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Cellular distributions of monocarboxylate transporters: a review

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ABSTRACT

Lactate and ketone bodies play important roles as alternative energy substrates, especially in conditions with a decreased utility of glucose. Short-chain fatty acids (acetate, propionate, and butyrate), produced by bacterial fermentation, supply most of the energy substrates in ruminants such as the cow and sheep. These monocarboxylates are transfered through the plasma membrane by proton-coupled monocarboxylate transporters (MCTs) and sodium-coupled MCTs (SMCTs). To reveal the metabolism and functional significance of monocarboxylates, the cellular localization of MCTs and SMCTs together with the expressed intensities holds great importance. This paper reviews the immunohistochemical localization of SMCTs and major MCT subtypes throughout the mammalian body. MCTs and SMCTs display a selective membrane-bound localization with porality. In contrast to the limited expression of SMCTs in the intestine and kidney, MCTs display a broader distribution pattern than GLUTs. The brain, kidney, placenta, and male genital tract express multiple subtypes of the MCT family. Determination of the cellular localization of MCTs is most controversial in the brain, possibly due to regional differences and the transcriptional modification of MCT proteins. Information on the localization of MCTs and SMCTs aids in understanding the nutrient absorption and metabolism throughout the mammalian body. In some cases, the body may use monocarboxylates as signal molecules, like hormones.

Intermediate metabolites including lactate, pyruvate, and ketone bodies provide immediate fuels and materials for conversion into glucose and fatty acids in most cells. Short-chain fatty acids (acetate, propionate, and butyrate), structurally related to the intermediate metabolites, are produced by the fermentation of dietary fibers in the lumen of the large intestine and absorbed via the epithelium. All these organic acids containing 2–4 carbon atoms and one carboxyl terminal are collectively called monocarboxylates. There is an idea that animals have evolved over a long time with the establishment of two energy systems using monocarboxylates and hexoses. Although animal cells can alternatively use the energy substrates according to specific conditions, their fundamental energy source may be monocarboxylates, which are able to be produced without oxygen consumption. Animals specialized with a high dependency on monocarboxylates are ruminants such as cattle and sheep, which obtain a large amount of energy from acetate produced by microorganisms in the huge forestomach, the rumen. The dependency on acetate is estimated to be more than 70% of the total energy substrates in the ruminant. As a result, the plasma glucose levels in ruminants are very low, whereas the concentration of short-chain fatty acids (SCFAs) in the blood is high as compared with non-ruminants. Even in non-ruminants, neonates preferentially utilize monocarboxylates for energy sources and the biosynthesis of lipids and amino acids. Moreover, monocarboxylates become essential fuels when the availability of glucose is low, such as in diabetes and during long starvation (63, 154). It is

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worth noting that the anaerobic glycolysis produces ATP much more quickly than aerobic respiration under normal conditions (72).

The regulated transfer and handling of monocarboxylates require membrane-bound transporters specific for substrates. Monocarboxylate transporters, MCTs, were first identified in 1994 and 1995 (38, 39). MCTs are membrane-bound channels that transport lactate, ketone bodies, and other monocarboxylates, along with protons (pH), down their concentration gradient. The MCTs facilitate the 1 : 1 exchange of monocarboxylate and proton across the plasma membrane. The direction of transport is determined by the relative intra- and extracellular concentrations of monocarboxylates and hydrogen ions (38, 54, 111). Formate, one of the SCFAs, is a very poor substrate while dicarboxylates and tricarboxylates are not transported by MCTs.

The oxygen-independent glycolysis produces lactate, which should not be excreted out of tissues but instead utilized by oxidative cells as an important energy substrate. The exchange of lactate between neighboring cells is often called the "lactate shuttle" (13). Representative lactate shuttles are constructed between astrocytes and neurons (5, 106), white (glycolytic) and red (oxidative) skeletal muscle fibers (158), Sertoli cells and germ cells (10), and hypoxic tumor cells and perivascular tumor cells (29). The complete knockout of MCT1 is embryonically lethal, possibly due to the deficient transfer of lactate via placental MCT1. Ketone bodies often serve as the major circulating energy source, especially during fasting. Ketoacidosis occurs when ketone formation exceeds ketone utilization. The MCT1-mediated transport of ketone bodies is also important to maintain an acid-base balance. Genetic analysis of patients with ketoacidosis has revealed a mutation of the gene encoding MCT1 (152). In accordance with the energy demand and crititical metabolism, MCTs show an extremely broad distribution as compared with glucose transporters, GLUTs. GLUT1, a predominant GLUT subtype, is mainly expressed in "the blood-tissue barriers" such as the brain endothelium (blood-brain barrier), perineurium (blood-nerve barrier), epithelial cells in the choroid plexus (blood-cerebrospinal fluid barrier), retinal pigment epithelium (blood-retinal barrier), and cytotrophoblasts (placental barrier) (140). Essentially all of these barriers simultaneouly express MCT1 and other MCT subtypes.

Another type of transporter for monocarboxylates, SLC5A8, was identified in 2003 (87). Since its transport via SLC5A8 is coupled with Na^+ (19, 96), it has been termed a Sodium-dependent MonoCarboxylate Transporter (SMCT). This transporter is an apical type at the cellular level—in contrast to MCTs, which are basically a basolateral type. It functions in absorbing monocarboxylates in the luminal space of the intestine and urinary tubules. Accordingly, the tissues predominantly expressing SMCTs are the intestine and kidney. SMCT also functions as a drug transporter with the potential to modulate the intestinal absorption and pharmacokinetics of various monocarboxylic drugs. In contrast to MCTs, which function as H^+-coupled electroneutral transporters, SMCT functions as a Na^+-coupled electrogenic transporter and induces water movement.

The present paper reviews the cellular localization of MCTs and SMCTs shown mainly by immunohistochemistry. For their biochemical and functional aspects, detailed reviews are available for both MCTs (28, 52, 53, 55, 56, 94, 111) and SMCTs (37, 50).

Monocarboxylate transporter members

The amino acid sequence alignment shows that MCT family members (SLC16) have twelve highly conserved transmembrane domains and indicates that the isoforms work as a gate for lactate transition with plasma membrane-bound form. Multiple MCT-related sequences of more than 14 subtypes have been identified, although only MCT1–MCT4, MCT8, and MCT10 have been functionally characterized (53, 55). Among them, MCT8 is a high affinity thyroid hormone transporter, and MCT10 (TAT1) is an aromatic acid transporter (155). MCT1 and MCT2 have been earliest and most extensively characterized. These transporters are 60% identical and encode proteins having approximately 490 amino acids and 12 putative transmembrane segments. MCT3 has a 43% and 45% sequence identity with MCT1 and MCT2, respectively. MCT4 is more closely related to MCT3 (68% similar residues) than to the other isoforms. The majority of tissues analyzed in the mouse and rat express MCT1, the best-characterized isoform. MCT1 particularly is expressed in cells which use ketone bodies for energy production but which also release lactate under certain conditions. The high-affinity isoform MCT2 is mostly expressed in cell types which use lactate, such as hepatocytes and neurons. MCT3 has been localized exclusively in the retinal pigment epithelium. On the other hand, low affinity MCT4 is particularly evident in tissues with higher glycolytic rates, such as white skeletal muscle (158) and astrocytes (7, 120). Its characteristics suggest an adaptation to the release of lactate in glycolytic cells. Several cells and tissues display the co-localization of MCT4

with MCT1.

In 2004, SLC5A8 (SMCT1), a 69-kDa protein, was found to code for a Na^+-coupled transporter for SCFAs, lactate, and pyruvate but not for di-carboxylate succinate or tri-carboxylate citrate. Soon afterwards, another type of SMCT2 (SLC5A12) with a 59% identity with SMCT1 at the amino acid level was identified (134). Both of these are abundant in the intestine and kidney and localized in the brush border, being apical types of transporters.

MCT1–MCT4 are localized along either the basolateral or apical membrane in most cells with polarity. The intracellular localization of MCT is important for considering the movement and utility of incorporated monocarboxylates. It is generally considered that MCT is also required for their movement into mitochondria. The MCT1 present in the inner mitochondrial membrane functions in the transport of lactate or pyruvate into the mitochondrial matrix in skeletal and cardiac muscle (12, 15, 61). However, most immunohistochemical studies have failed to demonstrate MCTs in mitochondria at the electron microscopic level (71) (also our unpublished data). At the light microscopic level, MCT1 signals have overlapped with a mitochondrial inner membrane protein (COX) in the rat skeletal muscle (60). The transport of monocarboxylates, mainly pyruvate, may be mediated by another proton-linked carrier known as the Mitochondrial Pyruvate Carrier (MPC) (54).

Technical aspects of histochemical demonstration

For the histochemical demonstration of MCTs and SMCTs, there are several types of tissue sections available: cryostat sections, paraffin sections, vibratome sections, or free-floating sections. Usually, cryostat (frozen) and vibratome sections are superior for the preservation of antigenicity rather than conventional paraffin sections in immunohistochemistry. Still, we were able to obtain sufficient stainability for MCTs using paraffin sections, due to their condensed localization along the plasma membrane and intense antigenicity. The effects of fixation on the stainability seem to be slight when immunostaining MCTs and SMCTs; we have been able to obtain excellent staining results in the fixation with the most representative fixative, 4% paraformaldehyde equivalent to 10% formalin. Picric acid is generally believed to enhance the antigenicity in the fixation process. Therefore, we routinely use Bouin's fluid for paraffin sections and Zamboni's fixative for cryostat sections and immuno-electron microscopy. On the basis of immunostaining results for MCT in the skeletal muscle, Hashimoto *et al.* (60) have ascribed the results of unstaining to the fixation itself: immunoreactivities of MCT1 and MCT4 are blocked in the formalin-fixed sections in contrast to unfixed sections. However, the different or unsuccessful stainability may be more likely due to antibody specificity, animal species, or types of sections.

The selection of antibodies is highly important for immunohistochemical analysis. We often experience a discordance of antibodies available between immunoblotting and immunohistochemistry. We also must pay attention to the species differences in the use of an antibody. For example, a world-wide commersial antibody against the carboxy-terminal of rat MCT1 was able to stain tissues from many animal species including the mouse, whereas an MCT2 antibody raised against the carboxy-terminal of rat MCT2 (N<u>T</u>HNPPSDRDKES<u>S</u>I) supplied from the same company could not detect murine MCT2 (corresponding sequence: N<u>A</u>HNPPSDRDKES<u>N</u>I, in which two amino acids are substituted in mouse MCT2). Checking available antibodies by the use of positive controls is recommended. As a positive control for each subtype of MCTs, the large intestine is suitable for MCT1, the kidney and stomach for MCT2, the retina for MCT3, and the skeletal muscle for MCT4 (and MCT1). Tissues adequate for the positive control of SMCTs are the intestine and kidney. The specificity of antibodies should be checked also by immunoblotting. However, molecular sizes of immunoreactive bands change according to the tissues and animals, possibly due to the post-transcriptional modification. Although an absorption test by preincubation of the antibody with corresponding antigen is indispensable for checking the specificity of immunoreactions on sections, it is sometimes difficult to obtain the antigens. *In situ* hybridization analyses using RNA probes or synthetic oligonucleotide DNA probes are useful for confirming the immunoreaction at mRNA levels. Fortunately, the gene expression of MCTs and SMCTs is at levels high enough to be detected by the *in situ* hybridization methods.

Before discussing the specificity of an immunoreaction, the selective localization of immunoreactivities along the cell membrane is essential in cases of MCTs and SMCTs. Furthermore, MCTs require chaperone proteins for a correct expression. Basigin, also known as the extracellular matrix metalloproteinase inducer (EMMPRIN) or cluster of differentiation 147 (CD147), colocalizes with MCTs (Fig. 1). Basigin is an accessory glycoprotein that assists the delivery of MCT1 and MCT4 to the plasma mem-

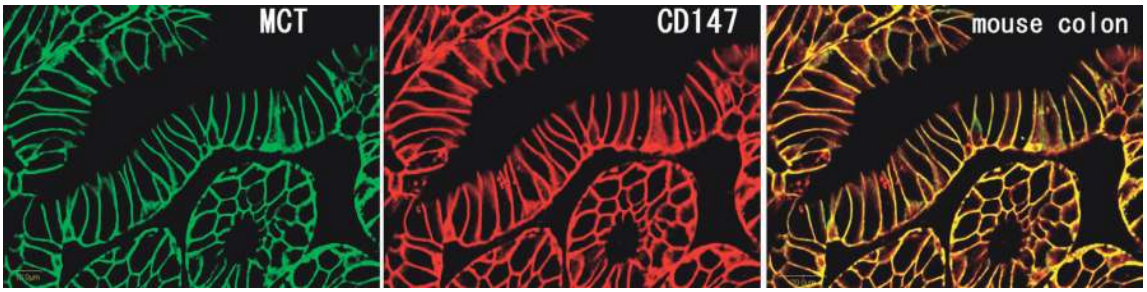

Fig. 1 Double immunostaining for MCT1 and CD147 in the mouse colon. The immunoreactivities of MCT1 (green) and CD147 (red) perfectly colocalize on a single section.

brane required for their folding, stability, membrane expression, and functionality (53, 79, 109). MCT2 associates with gp-70 (embigen), a homolog of CD147. The immunostaining of CD147 and gp-70 can confirm the localization of MCTs in many cases, although CD147 is not always associated with MCTs, such as in the cardiac muscle, pancreas, and small intestine.

Gastrointestinal tract, liver, and pancreas

Plant-derived dietary fiber and undigested carbohydrates are fermented by resident microflora mainly in the large intestine and produce SCFAs: acetate, propionate, and butyrate. The SCFAs in the bowel lumen have some direct effects on the intestinal wall, including the stimulation of colonic blood flow and of fluid and electrolyte uptake (150). Butyrate also functions as an energy source for epithelial cells in the large intestine and promotes the differentiation of enterocytes (14, 98). The uptake of a considerable amount of SCFAs in the large intestine occurs by the simple diffusion of the unionized form across the cell membrane; the remainder occurs by the active cellular uptake of ionized SCFAs together with inorganic protons, such as Na^+, K^+, and H^+ (31).

As a transporter of monocarboxylates including SCFAs, MCT1 was first identified and shown to be localized in gut epithelial cells as well as the heart and skeletal muscle (38). The original study by Garcia *et al.* (38) demonstrated immunohistochemically the selective localization of MCT1 at the basolateral membrane of epithelial cells in the hamster gut, suggesting a role for MCT1 in the transport of monocarboxylates from the epithelium to the internal miliue. However, many subsequent studies using immunohistochemistry and the immunoblotting of purified membrane fractions have reported the predominant localization of MCT1 to be in the luminal/apical membrane of intestinal epithelial cells in humans (44, 123), pigs (123), and rats (145) (for reviews, 22, 50). In contrast, our (66) and other immunostaining studies (78, 128) confirmed the basolateral localization of MCT1 in the epithelial cells of the mouse, rat, dog, bovine and human intestine (Fig. 2a). The discrepant findings on the subcellular localization of MCT1 may be due to different staining conditions, including the specificity of antibodies and preparation of tissue sections. The co-localization of CD147 with MCT1 strongly supports the concept of the basolateral localization of MCT1 (Fig. 1).

MCT1 in the gut is broadly distributed from the stomach to the rectum; however, the expression intensity is higher in the large intestine of most mammals, being highest in the cecum in the case of rodents (38, 39, 66). Such expression patterns of MCT1 in the large intestine are reasonable since bacterial fermentation occurs predominantly in the proximal regions of the large intestine, especially in the cecum of rodents (31). Interestingly, the bovine and ovine digestive tract abundantly displays its expression in the forestomach as well as the large intestine (75). The forestomach in ruminants is known to be the most active site for SCFA production, especially acetate, while very low levels of SCFAs exist in the glandular stomach and small intestine of these animals. The forestomach of ruminants is covered by the stratified epithelium, where the basal half layer of the epithelium expresses MCT1 on the entire cell membrane. In most mammals, absorptive epithelial cells (columnar cells) of the small intestine also express a considerable amount of MCT1 basolaterally with different intensities according to the location (villi versus crypts). The basolateral localization of MCT1 in the small intestine must be related to the expression of SMCT2, as mentioned below.

The energy requirement of cells increases during cell dividing and proliferation. Highly proliferating tumor cells switch from oxidative phosphorylation to

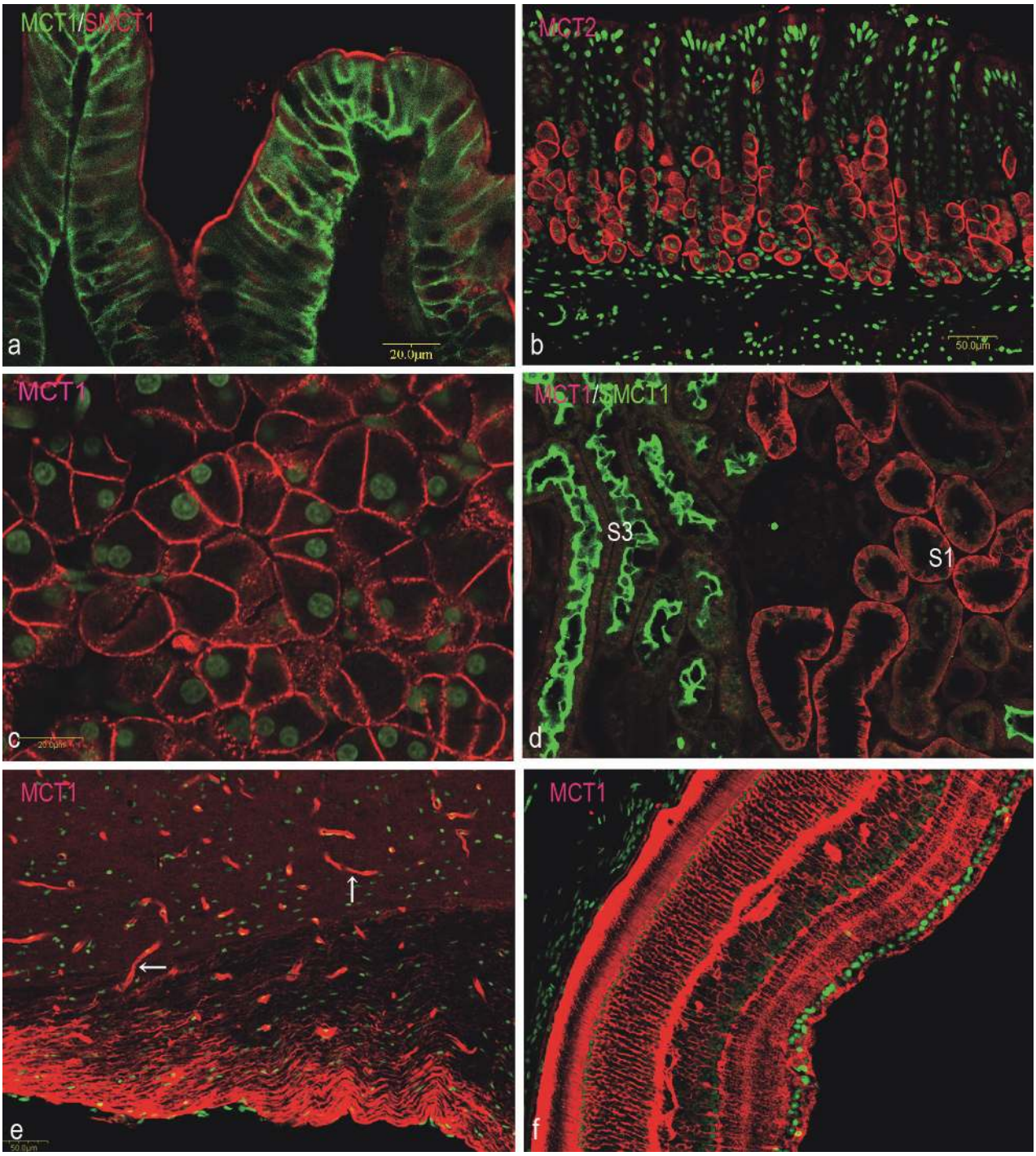

Fig. 2 Immunostaining of MCT1, MCT2, and SMCT1 in mouse and rat tissues. In the mouse cecum (a), MCT1 immunoreactivity is localized along the basolateral membrane while SMCT1 is restricted to the brush border of epithelial cells. Parietal cells of the stomach (b) are unique for an intense expression of MCT2 in the gastrointestinal tract. The mouse pancreas displays a selective expression of MCT1 along the basolateral membrane of acinar cells (c). Double staining of MCT1 (red) and SMCT1 (green) in the rat kidney shows a differential distribution of MCT1 in the S1 segment and SMCT1 in S3 segment, respectively (d). In the mouse brain (e), the immunoreactivity of MCT1 is seen in the blood vessels (arrows) and within the neuropils. The retina is rich in MCT1 expression (f); the apical side of the pigmented epithelium, Müller cell's processes, and vascular elements are intensely immunolabeled. The nuclei are stained green with SYTOX (b, c, e, f).

glycolysis for energy production, referred to as the Warburg effect. Similar to tumor cells, normally proliferating cells may prefer to use monocarboxylates rather than glucose. In the small intestine, crypts largely consisting of proliferating and immature cells express MCT1 more intensely, especially the dividing cells (144). A similarly intensified expression of MCT1 is found at the proliferating zone of the stomach and at the basal layer of the esophagus.

Reportedly, MCT2 in the digestive tract of hamsters is expressed mainly in the stomach, where parietal cells are the cellular source of production (Fig. 2b) and the expression levels of MCT2 are low in the other parts of the gut (38, 39). Our quantitative PCR analysis confirmed the restricted distribution of MCT2 in the glandular stomach (146). Immunostaining detects an intense localization of MCT2 along the basolateral membrane of parietal cells, in accordance with an *in situ* hybridization study at the mRNA level. This finding, together with a lack of GLUT1 expression in parietal cells, suggests that parietal cells with high energy demand use monocarboxylaye as a main energy substrate.

A Western blot analysis of the human small and large intestine (44) has described the intense expression of MCT4 and MCT5 as well as MCT1 in the distal colon, with less intense expressions of all three subtypes in the proximal colon and ileum. In the immunoblotting study by the same research group, MCT3 was present at a relatively low level compared with other isoforms, but significant immunoreactive bands for MCT3 were detected in the human small intestine and colon. A Northern blot analysis of the human small and large intestine has demonstrated an intense expression of MCT8 (XPCT) comparable to the expression of MCT1 (118). However, our *in situ* hybridization study failed to obtain any visible signals for MCT3, MCT4, MCT5, or MCT8 throughout the digestive tract of the mouse. Our survey in the mouse indicates that MCT1 is a predominant subtype expressed in the gastrointestinal tract, except for a rich amount of MCT2 in the glandular stomach. The epithelial cells in the mouse small intestine express faintly immunoreactive MCT4 along the basolateral membrane. These findings are supported by our real-time PCR analysis in the mouse (146). Cell types other than epithelial cells expressing MCTs include smooth muscle cells (MCT1) throughout the gut (Fig. 5d) and some endocrine cells (MCT1), the latter being abundant in the glandular stomach.

The liver parenchyma preferentially expresses MCT2 rather than MCT1 in the hamster (38, 39); hepatocytes possess MCT2 along cell membrane, while no immunoreactivity for MCT1 is detectable in the liver. In adult mice, hepatocytes express MCT1 at a considerable level, with a predominant immunolabeling of the plasma membrane on the sinusoidal side. In neonates, the immunoreactivity for MCT1 becomes more intense in the hepatocytes of mice and rats, indicating the involvement of monocaboxylates in the energy supply during suckling stages (144). However, MCT2 immunoreactivity is low or undetectable in the liver of adult mice and rats, in contrast to the reported positive finding in the hamster liver, showing a marked species difference. MCT2 has been indicated to exhibit a considerable species differences in tissue expression, unlike MCT1, MCT3, and MCT4. SCFAs stimulate pancreatic exocrine secretion (21, 58), possibly due to a direct effect on acinar cells. Zhao *et al.* (161) have detected MCT1 immunoreactivity in the cell membrane of pancreatic acinar cells in the rat but not in islet cells. A similar immunoreactivity for MCT1 is reproduced in the pancreas of mice (our unpublished data); the immunoreactivity is restricted to the basolateral membrane of acinar cells, without any signals in the luminal membrane (Fig. 2c).

SMCTs: counterpart of MCTs in the intestine

SMCT1 has been identified as a Na^+-coupled cotransporter for SCFAs, lactate, and nicotinate (19, 96). Affinities of SMCT1 for its major substrates lie in the following order: butyrate > propionate > lactate >> acetate (96). The down-regulation of SMCT1 expression in a variety of cancers including colon cancer suggests its tumor suppressive function; this can partially explain the anti-tumourigenic effects of butyrate (37, 105). Transcripts of SMCT1 in mice are found throughout the large intestine, with the most intense expression in the distal colon, followed by the cecum. Another site showing an intense expression of SMCT1 mRNA is the ileum, where the expression is restricted to the distal segments (141) (Fig. 3). Through immunohistochemical studies (66, 141), we have revealed the selective localization of SMCT1 on the brush border in the villi of the terminal ileum and crypts of the large intestine in mice. In the cecum (Fig. 2a), intense immunoreactivity for SMCT1 is found in the brush border at the middle part of the crypts, while the MCT1 antibody labels the basolateral membrane of epithelial cells extensively in both the surface epithelium and crypts. Crypt cells show a more or less dual expression of the two transporters, but the surface epithelium tends to be devoid of SMCT1 immunoreactivity.

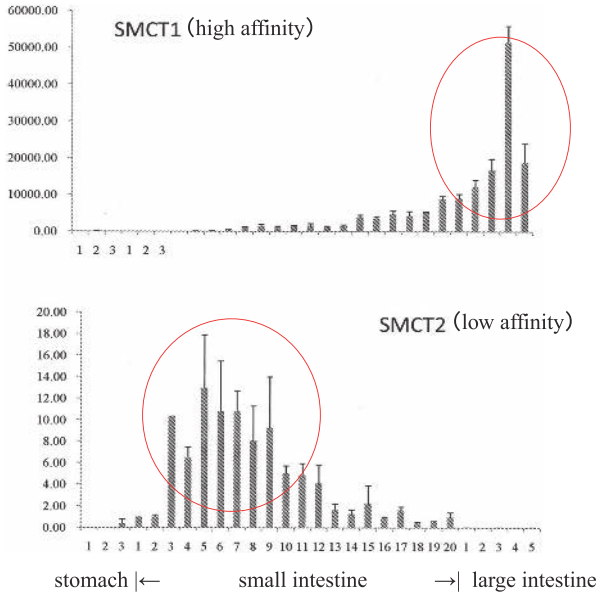

Fig. 3 The gene expression of SMCT1 and SMCT2 in the gastrointestinal tract of mice analysed by a real-time PCR method (146). The stomach, small intestine, and large intestine were segmented into three, twenty, and five sections, respectively.

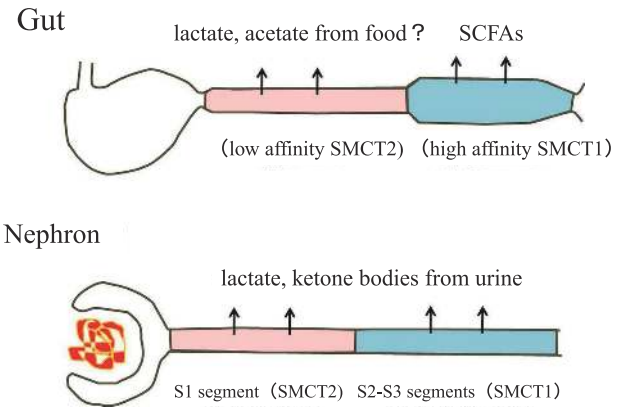

Fig. 4 A scheme showing the differential distribution of SMCT1 and SMCT2 in the gut and urinary tubules.

Thus, the distinct coexistence of the two transporters at the cellular level is not found throughout the epithelium of the large intestine.

The higher expression of SMCT1 in the large intestine should be related to the active production of SCFAs produced by microflola. MCT (MCT1) and SMCT1 in the large intestine are coordinated in the uptake and transfer of SCFAs, which are used as a fuel for gut epithelial cells and the whole body. It is worth noting that the Na^+: substrate stoichiometry is 4 : 1, namely the epithelium absorbs an amount of water with Na^+ and SCFAs. This is helpful for the hardening of feces and reducing the volume. Accordingly, the expression levels of SMCT1 in the distal half of the colo-rectum are higher compared with those in the proximal half. In the small intestine, the intense expression of SMCT1 is restricted to the terminal ileum in mice. It remains unknown whether the restricted expression reflects the production of SCFAs *in loco* or the backflow of SCFAs from the cecum.

The intestine and kidney express a low-affinity SMCT (SMCT2) with a sequence homology of 59% identity in the amino acid sequence against SMCT1. The transport by SMCT2 seems to be electroneutral with a Na^+: monocarboxylate stoichiometry of 1 : 1. Transcripts of SMCT2 are detectable in the small intestine of mice but not in the large intestine (134). Semi-quantitative RT-PCR in mice also reveals a predominant expression in the small intestine, with the highest expression in the proximal one third (146) (Fig. 3). In a sense, the expression patterns of SMCT1 and SMCT2 along the long axis of the gut are complementary; SMCT2 and SMCT1 predominate in the small intestine and large intestine, respectively. Since SMCT2 has a main productive site in the proximal small intestine with minimal bacterial fermentation, the function of SMCT2 may be to absorb acetate, lactate, and other monocarboxylates from drinks and diets such as milk and fermented products (134). The transport capacity of SMCT2 is higher compared with that of SMCT1. Thus, low affinity/high capacity SMCT is arranged in the small intestine, while the high affinity SMCT is present in the large intestine (Fig. 4). In the rat intestine, SMCT1 is particularly expressed in the brush border of the duodenum, with MCT1 and MCT4 in the basolateral membrane (73). This expression profile is useful for the rapid absorption of acetate and stimulation of duodenal or pancreatic HCO_3^- secretion.

The kidney

The kidney is active in the uptake of monocarboxylates from the glomerular filtrate against energy loss and for gluconeogenesis; thus, the reabsorption rate of lactate in the mammalian kidney is very high (>95%). In 2004, SMCT1 was shown to be responsible for the active Na^+-coupled reabsorption of lactate in the mammalian kidney (19, 46). Northern blot analyses of the mouse SMCT1 have demonstrated its abundant but selective expression in the kidney besides the intestine (46, 47). Immunohistochemically, SMCT1 in the mouse and rat kidney is largely restricted to the cortex and the outer stripe

of the outer medulla, in agreement with the expression pattern of SMCT1 mRNA. Its expression is not found in S1 segment of the proximal tubules but starts to appear in S2 up to S3 (S3: the proximal straight tubules) (141, 159) (Fig. 2d). Intense SMCT1 immunoreactivity is localized at the brush border but not in the other parts of cells. Genetic deletion of SMCT1 results in a significant increase in urinary lactate levels (32, 147). Another type of SMCT (SMCT2) mediates the sodium-dependent transport of various monocarboxylates with low affinity. Renal SMCT2 is localized at the brush border from the beginning of proximal tubules with decreasing expression toward the S3 segment (48). Thus, the proximal convoluted tubules respectively provide low and high affinity transporters in the upper and lower proximal tubules, as does the intestine (Fig. 4). The arrangement of two SMCTs with differing affinities to substrates and differing capacities in the kidney is comparable to that of Na^+-dependent glucose transporter SGLTs. In the kidney of mammals—including humans, the low-affinity/high-capacity Na^+-glucose cotransporter (SGLT2) mediates the bulk of glucose reabsorption in the early part of the proximal convoluted tubules (S1 or S1/S2), whereas the high-affinity/low-capacity SGLT1 in the distal part of proximal tubules (S3) reduces glucose in the urine to very low levels (156). Therefore, it can be concluded that the urinary tubules reabsorb the two major energy substrates using the Na^+-coupled uptaking system.

As for the MCT family, early immunohistochemical studies have reported discrepant cellular localization of MCT1 and MCT2 in the mammalian kidney. MCT1 in the hamster kidney has been reported by the same research group to exist in both proximal and distal convoluted tubules (38) or proximal convoluted tubules (39). The reported localization of MCT2 in the kidney is also controversial among researchers—in this case—between animal species. MCT2 immunoreactivity in the hamster kidney is detected in the collecting ducts of the inner medulla (39), while in the rat kidney it exists in the medullary thick ascending limbs of Henle's loop, with a weak immunoreactivity in the collecting ducts (27). Among the other MCT subtypes, the existence of MCT5, MCT6, MCT8, MCT9, MCT10 (or TAT1) and MCT12 has been shown in the kidney (33, 55), but their cellular localization and expression intensities remain unknown.

We, therefore, tried to confirm the expression intensities and sites of MCTs in mice (159). First, we compared mRNA expressions of various MCT subtypes (MCT1 to MCT12) by an *in situ* hybridization technique (159); four subtypes (MCT2, MCT5, MCT8, and MCT9) displayed comparatively intense signals of mRNA, with the most intense expression being for MCT2 and MCT8. Weak but significant signals were detected for MCT1, MCT10, and MCT12. These expression intensities largely agreed with a genome-wide gene expression analysis by Nowik *et al.* (102). Immunohistochemically, MCT1 is exclusive to the most proximal part of the proximal convoluted tubules (S1) in the mouse kidney. The staining profiles of MCT1 in the rat kidney are essentially identical to those in the mouse, but the MCT1 immunoreactivity in S1 is more intense than that in the mouse (159) (Fig. 2d). In both animals, the basal infoldings of epithelial cells in the S1 segment are intensely labeled with the MCT1 antibody. MCT2 immunoreactivity in the kidney predominates in the inner stripe of the outer medulla in both animals, corresponding to the thick ascending limbs of Henle's loop, where again the basal infoldings are immunolabeled. The MCT2 immunoreactivity further extends to the distal convoluted tubules and collecting ducts in the rat. MCT1 and MCT2 may act as an efflux pathway of the reabsored lactate toward the blood; they also may be involved in taking up lactate or pyruvate from the circulation for gluconeogenesis and β-oxidation. MCT8, an efficient transporter for thyroid hormones, is abundantly expressed throughout the proximal tubule on the basolateral side of epithelial cells in mice (4).

The brain

Neurons require lactate as an energy substrate under activated conditions, and this lactate is supplied largely by adjacent glial cells, being representative of the metabolic coupling. Moreover, lactate and ketone bodies serve as predominant energy sources—rather than glucose—for the brain during the neonatal period. Although the brains of mature animals rely increasingly on glucose for energy, monocarboxylates represent alternative energy substrates under particular conditions of a decreased utility of glucose, including diabetes and prolonged starvation. For the transfer of monocarboxylates, especially lactate, the brain tissue utilizes at least three different MCTs: MCT1, MCT2, and MCT4 (130). However, findings concerning the expression sites of MCTs in the brain fail to coincide among immunohistochemical studies using the antibodies from different sources, various types of sections (frozen, vibratome, paraffin, free-floating or enzyme-pretreated), and different animal species (111). The regional differences

in immunolabeling in the brain, except for MCT1 in the vascular endothelium, also make it difficult for a general consensus concerning their cellular localization. Furthermore, regulation of MCT expression occurs at the translational level, causing another discrepancy in the distribution of MCT mRNA and protein in the brain (111).

Many studies have detected a consistent immunoreactivity of MCT1 in the vascular endothelium, ependymal cells, and superficial glial limiting membrane (40, 42, 112), as we confirmed (Fig. 2e). There is also accordance that the expression of MCT1 is more intense in suckling neonates than adult rodents, especially in the vascular endothelium (40, 106). This developmental change can account for the metabolic adaptations occurring in the brain during the suckling period, when ketone bodies derived from milk are major energy sources (101, 106, 154). In adult rats, a ketogenic diet has been found to induce brain MCT1 expression (85). MCT1 is also the primary isoform of glia, whereas MCT2 has a more neuronal localization (112). Long cytoplasmic processes, which radiate off the brain surface or connect with the superficial glial limiting membrane, are intensely immunolabeled for MCT1 in some regions of the brain, suggesting the MCT1 expression in astrocytes. Astrocyte foot processes adjacent to capillaries (the perivascular glial limiting membrane) display an MCT1 immunoreactivity (18, 84). It is worth noting that MCT1-immunoreactive astrocytes including cell bodies are restricted to some areas of the adult brain (40, 57). Recently, it has become commonly accepted that a main MCT subtype expressed by astrocytes is MCT4. An analysis of transgenic mice carrying the fluorescent reporter has indicated that MCT1, MCT2, and MCT4 are predominantly expressed, respectively, in oligodendrocytes, neurons, and astrocytes (83). Lactate has been proposed as a fuel for proper myelination by oligodendrocytes (126). Disrupting the transfer of lactate from oligodendrocytes to axons through MCT1 causes axonal damage (83).

There are more discrepant findings for immunoreactive MCT2 in the literature. Several studies in rodents have demonstrated that MCT2 is predominantly expressed in ependymal cells and astrocytic processes, including the perivascular and surface glial limiting membrane in rats (42, 120). On the other hand, the murine brain shows a broader distribution of MCT2 in choroid plexuses, blood vessels, and some neurons; subsets of astrocytes (S100 protein or GFAP-positive) express MCT1 while neuronal processes instead contain MCT2 in the adult mouse brain (18, 110, 112). Although MCT2 is strongly expressed by astrocytes in the rat brain (42, 120), astrocytes in the mouse brain do not significantly express MCT2 (112). Nevertheless, *in situ* hybridization analyses of the mouse brain have characterized the neuronal expression of MCT2 in the cerebral cortex, hippocampus, and cerebellar cortex, including the Purkinje cell layer (81, 106, 154). Another immunostaining study of the rat brain for MCT2 has detected positive reactivities of neurons in the hippocampus, thalamus, olfactory bulb, and cerebellum (7, 110, 120). In accordance with this, an analysis of primary cultured cells gave evidence of a cell-specific localization of MCTs with an enrichment of MCT1 in astrocytes and of MCT2 in neurons (11), though another similar analysis of the rat brain has shown that all cultured cortical astrocytes express MCT2 as well as MCT1 (57). Accumulating evidence indicates that part of the MCT2 expression is associated with synapses. Some studies have focused on the MCT2 localization along the post-synaptic density of excitatory synapses (7, 8). The major role in the post-synaptic density has been related to the maintenance of energy substrate availability upon excitation of post-synaptic cells (8). Recent studies suggest that MCT2 is critical in long-term memory, as the expression of MCT2 decreased in a rat model of Alzheimer's disease (89, 137).

MCT4 is present in astrocytes and involved in lactate shuttling from glia to neurons expressing MCT2. MCT4 is preferentially expressed in astrocytes including the astrocytic processes that ensheath the synapse and blood vessels (7, 120) although the immunolabeling is also restricted to some regions and not so intense in reaction.

It has been described how SMCTs are expressed in the brain and retina (93). The expression pattern of SMCT1 in the brain overlaps with that of MCT2, a possible neuron-specific MCT, while the low-affinity SMCT2 is expressed specifically in astrocytes (93). A similar expression pattern of SMCTs is also seen in the retina (93).

The peripheral nervous system

Many studies in the brain have suggested that MCTs expressed in the vascular endothelium, glia, and other elements are critically important for supplying lactate and ketone bodies to neurons and axons. In contrast, a few studies have focused on the pathway for energy supply to the peripheral nerves. According to recent studies, peripheral nerves also consume lactate—particularly during regeneration when they require greater energy (97). Peripheral nerves ex-

press GLUT1 in both endoneurial blood vessels and the perineurium (34, 41, 99) and utilize glucose as a major energy substrate, as dose the brain. However, under conditions of a reduced utilization of glucose, the brain must depend upon monocarboxylates such as ketone bodies and lactate, being accompanied by an elevated expression of MCT1 in the blood-brain barrier. In the peripheral nerves, MCT1 immunoreactivity is found in the perineurial sheath and colocalized with GLUT1 (Fig. 5a, b), while the endoneurial blood vessels express GLUT1 only (142). Ultrastructually, the MCT1 and GLUT1 immunoreactivities in the thick perineurium show an intensity gradient decreasing towards the innermost layer. In neonates, the MCT1 immunoreactivity in the perineurium is intense, while the GLUT1 immunoreactivity is faint or absent (142). These findings suggest that peripheral nerves depend on monocarboxylates as one of the major energy sources and that MCT1 in the perineurium is responsible for the supply of monocarboxylates to nerve fibers and Schwann cells, possibly from outside the perineurium. If surrounding non-vascular tissues directly supply the monocarboxylates, they may be lactate produced by glycolysis in skeletal muscles or ketone bodies released from adipose tissues, which are rich around nerve fasciculi.

The localization of GLUT1 in peripheral nerve bundles indicates the dual supply of glucose to individual nerve fibers via both endoneurial blood vessels and the perineurium. The endoneurial capillaries have been recognized a main route for the uni-directional transport of glucose by some researchers (121). This notion, however, is refused by the following morphological findings. First, only a small proportion of the endoneurial blood vessels are positive for GLUT1 in the adult human nerves, in contrast to the intense and consistent immunoreactivity of GLUT1 in the perineurium (99). Second, nerve fasciculi of smaller diameters do not contain any GLUT1-expressing blood vessels in the endoneurial space. On the other hand, the presence of MCT1 with a limited localization to the perineurium suggests the supply of monocarboxylates to nerve fibers and Schwann cells only via the trans-perineurial route. The dynamic switching of energy transporters from MCT1 to GLUT1 is also seen in the perineurium of peripheral nerves during developmental stages. Stark *et al.* (135) have reported the lack of GLUT1 in the perineurium of peripheral nerves in P0 and P14 rats, in contrast to its intense expression in adult rats. In accordance with this, nerves in murine neonates at P5 and P10 were respectively negative and faintly immunoreactive for GLUT1 but intensely positive for MCT1 in these stages (142).

Lactate is proposed as a fuel source for proper myelination in the central nervous system (126). Disrupting the transfer of lactate from oligodendrocytes to axons through MCT1 causes axonal damage (83). In the peripheral nervous system, myelinated Schwann cells express MCT1 and MCT4 (26, 97); MCT1 is present in regions of noncompacted myelin such as Schmidt-Lanterman incisures and paranodal regions, both of which provide special routes for moving metabolites. Using sensory neurons cocultured with Schwann cells, Domènech-Estévez E *et al.* (26) have observed a change of myelination with a down-regulation of Schwann cell-specific MCT1, proposing that MCTs mediate axon-glial metabolic interactions. Only a 50% decline in MCT1 in MCT1-heterozygous null mice dramatically delays the regeneration of peripheral nerves including remyelination by Schwann cells (97).

The retina and other ocular tissues

In the retina, lactate is one of the major nutrients for rods and cones of photoreceptor cells. The retina produces more lactate than any other tissue, and the lactate production is particularly prominent in the outer retina containing numerous photoreceptor cells. It is generally believed that neurotransmission in the inner retina is largely supported by glycolysis, whereas phototransduction in the outer retina is supported by oxidative processes. In studies using isolated photoreceptor cells, lactate was a better substrate for the mitochondrial oxidative metabolism than glucose (115). The first report on MCT molecules in the retina has demonstrated the unique expression of MCT3 (REMP: retinal epithelial membrane protein) in the retinal pigment epithelium of the chick and rat (108, 160). There is a consensus that MCT3 and MCT1 are localized respectively in the basal membrane and the apical membrane of retinal pigment epithelium, effecting the transfer of H^+-lactate across the pigmented epithelium between the outer retina and the blood vessels (6, 17, 43, 108). In some conditions, an excess of lactate may be extruded through the pigment epithelium by the combination of MCT1 and MCT3.

Photoreceptor cells continuously shed the periphery of their outer segment, which is then phagocytosed by the adjacent pigment epithelial cells. The digestion of the outer segment is indispensable for the recycling of rhodopsin and also provides a rich energy source as fatty acids. The fatty acids are converted to β-hydroxybutyrate, which is transported

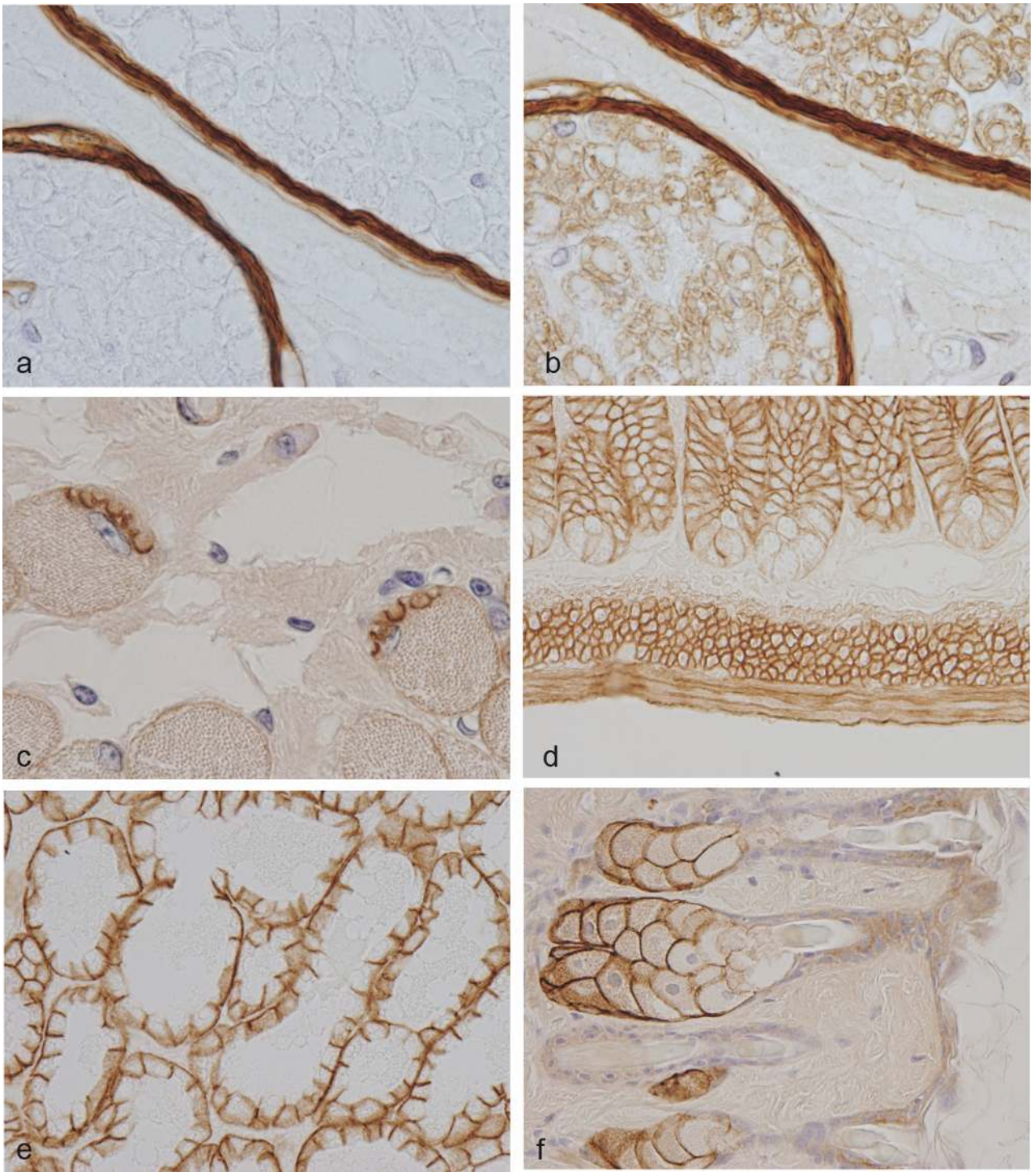

Fig. 5 MCT1 immunoreactivities visualized by the avidin-biotin complex method. In Fig. 5a and b, two serial sections from the mouse sciatic nerve are stained for GLUT1 (a) and MCT1 (b). The stainability of the perineurium is almost identical between GLUT1 and MCT1. Immunostaining of MCT1 in the skeletal muscle displays peculiar structures at the periphery of skeletal muscle fibers, possibly corresponding to the neuromuscular junction (c). In the small intestine (d), the cell membrane of smooth muscle fibers and of crypt epithelium are immunoreactive for MCT1. Note the lack of MCT1 immunoreactivity in Paneth cells at the bottom of crypts. The mammary gland is rich in MCT1 immunoreactivity; the basolateral membrane of secretory portion is selectively immunolabeled (e). In sebaceous glands (f), an intense labeling for MCT1 is seen along the entire length of the plasma membrane of alveolar cells.

across the apical membrane of the pigment epithelium via MCT1 and enters photoreceptor cells via MCT1 and MCT7, indicating metabolic shuttling via MCTs (1). Philp *et al.* (109) have reported that basigin (CD147) is necessary for the targeting of MCTs to the plasma membrane and is essential for normal retinal function. Immunohistochemically, basigin is condensed in both the apical and basal plasma membrane of the pigment epithelium. GLUT1 expressed in both the apical and basolateral membranes can transport glucose across the retinal pigment epithelium to the outer retina. The dense arrangement of the two nutrient transporters in the pigment epithelium is related to the lack of a vascular system in the outer retina, and support the notion that the pigment epithelium functions as blood-retinal barrier.

Beside the pigmented epithelium, the retinal MCT1 is broadly distributed in the photoreceptor inner segments, the outer nuclear layer, and the inner/outer plexiform layer (43) (Fig. 2f). The wide distribution of MCT1 in the retina is comparable with that of GLUT1 (6, 43). At cellular levels, immunostaining for MCT1 labels Müller cells and the vascular endothelium in the retina of the adult rat, the latter being the primary site in neonates (6, 17). The intense localization of MCT1 in Müller cells supports the concept of metabolic coupling (i.e. exchange of lactate) between Müller cells and neurons/photoreceptors. *In vitro* studies have identified the Müller cells as the source of the lactate utilized by photoreceptor cells (115). This finding is consistent with the idea that Müller cells do contain low numbers of mitochondria (rich in glycogen particles) and release lactate as energy metabolites for photoreceptor cells (115). This would be analogous to the transport of lactate between astroglia/oligodendroglia and neurons in the brain. According to Gerhart *et al.* (43), rat MCT2 is present in all layers between the inner and outer limiting membranes, where only glial cells (Müller cells) are the cellular source. However, this finding is not supported by other immunohistochemical studies in the rat retina (6, 17). MCT4 immunoreactivity has been reported to be localized from the outer plexiform layer to inner plexiform layer in 28-day-old rats (6). In another study by Chidlow *et al.* (17), MCT4 immunoreactivity is detectable in Müller cells and the two plexiform layers in the rat, but its intensity is faint as compared with MCT1 and MCT3 in the retina.

Our survey by an *in situ* hybridization technique detected intense signals of MCT1 and MCT3 among the four major MCT subtypes in the mouse retina. Both signals appear intense in the retinal pigment epithelium, with weak and diffuse signals in the outer and inner granular layers. Immunohistochemistry for MCT1 yielded the same staining patterns in the retina of the mouse and rat. Immunoreactivity for MCT1 is intense in the apical microvillous region of the pigment epithelium, the inner segment of photoreceptor cells, and the vascular endothelium of the inner retina (Fig. 2f). Outer and inner plexiform layers, especially the outer one, show an intense immunoreactivity for MCT1. Müller cell's processes running perpendicularly in the outer granular layer are immunolabeled together with the inner limiting membranes of Müller cells. A weaker but significant immunoreactivity of MCT4 is also present in Müller cells.

Beyond the retina, the epithelium of the iris and non-pigmented epithelium of the ciliary body have been reported to be immunoreactive to MCT1 and MCT2 antibodies, whereas no immunoreactivity for MCT3 and MCT4 is detectable in either tissue (17). Our immunostaining confirmed that the posterior epithelium of the iris and inner non-pigment epithelium of the ciliary body are immunoreactive for MCT1, mainly at the lateral and apical plasma membrane, though the immunoreactivity in the ciliary process is more intense than that in the iris. In contrast to the pigment epithelium of the retina, the pigment epithelia of the iris and ciliary body are devoid of immunoreactivity for MCT1. The corneal endothelium does contain at least some MCT1 immunoreactivity in the mouse. The harderian gland, especially developed in rodents, is a lipid-secreting lacrimal gland behind the ocular bulb and intensely expresses MCT1 along the basolateral plasma membrane (143). This may reflect a great demand for monocarboxylates as materials for the production of lipids.

The muscle

Monocarboxylate transporters would have their most obvious value in the skeletal muscle, which produces large quantities of lactate during strenuous exercise. Skeletal muscles are classified into three types depending on their myoglobulin and mitochondrial content: slow oxidative (SO), fast oxidative/glycolytic (FOG, intermediate type), and fast glycolytic fibers (FG). The membrane-bound expression of MCT1 in the skeletal muscles was first reported in SO and FOG fibers of the hamster (38, 39). In the human muscle, MCT1 expression is also high in type I oxydative fibers (SO), with modest to low expression in type IIa (FOG) fibers (30, 103, 104). The restricted expression of MCT1 to oxidative

muscle fibers suggests that MCT1 plays a role in lactate uptake rather than in lactate excretion. In contrast, MCT4 is abundant in FG and FOG fibers in the rat (60, 158) and in type IIx/b fibers in the human (30, 104, 113). Thus, the predominant role of low-affinity MCT4 is cellular lactate extrusion from glycolytic muscle fibers (25, 158). MCT2, a high-affinity pyruvate transporter, is not abundant in the skeletal muscle of rats (60, 69) and is predominantly found in oxidative fibers (SO) (30, 39). At present, there is an accepted idea that MCT1 and MCT4 are expressed respectively in oxidative (SO and FOG) fibers and glycolytic (FG and FOG) fibers, with MCT2 being a minor transporter. According to Hashimoto *et al.* (60), the identification of muscle types expressing MCTs may be more or less influenced by the fixation conditions (fixed or unfixed), specificity of the antibodies, and animal species examined (60).

The muscle fiber type-specific arrangement of MCTs supports the idea that lactate is both a major fuel for oxidative muscle fibers and a major metabolic end product in glycolytic muscles. The production of lactate by one tissue and use by another is representative of the lactate shuttle: the mosaic distribution pattern of oxidative and glycolytic fibers is favorable for the exchange of lactate. Exercise may produce greater increases in MCT1 than MCT4 expression (148). On the other hand, denervation for 3 weeks brings about a significant decrease in the expression of MCT4 as well as MCT1 (158). Moreover, skeletal muscle regeneration down-regulates MCT1 expression, being consistent with the switching of skeletal muscle to a more anaerobic energy profile during proliferation (157). Interestingly, GLUT4, a muscular type of GLUT, is enriched in oxidative fibers (91), and the co-expression of GLUT4 and MCT1 in this type of fiber (60) indicates that oxidative fibers are provided with uptake systems of two major energy sources.

Our immunostaining for MCT1 visualized a special structure at the neuromuscular junction in mice (Fig. 5c). The linear positive reaction may correspond to the postsynaptic membrane on the skeletal muscle. Released acetylcholine is degraded to form choline and acetate, the former being incorporated by presynaptic choline transporter 1 (CHT1) for recycling. Although the mechanism for the removal of acetate from the synaptic cleft is unknown, the MCT1 expressed in the postsynaptic membrane may influence the removal of acetate.

The predominant energy substrate for the heart in a normal metabolism may be monocarboxylates and/or fatty acids, whereas the glucose metabolism becomes more prevalent during insulin stimulation and ischemia (23). GLUT1 is primarily responsible for basal glucose uptake but has a low expression level in the heart. GLUT4 is a major cardiac transporter of glucose and translocates from the sarcoplasma to myocyte surface membranes, thus resulting in an increase in the transporter activity in response to various stimuli, including insulin and ischemia (23, 132). Our *in situ* hybridization analysis in the mouse showed that the expression of MCT1 predominates instead of GLUTs in the heart under normal conditions. Recently, pluripotent stem cell-derived cardiomyocytes were shown to prefer lactate to glucose for cell survival (149). An earlier immunostaining of MCT1 has demonstrated a condensed localization of MCT1 in the plasma membrane of cardiocytes, including the intercalated disks in normal conditions (38, 70) (Fig. 6a). Immunoblotting of the human heart has shown the expression of MCT4 as well as MCT1, although no signals of MCT4 can be detected in the rat heart (158). In the rat, MCT4 is expressed in the neonatal heart, which is more glycolytic in its energy metabolism than the adult heart (62).

Essentially all smooth muscle fibers express MCT1, including the gastrointestinal tract (Fig. 5d), female genital tract, and blood vessels. The vascular smooth muscle in the ocular tissues has been reported to be immunoreactive for the MCT4 antibody (17). MCTs in the smooth muscle have not attracted the attention of researchers as compared with MCTs in the skeletal muscle. Experimental evidence in large and small arteries has demonstrated that lactate at a physiological concentration causes vasodilation and increases blood flow *in vitro* and *in vivo*. Furthermore, it has been proposed that lactate released from the retinal neuronal and glial elements serves an essential signal for modulating retinal microvascular tone via the stimulation of NO synthase and subsequent activation of guanylyl cyclase (64). A blockade of MCT by use of a transporter inhibitor (CHC) nearly abolished lactate-induced vasodilation, suggesting the involvement of MCT and/or its function as a "lactormone" (64). The expression of MCT1 has been examined in a cell line of human aortic smooth muscle cell (HASMC); a pressure stress up-regulated MCT1 expression on the plasma membrane of HASMC but not cytoplasmic MCT1 (103).

Lipogenetic tissues

Human adipocytes are known to express MCT1, MCT2, and MCT4, and the expression of MCT1 and MCT4 is stimulated by hypoxia (107). Rat adi-

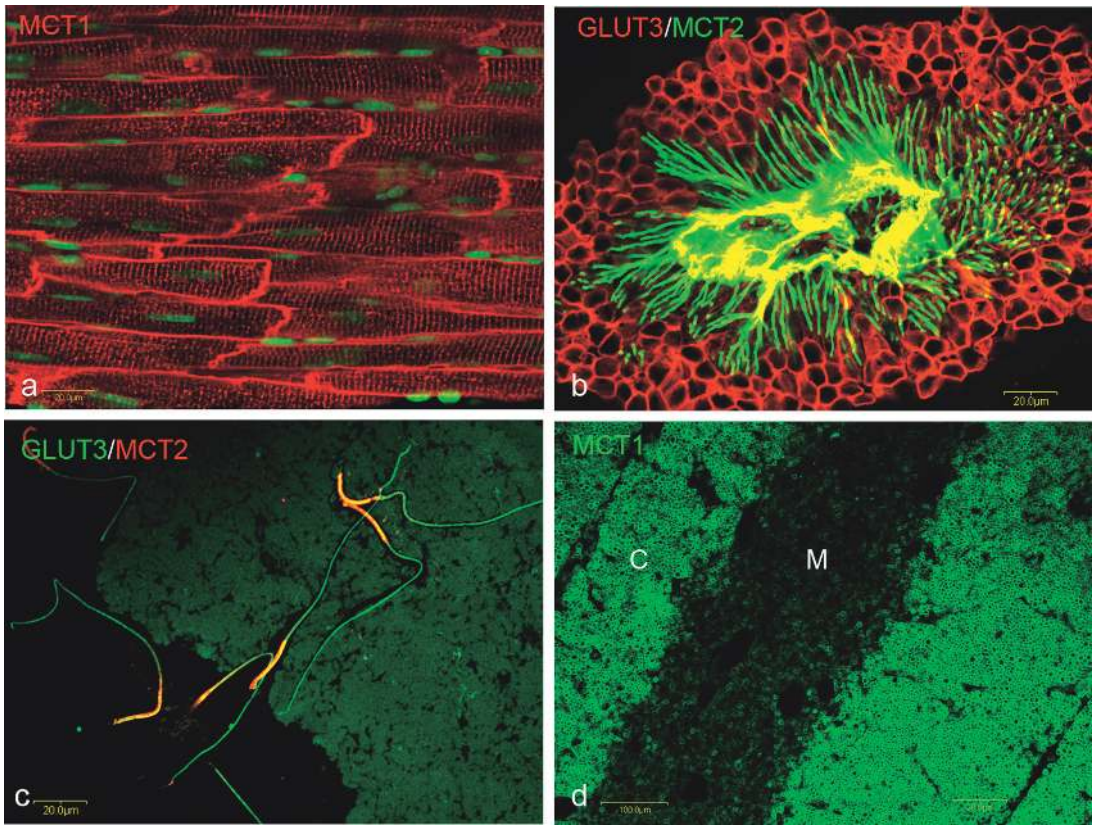

Fig. 6 Immunostaining of MCTs and GLUT3 in mouse tissues. The plasma membrane of cardiocytes including intercalated discs are heavily immunolabeled for MCT1 (a). Nuclei are stained green with SYTOX. In the testis, GLUT3 is the major GLUT member which is expressed in germ cells (spermatocyes and round spermatids) (red in Fig. 6b) and also tails of elongated spermatids and sperm. Testicular MCT2 expression (green in Fig. 6b) is restricted to elongated spermatids. In matured sperm (c), GLUT3 is expressed along the entire length of sperm tail (green in Fig. 6c) while signals of MCT2 (red) are restricted to the middle piece. The thymus expresses MCT1 only in the cortex (C) (d). M: medulla

pocytes express MCT1 on the plasma membrane; the expression level markedly decreases in a diabetic condition (51). It is generally considered that MCT1 in adipose cells is related to lactate production and whole body lactate metabolism. On the other hand, MCTs in some lipogenetic tissues are involved in the production of steroids and medium/long-chain fatty acids (143). Acetate, ketone bodies, and other monocarboxylates can serve as materials for lipogenesis. MCT1 in adults under normal conditions may be useful for the uptake of these materials for lipogenesis (125), as supported by cases of sebaceous glands and mammary glands (143) (Fig. 5e, f).

Brown adipose tissue (BAT) as well as white adipose cells express MCT1, the expression level being much higher than white adipose tissue (67). Because of the extremely rich existence of mitochondria in BAT, MCT1 in BAT may play a leading role in the uptake of ketone bodies and other monocarboxylates but not their release into circulation. Among GLUT1–GLUT5, GLUT4 is a predominant type expressed in BAT, as reported previously (127). The signals for MCT1 are more intense in fetal and early neonatal stages than in adults, with a similar tendency for GLUT4 (67). MCT1 in BAT may be involved in the uptake of the monocarboxylates as energy sources and/or substrates of thermogenesis. The abundant supply of ketone bodies in neonates is supplemented by ketone bodies derived from milk lipid. The expression level in the BAT of adult rodents increases under cold conditions (67) and exercise (24), although the increased activity could be related to the stimulated uptake of lactate to avoid systemic acidosis.

The male genital tract

The testis and sperm abundantly express some types of MCTs. The testicular tissues of rats contain immunoreactivities for MCT1, MCT2, and MCT4 (10), and a Northern blotting analysis of rat tissues has

detected the most abundant expression of MCT2 in the testis (69). The first report by Galcia *et al.* (38) documented that the MCT1 antibody labeled the heads of maturing sperm in the seminiferous tubule and of the sperm in the caput of the ductus epididymis in hamsters. The MCT1 immunoreactivity of the sperm disappears in the cauda epididymis; instead, the stereocilia of the epididymal epithelium are strongly immunopositive for MCT1. In contrast to the reported localization of MCT1 at the head of maturing/matured sperm in the hamster, our immunostaining of the murine testis failed to confirm the selective localization of MCT1 at the sperm heads. Although the expression profiles of MCT1 and MCT2 in the seminiferous epithelium of the mouse and rat are controversial among researchers, MCT1 must be present at the cell surface of germ cells at different stages, especially spermatogonia (45, 80). An intense mRNA expression of MCT2 is detected in round spermatids, and MCT2 immunoreactivity is localized in elongated spermatids and sperm (80) (Fig. 6b). Among GLUTs, GLUT3 is predominant in the seminiferous epithelium, but spermatogonia are lacking in GLUT3. The sufficient expression of GLUT3 in the spermatocytes is achieved during the subsequent stages of spermatogenesis, thus providing mature sperm with GLUT3 to utilize glucose for successful fertilization (131, 151) (Fig. 6b, c). Spermatogonia in the mouse and rat testis are provided with only MCT1, simply indicating that the main energy sources of germ cells change from monocarboxylates to glucose or other hexoses (80).

MCT2 is expressed exclusively on sperm tails in both the seminiferous tubule and distal epididymis (39). The predominant localization of MCT2 in the tails of sperm is consistent among researchers: MCT2 is localized specifically in the distal part of sperm tails (9, 10, 16, 39, 45) (Fig. 6b). Electron microscopically, MCT2 is present along the cell membrane of the principal and terminal pieces in sperm residing in seminiferous tubules of the rat and mouse (9, 16). Interestingly, the localization of MCT2 translocates to the middle piece of the sperm during maturation (16, 39, 90) (Fig. 6c). In contrast, sperm continue to express GLUT3 along the entire length of their tails.

The localization of MCT1 on the microvillous surface (termed stereocilia) of the epididymal epithelium is indeed unique (38). However, this expression is not supported by mRNA expression and the immunostaining of CD147. The epididymis is an organ rich in the expression of other MCT subsets. Our *in situ* hybridization analysis has revealed region-specific expressions of MCTs—MCT8, MCT10, MCT11, and MCT12—although their functional significance remains unknown.

It is important to determine whether Sertoli cells express MCTs to support germ cells, as compared with astrocytes which express MCT1 and MCT4 and supply lactate to adjacent neuronal elements. Although there is evidence that lactate has a crucial role in spermatogenesis (20, 36, 49), the findings concerning MCTs in Sertoli cells remain controversial. Studies using cultured rat Sertoli cells have detected the expression of MCT1, MCT2, and MCT4 by a Northern blot analysis (35, 36). Mannowetz *et al.* (90) have reported the localization of MCT2 in the cytoplasm of murine Sertoli cells as well as sperm tails. However, our immunohistochemical examination failed to confirm the distinct localization of MCTs (MCT1, MCT2, and MCT4) in the mouse and rat Sertoli cells. Leydig cells (interstitial cells) also express MCT1 intensely, possibly due to the uptake of monocarboxylates (acetate) for the biosynthesis of teststerone.

The female genital organ

The female genital tract contains abundant lactate in the luminal fluids, where its concentration is much higher than that in the blood. The follicular fluid in the ovary is a potential source of many nutrients for growing oocytes, since the ovarian follicles lack blood vessels. Due to active glycogenesis in granulosa cells with a high activity of lactate dehydrogenase (LDH), the follicular fluid maintains higher lactate concentrations than the oviductal and uterine fluids (59). Intense signals for LDH-A mRNA are present only in the granulosa of antral follicles, being especially intense in the cumulus cells (82, 136), while LDH-B is abundantly expressed in oocytes at earlier stages of growing follicles, including the primary follicles. The glycolytic products of granulosa such as lactate and pyruvate mediate normal oocyte maturation. The oviductal lactate, possibly secreted from the oviductal epithelium, is important for the energy supply of ovulated eggs and zygotes and for the control of fertilization.

In accordance with the importance of monocarboxylates, MCTs are expressed throughout the female genital tract. The granulosa is a predominant expression site of MCT1 in the mouse ovary (82). Oocytes may consume lactate by converting it to pyruvate. Therefore, the lactate shuttle is established between cumulus cells and oocytes, similar to testicular Sertoli cells and spermatogenic cells. In the oviduct, the mucosal epithelium is immunoreactive

for MCT1 and MCT4, the former predominant in type (82). The basolateral membrane of ciliated cells in the infundibulum and ampulla is selectively immunolabeled for both MCTs. Since the ciliated cells are scarce in the isthmus region of the oviduct, the expression of MCTs conspicuously decreases in intensity and extension. Using MCT1, the oviductal-ciliated cells can incorporate lactate produced by adjacent non-ciliated cells with higher glycolytic activity and use it as an energy source, possibly for ciliary movement. Furthermore, follicle-derived lactate released at ovulation may function as a signaling molecule, namely lactormone (13). The ciliated cells of the oviduct are lacking in GLUT1, whose immunoreactivity is restricted to the basolateral membrane of grouped non-ciliated cells (82). Zygotes and early embryos utilize lactate and pyruvate before implantation, while glucose is a major energy source from the blastocyst stage onwards (74, 138). In the uterus, both epithelial cells facing the lumen of the uterus and uterine gland cells express MCT1 but not MCT4 along their basolateral membrane. The expression of MCT1 corresponds to the intense expression of LDH-A in the uterine epithelium, suggesting that these epithelial cells are the main source of lactate production.

In the human and rodent placenta, arborized villi or complex labyrinths derived from the fetal tissues directly contact the maternal blood. Continuous cell layers of syncytiotrophoblasts cover the placental villi along the entire length, forming an epithelial barrier together with the intravillous vascular wall against the maternal immune system (the blood-placental barrier). Therefore, transport systems for nutrients are indispensable on the barrier, like the blood-brain barrier. The syncytiotrophoblasts in humans and rodents are provided with GLUT1 in both the apical microvillous membrane facing the maternal blood and the basal plasma membrane facing the fetal circulation (129, 139). Among MCT members, MCT1 is predominant, and significant expressions of MCT4, MCT9, MCT10, and MCT12 could be detected by our *in situ* hybridization analysis in the mouse (100). Furthermore, Northern blot and quantitative PCR analyses have demonstrated significant mRNA expressions of MCT1, MCT4–6, MCT8, and MCT10 (TAT-1) in the human placenta. The placenta is another organ with multiple expressions of MCT members comparable to the intestine (44) and kidney (159). Noteworthily, the subcellular localization of MCT1 and MCT4 is differentiated on the maternal or fetal sides of the trophoblast in the mouse, suggesting the transport of monocarboxylates from the maternal side to the fetal side (86). This placental MCT expression might play a key role in the transfer of lactate and ketone bodies from the maternal circulation and ensure a proper substrate supply for embryonic development. However, the human placenta displays the reverse topology of two transporters: MCT1 and MCT4 are arranged respectively to the basal plasma and the apical plasma membrane facing the maternal blood (100). Although the reverse arrangement of these two MCTs between humans and mice remains unknown, it may be important when considering the direction of transport as well as the functional significance of MCTs.

The skin including sebaceous gland and mammary gland

The epidermis of the skin intensely expresses MCT1 along the basal layer, where GLUT1 with a sufficient expression is also provided. The cellular localization along the entire length of the plasma membrane is common between the two transporters. These transporters must supply nutrients derived from the circulation for cell division and proliferation in the epidermis, a representative tissue consistently renewing itself (144).

Acetate, lactate, and other monocarboxylates can serve as materials for lipogenesis via acetyl-CoA. In an immunohistochemical survey of MCTs, we found an intense expression of MCT1 in the mammary gland and sebaceous gland (143). The mammary gland is a modified sweat gland whose secretion contains lipid as a major component: the total lipid contents are evaluated at 10–22% in rodents. Milk contains medium-chain fatty acids which occupy 20–50% and are synthesized in mammary gland cells using glucose and monocarboxylates—mainly acetate—as materials. Secretory portions of mammary glands are simply composed of one type of secretory cell and intensely express MCT1 at the basolateral cell membrane (Fig. 5e). The expression intensity of MCT1 at the mRNA level is closely related to the lactating activity, with the highest expression during active lactation (143). Acetyl-CoA carboxylase-1 is abundant in lipogenic tissues and regulates the *de novo* synthesis of long-chain fatty acids. An extremely intense mRNA expression of this enzyme is found in the mammary gland. The selective expression of MCT1 with a higher activity of acetyl-CoA carboxylase-1 in the mammary glands suggests the involvement of MCT1 in lipogenesis, at least in the synthesis of medium-chain and long-chain fatty acids. Kirat and Kato (76) have detected expressions of MCT1, MCT2, MCT4, and MCT8

by immunoblotting and RT-PCR in the mammary gland of cows; MCT1 and MCT2 have been described as localized at the basolateral membrane of alveolar epithelial cells with the expression of MCT4 at the apical side. Unfortunately, immunostaining of CD147 in cows failed to confirm the cellular localization of MCTs (76). Our *in situ* hybridization analysis in the murine mammary gland could not detect any significant mRNA expression of MCT2 or MCT4.

Hair-associated sebaceous glands and free sebaceous glands including meibomian and preputial glands exhibit an intense labeling for MCT1 immunoreactivity along the entire length of the plasma membrane of alveolar cells (143) (Fig. 5f). The sebaceous gland cells gradually fill with lipid droplets and secrete the products in a holocrine manner. According to the degeneration process, the MCT1 immunoreactivity on the cell membrane decreases in intensity and finally disappears. The active lipogenesis in the holocrine cells indicates the simple role of MCTs in the uptake of monocarboxylates for production of the oily secretions.

Lymphoid tissues and blood cells

The expression of MCTs in the lymphoid organs and immune systems is largely unknown. A study by our research group has reported a selective immunoreactivity of MCT1 in reticular cells in both medullary sinuses and marginal (subcapsular) sinuses of the murine mesenteric lymph node (162). When exogenous particles were injected via Peyer's patches, the MCT1-positive sinus reticular cells of the mesenteric lymph node actively ingested carboxylate-modified fluorescent latex particles of 20 nm in size (124). The uptake activity of the reticular cells was slight against non-modified exogenous particles, such as carbon particles, under normal conditions. The difference between carboxy-modified particles and non-modified carbon particles may be partially due to the affinity of MCT1 to the carboxy moiety. This function may be important for the uptake of waste products and pathogens, both frequently possessing carboxyl terminals in the molecule. Among the several functions of CD147 is the stimulation of hyaluronan synthesis (92). CD147 has been shown to interact with the hyaluronan receptors, LYVE-1 (119) and CD44. Co-localization of LYVE-1 with MCT1 (but not CD147) is seen in the reticular cells in the lymph node of mice (162), suggesting a novel role for MCTs in the elimination of virus particles and other microorganisms.

Before the molecular cloning and identification of MCT molecules, Pool and Halestrap succeeded in the identification of a lactate transporter in rat, rabbit, and guinea pig erythrocytes (116, 117). The MCT1 is present in human serum and is considered to function in buffering or carrying the lactate in the circulation, although the source remains unknown (65). Only MCT1 is present in erythrocytes (30); immunohistochemically, red blood cells are immunolabeled for MCT1 under normal conditions. The MCT1 antibody more intensely labels the plasma membrane of immature red blood cells containing a nucleus, as seen in the bone marrow and placenta of the mouse. It is noteworthy that the bone marrow displays an intense expression of MCT1, in contrast to a faint expression of GLUTs (144). The plasma membrane of hematopoietic cells, mainly erythrocytic progenitor cells, is selectively immunolabeled for MCT1 in the murine bone marrow (144).

Sustained exercise releases a great amount of lactate into the blood to induce a lactate influx from the plasma to erythrocytes (133). Training is known to elevate MCT1 concentration of skeletal muscle and heart depending on the intensity of training (3, 113). The MCT1 concentration in erythrocytes of the rat has been reported to increase by about 30% by daily swimming for one hour (5 days/week) (2).

Three subtypes of MCTs—MCT1, MCT2, and MCT4—are detected in all types of leukocytes (granulocytes, lymphocytes, and monocytes) in human blood (95). MCT4 is the only MCT expressed in human white blood cells, which are also highly glycolytic lactate exporters (158). Research for low molecular-weight compounds with immunosuppressive activities has found new drugs effective for the graft-versus-host (GVH) response. Interestingly, the new compounds are found to bind MCT1 as the receptor and effector (104). In the lymphoid organs, immunostaining of the thymus demonstrates an intense reactivity for MCT1 only in the cortex; the medulla is largely free from immunolabeling (Fig. 6d). Further studies are required for the identification of MCT-expressing lymphocytes and their distribution in hematopoietic and lymphoid tissues.

Other tissues: the lung, mesothelium, endocrine organs, and tumor cells

The lung displays an intense immunoreactivity for MCT1 in the tracheal and bronchiolar epithelium up to the terminal of the airway with basolateral labeling and possibly in type II alveolar epithelial cells (our unpublished data). A moderately intense immunoreactivity for MCT4 is also found in the basolateral membrane of tracheal/bronchiolar epithelia, being

co-localized with MCT1. The mesothelium, such as the peritoneal cavity on both the visceral and parietal sides, shows an intense immunoreactivity for MCT1 along the basolateral membrane.

Most endocrine organs are devoid of MCTs. Besides some endocrine cells in the stomach, the adrenal gland of mouse and cattle contains a distinct immunoreactivity for MCT1 in the plasma membrane of the zona glomerulosa (77; and our unpublished data). A similar membrane-bound localization of MCTs is reported to be found for MCT2 and MCT4 only in the adrenal gland of cattle (77).

Cancer cells produce large amounts of lactate regardless of the availability of oxgen; hence, their metabolism is often referred to as "aerobic glycolysis" (153). This aerobic glycolysis supports mitochondrial oxidative phosphorylation and supplies lactate to adjacent cells which express MCT1 for uptake (29). Most cancer cell lines express MCT1 more intensely than GLUTs. Eleven human lung cancer cell lines examined have expressed MCT1 and MCT4, and a knockdown of these MCTs significantly has reduced the migration of cancer cells (68). Our preliminary examination of human tumor tissues also indicated that most tumors express MCT1 rather than GLUT1. Interestingly, CD147 is highly enriched on the surfaces of most malignant cancer cell types, whereas the expression in normal tissues is usually low (88, 122).

Conclusion

It is worth noting that MCTs display a broader distribution than GLUTs, suggesting that monocarboxylates are more fundamental energy substrates than glucose. This is likely related to the predominant expression of MCTs in the fetus and tumor cell lines. The production of monocarboxylates does not need oxygen; therefore, they may be evolutionally older (more primitive) energy substrates than glucose since the concentration of O_2 in the atmosphere might have been low in ancient times. Although glycolysis is not considered to be effective for the production of ATP, the speed of the reaction is much higher than with the use of the TCA cycle. Finally, the cellular localization of MCTs is important for considering the metabolism and utility of monocarboxylates as well as for elucidation of metabolic pathogenesis and the development of drugs.

REFERENCES

1. Adijanto J, Du J, Moffat C, Seifert EL, Hurle JB and Philp NJ (2014) The retinal pigment epithelium utilizes fatty acids for ketogenesis. Implications for metabolic coupling with the outer retina. *J Biol Chem* **289**, 20570–20582.
2. Aoi W, Iwashita S, Fujie M and Suzuki M (2004) Sustained swimming increases erythrocyte MCT1 during erythropoiesis and ability to regulate pH homeostasis in rat. *Int J Sports Med* **25**, 339–344.
3. Baker SK, McCullagh KJ and Bonen A (1988) Training intensity-dependent and tissue-specific increases in lactate uptake and MCT-1 in heart and muscle. *J Appl Physiol* **84**, 987–994.
4. Becker HM, Mohebbi N, Perna A, Ganapathy V, Capasso G and Wagner CA (2010) Localization of members of MCT monocarboxylate transporter family Slc16 in the kidney and regulation during metabolic acidosis. *Am J Physiol Renal Physiol* **299**, F141–F154.
5. Bergersen LH (2007) Is lactate food for neurons? Comparison of monocarboxylate transporter subtypes in brain and muscle. *Neuroscience* **145**, 11–19.
6. Bergersen L, Jóhannsson E, Veruki ML, Nagelhus EA, Halestrap A, Sejersted OM and Ottersen OP (1999) Cellular and subcellular expression of monocarboxylate transporters in the pigment epithelium and retina of the rat. *Neuroscience* **90**, 319–331.
7. Bergersen L, Wæhaug O, Helm J, Thomas M, Laake P, Davies AJ, Wilson MC, Halestrap AP and Ottersen OP (2001) A novel postsynaptic density protein: the monocarboxylate transporter MCT2 is co-localized with delta-glutamate receptors in postsynaptic densities of parallel fibre-Purkinje cell synapses. *Exp Brain Res* **136**, 523–534.
8. Bergersen LH, Magistretti PJ and Pellerin L (2005) Selective postsynaptic co-localization of MCT2 with AMPA receptor GluR2/3 subunits at excitatory synapses exhibiting AMPA receptor trafficking. *Cereb Cortex* **15**, 361–370.
9. Boussouar F, Mauduit C, Tabone E, Pellerin L, Magistretti PJ and Benahmed M (2003) Developmental and hormonal regulation of the monocarboxylate transporter 2 (MCT2) expression in the mouse germ cells. *Biol Reprod* **69**, 1069–1078.
10. Brauchi S, Rauch MC, Alfaro IE, Cea C, Concha II, Benos DJ and Reyes JG (2005) Kinetics, molecular basis, and differentiation of L-lactate transport in spermatogenic cells. *Am J Physiol Cell Physiol* **288**, C523–C534.
11. Bröer S, Rahman B, Pellegri G, Pellerin L, Martin J-L, Verleysdonk S, Hamprecht B and Magistretti PJ (1997) Comparison of lactate transport in astroglial cells and monocarboxylate transporter 1 (MCT 1) expressing *Xenopus laevis* oocytes. *J Biol Chem* **272**, 30096–30102.
12. Brooks GA, Brown MA, Butz CE, Sicurello JP and Dubouchaud H (1999) Cardiac and skeletal muscle mitochondria have a monocarboxylate transporter MCT1. *J Appl Physiol* **87**, 1713–1718.
13. Brooks GA (2009) Cell-cell and intracellular lactate shuttles. *J Physiol* **587**, 5591–5600.
14. Bugaut M (1987) Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comp Biochem Physiol B* **86**, 439–472.
15. Butz CE, McClelland GB and Brooks GA (2004) MCT1 confirmed in rat striated muscle mitochondria. *J Appl Physiol* **97**, 1059–1066.
16. Chen C, Maekawa M, Yamatoya K, Nozaki M, Ito C, Iwanaga T and Toshimori K (2015) Interaction between basigin and monocarboxylate transporter 2 in the mouse testes and spermatozoa. *Asian J Androl*, in press
17. Chidlow G, Wood JPM, Graham M and Osborne NN (2005) Expression of monocarboxylate transporters in rat ocular

tissues. *Am J Physiol Cell Physiol* **288**, C416–C428.
18. Chiry O, Pellerin L, Monnet-Tschudi F, Fishbein WN, Merezhinskaya N, Magistretti PJ and Clarke S (2006) Expression of the monocarboxylate transporter MCT1 in the adult human brain cortex. *Brain Res* **1070**, 65–70.
19. Coady MJ, Chang M-H, Charron FM, Plata C, Wallendorff B, Sah JF, Markowitz SD, Romero MF and Lapointe J-Y (2004) The human tumor suppressor gene SLC5A8 expresses a Na^+-monocarboxylate cotransporter. *J Physiol* **557**, 719–731.
20. Courtens JL and Plöen L (1999) Improvement of spermatogenesis in adult cryptorchid rat testis by intratesticular infusion of lactate. *Biol Reprod* **61**, 154–161.
21. Croom WJ, Bull LS and Taylor IL (1992) Regulation of pancreatic exocrine secretion in ruminants: a review. *J Nutr* **122**, 191–202.
22. Cuff MA and Shirazi-Beechey SP (2004) The importance of butyrate transport to the regulation of gene expression in the colonic epithelium. *Biochem Soc Trans* **32**, 1100–1102.
23. Davey KAB, Garlick PB, Warley A and Southworth R (2007) Immunogold labeling study of the distribution of GLUT-1 and GLUT-4 in cardiac tissue following stimulation by insulin or ischemia. *Am J Physiol Heart Circ Physiol* **292**, H2009–H2019.
24. De Matteis R, Lucertini F, Guescini M, Polidori E, Zeppa S, Stocchi V, Cinti S and Cuppini R (2013) Exercise as a new physiological stimulus for brown adipose tissue activity. *Nutr Metab Cardiovasc Dis* **23**, 582–590.
25. Dimmer KS, Friedrich B, Lang F, Deitmer JW and Bröer S (2000) The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. *Biochem J* **350**, 219–227.
26. Domènech-Estévez E, Baloui H, Repond C, Rosafio K, Médard JJ, Tricaud N, Pellerin L and Chrast R (2015) Distribution of monocarboxylate transporters in the peripheral nervous system suggests putative roles in lactate shuttling and myelination. *J Neurosci* **35**, 4151–4156.
27. Eladari D, Chambrey R, Irinopoulou T, Leviel F, Pezy F, Bruneval P, Paillard M and Podevin R-A (1999) Polarized expression of different monocarboxylate transporters in rat medullary thick limbs of Henle. *J Biol Chem* **274**, 28420–28426.
28. Enerson BE and Drewes LR (2003) Molecular features, regulation, and function of monocarboxylate transporters: implications for drug delivery. *J Pharm Sci* **92**, 1531–1544.
29. Feron O (2009) Pyruvate into lactate and back: from Warburg effect to symbiotic energy fuel exchange in cancer cells. *Radiother Oncol* **92**, 329–333.
30. Fishbein WN, Merezhinskaya N and Foellmer JW (2002) Relative distribution of three major lactate transporters in frozen human tissues and their localization in unfixed skeletal muscle. *Muscle Nerve* **26**, 101–112.
31. Fleming SE, Choi SY and Fitch MD (1991) Absorption of short-chain fatty acids from the rat cecum in vivo. *J Nutr* **121**, 1787–1797.
32. Frank H, Gröger N, Diener M, Becker C, Braun T and Boettger T (2008) Lactaturia and loss of sodium-dependent lactate uptake in the colon of SLC5A8-deficient mice. *J Biol Chem* **283**, 24729–24737.
33. Friesema ECH, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP and Visser TJ (2003) Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J Biol Chem* **278**, 40128–40135.
34. Froehner SC, Davies A, Baldwin SA and Lienhard GE (1988) The blood-nerve barrier is rich in glucose transporter. *J Neurocytol* **17**, 173–178.
35. Galardo MN, Riera MF, Pellizzari EH, Cigorraga SB and Meroni SB (2007) The AMP-activated protein kinase activator, 5-aminoimidazole-4-carboxamide-1b-D-ribonucleoside, regulates lactate production in rat Sertoli cells. *J Mol Endocrinol* **39**, 279–288.
36. Galardo MN, Regueira M, Riera MF, Pellizzari EH, Cigorraga SB and Meroni SB (2014) Lactate regulates rat male germ cell function through oxygen species. *PloS One* **9**, e88024.
37. Ganapathy V, Thangaraju M, Gopal E, Martin PM, Itagaki S, Miyauchi S and Prasad PD (2008) Sodium-coupled monocarboxylate transporters in normal tissues and in cancer. *AAPS J* **10**, 193–199.
38. Garcia CK, Goldstein JL, Pathak RK, Anderson RGW and Brown MS (1994) Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell* **76**, 865–873.
39. Garcia CK, Brown MS, Pathak RK and Goldstein JL (1995) cDNA cloning of MCT2, a second monocarboxylates transporter expressed in different cells than MCT1. *J Biol Chem* **270**, 1843–1849.
40. Gerhart DZ, Enerson BE, Zhdankina OY, Leino RL and Drewes LR (1997) Expression of monocarboxylate transporter MCT1 by brain endothelium and glia in adult and suckling rats. *Am J Physiol* **273** (*Endocrinol Metab* 36), E207–E213.
41. Gerhart DZ and Drewes LR (1990) Glucose transporters at the blood-nerve barrier are associated with perineurial cells and endoneurial microvessels. *Brain Res* **508**, 46–50.
42. Gerhart DZ, Enerson BE, Zhdankina OY, Leino RL and Drewes LR (1998) Expression of the monocarboxylate transporter MCT2 by rat brain glia. *Glia* **22**, 272–281.
43. Gerhart DZ, Leino RL and Drewes LR (1999) Distribution of monocarboxylate transporters MCT1 and MCT2 in rat retina. *Neuroscience* **92**, 367–375.
44. Gill RK, Saksena S, Alrefai WA, Sarwar Z, Goldstein JL, Carroll RE, Ramaswamy K and Dudeja PK (2005) Expression of membrane localization of MCT isoforms along the length of the human intestine. *Am J Physiol Cell Physiol* **289**, C846–C852.
45. Goddard I, Florin A, Mauduit C, Tabone E, Contard P, Bars R, Chuzel F and Benahmed M (2003) Alteration of lactate production and transport in the adult rat testis exposed in utero to flutamide. *Mol Cell Endocrinol* **206**, 137–146.
46. Gopal E, Fei Y-J, Sugawara M, Miyauchi S, Zhuang L, Martin P, Smith SB, Prasad PD and Ganapathy V (2004) Expression of slc5a8 in kidney and its role in Na^+-coupled transport of lactate. *J Biol Chem* **279**, 44522–44532.
47. Gopal E, Fei Y-J, Miyauchi S, Zhuang L, Prasad PD and Ganapathy V (2005) Sodium-coupled and electrogenic transport of B-complex vitamin nicotinic acid by slc5a8, a member of the Na/glucose co-transporter gene family. *Biochem J* **388**, 309–316.
48. Gopal E, Umapathy NS, Martin PM, Ananth S, Gnana-Prakasam JP, Becker H, Wagner CA, Ganapathy V and Prasad PD (2007) Cloning and functional characterization of human SMCT (SLC5A12) and expression pattern of the transporter in kidney. *Biochim Biophys Acta* **1768**, 2690–2697.
49. Grootegoed JA and Den Boer PJ (1989) Energy metabolism in spermatids: a review. In *Cellular and Molecullar Events in Spermiogenesis* (Hamilton DW, Waites GHM, eds), pp193–215, Cambridge University Press, Cambridge.
50. Gupta N, Martin PM, Prasad PD and Ganapathy V (2006)

SLC5A8 (SMCT1)-mediated transport of butyrate forms the basis for the tumor suppressive function of the transporter. *Life Sci* **78**, 2419–2425.
51. Hajduch E, Heyes RR, Watt PW and Hundal HS (2000) Lactate transport in rat adipocytes: identification of monocarboxylate transporter 1 (MCT1) and its modulation during streptozotocin-induced diabetes. *FEBS Lett* **479**, 89–92.
52. Halestrap AP (2012) The monocarboxylate transporter family—structure and functional characterization. *IUBMB Life* **64**, 1–9.
53. Halestrap AP (2013) The SLC gene family—structure, role and regulation in health and disease. *Mol Aspects Med* **34**, 337–349.
54. Halestrap AP (2013) Monocarboxylic acid transport. *Compr Physiol* **3**, 1611–1643.
55. Halestrap AP and Meredith D (2004) The SLC16 gene family—from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflugers Arch* **447**, 619–628.
56. Halestrap AP and Price NT (1999) The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. *Biochem J* **343**, 281–299.
57. Hanu R, McKenna M, O'Neill A, Resneck WG and Bloch RJ (2000) Monocarboxylic acid transporters, MCT1 and MCT2, in cortical astrocytes in vitro and in vivo. *Am J Physiol Cell Physiol* **278**, C921–C930.
58. Harada E and Kato S (1983) Effect of short-chain fatty acids on the secretory response of ovine exocrine pancreas. *Am J Physiol* **244** (*Gastrointest Liver Physiol* **7**), G284–G290.
59. Harris SE, Gopichandran N, Picton HM, Leese HJ and Orsi NM (2005) Nutrient concentrations in murine follicular fluid and the female reproductive tract. *Theriogenology* **64**, 992–1006.
60. Hashimoto T, Masuda S, Taguchi S and Brooks GA (2005) Immunohistochemical analysis of MCT1, MCT2 and MCT4 expression in rat plantaris muscle. *J Physiol* **567**, 121–129.
61. Hashimoto T, Hussien R and Brooks GA (2006) Colocalization of MCT1, CD147, and LDH in mitochondrial inner membrane of L6 muscle cells: Evidence of a mitochondrial lactate oxidation complex. *Am J Physiol* **290**, E1237–E1244.
62. Hatta H, Tonouchi M, Miskovic D, Wang YX, Heikkila JJ and Bonen A (2001) Tissue-specific and isoform-specific changes in MCT1 and MCT4 in heart and soleus muscle during a 1-yr period. *Am J Physiol* **281**, E749–E756.
63. Hawkins RA, Mans AM and Davis DW (1986) Regional ketone body utilization by rat brain in starvation and diabetes. *Am J Physiol* **250** (*Endocrinol Metab* **13**), E169–E178.
64. Hein TW, Xu W and Kuo L (2006) Dilation of retinal arterioles in response to lactate: role of nitric oxide, guanylyl cyclase, and ATP-sensitive potassium channels. *Invest Opthalmol Vis Sci* **47**, 693–699.
65. Iizuka K, Morita N, Nagai T, Hanada A, Okita K, Yonezawa K, Murakami T, Kitabatake A and Kawaguchi H (2003) A 44-kDa of protein identical to the N-terminal amino acid sequence of MCT1 in human circulation. *Mol Cell Biochem* **248**, 217–223.
66. Iwanaga T, Takebe K, Kato I, Karaki S-I and Kuwahara A (2006) Cellular expression of monocarboxylate transporters (MCT) in the digestive tract of the mouse, rat, and humans, with special reference to slc5a8. *Biomed Res (Tokyo)* **27**, 243–254.
67. Iwanaga T, Kuchiiwa T and Saito M (2009) Histochemical demonstration of monocarboxylate transporters in mouse brown adipose tissue. *Biomed Res (Tokyo)* **30**, 217–225.
68. Izumi H, Takahashi M, Uramoto H, Nakayama Y, Oyama T, Wang K-Y, Sasaguri Y, Nishizawa S and Kohno K (2011) Monocarboxylate transporters 1 and 4 are involved in the invasion activity of human lung cancer cells. *Cancer Sci* **102**, 1007–1013.
69. Jackson VN, Price NT, Carpenter L and Halestrap AP (1997) Cloning of the monocarboxylate transporter isoform MCT2 from rat testis provides evidence that expression in tissues is species-specific and may involve post-transcriptional regulation. *Biochem J* **324**, 447–453.
70. Jóhannsson E, Nagelhus EA, McCullagh KJ, Sejersted OM, Blackstad TW, Bonen A and Ottersen OP (1997) Cellular and subcellular expression of the monocarboxylate transporter MCT1 in rat heart. A high-resolution immunogold analysis. *Circ Res* **80**, 400–407.
71. Jóhannsson E, Lunde PK, Heddle C, Sjaastad I, Thomas MJ, Bergersen L, Halestrap AP, Blackstad TW, Ottersen OP and Sejersted OM (2001) Upregulation of the cardiac monocarboxylate transporter MCT1 in a rat model of congestive heart failure. *Circulation* **104**, 729–734.
72. Kainuma E, Watanabe M, Tomiyama-Miyaji C, Inoue M, Kuwano Y, Ren HW and Abo T (2009) Proposal of alternative mechanism responsible for the function of high-speed swimsuits. *Biomed Res (Tokyo)* **30**, 69–70.
73. Kaji I, Iwanaga T, Watanabe M, Guth PH, Engel E, Kaunitz JD and Akiba Y (2015) SCFA transport in rat duodenum. *Am J Physiol Gastrointest Liver Physiol* **308**, G188–G197.
74. Kim JH, Funahashi H, Niwa K and Okuda K (1993) Glucose equipment at different developmental stages of in vitro fertilized bovine embryos cultured in semi-defined medium. *Theriogenology* **39**, 875–886.
75. Kirat D, Inoue H, Iwano H, Hirayama K, Yokota H, Taniyama H and Kato S (2005) Expression and distribution of monocarboxylate transporter 1 (MCT1) in the gastrointestinal tract of calves. *Res Vet Sci* **79**, 45–50.
76. Kirat D and Kato S (2009) Monocarboxylate transporter genes in the mammary gland of lactating cows. *Histochem Cell Biol* **132**, 447–455.
77. Kirat D, Sallam K, Hayahsi H, Miyasho T and Kato S (2009) Presence of ten isoforms of monocarboxylate transporter (MCT) family in the bovine adrenal gland. *Mol Cell Endocrinol* **298**, 89–100.
78. Kirat D, Kondo K, Shimada R and Kato S (2009) Dietary pectin up-regulates monocarboxylate transporter 1 in the rat gastrointestinal tract. *Exp Physiol* **94**, 422–433.
79. Kirk P, Wilson MC, Heddle C, Brown MH, Barclay AN and Halestrap AP (2000) CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. *EMBO J* **19**, 3896–3904.
80. Kishimoto A, Ishiguro-Oonuma T, Takahashi R, Maekawa M, Toshimori K, Watanabe M and Iwanaga T (2015) Immunohistochemical localization of GLUT3, MCT1, and MCT2 in the testes of mice and rats: the use of different energy sources in spermatogenesis. *Biomed Res (Tokyo)* **36**, 225–234.
81. Koehler-Stec EM, Simpson IA, Vannucci SJ, Landschulz KT and Landschulz WH (1998) Monocarboxylate transporter expression in mouse brain. *Am J Physiol* **275**, E516–E524.
82. Kuchiiwa T, Nio-Kobayashi J, Takahashi-Iwanaga H, Yajima T and Iwanaga T (2011) Cellular expression of monocarboxylate transporters in the female reproductive system of mice: implications for genital lactate shuttle. *Histochem Cell Biol* **135**, 351–360.

83. Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang PW, Pellerin L, Magistretti PJ and Rothstein JD (2012) Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* **487**, 443–448.
84. Leino RL, Gerhart DZ and Drewes LR (1999) Monocarboxylate transporter (MCT1) abundance in brains of suckling and adult rats: a quantitative electron microscopic immunogold study. *Dev Brain Res* **113**, 47–54.
85. Leino RL, Gerhart DZ, Duelli R, Enerson BE and Drewes LR (2001) Diet-induced ketosis increases monocarboxylate transporter (MCT1) levels in rat brain. *Neurochem Int* **38**, 519–527.
86. Lengacher S, Nehiri-Sitayeb T, Steiner N, Carneiro L, Favrod C, Preitner F, Thorens B, Stehle J-C, Dix L, Pralong F, Magistretti PJ and Pellerin L (2013) Resistance to diet-induced obesity and associated metabolic perturbations in haploinsufficient monocarboxylate transporter 1 mice. *ProS One* **8**, e82505.
87. Li H, Myeroff L, Smiraglia D, Romero MF, Pretlow TP, Kasturi L, Lutterbaugh J, Rerko RM, Casey G, Issa J-P, Willis J, Willson JKV, Plass C and Markowitz SD (2003) SLC5A8, a sodium transporter, is a tumor suppressor gene silenced by methylation in human colon aberrant crypt foci and cancers. *Proc Natl Acad Sci USA* **100**, 8412–8417.
88. Li Y, Xu J, Chen L, Zhong WD, Zhang Z, Mi L, Zhang Y, Liao CG, Bian HJ, Jiang JL, Yang XM, Li XY, Fan CM, Zhu P, Fu L and Chen ZN (2009) HAb18G (CD147), a cancer-associated biomarker and its role in cancer detection. *Histopathology* **54**, 677–687.
89. Lu W, Huang J, Sun S, Huang S, Gan S, Xu J, Yang M, Xu S and Jiang X (2015) Changes in lactate content and monocarboxylate transporter 2 expression in $Ab_{25\text{-}35}$-treated rat model of Alzheimer's disease. *Neurol Sci* **36**, 871–876.
90. Mannowetz N, Wandernoth P and Wennemuth G (2012) Basigin interacts with both MCT1 and MCT2 in murine spermatozoa. *J Cell Physiol* **227**, 2154–2162.
91. Marette A, Richardson JM, Ramlal T, Balon TW, Vranic M, Pessin JE and Klip A (1992) Abundance, localization, and insulin-induced translocation of glucose transporters in red and white muscle. *Am J Physiol* **263**, C443–452.
92. Marieb EA, Zoltan-Jones A, Li R, Misra S, Ghatak S, Cao J, Zucker S and Toole BP (2004) Emmprin promotes anchorage-independent growth in human mammary carcinoma cells by stimulating hyaluronan production. *Cancer Res* **64**, 1229–1232.
93. Martin PM, Gopal E, Ananth S, Zhuang L, Itagaki S, Prasad BM, Smith SB, Prasad PD and Ganapathy V (2006) Identity of SMCT (SLC5A8) as a neuron-specific Na^+-coupled transporter for active uptake of L-lactate and ketone bodies in the brain. *J Neurochem* **98**, 279–288.
94. Meredith D and Christian HC (2008) The SLC16 monocarboxylate transporter family. *Xenobiotica* **38**, 1072–1106.
95. Merezhinskaya N, Ogunwuyi SA, Mullick FG and Fishbein WN (2004) Presence and localization of three lactic acid transporters (MCT1, -2, and -4) in separated human granulocytes, lymphocytes, and monocytes. *J Histochem Cytochem* **52**, 1483–1493.
96. Miyauchi S, Gopal E, Fei Y-J and Ganapathy V (2004) Functional identification of SLC5A8, a tumor suppressor downregulated in colon cancer, as a Na^+-coupled transporter for short-chain fatty acids. *J Biol Chem* **279**, 13293–13296.
97. Morrison BM, Tsingalia A, Vidensky S, Lee Y, Jin L, Farah MH, Lengacher S, Magistretti PJ, Pellerin L and Rothstein JD (2015) Deficiency in monocarboxylate transporter 1 (MCT1) in mice delays regeneration of peripheral nerves following sciatic nerve crush. *Exp Neurol* **263**, 325–338.
98. Mortensen PB and Clausen MR (1996) Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease. *Scand J Gastroenterol* Suppl **216**, 132–148.
99. Muona P, Jaakkola S, Salonen V and Peltonen J (1993) Expression of glucose transporter 1 in adult and developing human peripheral nerve. *Diabetologia* **36**, 133–140.
100. Nagai A, Takebe K, Nio-Kobayashi J, Takahashi-Iwanaga H and Iwanaga T (2010) Cellular expression of the monocarboxylate transporter (MCT) family in the placenta of mice. *Placenta* **31**, 126–133.
101. Nehlig A and Pereira de Vasconcelos A (1993) Glucose and ketone body utilization by the brain of neonatal rats. *Prog Neurobiol* **40**, 163–221.
102. Nowik M, Lecca MR, Velic A, Rehrauer H, Brändli AW and Wagner CA (2008) Genome-wide gene expression profiling reveals renal genes regulated during metabolic acidosis. *Physiol Genomics* **32**, 322–334.
103. Oikawa K, Iizuka K, Murakami T, Nagai T, Okita K, Yonezawa K, Kitabatake A and Kawaguchi H (2004) Pure pressure stress increased monocarboxylate transporter in human aortic smooth muscle cell membrane. *Mol Cell Biochem* **259**, 151–156.
104. Påhlman C, Qi Z, Murray CM, Ferguson D, Bundick RV, Donald DK and Ekberg H (2013) Immunosuppressive properties of a series of novel inhibitors of the monocarboxylate transporter MCT1. *Transpl Int* **26**, 22–29.
105. Paroder V, Spencer SR, Paroder M, Arango D, Schwartz S Jr, Mariadason JM, Augenlicht LH, Eskandari S and Carrasco N (2006) Na^+/monocarboxylate transport (SMCT) protein expression correlates with survival in colon cancer: Molecular characterization of SMCT. *Proc Natl Acad Sci USA* **103**, 7270–7275.
106. Pellerin L, Pellegri G, Martin J-L and Magistretti PJ (1998) Expression of monocarboxylate transporter mRNAs in mouse brain: Support for a distinct role of lactate as an energy substrate for the neonatal vs. adult brain. *Proc Natl Acad Sci USA* **95**, 3990–3995.
107. Pérez de Heredia F, Wood IS and Trayhurn P (2010) Hypoxia stimulates lactate release and modulates monocarboxylate transporter (MCT1, MCT2, and MCT4) expression in human adipocytes. *Pflugers Arch* **459**, 509–518.
108. Philp NJ, Yoon H and Grollman EF (1998) Monocarboxylate transporter MCT1 is located in the apical membrane and MCT3 in the basal membrane of rat RPE. *Am J Physiol* **274** (*Regulatory Integrative Comp Physiol* **43**), R1824–R1828.
109. Philp NJ, Ochrietor JD, Rudoy C, Muramatsu T and Linser PJ (2003) Loss of MCT1, MCT3, and MCT4 expression in the retinal pigment epithelium and neural retina of the 5A11/basigin-null mouse. *Invest Opthalmol Vis Sci* **44**, 1305–1311.
110. Pierre K, Magistretti PJ and Pellerin L (2002) MCT2 is a major neuronal monocarboxylate transporter in the adult mouse brain. *J Cereb Blood Flow Metab* **22**, 586–595.
111. Pierre K and Pellerin L (2005) Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *J Neurochem* **94**, 1–14.
112. Pierre K, Pellerin L, Debernardi R, Riederer BM and Magistretti PJ (2000) Cell-specific localization of monocarboxylate transporters, MCT1 and MCT2, in the adult mouse brain revealed by double immunohistochemical labeling and con-

focal microscopy. *Neuroscience* **100**, 617–627.
113. Pilegaard H, Domino K, Noland T, Juel C, Hellsten Y, Halestrap AP and Bangsbo J (1999) Effect of high-intensity exersize training on lactate/H^+ transport capacity in human skeletal muscle. *Am J Physiol (Endocrinol Metab 39)* **276**, 255–261.
114. Pilegaard H, Terzis G, Halestrap A and Juel C (1999) Distribution of the lactate/H^+ transporter isoforms MCT1 and MCT4 in human skeletal muscle. *Am J Physiol* **276** (*Endocrinol Metab* **39**), E843–E848.
115. Poitry-Yamate CL, Poitry S and Tsacopoulos M (1995) Lactate released by Müller glial cells is metabolized by photoreceptors from mammalian retina. *J Neurosci* **15**, 5179–5191.
116. Poole RC and Halestrap AP(1992) Identification and partial purification of the erythrocyte L-lactate transporter. *Biochem J* **283**, 855–862.
117. Poole RC and Halestrap A (1993) Transport of lactate and other monocarboxylates across mammalian plasma membrane. *Am J Physiol* **264**, C761-C782.
118. Price NT, Jackson VN and Halestrap AP (1998) Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *Biochem J* **329**, 321–328.
119. Qin Z, Dai L, Bratoeva M, Slomiany MG, Toole BP and Parsons C (2011) Cooperative roles for emmprin and LYVE-1 in the regulation of chemoresistance for primary effusion lymphoma. *Leukemia* **25**, 1598–1609.
120. Rafiki A, Boulland JL, Halestrap AP, Ottersen OP and Bergersen L (2003) Highly differential expression of the monocarboxylate transporters MCT2 and MCT4 in the developing rat brain. *Neuroscience* **122**, 677–688.
121. Rechthand E, Smith QR and Rapoport SI (1985) Facilitated transport of glucose from blood into peripheral nerve. *J Neurochem* **45**, 957–964.
122. Riethdorf S, Reimers N, Assmann V, Kornfeld J-W, Terracciano L, Sauter G and Pantel K (2006) High incidence of EMMPRIN expression in human tumors. *Int J Cancer* **119**, 1800–1810.
123. Ritzhaupt A, Wood IS, Ellis A, Hosie KB and Shirazi-Beechey SP (1998) Identification and characterization of a monocarboxylate transporter (MCT1) in pig and human colon: its potential to transport L-lactate as well as butyrate. *J Physiol* **513**, 719–732.
124. Rung-ruangkijkrai T, Fujikura D, Kitamura H, Saito M and Iwanaga T (2004) The expression of *src*-suppressed C kinase substrate (SSeCKS) and uptake of exogenous particles in endothelial and reticular cells. *Arch Histol Cytol* **67**, 135–147.
125. Saggerson ED, McAllister TW and Baht HS (1988) Lipogenesis in rat brown adipocytes. Effects of insulin and noradrenaline, contributions from glucose and lactate as precursors and comparisons with white adipocytes. *Biochem J* **251**, 701–709.
126. Sánchez-Abarca LI, Tabernero A and Medina JM (2001) Oligodendrocytes use lactate as a source of energy and as a precurssor of lipids. *Glia* **36**, 321–329.
127. Shimizu Y, Nikami H, Tsukazaki K, Machado UF, Yano H, Seino Y and Saito M (1993) Increased expression of glucose transporter GLUT-4 in brown adipose tissue of fasted rats after cold exposure. *Am J Physiol* **264**, E890–E895.
128. Shimoyama Y, Kirat D, Akihara Y, Kawasako K, Komine M, Hirayama K, Matsuda K, Okamoto M, Iwano H, Kato S and Taniyama H (2007) Expression of monocarboxylate transporter (MCT1) in the dog intestine. *J Vet Med Sci* **69**, 599–604.
129. Shin B-C, Fujikura K, Suzuki T, Tanaka S and Takata K (1997) Glucose transporter GLUT3 in the rat placental barrier: a possible machinery for the transplacental transfer of glucose. *Endocrinology* **138**, 3997–4004.
130. Simpson IA, Carruthers A and Vannucci SJ (2007) Supply and demand in cerebral energy metabolism: the role of nutrient transporters. *J Cerb Blood Flow Metab* **27**, 1766–1791.
131. Simpson IA, Dwyer D, Malide D, Moley KH, Travis A and Vannucci SJ (2008) The facilitative glucose transporter GLUT3: 20 years of distinction. *Am J Physiol Endocrinol Metab* **295**, E242–E253.
132. Slot JW, Geuze HJ, Gigengack S, James DE and Lienhard GE (1991) Translocation of the glucose transporter GLUT4 in cardiac myocytes of the rat. *Proc Natl Acad Sci USA* **88**, 7815–7819.
133. Smith JA, Telford RD, Kolbuch-Braddon M and Weidemann MJ (1997) Lactate/H^+ uptake by red blood cells during exercise alters their physical properties. *Eur J Appl Physiol* **75**, 54–61.
134. Srinivas SR, Gopal E, Zhuang L, Itagaki S, Martin PM, Fei Y-J, Ganapathy V and Prasad PD (2005) Cloning and functional identification of slc5a12 as a sodium-coupled low-affinity transporter for monocarboxylates (SMCT2). *Biochem J* **392**, 655–664.
135. Stark B, Carlstedt T, Cullheim S and Risling M (2000) Developmental and lesion-induced changes in the distribution of the glucose transporter Glut-1 in the central and peripheral nervous system. *Exp Brain Res* **131**, 74–84.
136. Sugiura K, Pendola FL and Eppig JJ (2005) Oocyte control of metabolic cooperativity between oocytes and companion granulosa cells: energy metabolism. *Dev Biol* **279**, 20–30.
137. Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magisttretti PJ and Alberini CM (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* **144**, 810–823.
138. Takahashi Y and First NL (1992) In vitro development of bovine one-cell embryos: influence of glucose, lactate, pyruvate, amino acids and vitamins. *Theriogenology* **37**, 963–978.
139. Takata K and Hirano H (1997) Mechanism of glucose transport across the human and rat placental barrier: a review. *Microsc Res Tech* **38**, 145–152.
140. Takata K, Hirano H and Kasahara M (1997) Transport of glucose across the blood-tissue barriers. *Int Rev Cytol* **172**, 1–53.
141. Takebe K, Nio J, Morimastu M, Karaki S-I, Kuwahara A, Kato I and Iwanaga T (2005) Histochemical demonstration of a Na^+-coupled transporter for short-chain fatty acids (Slc5a8) in the intestine and kidney of the mouse. *Biomed Res (Tokyo)* **26**, 213–221.
142. Takebe K, Nio-Kobayashi J, Takahashi-Iwanaga H and Iwanaga T (2008) Histochemical demonstration of a monocarboxylate transporter in the mouse perineurium with special reference to GLUT1. *Biomed Res (Tokyo)* **29**, 297–306.
143. Takebe K, Nio-Kobayashi J, Takahashi-Iwanaga H, Yajima T and Iwanaga T (2009) Cellular expression of a monocarboxylate transporter (MCT1) in the mammary gland and sebaceous gland of mice. *Histochem Cell Biol* **131**, 401–409.
144. Takebe K, Takahashi-Iwanaga H and Iwanaga T (2011) Intensified expressions of a monocarboxylate transporter in consistently renewing tissues of the mouse. *Biomed Res*

(Tokyo) **32**, 293–301.
145. Tamai I, Sai Y, Ono A, Kido Y, Yabuuchi H, Takanaga H, Satoh E, Ogihara T, Amano O, Izeki S and Tsuji A (1999) Immunohistochemical and functional characterization of pH-dependent intestinal absorption of weak organic acids by the monocarboxylic acid transporter MCT1. *J Pharm Pharmacol* **51**, 1113–1121.
146. Teramae H, Yoshikawa T, Inoue R, Ushida K, Takebe K, Nio-Kobayashi J and Iwanaga T (2010) The cellular expression of SMCT2 and its comparison with other transporters for monocarboxylates in the mouse digestive tract. *Biomed Res (Tokyo)* **31**, 239–249.
147. Thangaraju M, Ananth S, Martin PM, Roon P, Smith SB, Sterneck E, Prasad PD and Ganapathy V (2006) c/ebpdelta Null mouse as a model for the double knock-out of slc5a8 and alc5a12 in kidney. *J Biol Chem* **281**, 26769–26773.
148. Thomas C, Bishop DJ, Lambert K, Mercier J and Brooks GA (2012) Effects of acute and chronic exercise on sarcolemmal MCT1 and MCT4 contents in human skeletal muscles: current status. *Am J Physiol Regul Integr Comp Physiol* **302**, R1–R14.
149. Tohyama S, Hattori F, Sano M, Hishiki T, Nagahata Y *et al*. (2013) Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. *Cell Stem Cell* **12**, 127–137.
150. Topping D and Clifton PM (2001) Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* **81**, 1031–1064.
151. Urner F and Sakkas D (1999) A possible role for the pentose phosphate pathway of spermatozoa in gamete fusion in the mouse. *Biol Reprod* **60**, 733–739.
152. van Hasselt PM, Ferdinandusse S, Monroe GR, Ruiter JP, Turkenburg M, *et al*. (2014) Monocarboxylate transporter 1 deficiency and ketone utilization. *N Eng J Med* **371**, 1900–1907.
153. Vander Heiden MG, Cantley LC and Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* **324**, 1029–1033.
154. Vannucci SJ and Simpson IA (2003) Developmental switch in brain nutrient transporter expression in the rat. *Am J Physiol Endocrinol Metab* **285**, E1127–E1134.
155. Visser WE, Friesema EC and Visser TJ (2011) Minireview: Thyroid hormone transporters: The knowns and the unknowns. *Mol Endocrinol* **25**, 1–14.
156. Vrhovac I, Balen Eror D, Klessen D, Burger C, Breljak D *et al*. (2015) Localization of Na^+-D-glucose cotransporters SGLT1 and SGLT2 in human kidney and of SGLT1 in human small intestine, liver, lung, and heart. *Pflugers Arch* **467**, 1881–1898.
157. Washington TA, Brown L, Smith DA, Davis G, Baum J and Bottje W (2013) Monocarboxylate transporter expression at the onset of skeletal muscle regeneration. *Physiol Rep* **1**, e00075.
158. Wilson MC, Jackson VN, Heddle C, Price NT, Pilegaard H, Juel C, Bonen A, Montgomery I, Hutter OF and Halestrap AP (1998) Lactic acid efflux from white skeletal muscle is catalyzed by the monocarboxylate transporter isoform MCT3. *J Biol Chem* **273**, 15920–15926.
159. Yanase H, Takebe K, Nio-Kobayashi J, Takahashi-Iwanaga H and Iwanaga T (2008) Cellular localization of a sodium-dependent monocarboxylate transporter (Slc5a8) and the MCT family in the mouse kidney. *Histochem Cell Biol* **130**, 957–966.
160. Yoon H, Fanelli A, Grollman EF and Philp NJ (1997) Identification of a unique monocarboxylate transporter (MCT3) in retinal pigment epithelium. *Biochem Biophys Res Commun* **234**, 90–94.
161. Zhao C, Wilson MC, Schuit F, Halestrap AP and Rutter GA (2001) Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. *Diabetes* **50**, 361–366.
162. Zheng M, Ishiguro-Oonuma T and Iwanaga T (2014) Expresion of a monocarboxylate transporter in reticular cells of the mouse lymph node and its involvement in the uptake of exogenous particles. *Biomed Res (Tokyo)* **35**, 85–90.