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A Review of the Metabolic Origins of Milk Fatty Acids

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Abstract

Milk fat and its fatty acid profile are important determinants of the technological, sensorial, and nutritional properties of milk and dairy products. The two major processes contributing to the presence of fatty acids in ruminant milk are the mammary lipogenesis and the lipid metabolism in the rumen. Among fatty acids, 4:0 to 12:0, almost all 14:0 and about a half of 16:0 in milk fat derive from *de novo* synthesis within the mammary gland. *De novo* synthesis utilizes as precursors acetate and butyrate produced through carbohydrates ruminal fermentation and involves acetyl-CoA carboxylase and fatty acid synthetase as key enzymes. The rest of 16:0 and all of the long-chain fatty acids derive from mammary uptake of circulating lipoproteins and nonesterified fatty acids that originate from digestive absorption of lipids and body fat mobilization. Further, long-chain fatty acids as well as medium-chain fatty acids entering the mammary gland can be desaturated via Δ-9 desaturase, an enzyme that acts by adding a *cis*-9-double bond on the fatty acid chain. Moreover, ruminal biohydrogenation of dietary unsaturated fatty acids results in the formation of numerous fatty acids available for incorporation into milk fat. Ruminal biohydrogenation is performed by rumen microbial population as a means of protection against the toxic effects of polyunsaturated fatty acids. Within the rumen microorganisms, bacteria are principally responsible for ruminal biohydrogenation when compared to protozoa and anaerobic fungi.

Keywords: biohydrogenation, fatty acids, lipogenesis, milk

Introduction

Milk fat is one of the most important components of milk quality, influencing the technological, sensorial, and nutritional properties of milk and dairy products (Cozma *et al.*, 2013). Milk fat consist predominantly of triacylglycerides (TAG) (> 95% of total milk lipids) containing more than 400 individual fatty acids (FA), most of which are present in amounts of <1% of total lipids. Only saturated fatty acids of chain lengths from 4 to 18 carbon atoms, *cis*-9-16:1, *cis*-9-18:1, *trans*-18:1, and 18:3 n-3 are present in amounts greater than 1% in milk fat (Jensen *et al.*, 2002). As numerous *in vitro* and *in vivo* studies have shown potential positive effects of milk FA on human health (Shingfield *et al.*, 2008; Mills *et al.*, 2011), it is essential to understand the metabolic origins of FA in milk. Therefore, the aim of this paper was to review the two major processes underlying the origin of FA in ruminant milk: the mammary lipogenesis and the lipid metabolism in the rumen.

Mammary lipogenesis

Milk FA originate from two sources: the uptake from circulation of preformed FA (ca. 60%) and *de novo* synthesis within the mammary gland (ca. 40%) (Chilliard *et al.*, 2000). Precursors for *de novo* FA synthesis are acetate and butyrate, volatile FA produced during microbial fermentation of cellulose and hemicellulose in the rumen. Butyrate is converted to β-hydroxybutyrate in the rumen wall (Jensen, 2002). The mammary gland uses acetate and β-hydroxybutyrate for the synthesis of 4:0 to 12:0 FA, almost all of myristic acid (14:0) (cca. 95%) and about a half of palmitic acid (16:0) in milk fat (Shingfield *et al.*, 2013). The rest of 16:0 and all of the long-chain FA (LCFA) derive from mammary uptake of circulating TAG-rich lipoproteins (very low-density lipoproteins and chylomicrons), and plasma albumin bound nonesterified FA (NEFA) that originate from intestinal absorption of lipids and body fat mobilization (Bauman and Griinari, 2003; Shingfield *et al.*, 2010). Mammary lipoprotein lipase allows TAG hydrolysis and NEFA uptake by the mammary

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gland (Chilliard and Ferlay, 2004). Milk fat contains as well odd- and branched-chain FA, which are largely synthesized by rumen bacteria. However, there is evidence that 15:0 and 17:0 secreted in milk are also synthesized *de novo* from propionate in ruminant tissues, including the mammary gland (Vlaemink *et al.*, 2006).

De novo synthesis

De novo synthesis of milk FA involves two key enzymes: acetyl-CoA carboxylase and fatty acid synthetase (Chilliard and Ferlay, 2004). Acetate and β-hydroxybutyrate contribute in an equall manner to the initial unit of four carbon atoms. Acetate is transformed to acetyl-CoA and used for the chain length extension of synthesized FA via the malonyl-CoA pathway, whilst β-hydroxybutyrate is activated to butyryl-CoA and then incorporated (Shinglfield *et al.*, 2010). Acetyl-CoA carboxylase catalyses the formation of malonyl-CoA from acetate, whereas fatty acid synthetase catalyses the condensation cycles of malonyl-CoA with acetyl-CoA or butyryl-CoA (Chilliard *et al.*, 2000). LCFA containing 16 or more carbon atoms are known to lower mammary FA synthesis in cow or goat mammary epithelial cells *in vitro* due to direct inhibitory effects on acetyl-CoA carboxylase. The inhibitory effects have shown to be more pronounced when FA contain a longer carbon chain and/or have higher degree of unsaturation (Barber *et al.*, 1997). This phenomenon explains the decrease in the concentrations of medium-chain FA in milk fat following an increase in the supply of LCFA to the mammary gland through diet or body fat mobilization (Chilliard *et al.*, 2000).

Uptake from circulation of preformed FA

In ruminants, FA in milk fats that are taken up from circulation are derived mostly from the digestive absorption of dietary and microbial FA (Bauman and Griinari, 2001). When reaching the intestine, these FA are usually in the unesterified form. They are absorbed in the duodenum, esterified in the enterocyte, and used in conjunction with phospholipids and cholesterol esters in the assembly of very low-density lipoproteins and chylomicrons that pass into the peripheral blood (Vernon and Flint, 1988). Before esterification, stearic acid (18:0) can be desaturated to oleic acid (*cis*-9-18:1) within the enterocyte, but only to a limited extent (Bickerstaffe *et al.*, 1972). The remainder of the circulating FA originates from the mobilization of body fat reserves, which typically accounts for less than 10% of milk FA (Shingfield and Griinari, 2007). Nevertheless, the contribution from mobilized FA increases when cows are in early lactation and/or in negative energy balance (Bauman and Griinari, 2003). As 18:0 and *cis*-9-18:1 are the main FA stored in ruminant adipose tissue, body fat mobilization induces a sharp increase in these FA concentrations in milk (Chilliard *et al.*, 2003).

Δ-9 desaturation

LCFA entering the mammary secretory cells can be desaturated, whereas preformed FA cannot undergo elongation (e.g. 16:0 to 18:0) within the mammary gland (Chilliard and Ferlay, 2000). Mammary secretory cells contain the enzyme Δ-9 desaturase that acts by adding a *cis*-9-double bond on the FA chain (Shingfield *et al.*, 2008). Δ-9 desaturase activity in the ruminant mammary gland is assumed to occur as a mechanism to ensure the liquidity of milk for efficient utilization by the offspring (Timmen and Patton, 1988). In this respect, the mammary gland transforms 18:0 into *cis*-9-18:1 and contributes to 60% to 80% of the entire amount of oleic acid secreted in milk (Glasser *et al.*, 2007; Shingfield *et al.*, 2013). Likewise, the activity of Δ-9 desaturase is estimated to contribute to 90% of *cis*-9-14:1 and 50% of *cis*-9-16:1 in milk fat (Mosley and McGuire, 2007). Other FA shorter than 18 carbon chain length, such as 10:0, 12:0, 14:0, 15:0 and 17:0, can also be used as substrates for Δ-9 desaturase (Shingfield *et al.*, 2010). Moreover, is estimated that 25% of the vaccenic acid (*trans*-11-18:1) formed in the rumen is desaturated in the mammary gland to rumenic acid (*cis*-9,*trans*-11-18:2), the main isomer of conjugated linoleic acid in milk (Mosley *et al.*, 2006). Mammary endogenous synthesis from *trans*-11-18:1 is responsible for 70% to 95% of the milk *cis*-9,*trans*-11-18:2 (Shingfield *et al.*, 2013).

Triacylglycerides formation

The above mentioned metabolic pathways (*de novo* synthesis, uptake from circulation and desaturation) allow the formation of a pool of FA further used to form TAG through glycerol esterification (Chilliard and Ferlay, 2004). The central carbon atom of TAG (sn-2) shows chirality, resulting in an asymmetrical TAG molecule, if two different FA are in the primary positions (sn-1 and sn-3) of the molecule (Kontkanen *et al.*, 2011). The distribution of FA within TAG synthesis is not random. 8:0, 10:0, 12:0 and 14:0 FA are preferentially esterified at sn-2 position, 18:0 is preferentially esterified at sn-1 position, whereas the distribution of 16:0 between sn-1 and sn-2 positions is equal. Short-chain FA (4:0 and 6:0) and *cis*-9-18:1 are more abundant in the sn-3 position of TAG (Jensen, 2002). FA asymmetrical arrangement on the glycerol molecule, as for example the preferential esterification of short-chain FA and oleic acid on the sn-3 position, has an important influence on the physical properties of milk fat. It decreases milk fat melting point at or below the body temperature of the cow (39°C), thus ensuring its fluidity (Timmen and Patton, 1988).

Rumen lipid metabolism

Diets consumed by ruminants generally contain between 20 and 40 g lipid/kg dry matter, with a high proportion of polyunsaturated FA (PUFA) (Shingfield *et*

al., 2010). The predominant PUFA in ruminant diets are linoleic acid (LA, 18:2 n-6 or *cis*-9,*cis*-12-18:2) and linolenic acid (ALA, 18:3 n-3 or *cis*-9,*cis*-12,*cis*-15-18:3), derived from forages, cereals, and oil seeds. Moreover, some oil seeds provide monounsaturated FA (MUFA) (mainly *cis*-9-18:1), whereas marine products (fish oil, algae) provide long-chain PUFA (mainly 20:5 n-3 (eicosapentaenoic acid, EPA) and 22:6 n-3 (docosahexaenoic acid, DHA)) (Chilliard *et al.*, 2007).

Lipolysis and biohydrogenation of dietary lipids

On entering the rumen, hydrolysis of the ester linkages found in TAG, phospholipids, and glycolipids is the initial transformation dietary lipids undergo (Lock and Bauman, 2004). Following this lipolysis carried out by lipases produced by rumen bacteria, NEFA are released into the rumen, adsorbed onto feed particles and hydrogenated or incorporated directly into bacterial lipids (Shingfield *et al.*, 2008). The second major step in dietary lipids metabolism is the ruminal biohydrogenation (RBH) of unsaturated NEFA (Lock and Bauman, 2004). For most diets, RBH averages 80% for LA and 92% for ALA (Doreau and Ferlay, 1994). The major pathways of RBH have been established as a result of numerous *in vitro* and *in vivo* studies. Metabolism of LA and ALA starts with the isomerization of the *cis*-12 double bond and the formation of a conjugated 18:2 or 18:3 FA, respectively. Conjugated products are further hydrogenated into *trans*-11-18:1 and then into 18:0 as the final end product (Harfoot and Hazlewood, 1997). The final hydrogenation phase is rate-limiting, so that *trans*-18:1 intermediates can accumulate and leave the rumen (Shingfield *et al.*, 2010). RBH of dietary PUFA generates numerous FA intermediates that following formation in the rumen can be incorporated into milk fat (Shingfield *et al.*, 2008). The occurrence of a wide range of isomers of *trans*-18:1, 18:2 and 18:3 FA containing one or more *trans* double bonds suggests that the metabolic pathways of RBH are much complex than previously thought. Therefore, recent studies have been oriented toward the characterization of additional alternative pathways accounting for the formation of specific intermediates during FA biohydrogenation in the rumen (Shingfield *et al.*, 2010).

Regarding the RBH of *cis*-9-18:1, this FA is often shown to form directly 18:0 (Jenkins *et al.*, 2008). However, more recent *in vitro* studies reported that *cis*-9-18:1 metabolism results in the formation of hydroxystearic (10-OH 18:0) and ketostearic (10-O 18:0) acids and multiple *trans*-18:1 intermediates with double bond positions from carbon 6 to carbon 16 (Mosley *et al.*, 2002; Jenkins *et al.*, 2006). *Cis*-9-18:1 RBH typically varies between 58% and 87% (Shingfield *et al.*, 2010). Moreover, RBH also occurs on 20- and 22-carbon FA with more than three double bonds, such as EPA and DHA in fish oil and marine algae. The RBH of these FA is ample, but is usually not characterised by a complete saturation (Chilliard *et al.*, 2000). Incubation of EPA (*cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17-20:5) and DHA (*cis*-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19-22:6) in cultures of mixed ruminal microorganisms led to the disappearance of these two FA as well as to the accumulation of *trans*-18:1 (AbuGhazaleh and Jenkins, 2004). If consistent with pathways for LA and ALA RBH, the initial isomerization of EPA and DHA should produce isomers with five and six double bonds, including at least one *trans* double bond. Isomerization should be followed by hydrogenation to isomers with four and five double bonds (Jenkins *et al.*, 2008; Shingfield *et al.*, 2010). However, more research is required to elucidate the biochemical pathways of EPA and DHA metabolism in the rumen.

Microorganisms involved in rumen biohydrogenation

RBH involves only some species of the rumen microbial population, carrying out this process as a means of protection against the toxic effects of PUFA on microbial growth (Buccioni *et al.*, 2012). Several studies have shown that within the rumen microorganisms, bacteria are principally responsible for RBH when compared to protozoa and anaerobic fungi (Jenkins *et al.*, 2008). LA and ALA metabolism involves two groups of ruminal bacteria: Group A, which hydrogenates PUFA to *trans*-18:1 FA, and Group B, which hydrogenates *trans*-18:1 FA to 18:0 (Harfoot and Hazlewood, 1997). Nevertheless, more recent studies have reported that cellulolytic bacteria from *Butyrivibrio* group are of principal importance in RBH. *Butyrivibrio fibrisolvens* was identified to produce *cis*-9,*trans*-11-18:2 and *trans*-11-18:1 from LA, whilst is does not form 18:0 (Jenkins *et al.*, 2008). To the present, the rumen bacteria identified as having the capacity to produce 18:0 are *Butyrivibrio hungatei* and *Clostridium proteoclasticum*, reclassified as *Butyrivibrio proteoclasticus* (Van de Vossenberg and Joblin, 2003; Buccioni *et al.*, 2012). The contribution of protozoa to RBH has been suggested to be due to the activity of ingested or associated bacteria (Jenkins *et al.*, 2008). However, recent data indicate that ruminal protozoal cells contain proportionally more *cis*-9,*trans*-11-18:2 and *trans*-11-18:1 than ruminal bacteria. The most likely explanation is that protozoa do not form these FA, but play an important role in the uptake/protection of the intermediates of bacterial RBH (Devillard *et al.*, 2006). Moreover, an *in vitro* study demonstrated that rumen fungi have the capacity to biohydrogenate LA, with *Orpinomyces* fungus being the most active. RBH is slower in fungi than in bacteria and has *trans*-11-18:1 as the end product (Nam and Garnsworthy, 2007).

Conclusions

In this review, an overview of the most recent studies regarding the metabolic origins of milk FA has been presented. The revision has focused on mammary *de novo* synthesis, uptake of circulating lipids and Δ-9 desaturation as well as on rumen biohydrogenation as the main processes

contributing to the presence of FA in ruminant milk. Due to the importance of milk FA in human health, this review intends to be a guide to scientists who start in this challenging area of research.

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