • Mean age: 23 years	• The National Institutes of Heath • Best Foods • Kraft Inc. • Bertolli

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
USA	with two interventions Experimental period: 70 days								
Chan et al., 1991 Canada	Randomized crossover design with four interventions Experimental period: 18 days	1. 2. 3. 4.	6.5 5.3 7.1 6.4	18.7 18.3 8.4 9.9	7.4 8.5 16.8 16.1		• Initial: 8, final: 8 • One subject dropped out and was replaced	• 8 men • Age: 20-34 years	• Canola Council of Canada • Flax Council of Canada
Wardlaw et al., 1991 USA	Randomized parallel design with two interventions	1. 2.	6.7 6.7	21.1 8.6	10.6 21.1		• Initial: 34, final: 32 • Reason for loss: medication (n=1), unusual lipid values (n=1)	• Diet 1: 16 men • Diet 2: 16 men • Mean age: 33 years	• The Procter & Gamble Company

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 56 days								
Berry et al., 1991 Israel	Randomized crossover design with two interventions Experimental period: 84 days	1. 2.	8.0 7.1	15.9 6.2	7.5 16.0		• Initial: 26, final: 18 • Reason for loss: drop out (n=4), incomplete blood sampling (n=4)	• 18 men • Mean age: not reported	• The National Institutes of Health
Berry et al., 1992 Israel	Randomized crossover design with two interventions Experimental period: 84 days	1. 2.	6.6 4.7	16.6 6.8	7.5 5.7		• Initial: 25, final: 17 • Reason for loss: not reported	• 17 men • Age: 18-24 years	• The National Institutes of Health, Public Health Service

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Kris-Etherton et al., 1993 USA	Randomized crossover design with three interventions Experimental period: 26 days	1. 2. 3.	6.0 6.3 21.0	27.2 10.1 10.1	2.3 17.8 1.7		• Initial: 19, final: 18 • Reason for loss: not reported	• 18 men • Mean age: 26 years	• The American Cocoa Research Institute • The Pennsylvania Agricultural Experimental Station
Denke and Grundy, 1992 USA	Randomized crossover design with two interventions Experimental period: 21 days	1. 2.	2.6 18.9	29.1 15.4	6.0 3.8		• Initial: 14, final: 14 • No dropouts reported	• 14 men • Mean age: 63 years	• Southwestern Medical Foundation • Moss heart Foundation • Veterans' Affairs • National Heart, Lung, and Blood Institute
Bonanome et al., 1992	Randomized crossover design	1. 2.	9.6 9.6	28.8 4.8	4.8 28.8		• Initial: 11, final: 11 • No dropouts reported	• 11 men • Mean age: 22 years	• The European Economic Community

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Italy	with two interventions Experimental period: 21 days								
Judd et al., 1994 USA	Randomized crossover design with two interventions Experimental period: 42 days	1. 2.	14.0 20.1	16.4 10.9	5.9 5.8	0.7 0.7	• Initial: 64, final: 58 • Reason for loss: illness (n=1), no reason reported (n=1), other commitments (n=3), non-compliance (n=1)	• 29 men, 29 women • Mean age:43 years	• Institute of Shortening and Edible Oils and its member companies
Zock et al., 1994 The Netherlands	Randomized crossover design with two interventions	1. 2.	21.0 10.8	11.9 21.3	4.7 4.4	0.2 0.3	• Initial: 59, final: 59 • No dropouts reported	• 23 men and 36 women • Mean age: 29 years	• Foundation for Nutrition and Health Sciences

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 21 days								
Barr et al., 1992 USA	Randomized parallel design with two interventions Experimental period: 49 days	1. 2.	9.0 12.2	13.2 10.8	7.8 6.5		• Initial: 51, final: 48 • Reason for loss: illness (n=1), poor compliance (n=2) • 17 men received a diet that was not included in the meta-analysis	• Diet 1: 15 men • Diet 2: 16 men • Mean age: 25 years	• National Institutes of Health • Best Foods, Kraft Inc. • Bertolli
Ginsberg et al., 1994 USA	Randomized parallel design with two interventions Experimental period: 42 days	1. 2.	8.9 9.1	8.4 13.2	11.4 6.4		• Initial: 30, final: 30 • No dropouts reported • 12 men received a diet that was not included in the meta-analysis	• Diet 1: 9 men • Diet 2: 9 men • Mean age: 25 years	• National Institutes of Health • Best Foods, Kraft Inc. • Bertolli

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Judd et al., 1988 Marshall et al., 1986 USA	Randomized crossover design with two interventions Experimental period: 42 days	1. 2.	6.7 10.6	11.4 10.4	6.5 3.3		• Initial: 24, final: 23 • Reason for loss: personal	• 23 men • Age: 35-60 years	• Not reported
Sundram et al., 1995 Malaysia	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	6.0 13.0	17.5 14.3	7.7 4.1		• Initial: 24, final: 23 • Reason for loss: not reported	• 23 men • Mean age: 22 years	• Not reported
Iacona and Dougherty, 1991	Randomized crossover design	1. 2.	9.5 8.6	9.4 8.7	3.8 10.8		• Initial: 11, final: 11 • No dropouts reported	• 11 men • Mean age: 54 years	• Not reported

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
USA	with two interventions Experimental period: 40 days								
Lichtenstein et al, 1993 Lichtenstein et al, 1994 Lichtenstein et al, 1994 USA	Randomized crossover design with five interventions Experimental period: 32 days	1. 2. 3. 4. 5.	5.4 6.9 6.9 12.1 7.4	14.5 9.0 17.0 11.3 10.8	6.7 11.2 3.9 3.4 8.8		• Initial: 15, final: 14 • Reason for loss: scheduling problems (n=1)	• 6 men and 8 women • Mean age: 63 years	• US Department of Agriculture • National Institutes of Health • Uncle Bens, Inc
Marckmann et al., 1992 Denmark	Randomized crossover design with two interventions	1. 2.	15.4 13.5	11.8 8.2	6.0 4.7		• Initial: 13, final: 13 • No dropouts reported	• 6 men and 17 women • Mean age: 26 years	• The Danish Heart Foundation • The Danish Health Insurance Foundation • The Danish Agricultural and

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 14 days								Veterinary Research Council
Howard et al., 1995 USA	Randomized crossover design with four interventions Experimental period: 42 days	1. 2. 3. 4.	8.2 8.0 9.4 9.5	14.2 12.1 8.5 5.7	3.1 4.8 7.2 12.5		• Initial: 77, final: 63 • Reason for loss: employment obligations (n=4), poor compliance (n=9), loss of blood samples (n=1)	• 30 men and 33 women • Mean age: 46 years	• National Heart, Lung, and Blood Institute • Best Foods
Fielding et al., 1995 USA	Randomized parallel design with two interventions Experimental period: 28 days	1. 2.	10.3 15.3	16.5 15.4	8.5 5.8		• Initial: 48, final: 42 • Reason for loss: not reported (n=5), incomplete data (n=1)	• 42 men • Diet 1: 21 men • Diet 2: 21 men • Mean age: 29 years	• National Institutes of Health • Arteriosclerosis SCOR • National Dairy Promotion and Research Board

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Fielding et al., 1995 USA	Randomized parallel design with two interventions Experimental period: 28 days	1. 2.	10.0 16.7	14.9 12.7	9.9 4.7		• Initial: 48, final: 42 • Reason for loss: not reported (n=5), incomplete data (n=1)	• 42 men • Diet 1: 20 men • Diet 2: 22 men • Mean age: 29 years	• National Institutes of Health • Arteriosclerosis SCOR • National Dairy Promotion and Research Board
Cater et al., 1997 USA	Randomized crossover design with two interventions Experimental period: 21 days	1. 2.	23.3 5.7	18.4 39.8	6.0 4.9		• Initial: 9, final: 9 • No dropouts reported	• 9 men • Mean age: 66 years	• NIH Endocrinology and Metabolism Training Grant • NIH-NHLBI Clinical Investigator Award • National Institutes of Health
Tholstrup et al., 1998	Randomized crossover design	1. 2.	19.1 24.4	11.6 7.7	4.5 5.2	1.6 0.1	• Initial: 18, final: 18 • No dropouts reported	• 18 men • Mean age: 25 years	• The Danish Dairy Research Foundation • The Danish Research Development

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Denmark	with two interventions Experimental period: 28 days								Program for Food Technology
Mazier and Jones, 1997 Canada	Randomized crossover design with two interventions Experimental period: 13 days	1. 2.	11.0 10.9	24.0 9.2	4.1 17.9		• Initial: 9, final: 9 • No dropouts reported	• 9 men • Mean age: 26 years	• The Heart and Stroke Foundation of British Columbia and Yukon
Ginsberg et al., 1998 USA	Randomized crossover design with three interventions	1. 2. 3.	14.4 8.6 5.8	12.5 12.5 12.5	5.8 5.8 5.8		• Initial: 118, final: 103 • Reason for loss: not reported	• 46 men and 57 women • Mean age: 38 years	• National Heart, Lung, and Blood Institute • National Center for Research Resources

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 56 days								
Müller et al., 1998 Norway	Randomized crossover design with two interventions Experimental period: 17 days	1. 2.	12.5 7.3	11.4 11.4	5.5 9.8	0.1 0.2	• Initial: 30, final: 27 • Reason for loss: not reported (n=2), poor compliance (n=1)	• 27 women • Mean age: 27 years	• The Nordic Industrial Fund • Mills DA
Hunter et al., 2000 United Kingdom	Randomized crossover design with two interventions Experimental period: 14 days	1. 2.	6.8 7.3	25.0 14.4	4.5 14.4		• Initial: 9, final: 6 • Reason for loss: not reported	• 6 men • Mean age: 28 years	• Ministry of Agriculture, Food and Fisheries • Scottish Executive Rural Affairs Department

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Judd et al., 2002 Baer et al., 2004 USA	Randomized crossover design with three interventions Experimental period: 35 days	1. 2. 3.	12.8 12.6 20.8	10.5 17.6 10.5	3.8 3.8 4.2	0.2 0.1 0.2	• Initial: 54, final: 50 • Reason for loss: not reported (n=3), poor compliance (n=1)	• 50 men • Mean age: 42 years	• Technical Committee on Dietary Lipids, International Life Sciences Institute
Vega-López et al., 2006	Randomized crossover design with two interventions Experimental period: 35 days	1. 2.	14.8 6.4	10.9 15.4	3.5 8.7	0.6 1.0	• Initial: 15, final: 15 • No dropouts reported	• 5 men and 10 women • Mean age: 64 years	• National Institutes of Health / US Department of Agriculture
Lichtenstein et al., 1999	Randomized crossover design	1. 2.	7.3 8.6	8.1 8.1	12.5 13.5	0.6 0.9	• Initial: 36, final: 36 • No dropouts reported	• 18 men and 18 women • Mean age: 63 years	• National Institutes of Health • US Department of Agriculture

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
USA	with two interventions Experimental period: 35 days								
Lovejoy et al., 2002 USA	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	5.9 10.9	14.7 8.8	6.3 6.4	0.0 0.0	• Initial: 31, final: 25 • Reason for loss: not reported	• 12 men and 13 women • Mean age: 28 years	• US Department of Agriculture
Berglund et al., 2007 USA	Randomized crossover design with three interventions	1. 2. 3.	15.0 8.4 7.7	13.8 20.0 14.9	5.6 6.0 5.3		• Initial: 110, final: 85 • Reason for loss: not reported	• 52 men and 33 women • Mean age: 36 years	• National Institutes of Health

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 49 days								
Binkoski et al., 2005 USA	Randomized crossover design with three interventions Experimental period: 28 days	1. 2. 3.	10.8 8.0 7.6	14.3 16.5 13.6	7.5 4.1 7.4		• Initial: 31, final: 31 • No dropouts reported	• 12 men and 19 women • Mean age: 46 years	• National Institutes of Health • National Sunflower Association
Castro et al., 2000 Spain	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	9.4 8.6	24.3 24.8	4.3 4.7		• Initial: 22, final: 21 • Reason for loss: illness (n=1)	• 21 men • Mean age: 23 years	• Investigaciones de la Seguridad Social • Koype Co

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Kris-Etherton et al., 1999 USA	Randomized crossover design with four interventions Experimental period: 24 days	1. 2. 3. 4.	6.7 6.7 6.7 7.7	11.5 20.2 16.3 17.3	5.8 5.8 8.6 9.6		• Initial: 26, final: 22 • Reason for loss: poor compliance (n=2), moved outside the area (n=2)	• 9 men and 13 women • Mean age: 34 years	• The Peanut Institute
Nielsen et al., 2002 Denmark	Randomized crossover design with three interventions Experimental period: 21 days	1. 2. 3.	10.5 11.5 11.5	14.5 16.9 7.6	6.5 2.3 11.7		• Initial: 18, final: 18 • No dropouts reported	• 18 men • Mean age: 24 years	• Not reported
Poppitt et al., 2002	Randomized crossover design	1. 2.	19.2 14.4	5.8 7.7	13.4 15.4		• Initial: 20, final: 20 • No dropouts reported	• 20 men • Mean age: Not reported	• New Zealand Dairy Board • Auckland Uniservices • Maurice & Phyllis Paykel Trust

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
New Zealand	with two interventions Experimental period: 21 days								
Rajaram et al., 2001 USA	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	8.2 8.8	11.0 18.9	6.3 10.7		• Initial: 24, final: 23 • Reason for loss: not reported	• 14 men and 9 women • Mean age: Not reported	• National Pecan Sellers Association
Sanders et al., 2003 United Kingdom	Randomized crossover design with two interventions	1. 2.	9.8 10.5	19.9 12.3	6.3 6.1	0.1 0.1	• Initial: 36, final: 29 • Reason for loss: personal reasons (n=7)	• 29 men • Mean age: 24 years	• Ministry of Agriculture, Food and Fisheries • The Medical Research Council

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 14 days								
Wagner et al., 2001 Austria	Randomized crossover design with two interventions Experimental period: 14 days	1. 2.	8.5 8.4	9.8 14.5	11.5 6.9		• Initial: 28, final: 28 • No dropouts reported	• 28 men • Mean age: 24 years	• Not reported
Kratz et al., 2002 Germany	Randomized parallel design with three interventions Experimental period: 28 days	1. 2. 3.	9.1 10.7 10.0	19.3 23.3 8.7	9.0 3.4 18.5		• Initial: 69, final: 58 • Reason for loss: illness (n=6), poor compliance (n=5)	• Diet 1: 10 men and 8 women • Diet 2: 11 men and 9 women • Diet 3: 10 men and 10 women • Mean age: 26 years	• Central Marketing Agency of the German Agricultural Industry • The German Union for the Promotion of Oil and Protein Plants • The Austrian Science Foundation • The Brökelmann Ölmühle Company

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Lichtenstein et al., 2006 USA	Randomized crossover design with four interventions Experimental period: 35 days	1. 2. 3. 4.	6.5 4.9 5.8 6.8	6.3 6.1 18.8 6.7	12.3 14.1 2.3 13.2	0.6 0.6 0.3 0.5	• Initial: 42 (including 10 replacers), final: 30 • Reason for loss: time constraints (n=3), poor compliance (n=4), change in medical status (n=2), loss of medical insurance (n=1), moved out of the state (n=1), or dislike of the food (n=1)	• 14 men and 16 women • Mean age: 63 years	• The National Institutes of Health • US Department of Agriculture
Motard-Belanger et al., 2008 Canada	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	18.5 18.3	11.8 11.8	4.6 4.4	0.8 1.5	• Initial: 48, final: 38 • Reason for loss: not reported	• 38 men • Mean age: 33 years	• Dairy Farmers of Canada • Novalait Inc • Natural Sciences and Engineering Research Council of Canada

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Rajaram et al., 2009 USA	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	9.4 8.0	9.4 8.0	4.3 10.8	1.0 0.8	• Initial: 27, final: 25 • Reason for loss: time constraints (n=2)	• 14 men and 11 women • Age: 23-65 years	• California Walnut Commission
Gillingham et al., 2011 Canada	Randomized crossover design with three interventions Experimental period: 28 days	1. 2. 3.	11.2 5.6 6.1	16.1 22.9 15.9	6.5 5.7 12.3		• Initial: 39, final: 36 • Reason for loss: relocation of residence (n=2), work-related issues (n=1)	• 13 men and 23 women • Mean age: 48 years	• Flax Canada 2015 • Canola Council of Canada • Agri-Food Research & Development Initiative
Iggman et al., 2011	Randomized crossover design	1. 2.	19.6 7.9	11.1 17.4	3.9 9.6		• Initial: 20, final: 20 • No dropouts reported	• 14 men and 6 women • Mean age: 51 years	• Not reported

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Sweden	with two interventions Experimental period: 21 days								
Marin et al., 2011 Spain	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	8.8 8.8	13.0 23.4	5.0 4.6		• Initial: 59, final: 59 • No dropouts reported	• 31 men and 28 women • Mean age: 21 years	• Ministerio de Ciencia e Innovacion / Spanish Ministry of Health • CIBER Fisiopatologia de la Obesidad y Nutricion • Consejeria de Innovacion • Consejeria de Salud
Roussell et al., 2012 USA	Randomized crossover design with two interventions	1. 2.	6.0 6.0	9.0 11.0	8.0 7.0		• Initial: 42, final: 36 • Reason for loss: job change (n=1), illness (n=1), poor compliance (n=4)	• 15 men and 21 women • Mean age: 50 years	• Beef Checkoff Program • National Institutes of Health

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
	Experimental period: 35 days								
Zhao et al., 2004 USA	Randomized crossover design with three interventions Experimental period: 42 days	1. 2. 3.	12.7 8.5 8.2	13.2 12.2 12.3	8.7 16.4 17.2		• Initial: 23, final: 23 • No dropouts reported	• 20 men and 3 women • Mean age: 50 years	• California Walnut Commission • Walnut Marketing Board
Sabaté et al., 2003 USA	Randomized crossover design with three interventions Experimental period: 28 days	1. 2. 3.	8.2 8.0 7.7	12.1 16.5 19.4	6.2 7.5 8.7		• Initial: 27, final: 25 • Reason for loss: poor compliance (n=2)	• 14 men and 11 women • Mean age: 41 years	• Almond Board of California

Reference and country	Study design	Composition					Participants		Funding
		Diet	S	M	P	T			
Curb et al., 2000 USA	Randomized crossover design with three interventions Experimental period: 30 days	1. 2. 3.	13.4 8.6 8.6	11.5 14.4 19.2	8.6 6.7 5.8		• Initial: 34, final: 30 • Reason for loss: not reported	• 15 men and 15 women • Age: 18-53 years	• US Army Medical Research Acquisition Activity
Lacroix et al., 2012 Canada	Randomized crossover design with two interventions Experimental period: 28 days	1. 2.	9.9 10.3	14.2 12.8	5.9 5.8	0.6 1.8	• Initial: 72, final: 61 • Reason for loss: protocol too demanding (n=8), change of menopausal status (n=2), missing data (n=1)	• 61 women • Mean age: 64 years	• Dairy Farmers of Canada • Dairy Australia • Agriculture and Agri-Food Canada • The Canadian Dairy Commission

S: Saturated fatty acids

M: *Cis*-monounsaturated fatty acids

P: *Cis*-polyunsaturated fatty acids

T: *Trans* fatty acids

Annex 4: Estimated multiple regression equations for the mean changes in serum lipids and lipoproteins when one percent of energy in the diet from carbohydrates is replaced isocalorically by saturated fatty acids (Carb → SFA), by *cis*-monounsaturated fatty acids (Carb → MUFA) or by *cis*-polyunsaturated fatty acids (Carb → PUFA): impact of baseline levels

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
Below median					
ΔTotal cholesterol	mmol/L	0.035	-0.007	-0.022	82/37/1060
95% CI		0.023 to 0.048	-0.017 to 0.004	-0.033 to -0.010	
P-value		<0.001	0.220	0.001	
Above median					
ΔTotal cholesterol	mmol/L	0.050	-0.004	-0.024	95/37/1112
95% CI		0.043 to 0.057	-0.010 to 0.002	-0.031 to -0.018	
P-value		<0.001	0.201	<0.001	
Below median					
ΔLDL-cholesterol	mmol/L	0.029	-0.009	-0.018	79/35/1026
95% CI		0.020 to 0.039	-0.017 to -0.001	-0.027 to -0.008	
P-value		<0.001	0.021	<0.001	
Above median					
ΔLDL-cholesterol	mmol/L	0.041	-0.008	-0.024	86/34/1000
95% CI		0.032 to 0.049	-0.016 to 0.000	-0.033 to -0.016	
P-value		<0.001	0.045	<0.001	
Below median					
ΔHDL-cholesterol	mmol/L	0.008	0.007	0.005	81/34/789
95% CI		0.005 to 0.011	0.005 to 0.010	0.002 to 0.00	

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
P-value		<0.001	<0.001	0.001	
Above median					
ΔHDL-cholesterol	mmol/L	.013	0.008	0.006	82/34/1228
95% CI		0.011 to 0.016	0.006 to 0.010	0.003 to 0.008	
P-value		<0.001	<0.001	<0.001	
Below median					
ΔTriacylglycerol	mmol/L	-0.011	-0.013	-0.020	83/36/1102
95% CI		-0.015 to -0.006	-0.017 to -0.009	-0.025 to -0.015	
P-value		0.001	<0.001	<0.001	
Above median					
ΔTriacylglycerol	mmol/L	-0.013	-0.016	-0.022	89/36/1054
95% CI		-0.019 to -0.007	-0.021 to -0.011	-0.028 to -0.017	
P-value		<0.001	<0.001	<0.001	
Below median					
ΔTotal to HDL-cholesterol		0.002	-0.026	-0.035	76/33/1041
95% CI		-0.008 to 0.012	-0.034 to -0.018	-0.044 to -0.025	
P-value		0.695	<0.001	<0.001	
Above median					
ΔTotal to HDL-cholesterol		-0.006	-0.032	-0.038	83/33/949
95% CI		-0.016 to 0.004	-0.041 to -0.022	-0.048 to -0.028	
P-value		0.246	<0.001	<0.001	

The median level when subjects consumed a standardized fat-free diet was for total cholesterol 4.45 mmol/L, for LDL-cholesterol 2.89 mmol/L, for HDL-cholesterol 0.97 mmol/L, for triacylglycerol 1.48 mmol/L, and for the total to HDL-cholesterol ratio 4.36.

The 95 percent confidence intervals (CI) refer to the regression coefficients on the preceding line.

No: Number of diets/number of studies/number of subjects.

Annex 5: Estimated multiple regression equations for the mean changes in serum lipids and lipoproteins when one percent of energy in the diet from carbohydrates is replaced isocalorically by saturated fatty acids (Carb → SFA), by *cis*-monounsaturated fatty acids (Carb → MUFA) or by *cis*-polyunsaturated fatty acids (Carb → PUFA). Studies using liquid formula diets were excluded

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
ΔTotal cholesterol	mmol/L	0.046	-0.004	-0.022	166/69/2116
95% CI		0.039 to 0.052	-0.010 to 0.001	-0.029 to -0.016	
P-value		<0.001	0.133	<0.001	
ΔLDL-cholesterol	mmol/L	0.037	-0.009	-0.022	154/64/1970
95% CI		0.031 to 0.044	-0.014 to -0.003	-0.029 to -0.016	
P-value		<0.001	0.004	<0.001	
ΔHDL-cholesterol	mmol/L	0.011	0.008	0.006	152/63/1961
95% CI		0.010 to 0.013	0.006 to 0.010	0.004 to 0.007	
P-value		<0.001	<0.001	<0.001	
ΔTriacylglycerol	mmol/L	-0.012	-0.015	-0.021	163/68/2107
95% CI		-0.016 to -0.008	-0.018 to -0.011	-0.025 to -0.018	
P-value		<0.001	<0.001	<0.001	
ΔTotal to HDL-cholesterol		-0.002	-0.028	-0.037	150/62/1941
95% CI		-0.009 to 0.004	-0.034 to -0.022	-0.044 to -0.030	
P-value		0.485	<0.001	<0.001	

The 95 percent confidence intervals (CI) refer to the regression coefficients on the preceding line.

No: Number of diets/number of studies/number of subjects.

Annex 6: Estimated multiple regression equations for the mean changes in serum lipids and lipoproteins when one percent of energy in the diet from carbohydrates in the diet is replaced isocalorically by saturated fatty acids (Carb → SFA), by *cis*-monounsaturated fatty acids (Carb → MUFA) or by *cis*-polyunsaturated fatty acids (Carb → PUFA) stratified for year of publication

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
Published before 1993					
ΔTotal cholesterol	mmol/L	0.045	-0.005	-0.018	77/34/819
95% CI		0.035 to 0.054	-0.012 to 0.001	-0.026 to -0.011	
P-value		<0.001	0.120	<0.001	
Published in 1993 or later					
ΔTotal cholesterol	mmol/L	0.045	-0.005	-0.030	100/40/1353
95% CI		0.036 to 0.054	-0.013 to 0.004	-0.040 to -0.020	
P-value		<0.001	0.277	<0.001	
Published before 1993					
ΔLDL-cholesterol	mmol/L	0.035	-0.011	-0.019	69/31/757
95% CI		0.024 to 0.046	-0.019 to -0.003	-0.028 to -0.010	
P-value		<0.001	0.011	<0.001	
Published in 1993 or later					
ΔLDL-cholesterol	mmol/L	0.038	-0.007	-0.027	96/38/1269
95% CI		0.030 to 0.046	-0.014 to 0.000	-0.036 to -0.018	
P-value		<0.001	0.069	<0.001	

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
Published before 1993					
ΔHDL-cholesterol	mmol/L	0.011	0.009	0.006	69/30/748
95% CI		0.007 to 0.014	0.006 to 0.011	0.003 to 0.009	
P-value		<0.001	<0.001	<0.001	
Published in 1993 or later					
ΔHDL-cholesterol	mmol/L	0.012	0.008	0.005	96/38/1269
95% CI		0.010 to 0.014	0.006 to 0.010	0.002 to 0.007	
P-value		<0.001	<0.001	0.001	
Published before 1993					
ΔTriacylglycerol	mmol/L	-0.014	-0.016	-0.023	72/32/803
95% CI		-0.019 to -0.009	-0.020 to -0.012	-0.027 to -0.019	
P-value		<0.001	<0.001	<0.001	
Published in 1993 or later					
ΔTriacylglycerol	mmol/L	-0.010	-0.013	-0.020	100/40/1353
95% CI		-0.015 to -0.004	-0.019 to -0.008	-0.026 to -0.013	
P-value		0.002	<0.001	<0.001	
Published before 1993					
ΔTotal to HDL-cholesterol		0.003	-0.029	-0.032	65/29/741
95% CI		-0.008 to 0.015	-0.038 to -0.020	-0.042 to -0.033	
P-value		0.543	<0.001	<0.001	
Published in 1993 or later					

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
ΔTotal to HDL-cholesterol		-0.004	-0.028	-0.039	94/37/1249
95% CI		-0.013 to 0.005	-0.036 to -0.020	-0.049 to -0.029	
P-value		0.344	<0.001	<0.001	

The 95 percent confidence intervals (CI) refer to the regression coefficients on the preceding line.

No: Number of diets/number of studies/number of subjects.

Annex 7: Estimated multiple regression equations for the mean changes in serum lipids and lipoproteins when one percent of energy in the diet from carbohydrates in the diet is replaced isocalorically by saturated fatty acids (Carb → SFA), by *cis*-monounsaturated fatty acids (Carb → MUFA) or by *cis*-polyunsaturated fatty acids (Carb → PUFA) stratified for "not funded by industrial parties" vs. those of studies "funded by at least 1 industrial party".

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
No industrial funding					
ΔTotal cholesterol	mmol/L	0.046	-0.003	-0.015	78/34/1091
95% CI		0.040 to 0.053	-0.009 to 0.002	-0.021 to -0.008	
P-value		<0.001	0.222	<0.001	
Industrial funding					
ΔTotal cholesterol	mmol/L	0.037	-0.013	-0.038	81/32/935
95% CI		0.026 to 0.048	-0.022 to -0.005	-0.047 to -0.028	
P-value		<0.001	0.003	<0.001	
No industrial funding					
ΔLDL-cholesterol	mmol/L	0.038	-0.006	-0.013	70/31/1029
95% CI		0.031 to 0.045	-0.012 to 0.000	-0.020 to -0.006	
P-value		<0.001	0.043	0.001	
Industrial funding					
ΔLDL-cholesterol	mmol/L	0.035	-0.014	-0.032	77/30/851
95% CI		0.025 to 0.045	-0.021 to -0.006	-0.040 to -0.024	
P-value		<0.001	0.001	<0.001	
No industrial funding					

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
ΔHDL-cholesterol	mmol/L	0.013	0.008	0.006	68/30/1020
95% CI		0.010 to 0.015	0.006 to 0.011	0.004 to 0.009	
P-value		<0.001	<0.001	<0.001	
Industrial funding					
ΔHDL-cholesterol	mmol/L	0.010	0.008	0.005	77/30/851
95% CI		0.006 to 0.014	0.005 to 0.011	0.001 to 0.008	
P-value		<0.001	<0.001	0.006	
No industrial funding					
ΔTriacylglycerol	mmol/L	-0.013	-0.016	-0.021	75/33/1082
95% CI		-0.017 to -0.008	-0.019 to -0.012	-0.025 to -0.016	
P-value		<0.001	<0.001	<0.001	
Industrial funding					
ΔTriacylglycerol	mmol/L	-0.008	-0.012	-0.020	79/31/928
95% CI		-0.017 to 0.000	-0.019 to -0.004	-0.028 to -0.012	
P-value		0.059	0.002	<0.001	
No industrial funding					
ΔTotal to HDL-cholesterol		-0.003	-0.028	-0.030	68/30/1020
95% CI		-0.010 to 0.004	-0.034 to -0.021	-0.037 to -0.022	
P-value		0.432	<0.001	<0.001	
Industrial funding					
ΔTotal to HDL-cholesterol		0.001	-0.030	-0.041	75/29/844
95% CI		-0.010 to 0.012	-0.039 to -0.021	-0.051 to -0.032	

Lipid or lipoprotein	Unit	Change per percent of energy replaced			No
		Carb → SFA	Carb → MUFA	Carb → PUFA	
P-value		0.861	<0.001	<0.001	

The 95 percent confidence intervals (CI) refer to the regression coefficients on the preceding line.

No: Number of diets/number of studies/number of subjects.