Short- and medium-chain fatty acids in the energy metabolism – the cellular perspective

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Running title
Short-and medium-chain fatty acids energy metabolism

Abbreviations: AMPK, AMP-dependent kinase; LCFAs, long-chain fatty acids; MCFAs, medium-chain fatty acids; ROS, reactive oxygen species; SCFAs, short-chain fatty acids

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Abstract:

Short- and medium-chain fatty acids (SCFAs and MCFAs), independently of their cellular signalling functions, are important substrates of the energy metabolism and anabolic processes in mammals. SCFAs are mostly generated by colonic bacteria and are predominantly metabolized by enterocytes and liver, whereas MCFAs arise mostly from dietary triglycerides, among them milk and dairy products.

A common feature of SCFAs and MCFAs is their carnitine-independent uptake and intramitochondrial activation to acyl-CoA thioesters. Contrary to long-chain fatty acids, the cellular metabolism of SCFAs and MCFAs depends in a lesser extent on fatty acid-binding proteins. SCFAs and MCFAs modulate tissue metabolism of carbohydrates and lipids as manifested by mostly inhibitory effect on glycolysis and stimulation of lipogenesis or gluconeogenesis.

SCFAs and MCFAs exert in mitochondria no or only weak protonophoric and lytic activities and do not significantly impair the electron transport in the respiratory chain. SCFAs and MCFAs modulate mitochondrial energy production by two mechanism: they provide reducing equivalents to the respiratory chain and partly decrease efficacy of the oxidative ATP synthesis.

Supplementary keywords: short chain fatty acids, medium chain fatty acids, mitochondria, energy metabolism
Short- and medium-chain fatty acids (SCFAs and MCFAs), along with more abundant long-chain fatty acids (LCFAs), are natural compounds present in both animal and plant tissues that participate in cell metabolism. SCFAs and MCFAs are also important food constituents, where they are mostly in the form of triglycerides in some plant oils and milk (1). Nevertheless, bacterial fermentation of amylase-resistant starch and non-starch polysaccharides in the gut is probably the most important source of SCFAs in human and most mammalian species (2-4).

Along with their role as energy-supplying fuel, SCFAs and MCFAs exhibit various regulatory and signalling functions. Butyrate and other SCFAs are known to induce apoptosis under specific conditions and thus to control cell proliferation (5,6). Currently, increasing attention is given to SCFAs with respect to their putative role in the pathogenesis of allergies as well as autoimmune, metabolic and neurological diseases [reviewed in (7)]. In the last two decades, the role of MCFAs as agonists of peroxisome proliferator-activated receptors has also been characterized (8). Moreover, accumulating evidence indicates that SCFAs generated by the gut microbiota exert influence on food intake, thereby regulating energy homeostasis and body weight [reviewed in (9-11)]. SCFAs and MCFAs also play an important role in the intracellular signalling and contribute to the regulation of cell metabolism [reviewed in (12-16)]. Finally, MCFAs and SCFAs can control cell death and survival (17-20). These important regulatory functions of MCFAs and SCFAs and their implications to human health and pathologies are subject of a number of excellent comprehensive reviews (1,7,21,22). Here, we want to concentrate on some peculiarities of the metabolic features of SCFAs and MCFAs that differ from those of LCFAs and to sum up the current understanding of their role in the cellular energy metabolism. Some aspects of SCFA and MCFA participation in energy-dependent mitochondrial processes have already been briefly reviewed by ourselves previously (23).

DEFINITION AND PHYSICOCHEMICAL PROPERTIES

SCFAs and MCFAs, being monocarboxylic acids with hydrocarbon chain length of 1 to 12 total carbon atoms, are abundant in nature, although they are present in plant and animal material at much smaller quantities than LCFAs (24,25). Fatty acids of total carbon atom numbers from 1 to 6 are usually classified as SCFAs, whereas those of 7 to 12 carbon atoms are defined as MCFAs. Fatty acids
with shorter chain, up to 9 total carbon atoms, are liquid at room temperature [Table 1, (26)]. The odor of the first members is pungent, whereas that of the higher members is rancid or none. The lipophilicity of SCFAs and MCFAs measured as partition of the free acid between water and heptane gradually increases with increasing the carbon atom chain length so that MCFAs become comparable in this aspect to LCFAs (27-30). Due to their lower lipophilicity as compared to LCFAs, SCFAs do not form micellar structures and do not participate in the formation of biological membranes (31). SCFAs and MCFAs are weak acids, with pKₐ values around 4.8, except of formic acid whose pKₐ is by about one unit lower (Table 1). Thus, their alkali metal salts are considerably hydrolyzed in aqueous solutions. Water-soluble members of the family have a high tendency to form bimolecular associates in water solution. Interestingly, incorporation of SCFAs and MCFAs into bilayer membranes is known to increase their pKₐ values similarly as in case of LCFAs (32,33).

ORIGIN OF MCFAS AND SCFAS

In human, the major source of SCFAs is the fermentation of dietary fiber and undigested saccharides in the gut by colonic anaerobic bacteria [(2); reviewed in (3,4,7)]. Acetate is mainly formed by reductive methylation of CO₂ (34). There are two main routes producing propionate by colonic bacteria. According to the methylmalonic-CoA pathway (also called dicarboxylic pathway), propionate is generated from lactate, supplied by lactate fermenting bacteria. In short, lactate is taken up by propionic bacteria and thereafter dehydrogenated to pyruvate, which becomes carboxylated by methylmalonyl-CoA-carboxyl transferase to oxaloacetate. Subsequently, the latter is converted to propionate through a four-carbon pathway, consisting of the intermediates malate, fumarate, succinate and methylmalonyl-CoA (34). It should be noted, that this pathway generates, in addition to propionate, one acetate molecule per two molecules of propionate. Other bacteria, such as Clostridium propionicum and Megasphaera elsdenii, produce propionate easily from lactate (35,36). According to this route, the CoA ester of lactate (lactoyl-CoA) is converted via acryloyl-CoA to propionyl-CoA, which becomes subsequently hydrolysed to propionic acid (the acrylate pathway) (34). Butyrate is formed by the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA, followed by the reductive conversion of acetoacetyl-CoA to butyryl-CoA (37). According to an estimation, acetate,
propionate, and butyrate are formed in the human colon at a ratio of about 3:1:1 (38,39). In an in vivo study, the rate of SCFAs release by the gut to the circulatory system amounts to about 35 µmol/kg body weight/h (40). The highest concentrations (70-140 mM) were found in the proximal colon (2). SCFAs may contribute to approximately 10% of the total human caloric uptake (4).

For newborn mammals, including human babies, mother’s milk constitutes an important source of MCFAs and SCFAs that are present mainly in the form of triglycerides and phospholipids. For example, the content of MCFAs (C\textsubscript{7:0} – C\textsubscript{12:0}) amounts to 6% - 17% and to 9% - 28% of all fatty acids in bovine (1) and human (41,42) milk, respectively. Cow milk and milk products remain the main dietary source of SCFAs, mainly butyric acid, in adult humans. Other natural sources of MCFAs and SCFAs are coconut oil and palm kernel oil [(1) and references therein]. In comparison to triglycerides containing LCFAs, those containing MCFAs are more rapidly hydrolyzed in the intestinal tract and do not become incorporated into chylomicrons. SCFAs and MCFAs are transported by portal bloodstream to the liver, where they are readily metabolized (21).

SCFAs and MCFAs can also be formed in mammalian and human tissues, mainly liver. Thus, the peroxisomal β-oxidation of LCFAs produces chain-shortened acyl-CoAs (43) that can be hydrolyzed inside peroxisomes by distinct acyl-CoA thioesterases and released into the cytosol. In addition, peroxisomes are also equipped with carnitine-acetyl and carnitine-octanoyl transferases and thus shortened acyl-CoAs are converted into carnitine esters for the supply to mitochondria [reviewed in (44)].

Under pathological conditions, for example in inborn medium-chain acyl-CoA dehydrogenase deficiency, octanoate and decanoate accumulate to considerable amounts in tissues (45) resulting in an impairment of functioning of the mitochondrial respiratory complexes (46). These disorders are often accompanied by elevated urinary excretion of dicarboxylic MCFAs (mainly adipic, suberic and sebacic acids) that apparently originate from microsomal ω-oxidation of corresponding medium-chain acyl-CoAs (47,48). Based on their rapid absorption, triglycerides of MCFAs were introduced as a quickly available energy source in clinical nutrition in the middle of the last century. Emulsions enriched with MCFA-containing triglycerides were applied to patients suffering from various forms of impaired digestion of normal LCFA-containing triglycerides (1). In addition, due to the rapid transport
of MCFAs from the gut to the liver, breath tests were developed for non-invasive clinical diagnosis using $^{13}$C-labeled octanoate (49,50). Thereby, specific hepatic pathways as well as the speed of gastric emptying can be measured [reviewed in (51)].

PRINCIPLES OF METABOLISM IN ANIMAL TISSUES

**Transport from the gut to the liver**

Butyrate is taken up by enterocytes presumably by means of the monocarboxylate transporter 1 (MCT-1) and the sodium-coupled monocarboxylate transporter 1 (SMCT-1) [reviewed in (52,53)]. Butyrate is used by these cells mostly as fuel. According to other authors, SCFAs are also absorbed from the intestine lumen by an exchange with Cl$^-$ (54) and/or HCO$_3^-$ (55). More recently, however, the non-ionic diffusion of protonated SCFAs and MCFAs across the colon epithelium has been favored (56). The latter mechanism is also supported by studies in model systems (30). According to this mechanism the intestinal absorption of SCFAs depends on pH, while slight acidification of luminal pH, possibly by bacterial metabolic activity, increases the prevalence of the protonated form of SCFAs. Otherwise, the transport of SCFAs from enterocytes into the blood might be driven by anion exchange. Thus, it seems likely that the transport across the basolateral membrane is based on the anionic form of SCFAs against HCO$_3^-$ (57). Butyrate as well as other SCFAs and MCFAs that have not been utilized by enterocytes are transported by the portal vein to liver (40,58) and metabolized by hepatocytes.

In contrast to LCFAs, which are esterified to triglycerides in enterocytes, incorporated into chylomicrons and then enter the lymphatic system, SCFAs and MCFAs from the intestinal tract enter the portal vein as free acids. There, MCFAs become partly bound to plasma albumin. The proportion between albumin-bound and free MCFAs increases with increasing chain length, so that the first equilibrium constant (i.e. for the strongest binding site) between the albumin-bound and the free forms increases from $1.5 \times 10^4$ for hexanoate, through $3.4 \times 10^4$ for octanoate and $10^5$ for decanoate, up to $2.4 \times 10^6$ for laureate (59,60). It has to be remembered that LCFAs are present in circulating blood practically totally bound to plasma albumin with the first equilibrium constant of the order of $10^7$ to $10^9$ (60). The subsequent uptake of SCFAs and MCFAs, at least the lower members of that group, by
liver and muscle cells as well as other tissues is independent of fatty acid binding proteins (1). Similarly, their uptake by cells requires neither fatty acid transport proteins, nor plasma membrane-embedded fatty acid translocase, nor cytosolic fatty acid-binding proteins. These observations provide a likely explanation why octanoate oxidation by isolated hepatocytes is about five times faster than that of oleate (61). Moreover, the intracellular metabolism of SCFAs and MCFAs seems to require no or much less fatty acid-binding proteins (62,63). In contrast, LCFAs require fatty acid-binding proteins for their cellular uptake, intracellular transport, regulatory functions and metabolism [reviewed in (63)]. Binding of free LCFAs or LCFA-CoA-esters to fatty acid binding proteins also minimizes their toxic effects, for example such as the lytic property or enzyme inhibition (63). Interestingly, in rats deficient in one of the fatty acid transport proteins (CD36 protein), feeding with SCFA- and MCFA-rich diet eliminated the increased glucose uptake, hyperinsulinemia and heart hypertrophy (64). Similarly, in CD36-deficient mice octanoate alleviated poor heart ischemic tolerance (65). These observations may have important implications to human medicine.

**Fuelling the tissue energy metabolism**

The utilization by different tissues of acetate formed by intestinal bacteria greatly differs between ruminants and non-ruminants [(4) and references therein]. Acetate is also endogenously generated in adult humans by ethanol oxidation, which operates mainly in liver (66). Thus, it has been shown that ethanol oxidation could result in a 20-fold increase of the acetate level in peripheral blood (67). In addition, net acetate generation has been found during fatty acid oxidation in perfused rat liver (68). Formed acetate results mainly from the operation of acetyl-CoA hydrolase (acyetyl-CoA deacylase), which in rat liver has been found predominantly in the mitochondrial matrix (69, 70). Because acetyl-CoA hydrolase is inhibited by free CoASH (K<sub>i</sub>=17 µM), the level of free CoASH has to be strongly lowered before hydrolase can produce free acetate from acetyl-CoA (70). On the other hand, free acetate produced in liver by oxidation of ethanol or as byproduct of ketogenesis is barely oxidized in this organ. This is because of high K<sub>m</sub> of hepatic mitochondrial acetyl-CoA synthetase (71) or its absence in these organelles [(72), see also the next paragraph]. Acetate can be, however,
transported by circulation to other organs, e.g. the heart and skeletal muscles, where $K_m$ of mitochondrial acetyl-CoA synthetase is much lower and where it can be utilized as fuel (70).

Activation of SCFAs occurs in the liver and several other tissues by acyl-CoA synthetases (72). These enzymes are located in the cytosol as well as in the mitochondrial matrix, where they are loosely bound to the inner mitochondrial membrane. In mammals, acetate is activated to acetyl-CoA by two different acetyl-CoA synthetases, of which one (AceCS1) is cytosolic (78 kDa, $K_m=0.11$ mM) and the other one (AceCS2) mitochondrial (71 kDa, $K_m=0.06$) (73). According to these authors (73), AceCS2 is present in a wide range of tissues, with the highest level in heart (bovine and rodent), but essentially absent in liver. In contrast, earlier investigations (74-76) demonstrated the presence of acetyl-CoA synthetase activity in hepatocytes in both mitochondria (20-50%) and the cytosol (50-80%).

Acetate is an important fuel during fasting as evidenced by the observation that in skeletal muscles of mice lacking AceCS2 the ATP content declined to 50% in comparison to wild-type mice (77). Interestingly, the activity of cytosolic and mitochondrial acetyl-CoA synthetases are regulated by a reversible acetylation. Furthermore, this process is under the control of NAD$^+$-dependent deacetylases sirtuin 1 and sirtuin 3 [reviewed in (78)]. Sirtuin 1 is a nuclear and cytoplasmic enzyme, whereas sirtuin 3 is predominantly located within mitochondria. In summary, in mammals acetate plays not only an important role in energy homeostasis but also, as a substrate for sirtuins, it is also involved in the regulation of gene silencing and aging processes (78).

In contrast to LCFAs that are activated to acyl-CoAs in the cytosol and must be transferred to the mitochondrial interior via the carnitine shuttle, SCFAs and MCFAs, at least those of carbon atom number up to $C_{9}$, permeate the inner mitochondrial membrane in the non-esterified form and are activated to their CoA-derivatives in the mitochondrial matrix. Localization of medium chain acyl-CoA synthetase in the mitochondrial matrix has been first described already in the late 60’s of the past century [(79); for more recent reports see (80, 81)]. The latter property may have important metabolic consequences under specific conditions as will be discussed further. SCFAs and MCFAs activated inside mitochondria are used as substrates in mitochondrial β-oxidation and the citric acid cycle. Interestingly, it has been demonstrated using the perfusion technique that in rat liver and heart
octanoate can also undergo peroxisomal β-oxidation, thereby delivering acetyl-CoA to the cytosol (82, 83).

As the energy source for tissue metabolism, triglycerides of MCFAs have several advantages compared to those of LCFAs. Firstly, they are more rapidly digested and the liberated MCFAs are more quickly absorbed in the intestinal lumen (21,84). Secondly, tissue metabolism of SCFAs and partly of MCFAs does not depend on proteins for binding, transport and transmembrane translocation (see the preceding sub-chapter). Therefore, they can serve as better energy-providing fuel than LCFAs, especially under pathological conditions, as exemplified by severe inflammation (85). Finally, MCFAs, having a slightly lower energy content than LCFAs (8.4 instead of 9.2 kcal/g), reduce body fat mass and enhance the insulin sensitivity of tissues [reviewed in (1,21,22,62)].

As said before, SCFAs and MCFAs are transported by blood from the alimentary tract to the liver where they are metabolized, and therefore they are not stored in the adipose tissue. Only by prolonged feeding rats with portacaval anastomoses (blood circulation overpassing the liver) diets containing MCFAs, the group of van Itallie (86) succeeded to significantly enrich the tissue depot lipids in triglycerides containing higher MCFAs (C₈, C₉ and C₁₀). General features of the whole-body metabolism and physiological functions of MCFAs, in particular octanoate, the most abundant MCFAs, have been recently summarized (87,88). Like other MCFAs, and in contrast to LCFAs, octanoate is rapidly degraded and is stored as triglyceride in the adipose or other tissues only in a very low extent. Octanoate as a fuel for the energy metabolism in mammals has been studied in high-energy requiring tissues such as skeletal muscle, heart, liver and brain (89-94). Concerning the latter organ, it is important to remember that SCFAs and MCFAs are able to permeate the blood-brain barrier (95).

The effects of SCFAs and MCFAs on the hepatic energy metabolism were studied mostly either by the perfusion technique of isolated rat liver (89,91-93,96-98) or by incubation experiments with isolated hepatocytes (99-101). In summary, these studies have shown that addition of octanoate and, to a lesser extent, butyrate enhances oxygen consumption compared to incubations with the Krebs-Henseleit buffer supplemented with pyruvate or lactate as energy supplying substrates. Both the stimulation of oxygen consumption and the associated increase of the cellular level of NAD(P)H (102)
indicate that these fatty acids effectively supply reducing equivalents (NADH, FADH$_2$) to the mitochondrial respiratory chain. In addition, octanoate raised the mitochondrial energization, an observation based on the in situ measurement of the mitochondrial membrane potential ($\Delta\Psi_m$) (101). Energization by octanoate of hepatocytes oxidizing pyruvate plus lactate was also manifest (100). However, in contrast to LCFAs (e.g. oleate), octanoate significantly raised the AMP level in the tissue (89,100). It has also been reported that feeding rats with MCFA-rich diet enhances skeletal muscle mitochondrial oxidative capacity (62,103), an observation which is partly attributed to an increased activity of citrate synthase (62).

Because MCFA-containing triglycerides are rapidly digested in the intestine, rapidly taken up by enterocytes and are not incorporation into chylomicrons, they are ideal energy-delivering nutrients in clinical situations, where the digestion and/or absorption of LCFA-containing triglycerides is impaired or a rapid energy uptake by the body is required. For this reason, MCFA-containing triglycerides have been used for the nutrition of patients with inherited LCFA $\beta$-oxidation disorders (104). While increasing evidence indicates that the diseased heart suffers from energy deficiency, fuelling the myocardial energy metabolism with MCFAs has been proposed as metabolic therapy for treating patients suffering from certain cardiomyopathies (87). For this treatment, MCFAs with odd carbon-atom numbers appeared superior compared to those with even carbon-atom numbers (105).

Modulation of carbohydrate and lipid metabolism

Contrary to LCFAs, the oxidation of MCFAs is not affected by the carbohydrate content in the diet. Thus, it has been reported that adaptation of adult rats to low-fat high-carbohydrate or high-fat low-carbohydrate diet does not change the rate of octanoate oxidation measured in hepatocytes. In contrast, oleate oxidation declined by 50% in rats adapted to low-fat high-carbohydrate diet (61). Furthermore, MCFAs derived from digestion of MCFA-containing triglycerides are predominantly degraded by the hepatic mitochondrial $\beta$-oxidation. Excess of formed acetyl-CoA is used for the synthesis of ketone bodies (mostly acetoacetate and $\beta$-hydroxybutyrate), which are delivered as fuel to non-hepatic tissues (21,22). MCFA-containing triglycerides are preferentially hydrolyzed compared to those containing LCFAs and liberated MCFAs are also preferentially oxidized in organs, mostly heart,
muscles, kidneys, and liver (93, 106). In vitro studies on isolated hepatocytes and perfused rat liver have shown that SCFAs and MCFAs modulate the hepatic metabolism of carbohydrates and lipids. Thus, butyrate and octanoate inhibit glycolysis (107, 108) and thereby exert the “glucose sparing activity”. In contrast, anabolic pathways of glucose formation (100,109-111) and lipogenesis (107,112) become stimulated. For illustration, glucose formation by hepatocytes fed with pyruvate plus lactate as gluconeogenic precursors is about 2-fold stimulated by octanoate (100). In contrast to inhibiting glycolysis in hepatocytes, decanoate, but not octanoate, has been found to stimulate glycolysis in astrocytes, thus resulting in an enhanced release of lactate into the extracellular space (113). Since lactate is considered a key energy source for neurons, the astrocyte/neuron lactate shuttle supplies this substrate to neighbouring neurons.

Generally, it has been discussed that mitochondrial matrix enzymes, pyruvate carboxylase and the pyruvate dehydrogenase complex, are regulated by the ratios of acetyl-CoA/CoA, ATP/ADP and NADH/NAD\(^+\) and, in addition, by pyruvate concentration. On the other hand, however, the fatty acid (octanoate, palmitate)-induced increase of pyruvate flux through both enzymes has been explained exclusively by an increased uptake of pyruvate into the mitochondrial matrix compartment (109,111). It has been argued that the formation of acetoacetate from fatty acids drives pyruvate uptake across the inner mitochondrial membrane. Therefore, there is reason to hypothesize that SCFAs and MCFAs play a supporting role in the utilization of physiological low concentrations of pyruvate or lactate for glucose generation (104). Nevertheless, acceleration of pyruvate uptake is not sufficient to explain the huge stimulation by fatty acids of glucose generation with aspartate plus glycerol as gluconeogenic precursors (110). Such stimulation is generally attributed to the generation of acetyl-CoA (allosteric effector of pyruvate carboxylase) and reducing equivalents (114), the latter promoting formation of glyceraldehyde-3-phosphate. It is also worthy to remember that pyruvate carboxylation in isolated rat liver mitochondria is strongly stimulated by L-octanoylcarnitine, whereas non-esterified butyrate and octanoate exert a strong inhibition (100). In addition, octanoate exerts a short-term dual regulatory effect on the hepatic fatty acid synthesis, namely stimulation in the low concentration range (up to 1 mM) and inhibition at higher concentrations (107,112). The stimulation of lipogenesis has been attributed to the activation of acetyl-CoA carboxylase, presumably by a covalent modification of the
enzyme. Moreover, studies with digitonin-permeabilized hepatocytes have shown that stimulation of the acetyl-CoA carboxylase activity depends on the chain length of the fatty acid (112). The stimulation magnitude increased from capronic (C₆:0) to lauric (C₁₂:0) acids, but decreased with fatty acids of longer chain length. Malonyl-CoA, the product of the cytosolic acetyl-CoA-carboxylase reaction, acts as a substrate for fatty acid synthesis but also as an inhibitor of carnitine palmitoyltransferase I.

There is an ongoing discussion that SCFAs and MCFAs activate hepatic AMP-dependent kinase (AMPK) [reviewed in (115) and references therein]. Generally, AMPK activation inhibits ATP-utilizing processes in the cell and stimulates those that produce ATP. Being a cytosolic enzyme, AMPK is activated by elevation of cytosolic AMP. Consequently, the mechanism underlying the SCFA/MCFA activation of AMPK is not clear, since the activation of SCFAs and MCFAs to their acyl-CoA esters raises the intramitochondrial AMP level. Interestingly, a recent study with mouse L6 myotubes suggests that AMPK can also be activated without alteration of the cytosolic AMP/ATP ratio. According to this suggestion the activation mechanism of AMPK by MCFAs is mediated by extracellular Ca²⁺-dependent Ca²⁺/calmodulin-dependent kinase kinase β (116). Other reported effects of SCFAs and MCFAs on the anabolic pathways are the inhibition of triglyceride synthesis in adipocytes (117) and a sparing effect on the hepatic glycogen storage (118). The latter activity is attributed to the competition between fatty acid and glucose oxidation.

The fact that MCFAs with odd-chain and even-chain hydrocarbon skeletons exert different effects on the cell energy metabolism is of particular interest and practical importance (119, 120). In contrast to even-chain MCFAs, β-oxidation of odd-chain MCFAs generates acetyl-CoA and, in addition, propionyl-CoA, which is anaplerotic for the citric acid cycle. Propionyl-CoA can enter the citric acid cycle after its conversion into succinate. The anaplerotic function of odd-chain MCFAs seems to be crucial for maintenance of the level of citric acid cycle metabolites in various tissues. This biochemical background explains the proposed use of the MCFA-derived triheptanoin (glycerol triheptanoate) as an anaplerotic drug (121, 122) for the treatment of cardiomyopathies in long-chain fat oxidation disorders (105) and pyruvate carboxylase deficiency (123). This anaplerotic function of odd-chain MCFAs is also important during episodes of epilepsy when the neurons become excessively
excited and thereby release increased amounts of glutamate (124,125). It is assumed that glutamate release is likely to decrease the level of citric acid cycle metabolites and thereby declines the oxidation of acetyl-CoA by mitochondria. Indeed, it has been recently shown that triheptanoin partially restores the level of citric acid cycle metabolites in an epileptic animal model (126). Triheptanoin is also able to attenuate harmful side effects associated with ischemic stroke (127). For illustration, when mice were exposed to transient ischemia, triheptanoin reduced the extracellular level of glutamate released in the mouse striatum, maintained the cellular ATP content at the desired level and prevented a decline of the respiratory activity of isolated brain mitochondria. The latter findings strongly suggest that the mitochondrial ATP regeneration is a target of the trihepatoin action (127). It is also worthwhile to mention that, in sharp contrast to butyrate and octanoate, the odd-chain SCFA propionate has no inhibitory effect on glycolysis and does not stimulate ketogenesis (108). Similarly to propionyl-CoA formed by the cellular degradation of odd-chain fatty acids, external propionate supplies the gluconeogenic pathway with its hydrocarbon skeleton, an activity that is mostly observed in ruminants [reviewed in (128)].

POTENTIAL ADVERS EFFECTS

Energy coupling

LCFAs are long known as mild uncouplers of oxidative phosphorylation due to their protonophoric effect on the inner mitochondrial membrane [reviewed in (23)]. The mechanism of this effect has been comprehensively explained by Skulachev (129,130) who showed that LCFA anions can be transferred across the inner mitochondrial membrane by adenine nucleotide translocase, whereas the non-dissociated fatty acid molecules can move across the membrane by flip-flop mechanism. As effect, a net proton transfer occurs. Subsequent studies have shown that LCFA anions can also be transferred across the mitochondrial inner membrane by a number of other mitochondrial inner membrane anion carriers [reviewed in (131,132)]. This protonophoric effect decreases the electrochemical proton gradient across the inner membrane, thereby decreasing the efficiency of oxidative phosphorylation. Such activity of LCFAs has been repeatedly reported in vitro with isolated mitochondria and there is evidence that protonophoric uncoupling can also operate in vivo after
hypoxia/reperfusion or high fat diet (133,134). With isolated mitochondria, uncoupling by LCFAs can easily be quantified as an increase in the resting state respiration by micromolar concentrations of these acids. In contrast to LCFAs, the ability to stimulate the resting state respiration by SCFAs or MCFAs is either oligomycin-sensitive (C₄ to C₈) or weaker, even when applied at millimolar concentrations (100,132,135,136). Furthermore, addition of octanoate or decanoate (at 100 or 300 µM concentration) to cultured neurons or astrocytes did not stimulate their respiration (113).

Based on the fatty acid cycling hypothesis, this difference between LCFAs on one side and MCFAs and SCFAs on the other side can be discussed in two aspects, namely in terms of (i) varying permeation rate of fatty acids across the mitochondrial inner membrane and (ii) various affinities of fatty acid anions to mitochondrial anionic carriers depending on the fatty acid chain length. In this context it can be expected that SCFAs and MCFAs exhibit lower solubility in the lipid core of the inner mitochondrial membrane because of their lower lipophilicity. In fact, it has been shown (30) that the permeation of fatty acids across phosphatidylethanolamine bilayers depends on their partition coefficient between hexadecane and water and the latter decreases with decreasing hydrocarbon chain length (137). In addition, there is reason to speculate that the binding of the anionic forms of SCFAs and MCFAs to the mitochondrial anion carries is lower than those of LCFAs. Such a view is supported by the observation that inhibition of the adenine nucleotide carrier by acyl-CoA thioesters declines with their hydrocarbon chain length (138). Nevertheless, there is evidence, mostly from studies on isolated liver cells and perfused liver, that SCFAs and MCFAs can initiate ATP wastage (89,96,97,99,100). It has been speculated (100) that this effect may be associated with a futile cycling between the esterified forms of SCFAs or MCFAs and their acyl-CoA thioesters. This would result in a net ATP utilization. Thus, a high rate of acetyl-CoA hydrolysis in rat hepatocytes, its stimulation by increasing acetate concentrations and substrate cycling between acetate and acetyl-CoA have been suggested (139-141). In the case of acetate this cycling is likely to occur in the cytoplasm of hepatocytes and may account for as much as 1% of the total heat production (141).

Butyrate and octanoate are known to stimulate oxygen uptake in perfused liver and isolated hepatocytes, to raise the energization of mitochondria and to support ATP-dependent glucose generation (100,101,109). However, it has been repeatedly observed that these two fatty acids increase
oxygen uptake and dramatically lower the ATP/O ratio when added to the incubation or perfusion media supplemented with pyruvate plus lactate or lactate alone (89,96,97,100), thus pointing to an impairment of the energy generation. The mechanism of this effect could not be explained by protonophoric uncoupling of oxidative phosphorylation by these SCFAs and MCFAs, as the latter was characteristic for LCFAs (see the preceding chapter). The main argument against such a mechanism was the observation that oligomycin, a well known inhibitor of mitochondrial ATPase/ATP synthase, abolished most of the enhanced hepatic oxygen consumption (89,99) as well as the octanoate-stimulated oxygen uptake by isolated rat liver mitochondria (100). Other authors attributed the impairment of energy metabolism by butyrate and octanoate to the stimulation of Na\(^+\)/K\(^+\)-ATPase (99) or to an increase in the FADH\(_2\)/NADH ratio due to β-oxidation (96). However, these explanations seem unlikely since a similarly enhanced oxygen uptake in the presence of LCFAs was not sensitive to oligomycin. A further clue seemed to be a stationary elevated AMP level, which was not observed with LCFAs (89,100,142-144). This pointed to an increased turnover of ATP within mitochondria rather than to its impaired production. In addition, this putative ATP turnover competed with intramitochondrial ATP-dependent reactions, i.e., pyruvate carboxylation (100) and citrulline synthesis (144). We have shown (100) that this phenomenon is due to enhanced activation of octanoate within the inner mitochondrial compartment, accompanied by utilization of two high-energy bonds per each molecule of octanoyl-CoA formed. Because both octanoyl-AMP and octanoyl-CoA could be partly hydrolyzed within the mitochondrial matrix, a futile cycle appeared that was responsible for the increased intramitochondrial ATP consumption that resulted in lowering the mitochondrial membrane potential and thus increasing oxygen uptake (Fig. 1). This mechanism may prevent a drastic depletion of intramitochondrial free CoA under high supply of SCFAs and MCFAs with the portal vein. In addition, the octanoate activation-associated increased AMP level decreases the intramitochondrial pool of exchangeable adenine nucleotides ATP and ADP, an event that slows down the operation rate of the adenine nucleotide translocase and thereby enhances the control strength of this transporter on the total flux of oxidative ATP generation (145).

These specific properties of SCFAs and MCFAs may explain the well-known facts that diets rich in these fatty acids increase energy expenditure and decrease adiposity (146-148). It has been
reported (149,150) that MCFAs, in contrast to LCFAs, contribute to maintain a high sensitivity of muscle cells to insulin. This view has, however, not been confirmed by other authors (151). It is also worthy to note that CoA esters of SCFAs and MCFAs accumulate in tissues at various pathological situations such as the Reye syndrome (152). Furthermore, it has been reported (153) that octanoyl-CoA at low millimolar concentrations exerts a strong inhibition on complex III activity of the respiratory chain.

**Generation of reactive oxygen species**

Oxygen consumption by mitochondria is accompanied by the generation of reactive oxygen species (ROS), of which β-oxidation is the most important source (154-157). Theoretically, a one-electron transfer to molecular oxygen, thereby forming superoxide, can take place from complex I of the respiratory chain as well as from acyl-CoA dehydrogenase, the electron transfer flavoprotein (ETF), the ETF-ubiquinone oxidoreductase, and complex III (Fig. 2, more details are given in the legend). Indeed, it has been demonstrated for skeletal muscle mitochondria that several sites are involved in the β-oxidation-linked superoxide generation (158). H$_2$O$_2$ release by mitochondria from (rat) skeletal muscle, heart, and liver was measured with carnitine derivatives of palmitate, octanoate, and butyrate as substrates (157,159). These studies have shown that the β-oxidation-associated ROS generation is similar with LCFAs and MCFAs (157,159). In contrast, it has been reported that C2C12 myotubes treated with capric (C$_{10}$) or lauric (C$_{12}$) acid generate less ROS than treated with LCFAs myristic or palmitic acid, whereas the oxygen consumption is higher with MCFAs than with LCFAs (149). These authors speculate that this decrease in ROS production might be attributed to an increased expression of uncoupling protein-3 by MCFAs. However, other authors (160) did not observe increased expression of uncoupling protein-3 in hearts of rats fed MCFA-rich diet.

Along with their direct role in ROS generation as electron donors to the respiratory chain in β-oxidation, fatty acids also play an indirect effect on superoxide production due to modifying both the rate of the electron flux along the respiratory chain and the degree of energy coupling. As discussed by ourselves in detail elsewhere (161), LCFAs potentiate ROS generation due to their weak inhibition of the electron flow at the levels of complexes I and III, most likely by interaction within the complex
subunit structure, and between complexes III and IV due to the release of cytochrome c from the inner membrane. These effects occur in ROS generation accompanying the so-called forward mode of electron transport. On the other hand, due to the protonophoric action of LCFAs on the inner mitochondrial membrane (“mild uncoupling effect”), they strongly decrease ROS generation in the reverse mode of electron transport (161). Contrary to this, SCFAs and MCFAs, at least lower members of the latter, at low physiological levels neither affect functioning of the electron transport chain nor exert a protonophoric effect on the inner mitochondrial membrane. On the other side, however, excessive accumulation of MCFAs that occurs under inborn medium-chain acyl-CoA dehydrogenase deficiency (45) and is connected with an impairment of the mitochondrial respiratory chain complexes (46) may lead to increased ROS production. This results in an increased lipid peroxidation, generation of protein carbonyls (as peroxidation products) and a decrease in the non-enzymatic antioxidant defense (162).

CONCLUDING REMARKS

Although SCFAs and MCFAs, compared to LCFAs, constitute a minor component of human and most mammalian diets, they play important roles both as nutrients and metabolic regulators. In addition to their content in food, a large proportion of SCFAs is contributed by the intestinal microflora by fermentation of otherwise undecomposed food constituents, mostly undigested carbohydrates. As conclusion, a proper maintenance of gut microflora is important both for better utilization of food constituents and as a source of molecules important as metabolic regulators.

During the past three or four decades, multiple roles of SCFAs and MCFAs have been uncovered within the cellular and whole body metabolism. Along with their function as “fuels” for the oxidative generation of ATP, SCFAs and MCFAs supply anabolic pathways (gluconeogenesis and lipogenesis) with carbon-containing precursor molecules and contribute to the regulation of the cell metabolism by triggering signalling pathways. Thus, MCFAs and, in particular, SCFAs play an important role in a proper balance between lipogenesis and oxidative degradation of fatty acids. Many of these multiple mechanisms of SCFAs and MCFAs still await full elucidation.
REFERENCES


Legends to Figures

Fig. 1. Uncoupling by LCFAs and pseudo-uncoupling by SCFAs and MCFAs of energized mitochondria.
A. Real protonophoric uncoupling by long-chain fatty acids. Undissociated LCFAs undergo spontaneous flip-flop movements across the inner mitochondrial membrane. In the alkaline environment at the inner (matrix) side of the membrane, they undergo dissociation to proton (H⁺) and the fatty acid anion (RCOO⁻), which is subsequently transported by the adenine nucleotide transporter (ANT) and other mitochondrial anion carriers back to the external side of the membrane. Here, the LCFA anion becomes re-protonated and can undergo another flip-flop transfer.

B. Pseudo-uncoupling by short- and medium-chain fatty acids. SCFAs and MCFAs are activated to their CoA thioesters in the mitochondrial matrix compartment. This process utilizes ATP and releases AMP and pyrophosphate (PPᵢ). AMP can subsequently react with ATP yielding two molecules of ADP that are re-phosphorylated at the expense of the mitochondrial transmembrane potential (Δψₘ) thus producing an uncoupling-like effect. In addition, both acyl-AMP and acyl-CoA are subject to slow hydrolysis thus increasing AMP production and futile energy utilization.

Fig. 2. Electron transfer from fatty acids to complex III during β-oxidation and possible sites of superoxide generation.
Shown is a simplified scheme summarizing the sites of superoxide generation supported by the mitochondrial degradation of fatty acid thioesters. Electrons are donated from the first enzyme of the β-oxidation pathway, acyl-CoA dehydrogenase (Acyl-CoA-DH), and are transmitted via the electron-transfer protein (ETF) to electron-transferring ubiquinone oxidoreductase (ETF-QOR). ETF-QOR reduces ubiquinone (Q) to ubiquinole (QH₂). Finally, ubiquinole becomes oxidized to ubiquinone and subsequently electrons move to complex III. 3-Hydroxyacyl-CoA dehydrogenase (HO-CoA-DH), the third enzyme of the β-oxidation pathway, which oxidizes HO-acyl-CoA to keto-acyl-CoA, donates electrons directly to complex I. Sites of superoxide generation are indicated in red.
Table 1. Nomenclature and basic physical properties of short- and medium-chain fatty acids.

Data from ref. 21.

<table>
<thead>
<tr>
<th>Number of carbon atoms</th>
<th>Systematic name</th>
<th>Common name</th>
<th>Formula</th>
<th>Melting temperature (°C)</th>
<th>Boiling temperature (°C)</th>
<th>pKₐ</th>
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<tbody>
<tr>
<td>1</td>
<td>Methanoic acid</td>
<td>Formic acid</td>
<td>HCOOH</td>
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<td>100.8</td>
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<td>2</td>
<td>Ethanoic acid</td>
<td>Acetic acid</td>
<td>CH₃COOH</td>
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<td>118.1</td>
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<td>3</td>
<td>Propanoic acid</td>
<td>Propionic acid</td>
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<td>140.9</td>
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<td>4</td>
<td>Butanoic acid</td>
<td>Butyric acid</td>
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<td>162.5</td>
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<tr>
<td>5</td>
<td>Pentanoic acid</td>
<td>Valeric acid</td>
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<td>-34.5</td>
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<td>6</td>
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<td>Caproic acid</td>
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<td>205</td>
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<tr>
<td>7</td>
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<td>Oenanthic acid</td>
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<td>-7.5</td>
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<td>4.84</td>
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<tr>
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<td>Caprylic acid</td>
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<td>Pelargonic acid</td>
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<td>Capric acid</td>
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<td>Undecylic acid</td>
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<tr>
<td>12</td>
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<td>Lauric acid</td>
<td>CH₃(CH₂)₁₀COOH</td>
<td>44</td>
<td>225&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> At reduced pressure of 100 mm Hg.
Fig. 1

A Uncoupling by ANT-mediated fatty acid cycling

Matrix compartment

\[ \Delta \]

Intermembrane

H^+ + COOH \rightarrow COO- + H^+

B Pseudo-uncoupling by ATP

ATP \rightarrow RCOOH \rightarrow Acyl-AMP \rightarrow Acyl-CoA \rightarrow oxidation

PP_i \rightarrow AMP \rightarrow 2 ADP

Partial hydrolysis

Partial hydrolysis
Fig. 2