Monocarboxylate Transporters: Role in Volatile Fatty Acid Transport and Factors Influencing Gene Expression (Postprint)

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Abstract

Feedstuffs undergo extensive microbial fermentation in the animal digestive tract, generating large quantities of volatile fatty acids that serve as an energy source for the animal organism. Monocarboxylate transporters (MCT) play a crucial role in the intestinal absorption and transport of volatile fatty acids in animals, and in-depth investigation of MCT is therefore of significant importance for elucidating the mechanisms underlying volatile fatty acid absorption and transport. This review summarizes the transport mechanisms of volatile fatty acids, MCT gene expression and tissue distribution, factors influencing MCT gene expression, and their underlying mechanisms.

Full Text

Monocarboxylate Transporters: Function in Volatile Fatty Acid Transport and Gene Expression Influencing Factors

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Abstract: Dietary carbohydrates fermented by microorganisms in the animal digestive tract generate large amounts of volatile fatty acids (VFAs) that provide energy for the animal. Monocarboxylate transporters (MCTs) play a crucial role in the absorption and transport of VFAs in the intestinal tract. In-depth research on MCTs is essential for elucidating the mechanisms underlying VFA absorption and transport in animals. This review summarizes the transport mechanisms of VFAs, MCT gene expression and tissue distribution, and the factors influencing MCT gene expression along with their underlying mechanisms.

Keywords: volatile fatty acid; monocarboxylate transporter; transport mechanism; CD147

Dietary carbohydrates fermented by microorganisms in the digestive tract produce substantial volatile fatty acids that serve multiple functions in animals, including providing energy for tissue cells, promoting water and sodium absorption, regulating endocrine activity, stimulating epithelial tissue development and proliferation, maintaining gastrointestinal microecological balance, and exerting anti-inflammatory effects [1]. In ruminants, VFAs and their derivatives also provide essential precursors for milk production, with approximately 50% of milk fat synthesized *de novo* from acetate and β-hydroxybutyrate (BHBA) delivered via arterial blood [2]. Monocarboxylate transporters (MCTs), members of the solute carrier family 16 (SLC16), function as proton-coupled transporters that play vital roles in cellular metabolism [3]. To date, 14 MCT-related sequences have been identified in mammals through sequence homology, though only seven isoforms have confirmed functions, and only MCT1, MCT2, and MCT4 are verified to transport VFAs [4]. In the animal gastrointestinal tract, MCTs are important for transporting lactate and other monocarboxylates such as butyrate, acetate, and propionate, with VFA transport in the rumen of ruminants being particularly dependent on MCT participation [5]. Notably, MCT1 and MCT4 require the assistance of the accessory protein CD147 to perform their normal biological functions [6]. CD147 is a widely distributed cell surface glycoprotein containing a single transmembrane domain that facilitates the proper localization of MCT1 and MCT4 to the plasma membrane [7].

1 Transport Mechanisms of Volatile Fatty Acids

Gäbel et al. [8] demonstrated that ruminal VFA absorption occurs through a two-step process: (1) uptake across the apical membrane of ruminal epithelial cells from the rumen lumen, and (2) efflux across the basolateral membrane into the bloodstream. VFAs enter ruminal epithelial cells via distinct pathways depending on pH conditions. Under low pH conditions, VFAs in their protonated form (HVFA) cross the cell membrane through passive diffusion coupled with protons, with absorption rates determined by pH at the apical membrane surface. Under high pH conditions, the ion cotransporters MCT1 and MCT4 facilitate the cotransport of hydrogen ions (H^+) and monocarboxylate anions in a 1:1 stoichiometry [9]. The passively diffused HVFA rapidly dissociates into ionized VFA (VFA^-) and H^+ within the cell (Figure 1 [Figure 1: see original paper]). The increased intracellular H^+ concentration drives the efflux of short-chain VFAs such as acetate and propionate across the basolateral membrane via MCT1 into the bloodstream, while long-chain VFAs like butyrate are metabolized into ketone bodies and β-hydroxybutyrate within the cytoplasm before being transported across the basolateral membrane by MCTs [10].

2 Tissue Distribution of MCTs

2.1 Distribution in the Gastrointestinal Tract

Kirat et al. [11–12] reported differential distribution of MCT1 and MCT4 genes and proteins throughout the ruminant digestive tract, with expression levels following the pattern: rumen ≥ reticulum > omasum > cecum > proximal colon > distal colon > abomasum > small intestine. In the forestomach, MCT1 primarily localizes to the basolateral and spinous cell layers of ruminal epithelium, whereas MCT4 is mainly found in the stratum corneum and granulosum. In the abomasum, MCT4 localizes predominantly to the basolateral membrane of epithelial cells. In the small intestine, MCT4 is present in both the brush border membrane and basolateral membrane, while in the large intestine, MCT4 localizes to the basolateral membrane of crypt epithelial cells and the apical membrane of surface epithelial cells. The accessory protein CD147 for MCT1 has also been detected throughout the ruminant gastrointestinal tract [11,13-15]. Expression levels of MCT1 and MCT4 are lower in pre-ruminant calves compared to adult cattle and correlate positively with VFA concentrations in the gastrointestinal tract, further supporting the role of MCT1 in VFA transport [16]. Additionally, Koho et al. [17] found regional differences in MCT1 and MCT4 expression within the rumen, with highest levels in the ventral rumen wall, followed by the rumen vestibule, dorsal caudal blind sac, and dorsal cranial blind sac.

2.2 Mammary Gland

In bovine mammary tissue, MCT1 and MCT2 localize primarily to the basolateral membrane of mammary epithelial cells, while MCT4 is found at the apical membrane. This distribution pattern suggests that acetate and β-hydroxybutyrate required for *de novo* fatty acid synthesis in mammary epithelial cells are transported across the basolateral membrane via MCT1 and MCT2, while newly synthesized fatty acids within the cells are transported into the alveolar lumen by MCT4 at the apical membrane [2].

2.3 Pancreas

Immunohistochemical analysis revealed that MCT1, MCT2, and MCT4 in bovine pancreas localize mainly to the islets of Langerhans, with no detectable expression in pancreatic acinar cells. This contrasts with monogastric animals, where MCT1 is primarily expressed in pancreatic acini with minimal presence in islets and β-cells. Postprandial increases in blood insulin and glucagon concentrations in ruminants correlate with circulating VFA levels [18]. Although pancreatic exocrine enzyme secretion is regulated by VFAs, the absence of MCT family members in acinar cells suggests that alternative transporters may mediate VFA uptake in these cells.

2.4 Adrenal Gland

In bovine adrenal cortex, MCT1, MCT2, and MCT4 are predominantly expressed in the cell membranes of the zona glomerulosa, with minimal or no expression in the zona fasciculata and zona reticularis. In the adrenal medulla, MCT1 is primarily localized to the cytoplasm of inner medullary cells, while MCT2 and MCT4 show low expression levels. The adrenal cortex synthesizes various steroid hormones, and cholesterol serves as both a membrane component and a precursor for steroid hormone synthesis. Since acetate is an important source for *de novo* cholesterol synthesis in the adrenal gland, the presence of MCTs as VFA transporters is expected [19].

2.5 Liver

MCT1 localizes primarily to the sinusoidal surface of hepatocytes. Approximately 80% of glucose required for ruminant metabolism is produced through hepatic gluconeogenesis, with propionate serving as the major precursor. Hepatocytes transport propionate into the cell via MCT1 on the plasma membrane to participate in gluconeogenesis. Studies in pre-ruminant animals have found minimal MCT1 expression in the liver, further confirming the specific role of MCT1 in VFA transport [20].

3 Factors Influencing MCT Expression and Regulatory Mechanisms

MCT expression is regulated by multiple factors including diet, hormones, developmental stage, and substrate availability, which can exert either upregulating or downregulating effects.

3.1 Dietary Factors

Kuzinski et al. [21] demonstrated in goats that mixed hay/concentrate feeding upregulated both MCT1 mRNA and protein expression in ruminal epithelial cells compared to hay fed *ad libitum*, indicating that high-energy diets enhance MCT1 expression. Under mixed feeding regimens, rapid ruminal fermentation produces large quantities of VFAs, and increased MCT1 expression facilitates accelerated VFA removal from the rumen while stabilizing pH to protect ruminal structure and function. In weaned piglets, Metzler et al. [22] found that dietary supplementation with 8.95% oat β-glucan increased cecal MCT1 expression by 40%, with MCT1 levels showing strong positive correlation with butyrate and total VFA concentrations in the digestive tract ($r = 0.99$, $P < 0.001$). Oat β-glucan increases total VFA concentrations, particularly butyrate and valerate, in the stomach and colon. Furthermore, Metzler et al. [23] observed that goats fed a 60% grain diet showed 45% upregulation of MCT1 and 28% downregulation of MCT4 in ruminal epithelium compared to 0% and 30% grain groups, with similar expression patterns in the colon. The decreased MCT4 expression in high-grain diets may balance the increased VFA uptake via passive diffusion

and VFA^-/bicarbonate (HCO_3^-) exchange, while elevated MCT1 expression reduces intracellular acid load and accelerates VFA efflux to provide energy.

3.2 Hormonal Regulation

In vitro studies using human intestinal epithelial Caco-2 cells demonstrated that somatostatin (SST) enhances butyrate uptake through SST receptor subtype 2 and p38 protein kinase-mediated pathways. This increased uptake results from SST-induced upregulation of MCT1 and CD147 levels on the intestinal epithelial cell membrane and enhanced synergistic interaction between MCT1 and CD147. The anti-diarrheal effect of SST on intestinal electrolyte absorption may be attributed to increased MCT1 expression and subsequent enhanced VFA absorption [24].

3.3 Developmental Stage

Koho et al. [17] reported that MCT1 and CD147 expression levels in goat ruminal epithelium increased progressively with age (from birth to 8 weeks), showing strong positive correlation. Conversely, MCT1 expression in the duodenum decreased with age during the same period. The digestive tract of newborn ruminants resembles that of monogastric animals, with colostrum bypassing the rumen via the esophageal groove for direct digestion and absorption in the abomasum. Consequently, VFA concentrations are initially high in the abomasum and duodenum but increase in the rumen following microbial colonization and initiation of solid feed intake, which stimulates MCT expression. These findings strongly support the role of MCT1 in transporting both VFAs and lactose in the gastrointestinal tract. Pfannkuche et al. [25] further demonstrated that in newborn calves, MCT1 protein in immature ruminal epithelial cells initially localizes to the luminal surface within 24 hours of birth, but by day 4 postpartum, MCT1 relocates to the blood-facing side with concurrent increases in protein abundance. Since butyrate is crucial for ruminal epithelial development and blood lactate levels are high in newborn calves, increased MCT1 expression on the blood-facing side facilitates lactate uptake, which is subsequently metabolized to butyrate within epithelial cells to provide energy for ruminal growth. The mechanisms underlying developmental changes in MCT1 expression remain complex and not fully elucidated, though hormonal regulation is hypothesized to play a role.

3.4 Substrate Concentration

Cuff et al. [26] demonstrated that in cultured intestinal epithelial cells (AA/C1), both MCT1 mRNA and protein expression increased with butyrate sodium concentration (0–5 mmol/L) and incubation time (0, 6, 12, 24, 48, 72 h). Radiolabeling studies revealed that the maximum velocity of [U-^{14}C]-butyrate sodium uptake increased significantly without changes in the Michaelis constant, indicating that enhanced butyrate absorption results from increased MCT1 abundance on the cell membrane rather than altered transporter affinity. Butyrate-induced

upregulation of MCT1 expression in intestinal epithelial cells increases butyrate uptake, which helps maintain intestinal homeostasis and provides energy for epithelial cells. In contrast, Malhi et al. [27] found that butyrate infusion in goats increased MCT4 expression in ruminal epithelial cells by 135% without significantly affecting MCT1 levels, though the underlying mechanism remains unclear and requires further investigation.

3.5 CD147 Regulation

CD147 regulates MCT function through protein-protein interactions. The inhibitor *p*-chloromercuribenzoic acid disrupts MCT1 and MCT4 activity by breaking the active disulfide bonds in CD147 immunoglobulin domains, thereby dissociating the interaction between MCT1/MCT4 and CD147 [12]. Philp et al. [28] demonstrated that knockout of CD147 in mouse retinal cells resulted in failure of MCT1, MCT3, and MCT4 to properly localize to the plasma membrane and function normally, despite normal MCT gene expression.

MCTs are widely expressed in the intestinal tract and various tissues of animals and represent critical factors for VFA absorption and transport with broad substrate specificity. Numerous studies indicate that VFA absorption and transport can be modulated by regulating MCT activity. However, the precise mechanisms by which factors such as SST, dietary protein type and quantity, animal developmental processes, and substrate concentrations regulate MCT expression remain to be fully elucidated. Therefore, further investigation into the regulatory mechanisms of MCT gene expression is essential for advancing our understanding of VFA absorption and transport mechanisms in animals.

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