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Abstract

Commonly held dietary guidelines discourage full-fat dairy consumption due its to high levels of saturated fatty acids, which are believed to aversely influence cardiovascular disease risk. Cardiovascular diseases cause more than one third of deaths in the developed world. With dairy often providing a large part of energy and nutrients in Western diets, research into its effects on human health is warranted.

Several recent studies have suggested a protective role of dairy in the development of cardiometabolic disease. However, many of these have used imprecise methods of dietary assessment, based on questionnaires or interviews. Biomarkers are emerging as a means of evaluating diet in a more objective way.

Currently used dairy fat biomarkers are minor fatty acid constituents of dairy that preferably are unique to dairy and not influenced by endogenous metabolism. These compounds can be measured in blood or adipose tissue samples, and can be good indicators of short (weeks) and long (years) term intakes, depending on the sampling medium. The most reliable indicator of dairy fat intake seems to be pentadecanoic acid (C15:0), however research in this area is somewhat lacking.

Studies where these biomarkers have been evaluated in relation to cardiometabolic disease endpoints (incident cardiovascular disease or type-2 diabetes) have generally found dairy to elicit a protective effect. Cross-sectional studies evaluating indices of the metabolic syndrome suggest that dairy fat may slow down disease progression.

Further standardization of methodologies and validation of biomarker concentrations in relation to intake could improve reliability of biomarker-based dietary assessments. Furthermore, combining chemical and traditional methods could provide even better precision than using either method alone.

Sammanfattning

Många av dagens kostråd avråder från konsumtion av fullfeta mejeriprodukter p.g.a. höga halter mättade fettsyror, vilka anses påverka kardiovaskulär hälsa negativt. Hjärt–kärlsjukdomar orsakar mer än en tredjedel av alla dödsfall i den industrialiserade världen. Då mejeriprodukter ofta bidrar med en betydande del av energi och näringsämnen i den västerländska kosten är det motiverat att studera dess effekter på human hälsa.

Ett antal studier har de senaste åren funnit resultat som antytt att konsumtion av mejeriprodukter kan ha en skyddande inverkan på risken för insjuknande i hjärt–kärlsjukdom, men många av dessa har använt osäkra metoder för att mäta deltagarnas kostvanor. Mer precisa mätningar kan göras med biomarkörer, något som ökat i användning under senare tid.

De biomarkörer för mjölkfettintag som används i dag är fettsyror som förekommer i låga halter i mjölken, och som bör vara både unika för mejeriprodukter och inte påverkade av endogen metabolism. Dessa ämnen kan mätas i blod eller i fettvävnad, och kan vara goda indikatorer för konsumtion över korta (veckor) eller långa (år) perioder. En av de mest tillförlitliga markörerna anses vara pentadekansyra (C15:0), men ytterligare forskning behövs för att säkerställa detta.

Studier som utvärderat dessa biomarkörer i förhållande till kliniska ändpunkter (incidens av hjärt–kärlsjukdom eller diabetes typ 2) har generellt visat på en skyddande effekt. Tvärsnittsstudier som undersökt olika aspekter av metabola syndromet antyder att mjölkfettskonsumtion kan fördröja dess utveckling.

Ytterligare standardisering av metoder och validering av samband mellan biomarkörer och intag skulle kunna förbättra precisionen hos kemiska kostskattningsmetoder. Precisionen skulle ytterligare kunna förbättras om kemiska och traditionella metoder kombinerades.

Acronyms and abbreviations

BCAA	Branched-chain amino acid
CE	Cholesteryl ester
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
FA	Fatty acid
FFQ	Food frequency questionnaire
HDL-C	High density lipoprotein cholesterol
HR	Hazard ratio
MetS	Metabolic syndrome
MI	Myocardial infarction
OR	Odds ratio
PL	Phospholipid
RR	Relative risk
rTFA	Ruminant *trans* fatty acid
SFA	Saturated fatty acids
T2D	Type-2 diabetes
TC	Total cholesterol
TAG	Triacylglycerol

Contents

1 Introduction

Milk and dairy products are important sources of energy and nutrients in large parts of the Western world. In a Swedish study conducted in 2010–2011 (Amcoff et al., 2014), dairy products (excluding butter, and dairy used in cooking) provided 12 % of calories and 11 % of protein present in the diet. Additionally, dairy products supplied almost a fifth of the average daily intake of protein, and was a rich source of several vitamins and minerals including vitamins A and D, calcium, and phosphorus.

Dairy is also a rich source of saturated fatty acids (SFA), with dairy products and cheese supplying 25 % of the SFA intake (ibid.). However, since this value neither accounts for butter nor for dairy products used for cooking, the true amount is likely higher. Because SFA is generally considered harmful to cardiovascular and metabolic health, many Western dietary guidelines have focused on encouraging the substitution of reduced-fat dairy products for full-fat varieties. Choosing reduced-fat varieties is seen a means of reducing the consumption of saturated fat without adversely affecting intakes of other nutrients (Nordic Council of Ministers, 2014; U.S. Department of Agriculture and U.S. Department of Health and Human

Services, 2010; Krauss et al., 2000).

The metabolic syndrome (MetS) is a cluster of risk factors for heart disease and diabetes, which includes high blood pressure, dyslipidemia (high triglycerides and low HDL-C), raised fasting glucose, and central obesity (Alberti et al., 2009). Persons with MetS are at higher risk of heart disease and diabetes (ibid.), implying a common etiology for these diseases. Therefore, these diseases (coronary heart disease (CHD), stroke, and type-2 diabetes (T2D)) are often known as cardiometabolic diseases, encompassing both cardiovascular and metabolic disease. Strengthening the idea of a common etiology is the fact that association between an important component of MetS, raised fasting glucose, is part of the diagnostic criteria for T2D (American Diabetes Association, 2008), but MetS is also a risk factor for CVD independently of T2D (Malik et al., 2004).

Researching the impact of dairy consumption on human health is a complex task, fraught with methodological issues. Practical and financial considerations limit the feasibility of conducting large-scale randomized controlled trials, and studies of this kind generally measure surrogate markers, e.g. blood lipid profiles, and not clinical endpoints such as incident heart disease. Prospective cohort studies can use clinical endpoints, but the assessment of subjects' diets is generally imprecise (especially over extended periods of time). Due to these issues, the interest in using biomarkers, specific chemical indicators of diet, has increased among nutritional epidemiologists (Van Dam and Hunter, 2012).

The aim of this thesis is, first of all, to provide an overview of the use of biomarkers in studies of dairy intake and, secondly, to summarize the findings of studies that employ biomarkers to elucidate how dairy intake relates to outcomes of cardiometabolic health.

2 Dairy biomarkers

2.1 Biomarkers versus traditional methods of diet assessment

When using epidemiological methods to determine the influence of dietary factors on human health, the accuracy of dietary assessment is of the utmost importance. Potential systematic biases influencing the validity of dietary reporting by subjects include over- and underestimation of intake, subjects adjusting reported intake in accordance with what one believes is healthy or is "what the investigator wants to hear", and difficulty in remembering food choices over extended periods of time (Willett, 2012). Even the most comprehensive method of assessment, the weighed food record, where sub-

jects weigh all food consumed, may not be applicable for ascertainment of long-term intakes due to the high practical overhead associated with subjects weighing all their food.

Dietary biomarkers have their limitations as well. Subjects' levels of biomarkers can be influenced by many factors, including genetics, disease status, age, nutrient bioavailability, the specific depot that is being sampled, etc. (Hodson et al., 2008; Van Dam and Hunter, 2012; Arab, 2003). Optimally, biomarkers should be combined with other methods of measuring dietary intake to strengthen the conclusions and minimize bias, since measurement errors inherent to either method arise independently of each other (Van Dam and Hunter, 2012; Baylin et al., 2002).

2.2 Compounds used as biomarkers

The most common biomarkers used for assessing dairy intake are the odd-numbered saturated fatty acids (FA) pentadecanoic acid (15:0) and margaric acid (17:0). These FAs are microbially synthesized in the rumen and passed on to the milk, and they cannot be endogenously synthesized by humans. Several studies have found 15:0 to be a valid biomarker (Wolk et al., 1998; Smedman et al., 1999; Wolk et al., 2001), but correlations have generally been weaker and less consistent for 17:0 (Nestel et al., 2014; Jacobs et al., 2014).

Myristic acid (14:0) has been suggested as a dairy fat biomarker that is easier to measure, due to the higher concentrations normally present in human FA pools. 14:0 is abundant in dairy and ruminant fat, but is otherwise scarce in the food supply. Although dietary intake of this FA may thus correlate with dairy fat consumption, circulating (i.e. present in blood) or adipose (fat) tissue levels may not. Being an even-chained FA, endogenous synthesis can be an important confounder. Wolk et al. (2001) found 14:0 to be an adequate biomarker in adipose tissue but not in cholesteryl ester (CE) and phospholipids (PL), and several other studies have reported inconsistent results when comparing 14:0 and 15:0 to the same outcomes (c.f. Perreault et al. (2014); de Oliveira Otto et al. (2013); Iggman et al. (2010)). Since endogenous synthesis can be upregulated in metabolic disease, as evidenced by elevated triglyceride levels, the use of this biomarker can be problematic when studying cardiometabolic outcomes or risk factors.

Another marker that has been evaluated in a number of studies (c.f. Mozaffarian et al. (2010, 2013); de Oliveira Otto et al. (2013)) is the ruminant *trans* fatty acid (rTFA) *trans*-palmitoleic acid (*trans*-16:1n–7), which cannot be synthesized endogenously. However, this fatty acid can also be formed by partial hydrogenation of vegetable oils, which can limit its validity as a

biomarker. In a study on the Multi-ethnic Study of Atherosclerosis (MESA) cohort, *trans*-16:1n–7 was found to be more strongly associated with french fry consumption than with any other food (de Oliveira Otto et al., 2013).

Other minor FA constituents have also been used. Conjugated linoleic acid (conjugated 18:2; CLA) isomers were validated in a Swedish population (Jiang et al., 1999), where the $9c, 11t$ isomer was found to correlate more strongly with dairy fat intake than with 15:0 in adipose tissue. Smit et al. (2010) used this isomer as a biomarker for the intake of dairy fat from cows grazing on pasture, as opposed to silage or grain.

Recently, *trans*-18:1 isomers have been demonstrated to correlate with milk consumption in Chinese subjects (Zong et al., 2014; Yu et al., 2012). As with other *trans* FAs, hydrogenated vegetable oils are likely confounders here as well (Micha et al., 2010).

2.3 The use of tissues and blood for sampling

The biomarkers described above are fatty acid components of dairy fat. For biomarker analysis, either adipose tissue or blood samples are taken from the subjects. Adipose tissue is believed to be the best indicator of long-term intake, with studies estimating $t_{1/2}$ values of 6–24 months for adipose tissue triacylglycerol (TAG) (Hodson et al., 2008).

Serum or plasma samples are generally analyzed for FA composition of free or total PL, erythrocytes (red blood cells), non-esterified fatty acids (NEFA), TAG or CE FAs. An issue with sampling serum FA composition is that it likely does not correlate with long-term intake as closely as samples from adipose tissue do. Even erythrocytes, previously believed to reflect 120-day average intakes (Arab, 2003), have been demonstrated to alter their membrane FA composition in response to diet in a matter of days (Hodson et al., 2008). In a study by Wolk et al. (2001), weaker correlations were found for serum PL and cholesteryl esters and dietary dairy fat intake than for adipose tissue FAs, especially for 14:0. In a recent study (Hodson et al., 2014), plasma CE, TAG, PL and erythrocytes were found to be equally valid indicators of 15:0 over a similar time period, equilibrating after two weeks on a high-SFA diet.

Blood sampling has been considered less invasive than adipose tissue sampling, due to the requirement of a biopsy when performing the latter. However, Beynen and Katan (1985) has reported on the use of a less invasive method for sampling adipose tissue. In this method, a “mini biopsy” is performed via aspiration into a 1.5 mm diameter needle, thereby reducing intrusiveness and lessening requirements for operator training. This method should be considered when more reliable measures of long-term dairy fat

intake are needed, if the study protocol allows it.

2.4 Other methodological considerations

Although less representative of long-term intake than adipose tissue samples, serum samples can be used for nested case–control studies. In this type of study, blood is drawn from all subjects in a large cohort, but samples are analyzed only after a certain number of cases are present in the cohort, or after a predetermined period of time. At this time, risk factor matched controls are chosen from the cohort according to a certain case:control ratio, often 1:2. The use of biomarkers makes accurate retrospective dietary assessment feasible, even for deceased subjects. *Post hoc* analysis of blood samples also reduces laboratory costs.

It is worth noting that measures of dairy intake can differ between traditional and biomarker-based methods of dietary assessment in a number of ways. First, dairy biomarkers are indicative of total dairy fat consumption, which may or may not reflect total dairy intake. This also means that high-fat products will influence biomarker concentrations more than low-fat products. Second, since these biomarkers are measures of total dairy fat intake, food items which are normally hard to quantify using traditional methods, e.g. cheese on pizza or butter in mashed potatoes, will influence biomarker concentrations as well. Third, levels of individual fatty acids in dairy are dependent on animal husbandry practices, primarily the type of feed used (Kratz et al., 2013). Finally, fatty acid biomarkers are a relative measure of fatty acid intake, and cannot be used for quantitative assessment.

Dairy biomarkers are present not only in dairy, but also in adipose tissue and intramuscular fat of ruminants. Thus, dairy is not the only dietary source for these FAs. However, since the intake of dairy fat generally far exceeds the intake of ruminant fat from meat in most Western countries, the risk of confounding is likely minor. Although not usually an issue in Western populations, one should take other dietary sources into account when examining populations with other dietary habits.

3 Bioactive compounds in dairy products

Bioactive compounds are compounds which have an effect on living tissue. This is a very broad concept, especially since any type of food can arguably fall under this definition, so for practical purposes, *bioactivity* will be referred to as the effects caused beyond those that could be expected from metabolism alone.

A large part of dairy fat is made up of SFA. For several decades, SFA consumption has been discouraged by policymakers and nutritionists, due to its observed correlation with CVD (Keys et al., 1986). However, recent meta-analyses of prospective cohort studies have detected either inverse or neutral associations between SFA consumption and CVD (Siri-Tarino et al., 2010; Chowdhury et al., 2014). Additionally, recent publications have found inverse associations between dairy consumption and CVD (Soedamah-Muthu et al., 2011), as well as for incident T2D (Gao et al., 2013). Moreover, physiological effects of SFA vary with chain length. For example, medium-chain fatty acids (C_5–C_{12}) are taken up via the portal vein and are preferentially β-oxidized in the liver (Bach and Babayan, 1982). Short chain (C_2–C_4) SFAs are absorbed by enterocytes and provide energy as well as downregulating inflammatory pathways in these cells (Kratz et al., 2013).

More than 400 different individual FAs have been identified in dairy (Jensen, 2002), of which some have been demonstrated to exhibit physiological activity. Examples of bioactive fatty acids in dairy are conjugated linoleic acids (in particular the $9c, 11t$ and $10t, 12c$ isomers), rTFAs, branched-chain fatty acids such as phytanic acid (Kratz et al., 2013), and *trans*-16:1 n–7 (Mozaffarian et al., 2010, 2013). Due to the large number of identified FAs, there are likely several that have yet to be classified as bioactive.

Dairy provides around 11 % of total protein in the Swedish diet (Amcoff et al., 2014), again only counting direct consumption. Dairy protein is highly bioavailable, and may benefit cardiometabolic health in several ways. It has been shown to improve metabolic risk factors such as hypertension (high blood pressure) and dyslipidemia (abnormal blood lipids) (McGregor and Poppitt, 2013). In addition, protein consumption, especially branched-chain amino acids (BCAA) and in particular leucine, is known to stimulate protein synthesis and improve body composition, which can be of importance for preventing the development of MetS (ibid.). In a recent study comparing the effects of diet on weight regain after weight loss, Bendtsen et al. (2014) found a non-significant ($p = 0.08$) association between dairy protein intake and weight regain. Although this effect was attenuated after adjusting for total protein intake, suggesting that the source of protein was not the determinant for weight regain in this study, it is plausible that regular dairy consumption is associated with higher protein intake.

Calcium and vitamin D, which are present in many dairy products, are also potentially beneficial to cardiometabolic health. In a review of the influence of these nutrients on energy balance, Soares et al. (2014) concluded that both nutrients influence human energy regulation positively. According to the authors, the evidence for the beneficial effects of vitamin D is largely

based on cross-sectional studies showing an inverse correlation between vitamin D status and obesity, which could be confounded by dilution of the vitamin into a larger adipose tissue mass. In a meta-analysis of randomized controlled trials, Onakpoya et al. (2011) found that calcium supplementation of 1000–1500 mg day^{-1} led to small but significant changes in body weight and body fat, with trials ranging from 6–24 months in duration. Calcium-induced fat loss also seems to favor visceral fat depots (Soares et al., 2014), which can be of certain importance for preventing cardiometabolic disease. In a meta-analysis of prospective studies and randomized trials conducted by Wang (2010), vitamin D, but not calcium, was found to be associated with a reduced risk of developing cardiovascular disease.

Dairy products are diverse, owing to the versatility of milk. Milk can be consumed whole, containing protein, fat, and carbohydrate along with minerals and vitamins in more or less the original proportions. More often, dairy is processed into one of many end products. For example, separation by centrifugation renders skim milk and cream, with each fraction containing any potentially bioactive compound associated with either phase (hydro- or lipophilic). In cheesemaking, dairy proteins are separated based on coagulation properties, thus cheese contains only the coagulative casein protein fraction. The whey fraction is then used in products such as protein powder or ice cream. Fermentation further alters the nutritional and chemical composition. Due to this diversity, the health effects of dairy can differ by the type of product consumed, further complicating research into any potential health effects.

A thorough review of all known bioactive compounds potentially present in dairy products is outside of the scope of this thesis, but there are several reviews published on the subject, c.f. Haug et al. (2007); Kratz et al. (2013). In addition to process-induced changes in composition, the issue of the bioactive properties of milk is further complicated by the fact that levels of many compounds, in either individual products or total intake, can be influenced by factors such as feeding regime, genetics, culture and climate (via a preference for certain products over others), etc. However, this section serves to demonstrate the nutritional heterogeneity of these products. One must also take into account that at least some compounds can be beneficial to health only where an insufficiency is present, as can be the case for several vitamins and minerals.

4 Studies evaluating dairy biomarkers

4.1 Study selection

To find pertinent studies, MEDLINE was searched using the terms “fatty acid composition”, “dairy”, “biomarkers”, and names of relevant biomarker compounds in combination with “cardiovascular”, “coronary”, and “diabetes”. Moreover, review articles and meta-analyses on either dairy consumption or circulating fatty acid composition and cardiometabolic disease outcomes were identified, and their reference lists examined for relevant studies.

A similar strategy was used for finding studies relevant to different aspects of the metabolic syndrome. However, due to the many facets of metabolic health that can be measured, an exhaustive list of such studies is outside the scope of this thesis. Since most studies of this kind are cross-sectional in design, one cannot infer causality from their results. Rather, the studies have been selected to be representative of the literature on the subject as a whole, and as a basis for discussing the results from studies using clinical endpoints presented below.

4.2 Dairy biomarkers and metabolic health

As described above, the diagnosis of MetS is based on metabolic and anthropometric measures that exist on a continuum. Therefore, emergence of disease within subjects can be tracked, as opposed to simply estimating the increased risk of disease.

Nine studies measuring both dairy biomarkers and indices of cardiometabolic health are included in this overview (Table 1). All experiments were cross-sectional in design, which means that they cannot be used for deducing causality.

Two publications evaluated the correlation between dairy fat biomarkers and prevalence of MetS. Maruyama et al. (2008) examined the FA composition of plasma PL in 165 males aged 40–59. Subjects receiving medication for MetS-related complications were excluded from this study. Out of 165 subjects, 27 were subsequently diagnosed as having untreated MetS. Levels of 15:0 and 17:0 were lower in subjects with MetS, compared to MetS-free subjects. Additionally, negative correlations were found between plasma 17:0 and HDL-C, body fat percentage, and triglycerides. Mayneris-Perxachs et al. (2014) found a strong positive correlation for plasma 14:0 and MetS prevalence, but this correlation was not present for 17:0. The aim of the latter study was not explicitly to measure dairy biomarkers, which explains the use of the less reliable markers 14:0 and 17:0.

Table 1: Studies examining dairy biomarkers and indices of metabolic health. Abbreviations: FA, fatty acid; LH, lean healthy; MetS, metabolic syndrome; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; NEFA, non-esterified fatty acid; PL, phospholipid

Citation	Biomarkers	Outcomes	Subjects	Main findings
Maruyama et al. (2008)	PL 14:0, 15:0 and 17:0 in serum.	FA profiles of subjects with and without previously undiagnosed MetS.	Males aged 40–59 years, with ($n = 27$) and without ($n = 138$) MetS.	15:0 and 17:0 concentrations were lower in subjects with MetS.
Mozaffarian et al. (2010)	PL *t*-16:1n–7 in plasma.	Cardiometabolic risk markers, incident T2D.	3736 adults.	*t*-16:1n–7 was associated with favorable changes in cardiometabolic risk markers.
Iggman et al. (2010)	Multiple FAs in adipose tissue.	Insulin sensitivity.	795 men, mean age 71 years.	14:0 and 17:0, but not 15:0, correlated positively with insulin sensitivity.
Wang et al. (2011)	PL 15:0 and 17:0 in serum.	Markers of inflammation and oxidative stress.	305 adolescents, mean age 15 years.	Most dairy biomarkers were associated with lower levels of inflammation in obese subjects. Only IL-6 was associated with dairy biomarkers in all subjects.
Kratz et al. (2014)	PL and NEFA 15:0, 17:0, and *t*-16:1n–7 in plasma.	Glucose tolerance.	17 men and women with NAFLD, 15 age- and BMI-matched controls.	Several biomarkers were associated with increased glucose tolerance.
Jacobs et al. (2014)	15:0, 17:0, and *t*-16:1n–7 in erythrocytes.	Markers of dyslipidemia.	1759 random subjects from EPIC-Potsdam study, age 35–64.	Weak or no association for most dairy biomarkers and dyslipidemia.
Mayneris-Perxachs et al. (2014)	NEFA 14:0 and 17:0 in plasma.	Presence of MetS.	427 subjects aged 55–80 years.	Strong positive correlation between 14:0 and MetS, 17:0 neutral.
Nestel et al. (2014)	PL 14:0, 15:0, *t*-16:1n–7, 17:0, and *t*-18:1n–7 in plasma.	Insulin sensitivity.	86 overweight and obese subjects with metabolic syndrome.	Dairy biomarkers were associated with favorable measures of insulin sensitivity.
Yu et al. (2012)	*t*-18:1 in erythrocytes.	Several indices of cardiometabolic health, prevalence of T2D.	3107 subjects aged 50–70 y.	*t*-18:1 was inversely correlated with all indices of cardiometabolic health except hypertension.
Perreault et al. (2014)	Total 14:0, 15:0, 17:0, and *t*-18:1n–7 in serum.	FA profiles of unhealthy vs. healthy subjects.	LH, MHO and MUO subjects ($n = 10$ per group).	Significantly higher concentration of 14:0 in MUO than LH or MHO.

Insulin sensitivity and glucose tolerance are important factors of MetS. Three studies that examined relationships between dairy biomarkers and these factors are included in this overview. Iggman et al. (2010) examined adipose tissue 14:0, 15:0, and 17:0 in 795 older Swedish men, and compared it to insulin sensitivity as measured by the "gold standard" euglycemic clamp method (Kratz et al., 2013). In the fully adjusted model (BMI, smoking,

alcohol intake and physical activity), 14:0 and 17:0, but not 15:0, were associated with increased insulin sensitivity, but the authors mention that there might have been methodological errors which could have influenced their measurements. In a recent study, Kratz et al. (2014) measured insulin sensitivity in 17 subjects with non-alcoholic fatty liver disease (NAFLD; an affliction closely related to MetS) and 15 age- and BMI-matched controls, and examined correlations with 15:0, 17:0 and *trans*-16:1n–7 in plasma PL and NEFA. In this investigation, all dairy biomarkers in PL were found to be associated with favorable changes in glucose metabolism. In the third study (Nestel et al., 2014), glucose tolerance was measured in 86 overweight and obese subjects with MetS. Most dairy biomarkers (15:0, *trans*-16:1n–7, and *trans*-18:1n–7, but not 17:0) were found to be positively correlated with glucose tolerance.

Other researchers have looked at a number of components of MetS in relation to dairy biomarkers. Mozaffarian et al. (2010) measured plasma PL levels of *t*-16:1n–7 in 3736 adults aged 65 or older, and evaluated correlations with markers of metabolic health. Comparing extreme quintiles of *trans*-16:1n–7 levels, subjects in the highest quintile had lower waist circumference ($p = 0.009$), lower total cholesterol (TC):HDL-C ratio ($p < 0.001$), less insulin resistance ($p < 0.001$), and lower levels of C-reactive protein (CRP; a marker of inflammation, $p = 0.050$). These findings suggest better metabolic health in the highest quintile of dairy intake. Measures of blood glucose, insulin concentrations and triglycerides were also favorable for the highest quintile, though bias is likely present due the fact that many samples were taken when the subjects were not in a fasting state.

Jacobs et al. (2014) examined correlations between dairy biomarkers (15:0, 17:0 and *t*-16:1n–7) in erythrocytes and a number of markers for dyslipidemia in 655 men and 1104 women. The results of this study suggest that dairy intake is not correlated with most aspects of dyslipidemia in men, but that several markers correlate with a favorable lipidemic profile in women. However, many measures of dyslipidemia are influenced by fasting status, which was not consistent among subjects in this study. Overall, the findings were inconclusive regarding dairy biomarkers, which might be at least in part attributable to the pronounced heterogeneity between the male and female groups (Jacobs et al., 2014, Table 1), as well as heterogenous fasting status (which was not included in multivariate analyses). Additionally, 25 % of the subjects were excluded due to "implausible" values of erythrocyte FAs, suggesting further methodological issues.

An uncommon biomarker, *trans*-18:1 (all isomers), was used in a relatively large study ($n = 3107$) conducted on a Chinese population (Yu et al.,

2012). The authors evaluated associations between extreme quintiles of erythrocyte *trans*-18:1 and several indices of cardiometabolic health, including markers of dyslipidemia, insulin resistance, hypertension, and central obesity, as well as the prevalence of T2D. For extreme quartiles, all indices except hypertension were inversely correlated with erythrocyte *trans*-18:1 concentrations.

Finally, in a recent study by Perreault et al. (2014), serum FA profiles of groups of lean healthy (LH), metabolically healthy obese (MHO), and metabolically unhealthy obese (MUO; $n = 10$ for each group) were measured. MHO and MUO groups did not differ in anthropometric measurements, but were distinguished by cardiometabolic risk profiles. FA biomarkers of interest to this thesis were 14:0 and 15:0. Only 14:0 showed a consistent trend, with elevated levels in the MUO group compared to both LH and MHO. Since 14:0 might be confounded by metabolic disease, these results might be irrelevant to the question at hand, especially considering the small number of subjects.

4.3 Cardiovascular disease outcomes

Cardiovascular diseases (CVD) are diseases of the heart and circulatory systems, and include both CHD and stroke, as well as heart failure (HF). CHD diseases share a common etiology in the inability of the coronary arteries to deliver sufficient oxygen to the heart, commonly due to atherosclerosis. A common type of CHD is myocardial infarction (MI), commonly known as a heart attack. Strokes are caused by a temporary lack of oxygen supply to parts of the brain, often stemming from either blocked arteries or brain hemorrhages.

Ten studies were found that evaluated associations between biomarkers of dairy fat intake and cardiac event endpoints. Of these, two were prospective cohort studies and eight were nested case–control studies. All except two used plasma or serum biomarkers, and all except one measured 15:0 concentrations (Table 2).

Of the nine studies measuring 15:0 concentrations, five found inverse correlations for cardiac events, and three did not find any significant correlations. In one study (Sun et al., 2007), plasma 15:0 was found to be strongly associated with CHD in the fully-adjusted model (OR for extreme tertiles: 2.36, 95% CI: 1.16–4.78, $p_{trend} = 0.03$). However, this association was not found for erythrocytes (OR: 0.93, 95% CI: 0.42–2.05). Additionally, 17:0 was associated with a protective effect in erythrocytes, but not in plasma. It is likely that the discrepancy between plasma and erythrocyte biomarker associations is an artifact stemming from a methodological problem, likely

Table 2: Studies examining dairy biomarkers and incident cardiovascular disease. Abbreviations: CE, cholesteryl ester; CHD, coronary heart disease; CLA, conjugated linoleic acid; CVD, cardiovascular disease; HF, heart failure; MI, myocardial infarction; OR, odds ratio; PL, phospholipid

Citation	Biomarkers	Outcomes	Subjects	Main findings
Aslibekyan et al. (2012)	15:0 and 17:0 in adipose tissue.	Incident first MI.	1815 cases, 1815 matched controls.	No significant correlations.
de Oliveira Otto et al. (2013)	PL 14:0, 15:0, and t-16:1n-7 in plasma.	Incident CVD.	2837 (cohort), 189 cases.	Inverse correlation between 15:0 and CVD.
Khaw et al. (2012)	Odd chain SFA composition in plasma PL.	Incident CHD.	2424 cases, 4930 matched controls.	Inverse correlation between 15:0, 17:0 and CHD.
Warensjö et al. (2010)	PL 15:0 and 17:0 in serum.	Incident MI.	444 cases, 556 controls.	No significant associations.
Smit et al. (2010)	$9c,11t$-CLA in adipose tissue.	Incident MI.	1813 cases, 1813 matched controls.	OR for extreme quintiles = 0.51.
Warensjö et al. (2009)	PL 15:0 and 17:0 in plasma.	Incident stroke.	129 cases, 257 matched controls.	17:0 and (15:0 + 17:0) were inversely associated with incident stroke.
Yamagishi et al. (2008)	PL and CE 14:0, 15:0, and 17:0 in plasma.	Incident HF.	3592 (cohort), 197 cases.	15:0 and 17:0 in PL were negatively associated with HF, whereas 14:0 in CE was positively associated, but results for 15:0 and 17:0 were not significant after multivariate adjustment.
Sun et al. (2007)	15:0, 17:0, and t-16:1n-7 in plasma and erythrocytes.	Incident CHD.	166 cases, 327 matched controls.	15:0 in plasma, but not in erythrocytes, correlated positively with incident CHD. 17:0 in erythrocytes was inversely correlated.
Biong et al. (2006)	14:0, 15:0, and 17:0 in adipose tissue.	Incident first MI.	99 cases, 98 matched controls.	15:0 inversely associated with incident first MI.
Warensjö et al. (2004)	CE and PL 15:0 and 17:0 in serum.	Incident first MI.	78 cases, 156 matched controls.	15:0 and 17:0 in serum PL were inversely associated with incident first MI.

due in part to the heterogenous fasting status among subjects.

Smit et al. (2010) measured $9c,11t$-CLA in adipose tissue. The authors state that this biomarker is indicative of pasture-fed dairy consumption, however, no measures of 15:0 were performed to separate putatively CLA-rich products from conventional dairy. In this study, adipose tissue CLA was strongly and significantly associated with reduced risk of MI (OR for extreme quintiles: 0.51, 95% CI: 0.36–0.71, $p < 0.0001$), as well as with total dairy intake ($p < 0.0001$).

Eight of the studies measured 17:0 concentrations. Of these, five found inverse correlations with cardiac events, and three found no significant cor-

relations. It is worth noting that although 17:0 is generally less strongly correlated with dairy fat intake, it is often generally found to be inversely associated with CVD risk, perhaps indicative of unknown metabolic processes.

Regarding associations for *t*-16:1n–7, associations were generally weak. Sun et al. (2007) found a weakly significant ($p_{trend} = 0.04$) inverse correlation for *t*-16:1n–7 in plasma and CHD in the basic multivariate model, but this association lost significance in the fully adjusted model. Additionally, due to the issues described above, the ambiguous results found in this study could likely be influenced by fasting status. In the MESA cohort, no significant association was found, but the authors state that *t*-16:1n–7 was likely a marker for intake of partially hydrogenated vegetable oils in these subjects, as indicated by FFQ results (de Oliveira Otto et al., 2013).

4.4 Diabetes outcomes

Type-2 diabetes (T2D) is a disease of impaired glucose tolerance, resulting either from reduced production of or from impaired sensitivity to insulin. The disease is diagnosed as either having a plasma fasting glucose level of ≥ 7.0 mmol l^{-1}, or a plasma glucose level of ≥ 11.1 mmol l^{-1} two hours after an oral glucose tolerance test (American Diabetes Association, 2008).

Three studies were identified that employed dairy biomarkers and used incident T2D as a main outcome. All found strong significant inverse associations between dairy biomarkers and incident T2D (Table 3).

Table 3: Studies examining dairy biomarkers and incident type-2 diabetes. Abbreviations: PL, phospholipid; T2D, type-2 diabetes

Citation	Biomarkers	Outcomes	Subjects	Main findings
Mozaffarian et al. (2010)	*t*-16:1n-7 in plasma PL.	Incident T2D.	3736 (cohort), 304 cases.	Significant inverse correlation for *t*-16:1n-7 and incident T2D.
Mozaffarian et al. (2013)	*t*-16:1n-7 in plasma PL.	Incident T2D.	2281 (cohort), 205 cases.	Significant inverse correlation for *t*-16:1n-7 and incident T2D.
Zong et al. (2014)	t-18:1 in erythrocytes.	Incident T2D.	2091 (cohort), 504 cases.	Significant inverse association for *t*-18:1 and incident T2D.

In two studies, both conducted by Mozaffarian et al. (2010, 2013), *t*-16:1n–7 levels were measured in plasma PL. In a meta-analysis including subjects from both studies (Mozaffarian et al., 2013), the HR for extreme quintiles was 0.44 (95% CI: 0.31–0.63, $p_{trend} < 0.001$).

In a recent article, Zong et al. (2014) reports on erythrocyte *t*-18:1 concentrations in a cohort of middle-aged and older Chinese subjects. In this cohort, dairy consumption was uncommon, with only 57.5% of participants reporting any dairy consumption at all. *t*-18:1 was associated with total dairy consumption, and the RR for T2D for extreme quartiles was 0.82 (95% CI: 0.65–1.04, $p_{trend} = 0.02$).

5 Discussion

A number of recent reviews and meta-analyses have found either inverse or neutral associations for dairy consumption and cardiometabolic disease outcomes (Kratz et al., 2013; Aune et al., 2013; Soedamah-Muthu et al., 2011). However, many of the underlying studies have used FFQs for diet assessment.

Unlike in drug trials, it is not possible to design a properly controlled, randomized, double-blind trial examining the effects of dairy consumption on human health. By definition, an isocaloric non-dairy control food cannot be metabolically inert, and likely cannot be designed to have the sensory properties required for proper blinding. Therefore, evidence from biomarker-based studies should be considered very high-grade, especially when used in studies of prospective cohort or nested case–control designs.

Table 4: Summary of findings from studies comparing dairy biomarkers and clinical endpoints. Individual biomarkers have been broken out for CVD (15:0 and 17:0). *Positive* refers to reduced risk of disease. Abbreviations: CVD, cardiovascular disease; T2D, type-2 diabetes

Endpoint	Positive	Neutral	Negative
CVD	7	2	1
15:0	5	3	1
17:0	5	3	0
T2D	3	0	0

In the studies presented above, most found favorable correlations between dairy biomarkers and clinical endpoints relating to cardiometabolic health (see Table 4). Although biomarkers can be regarded as more objective measurements of dairy fat intake than questionnaire- or interview-based methods, the methods used when employing this technique would benefit from increased standardization, both regarding sampling sites and biomarkers. Adipose tissue samples are the best indicators of long-term intake, how-

ever, in subjects with stable diets plasma PL samples are likely satisfactorily valid as well, as long as the samples are taken after proper fasting.

Regarding the choice of biomarker, 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, as well as in controlled feeding trials (Hodson et al., 2014). Other biomarkers have also exhibited both reliability and validity, but heterogeneity complicates meta-analyses and other comparisons between studies. Due to the many inconsistencies described above, 14:0 should not be considered a biomarker of dairy fat intake. Endogenous synthesis, often upregulated in subjects exhibiting poor metabolic health, as evident by higher triglycerides, is likely a major confounder for this biomarker.

Due to the inconsistent results found in several studies, the use of 17:0 as a biomarker for dairy fat intake should be discouraged. The earliest reports on using odd-chain SFAs as biomarkers reported weaker correlations for this biomarker as well as for 15:0 + 17:0 combined, compared to 15:0 alone.

Although biomarkers are likely more objective measures of diet than the commonly used FFQ and food diary methods, they are not free of confounding. Changing one aspect of ones diet invariably alters another. Consumption of dairy can be associated with other dietary or lifestyle patterns that also influence cardiometabolic health. These confounders can be culture-specific and heterogeneous. For example, high dairy consumption can result from the consumption of cheese-rich processed food or ice cream in cultures lacking a tradition of dairy consumption in its unprocessed form, while at the same time being indicative of a traditional-type diet in countries where dairy has historically been a major part of the cuisine.

Most of the studies included in this review have shown either protective or neutral effects of dairy biomarkers in regards to clinical cardiometabolic endpoints. Moreover, many studies have found positive or neutral correlations between dairy biomarkers and different aspects of MetS. Although the body of evidence is still quite small, it does not support the commonly stated recommendation of replacing full-fat dairy products with low-fat varieties. The complex and diverse nature of dairy products, as well as the conflicting evidence presented above, suggests that dairy cannot simply be reduced to a vehicle for SFAs.

Future research into dairy biomarkers could focus on at least a few important aspects. First, for any large cohort where blood samples have been stored and cardiometabolic endpoints followed up, dairy biomarkers should be measured, and nested case–control studies should be conducted for these endpoints. Second, to ascertain whether the link between dairy

biomarkers and improved metabolic health is causal, proper longitudinal studies where blood samples are taken at several time points can be used to deduce which, if any, factors of MetS are influenced by dairy consumption. Finally, the reliability and validity of 15:0 as well as other biomarkers needs to be researched further, since most validation studies have used imprecise methods of dietary assessment such as FFQs and interviews. Although practicality and cost would limit the feasibility of validating adipose tissue biomarkers under controlled conditions, such a study would be of great value as well. Finally, the combination of biomarkers with other methods of dietary assessment could provide improved means of measuring food intakes, since combining two methods where errors occur independently of each other can increase precision (Baylin et al., 2002). However, such a method would likely be more expensive and time-consuming (ibid.), and a standard protocol for such combined assessments remains to be developed.

Furthermore, future research into specific bioactive compounds present in dairy products could be used as a basis for determining bovine breeding strategies and optimizing manufacturing processes, as well as for identifying potential targets for drug research.

6 Conclusion

The use of biomarkers for assessing dairy fat intake is growing among nutritional epidemiologists, promising accurate and objective measures of dietary intake, both current and retrospective. However, there are many facets of dairy biomarker research that remain under-explored. Among these are more stringent validations of several biomarkers, standardization of several aspects such as the choice of compounds and FA pools used for sampling, and development of a "gold standard" protocol employing several measures of diet for increased statistical power.

Summarizing the evidence to date, a vast majority of prospective cohort studies have found dairy fat consumption, as indicated by biomarkers, to be either neutral or beneficial in regards to cardiometabolic health. In addition, many cross-sectional studies have found beneficial associations between these biomarkers and indices of cardiometabolic health. This stands in contrast to prevalent dietary guidelines, that emphasize the choice of low-fat dairy products where possible. Further research is needed to determine whether these recommendations are conducive to public health.

References

Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J. C., James, W. P. T., Loria, C. M., and Smith, S. C. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity, *Circulation*, Lippincott Williams & Wilkins, pp. 1640–1645.

Amcoff, E., Edberg, A., Enghardt Barbieri, H., Lindroos, A. K., Nälsén, C., Pearson, M., and Warensjö Lemming, E. (2014). *Riksmaten – vuxna 2010–11*, Livsmedels- och näringsintag bland vuxna i Sverige, Livsmedelsverket, Uppsala.

American Diabetes Association (2008). Diagnosis and classification of diabetes mellitus, *Diabetes Care* **31 Suppl 1**: S55–60.

Arab, L. (2003). Biomarkers of fat and fatty acid intake, *The Journal of Nutrition* **133 Suppl 3**: 925S–932S.

Aslibekyan, S., Campos, H., and Baylin, A. (2012). Biomarkers of dairy intake and the risk of heart disease, *Nutrition, Metabolism, and Cardiovascular Diseases* **22**(12): 1039–1045.

Aune, D., Norat, T., Romundstad, P., and Vatten, L. J. (2013). Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies, *American Journal of Clinical Nutrition* **98**(4): 1066–1083.

Bach, A. C., and Babayan, V. K. (1982). Medium-chain triglycerides: an update, *American Journal of Clinical Nutrition* **36**(5): 950–962.

Baylin, A., Kabagambe, E. K., Siles, X., and Campos, H. (2002). Adipose tissue biomarkers of fatty acid intake, *American Journal of Clinical Nutrition* **76**(4): 750–757.

Bendtsen, L. Q., Lorenzen, J. K., Larsen, T. M., van Baak, M., Papadaki, A., Martinez, J. A., Handjieva-Darlenska, T., Jebb, S. A., Kunešová, M., Pfeiffer, A. F. H., Saris, W. H. M., Astrup, A., and Raben, A. (2014). Associations between dairy protein intake and body weight and risk markers of diabetes and CVD during weight maintenance, *The British Journal of Nutrition* **111**(5): 944–953.

Beynen, A. C., and Katan, M. B. (1985). Rapid sampling and long-term storage of subcutaneous adipose-tissue biopsies for determination of fatty acid composition, *American Journal of Clinical Nutrition* **42**(2): 317–322.

Biong, A. S., d, M. B. V. o., Ringstad, J., Thelle, D. S., and Pedersen, J. I. (2006). Intake of milk fat, reflected in adipose tissue fatty acids and risk of myocardial infarction: a case–control study, *European Journal of Clinical Nutrition* **60**(2): 236–244.

Chowdhury, R., Warnakula, S., Kunutsor, S., Crowe, F., Ward, H. A., Johnson, L., Franco, O. H., Butterworth, A. S., Forouhi, N. G., Thompson, S. G., Khaw, K.-T., Mozaffarian, D., Danesh, J., and Di Angelantonio, E. (2014). Association of Dietary, Circulating, and Supplement Fatty Acids With Coronary Risk: A Systematic Review and Meta-analysis, *Annals of Internal Medicine* **160**(6): 398–406.

de Oliveira Otto, M. C., Nettleton, J. A., Lemaitre, R. N., Steffen, L. M., Kromhout, D., Rich, S. S., Tsai, M. Y., Jacobs, D. R., and Mozaffarian, D. (2013). Biomarkers of dairy fatty acids and risk of cardiovascular disease in the multi-ethnic study of atherosclerosis, *Journal of the American Heart Association* **2**(4): e000092.

Gao, D., Ning, N., Wang, C., Wang, Y., Li, Q., Meng, Z., Liu, Y., and Li, Q. (2013). Dairy products consumption and risk of type 2 diabetes: systematic review and dose-response meta-analysis, *PLoS ONE* **8**(9): e73965.

Haug, A., Høstmark, A. T., and Harstad, O. M. (2007). Bovine milk in human nutrition – a review, *Lipids in Health and Disease* **6**(1): 25.

Hodson, L., Eyles, H. C., McLachlan, K. J., Bell, M. L., Green, T. J., and Skeaff, C. M. (2014). Plasma and erythrocyte fatty acids reflect intakes of saturated and n-6 PUFA within a similar time frame, *The Journal of Nutrition* **144**(1): 33–41.

Hodson, L., Skeaff, C. M., and Fielding, B. A. (2008). Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake, *Progress in Lipid Research* **47**(5): 348–380.

Iggman, D., Ärnlöv, J., Vessby, B., Cederholm, T., Sjögren, P., and Risérus, U. (2010). Adipose tissue fatty acids and insulin sensitivity in elderly men, *Diabetologia* **53**(5): 850–857.

Jacobs, S., Schiller, K., Jansen, E., Fritsche, A., Weikert, C., di Giuseppe, R., Boeing, H., Schulze, M. B., and Kröger, J. (2014). Association between

erythrocyte membrane fatty acids and biomarkers of dyslipidemia in the EPIC-Potsdam study, *European Journal of Clinical Nutrition* **68**: 517–525.

Jensen, R. G. (2002). The composition of bovine milk lipids: January 1995 to December 2000, *Journal of Dairy Science* **85**(2): 295–350.

Jiang, J., Wolk, A., and Vessby, B. (1999). Relation between the intake of milk fat and the occurrence of conjugated linoleic acid in human adipose tissue, *American Journal of Clinical Nutrition* **70**(1): 21–27.

Keys, A., Menotti, A., Karvonen, M. J., Aravanis, C., Blackburn, H., Buzina, R., Djordevic, B. S., Dontas, A. S., Fidanza, F., and Keys, M. H. (1986). The diet and 15-year death rate in the seven countries study, *American Journal of Epidemiology* **124**(6): 903–915.

Khaw, K.-T., Friesen, M. D., Riboli, E., Luben, R., and Wareham, N. (2012). Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the EPIC-Norfolk prospective study, *PLoS Medicine* **9**(7): e1001255.

Kratz, M., Baars, T., and Guyenet, S. (2013). The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease, *European Journal of Nutrition* **52**(1): 1–24.

Kratz, M., Marcovina, S., Nelson, J. E., Yeh, M. M., Kowdley, K. V., Callahan, H. S., Song, X., Di, C., and Utzschneider, K. M. (2014). Dairy fat intake is associated with glucose tolerance, hepatic and systemic insulin sensitivity, and liver fat but not β-cell function in humans, *American Journal of Clinical Nutrition* **99**(6): 1385–1396.

Krauss, R. M., Eckel, R. H., Howard, B., Appel, L. J., Daniels, S. R., Deckelbaum, R. J., Erdman, J. W., Kris-Etherton, P., Goldberg, I. J., Kotchen, T. A., Lichtenstein, A. H., Mitch, W. E., Mullis, R., Robinson, K., Wylie-Rosett, J., St Jeor, S., Suttie, J., Tribble, D. L., and Bazzarre, T. L. (2000). AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association.

Malik, S., Wong, N. D., Franklin, S. S., Kamath, T. V., L'Italien, G. J., Pio, J. R., and Williams, G. R. (2004). Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults, *Circulation* **110**(10): 1245–1250.

Maruyama, C., Yoneyama, M., Suyama, N., Yoshimi, K., Teramoto, A., Sakaki, Y., Suto, Y., Takahashi, K., Araki, R., Ishizaka, Y., Yamakado, M., and Teramoto, T. (2008). Differences in serum phospholipid fatty acid compositions and estimated desaturase activities between Japanese men with and without metabolic syndrome, *Journal of Atherosclerosis and Thrombosis* **15**(6): 306–313.

Mayneris-Perxachs, J., Guerendiain, M., Castellote, A. I., Estruch, R., Covas, M. I., Fitó, M., Salas-Salvadó, J., Martínez-González, M. A., Aros, F., Lamuela-Raventós, R. M., and López-Sabater, M. C. (2014). Plasma fatty acid composition, estimated desaturase activities, and their relation with the metabolic syndrome in a population at high risk of cardiovascular disease, *Clinical Nutrition* **33**(1): 90–97.

McGregor, R. A., and Poppitt, S. D. (2013). Milk protein for improved metabolic health: a review of the evidence, *Nutrition & Metabolism* **10**(1): 46.

Micha, R., King, I. B., Lemaitre, R. N., Rimm, E. B., Sacks, F., Song, X., Siscovick, D. S., and Mozaffarian, D. (2010). Food sources of individual plasma phospholipid trans fatty acid isomers: the Cardiovascular Health Study, *American Journal of Clinical Nutrition* **91**(4): 883–893.

Mozaffarian, D., Cao, H., King, I. B., Lemaitre, R. N., Song, X., Siscovick, D. S., and Hotamisligil, G. S. (2010). Trans-Palmitoleic Acid, Metabolic Risk Factors, and New-Onset Diabetes in U.S. Adults, *Annals of Internal Medicine* **153**(12): 790–799.

Mozaffarian, D., de Oliveira Otto, M. C., Lemaitre, R. N., Fretts, A. M., Hotamisligil, G., Tsai, M. Y., Siscovick, D. S., and Nettleton, J. A. (2013). trans-Palmitoleic acid, other dairy fat biomarkers, and incident diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA), *American Journal of Clinical Nutrition* **97**(4): 854–861.

Nestel, P. J., Straznicky, N., Mellett, N. A., Wong, G., De Souza, D. P., Tull, D. L., Barlow, C. K., Grima, M. T., and Meikle, P. J. (2014). Specific plasma lipid classes and phospholipid fatty acids indicative of dairy food consumption associate with insulin sensitivity, *American Journal of Clinical Nutrition* **99**(1): 46–53.

Nordic Council of Ministers (2014). *Nordic Nutrition Recommendations 2012*, Integrating nutrition and physical activity, Nordic Council of Ministers.

Onakpoya, I. J., Perry, R., Zhang, J., and Ernst, E. (2011). Efficacy of calcium supplementation for management of overweight and obesity: systematic review of randomized clinical trials, *Nutrition Reviews* **69**(6): 335–343.

Perreault, M., Zulyniak, M. A., Badoud, F., Stephenson, S., Badawi, A., Buchholz, A., and Mutch, D. M. (2014). A distinct fatty acid profile underlies the reduced inflammatory state of metabolically healthy obese individuals, *PLoS ONE* **9**(2): e88539.

Siri-Tarino, P. W., Sun, Q., Hu, F. B., and Krauss, R. M. (2010). Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease, *American Journal of Clinical Nutrition* **91**(3): 535–546.

Smedman, A. E., Gustafsson, I. B., Berglund, L. G., and Vessby, B. O. (1999). Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors, *American Journal of Clinical Nutrition* **69**(1): 22–29.

Smit, L. A., Baylin, A., and Campos, H. (2010). Conjugated linoleic acid in adipose tissue and risk of myocardial infarction, *American Journal of Clinical Nutrition* **92**(1): 34–40.

Soares, M., Pathak, K., and Calton, E. (2014). Calcium and vitamin D in the regulation of energy balance: where do we stand?, *International Journal of Molecular Sciences* **15**(3): 4938–4945.

Soedamah-Muthu, S. S., Ding, E. L., Al-Delaimy, W. K., Hu, F. B., Engberink, M. F., Willett, W. C., and Geleijnse, J. M. (2011). Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies, *American Journal of Clinical Nutrition* **93**(1): 158–171.

Sun, Q., Ma, J., Campos, H., and Hu, F. B. (2007). Plasma and erythrocyte biomarkers of dairy fat intake and risk of ischemic heart disease, *American Journal of Clinical Nutrition* **86**(4): 929–937.

U.S. Department of Agriculture, and U.S. Department of Health and Human Services (2010). *Dietary Guidelines for Americans*, U.S. Government Printing Office.

Van Dam, R. M., and Hunter, D. (2012). Biochemical Indicators of Dietary Intake, *Nutritional Epidemiology*, Oxford University Press, pp. 150–212.

Wang, H., Steffen, L. M., Vessby, B., Basu, S., Steinberger, J., Moran, A., Jacobs, D. R., Hong, C. P., and Sinaiko, A. R. (2011). Obesity Modifies the Relations Between Serum Markers of Dairy Fats and Inflammation and Oxidative Stress Among Adolescents, *Obesity* **19**(12): 2404–2410.

Wang, L. (2010). Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events, *Annals of Internal Medicine* **152**(5): 315–323.

Warensjö, E., Jansson, J.-H., Berglund, L., Boman, K., Ahrén, B., Weinehall, L., Lindahl, B., Hallmans, G., and Vessby, B. (2004). Estimated intake of milk fat is negatively associated with cardiovascular risk factors and does not increase the risk of a first acute myocardial infarction. A prospective case-control study, *The British Journal of Nutrition* **91**(4): 635–642.

Warensjö, E., Jansson, J.-H., Cederholm, T., Boman, K., Eliasson, M., Hallmans, G., Johansson, I., and Sjögren, P. (2010). Biomarkers of milk fat and the risk of myocardial infarction in men and women: a prospective, matched case-control study, *American Journal of Clinical Nutrition* **92**(1): 194–202.

Warensjö, E., Smedman, A., Stegmayr, B., Hallmans, G., Weinehall, L., Vessby, B., and Johansson, I. (2009). Stroke and plasma markers of milk fat intake–a prospective nested case-control study, *Nutrition Journal* **8**: 21.

Willett, W. (2012). Correction for the Effects of Measurement Error, *Nutritional Epidemiology*, Oxford University Press, pp. 287–304.

Wolk, A., Furuheim, M., and Vessby, B. (2001). Fatty acid composition of adipose tissue and serum lipids are valid biological markers of dairy fat intake in men, *The Journal of Nutrition* **131**(3): 828–833.

Wolk, A., Vessby, B., Ljung, H., and Barrefors, P. (1998). Evaluation of a biological marker of dairy fat intake, *American Journal of Clinical Nutrition* **68**(2): 291–295.

Yamagishi, K., Nettleton, J. A., and Folsom, A. R. (2008). Plasma fatty acid composition and incident heart failure in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study, *American Heart Journal* **156**(5): 965–974.

Yu, D. X., Sun, Q., Ye, X. W., Pan, A., Zong, G., Zhou, Y. H., Li, H. X., Hu, F. B., and Lin, X. (2012). Erythrocyte trans-fatty acids, type 2 diabetes and

cardiovascular risk factors in middle-aged and older Chinese individuals, *Diabetologia* **55**(11): 2954–2962.

Zong, G., Sun, Q., Yu, D., Zhu, J., Sun, L., Ye, X., Li, H., Jin, Q., Zheng, H., Hu, F. B., and Lin, X. (2014). Dairy consumption, type 2 diabetes, and changes in cardiometabolic traits: a prospective cohort study of middle-aged and older Chinese in Beijing and Shanghai, *Diabetes Care* **37**(1): 56–63.