Ruminant fatty acids in adipose tissue and blood lipid fractions as biomarkers of dairy consumption

Bachelor Thesis
Global Nutrition and Health-Lifestyle and Health Education

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Abstract

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Background: The relation between dairy products intake and the risk of cardiovascular diseases is controversial. These controversial results could partly be explained by the inherent limitations of the traditional dietary assessment methods. For this reason, there is an increasing interest in identifying and using biological markers of dairy consumption as they can provide a more objective and precise measure.

Objective: The aim of this thesis was to investigate if compositions of ruminant fatty acids in blood lipid fractions and adipose tissue are valid biomarkers of dairy consumption.

Method: A systematic search of two electronic databases, PubMed and Scopus, was conducted to find relevant existing literature using terms such as biological markers, dairy products, fatty acids, adipose tissue, blood and questionnaire.

Results: Systematic review of 15 studies showed that among investigated ruminant fatty acids, pentadecanoic acid (15:0) content in adipose tissue, total plasma, plasma cholesterol esters and plasma phospholipids were the best correlates of total dairy fats intake. None of the ruminant fatty acids were found to reasonably correlate with the intake of total dairy products.

Conclusion: The use of pentadecanoic acid (15:0) concentration of adipose tissue and blood lipid fractions (except erythrocytes) may be suggested as useful indicators of total dairy fats intake although their validity remains to be determined. This thesis implies the need of well-controlled feeding studies to better investigate the validity of ruminant fatty acids as biomarkers of dairy consumption.
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1. Introduction

1.1. Background

Cardiovascular diseases (CVDs) are now the number one cause of deaths worldwide (World Health Organisation, 2015). CVDs are a group of disorders concerning the heart and blood vessels. The most common CVDs are coronary heart disease, cerebrovascular disease, and peripheral vascular disease, which are caused by atherosclerosis (Mendis, Puska & Norrving, 2011, p.3). Atherosclerosis is the narrowing of blood vessels (arteries) due to the buildup of fatty material and cholesterol deposits. As a consequence of atherosclerosis, the fatty deposits (plaques) may rupture, resulting in the formation of blood clots, which can therefore cut off blood flow to the heart, causing heart attack, or to the brain, causing stroke (ibid). According to World Health Organization (2015), 17.5 million people have died from CVDs in 2012 with an estimated 7.4 million of deaths due to coronary heart disease and 6.7 million due to stroke.

Atherosclerosis is promoted by a number of risk factors such as physical inactivity, unhealthy diet (rich in salt, fat (particularly saturated fat, trans-fat, and cholesterol) or calories), hypertension, raised blood lipids, obesity, etc. (Mendis et al., 2011, p.3, 23). In particular, the role of diet is of great importance in the development of CVDs. A high intake of saturated fat and trans-fat has been linked to an increased risk of CVDs by means of increasing blood levels of low-density lipoprotein cholesterol (LDL-C) (Huth & Park, 2012). The consumption of dairy products, in particular, has often been suggested as a potential risk factor for CVDs as they are characterized by a high content of saturated fatty acids and cholesterol (Tholstrup, 2006). There are, however, reports of the existence of an inverse association between dairy consumption and the risk of CVDs (Ness, Smith & Hart, 2001). Therefore, the role of dairy products in the cardiovascular health is controversial.

The possible explanation for the inconclusive results could be related to the inherent limitations within the usage of traditional dietary assessment methods, such as food frequency questionnaire (FFQ), 24-hour dietary recall (24-HDR) or food/diet record (FR/DR), for the measurement of dietary intake (Hodson, Skeaff & Fielding, 2008). As assessment of dietary intake using these traditional methods requires the participants to recall their food and beverages intake over a period of time, these techniques can be prone to recall bias due to reliance on memory. Moreover, in the case of recording the habitual diet, there could be an unwillingness to report the actual intake by respondents, a practice, which is more likely to happen among overweight individuals (ibid). Participants are also known to
change their usual diet during the period of recording, consciously or sub-consciously (ibid). Interviewer bias, errors related to the used food composition database, imprecise portion size estimations etc. could be considered as other limitations for these commonly used dietary methods (ibid).

In light of these limitations, there has been an interest in identifying and using nutrients compositions of body’s different compartments as biomarkers of dietary intake. Biomarkers are believed to be objective and more precise measure of dietary intakes than traditional methods as they do not rely on self-reports, memory, or interviewer bias (Biong, Berstad & Pedersen, 2009). Fatty acids compositions of adipose tissue and blood lipid fractions are of great interest as biomarkers of dietary fat intake (Hodson et al., 2008). Fatty acids that cannot be synthesized endogenously are considered the best markers of dietary fat intake. These include polyunsaturated n-3 and n-6 fatty acids, trans fatty acids, and odd-numbered and branched-chain fatty acids (Willett, 2013 p.182).

1.1.1. Biomarkers of dairy fat intake

The odd-numbered saturated fatty acids pentadecanoic acid (15:0) and heptadecanoic acid (17:0) have been proposed as biomarkers for intake of dairy fat. These fatty acids are virtually specific for dairy fat because they cannot be produced in the human body (due to having an odd number of carbon atoms) and are principally derived from ruminant sources as they are synthesized by intestinal bacterial flora of ruminants (Willett, 2013 p.190).

Palmitoleic acid (trans 16:1n-7) could also be used as a biomarker of dairy fat intake (ibid). Likewise, this fatty acid cannot be synthesized endogenously and is predominantly derived from naturally occurring dairy/ruminant trans-fat (Mozaffarian et al., 2012).

Another fatty acid that might function as a biomarker for dairy fat intake is myristic acid (14:0), a saturated fatty acid that exists in substantial amounts in dairy products. However, this fatty acid can be produced endogenously, which thus limits its appeal as a biomarker of dairy fat intake (Biong et al., 2009).

trans 18:1 isomers have been considered as other markers of dairy fat intake. In addition to natural or ruminant sources, these fatty acids are partially derived from unnaturally occurring hydrogenated oils, which makes them less useful as biomarkers (Yu et al., 2012). Particularly, trans 18:1n-7 (vacceneic acid) can possess the characteristics of a potential biomarker as it is the major trans isomer in dairy fat
Recently, phytanic acid, the 20 carbon branched chain fatty acid, has been shown to be correlated with dairy fat intake (Hodson et al., 2008). This fatty acid is found predominantly in the ruminant sources. Phytanic acid is derived from phytol, which is produced from the breakdown of chlorophyll in the gastrointestinal system of ruminants. Phytol cannot be produced in the human gut (Allen et al., 2008).

1.1.2. Biological constituents used to reflect dairy fat intake

Dietary fat intake and its fatty acids compositions are reflected by the fatty acids in the body’s different compartments and at different points in time (Arab & Akbar, 2002).

Fatty acid composition of adipose tissue is considered as an ideal measure to reflect the long-term dietary fat intake due to the slow turnover time of the lipids in this tissue (a half-life between 1 and 2 years) (Willett, 2013, p.185). In using adipose tissue specimen, the fatty acid composition of either total lipids or triglycerides (TGs) is measured due to the 99% contribution of TGs to adipose tissue while cholesterol (0.3%) and phospholipids (PLs) (less than 0.1%) make minor contributions. Thus, these two measures appear to give similar results (Hodson et al., 2008).

Of the blood lipid fractions, TGs are not considered as appropriate biomarkers of habitual intake of dietary fat because they are influenced by the fat consumed in the recent meals, reflecting only the short-term dietary fat intake (Saadatian-Elahi et al., 2009), whereas, the fatty acid compositions of PLs and cholesterol esters (CEs) in plasma, serum, or erythrocytes membrane reflect the medium-term (weeks to months) dietary fat intake. Therefore, they can be better markers of usual dietary habits (Saadatian-Elahi et al., 2009 & Lagström et al., 1998). Total plasma or total serum and whole blood pool can also be used for the measurement of fatty acid biomarkers as alternatives to plasma or serum lipid fractions (Willett, 2013, p.186). Total plasma or total serum is a mixture of all lipid fractions including PL, TG, CE and non-esterified fatty acids (NEFA) and whole blood consists of plasma (54% by volume) and circulating cells (46% by volume), with erythrocytes making major contribution (ibid).

1.2. Research question

Before a fatty acid biomarker is used as a measure of dairy consumption in the assessment of the potential role of dairy consumption in the development of CVDs, its validity should be assessed. The
aim of this thesis was to investigate if compositions of ruminant fatty acids in blood lipid fractions and adipose tissue are valid biomarkers of dairy consumption.

1.3. Clarification of Key Words and Delimitation of the Thesis

“The term "biomarker" is used in a broad sense to include almost any measurement reflecting an interaction between a biological system and a chemical, physical or biological environmental” (Gârban, Gârban & Ghibu, 2006). However, a nutritional biomarker, which is the focus here, refers to “any biological specimen that is an indicator of nutritional status with respect to intake or metabolism of dietary constituents” (Potischman & Freudenheim, 2003). In this thesis, I investigated only the validity of fatty acids from ruminant sources as biomarkers of dairy consumption, not the validity of other fatty acids or other components of dairy products such as potassium or calcium. Fatty acids are long chains of carbon surrounded by hydrogen molecules with an acid group (carboxyl) at one end and a methyl group at the other. Fatty acids, which are found in lipid molecules are either saturated or unsaturated (Byrd-Bredbenner, Moe, Beshgetoor, & Berning, 2009, p.7). Although not all of the investigated fatty acids here in this thesis are derived exclusively from ruminant sources, they are known that either exist in substantial amounts in dairy products or are predominantly derived from ruminant sources.

A biomarker is considered valid when it correlates strongly with the dietary intake of the food/nutrient of interest for which it is serving as an indicator (Strimbu & Tavel, 2011). In this thesis, interpretation of the strength of the correlation coefficients between the measures of dairy consumption and the fatty acid concentrations in body tissues is based on the rule of thumb presented by Mukaka (2009) (Table 1).

Table 1. Rule of Thumb for Interpreting the Size of a Correlation Coefficient (Mukaka, 2009)

<table>
<thead>
<tr>
<th>Size of correlation</th>
<th>Interpretation</th>
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<tr>
<td>0.90 to 1.00 (-0.90 to -1.00)</td>
<td>Very high positive (negative) correlation</td>
</tr>
<tr>
<td>0.70 to 0.90 (-0.70 to -0.90)</td>
<td>High positive (negative) correlation</td>
</tr>
<tr>
<td>0.50 to 0.70 (-0.50 to -0.70)</td>
<td>Moderate positive (negative) correlation</td>
</tr>
<tr>
<td>0.30 to 0.50 (-0.30 to -0.50)</td>
<td>Low positive (negative) correlation</td>
</tr>
<tr>
<td>0.00 to 0.30 (0.00 to -0.30)</td>
<td>Negligible correlation</td>
</tr>
</tbody>
</table>

In this thesis, the type of biological samples used for the measurement of fatty acid compositions is
limited to adipose tissue and blood fractions as they are the most commonly used/reported samples for this aim in the existing literatures (Hodson et al., 2008). Thus, the investigation of other specimen types such as skin, breast milk, sperm, cheek cells, urine or others is not covered here. Adipose tissue is the loose connective tissue that is considered as the major storage site of fatty acids in the body (Longenbaker, 2011, p.69; Denniston, Topping & Caret, 2011, p.816). Among the major fat depots: lower-body, upper-body subcutaneous fat and intra-abdominal/visceral fat, upper body and lower body subcutaneous fat comprise the majority of total body fat and are the most commonly used sample for the measurement of adipose tissue fatty acids in the existing literatures (Ebbert & Jensen, 2013; Hodson et al., 2008). This thesis also focuses on the use of these two fat depots. In regard to the blood sample, as the other specimen of interest here, the focus is limited to whole blood, total plasma or total serum, erythrocytes and also different fractions of plasma or serum including TG, PL, CE, and NEFA, similarly because they are the most commonly used specimens for the measurement of blood fatty acids in the existing literatures (Hodson et al., 2008). Note, blood plasma and serum are both used for the measurement of fatty acids concentrations by investigators (Hodson et al., 2008). These two blood components are similar in all of their compositions except clotting factors (fibrinogen), which are present only in plasma (Yu et al., 2011). Therefore, for the sake of simplicity, the term “plasma” is used throughout the thesis, although some studies have measured the fatty acids compositions of serum. Dairy products are referred to the food items produced from the milk of ruminants such as fresh milk, cheese, cream, butter, ice cream, and yogurt (Council of Ministers, 2014, p.107). In this thesis, the validity of ruminant fatty acids as potential biomarkers was investigated for the intake of both total dairy products and total dairy fat.

1.4. Structure of the assignment

A description of the topic of concern, a background of different aspects of the subject matter, and the aim of the thesis were presented so far. In the following part, the type of the method used to address the research question and the reasons for choosing it will be presented. This is followed by a detailed description of the planned procedure to collect information. Next, the results will be presented including a display of the relevant data in a table. Thereafter, the results will be discussed in details. At the end, a conclusion will be posed and subsequently my consideration on how to apply the knowledge gained into a practical setting and new perspectives of the subject matter are presented.
1.5. Choice of method

Due to a limited time and lack of human and financial resources, it was not doable to conduct a new clinical research such as a controlled feeding study for this project. Instead, I systematically reviewed the scientific literature to identify relevant studies examining the correlation between the dietary intake of total dairy products or dairy fat and the fatty acids compositions of adipose tissue and blood lipid fractions. Systematic review helps to have a comprehensive overview of all relevant studies in the topic of interest (Green & Higgins, 2011). It is considered a valuable method as it objectively collates and summarizes a large body of evidence (Gopalakrishnan & Ganeshkumar, 2013). As another strength, the diversity of reviewed studies can improve the generalizability of the results since studies often have different eligibility criteria for participants or use different study designs or measurement methods (Mulrow, 1994). Moreover, as it is less time required and less costly to review prior studies than conducting a new study, systematic review can be considered an efficient method (ibid). However, there is the risk of publication bias when using the systematic review as your work method. Publication bias, a form of selection bias, refers to a systemic bias that could exist in the published literature of a field due to over-reporting of positive results compare to neutral or negative results, thus, making the published literature not a good representative of all the completed studies (Schlosser, 2007).

2. Work Method

For this systematic review, a search for relevant literature was performed on the databases of “PubMed” and “Scopus”. In the PubMed, the search strategy was based on two different combinations of the relevant MeSH (Medical Subject Headings) terms: 1- “Biological Markers/blood” AND “Dairy Products” AND “Fatty Acids/analysis” and 2- “Dairy Products” AND “Biological Markers” AND “Fatty Acids/analysis” AND “Adipose Tissue”. In addition, I looked for additional studies in the Scopus database using the following terms: “Dairy products intake” AND “Biological markers” AND “Fatty acids” AND “Adipose tissue” OR “Blood” AND “Questionnaire”.

Any study that had investigated the correlation between consumption of total dairy products and/or total dairy fat, assessed by traditional dietary measurement methods, and the ruminant fatty acids compositions in adipose tissue and/or blood fractions, was considered to be eligible for inclusion. However, studies only investigating the association between total dairy products and/or total dairy fat consumption and specific diseases using ruminant fatty acid biomarkers as dietary assessment method
without assessing their validity were not included. Other exclusion criteria included non-English language studies, animal studies, full-text unavailability, reviews, meta-analysis and letter to the editors.

3. Results

A flow diagram of study screening and selection process is reported in Figure 1. Briefly, the initial search yielded a total number of 73 studies (55 studies from the first search strategy on PubMed, 5 from the second strategy on PubMed and 13 studies from Scopus). After removing 8 duplicates, the title and abstracts of the 65 remained studies were screened to identify relevant studies for full review. Full texts of 17 studies were retrieved, of which two studies got excluded after reading the full text. In all, a total of 15 studies were identified to be relevant to the focus of this thesis and got included.

The characteristics of the included studies are summarized in Table 2. A brief description of the study design, participant characteristics, the type of investigated biomarkers and body compartments used for their measurement, methods for assessing dairy consumption, statistical analysis and the results of each study are included in the Table 2. The 15 studies were published between 1999 and 2015. Studies were from the USA (n = 6), Sweden (n = 2), Norway (n = 1), Costa Rica (n = 1), France (n = 2), Australia (n = 1), Canada (n = 2) and China (n = 1). Study sizes ranged between 62 and 2837. Most studies involved adult participants with an age range between 21 and 84 years and only one study involved children. Three studies involved men only, two involved women only, and 10 studies consisted of both sexes. 13 studies assessed the validity of 15:0 fatty acid as a biomarker for dairy consumption, 10 studies assessed the 17:0, six studies assessed the 14:0, five studies assessed the trans 16:1-n, one study assessed the phytanic acid and one assessed the trans isomer 18:1. Eight studies assessed the validity of potential biomarkers for total dairy products intake, six studies assessed the validity of biomarkers for total dairy fat intake, and only one study undertook the investigation for both total dairy products and total dairy fat intake.
Figure 1. Study flow diagram

Records identified through **PubMed** (the two ways) 
\(n = 60\)

Records identified through **Scopus** 
\(n = 13\)

Records removed after duplicates 
\(n = 8\)

Records screened by title 
\(n = 65\)

Records excluded based on title 
\(n = 34\)

Abstract of studies retrieved (n=31)

Records excluded based on abstracts 
\(n = 14\)
Reasons for exclusion:
- not an investigation study of the association between the two variables of interest
- letters to the editors
- review articles

Full-text articles assessed for eligibility 
\(n = 17\)

Full-text articles excluded 
\(n = 2\)
Reason for exclusion:
- Because the reported correlation was between fatty acids intake (not total dairy products/fats intake) and fatty acids compositions in body

Studies included in the review 
\(n = 15\)
3.1. Pentadecanoic acid (15:0)

3.1.1. Total dairy products intake

Nine studies assessed the validity of 15:0 fatty acid as a biomarker of total dairy products intake measured by FFQ (Giovannelli et al., 2013; Abdullah et al., 2015; Thiébaut et al., 2008; Sun, Ma, Campos, and Hu, 2007; Baylin, Kabagambe, Siles, and Campos, 2002; de Oliveira Otto et al., 2013 & Santaren et al., 2014; Yakoob et al., 2014; Mozaffarian et al., 2013). Except for one study, which found a weak correlation (r=0.31) (Baylin et al., 2002), the rest of the studies showed a negligible correlation in a range between r=-0.03 to r=0.29. Note, in Mozaffarian et al., (2013) and Yakoob et al., (2014), no correlation was presented for total dairy products intake; instead, correlations were presented for whole-fat and low-fat dairy products, which are included in this range. The only randomized clinical trial (cross-over) included in this review (Abdullah et al., 2015), in which the intervention group was provided with some dairy products including low-fat (1% fat) milk, low-fat (1.5% fat) creamy stirred yogurt, and regular (34% fat) cheddar cheese and was asked to incorporate 3 servings/d of these products into their diet, showed negligible correlation for the association between total dairy products intake and plasma content of 15:0, similar result to the rest of the included studies with observational design. In the study by Giovannelli and colleges (2013), in addition to FFQ, the validity of total plasma 15:0 fatty acid biomarker was assessed against FR estimates of dairy products intake, which also showed a negligible correlation (r=0.03). In the correlations between dairy products intake measured by FFQ and 15:0 content of body tissues, no considerable differences were observed between different specimens used: r=0.31 for adipose tissue (being the highest correlation), a range between r= 0.11 to r=0.29 for total plasma, r=0.13 to r=0.22 for plasma PLs, and r= 0.01 to r=0.23 for erythrocytes.

3.1.2. Total dairy fat intake

Six studies assessed the validity of 15:0 fatty acid as a biomarker for total dairy fat intake (Sun et al., 2007; Smedman, Gustafsson, Berglund & Vessby, 1999; Brevik, Veierød, Drevonand & Andersen, 2005; Wolk, Furuheim & Vessby, 2001; Golley & Hendrie, 2014 & Yakoob et al., 2014) and all found a correlation ranging from r= 0.22 to r=0.74. In the correlations, substantial differences were observed considering the type of the specimen used to measure 15:0 fatty acid composition and the type of the dietary assessment method used to measure total dairy fat intake. For adipose tissue concentration of 15:0 fatty acid, the correlation with total dairy fat intake was r=0.28 when measured by FFQ, r=0.52 by
14-day WRs, r=0.71 by 2×7-day DRs, and r=0.74 by 14×24-HDRs. For total plasma concentration of 15:0, the correlation was between r=0.22 to r=0.36 for FFQ, r=0.43 for 14-day WRs, and r=0.46 for 3×24-HDRs. For plasma CE s concentration of 15:0, the correlation was r=0.46 for 7-day FRs, r=0.47 for 2 7-day DRs and r=0.45 for 14×24-HDRs. For plasma PLs concentration of 15:0, the correlation was between r=0.34 for 7-day FRs and r=0.53 for 2×7-day DRs and r=0.50 for 14×24-HDRs. And for erythrocytes concentration of 15:0, the correlation ranged between r=0.02 to r=0.30 for the FFQ measurement of total dairy fat intake.

3.2. Heptadecanoic acid (17:0)

3.2.1. Total dairy products intake

Seven studies assessed the association of 17:0 fatty acid biomarker with total dairy products intake measured by FFQ (Giovannelli et al., 2013; Abdullah et al., 2015; Thiebaut et al., 2008, Baylin et al., 2002; Sun et al., 2007; Yakoob et al., 2014 & Kataria et al., 2015). The highest correlation was a weak correlation, r=0.31, found by Baylin et al., (2002), while the rest of the studies only showed negligible correlations in a range between r=-0.01 to r=0.25 (Abdullah et al., 2015; Thiebaut et al., 2008; Sun et al., 2007; Yakoob et al., 2014; Giovannelli et al., 2013 & Kataria et al., 2015). Note, this range includes the corresponding correlation for whole-fat and low-fat dairy products for Yakoob et al., study (2014), instead of total dairy products intake. The randomized cross-over clinical trial conducted by Abdullah and collages (2015), showed negligible correlation for the association between total dairy products intake and plasma content of 17:0, similar to the rest of the included studies with observational design. In the study by Giovannelli and colleges (2013), the validity of total plasma 17:0 fatty acid biomarker was assessed against FR estimates of total dairy products intake, in addition to FFQ measures, which also showed a negligible correlation (r=0.09). Considering the different specimens used for measuring 17:0 content, no substantial differences were observed in the correlations: r=0.31 for adipose tissue (the highest correlation), a range between r=-0.01 to r=0.25 for total plasma, r=0.10 for plasma PLs, and r=-0.01 to r=0.14 for erythrocytes.

3.2.2. Total dairy fat intake

Five studies assessed the validity of 17:0 fatty acid as a biomarker for total dairy fat intake (Golley & Hendrie, 2014; Brevik et al., 2005; Yakoob et al., 2014; Sun et al., 2007 & Wolk et al., 2001), for
which only negligible (r=-0.05 to r=0.25) to weak (r=0.36 to r=0.47) correlations were observed. For plasma CEs and PLs concentration of 17:0 fatty acid, the correlation with total dairy fat intake was r=0.40 and r=0.36, respectively, when measured by 2×7-day DRs, and r=0.47 and r=0.39, respectively, when measured by 14×24-HDRs (Wolk et al., 2001). However, for the other used specimens, only negligible correlations were observed. For adipose tissue concentration of 17:0, the correlation with total dairy fat intake was r=0.03 when measured by FFQ, r=0.07 by 14-day WRs, r=0.16 by 2×7-day DRs and r=0.23 by 14×24-HDRs (Brevik et al., 2005 & Wolk et al., 2001). For total plasma, the corresponding correlation was r=0.25 for 3×24-HDRs and between r=-0.10 to r=0.21 for FFQ (Golley & Hendrie, 2014; Brevik et al., 2005; Yakoob et al., 2014 & Sun et al., 2007). And for erythrocytes concentration of 17:0, the corresponding correlation ranged between r=-0.05 to r=0.16 for the FFQ measurement of total dairy fat intake (Yakoob et al., 2014 & Sun et al., 2007).

3.3. Myristic acid (14:0)

3.3.1. Total dairy products intake

Four studies assessed the association of 14:0 fatty acid biomarker with total dairy products intake measured by FFQ (de Oliveira Otto et al., 2013; Sun et al., 2007; Mozzafarina et al., 2013 & Yakoob et al., 2014), which all showed a negligible correlation (range r=-0.01 to r=0.17). Note, this range includes the correlations related to whole-fat and low-fat dairy products for Mozaffarian et al., (2013) and Yakoob et al., (2014) as there was not any reported value for total dairy products intake. No substantial differences were observed in the correlations, considering the type of the specimen used for measuring 14:0 content (total plasma: r=0.04 to r=0.17, plasma PLs: r=0.09 to r=0.14, and erythrocytes: r=-0.01 to r=0.11).

3.3.2. Total dairy fat intake

The validity of 14:0 as a biomarker for total dairy fat intake was assessed in four studies, from which, one found weak to moderate correlations ranging from r=0.30 to r=0.64 (Wolk, 2001), while the other three only showed negligible correlations (range r=-0.01 to r=0.24) (Golley & Hendrie, 2014; Sun et al., 2007 & Yakoob et al., 2014). The adipose tissue content of 14:0 correlated moderately (r=0.64) with total dairy fat intake measured by either 2×7-day DRs or 14×24-HDRs (Wolk et al., 2001). In the same study, similar weak correlations were observed for the association of plasma CEs and PLs
concentration of 14:0 with total dairy fat intake measured by either 2×7-day DRs ($r=0.30$ for each) or 14×24-HDRs ($r=0.30$ and $r=0.34$ respectively). However, for total plasma and erythrocytes concentrations of 14:0, the correlation with total dairy fat intake measured by FFQ was negligible ranging from $r=0.08$ to $r=0.24$ and $r=-0.01$ to $r=0.16$ respectively (Golley & Hendrie, 2014; Sun et al., 2007 & Yakoob et al., 2014).

3.4. trans 16:1n-7

3.4.1. Total dairy products intake

Five studies assessed the association of trans 16:1n-7 fatty acid biomarker with total dairy products intake measured by FFQ, which all showed a negligible correlation (range $r=0.07$ to $r=0.20$) (Sun et al., 2007; de Oliveira Otto et al., 2013; Santaren et al., 2014; Mozaffarian et al., 2013 & Yakoob et al., 2014). Note, this range includes the correlations related to whole-fat and low-fat dairy products for Mozaffarian et al., (2013) and Yakoob et al., (2014). No substantial differences were observed in the correlations, considering the type of the specimen used for measuring 14:0 content (total plasma: $r=0.07$ to $r=0.20$, plasma PL: $r=0.07$ to $r=0.15$, erythrocytes: $r=0.09$ to $r=0.18$).

3.4.2. Total dairy fat intake

Two studies assessed the validity of trans 16:1n-7 fatty acid as a biomarker for total dairy fat intake measured by FFQ. In Sun et al., (2007), the proportion of trans 16:1n-7 in both total plasma and erythrocytes was weakly correlated with total dairy fat intake ($r=0.30$ and $r=0.32$ respectively), while Yakoob et al., (2014) showed negligible values for the corresponding correlation ($r=0.13$ for total plasma and $r=0.08$ for erythrocytes).

3.5. Phytanic acid

The validity of phytanic acid was assessed in only one study (Kataria et al., 2015), which showed no correlation between total dairy products intake and the plasma content of this fatty acid, however, high-fat dairy products intake was shown to be weakly correlated ($r=0.35$).
3.6. trans 18:1 isomers

The validity of trans 18:1 isomers as biomarkers was also assessed in only one study (Zong et al., 2015). The proportion of trans 18:1 isomers in erythrocytes weakly correlated with total dairy products intake ($r = 0.37$).
Table 2, Overview of the studies

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<tr>
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<tbody>
<tr>
<td>Study design</td>
<td>Cross-sectional study within the MONA LISA-NUT cross sectional survey</td>
<td>Cross-sectional study within a Prospective cohort survey</td>
<td>Cross-sectional study Setting: Norway</td>
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<tr>
<td>Setting: France</td>
<td></td>
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</tr>
<tr>
<td>Description of Participants</td>
<td>2,630 participants aged 35 to 65 (men=1,335 women=1,295)</td>
<td>62 participants, only men, 70-year old</td>
<td>125 Healthy men aged 21–55 years</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>15:0 &amp; 17:0 fatty acids</td>
<td>15:0 fatty acid</td>
<td>15:0 &amp; 17:0 fatty acids</td>
</tr>
<tr>
<td>Biological sample</td>
<td>Total plasma fatty acids were assessed by high-performance liquid chromatography a few days before completion of the food records</td>
<td>Plasma CEs and PLs fatty acid compositions were analyzed by gas–liquid chromatography</td>
<td>Adipose tissue (n= 110) and total plasma (n= 107) content of 15:0 and 17:0 were measured by gas-liquid chromatography at the start of the diet-recording period</td>
</tr>
<tr>
<td>Dietary assessment</td>
<td>Dietary intakes were measured with a FFQ and also via FR for 3 consecutive days</td>
<td>7-day FR was used to assess dietary intakes (the dietary assessment and the clinical examination were carried out within 3 weeks)</td>
<td>Dietary intakes were measured by FFQ and 14-day WRs</td>
</tr>
<tr>
<td>Statistical method</td>
<td>Association between plasma content of 15:0 and 17:0 and dairy products intake assessed by FFQ and between plasma content of 15:0 and 17:0 and dairy products intake assessed by food records were examined by Crude Pearson correlation coefficients</td>
<td>Associations between 15:0 content of plasma CEs and PLs and total dairy fat intake were examined by Spearman’s rank correlation analysis adjusted for energy intake and physical activity</td>
<td>Pearson’s correlation coefficients were calculated for the relationship between the dairy fat intake as assessed by the different methods and the fatty acids compositions in adipose tissue as well as in total plasma</td>
</tr>
<tr>
<td>Results</td>
<td>-Correlations between plasma fatty acid levels and the intake of total dairy products estimated by FFQ: r=-0.03 (95% CI -0.11 to -0.06) for 15:0 and r=-0.01 (95% CI -0.09 to -0.08) for 17:0</td>
<td>-Correlations between proportion of 15:0 in plasma CEs and total dairy fat intake: r=0.46 (P&lt;0.0001)</td>
<td>-Based on FFQ: correlations between total dairy fat intake and the concentration of 15:0 and 17:0 were r=0.28 (P&lt;0.01) and r= 0.03 (not significant), respectively, in adipose tissue and r=0.28 (P&lt;0.01) and r=0.10 (not significant), respectively, in total plasma</td>
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<tr>
<td></td>
<td>-Correlations between plasma fatty acid levels and the intake of total dairy products estimated by FRs: r=0.30 (95% CI -0.05 to -0.11) for 15:0 and r=0.09 (95% CI 0.00 to -0.19) for 17:0</td>
<td>-Correlations between proportion of 15:0 in plasma PLs and total dairy fat intake: r=0.34 (P=0.008)</td>
<td>-Based on 14-day WRs: correlations between total dairy fat intake and the concentration of 15:0 and 17:0 were r=0.52 (P&lt;0.01) and r=0.07 (not significant), respectively, in adipose tissue and r=0.43 (P&lt;0.01) and r=0.05 (not significant), respectively, in total plasma</td>
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<tr>
<td>Study design</td>
<td>Case-control study within E3N-EPIC cohort Setting: France</td>
<td>Cross-sectional study within a case-control study of diet and heart disease Setting: Costa Rica</td>
<td>Prospective cohort study within MESA cohort study (Multi-Ethnic Study of Atherosclerosis) Setting: USA</td>
</tr>
<tr>
<td>Description of Participants</td>
<td>1,114 healthy women aged 40-65 years</td>
<td>367 men (control subjects) with a mean (± SD) age of 56 ± 11 y and 136 women (control subjects) with a mean age of 60±10 y.</td>
<td>2617 participants aged 45–84 y, men= 46.7% and women= 53.3%, and race-ethnicity was fairly evenly divided.</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>15:0 and 17:0 fatty acids</td>
<td>15:0 and 17:0 fatty acids</td>
<td>14:0, 15:0 and trans 16:1n-7 fatty acids</td>
</tr>
<tr>
<td>Biological sample</td>
<td>Plasma PLs fatty acid compositions were assessed by capillary gas chromatography (blood samples were collected in 1995–1998)</td>
<td>Subcutaneous adipose tissue fatty acids were identified by capillary gas chromatography</td>
<td>Plasma PLs fatty acids were measured and separated using gas chromatography quantification (blood sample was collected at the baseline)</td>
</tr>
<tr>
<td>Dietary assessment</td>
<td>Usual diet over the previous year was assessed through a validated FFQ in 1993–1995.</td>
<td>Dietary consumption was obtained using a validated FFQ</td>
<td>Usual dairy consumption over the past year was assessed at baseline using a FFQ.</td>
</tr>
<tr>
<td>Statistical method</td>
<td>Associations between plasma PLs fatty acids and dairy products intake were explored using partial Spearman correlation coefficients adjusted for age and BMI</td>
<td>The relationships between biomarkers concentration and total dairy products intake were explored using Pearson’s correlation coefficient after adjustment for age, sex, BMI, and smoking status</td>
<td>Associations between plasma PLs fatty acids and whole-fat dairy products as well as low-fat dairy products intake were evaluated using partial Spearman’s correlation coefficients, adjusted for age, sex, race-ethnicity, field center, and energy intake</td>
</tr>
</tbody>
</table>
| Results | Correlation between total dairy products intake and plasma PLs content of 15:0 and 17:0 were r = 0.13 and r = 0.10, respectively (P ≤ 0.005 for both) | Adipose tissue content of 15:0 and 17:0 equally correlated with total dairy products intake: r = 0.31 (P < 0.01) | -Correlations between total whole-fat dairy products intake and biomarkers concentrations: r=0.10 for 14:0, r=0.15 for 15:0, and r=0.15 for trans 16:1n-7 (all the P-values < 0.05)  
-Correlations between total low-fat dairy products intake and biomarkers concentrations: r=0.09 for 14:0, r=0.14 for 15:0 (P-values < 0.05 for both) but the correlation was not significant for trans 16:1n-7 (not shown) |
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<tbody>
<tr>
<td>Study design</td>
<td>Prospective cohort study within MESA cohort study (Multi-Ethnic Study of Atherosclerosis) Setting: USA</td>
<td>Prospective cohort study from IRAS cohort (Insulin Resistance Atherosclerosis Study) Setting: USA</td>
<td>Cross-sectional study Setting: USA</td>
</tr>
<tr>
<td>Description of Participants</td>
<td>2837 participants aged 45–84 y, men= 46.7% and women= 53.3%,</td>
<td>659 adults aged 40-60 y, men=297 and women=362</td>
<td>39 men with localized prostate cancer, with the mean age of 62.1 year</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>15:0, 14:0, and trans 16:1 n-7 fatty acids</td>
<td>15:0, and trans 16:1 n-7 fatty acids</td>
<td>Phytic acid &amp; 17:0 fatty acid</td>
</tr>
<tr>
<td>Biological sample</td>
<td>Plasma PLs fatty acids were measured and separated using gas chromatography quantification (blood sample was collected at the baseline)</td>
<td>Total plasma fatty acids were separated and quantified using gas chromatography (blood sample was collected at the baseline)</td>
<td>Total plasma fatty acids were measured by capillary gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>Dietary assessment</td>
<td>Usual dairy consumption over the past year was assessed at baseline using a FFQ</td>
<td>Usual dairy consumption over the previous year was assessed before baseline using a validated FFQ</td>
<td>Usual dietary intake over the previous year was assessed using FFQ</td>
</tr>
<tr>
<td>Statistical method</td>
<td>Associations between the plasma PLs fatty acids and total dairy products intake were assessed using Spearman correlations after adjustment for demographics, lifestyle and dietary factors</td>
<td>Correlation coefficients between 15:0 and trans 16:1n-7 with total dairy products intake were assessed, adjusted for energy intake</td>
<td>Associations between the two fatty acids and total dairy products intake were explored using Spearman rank correlation coefficients</td>
</tr>
<tr>
<td>Results</td>
<td>Correlations between total dairy products intake and plasma PLs contents of 15:0, 14:0, and trans 16:1n-7 were r=0.22, r=0.14, and r=0.07 respectively (all the P-values &lt; 0.05)</td>
<td>Correlation between total dairy products intake and 15:0 fatty acid was r = 0.20 (P &lt; 0.0001) but the correlation was not shown for trans 16:1n-7 (not significant)</td>
<td>No correlation between total dairy products intake and plasma content of phytanic acid and 17:0 was observed (data not shown); however, a weak correlation with high-fat dairy foods was found for phytanic acid: r=0.36 (P=0.04)</td>
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<tr>
<td><strong>Study design</strong></td>
<td>Prospective cohort study- from a population-based prospective study (Nutrition and Health of Aging Population) Setting: China</td>
<td>Cross-sectional study from a clinical randomized control trial (12-week intervention) -Intervention group: were advised to switch their dairy foods from regular-fat to reduced-fat items. -Control group: were advised to maintaining their usual dairy consumption</td>
<td>A randomised cross-over clinical trial- each dietary phase lasted 4 weeks (a 2-week run-in period and a 4- to 8-week washout period between the phases). DAIRY phase: 3 servings/d of conventional dairy (1% fat milk, 1.5% fat yogurt and 34% fat cheese). CONTROL phase: energy-matched control products (fruit and vegetable juice, cashews and a cookie)</td>
</tr>
<tr>
<td><strong>Description of Participants</strong></td>
<td>2,091 individuals, men and women, aged 50–70 years. Setting: Australia</td>
<td>114 healthy children of 4–13 years of age Setting: Australia</td>
<td>124 healthy individuals aged 18- 69 years, women=84 and men=40 Setting: Canada</td>
</tr>
<tr>
<td><strong>Biomarkers</strong></td>
<td>trans 18:1 isomers</td>
<td>14:0, 15:0 and 17:0 fatty acids</td>
<td>15:0 and 17:0 fatty acids</td>
</tr>
<tr>
<td><strong>Biological sample</strong></td>
<td>Erythrocytes fatty acids were analyzed using gas chromatography coupled with flame ion detector</td>
<td>Total plasma fatty acids were separated by gas-liquid chromatography</td>
<td>Total plasma fatty acids were assessed by standard methods at the end of each dietary phase</td>
</tr>
<tr>
<td><strong>Dietary assessment</strong></td>
<td>Baseline dairy consumption was assessed using FFQ</td>
<td>Dairy intake was assessed via 3× 24-HDRs</td>
<td>Compliance to the study’s dietary protocols was assessed using a validated FFQ</td>
</tr>
<tr>
<td><strong>Statistical method</strong></td>
<td>Association between total dairy products intake and erythrocytes trans 18:1 isomers was explored using Spearman correlation coefficients after controlling for age, sex, and total energy intake</td>
<td>The relationships between 14:0, 15:0 and 17:0 fatty acids and total dairy fat intake at baseline and at week 12 were explored using Pearson’s correlation coefficient</td>
<td>Association between total plasma content of 15:0 and 17:0 and dairy products intake were examined by Spearman’s correlation coefficient</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>Correlation between erythrocytes content of trans 18:1 isomers and total dairy products intake was r=0.37 (P &lt; 0.001)</td>
<td>-The correlations between total dairy fat intake and the plasma content of 14:0, 15:0 and 17:0 were r = 0.24 (P=0.01), r = 0.46 (P &lt; 0.01), and r=0.25 (P=0.01), respectively.</td>
<td>Correlations between plasma fatty acid levels and total dairy products intake: r = 0.27 (P&lt; 0.0001) for 15:0 and r =0.25 (P=0.0002) for 17:0</td>
</tr>
</tbody>
</table>

- The corresponding correlations following the intervention (at week 12) were all r < 0.15 (p > 0.05) but remained consistent with the baseline correlations for control group (data not shown in details for both)
|--------|-----------------------------|-------------------------------|---------------------|
| Study design | Prospective case-control study nested in the Nurses’ Health Study cohort  
Note: the validity of the biomarkers was only investigated in the control subjects  
Setting: USA | Cross-sectional study  
Setting: Sweden | Case-control study within two prospective cohort studies (Health Professionals Follow-Up Study (only men) & the Nurses’ Health Study (only women)).  
Setting: USA |
| Description of Participants | 313 women (free of cancer and CVD at the time of blood drawing) aged 30-55 years | 114 healthy men aged 40–76 years. | 594 participants (control subjects), women=472 with mean age of 61 ± 6.1 year and men=122 with mean age of 67.6 ± 7.8 year |
| Biomarkers | 14:0, 15:0, 17:0 and trans 16:1n-7 fatty acids | 14:0, 15:0, and 17:0 fatty acids | 14:0, 15:0, 17:0, and trans 16:1n-7 fatty acids |
| Biological sample | Total plasma and erythrocytes fatty acid concentrations were analyzed by gas-liquid chromatography (blood samples were collected In 1989–1990) | The fatty acids in the plasma CE s and PLs (n=104 for both) and in subcutaneous adipose tissue TGs (n=114) were separated by gas-liquid chromatography | Total plasma (n=577) and erythrocytes (n=559) fatty acids were measured using gas-liquid chromatography |
| Dietary assessment | Usual dairy consumption was assessed by semiquantitative FFQ in 1986 and 1990 | Dietary intakes were measured using 14×24-HDR interviews (distributed over one year) and 2×7-d DRs (kept for two 1-week periods, almost 6 month apart) | Usual dairy consumption over the previous year was assessed by FFQ |
| Statistical method | Relations between total plasma as well as erythrocytes concentrations of fatty acids and the intake of total dairy fat and dairy products were explored using Spearman partial correlation coefficients after adjustment for age at blood collection, BMI, current weight, smoking, fasting status, postmenopausal status, postmenopausal hormone use, and the periods during which the blood samples were assayed | Pearson’s product-moment correlations were calculated for the relationship between the total dairy fat intake and the proportions of 14:0, 15:0 and 17:0 fatty acids in adipose tissue as well as in plasma CE s and PL s | Associations between fatty acids biomarkers and total dairy fat intake, whole-fat and low-fat dairy products intake were assessed using correlation coefficients after adjustment for age, sex, BMI, smoking, fasting status at blood draw, date of blood collection, and consumption of total energy, total fat, whole-fat dairy (when low-fat dairy was evaluated) and low-fat dairy. |
| Results | Total plasma:  
-Correlations between average total dairy fat intake in 1986–1990 and biomarkers concentrations: r=0.21 for 14:0, r=0.36 for 15:0, r=0.21 for 17:0 and r=0.30 for trans 16:1n-7 (all the P-values < 0.01)  
-Correlations between average total dairy products intake in 1986–1990 and biomarkers concentrations: r=0.17 for 14:0, r=0.29 for 15:0, r=0.13 for 17:0 and r=0.20 for trans 16:1n-7 (all the P-values < 0.01). | Based on DRs:  
-Correlations between total dairy fat intake and the content of 14:0, 15:0 and 17:0 in adipose tissue were r = 0.64 (P < 0.001), r = 0.71 (P < 0.001), and r=0.16 (not significant) respectively  
-Corresponding correlations in plasma CE s: r = 0.30 (P < 0.01) for 14:0, r = 0.47 (P < 0.001) for 15:0, and r=0.40 (P < 0.01) for 17:0  
-Corresponding correlations in plasma PL s: r = | Total plasma:  
-Correlations between total dairy fat intake and biomarkers concentrations: r=0.22 for 15:0, r=0.16 for 17:0 and r=0.13 for trans 16:1n-7 (all p<0.05) and r=0.08 for 14:0 (not significant)  
-Correlations between whole-fat dairy products intake and biomarkers concentrations: r=0.19 for 15:0, r=0.12 for 17:0 and r=0.10 for trans 16:1n-7 (all p<0.05) and r=0.05 for 14:0 (not significant)  
-Correlations between low-fat dairy products intake and |
| Results (Continued) | Erythrocytes: Correlations between average total dairy fat intake in 1986–1990 and biomarkers concentrations: r=0.16 for 14:0, r=0.30 for 15:0, r=0.16 for 17:0 and r=0.32 for trans 16:1n-7 (all the P-values < 0.01). -Correlations between average total dairy products intake in 1986–1990 and biomarkers concentrations: r=0.11 for 14:0, r=0.23 for 15:0, r=0.14 for 17:0 and r=0.18 for trans 16:1n-7 (all the P-values < 0.01). Note: correlations of each fatty acids with average dairy fat/products intake in 1986–1990 were stronger than with 1990 intake alone. | 0.30 (P < 0.01) for 14:0, r = 0.53 (P < 0.001) for 15:0, and r=0.36 (P < 0.001) for 17:0. Based on 24-HDRs: -Correlations between total dairy fat intake and the content of 14:0, 15:0 and 17:0 in adipose tissue were r = 0.64 (P < 0.001), r = 0.74 (P < 0.001), and r=0.23 (P < 0.05) respectively. -Corresponding correlations in plasma CEs were r = 0.34 (P < 0.001) for 14:0, r = 0.45 (P < 0.001) for 15:0, and r=0.47 (P < 0.001) for 17:0. -Corresponding correlations in plasma PLs were r = 0.30 (P < 0.01) for 14:0, r = 0.50 (P < 0.001) for 15:0, and r=0.39 (P < 0.001) for 17:0. | biomarkers concentrations: r=0.11 for 15:0, r=0.11 for 17:0 (p<0.05 for both) and r=0.07 for trans 16:1n-7 and r=0.04 for 14:0 (both not significant). Erythrocytes: -Correlations between total dairy fat intake and biomarkers concentrations: r=-0.01 for 14:0, r=0.02 for 15:0, r=-0.05 for 17:0 and r=0.08 for trans 16:1n-7 (all not significant). -Correlations between whole-fat dairy products intake and biomarkers concentrations: r=0.01 for 14:0, r=0.07 for 15:0, r=-0.01 for 17:0 (all not significant) and r=0.09 for trans 16:1n-7 (all p<0.05). -Correlations between low-fat dairy products intake and biomarkers concentrations: r=-0.01 for 14:0, r=0.01 for 15:0, r=0.02 for 17:0 (all not significant for both) and r=0.10 for trans 16:1n-7 (p<0.05). |
4. Discussion

4.1. Summary of main findings

In this systematic review on the validity of ruminant fatty acids in body tissues as biomarkers of dairy consumption, 15:0 fatty acid content in adipose tissue, total plasma, plasma CEs and PLs were the best correlates of total dairy fat intake (range $r=0.22$ to $r=0.74$), with adipose tissue content of 15:0 being a better marker than the blood lipid fractions. The content of fatty acids 14:0, 17:0, trans 16:1n-7, phytanic acid and trans 18:1 isomers in body tissues showed weaker and also inconsistent correlations with the intake of total dairy fat intake. As biomarkers for total dairy products intake, the investigated ruminant fatty acids content in body tissues showed only negligible or weak correlations.

4.2. Methodological considerations

I undertook the electronic search on two different databases, PubMed and Scopus, using a broad and comprehensive search strategy, three different strategies, including all the relevant MeSH terms to identify as much as possible of the relevant studies, which can be considered as a strength for my work. It would have strengthened the work method if I had hand searched the bibliographies of retrieved studies as well as reviews on the related topic to identify additional studies as the level of indexing studies might have been superficial in the databases and some relevant studies might have been missed out (Green & Higgins, 2011), however I did not get to do this due to the limited time allocated to this project. The process of selecting studies including screening titles and abstracts as well as assessing the retrieved full text of potential studies can be subject to bias and errors if it is done by one individual (ibid), as it was the case in this systematic review, which, thus, can be considered as a limitation. In order to minimize the potential bias, it would have been better to have at least two reviewers involved in this process to independently assess the studies for inclusion.

The impact of measurement error on the collected data here may be minimized as I attempted to systematically extract all the data relevant to the research question from the included studies. The types of the data that had to be extracted from the studies were decided upon before starting the search. However, as with the steps of screening and
including studies, ideally, two investigators should have independently performed the data extraction in order to minimize bias (ibid), which was not possible in this work.

As a systematic review of published literature, publication bias that results from an over reporting of positive results compare to neutral or negative results is always a consideration (Schlosser, 2007). However, the potential impact of publication bias on the findings of this systematic review should be minimized as I found many neutral or negligible correlations between the variables of interest. However, this review was limited to papers that were written in English, which can make it subject to selection bias as some eligible studies in foreign language might have been missed out.

Owing to a broad search strategy, I was able to identify empirical evidences about most of the potential dairy fat biomarkers in question i.e. 14:0, 15:0, 17:0, trans 16:1n-7, trans 18:1 isomers and phytanic acid. In addition to different biomarkers, this systematic review included studies with different dietary assessment methods used as reference method in the investigation of the validity of biomarkers and also different body tissues used for the measurement of biomarkers, which can help to show variation in the correlations depending on the type of used dietary assessment method and body tissue. However, on the other hand, this variation in the use of dietary assessment methods and body tissues between the included studies can result in complexity in comparing the findings of studies, which makes it difficult to draw conclusions form the results.

As a reference for interpretation of the size or strength of the reported correlations by studies, I chose to use the rule of thumb presented by Mukaka (2009), as it seemed to be a sensible classification. However, this classification of the size of correlations might seem a bit strict compare to what is common in the nutritional epidemiology field. For instance, a correlation of 0.20 or 0.28 is considered as moderate according to Willett (2013, p.188), while a correlation < 0.30 is considered negligible based on the classification presented by Mukaka (2009).

Measurement errors in the assessment of dietary foods intake are known to bias the association between two variables (Gao et al., 2013). The results of this review were mainly based on data from observational studies, in which dairy products intake was self-reported assessed by dietary measurement methods. Therefore, it cannot be ignored that these measurement errors might have attenuated the reported correlations by studies
between the fatty acids biomarkers and the dietary intake of dairy products or dairy fat. It would have strengthen the results of my review if I could have identified some controlled feeding studies instead, in which the quality and quantity of consumed dairy products are known and therefore, a true correlation between biomarkers and consumed dairy products or dairy fat would be estimated. In addition, the reported correlations might have been influenced by biological variability and laboratory measurement errors, the two factors that may contribute to within-subject variation in measured fatty acids compositions in adipose tissue and blood fractions and therefore attenuate the estimated correlations (Wolk et al., 2001).

4.3. Interpretation of the results

Among different fatty acids characteristic of dairy fat, 15:0 is the most well-studied biomarker. The strongest and the most consistent correlations were observed between 15:0 fatty acid in body tissues and the intake of total dairy fat intake, which reflects the fact that this fatty acid is exclusively of exogenous origin with dairy products being the main dietary sources of. However, the corresponding correlations were shown to be quite weaker for 17:0 fatty acid, which has the similar characteristic as 15:0. Although 17:0 is the other odd-number fatty acid that is exclusively synthesized in the rumen of ruminants, however, it represents 0.61% of total milk fat compared to the 1.05% contribution of 15:0 to total milk fat (Wolk, Vessby, Ljung & Barrefors, 1998), which can partly explain its weaker correlations with the total dairy fat intake. The observed difference in the correlations may also be due to different metabolic routes of these two odd-numbered fatty acids in the human body as suggested by Sun et al., (2007).

The second highest correlation was observed between the proportion of 14:0 fatty acid in adipose tissue and total dairy fat intake measured by either 14×24-HDR or 2×DR (r=0.64 for both). This is in line with the finding of another study in which the 14:0 content in adipose tissue also moderately correlated (r=0.50) with the intake of total dairy fat intake based on FFQ (Biong et al., 2009). However, the use of this fatty acid as a biomarker for total dairy fat intake cannot be very promising due to its endogenous synthesis although this fatty acid exists in substantial amounts in dairy products and its measurement is easier compare to 15:0 and 17:0 fatty acids due to its higher content in
adipose tissue and plasma (Biong et al., 2009 & Wolk et al., 2001).

*trans* 16:1n-7 is another fatty acid of interest as biomarker of dairy consumption. However, the results of this review did not reveal reasonable correlations for this fatty acid. This fatty acid is known to have an exogenous origin with naturally occurring dairy/ruminant products being the main source of, which thus is expected to show a reasonable correlation with the intake of total dairy products or dairy fat intake (Saadatian-Elahi et al., 2009 & Mozaffarian et al., 2012). However, recently, it has been found that this fatty acid is not entirely diet derived but may be synthesized by the partial β-oxidation of dietary vaccenic acid (*trans*-18:1n–7) in the human body (Jaudszus et al., 2014), which can partly explain the observed weaker correlations for this fatty acid.

To my knowledge, the validity of total *trans* 18:1 isomers and phytanic acid as biomarkers of dairy consumption has not been studied as much as other fatty acids of interest. Through this systematic review, I was able to identify only one study on the validity assessment of each of the two fatty acids. Therefore, it is difficult to conclude if they can be used as biomarkers or not.

Zong et al., (2015) found a weak correlation between the proportions of total *trans* 18:1 isomers in erythrocytes and total dairy products intake (*r* = 0.37). Similarly, a weak (*β* =0.27) association has been reported between the content of *trans* 18:1 isomers in erythrocyte and dairy consumption by Yu and colleges (2012). These weak correlations can be related to the fact that *trans* 18:1 isomers also exist in partially hydrogenated oils (Kris-Etherton, 1995), which thus, does not make them promising biomarkers.

The content of phytanic acid in plasma was shown that is not correlated with the total dairy products intake in the only identified study by this review (Kataria et al., 2015). Nonetheless, a study that has investigated the validity of this fatty acid as a biomarker for total dairy fat intake, based on FFQ, shows a moderate correlation (*r*=0.68) (Allen et al., 2008). Taking this result into consideration, it can be suggested that phytanic acid is also specific to the dairy fat, similar to the rest of the biomarkers investigated in this review. It was also shown by Kataria et al., (2014) that phytanic acid showed a better correlation (*r*=0.35) with high-fat dairy products intake.

Overall, the results of this review showed that these fatty acids in either specimen (adipose tissue, total plasma, plasma CEs, plasma PLs, or erythrocytes) were correlated
with total dairy products intake less than with total dairy fat intake. This can be explained by the fact that these biomarkers are specific to dairy fat intake rather than overall dairy consumption as it was also reported by de Oliveira Otto and colleges (2013) that 15:0 content in plasma had the highest correlations with consumption of whole-fat diary foods such as cheese, butter, whole-fat milk and lowest correlations with consumption of low-fat cheese, low-fat milk or skim milk. Therefore, as low-fat dairy products may comprise a large part of the total dairy products consumption, these biomarkers, which are specific to dairy fat, would contribute to a weaker correlation with total dairy products intake.

This review did not find any consistent pattern of difference in the results by characteristics of the populations included in the studies such as age, sex, and geographic location. The only included study in this review with children as its participants (Golley & Hendrie, 2014), showed quite similar results with the rest of the studies on middle-aged and older participants for the investigation of the relation between fatty acids 14:0, 15:0 and 17:0 content in plasma and both total dairy products and total dairy fat intake measured by 3×24-HDRs. The small difference observed in the size of the correlations reported by these studies is more likely to be explained by differences in the used dietary assessment methods or body tissues rather than by differences in the age group of populations included. Similarly, the observed differences in the correlations of 14:0, 15:0 and 17:0 fatty acids and total dairy fat intake between studies with male participants (Smedman et al., 1999; Brevik et al., 2005 & Wolk et al., 2001) and studies with female participants (Sun et al., 2007) are not substantial after taking into account the different dietary assessment methods and body tissues used by these studies. Likewise, the difference in the setting (country) of studies is not expected to have contributed in the disparity of the results as the majority of the included studies have been undertaken in the western countries, except for one study conducted in China and meanwhile the only study investigating the validity of trans 18:1 isomers as biomarker of total dairy products intake. In the investigation of the validity of 15:0 fatty acid as a biomarker of total dairy fat intake, studies undertaken in Scandinavian countries (Smedman et al., 1999; Brevik et al., 2005 & Wolk et al., 2001), showed stronger correlations compared to the other three studies undertaken in the united states (Sun et al., 2007 & Yakoob et al., 2014) and Australia (Golley & Hendrie, 2014), however, they were the ones using multiple dietary
assessment methods to measure dairy consumption in contrast to FFQ or using adipose tissue to measure fatty acids compositions in contrast to blood fractions.

The evaluation of potential biomarkers as indicators of dietary intake depends on an accurate assessment of dietary intake (Hodson et al., 2008). However, this is a difficult task in itself as most of the nutritional epidemiological studies use self-reported dietary estimates, which are subject to measurement errors due to poor memory, imprecise portion size estimations, misreporting of different varieties of a food item (e.g. dairy products), social desirability, selective reporting, interviewer bias etc. (Mozaffarian et al., 2013 & Hodson et al., 2008). Furthermore, there should be a temporal relationship between the period of dietary assessment and the biomarkers measurement (Hodson et al., 2008). For example, when using adipose tissue to measure the fatty acid compositions, since adipose tissue has a half-life between 1 and 2 years, a dietary assessment method should be chosen that reflects the dietary intake in that time frame (ibid). Therefore, it cannot be expected to observe a strong correlation between the dietary intake of a special nutrient measured by a single 24-HDR and the adipose tissue content of a corresponding fatty acid, since a single 24-HDR measure reports the intake of one day while the adipose tissue reflects long-term intake. Instead, multiple 24-HDRs can be an ideal choice (Willett, 2013, p.49).

In the association between 15:0 fatty acid and total dairy fat intake, which was shown to have the highest and more consistent correlations, the strongest correlations were observed when composition of 15:0 was measured from adipose tissue and the total dairy fat intake was measured using repeated or multiple assessment methods. The results of this review showed that adipose tissue content of 15:0 was correlated, in descending order, with total dairy fat intake measured by 14×24-HDR (distributed over one year) (r=0.74), 2×7-day DRs (in six month apart) (r=0.71), 14-day WRs (split into five shorter periods by 1-week intervals distributed over two months) (r=0.52) and FFQ (r=0.28). This observation may not be surprising as 14 independent 24-HDR interviews distributed over 1 year, two 1-week period DRs performed six month apart and 14-day WRs distributed over two month are expected to give more accurate dietary information, minimizing error of day-to-day variation in intake, compared to FFQ estimates of total dairy fat intake, which can be subject to errors of averaging and misclassification of
dietary intake. Similarly, in the associations between blood lipid fractions content of 15:0 and total dairy fat intake, the highest correlations were related to the measurement of dairy consumption using repeated or multiple assessment methods (Golley & Hendrie, 2014; Brevik et al., 2005; Wolk et al., 2001 & Smedman et al., 1998) in contrast to FFQ (Sun et al., 2007; Yakoob et al., 2014 & Brevik et al., 2005).

Overall, FFQ estimates of total dairy products or total dairy fat intake appeared to be poorly correlated with all of the fatty acid biomarkers investigated here. Only negligible or very weak correlation was reported by the studies for the corresponding association. Even though FFQ can be used to measure habitual dietary intake in comparison to 24-HDR or FR/DR, which are based on the dietary intake of one or more specific days, it requires the participants’ ability to perform a cognitively complicated memory task to recall their dietary intake over a long period of time. The collected data from this dietary assessment method that relies on a generic memory provides an average estimation of dietary intake (Dietary assessment primer, 2015). Therefore, the observed very low correlations here for the FFQ estimates can be related to inaccuracies derived from the cognitive task of recalling and averaging long-term intake by individuals. However, because FFQ is less expensive and quicker than other dietary measurement methods, can be self-administered and has low burden for participants, most investigators tend to use these questionnaires. For the practical reasons, it is challenging to perform multiple 24-HDRs or DR/FR for a large number of participants although it is the optimal way to obtain accurate dietary information (Hodson et al., 2008).

As expected, the 15:0 fatty acid content of total plasma and its different lipid fractions showed a weaker correlation with total dairy fat intake compared to adipose tissue, which can be explained by the poor time integration of the fatty acids in plasma in comparison to a slow turnover rate of adipose tissue. However, in the correlations, no substantial differences were observed between total plasma and plasma CEs and PLs. The correlation observed in Brevik et al., (2005) between the total dairy fat intake and the proportion of 15:0 in total plasma was in the same magnitude as the associations between the total dairy fat intake and the proportion of 15:0 in CEs and PLs reported by Smedman and colleges (1999) and Wolk and colleges (2001). This indicates that total plasma 15:0 content might be an equally suitable indicator of total dairy fat intake as the 15:0
proportion of plasma CEs and PLs. It is reasonable to use total plasma fatty acids as it helps to decrease possible extra measurement errors derived from the task of separating plasma fractions (CE, PL, TG and NEFA) and also to avoid additional cost and effort (Willett, 2013 p.185).

Although it can be expected for the erythrocyte concentrations of fatty acids to be a better biomarker for dairy consumption than plasma due to its longer lifetime of about 120 days (Willett, 2013, p.159), the findings of this review did not support this assumption. The two included studies that investigated the validity of erythrocytes concentration of fatty acids 14:0, 15:0, 17:0, and trans 16:1-n as biomarkers of total dairy products or total dairy fat intake (Sun et al., 2007 & Yakoob et al., 2014), reported only negligible correlations (except for two very week correlations for 15:0 (r=0.30) and trans 16:1-n (r=0.32) with total dairy fat intake (Sun et al., 2007)) and to some extent lower correlations than total plasma concentrations of these fatty acids. For the erythrocytes concentration of biomarkers to properly reflect the dietary intake, the content of interest (e.g. fatty acid) should incorporate during erythropoiesis and remains static (not be transferred to plasma) throughout the 120 days; otherwise, its appeal as a biomarker will be decreased (Willett, 2013, p.159). In addition, as a potential reason for obtaining weaker correlations for erythrocytes than plasma, it was stated by Sun and colleagues (2007) that they had observed higher laboratory measurement errors for erythrocytes fatty acids than for plasma fatty acids.

Although biomarkers are considered as objective measures, it is assumed that studies of nutrient biomarkers may be subject to confounders. Other than the fatty acid composition of the consumed food, age, sex, physical activity, smoking, and genetic background are considered as other potential factors that may influence the fatty acid concentration of adipose tissue and blood fractions. However, the effect of these factors is not well studied and documented (Hodson et al., 2008). In the investigation of the validity of 15:0 fatty acid content in body tissues as a biomarker for total dairy fat intake, the observed disparity in the correlations does not appear to be explained by the effect of the potential confounders. For instance, the observed disparity in the correlations calculated by Sun et al., (2007) and Yakoob et al., (2014), between total plasma 15:0 consecration and total dairy fat intake measured by FFQ adjusted for age, sex, smoking, and BMI, and the
correlation calculated by Brevik et al., (2005), between total plasma 15:0 content and total dairy fat intake measured by 14-day WRs only adjusted for sex, appeared to decrease when FFQ was used by Brevik et al., (2007) (similar method to the other two studies) instead of 14-day WRs. Likewise, all four studies assessing the validity of 15:0 fatty acid concentration in total plasma, plasma CE$s$ or plasma PL$s$ as a biomarker of total dairy fat intake measured by more similar methods (i.e. multiple 24-HDR and repeated WR and DR), showed quite similar correlations although there was difference in their choice of confounders (Brevik et al., 2007; Smedman et al., 1999; Wolk et al., 2001 & Golley & Hendrie, 2014). Adjustment for physical activity was undertaken in only one study, which however was reported that the correlations remained unchanged after adjustment (Smedman et al., 1999).

5. Conclusion

According to the definition of validity proposed by Strimbu & Tavel (2011) (section 1.3.), none of the investigated ruminant fatty acids in this review can be considered as valid biomarkers for total dairy products or total dairy fat intake. Total dairy products intake correlated negligibly or weakly with the ruminant fatty acids content of adipose tissue and blood lipid fractions. The proportion of 15:0 fatty acid in adipose tissue and blood lipid fractions (except erythrocytes) was shown to be the best correlate of total dairy fat intake (range r= 0.22 to r=0.74). Accordingly, the use of adipose tissue and blood lipid fractions content of 15:0 fatty acid might be suggested as indicators of total dairy fat intake. Adipose tissue content of 15:0 was shown to be a better biomarker, however, in situations where adipose tissue is not available, the content of 15:0 in total plasma, plasma CE$s$ and PL$s$ might still be used as a biomarker. The content of fatty acids 14:0, 17:0, trans 16:1n-7, phytanic acid and trans 18:1 isomers in body tissues, however, showed weaker and also inconsistent correlations with the intake of total dairy fat intake, thus, they cannot be considered as promising indicators of the intake of total dairy fat.

6. Best practice consideration

As none of the potential biomarkers were found to be valid in this review for reflecting
the intake of total dairy products or dairy fat, it would be unwise to base conclusions about the associations between dairy products consumption and cardiovascular diseases on blood lipid fractions or adipose tissue concentrations of these biomarkers alone. Biomarkers and traditional dietary assessment methods are better to complement, rather than substitute for each other. A parallel application of biomarkers and traditional dietary assessment methods may provide the best measure of dairy consumption and thus a true estimate of the association between dairy consumption and cardiovascular diseases. However, a standard protocol for such combination of these two assessment methods remains to be developed.

7. Perspectives

To my knowledge, the majority of the existing evidences about the validity of potential biomarkers of dairy consumption rely on observational studies, in which dairy products intake is self-reported assessed by traditional dietary measurement methods. The validity of these fatty acid biomarkers needs to be further researched as the inherent measurement errors in these traditional dietary methods are known to bias the investigated association of interest (Gao et al., 2013). To this end, controlled feeding studies would be needed as ideal study design to estimate the true relation between dairy consumption and biomarkers concentrations of body tissues. Controlled feeding studies are of great value as in which the quantity and quality of dairy products intake are known (Hall & Most, 2005).

In addition, new potential biomarkers are of need to be explored to reflect total dairy products intake as the ruminant fatty acids were shown to be specific to dairy fat intake rather than overall dairy consumption. Considering the increasing varieties of the dairy products in the market today with various fat contents, other nutrients of dairy products, rather than fat, may be taken into consideration to explore better biomarkers to reflect the overall intake of dairy products.
**List of references**


American Heart Association, 2 (4), 1-11.


