Biomarkers of dairy fat intake and risk of cardiovascular disease: a systematic review and meta analysis of prospective studies

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Abstract

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Background: Circulating biomarkers of dairy fat provide objective measures of dairy fat intake and facilitate conclusions relevant to populations with different diets and susceptibility to cardiovascular diseases (CVD).

Objective: To assess the relationship between circulating pentadecanoic acid (15:0), heptadecanoic acid (17:0) and trans-palmitoleic acid (trans-16:1n-7) and the risk of CVD.

Methods: Pubmed, Medline and Embase were searched for prospective cohort studies of the relationship between biomarkers of dairy fat and CVD risk, which included coronary heart disease (CHD), stroke, heart failure and CVD mortality, supplemented by bibliographies of retrieved articles and previous reviews. For each study, relative risks (RR) and 95% confidence intervals (CI) were extracted and pooled with the random effect model.

Results: Thirteen studies involving 7,680 CVD cases were included. The pooled RRs of the risk of CVD for the top third vs. bottom third 15:0, 17:0 and trans-16:1n-7 level were 0.94 (95% CI: 0.77-1.15), 0.82 (95% CI: 0.68-0.99) and 0.82 (95% CI: 0.67-1.02), respectively. Subgroup analysis indicated that there were no associations between the concentration of 15:0 with CHD and stroke, but a negative relationship with heart failure (RR=0.72, 95% CI: 0.55-0.95). Null association was observed between circulating 17:0 and trans-16:1n-7 level and subtypes of CVD except for only one study which reported a negative relationship between 17:0 and heart failure.

Conclusion: Higher diary fat exposure is not associated with an increased risk of CVD.

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Key words: dairy fat; biomarker; cardiovascular diseases; pentadecanoic acid, heptadecanoic acid; trans-palmitoleic acid

Introduction

Consumption of whole milk and other high-fat dairy products have been postulated to be associated with the risk of cardiovascular disease (CVD) because of the high saturated fat content. It is estimated that dairy products (excluding butter) contribute to 24% of the saturated fat intake of the US diet (Huth et al., 2006); this figure is 25–30% in European countries (Givens, 2009). Based on these considerations, major dietary guidelines recommend low-fat dairy products and avoidance of whole-fat dairy (McGuire, 2016). However, the self-reported consumption of dairy foods has mixed associations with risk of CVD, without consistent different results for low-fat vs. whole-fat products (Huth et al., 2012). For instance, several studies suggest that yogurt may be beneficial for CVD (Goldbohm et al., 2011; Ivey et al., 2011), while some studies (Iso et al., 1999; Sonestedt et al., 2011), but not others (Goldbohm et al., 2011; Larsson et al., 2009), further suggest that cheese, which is highest in dairy fat, may also be protective. A recent meta analysis restricted to four cohort studies provided no convincing evidence that high fat dairy products are harmful to cardiovascular health, but low-dairy intake might be associated with a small but worthwhile reduction in stroke based on 6 cohort studies (Qin et al., 2015).

Compared with self-reported dietary estimates, circulating fatty acid biomarkers (in serum, plasma, erythrocyte membranes, or whole blood) such as pentadecanoic acid (15:0), heptadecanoic acid (17:0) and trans-palmitoleic acid (trans-16:1n-7) cannot be synthesized in

vivo and thus provide the best estimates of dairy fat consumption and help elucidate associations between dairy and cardiovascular health (Smedman et al., 1999; Wolk et al., 2001; Wolk et al., 1998). However, studies using biomarkers of dairy intake have also yielded inconsistent results. Several prospective studies have reported decreased risk of stroke or coronary heart disease (CHD) with greater levels of 15:0, 17:0 or trans-16:1n-7 (Otto et al., 2013; Warensjo et al., 2009; Yamagishi et al., 2008), Whereas other cohort studies reported they were positively associated with CHD or stroke (Malik et al., 2012; Sun et al., 2007). Many studies have also reported no significant association between those fatty acids and CVD (Khaw et al., 2012; Matthan et al., 2014; Warensjo et al., 2010; Yakoob et al., 2014). Therefore, the effects of dairy fat and specific dairy fatty acids on CVD risk remain unclear.

Given the mixed results and the potential for global impact of dairy fat intake on CVD outcomes, understanding these relationships is crucial for informing dietary guidelines. We performed a systematic review and meta-analysis of the current evidence for the association between dairy fat biomarker concentrations, with the risk of incidence of CVD.

SUBJECTS AND METHODS

Search strategy

A systematic literature search up to 16th September 2016 was conducted by using the PubMed, Embase databases and Medline with the following search terms: (“pentadecanoic acid” OR “heptadecanoic acid” OR “trans-palmitoleic acid” OR milk OR dairy OR “fatty acid*”)

AND (“serum” OR “plasma” OR “whole blood” OR “adipose tissue” OR “circulat*” OR “erythrocyte*” OR “red blood cell*”) AND (“cardiovascular disease” OR “myocardial infarction” OR “exp heart disease” OR “exp Vascular Diseases” OR “Cerebrovascular Disorders” OR “exp Brain Ischemia” OR “exp Carotid Artery Diseases” OR “exp Intracranial Arterial Diseases” OR coronary OR stroke OR death OR mortality OR mortalities OR fatal) AND (“cohort*” OR “nested” OR “prospective” OR “follow-up” OR “follow up” OR “longitudinal”). Further information was retrieved through a manual search of references from recent reviews (including meta-analyses) and relevant published original studies generated by PubMed, as well as searches in Google scholar (https://scholar.google.com.hk/).

Study selection

Two reviewers (JJL and QZ) independently extracted the data. Discrepancies were resolved by group discussions. The studies eligible for inclusion were prospective studies that examined the association between circulating concentrations of fatty acids at baseline and risk of CVD events during follow-up. Studies were included in this meta-analysis if they met the following criteria: (1) The study was a prospective cohort study, including case-cohort studies and nested case-control studies with a prospective design; (2) the exposure was circulating biomarker of dairy fat; and (3) the outcome was risk of CVD, including incidence of CHD, stroke, heart failure, and CVD mortality. Studies were excluded if (1) the study had a retrospective design; (2) the estimates were presented without standard errors or other information that allowed

calculation of standard errors; (3) participants were selected by confirmed medical conditions (such as diabetes or end-stage kidney disease); or (4) there were no adjustment for confounders. We restricted the search to studies written in English and human subjects. In case of multiple reports from the same study, we included the most recent and complete study. References of the retrieved articles were manually screened for additional eligible studies.

Outcomes

Outcomes of interest were: major cardiovascular events, defined as a myocardial infarction, stroke, heart failure, or cardiovascular death, separately and combined. CVD was defined as CHD or stroke [WHO International Classification of Diseases (ICD)-10 I60-69; http://www.who.int/classifications/icd/en] and other CVD, including heart failure (I50), cardiac arrest (I46), and sudden death (R96). CHD was defined as acute myocardial infarction, angina pectoris, and other ischemic heart diseases (as in ICD-10 I20-I25).

Data extraction

For each identified article, 2 investigators (JJL and QZ) independently extracted information on study characteristics (study name, authors, publication year), participant characteristics (location, number, sex, mean age and BMI), fatty acids assessment (fatty acids measures, blood sample, lipid fraction measured, storage temperature), CVD outcomes (specific endpoints, follow-up years, duration), analysis strategy (covariates), and multivariable-adjusted risk estimates, including data to calculate its precision, such as 95% CIs, SEs, or P values. We utilized

a 9-star system by Newcastle-Ottawa Scale for assessing the quality of studies (Wells GA et al., 2011). This scale awards a maximum of nine points to each study: four for selection of participants and measurement of exposure, two for comparability of cohorts on the basis of the design or analysis, and three for assessment of outcomes and adequacy of follow-ups.

Data synthesis

We used STATA/SE (version 11; Stata Corp LP, College Station, TX, USA) for statistical analysis. All statistical tests were 2-sided and used a significance level of $P < 0.05$. The included studies reported relative risks (RRs) or hazard ratios (HRs) for prospective cohorts. Individual studies reported risk estimates for dairy fatty acid biomarkers based on various categories (e.g, tertiles, quartiles, quintiles, or specific thresholds), per standard deviation change in exposure. To enable a consistent approach to meta-analysis, risk estimates for each study were transformed to involve comparisons between the top versus bottom third of fatty acid distribution by using methods previously described (Chene et al., 1996). In brief, log risk estimates were transformed assuming a normal distribution, with the conversion factor of 2.18 as the difference in the medians of the top and bottom thirds of the standard normal distribution; other conversions were used for differences in medians of extreme quartiles (2.54) or quintiles (2.80). The standard errors (SEs) of log RRs were calculated by using reported data on precision and were similarly standardized.

Statistical heterogeneity across individual studies was assessed by standard chi-square tests and the I^2 statistic(Higgins et al., 2002). For the I^2 metric, the values of 25%, 50%, and 75% correspond to cut-off points for low, moderate, and high degrees of heterogeneity. Summary RRs were calculated by pooling the study-specific estimate by using a random-effects model that included between-study heterogeneity. Forest plots were made for the relation. Analyses were further stratified to examine the difference in pooled RRs by sex (women and men), subtypes of CVD (CHD, stroke and heart failure), baseline age (≤61 and >61 years) and follow-up duration (≤8 and >8 years). We removed each single study from the meta-analyses and recalculated the summary association (the "leave one out" approach) (Greenhouse JB, 2009). We explored other methodological features through predefined meta-regression. These included baseline BMI, study year of recruitment, fatty acid measurement (gas chromatography or gas liquid chromatography), storage temperature and whether the results were adjusted for other circulating fatty acids. Funnel plot asymmetry was used to detect publication bias and Egger's regression test was applied to measure any asymmetry.

Results

Overall, the search yielded 3,934 reports (Figure 1). After dual review of abstracts and titles, we selected 387 articles for full-text dual review and determined that 13 studies met our inclusion criteria ((Khaw et al., 2012; Matsumoto et al., 2013; Matthan et al., 2014; Otto et al., 2013; Sun et al., 2007; Tokede et al., 2013; Warensjo et al., 2010; Warensjo et al., 2009; Yaemsiri

et al., 2013; Yakoob et al., 2014; Yamagishi et al., 2013; Yamagishi et al., 2008). A manual search of references cited by these studies did not yield new eligible articles. Among these 13 studies, Sun et al's report included data from two independent cohorts (Sun et al., 2007). Therefore, we included 14 comparisons in the meta-analysis.

Study characteristics

Among the 13 identified articles, 3 were prospective cohorts and 10 were prospective nested case-control studies (Table 1). Duration of follow-up for incidence of CVD ranged from 1.5 to 18.3 years and 7,680 participants-occurring incident cases of CVD. Three studies were conducted in Europe and ten in the United States. The outcome in 4 studies was risk of stroke, whereas the outcome in 6 studies was risk of CHD and in 3 was heart failure. Seven studies had scores of the NOS quality assessment of 9 and the others had scores of 8.

Circulating 15:0 and the CVD risk

A total of 12 studies reported the association between circulating 15:0 and total CVD risk. The pooled RR for the comparison of the top with the bottom tertiles was 0.94 (95% CI: 0.77-1.15; Figure 2) with high heterogeneity (I^2=58.0%). Stratified analyses were conducted according to the CVD subtypes, including CHD, stroke and heart failure; the association was only statistically significant for circulating 15:0 and heart failure (pooled RR from 2 prospective cohort studies: 0.72; 95% CI: 0.55-0.95; I^2=0.0%). For the risk of CHD and stroke, compared with the lowest category of circulating 15:0, the RRs of CHD and stroke were 1.08 (95% CI:

0.71-1.62; P for heterogeneity=0.002; I^2=73.7%) and 0.94 (95% CI: 0.76-1.17; P for heterogeneity=0.493; I^2=0.0%) for the highest category, respectively. The null association of 15:0 concentration with the risk of CVD was consistent in subgroup analysis by sex (women: RR=1.04 [95% CI: 0.67-1.63]; men: RR=1.11 [95% CI: 0.74-1.66]) , baseline age (≤ 61 years: RR=0.89 [95% CI: 0.67-1.19]; age >61 years (RR=1.01 [95% CI: 0.74, 1.38]) and follow-up duration (≤8 years: RR=0.94 [95% CI: 0.59-1.51]; >8 years: RR=0.95 [95% CI: 0.71-1.19]) (Table 2). Sensitivity analyses that 1 study at a time was removed and the rest analyzed revealed generally similar results.

Meta-regression analysis showed that the recruitment year (β=0.028, P=0.496), baseline BMI (β=-0.294, P=0.308), method for measuring fatty acids (β=0.160, P=0.628), storage temperature (β=-0.004, P=0.349) and the adjustment for other circulating fatty acids (β=-0.291, P=0.445) did not explain the high heterogeneity for circulating 15:0 and risk of CVD.

Circulating 17:0 and trans-16:1n-7 and the CVD risk

Seven studies with 8 cohorts reported RRs between circulating 17:0 and CVD, resulting in a decreased risk of 0.82 (95% CI: 0.68–0.99; P for heterogeneity=0.224; I^2=26.9%). Two of the 8 cohorts reported inverse association. No significant association between circulating 17:0 and subtypes of CVD were detected (Figure 3), and the results were similar when data were stratified by sex, baseline age and follow-up years (Table 2). No significant results were found in the meta-analysis of the four studies that reported data for circulating trans-16:1n-7 and CVD

(RR=0.82, 95% CI: 0.67–1.02) and the two studies of CHD (RR=0.94, 95% CI: 0.65–1.36) (Figure 4).

Publication bias

There was no evidence for publication bias on visual inspection of the funnel plot (Figure 5) as supported by the Begg's (P = 0.497-0.891) and Egger's tests (P = 0.760-0.944).

Discussion

The findings from this meta-analysis, based on 13 studies' participants and 7,680 CVD cases, including 4,863 CHD cases, 1,834 stroke cases, and 983 other CVD cases, do not support an adverse association of dairy fat consumption with incident CVD. We found no significant effects of circulating 15:0 on total CVD, CHD and stroke but a favorable benefit on heart failure. Null association was also observed in terms of trans-16:1n-7. By pooling 7 studies including 4,895 CVD cases, people in the top third of circulating 17:0 have 18% lower odds for the occurrence of CVD versus those in the bottom third.

Although saturated fat in dairy products may counteract potential beneficial effects of minerals (calcium, potassium, and magnesium), protein (casein and whey), and vitamins (riboflavin and vitamin B-12) on cardiovascular health, there are limited researches that have examined the potential differential effects of high- versus low-fat dairy consumption on heart disease and most of them reported no associations. In the meta-analysis of over 600,000 multiethnic adults, pooled results from a limited number of studies on the association between

total high-fat (n=4), and total low-fat (n=3) dairy consumption and CHD risk showed no significant association between total high-fat dairy and CHD (RR=1.04; 95% CI: 0.89-1.21; I^2=0%), and total low-fat dairy and CHD (RR=0.93; 95% CI:0.74-1.17; I^2=56%) (Soedamah-Muthu et al., 2011). A recent updated meta-analysis of Qin et al have documented that high-fat dairy consumption resulted in no significant difference in stroke risk (RR=0.95, 95%CI: 0.83-1.08, I^2=72.1%) or the CHD risk (RR=1.08, 95% CI: 0.99-1.17, I^2=0%); whereas an inverse association was observed between low-fat dairy intake and the incidence of stroke (RR=0.93, 95% CI:0.88-0.99, I^2=20%) but not CHD (RR=1.02, 95% CI:0.92-1.14, I^2=33.5%) (Qin et al., 2015). Moreover, by pooling seventeen cohort studies Aune et al have found that a high intake of total dairy and low-fat dairy but not high-fat dairy products were associated with a significant decrease in the risk of type 2 diabetes (Aune et al., 2013), a condition which may be linked to increased risk of CVD (Grundy et al., 1999). Nevertheless, reliance on self-reported dietary intakes poses substantial problems with validity, and the development of objective biomarkers to distinguish between different food-derived fatty acids represents a major advantage.

Two recent meta analysis have been conducted to established the relationship between biomarkers of fatty acids and coronary disease. A meta-analysis of case-control and cohort studies published up to May 2015, showed a summary relative risk of 0.99 (95%CI: 0.90-1.09; I^2=75%) for circulating 15:0 and of 0.97 (95%CI: 0.85–1.11; I^2=12%) for circulating 17:0 (de

Souza et al., 2015). Based on 4 prospective studies including 2283 cases, Chowdhury et al suggested a null association between blood levels of 15:0 and coronary disease (RR=0.94, 95%CI: 0.67-1.32; I^2=51%), but a negative relationship between circulating 17:0 and coronary disease (RR=0.77, 95%CI: 0.63-0.93; I^2=5%) (Chowdhury et al., 2014). Our results are in accordance with the most recent meta analysis of Chowdhury et al but are more precise than those two previous meta analysis because of a larger number of cases and the inclusion of only cohort studies.

Among the biomarkers evaluated, 15:0 is the most well-studied and has been shown to be reliably correlated to dairy intake as assessed by other methods in several studies (Otto et al., 2013), as well as in controlled feeding trials (Hodson et al., 2014). Thus, the association between circulating 15:0 and total CVD or its subtypes may provide a more credible witness for the relationship between dairy fat intake and CVD in comparison with other biomarkers. Although higher concentration of 17:0 related with lower risk of CVD, the pooling result was derived from only 7 studies. In addition, it was not possible to delineate the exact mechanism behind the relations or exclude a more beneficial lifestyle pattern in milk fat consumers. Covariate adjustments may result in changes in the magnitude and even the direction of the associations and thus lead to different conclusions. Although potential confounders such as age, BMI, and physical activity have been adjusted for in most individual studies, residual confounding by other dairy components such as calcium or vitamin D can neither be ruled out, and nor can the possible

effects of fermentation processes of dairy products. Interestingly, several recent larger prospective cohort studies including the Nurses' Health Study (Yakoob et al., 2016), Health Professionals Follow-Up Study (Yakoob et al., 2016), Multi-Ethnic Study of Atherosclerosis (Mozaffarian et al., 2013) and the Cardiovascular Health Study (Mozaffarian et al., 2010) have reported substantially beneficial effects of circulating trans-16:1n-7 on the diabetes, which shares many common risk factors with CVD (Grundy et al., 1999; Meigs, 2010). Those results also corroborate data from a prospective study of 3259 older participants at high cardiovascular risk by Kleberet al, which was excluded from our meta-analysis because all of their participants were already hospitalized for coronary angiography at baseline (Kleber et al., 2015). They found an inverse association of trans-16:1n-7 with adverse cardiac outcomes during 10 years of follow-up, with a hazard ratio of 0.63 (95%CI: 0.46-0.86) for the third tertile compared with the first tertile (Kleber et al., 2015). By contrast, in over 1300 Norwegian patients with suspected CHD, Borgeraas et al reported that serum trans 16:1n-7 level were not correlated with incident acute myocardial infarction, cardiovascular death and all-cause mortality (Borgeraas et al., 2016). In line with the study of Borgeraas et al and the four individual studies included in the present analysis, the pooled result of trans-16:1n-7 presented a null relationship between trans-16:1n-7 and CVD. But the number of studies may have been too small to allow a conclusive evaluation of the effect and further investigations are needed.

The meta analysis is strengthened by restricting analyses to prospective studies and the case–control studies nested within them, and excluding traditional case–control studies, which are prone to recall and interviewer bias. Several limitations also merit careful consideration. First, the results of any meta-analysis may be plagued by publication bias; nevertheless, only follow-up studies on CVD were considered. We also found no evidence of publication bias. Therefore, publication bias—if any—might have only weakly altered our findings. Second, although subgroup and meta-regression analysis failed to identify sex, age, duration of exposure, BMI, date of enrollment, fatty acid measurement, storage temperature or confounder adjustment as factors contributing to heterogeneity of the study results, the small number of studies included in the analysis preclude definitive conclusions of the effect of these different factors. Third, because most studies lacked serial assessment of fatty acids in the same persons, relative risks in published reports may have been prone to underestimation because of "regression dilution bias" (Hutcheon et al., 2010). Fourth, study participants were mostly whites, and race-ethnicity information was rarely reported, thus limiting the applicability of the reviewed evidence to other racial groups. Last, we did not conduct dose-response analysis for lack of available data. Further study should be performed to explore dose-response relation in general population hereafter.

In conclusion, our meta-analysis showed that there is insufficient evidence from prospective epidemiological studies to conclude that dairy fat is associated with an increased risk of CVD. However, the available data were not adequate for determining whether there are CHD or stroke

associations with dairy fatty acids subspecies in specific age and sex subgroups.

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Disclosure of interests

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Table 1 Characteristics of studies included in the meta-analysis.

First author	Year	Country	Study design	Cohort name	Year of baseline survey	Average follow-up (year)	No. of events	Gender (M/F)	Age (year)	BMI (kg/m^2)	Outcome	Fatty acids measures	Exposure Contrast	Blood sample	Lipid Fraction Measured	Storage temp	Covariates	NOS score
Otto	2013	USA	PC	MESA	2000-2002	7.0	146	47/53	61.5	27.9	CHD	GC	Q5 vs. Q1 15:0: 0.24 vs. 0.11% Trans 16:1n-7: 0.09 vs. 0.03 %	Plasma	Pf	-70°C	+++	9
Warensjö	2010	Sweden	NCC	NSHDS	1987-1999	3.6	444	62/38	55.6	26.0	MI	GLC	Per SD	Serum	Pf	-80°C	#	9
Yakoob	2014	USA	NCC	NHS	1989–1990	8.3	472	0/100	61.0	25.6	stroke	GLC	Per SD	Plasma	Total	-130°C	++++	8
		USA	NC	HPFS	1993–1	8.3	122	100/	67.6	26.2	stroke	GLC	Per SD	Plas	Total	-130°	++++	8

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Sun	2007	USA	NCC	NHS	1989–1990	3.0	166	0/100	60.5	25.9	IHD	GLC	T3 vs. T1 15:0: 0.21 vs. 0.11% 17:0: 0.36 vs. 0.25% Trans 16:1n-7: 0.20 vs. 0.11%	Plasma	Total	-130°C	++++	8
Yamagishi	2008	USA	PC	ARIC	1987-1989	14.5	195	47/53	53.7	26.9	heart failure	GLC	Q5 vs. Q1: not stated	Plasma	Cho/Pf	-80°C	+	9
Yamagishi	2013	USA	PC	ARIC	1987-1989	18.3	168	48/52	53.9	27.0	stroke	GLC	Q4 vs. Q1: not stated	Plasma	Cho/Pf	-80°C	+	9

Matthan	2014	USA	NCC	WHI-OS	1993-1998	4.5	1224	0/100	67.8	27.6	CHD	GC	Per SD	Plasma	Pf	-70°C	++#	9
Khaw	2012	UK	NCC	EPIC-Norfolk	1993–1997	13.0	2424	53/47	61.6	26.5	CHD	GC	Per SD	Plasma	Pf	-196°C	++++	9
Matsumoto	2013	USA	NCC	PHS	1982-1984	17.1	788	100/0	58.7	25.2	heart failure	GC	Q4 vs. Q1: not stated	Plasma	Pf	-80 °C	++++	8
Tokede	2013	USA	NCC	PHS	1982-1984	17.1	788	100/0	58.7	25.2	heart failure	GC	Q5 vs. Q1 Trans 16:1n-7: (0.109-0.219) vs. (0.018-0.062)	Plasma	Pf	-80°C	+#	8
Yaemsiri	2013	USA	NCC	WHI-OS	1993-1998	4.5	964	0/100	68.7	27.4	stroke	GC	Per SD	Serum	Total	-70°C	+#	8
Warensjo	2009	Sweden	NCC	MONICA/VIP	1985-1996	3.0	108	13/87	60.0	26.7	stroke	GLC	Per SD	Serum	Pf	-80°C	#	9

Malik	2012	USA	NCC	HPFS	1993-1994	11.0	459	100/0	67.6	26.2	CHD	GLC	T3 vs. T1: not stated	Plasma	Total	-130°C	++	8

M: males; F: females; PC: prospective cohort study; NCC: nested case-control study; MESA: Multi-Ethnic Study of Atherosclerosis; NSHDS: Northern Sweden Health and Disease Study; NHS: Nurses' Health Study; HPFS: Health Professionals Follow-up study; ARIC: Atherosclerosis Risk in Communities Study; WHI-OS: Women' s Health Initiative Observational Study; EPIC-NORFOLK: European Prospective Studies into Cancer-Norfolk; PHS: Physicians' Health Study; MONICA: Multinational Monitoring of trends and determinants in Cardiovascular disease; CVD: cardiovascular disease; IHD: ischemic heart disease; CHD: coronary heart disease; GC: gas chromatography; GLC: gas liquid chromatography; SD: standard deviation; Total: plasma total fatty acid fraction; Pf: Phospholipid fraction; Cho: cholesterol; temp: temperature; NOS: Newcastle Ottawa Scale

*Degree of covariate adjustment indicated by +: sociodemographics (eg, age, sex, race, education, income, etc.); ++: sociodemographics, and certain dietary variables (total energy, fiber intake etc.); +++: sociodemographics, certain dietary variables , and other circulating fatty acids; ++++: sociodemographics, certain dietary variables , other circulating fatty acids, and CVD risk factors (eg, BMI, smoking, alcohol intake, physical activity, family history, blood pressure, and blood lipids); # : CVD risk factors (eg, BMI, smoking, alcohol intake, physical activity, family history, blood pressure, and blood lipids); +#: sociodemographics and CVD risk factors; ++#: sociodemographics, certain dietary variables and CVD risk factor

Table 2 Subgroup analysis of circulating 15:0, 17:0 and Trans 16:1n-7.

	Studies (n)	Cases (n)	RR (95%CI)	P[a]	I^2 (%)	P[b]
15:0						
Sex						
Women	6	3005	1.04 (0.67, 1.63)	0.850	49.8	0.077
Men	5	1742	1.11 (0.74, 1.66)	0.627	52.7	0.076
Baseline age						
<61.0 years	6	1869	0.89 (0.67, 1.19)	0.432	52.9	0.059
⩾61.0 years	7	5811	1.01 (0.74, 1.38)	0.945	62.5	0.014
Follow-up duration						
≤8 years	6	3052	0.94 (0.59, 1.51)	0.807	68.4	0.007
>8 years	7	4628	0.95 (0.71, 1.19)	0.675	51.7	0.053
17:0						
Sex						
Women	5	1781	0.92 (0.58, 1.46)	0.781	52.7	0.076
Men	3	495	0.73 (0.46, 1.17)	0.192	0.0	0.640
Baseline age						
<61.0 years	4	913	0.72 (0.50, 1.02)	0.066	38.9	0.179

⩾61.0 years	3	3982	0.89 (0.72, 1.10)	0.292	15.4	0.315
Follow-up duration						
≤8 years	4	1682	0.86 (0.59, 1.24)	0.423	40.2	0.170
>8 years	3	3018	0.78 (0.60, 1.01)	0.062	36.9	0.191
Trans 16:1n-7						
Sex						
Women	2	760	0.82 (0.56, 1.21)	0.326	0.0	0.855
Men	1	1382	0.72 (0.47, 1.10)	0.131	15.9	0.275
Baseline age						
<61.0 years	2	954	0.79 (0.60, 1.04)	0.092	0.0	1.000
⩾61.0 years	2	740	0.88 (0.62, 1.24)	0.459	3.7	0.308
Follow-up duration						
≤8 years	2	312	0.94 (0.65, 1.36)	0.729	0.0	0.462
>8 years	2	1382	0.77 (0.60, 1.00)	0.054	0.0	0.820

RR: relative risk; CI: confidence interval;

[a]: p value of Z-test for significance of pooled RRs and 95% CIs;

[b]: p value of Q-test for between study heterogeneity test.

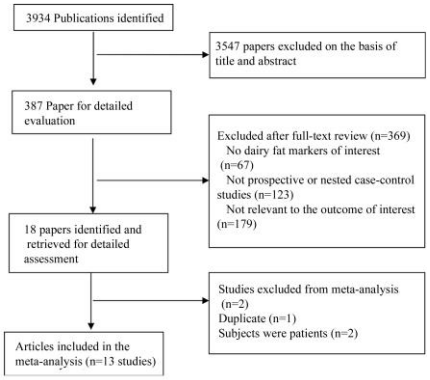

Figure 1 The flowchart of the study selection.

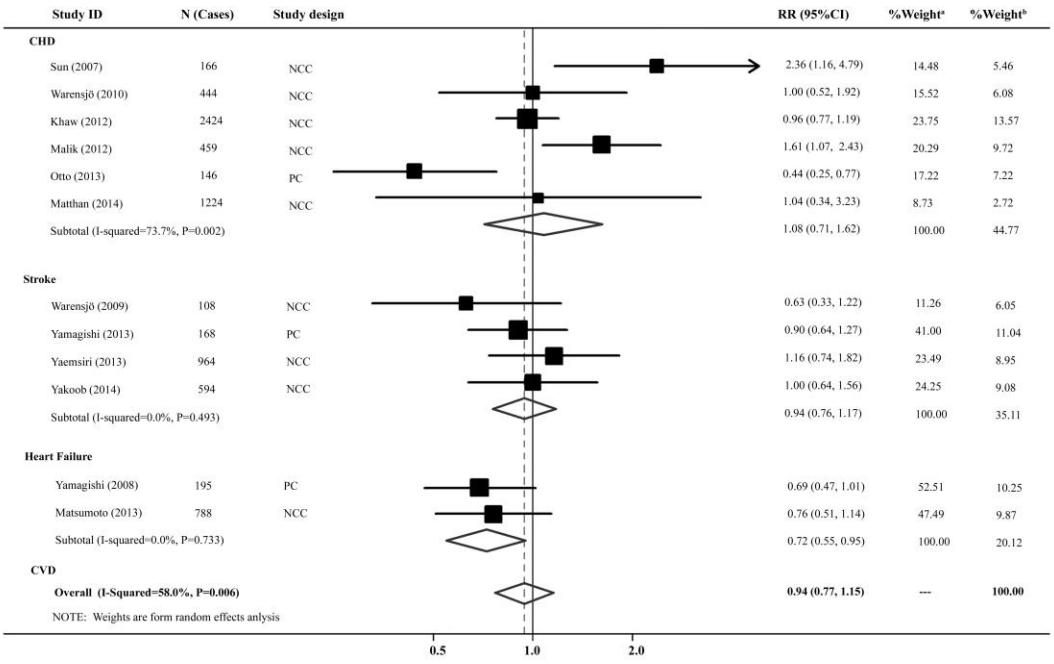

Figure 2 Risk estimates for subtypes and total CVD associated with greater circulating level of 15:0. RR: relative risk. Study-specific RRs and 95% CIs are visualized in squares (the square sizes are proportional to the weight of each study in the overall estimate). The diamond presents the pooled RR and a 95% CI. The percentage of heterogeneity because of between-study variation is shown by I^2. PC: prospective cohort study; NCC: nested case-control study. [a]: weight from subgroup analysis; [b]: weight from overall analysis.

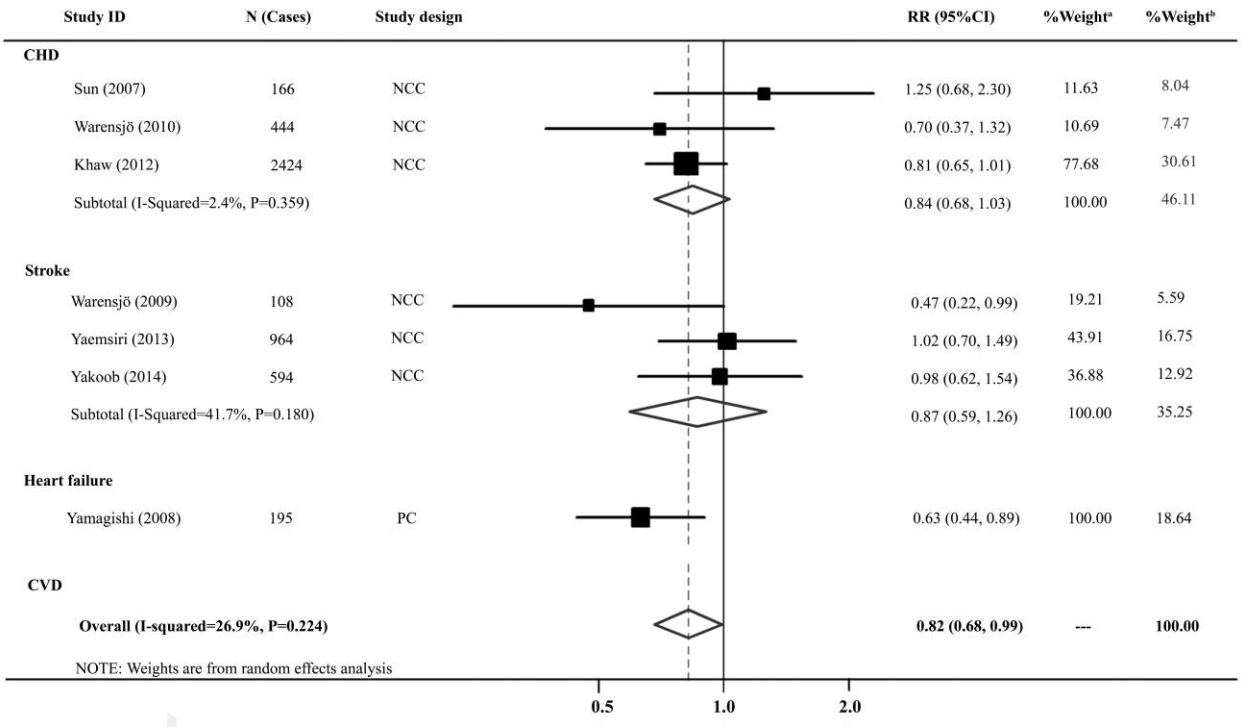

Figure 3 Risk estimates for subtypes and total CVD associated with greater circulating level of 17:0. RR: relative risk. Study-specific RRs and 95% CIs are visualized in squares (the square sizes are proportional to the weight of each study in the overall estimate). The diamond presents the pooled RR and a 95% CI. The percentage of heterogeneity because of between-study variation is shown by I^2. PC: prospective cohort study; NCC: nested case-control study. [a]: weight from subgroup analysis; [b]: weight from overall analysis.

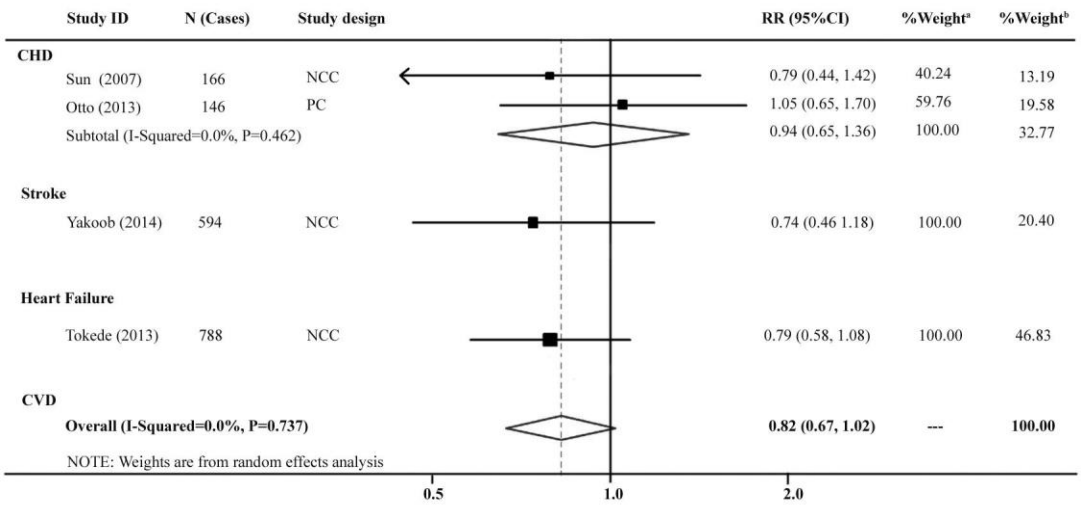

Figure 4 Risk estimates for subtypes and total CVD associated with greater circulating level of trans-16:1n-7. RR: relative risk. Study-specific RRs and 95% CIs are visualized in squares (the square sizes are proportional to the weight of each study in the overall estimate). The diamond presents the pooled RR and a 95% CI. The percentage of heterogeneity because of between-study variation is shown by I^2. PC: prospective cohort study; NCC: nested case-control study. [a]: weight from subgroup analysis; [b]: weight from overall analysis.

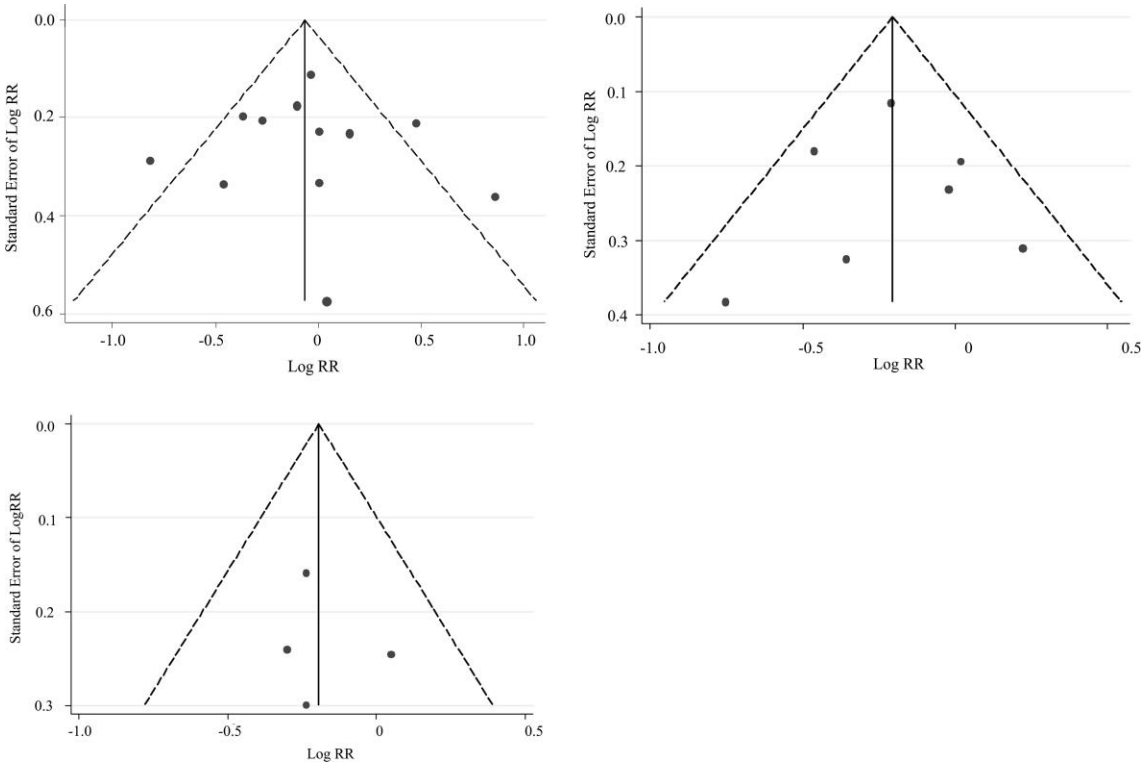

Figure 5 Funnel plot for prospective cohort studies of biomarkers of dairy fat and CVD incidence. The dotted lines show 95% confidence intervals around the overall summary estimate. RR: relative risk.