MILK FATTY ACIDS AND POTENTIAL HEALTH BENEFITS: AN UPDATED VISION

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ABSTRACT

Background: Milk fat intake is often associated with a high risk of suffering from cardiovascular disease (CVD) due to its high saturated fat content. However, not all saturated fatty acids (SFA) are equal and they present structural differences that promote distinct effects on the biological processes. In addition, there is a growing scientific consensus pointing to dairy fat as a natural source of bioactive components.

Scope and Approach: The present review provides the most recent knowledge on the bioactive properties of fatty acids detected in dairy products and their potential effects on consumer health. The metabolic processes that involve these fatty acids and serious chronic diseases such as CVD, obesity, diabetes or cancer are explained and discussed throughout the text based on *in vitro*, animal and human studies. Moreover, information gaps are highlighted to inspire further research in the field.

Key Findings and Conclusions: Recent investigations support that milk SFA should no longer be considered as a single group in terms of metabolism or negative effects in case of excess. Even they suggest that individual SFA possess specific properties associated with important physiological functions. Whole dairy products would also promote human health due to the presence of certain bioactive fatty acids. Among them, it is worth mentioning the maintenance of gut microbiota and weight control from short and medium-chain SFA, the essential role of branched-chain SFA in gut health at birth and the prevention of chronic inflammatory diseases by vaccenic and rumenic acids.

Keywords: bioactive lipids, dairy fat, human health, *trans* fat, conjugated linoleic acid

1. Introduction

Lipids are among the most important constituents of milk for nutritional and economic reasons. They are a good source of energy and provide unique sensory and physical attributes to dairy products. Milk fat is also carrier of the naturally present fat-soluble vitamins (A, D, E and K) as well as β-carotene, a pro-vitamin A carotenoid. The main lipids in dairy fat are the triacylglycerides (TAG), accounting for more than 98% of total fat, while the remaining 2% comprises diacylglycerides, monoacylglycerides, free fatty acids (FFA), phospholipids, sterols and hydrocarbons. TAG composition is extremely complex as more than 400 different fatty acids (FA) can be esterified in the three positions (*sn*-1, *sn*-2 and *sn*-3) of the glycerol backbone at different concentrations, which mainly depend on ruminant diet and its lactation stage (Jensen, 2002; Schroder & Vetter, 2013; Shingfield, Bonnet, & Scollan, 2013; Hanuš, Samková, Křížová, Hasoňová & Kala, 2018). The most abundant milk FA are reported in Table 1.

It is estimated that dairy products (excluding butter) contribute to 24% of the saturated fat intake of the USA diet and 25–30% in European countries (Liang, Zhou, Amakye, Su, & Zhang, 2017). The dogma that dietary saturated fatty acids (SFA) should be minimized to reduce the metabolic syndrome and cardiovascular disease (CVD) risk has dominated nutritional guidelines for decades and the high content of SFA in milk fat (about two-thirds of total FA) has been currently employed as argument to link dairy products consumption with an increased incidence of those pathologies. However, recent scientific studies do not justify the maintenance of those recommendations in a healthy population (Astrup et al., 2016; Lovegrove & Givens, 2016; Guo et al., 2017). Firstly, up-to-date research does not support an association between biomarkers of dairy

fat intake with risk of diabetes mellitus or CVD (Yakoob et al., 2016; Kleber, Delgado, Lorkowski, Marz, & Von Schacky, 2016; Liang et al. 2017). Furthermore, the observational evidence does not endorse the hypothesis that high-fat dairy foods contribute to metabolic syndrome or cardiovascular risk, and even indicate that fat dairy consumption within typical dietary patterns is inversely associated with this risk (Kratz, Baars, & Guyenet, 2013; Alexander et al., 2016; Thorning et al., 2017).

In the past, it was widely accepted that the intake of 12:0, 14:0 and 16:0 SFA, which are detected in relevant quantities in dairy fat, would seem to be unhealthy in excessive amounts. However, the matrix in which these SFA are contained may influence health outcomes. Recent research has shown that several dairy matrix components, mainly calcium, peptides, phosphorus and the milk fat globule membrane, modify blood lipid responses to SFA intake (Thorning et al., 2017). As a result, these FA formerly considered hypercholesterolemic (12:0, 14:0 and 16:0) would have no impact on cardiovascular health parameters when supplied in the dairy matrix. On the other hand, it is also well stablished that the amount of carbohydrates in the diet regulates the synthesis and metabolism of saturated fats in humans (Ruiz-Núñez, Dijck-Brouwer, & Muskiet, 2016). A high intake of carbohydrates would promote its initial utilization for energy generation, leading to SFA storage and promoting *de novo* lipogenesis, while a low-carbohydrate diet would cause oxidation of dietary SFA and reduce its storage.

At last, milk fat is a natural and almost exclusive source of certain bioactive FA with potential benefits on human health. Some of them are not found in our diets in significant amounts elsewhere and the consumption of low-fat or fat-free dairy products would limit their intake. For instance, dairy fat is almost the only source of butyric acid

(4:0), conjugated linoleic acid (CLA) as well as branched-chain FA in human diet. Although these FA constitute only a minor percentage in dairy fat, small amounts may still be biologically relevant, alone or within the context of the dairy matrix (Kratz et al., 2013). The present review aims to summarize the most recent knowledge of the bioactive properties of milk FA and their potential effect on consumer health.

2. Short and medium-chain saturated Fatty Acids

SFA of total C number from 2 to 6 are usually classified as short-chain saturated FA (SCSFA), whereas those from 7 to 12 C atoms are defined as medium-chain saturated FA (MCSFA). SCSFA and MCSFA are easily digestible and they show a low tendency to be stored in the adipose tissue. These FA are preferentially hydrolysed from the TAG molecules and transferred directly from the intestine to the bloodstream. Afterwards, they are transported as FFA to the liver where they are rapidly metabolized via mitochondrial β-oxidation without TAG resynthesis, indicating that those SFA are a quick energy source for active cells. SCSFA and MCSFA also contribute to the regulation of cell metabolism and play an important role in intracellular signalling (Schönfeld & Wojtczak, 2016).

Milk fat is the main source of SCSFA in the human diet since most food products, including ruminant meat, present SFA of longer chain length. SCFA can exert antimicrobial activities and affect the pathogenesis of a diverse range of diseases (Tan et al., 2014). They are esterified almost entirely at the *sn*-3 TAG position, which would have some implications in the human digestion process. When dairy fat is consumed, our lingual and gastric lipases preferentially hydrolyse the FA at the *sn-3* position, and therefore there is a selective release of SCSFA in the gastrointestinal tract.

As average, a 4% of the FA esterified to cow milk TAG corresponds to butyric acid (4:0) (Table 1) which is the most important SCFA in dairy foods and exert multiple functions in the organism (Figure 1). Its high bioavailability would be related to multiple functions in our organism. 4:0 is the primary energy source for intestinal epithelial cells and it has an essential role in the maintenance of colonic homeostasis and health (Hamer et al., 2008). Butyric acid has a prominent mission in preserving the physiological functions of the colonic mucosa, a barrier consisting of mainly mucins, which is considered the first line of defence against pathogens and harmful substances. Mucin secretion positively influences adhesion of probiotics such as bifidobacteria and lactobacilli, while inhibits the incorporation of pathogenic bacteria. In this line, *in vitro* studies using human specific cell lines have shown that the presence of butyrate stimulates the production of mucins through upregulation of their gene expression, which would be beneficial for the microbiota adherence to the mucus layer (Hatayama, Iwashita, Kuwajima, & Abe, 2008; Jung, Park, Jeon, & Han, 2015).

Low concentration of 4:0 can inhibit growth in a wide range of human cancer cell lines, mainly colon (Hamer et al., 2008). Firstly it was believed that central to the anti-cancer action of 4:0 was its ability to inhibit histone deacetylases, which results in histone hyperacetylation and destabilization of chromatin structure that facilitates activation of genes associated with cell growth (Davie, 2003). Subsequent studies have reported that butyrate could also exert other effects in colon cells in different stages of cancer development (Fung, Cosgrove, Lockett, Head, & Topping, 2012). On primary chemoprevention, butyrate reduces inflammatory processes by modulating genes involved in oxidative and metabolic stress in human colon cells (Scharlau et al., 2009).

Regarding secondary chemoprevention, 4:0 has shown anti-cancer activity through the induction of apoptosis (Zhang et al., 2010) as well as the suppression of cell proliferation and migration by different molecular mechanisms (Zuo, Lu, Zhou, Wei, & Wang, 2013). Despite these promising evidences, it should be noted that 4:0 is not an isolated metabolite and it surely acts in conjunction with other compounds from the gut flora and dietary fibre. Furthermore, there is a lack of information on the action of butyrate in human subjects as most of the studies are based on *in vitro* and animal model data. In this context, gut fermentation research is of special relevance, since it could better reflect *in vivo* exposure conditions than butyrate alone (Scharlau et al., 2009).

In contrast to SCSFA, MCSFA are preferentially esterified at positions *sn-1* and *sn-2* of the TAG that would help to explain its responses on the human body. Dietary MCSFA have been related with a suppression of fat deposition through enhanced thermogenesis and fat oxidation (Nagao & Yanagita, 2010). The consumption of MCSFA has also shown positive effects on weight control and lipid metabolism (Mumme & Stonehouse, 2015). More recently, Bohl, Bjørnshave, Larsen, Gregersen, & Hermansen (2017) have reported that obese adults following a diet naturally enriched in dairy MCSFA increased their lean body mass, which would protect against body fat gain, while no changes in insulin resistance, blood pressure or cholesterol concentrations were observed. Although the relationship between MCSFA intake and the decrease of fat accumulation is well documented, the metabolic reasons behind are still under debate. Amer et al. (2017) attributed the reduction of body fat to the ω-oxidation of MCSFA in the liver whereas Matualatupauw, Bohl, Gregersen, Hermansen, & Afman (2017) stated that the beneficial effects of MCSFA would not only be achieved in the liver. The latter

suggested that the adipose tissue could play a key role in mediating the effects of MCSFA, via increased gene expression, by preventing fat accumulation. Overall, these findings prove the usefulness of milk fat, which contains high amounts of MCSFA, for a healthy body weight management and would not justify the consumption of skimmed dairy products with slimming purposes.

3. Long-chain saturated Fatty Acids

An important part of the lipids found in daily diets and milk consist of long-chain saturated FA (LCFA), with more than 12 carbon molecules (14:0-18:0), which are preferentially stored as body fat. From the quantitative point of view, three of the four most abundant FA in ruminants (14:0, 16:0 and 18:0) are LCFA (Table 1). As stated above, LCSFA storage is influenced by the amount of dietary simple carbohydrates, therefore their impact on cardiovascular health would be closely related to the balance between SFA and carbohydrates in the diet (Ruiz-Núñez et al., 2016). In contrast to CVD promoting effects, 14:0 and 16:0 are directly involved in post-translational protein changes, namely N-terminal myristoylation and side-chain palmitoylation (Legrand & Rioux, 2015). Both biochemical mechanisms control important metabolic processes in the human body. The reversible attachment of 16:0 to the sulphur atom of cysteine facilitates protein-membrane interactions and the intracellular movement of proteins, whereas myristoylation embraces key components in intracellular signaling pathways, oncogenes, structural viral proteins and common constitutive eukaryotic proteins (Ruiz-Núñez et al., 2016).

Additional metabolic functions are exerted by 14:0 through the improvement of cardiovascular health parameters in humans. A moderate intake of myristic acid from

dairy products, located mainly at the *sn*-2 position of the TAG, has shown to enhance long-chain omega-3 FA levels (20:5 n-3 and 22:6 n-3) in plasma phospholipids (Dabadie, Peuchant, Bernard, Leruyet, & Mendy, 2005). Further research has also indicated that 14:0 from dairy fat would exert a beneficial effect on lipid biomarkers by increasing HDL cholesterol and decreasing TAG levels, without changes in LDL cholesterol (Dabadie, Peuchant, Motta, Bernard, & Mendy, 2008). As a whole, taking into account the latest advances on the effects of SFA from dairy fat, only an excess of dietary palmitic acid (i.e. more than 8–10% of daily energy) would remain a concern in terms of cholesterolemia and CVD risk (Legrand & Rioux, 2015).

The effects of stearic acid (18:0), which represents about 10% of total dairy fat (Table 1), are more related to *cis*-9 18:1 (oleic acid) than other SFA (Thijssen, Hornstra, & Mensink, 2005; Thijssen & Mensink, 2005). Oleic acid is the second most abundant FA in milk (Table 1), accounting for 15-25% of total fat. It is a well-known antiatherogenic agent and promotes positive effects on human health (Sales-Campos, De Souza, Peghini, Da Silva, & Cardoso, 2013). Based upon a systematic review, multiple lines of evidence from laboratory experiments and randomized trials suggest that 18:0 would be beneficial, or at least neutral, for CVD prevention. Moreover, 18:0 do not show any detrimental effect on human health when compared with other SFA (Hunter, Zhang, & Kris-Etherton, 2010).

4. Branched and odd chain saturated fatty acids

Branched-chain saturated fatty acids (BCSFA) are major lipids of bacterial membranes and they are present in dairy fat as a consequence of ruminal processes (Fievez, Colman, Castro-Montoya, Stefanov, & Vlaeminck, 2012). BCSFA constitute about 2%

of total FA in milk fat. The most abundant BCSFA are *iso*-16:0, *anteiso*-15:0, *iso*-17:0 and *anteiso*-17:0, although other minor BCSFA are also found in lower amounts (Table 2). BCSFA are primarily components of dairy and ruminant food products, and are absent from fish, chicken and pork. The mean BCFA intake of 500 mg/d in USA was delivered primarily from dairy (around two thirds) and beef food products. (Ran-Ressler et al. 2011a, Ran-Ressler, Bae, Lawrence, Wang, & Brenna, 2014). However, the information regarding BCSFA intake in other populations is still scarce and additional research would be required.

BCSFA are rare in human tissues but they are important bioactive components and possess an essential role in the gut. Ran-Ressler, Devapatla, Lawrence & Brenna (2008) reported that BCSFA are a major component of the late term fetal and newborn guts. They comprise about 30% of the FA present in vernix, the waxy coating that is unique to human infants and fetuses. The vernix suspended in amniotic fluid is normally swallowed by the fetus, increasingly as parturition approaches, and exposes the fetal gut to BCSFA at an early age. It has been observed that the newborn lumen in the gastrointestinal tract contains BCSFA, indicating that these FA provided by the vernix would support the colonization of specific microorganisms in the gut microbiome (Ran-Ressler et al., 2008). On the other hand, the substitution of dietary fat by BCSFA in neonatal rats has shown to alter the microbiota composition and to reduce the incidence of necrotising enterocolitis (Ran-Ressler et al., 2011b). Liu et al. (2017) also reported that human fetal intestinal epithelial cells would incorporate high levels of BCSFA into membrane phospholipids, and thus tend to modulate the biophysical properties of the membranes similarly to *cis* double bonds. The mechanisms involved are pending but BCSFA appears to interfere with the ability of SFA to pack tightly to form rigid, high-

melting point extended structures, and reduce the phase transition temperature of membrane phospholipids (Ran-Ressler et al., 2014).

Apart from the beneficial effects on gut microbiota health, BCSFA have also shown positive effects in several chronic diseases. BCSFA induce apoptosis in human breast cancer cells, and inhibit tumour growth in cultured cells and animal models (Wongtangthinharn, Oku, Iwasaki, & Toda, 2004). Cai et al. (2013) stated that *iso*-15:0 promotes inhibitory effects on T-cell lymphomas *in vivo* in mice. More recently, Mika et al. (2016) observed that low contents of *iso*-BCSFA may contribute to increase serum TAG levels in obese subjects, while Yan et al. (2017) showed for the first time the reduction of inflammatory markers in human cells by treatment with BCSFA. From a nutritional perspective, as BCSFA are absent or in very low amounts in food products other than ruminants, dairy fats could have a beneficial role in the moderation of inflammatory responses through dietary habits. The beneficial role of BCSFA from dairy products needs further research in regard to their promising effects on the maintenance and development of the gut microbiota and possibly other functions related to CVD, cancer, obesity, and inflammatory prevention.

Others researchers (Wongtangthinharn, Oku, Iwasaki, & Toda, 2004) reported that BCFA induce apoptosis in human breast cancer cells, and inhibit tumour growth in cultured cells and in a mouse model. *Iso*-15:0 also induced inhibitory effects on T-cell lymphomas *in vivo* in mice (Cai et al. 2013). More recently Mika et al. (2016) presented findings imply that lower contents of *iso*-BCFA may contribute to increased serum levels of TAG in subjects with obesity, while Yan et al. (2017) showed for the first time the reduction of inflammatory markers in human cells by treatment with BCFA,

although this decrease was less effective than 20:5 n-3 and 22:6 n-3. This observation in human intestinal epithelial cells suggested that dairy fats may play a role in the moderation of inflammatory responses. These data all point a possible beneficial role of dairy foods and warrant further study on previously neglected nutritional properties of BCFA that may be important for the maintenance and development of the microbiota, enterocyte health, skin and possibly other functions.

The last group of SFA detected in dairy products are odd-chain saturated fatty acids (OCSFA). The most abundant are pentadecanoic acid (15:0) and heptadecanoic acid (17:0), whose sum is about 1.5% of total fat, and have been widely used as biomarkers of dairy fat intake. However, it has been recently proposed that these FA may also be endogenously synthesized via α-oxidation (Jenkins, West, & Koulman, 2015) and could exert healthful effects in our body. Several studies have clearly shown an inverse association between 15:0 and 17:0 concentrations in human plasma phospholipids and risk of type 2 diabetes (Yakoob et al., 2016; Pfeuffer & Jaudszus, 2016; Risérus & Marklung, 2017). This was also observed in European populations with distinct dietary backgrounds (Forouhi et al., 2014). Additional research on CVD (Khaw, Friesen, Riboli, Luben, & Wareham, 2012; Otto et al., 2013; Liang et al., 2017) and other pathologies (Jenkins et al., 2015) showed that plasma concentrations of OCSFA would be associated with a lower disease risk. Nevertheless, research on dietary OCSFA effects and their potential mechanisms of action is still limited and warrant further study.

5. Trans fatty acids

In 1990, Mensink & Katan described for the first time an increased total and LDL-cholesterol levels in serum, with a simultaneous decrease of HDL-cholesterol, due to a diet rich in *trans* fatty acids (TFA). Since then, numerous clinical studies have confirmed their results and it has been stated that TFA would be responsible for an increased risk of heart disease more than any other macronutrient compared on a per-calorie basis (European Commission, 2015).

The greatest disadvantage of TFA lies in its capacity to disorder cell membranes. FA composition has a fundamental impact on membrane fluidity, and the rigid TFA chain can distort the phospholipids and thus negatively affect the membrane physical structure. The fluidity defines the extent to which molecules are transported and cell signals can be transmitted through the membrane. This fact shows wide metabolic implications in body functions and disease development, principally CVD.

The major source of TFA in human diet has been partially hydrogenated vegetable oils or fats (PHVO), but they also occur naturally in ruminant foods as a result of partial biohydrogenation of dietary PUFA by rumen microorganisms (Brouwer, Wanders, & Katan, 2013). As average, TFA contents in milk and dairy products are low, less than 5% of total FA, and nowadays the TFA daily energy intake is generally below 1% worldwide (Wanders, Zock, & Katan, 2017). The World Health Organization recommends to consume not more than 1% of TFA of daily energy as TFA (WHO, 2010), while the European Food Safety Authority advise that TFA intake should be as low as possible (European Commission, 2015). In any case, it is of vital importance to specify the origin of TFA when establishing statements as TFA effects depend on the

316 position of the double bond and specific TFA profiles are observed from different fat
317 sources (Figure 2).
318
319 Monoenes with 18 carbon atoms are the most prominent TFA in human diet. During the
320 industrial hydrogenation of vegetable oils or fats a wide range of monounsaturated TFA
321 are generated and the major isomers are *trans*-9 18:1 (elaidic acid) and *trans*-10 18:1
322 (Figure 2A). In contrast, the main TFA in milk fat is *trans*-11 18:1 (vaccenic acid, VA,
323 Figure 2B) and its contents can be influenced by the animal feeding regime (Shingfield
324 et al., 2013). Under conventional ruminant diets, milk VA percentage is around 40-50%
325 of total 18:1 TFA whereas elaidic acid and *trans*-10 18:1 are only present in small
326 amounts (5% and 10% on average, respectively, of total 18:1 TFA) (Shingfield et al.,
327 2008; Shingfield et al., 2013). Thus, the consumption of dairy fat would represent a
328 very low intake of *trans*-9 and *trans*-10 18:1 isomers while it is a good source of VA,
329 often described as the "natural TFA".
330
331 Many scientific evidences have recognized the unfavorable effects of TFA from
332 industrial PHVO on CVD risk factors whereas ruminant TFA have not been related with
333 such detrimental consequences. Denmark was a pioneer in the introduction of legal
334 limits for industrial *trans* fats. This regulation nearly eliminated TFA from the Danish
335 food supply and, as a result, their average industrial TFA intake is very low (0.01-0.03
336 g/day) and it has reduced deaths caused by CVD. In the 3 years after the legal limit was
337 implemented, mortality attributable to CVD decreased on average by about 14.2 deaths
338 per 100.000 people per year (European Commission, 2015).
339

It has been argued that the low negative impact of ruminant TFA in human health would be a consequence of the lower presence of this type of fat in our diet. A clinical trial reported that very high levels of VA (i.e. 10 times greater than the amount typically consumed) had similar effects to industrial TFA on CVD risk factors (Gebauer, Destaillats, Dionisi, Krauss, & Baer, 2015). Motard-Belanger et al., (2008) also observed that, whereas high dietary intake of TFA from any source adversely affected plasma LDL- and HDL-cholesterol, ruminant TFA intake in moderate amounts but exceeding normal intake had a neutral effect on CVD risk factors. Furthermore, most of the studies stated that the positive association between TFA consumption and the risk of CVD could be entirely explained by the intake of TFA from industrial PHVO and, at least, no changes were reported with increasing TFA from ruminant sources (Jakobsen, Overvad, Dyerberg, & Heitman, 2008; Gebauer et al. 2011; Bendsen, Christensen, Bartels, & Astrup, 2011). A recent review (Kuhnt, Degen, & Jahreis, 2016) relating to ruminant TFA alone makes clear that no convincing adverse physiological effect can be attributed to them and only extremely high ruminant TFA intakes would cause negative change in blood lipids. (Kuhnt, Degen, & Jahreis, 2016).

The previous statements are based in studies where both TFA groups are considered as a whole. However, for a very long time, health effects of TFA have ignored the role of individual isomers, likely because of their lack of commercial availability. In order to circumvent this problem, Turner et al. (2015) developed a methodology combining Ag^+ solid-phase extraction and Ag^+-HPLC to isolate individual TFA from ruminant fat. This procedure allowed them to obtain sufficient amount of each *trans* 18:1 to examine the physiological effects of individual isomers in cell culture models. Using mouse adipocytes, Vahmani et al. (2015a,b) described that 18:1 TFA isomers are metabolized

differently and have different lipogenic properties, in which the position of the double bond would play an essential role. VA and *trans*-13 18:1 showed the greatest delta-9 desaturase activity. In contrast, elaidic acid upregulated the expression of several lipogenic genes such as acetyl-CoA carboxylase, FA synthase, FA elongase and stearoyl-CoA desaturase, which it was not observed for either VA or *trans*-13 18:1. The effects of individual *trans*-18:1 isomers were also compared in the liver cells. *Trans*-6, *trans*-9 and *trans*-10 18:1 induced hepatic lipogenic and cholesterogenic gene expression that resulted in increased contents of TAG and cholesteryl esters. On the contrary, VA, *trans*-13, *trans*-14 and *trans*- 15 18:1 responded similarly to oleic acid or to an absence of TFA (Vahmani, Meadus, Duff, Rolland, & Dugan, 2017).

In 2009, Field, Blewett, Proctor & Vine compiled all the information published on the effects of VA in cell lines, animal models and humans. They concluded that epidemiological, clinical, and rodent studies to that date did not demonstrate a relationship between VA and CVD, insulin resistance, or inflammation. Since then, a number of positive health effects have been ascribed to VA. An acute oral safety study in rats of a dairy fat enriched in VA, representing 14% of total FA, significantly decreased TAG in plasma and did not show any detrimental metabolic effects or negatively influence toxicological parameters (Anadón et al., 2010). Another experiment in rats with metabolic syndrome fed a diet supplemented with 1% VA showed an upregulated intestinal expression of peroxisome proliferator-activator receptors (PPAR), which regulate the expression of genes controling cell cycle, lipid oxidation and energy balance (Wang et al., 2012). Furthermore, it has been reported that VA alleviated features of metabolic syndrome by attenuating ectopic lipid accumulation

in rats (Jacome-Sosa et al., 2014) and improved insulin secretion in rodent models of type 2 diabetes (Wang et al., 2016).

Apparently, the importance of VA lies in its role as precursor of the main CLA isomer, rumenic acid (RA, *cis*-9, *trans*-11 C18:2), one of the most relevant bioactive compounds present in milk fat (see next section). Initially, it was believed that RA endogenous synthesis only occurred in the ruminant mammary gland, but it has been demonstrated that RA is also synthesized via delta-9 desaturase in human tissues (Van Wijlen & Colombani, 2010). Besides, there are growing evidences suggesting the independent bioactivity of VA irrespective of its conversion to RA. For instance, VA significantly suppressed the proliferation of MCF-7 cells which would indicate that this isomer may exert a direct anti-carcinogenic effect on human mammary adenocarcinoma (Lim et al., 2014). Herrera-Meza et al. (2013) also reported beneficial effects on CVD risk biomarkers in hypertensive rats that followed a diet including milk fat naturally enriched in VA and RA, even when an inhibitor of the conversion of VA to RA was administered. Regarding inflammatory processes, Jaudszus et al. (2012) examined whether VA and RA inhibited pro-inflammatory cytokine production based on human peripheral blood mononuclear cells. Although the mechanism of both FA was similar, it was observed that the anti-inflammatory effect of VA was independent of RA. More recently, Jacome-Sosa et al. (2016) demonstrated that dietary supplementation with VA exerts a tissue-specific regulation of endocannabinoides (EC), lipid-derived messengers involved in energy metabolism and intestinal inflammation. These authors provided evidence that VA could act independently of RA by altering the availability of arachidonic acid, an EC precursor in membrane phospholipids, that would suppress intestinal inflammation (Figure 3). In sum, all these studies provide further insights into

414 the potential beneficial effects of VA by itself and suggest that enriching dairy products
415 with VA would not be negative for health.
416
417 *Trans* MUFA isomers other than 18:1 can also be found in milk fat in minor amounts
418 (Jensen, 2002) but their effects in human health are very little known. Only *trans*-9
419 16:1, a natural biomarker of milk fat, has been studied in some extent. This 16:1 isomer
420 has been inversely associated in humans with the incidence of diabetes (Mozaffarian et
421 al., 2010, 2013), mortality related to CVD and sudden cardiac death (Kleber et al.,
422 2016). These evidences encourage further research to cover this gap in knowledge in the
423 near future.
424
425 **6. Conjugated Linoleic Acid**
426 CLA is the acronym comprising a group of linoleic acid (*cis*-9 *cis*-12 18:2) isomers with
427 conjugated double bonds that differ in position and geometry (*cis* or *trans*). The source
428 of CLA in the human diet is almost exclusively through the consumption of ruminant
429 meats such as beef and lamb and, mainly, from high fat dairy products (Van Wijlen &
430 Colombani, 2010). RA, *cis*-9 *trans*-11 18:2, is quantitative the most important isomer
431 in dairy fat and it accounts for at least 75% of total CLA. Other CLA isomers are also
432 naturally present in the lipid fraction of dairy products but in very low amounts (about
433 0.5-1.0% of total FA). The 18:2 conjugated isomers in ruminant milks present double
434 bond positions from 7-9 to 12-14 and cover all geometric configurations (*cis-cis*, *cis-*
435 *trans*, *trans-cis* and *trans-trans*, Table 3).
436
437 Since the identification of CLA as a compound capable of inhibiting carcinogenesis
438 (Ha, Grimm, & Pariza, 1987), thousands of studies have been conducted in the

subsequent three decades and it has been related with multiple bioactive effects. There are more than 5.000 publications related to CLA only in the last ten years (Web of Science, April, 2018), which reflect the great scientific interest in these molecules. CLA exhibits both, *in vivo* and *in vitro*, antitumor, anti-atherosclerosis, anti-diabetic as well as antiobesity effects, and also modulates the immune system (Wahle, Heys, & Rotondo, 2004; Churruca, Fernández-Quintela, & Portillo, 2009; Dilzer & Park, 2012). As the information available is extremely vast, our review summarizes the general bioactivities of CLA and some isomer-specific effects. If more information is required, we recommend recent and inspiring reviews (Yang et al., 2015; Kim, Kim, Kim, & Park, 2016; Shokryazdan et al., 2017; Fuke & Nornberg, 2016) that would complete the present summary.

Most biological effects of CLA have been linked to two isomers (*cis*-9 *trans*-11 18:2 and *trans*-10 *cis*-12 18:2), although other minor CLA could also show benefitial activities. Since the 90s, nearly all the animal and human CLA studies were performed using synthetically prepared mixtures of RA and *trans*-10 *cis*-12 18:2 due to the high cost and difficulty to isolate each unique CLA isomer (De la Fuente, Luna, & Juárez, 2006). However, during the first decade of 2000, it was clear that RA and *trans*-10 *cis*-12 18:2 elicited different biological effects and their cell mechanisms and functions depend on a great number of variables (cell or tissue type, *in vivo* or *in vitro* assays, mammalian species or CLA concentration). Moreover, most of their beneficial properties should be attributed to the separate action of both FA (Wahle et al., 2004; Churruca et al., 2009). Together they showed anti-carcinogenic properties, but this activity was mediated by different pathways on apoptosis modulation, lipid metabolism and oncogene regulation (Kelley, Hubbard, & Erickson., 2007). It is important to

highlight that the evaluation of a substrate composed of two isomers often adds complexity to the investigations and may compromise the conclusions. Hopefully in the next decade, advances in CLA purification will accelerate *in vivo* investigations and will allow the scientific community to comprehend the biological mechanisms of each CLA isomer in the different diseases they have been related with.

Differences among RA and *trans*-10 *cis*-12 18:2 should be attributed to a mixture between the position of the double bonds and their unique associated geometry. *Trans*-10 *cis*-12 18:2 is more efficiently oxidized than RA (Churruca et al., 2009) because its double bonds are more exposed and, as a result, it is related to catabolic processes such as fat oxidation or lipolyis. In contrast, *cis*-9 *trans*-11 18:2 has been generally associated to anabolic and anti-inflammatory effects (Reynolds, & Roche, 2010; Ferlay, Bernard, Meynadier, & Malpuech-Brugere, 2017; Lordan & Zabetakis, 2017). Inflammation underlies the pathogenesis of many widespread diseases including CVD, obesity, diabetes, viral infections or cancer, and RA would be involved in the modulation of inflammatory molecules as prostaglandins, cytokines, thromboxanes, leukotrienes or immunoglobulins.

Regarding RA, whose intake is mainly from dairy products, a great number of positive effects on inflammatory responses have been demonstrated in *in vitro* and animal models (Viladomiu, Hontecillas, & Bassaganya-Riera, 2016). This antiinflamatory activity would be linked to two metabolic pathways occurring in the cell (Figure 3). In the first place, the *endoplasmic pathway* proposes that RA might alter eicosanoid formation, displacing both arachidonic acid (20:4 n-6) and its precursor linoleic acid (18:2 n-6) in phospholipids. As shown in Figure 3, RA and linoleic acid would compete

for the same enzymes in lipid metabolism. The presence of RA may hinder the cascade reactions of 18:2 n-6 and be converted into *cis*-6 *cis*-9 *trans*-11 18:3, *cis*-8 *cis*-11 *trans*-13 20:3 and conjugated arachidonic acid (*cis*-5 *cis*-8 *cis*-11 *trans*-13 20:4) thus, the occurrence of 20:4 n-6 and its derived pro-inflamatory eicosanoids would be diminished. It has been observed that RA can also compete with arachidonic acid for cyclooxygenases and lipoxygenases enzymes involved in the synthesis of protanglandins, tromboxanes and leukotrienes (Figure 3). As a whole, the inhibition of these downstream eicosanoid products from 20:4 n-6, which apparently are not formed from conjugated 20:4 n-6, would be related to the prevention of inflammation processes, vascularization, tumor promotion and immune response events (Belury, 2002, Yang et al., 2015). The other metabolic process, the *nucleous pathway*, postulates that RA would operate as a high affinity ligand for PPAR (Belury et al., 2002). The PPAR activation acts as an important negative regulator of inflammatory responses by altering the expression of target genes known to modulate inflamation, carcinogenesis, adiposity, diabetes and cardiovascular disease (Viladomiu et al. 2015; Yang et al., 2015).

The dose of RA in the diet is an important factor to consider in these biological processes. To date there are not many human dietary intervention studies evaluating the consumption of RA-enriched dairy products and the results obtained are diverse. Tricon et al. (2006) did not report significant changes in inflammatory biomarkers after the consumption of different dairy foods enriched in RA (1.4 g/d) while Sofi et al. (2010) detected a significant reduction of inflammatory cytokines with an intake of 0.14 g/d of RA from sheep cheeses. Penedo et al. (2013) have also suggested that RA may contribute to a reduction of inflammatory responses. They observed that healthy young

adults consuming a RA enriched butter (1 g/d) reduced the pro-inflamatory interleukins in the blood serum. More recently, Jaudszus et al. (2016) in a placebo-controlled pilot trial concluded that dietary supplementation with purified RA could modestly dampen the inflammatory response at the cellular level in children with allergic asthma. Although this information is extracted from the limited number of studies in humans, there are promising evidences suggesting that the consumption of RA would be useful to reduce inflammatory responses and prevent subsequent related diseases (Lordan & Zabetakis, 2017).

As far as the voluminous information on CLA is concerned, most of the preclinical data support CLA nutritional advantages but, unfortunately, not all health benefits observed in animal models have been well translated into human clinical studies. In addition, some adverse effects have been reported in regard to oxidative processes, glucose homeostasis or carcinogenesis (Reynolds & Roche, 2010; Kim et al., 2016; Shokryazdan et al. 2017). However, it is important to mention that most of these investigations are observed with synthetic CLA mixtures or the *trans*-10 *cis*-12 isomer alone, and not RA which is the predominant CLA isomer in dairy products.

One of the major limitations in human studies is that most of the research focused only on fat deposition, blood cells or plasma thus, unconclusive evidences were obtained for the effectiveness of CLA on human subjects (Benjamin, Prakasan, Sreedharan, Wright, & Spener, 2015). Furthermore CLA effects on serum lipid profile, blood glucose, insulin sensitivity, blood pressure and CVD risk factors need to be further investigated using different health conditions and hosts (Shokryazdan et al. 2017).

In comparison to the promising results observed in animal models, CLA effects on human trials have been limited. One possible explanation is that animal assays have been generally carried out with higher CLA concentrations. Humans consume between 15-400 mg of CLA per day on average (Bruen, Fitzsimons, & Belton, 2017) and this amount can reach 700-1100 mg per day if dairy products derive from animals under extensive feeding systems (Van Wijlen & Colombani, 2010). Unfortunately, CLA effects are only observed at higher doses in animal studies (Yang et al., 2015). In this concern, the recommended CLA dietary intake is still under scrutiny by the scientific community. There is still much CLA research ahead. Investigations dealing with effective doses, differences between CLA isomers and the holistic perspective of the whole CLA biological network will be especially welcome.

7. Conclusions

Milk fat is naturally rich in SFA and its intake has been notably reduced in the last decades. The consumer often perceives dairy fat with increased chances of suffering from CVD, metabolic syndrome or obesity and opts for fat-free dairy products in the market. However, this negative image is not supported by most recent scientific evidences. Biomarkers of milk fat consumption have been associated with a lower incidence of diabetes while they have not shown any negative effect on CVD. LCSFA intake, with the exception of 18:0, could be related with a higher CVD risk but only under isolated consumption in relevant amounts. Recent scientific contributions even suggest that individual 14:0 and 16:0 possess specific properties associated with important cell and biological functions.

On the other hand, there is a growing consensus pointing to milk fat as a source of natural bioactive components with beneficial effects on human health. Among other functions, butyric acid plays an essential role in the maintenance of microbiota health whereas MCSFA show beneficial effects on weight control and lipid metabolism. Other SFA, such as BCSFA support the colonization of specific microorganisms in the gut microbiome at birth and are also related with positive effects on inflammatory chronic diseases. Regarding RA, the main CLA isomer, and its precursor VA, they show bioactive properties by modulating inflammation. These FA would be involved in the prevention of cancer, obesity, diabetes and CVD. As several of the aforementioned FA are only included in human diet from ruminant fats, the incorporation of whole dairy products in our menu should be encouraged. This increase in the consumption of dairy fat would have a neutral effect or may even reduce CVD risk. Future research needs to establish which amount of dairy products is required to achieve positive health-effects.

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Figure 1. The role of butyric acid in health and disease.

Figure 2. Distribution of *trans* 18:1 isomers in partially hydrogenated vegetable oil (A) and bovine milk fat (B). Values on the x-axis indicate the position of the double bond. (Adapted from Shingfield et al., 2008. With permission).

Figure 3. Mechanisms of action of *cis*-9, *trans*-11 18:2 (rumenic acid, RA) in the cell metabolism. AA: araquidonic acid (20:4 n-6); COX: cycloxygenase; LA: linoleic acid (18:2 n-6); LOX: lipoxygenase; PPAR: peroxisome proliferator-activated regulator; VA: vaccenic acid (*trans*-11 18:1).

Table 1. Mean values of cow, sheep and goat milk fat main fatty acids (% in total fatty acid methyl esters). Data reported from De la Fuente et al. (2013)[1] and Alonso et al. (1999)[2].

Fatty Acid	Cow[1]	Sheep[1]	Goat[2]
4:0	3.9	3.5	2.2
6:0	2.5	2.9	2.4
8:0	1.5	2.6	2.7
10:0	3.2	7.8	10.0
12:0	3.6	4.4	5.0
14:0	11.1	10.4	9.8
15:0	1.2	1.0	0.7
16:0	27.9	25.9	28.2
17:0	0.6	0.6	0.7
18:0	12.2	9.6	8.9
cis 18:1	17.2	18.2	19.3
trans 18:1	3.9	2.9	2.1
18:2 n-6	1.4	2.3	3.2
18:3 n-3	1.0	0.8	0.4

Table 2. Branched-chain fatty acid (BCFA) profile in dairy products (% of total fatty acids). Data from Ran-Ressler et al. (2011a)[1], Shingfield et al. (2008)[2] and Ran-Ressler et al. (2014)[3]. NR: not reported.

	Milk[1]	Milk[2]	Butter[3]	Yogurt[3]	Cream[3]	Cheese[3]
iso 13:0	NR	0.04	NR	NR	NR	NR
anteiso 13:0	NR	0.08	NR	NR	NR	NR
iso 14:0	0.13	0.09	0.17	0.12-0.13	0.00-0.05	0.00-0.22
iso 15:0	0.13	0.22	0.01	0.14-0.15	0.00-0.11	0.02-0.42
anteiso 15:0	0.56	0.46	0.63	0.62-0.63	0.46-0.49	0.38-0.88
iso 16:0	0.31	0.21	0.34	0.29-0.30	0.24	0.00-1.18
iso 17:0	0.26	0.27	0.31	0.16-0.25	0.27-0.30	0.05-0.30
anteiso 17:0	0.61	0.50	0.38	0.56-0.59	0.36-0.37	0.29-0.61
iso 18:0	0.04	NR	<0.01	0.00-0.04	<0.01	0.00-0.09
TOTAL BCFA	2.04	1.87	1.84	1.75-2.01	1.37-1.52	1.41-2.73

Table 3. Minimum and maximum contents (percentage of total fatty acids) of individual conjugated linoleic acid isomers in cow, goat and sheep milk. Data reported from Ferlay et al. (2017)[1] and Shingfield et al. (2013)[2]. NR: not reported.

Isomer	Cow[1]	Goat[1]	Sheep[2]
cis-cis			
cis-9 *cis*-11	NR	0.001-0.002	NR
cis-trans			
cis-9 *trans*-11	0.586-1.186	0.685-0.828	0.386-0.960
cis-12 *trans*-14	0.001-0.006	0-0.002	0.001-0.001
trans-cis			
trans-7 *cis*-9	0.030-0.054	0.028-0.040	0.026-0.040
trans-8 *cis*-10	NR	0.009-0.019	0.020-0.020
trans-9 *cis*-11	0.008-0.250	0.002-0.026	0.010-0.020
trans-10 *cis*-12	0.004-0.070	0.001-0.008	0.001-0.010
trans-11 *cis*-13	0.012-0.120	0.007-0.028	0.010-0.020
trans-12 *cis*-14	NR	0.002-0.003	NR
trans-trans			
trans-7 *trans*-9	0.002-0.007	0.003-0.007	0.008-0.009
trans-8 *trans*-10	0.002-0.004	0.002-0.004	0.010-0.011
trans-9 *trans*-11	0.009-0.012	0.013-0.021	0.009-0.011
trans-10 *trans*-12	0.003-0.008	0.003-0.003	0.005-0.006
trans-11 *trans*-13	0.015-0.160	0.003-0.009	0.010-0.030
trans-12 *trans*-14	0.007-0.017	0.002-0.009	0.010-0.010