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The Synthesis of Milk Medium-Chain Fatty Acids in Mammary Gland

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Abstract

The fatty acid de novo synthesized in mammary gland is mainly catalyzed by fatty acid synthase (FASN) and acetyl CoA carboxylase (ACC), including all the short- and medium-chain fatty acid and half part of the palmitate in ruminants. However, the synthesis mechanism of medium-chain fatty acid among different species is different. In non-ruminants, a tissue-specific enzyme thioesterase II (TE II) can interact with TE I, which is a part of FASN, and terminate the elongation of fatty acids at about 10 carbons. However, in ruminants' mammary-gland acetyl/malonyl-CoA transferase (MAT) is predicted to be involved in the termination of medium-chain fatty acid without the presence of (TE II). A more exact understanding about the mechanism of synthesis of medium-chain fatty acid in different species is still unclear. This review gives the research development of synthesis mechanism of medium-chain fatty acid in mammary gland among different species.

**Keywords:** mammary gland, medium-chain fatty acid, FASN, ruminants

1. FASN and fatty acid synthesis

The composition of milk fatty acid varies greatly among different species. Long-chain fatty acid is the most abundant fatty acid (C14-C18) in guinea pigs, medium-chain fatty acid takes a large part of medium-chain fatty acid (C8-C10), and long-chain and short-chain (C4-C6) fatty acids are the most abundant in cow's milk [1]; however, in goat milk all carbon-chain fatty acids are observed including short-chain, medium-chain, and long-chain fatty acids [2]. The short-chain and medium-chain fatty acids are mainly de novo synthesized in mammary gland [3–5].

The synthesis of fatty acid in mammary gland is mainly catalyzed by fatty acid synthase (FASN) and acetyl CoA carboxylase (ACC). In mammalian cells, the functional form of FASN is a homodimer (MW ~540,000 Da) [6]. FASN is composed of seven domains, including b-ketoacyl synthase (KS), acetyl/malonyl-CoA transferase (MAT), b-hydroxyacyl dehydratase (DH), enoyl reductase (ER), b-ketoacyl reductase (KR), acyl-carrier protein (ACP), and thioesterase I (TE I) (**Figure 1**). The core region between the DH and ER domains has no catalytic activity. At first, the acyl moiety of acetyl-CoA (initiation substrate) is transferred to the ACP catalyzed by MAT to generate malonyl-CoA (elongation substrate). And then, the acyl moiety is momentarily transferred to KS, and transacylation of malonyl-CoA is catalyzed by MAT to ACP. Acetoacetyl-ACP is then generated with decarboxylative condensation catalyzed by KS. This process follows catalyzation by KR and DH, which are responsible for the NADPH-dependent reduction of the b-carbon, and the dehydration of b-hydroxyacyl-ACP to a,b-enoyl, respectively. And then, by the catalyzation of ER, a four-carbon acyl chain is produced from NADPH-dependent reduction of the enoyl. The subsequent elongation cycles are performed with the malonyl-CoA as two-carbon units. Lastly, TE I is responsible for the release of fatty acid, with a length of 16 carbons, from ACP [6].
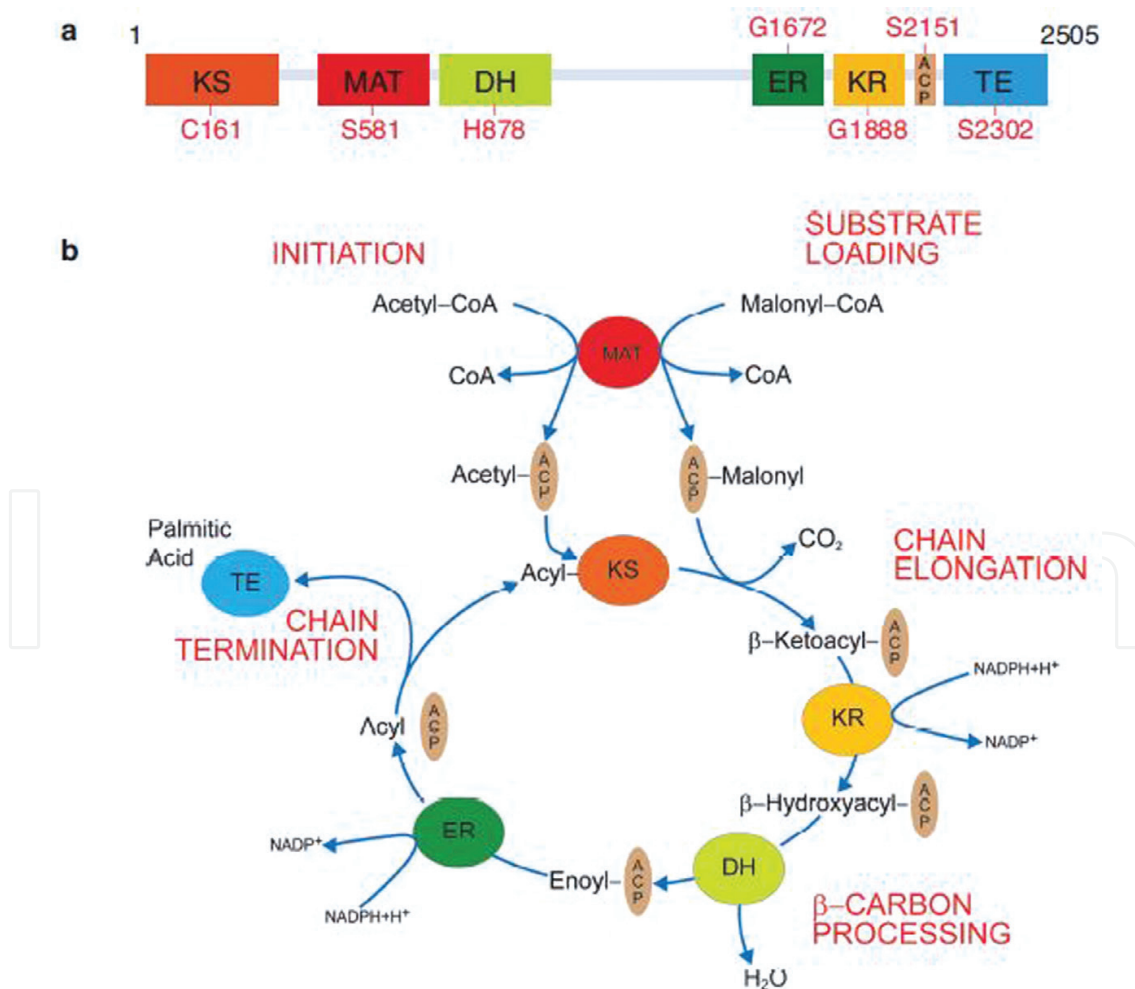

Figure 1. The structure of FASN gene and the fatty acid synthesis cycle. (a) The domain structure of FASN and the active site residues. (b) De novo fatty acid synthesis cycle catalyzed by domains of FASN.

FASN is the crucial enzyme for milk fat synthesis [7]. It plays an important role in the regulation of energy metabolism, cell membrane formation, signaling pathway regulation, and epigenetics [8]. By comparing the sequences of FASN in different species, including goat, sheep, cattle, human, chicken, and rats, FASN genes contain 42 exons in cattle and 43 exons in human and mice, and so on [9] (**Figure 2**).

FASN is also important for the regulation of the genes related to fatty acid synthesis in mammary gland. The inhibition of FASN with orlistat, a natural inhibitor of FASN, suppressed the expression of ACC, lipoprotein lipase (LPL), and heart-type fatty acid-binding protein (H-FABP) [10]. The inhibition of FASN in mammary cells by C75-mediated interference, a synthetic inhibitor of FASN activity, and short hairpin RNA-mediated interference significantly suppressed the deposition of triglyceride, decreased the expression of glycerol-3-phosphate acyltransferase (GPAT), 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT6), and diacylglycerol acyltransferase (DGAT2), which are important for cellular triglyceride synthesis. The inhibition of FASN also enhanced the expression of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), both of which are crucial for lipolysis. This is consistent with the markedly lower expression of genes related to lipid-droplet formation and secretion (hormone-sensitive lipase, TIP47; adipose differentiation-related protein, ADFP; xanthine oxidoreductase, XDH; butyrophilin 1A1, BTN1A1) [10].

Despite the effect of FASN inhibition on gene expression and lipid metabolism, the exact mechanism underlying the effect remains unclear. As a terminal enzyme for de novo fatty acid synthesis, previous studies indicated that FASN could help control lipid metabolism through indirect, hence, secondary effects. For instance, FASN could be involved in the control of fatty acid synthesis and oxidation through a direct effect on the concentration of malonyl-CoA, which was shown to help control lipid metabolism through the inhibition (allosteric) of β-oxidation by carnitine palmitoyltransferase 1 (CPT1) [11, 12]. In addition, FASN also helps generate ligands for transcription regulators, including peroxisome proliferator-activated receptors (PPARs) [13, 14], sterol-regulatory element-binding protein 1 (SREBP1), hepatocyte nuclear factor 4a (HNF4α), NF-E2-related factor-2 (NRF2), and Toll-like receptor 4 (TLR4) [14], all of which are important for lipid metabolism regulation. FASN also may affect protein activity indirectly, for example, endothelial nitric-oxide synthase, through palmitoylation [15]. Lastly, FASN may interact directly with caveolin-1 and lipid raft, which was involved in lipid secretion [16, 17]. However, the exact mechanism of how FASN affects the expression of genes related to milk fat synthesis remains unclear.
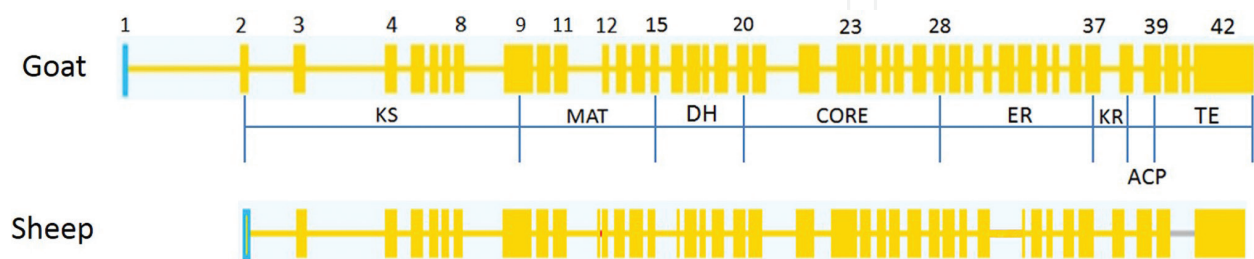
Figure 2. Prediction of exons and functional domains of FASN cloned from the goat mammary gland. Goat FASN gene includes 42 exons represented by seven function domains, in which three catalytic domains in the N-terminal section (KS, MAT, and DH) were separated by a core region from four C-terminal domains (ER, KR, ACP, and TE I). The sheep FASN gene contains 41 exons (XM 004013447).

2. The synthesis of medium-chain fatty acids in non-ruminants

The activity of hydrolase was necessary for the control of the length of the fatty acids produced by FASN with the main product of FASN being C16 palmitate. The thioesterase domain, TE I, catalyzes the termination step by hydrolyzing the thioester bond between palmitate and the 4'-phosphopantetheine moiety of the acyl-carrier protein (ACP) domain. Orlistat is a natural inhibitor of FASN, which was captured in the active sites of two thioesterase molecules as a stable acyl-enzyme intermediate and as the hydrolyzed product. The release of the thioesterase from FASN by limited proteolysis, however, results in the production of fatty acids containing 20–22 carbons [18]. Thus, the thioesterase domain is essential in regulating the length of the fatty acid chain.

Carey et al. [4, 19] purified the FASN protein from rabbit, mouse, and pig; the results showed that they just synthesized long-chain and short-chain fatty acids but not medium-chain fatty acids. However, with the help of TE II, FASN can produce all kinds of fatty acids, including C8, C10, C12, and so on [20, 21]. The medium-chain fatty acids synthesized de novo can be incorporated directly into triacylglycerol without the need of an activation step [22].

TE II is a tissue-specific enzyme independent of FASN. TE II can interact with TE I, which is a part of FASN, and terminate the elongation of fatty acids at about 10 carbons [23]. Decanoyl-CoA and decanoyl-pantetheine are the best substrates for TE II [24]. Ser101 and His237 of TE II are critical for the interaction. Asp236 of TE II enhances but is not essential for the reactivity of Ser101 and His237. For Leu262, it is involved in the interaction of TE II with TE I [23]. The interaction between TE II and FASN is stimulated by polyethylene glycol and suppressed by high concentrates of salt. Orlistat is a natural inhibitor and has a significant inhibition effect on FASN activity by curbing the binding of TE domain between FASN subunits [25]. TE II also can interact with P53 and is involved in the cancer development [25].

3. MAT and medium-chain fatty acid synthesis in ruminants

As a tissue-specific enzyme, TE II is only observed in non-ruminants. A similar enzyme is not present in goat mammary gland, although octanoic acid, decanoic acid, and dodecanoic acid amount to 20 mol% of the fatty acids synthesized in this tissue [26]. By contrast, goat mammary-gland fatty acid synthetase is by itself able to synthesize medium-chain fatty acids in the presence of the microsomal fraction and substrates for triacylglycerol synthesis [27]. Goat mammary-gland fatty acid synthetase exhibits both medium-chain thioesterase [21] and transacylase activity [28]. It seems that there are some differences about the activity of FASN between goat and other species. By treating the FASN protein of goat FASN with PMSF, an inhibitor of TE activity results in the termination of long-chain fatty acid synthesis, but not the medium-chain fatty acids. These results showed that there must be another hydrolase center in FASN protein. Hansen and Knudsen found that the supplementary of malonyl-CoA changed the proportion of medium-chain fatty acids [29]. Considering that the malonyl-CoA is transferred into the reaction by MAT activity, it was predicted that MAT may be involved

in the synthesis of medium-chain fatty acid. Engese et al. first found that MAT can transfer not only C2 but also C4, C6, and C10 fatty acids from CoA [28, 30], although the malonyl-CoA showed the strongest affinity with MAT. Supporting the results, the interference of FASN reduced the synthesis of C10:0 and C12:0, increased the content of C14:0, but without an effect on C16:0 and C18:0, in goat mammary epithelial cells [31]. These literatures indicate that the MAT of goat FASN may be responsible for the termination of short- and medium-chain fatty acids in mammary gland.

Rangan and Smith cloned the MAT protein from rat, and showed that MAT expressed in *Escherichia coli*, and refolded in vitro as a catalytically active malonyl-/acetyltransferase [32], similar as our previous study in goat expressed for goat mammary epithelial cells that the MAT domain is capable of folding correctly as an independent protein (unpublished). Replacement of the highly conserved residue His-683 with Ala reduced the activity by 99.95%, and the residual activity was relatively unaffected by diethyl pyrocarbonate. The rate of acylation of the active site serine residue was also reduced by several orders of magnitude in the His-683 to Ala mutant, indicating that His-683 plays an essential role in catalysis, likely by accepting a proton from the active site serine, thus increasing its nucleophilicity. In addition, Ser581 is also important for the activity of MAT. The Ser-581 to Ala mutant was completely inactive with either substrate. The Ser-581 to Cys mutant, however, retained approximately 1% of the activity of the "wild-type" enzyme compared with about 0.05% retained by His-683 to Ala mutation.

Actually, many other stimulants altered the relative content of medium-chain fatty acids. By treating the goat FASN with 5.2 mg/mL, albumin increases the content of C10 fatty acids relative to C12, while 10.4 mg/mL results in four times of C10 than C12 [33]. The supplementary of Malonyl-CoA enhances the synthesis of C12, similar as the effect of globin treatment. These treatments can only change the relative content among different kinds of medium-chain fatty acids, but not alter the total proportion of medium-chain fatty acids relative to long-chain fatty acids [33, 34].

The role of MAT is predicted to be critical for the goat medium-chain fatty acid synthesis; however, there is no direct evidence for the effect of MAT on fatty acid compositions. Although several genes have been proved to be involved in the regulation of fatty acid metabolism in mammary gland, how the activity of MAT, not for the whole FASN, on medium-chain fatty acid synthesis is controlled remains unclear. Recently, our study showed that malonyl-CoA is not only the elongation subtract for de novo fatty acid synthesis but also a regulatory for fatty acid deposition as triglyceride and fatty acid oxidation by inhibiting the activity of carnitine palmitoyl transferase 1 (CPT1), which is important for the transportation of long-chain fatty acids from cytoplasm to mitochondria [35]. Thus, it is the controller that directly interacts with MAT domain indicating that malonyl-CoA may be the balancer of lipid metabolism. The concentration of malonyl-CoA is mainly regulated by the activity of ACC and FASN, both of which are the terminates of lipid metabolism regulation. The hypothesis is that malonyl-CoA may be the indicator of fatty acid metabolism in mammary gland and may be the main mediator for the length of fatty acid responses to the environment around the mammary gland. This may be a good point for revealing the mechanism of fatty acid chain length determination in the future study.

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