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Acylcarnitines—old actors auditioning for new roles in metabolic physiology

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Abstract

Perturbations in metabolic pathways can cause substantial increases in plasma and tissue concentrations of long-chain acylcarnitines (LCACs). For example, the levels of LCACs and other acylcarnitines rise in the blood and muscle during exercise, as changes in tissue pools of acylcoenzyme A reflect accelerated fuel flux that is incompletely coupled to mitochondrial energy demand and capacity of the tricarboxylic acid cycle. This natural ebb and flow of acylcarnitine generation and accumulation contrasts with that of inherited fatty acid oxidation disorders (FAODs), cardiac ischaemia or type 2 diabetes mellitus. These conditions are characterized by very high (FAODs, ischaemia) or modestly increased (type 2 diabetes mellitus) tissue and blood levels of LCACs. Although specific plasma LCAC concentrations and chain-lengths are widely used as diagnostic markers of FAODs, research into the potential effects of excessive LCAC accumulation or the roles of acylcarnitines as physiological modulators of cell metabolism is lacking. Nevertheless, a growing body of evidence has highlighted possible effects of LCACs on disparate aspects of pathophysiology, such as cardiac ischaemia outcomes, insulin sensitivity and inflammation. This Review, therefore, aims to provide a theoretical framework for the potential consequences of tissue build-up of LCACs among persons with metabolic disorders.

Introduction

Acylcarnitines are intermediate oxidative metabolites that consist of a fatty acid esterified to a carnitine molecule.[1] They are generated by both mitochondrial and peroxisomal enzymes, including the carnitine palmitoytransferase 1 (CPT1) and carnitine O-palmitoyltransferase 2 (CPT2) enzymes, for the purpose of transporting long-chain fatty acids across the mitochondrial membrane for β-oxidation.[2]

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Author contributions

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Competing interests

The authors declare no competing interests.

Fatty acid oxidation disorders (FAODs) consist of over 20 autosomal recessive inherited metabolic diseases[2] affecting approximately 1 in every 9,000 births worldwide[3, 4]. Lesions in critical oxidative enzymes can lead to a reduction of complete combustion of fatty acids and an increase in intermediate lipid metabolites, including acylcarnitines

Thus, acylcarnitines have long been used as diagnostic biomarkers of inherited disorders of metabolism, because the type and concentration of plasma acylcarnitines qualitatively mimic tissue pools of acyl coenzyme A (CoA) located upstream and downstream of the enzymes specifically affected in these conditions[5]. Fatty acid oxidation disorders affecting enzymes involved in long-chain fatty acid catabolism can lead to accumulation of plasma and tissue long-chain acylcarnitines (LCACs) [2]. Research into the biology of LCACs conducted to date has covered a range of topics, including their use as biomarkers; metabolism via the carnitine O-palmitoyltransferase system; analyses of LCAC-mediated ion flux; LCAC-associated inflammation and insulin resistance; interaction with cell signalling proteins; and involvement in cellular stress responses.

By examining the published literature on LCACs, this Review aims to provide a working model for the mechanisms whereby LCACs might contribute to physiology and pathophysiology phenotypes associated with metabolic pathways.

Generation and transport of LCACs

Physiological pathways

Before any discussion regarding the potential impact of LCACs on cell physiology and pathophysiology, it is important to briefly consider the enzymes involved in the generation and transport of these metabolites. The carnitine O-acyltransferase system has been extensively reviewed elsewhere.[6] Here, we focus on the acylcarnitine generation and transport system as a whole.

Oxidation of long-chain fatty acids (LCFAs) is a unique process that involves the coordinated activity of several enzymes to convert lipid into usable energy. A key requirement is the transport of LCFAs across the cell membrane, enabling delivery to the mitochondria for combustion (Figure 1). The mitochondrial membrane is impermeable to both free and esterified CoA molecules and, consequently, LCFAs (>14 carbon atoms), which are essential cofactors for lipid metabolism, must be esterified to carnitine to form an acylcarnitine molecule that can be transported into the mitochondria.[7] The esterification of carnitine with fatty acyl-CoA yields an LCAC molecule plus free CoASH (CoA with sulfhydryl functional group); this process is catalysed by CPT1. Three tissue-specific isoforms of CPT1 have been identified in mammals; namely, CPT1a (liver), CPT1b (muscle) and CPT1c (brain). The mitochondrial carnitine/acylcarnitine carrier protein (CAC), which is thought to be in close proximity to CPT1 in the mitochondrial membrane space, facilitates the exchange of LCACs and carnitine across the inner mitochondrial membrane. Once transported to the inner leaflet of the mitochondrial membrane, LCACs are retroconverted by CPT2 to LCFA-CoA, which then enters the β-oxidation cycle to yield free carnitine. Of note, both CAC and CPT2 can catalyse both the forward and reverse reactions; the latter can also produce LCACs from intramitochondrial LCFA-CoA. Under certain conditions, when

LCFAs cannot be fully or efficiently metabolized, pools of intramitochondrial LCFA-CoA accumulate and drive conversion to their respective LCACs, which can be exported out of the mitochondria and ultimately the cell.[8, 9]

With respect to metabolite transport, CAC is member of the solute carrier family 25 (SLC25) group of membrane transport proteins.[10] Some data suggest that two specific members of the SLC22[11] and SLC16 group proteins could be responsible for cellular acylcarnitine efflux. SLC22A5 seems to play the dominant role in fatty acid oxidation.[12] For example, when SLC22A5 was isolated from renal cells and reconstituted into liposomes, it was found to act as both a Na-dependent carnitine co-transporter and an acylcarnitine antiport exchanger [11] By contrast, SLC16A9 (monocarboxylate transporter 9) might export acylcarnitines in a pH-dependent manner.[12] Interestingly, both SLC22 and SLC16 group proteins have the ability to transport amino acids, indicating additional roles of these proteins beyond acylcarnitine transport.[13, 14]

Inherited metabolic disorders

Exported acylcarnitines accumulate in the plasma and urine. Consequently, patterns of acylcarnitines in these biofluids can be used as diagnostic markers of inherited diseases of fatty acid and amino acid metabolism in which dysfunction of oxidative enzymes can lead to increases or decreases in the size of mitochondrial and cellular LCFA-CoA pools.[2, 15, 16]

Fatty acid oxidation disorders include inherited conditions, such as CPT2[17], medium-chain acyl Co-A dehydrogenase[18], very long-chain acyl Co-A dehydrogenase (VLCAD), and mitochondrial trifunctional protein deficiencies[19], that block or diminish activities of key enzymes involved in the transport and oxidation of fatty acids.[2] Some FAODs specifically affect enzymes required for the catabolism of LCFAs, including CPT2[17], CAC[20] and very long-chain acyl-CoA dehydrogenase (VLCAD)[21], as well enzymes of the mitochondrial trifunctional protein complex, such as trifunctional enzyme subunit alpha, mitochondrial (a.k.a. long-chain 3-hydroxyacyl-CoA dehydrogenase [LCHAD]) [22] (Figure 1 and Figure 1 inset). CPT2 disorders can lead to reduced CPT2 enzyme activity and increase the concentration of LCACs, especially C16-carnitine but also C18-carnitine, C18:1-carnitine and C18:2-carnitine. Plasma C16-carnitine concentration has been reported to increase more than five-fold in some CPT2-deficient newborns, reaching concentrations of 5–44 μM.[23, 24] This finding reflects the fact that CAC and CPT1 remain functional despite diminished downstream catabolism of LCACs when CPT2 activity is reduced. Very long-chain acyl-CoA dehydrogenase deficiency is marked by a decrease in VLCAD enzyme activity resulting in elevated plasma or urine levels of LCACs and other acylcarnitines (C12-carnitine, C12:1-carnitine, C14-carnitine and C14:1-carnitine).[24] Isolated deficiency and reduced protein activity of LCHAD and complete trifunctional protein complex deficiency can lead to increases in plasma and urine levels of long-chain hydroxy (OH) acylcarnitines, specifically C16-OH, C16:1-OH, C18-OH and C18-OH [16], with C16-OH plasma concentrations increasing to >4 μM.[24]

Clinical manifestations of FAODs are wide-ranging and include myopathies, peripheral neuropathy, dyslipidaemia, rhabdomyolysis and associated increases in plasma creatine kinase, which tend to be episodic in nature and typically manifest under catabolic conditions

in which lipolysis is triggered.[2] In many cases of FAOD, symptoms and clinical episodes can be evaded or reduced in severity by avoiding excess exercise, and through nutritional interventions that include medium-chain triglycerides that deliver medium-chain fatty acids as fuel that enters β-oxidation downstream of the affected enzymes for LCFA catabolism in FAOD.[25-27]

In summary, FAODs leading to LCAC accumulation share some clinical features that, when examining the totality of LCAC literature, could arise due to acylcarnitines as discussed below.

Bioactivity and possible lipotoxic effects of LCACs

Much of the FAOD literature has focused on the development of diagnostic tests based on mass spectrometry. By contrast, the remainder of this Review will offer new perspectives on the bioactivity of LCACs by highlighting the growing body of evidence to support the concept that these naturally-occurring lipid derivatives directly influence cell metabolism and health outcomes. As discussed below, LCACs can affect several biological processes, including cardiac function[28], ion balance[29], insulin signalling in muscle[30], inflammation[31], cellular stress[32], and outcomes related to modulation of protein kinase C.[33] Gaining insight into the physiological and pathophysiological actions of LCACs will fill a knowledge gap that might in turn improve understanding of the underlying causes and symptoms of FAODs and other metabolic diseases.

Effects on cardiac tissue

To date, much of the research on acylcarnitine bioactivity has been performed using cardiac cells and tissue because heart muscle derives ~50–70% of its energy needs from fatty acids.[34] However, both fuel and oxygen are required for the efficient and complete β-oxidation of LCFAs.

Hypoxia—Concentrations of LCACs in the sarcolemma increased in the range of 2-fold to 10-fold within minutes of inducing cardiac hypoxia (and hence blunting LCFA β-oxidation) in neonatal rat cardiomyocytes.[28] The observed increase in LCAC levels coincided with the cessation of cellular electrical activity by the cardiomyoctyes; however, this effect was rescued by incubation of the cells with a CPT1 inhibitor before induction of hypoxia.[18]

Conflicting reports have been published regarding the physiological consequences of LCAC accumulation.[35] Nonetheless, some studies have identified clear electrophysiological and cellular responses to elevated amounts of LCACs as a consequence of hypoxic conditions. For example, elevated LCAC levels and a twofold to threefold increase in the number of α-1 adrenergic receptors were detected in adult canine myocytes within 10 minutes of inducing hypoxia.[36] These effects were reversed when the cells were pre-incubated with a CPT1 inhibitor. This inhibition also prevented tachycardia and fibrillation when delivered by intravenous injection to cats 10 minutes before induction of hypoxia.[37] Even with the interpretive caveat that chemical inhibitors can sometimes influence off-target systems, these results support the concept that LCACs affect cardiac electrophysiology.

Membrane permeability and proteins—Treatment of human erythrocytes with 20 μM of D,L-C16-carnitine caused complete cell lysis within 15 minutes.[38] The researchers hypothesized that LCACs interact with the hydrophobic bonds that form between lipids and proteins in cell membranes. Langendorff perfusion of rat cardiomyocytes with C16-carnitine caused the release of two markers of membrane permeability (myoglobin and creatine kinase) at concentrations of 1.6 μM[39] and 5 μM,[40] respectively. Further work revealed that treatment with 5 μM C16-carnitine affected both isolated plasma membrane and the sarcoplasmic reticulum ATPases that regulate Na^+, K^+ and Ca^{2+} ion transport in canine[41] and rabbit[42] ventricular cardiomyocytes. While the exact mechanisms remain to be established, the net result was to increase intracellular Ca^{2+} concentrations, [43] possibly owing to efflux caused by sarcolemmal permeability [44] or through the sarcoplasmic reticulum.[45]

The activity of partially purified bovine heart Na^+,K^+ ATPase was inhibited by treatment with D,L-palmitoylcarnitine and L-palmitoylcarnitine at a half maximal inhibitory concentration of ~45 μM;[46] however, the physiological consequences of such inhibition in heart muscle are not clear. Ca^{2+} efflux from the sarcoplasmic reticulum was elicited by the direct stimulation by palmitoylcarnitine of the ryanodine receptor at concentrations as low as 5 μM, using purified sarcoplasmic reticulum from pig and rabbit skeletal muscle.[47] Cardiomyocytes were not tested in that study, but might be expected to respond similarly.[47] Additionally, as LCAC concentrations increased in the junctional sarcolemma of freshly isolated adult canine cardiomyocytes, decreased gap junctional conductance occurred following treatment with 5 μM exogenous L-C16-carnitine,[48] a possible link to cardiac dysfunction hypothetically through a decreased in cardiac conductance required for coordinated contraction.

Extracellular LCACs were also capable of directly modulating specific ion channels. For example, C16-carnitine at 20 μM reduced the K^+ current and depolarized resting membrane through effects on guinea pig cardiac inward-rectifying K^+ channels [49] and increased deactivation kinetics of the potassium voltage-gated channel subfamily H member 2 channel at 3 μM in a human embryonic kidney cell line.[50] The authors hypothesized these changes could lead to arrhythmias due to a reduction of action potential duration[50]. The LCAC 16-carnitine at a concentration of 1 μM activated a reconstituted pig L-type Ca^{2+} channel when applied to either the intracellular or extracellular side of the channel using the patch-clamp technique, indicating that the observed effects might be membrane-based rather than channel-specific.[51] Intracellular or extracellular application of LCACs (5 μM) via the voltage-clamp technique inhibited voltage-gated Ca^{2+} channels,[52] and slowed both the activation and inactivation of Na^+ currents,[29] in cultured cardiomyocytes isolated from guinea pig. Intriguingly, the observed changes to Ca^{2+} and Na^+ channels were reversed by washing the cells,[36, 37] suggesting that some of the effects were specific to the channels and not the membrane.

Mitochondria are proficient generators of LCACs; however, some evidence indicates that they can also be modulated by these metabolites in cardiomyocytes. The administration of 80 μM of D,L C16-carnitine decreased Ca^{2+} uptake in isolated rat cardiac mitochondria. [53] In isolated canine cardiac mitochondria, C16-carnitine (at concentrations as low as 3.3 μM)

elicited rapid mitochondrial Ca^{2+} efflux and oxidation of NADPH, effects that were abolished by pre-incubation with an inhibitor of mitochondrial Ca^{2+} uptake.[54] Further investigation revealed that 5-40 μM LCACs elicited Ca^{2+} efflux through effects on the Na^{+}, Ca^{2+} antiporter; higher concentrations of LCACs (50 μM) were associated with changes in mitochondrial membrane permeability and uncoupling of oxidation.[55]

Taken together, the data obtained from cardiac cells and mitochondria support the concept that changes in intracellular (and potentially extracellular) LCAC concentrations can affect heart function. The effects on cardiac activity and function could especially manifest during and following cardiac ischaemia, which is characterized by a marked in LCAC levels in the heart tissue.

Skeletal muscle and insulin resistance

Plasma and skeletal muscle concentrations of LCACs are modestly increased among persons with insulin-resistant obesity or type 2 diabetes mellitus. [56, 57-60, 46]. This increase is consistent with a hypothesis originally postulated from studies of insulin-resistant rodents and cultured myotubes.[61] The premise was that a mismatch can arise between fuel availability and the activity of the tricarboxylic acid cycle under conditions of excess lipid availability and/or incomplete fatty acid oxidation. This concept supports the observation of increased levels of LCACs among patients with type 2 diabetes mellitus.[57, 60, 62] LCFAs, or their chain-shortened derivatives that are unable to be fully combusted, accumulate in tissue and blood in the form of medium-chain acylcarnitines, LCACs, or acylglycines. LCFAs can also be converted to alternative metabolites such as diacylglycerol or ceramides.

Both diacylglycerol[63] and ceramides[64] have been implicated in the development of diabetic insulin resistance. Another hypothesis is that some acylcarnitines could negatively affect the insulin signalling pathway, perhaps through activation of inflammation.[57] Increases in inflammatory mediators, described below, have been shown to play a role in obesity-associated insulin resistance by inhibiting insulin receptor signalling.[65] Initially, much of the evidence to support this hypothesis that LCACs influence insulin resistance was correlative. An *in vitro* study demonstrated that co-incubation of a rat myotube cell line with fatty acids and carnitine decreased insulin-stimulated phosphorylation of the serine-threonine protein kinase Akt in a carnitine-dependent manner.[61] Akt signalling was restored following treatment with a CPT1 inhibitor, which led to reduced accumulation of LCACs.[61] Hepatic overexpression of malonyl-CoA decarboxylase (an enzyme that facilitates breakdown of malonyl-CoA, an inhibitor of CPT1) in rats increased insulin sensitivity in skeletal muscle (gastrocnemius) and modestly reduced muscle concentrations of LCACs.[66] Additionally, insulin resistance induced by a high-fat diet in mice was reversed by exercise, with a concomitant 2.8-fold rise in gastrocnemius peroxisome proliferator-activated receptor gamma coactivator 1-alpha expression over sedentary mice, and a drop in muscle LCAC accumulation to levels detected in mice fed standard chow.[67] Finally, whole-body knockout of nuclear receptor subfamily 4 group A member 1 (Nur77), a transcriptional regulator of glucose metabolism in the liver and muscle, led to an appreciable accumulation of LCACs in the gastrocnemius and induction of insulin resistance (as measured by decreased insulin-

receptor phosphorylation) in mice fed a high-fat diet compared with wild-type control mice.[68]

In other experiments, mouse or human myotubes were treated with exogenous C14-carnitine or C16-carnitine and both insulin-stimulated phosphorylation of Akt and glucose uptake were examined. LCAC concentrations of 5–10 μM blunted insulin-stimulated phosphorylation of Akt (mouse myotubes) and insulin-stimulated glucose uptake (human muscle cells).[30] Although plasma levels of LCACs are increased in obesity and type 2 diabetes mellitus,[57, 60] the concentrations are below those required to induce insulin resistance using *in vitro* cell models.[30] Nevertheless, localized tissue LCAC concentrations (both intracellular and extracellular) have yet to be fully determined in different disease states. Furthermore, it remains to be determined how extracellular administration of LCACs influences their concentrations in different cellular compartments. Tissue concentrations of all acylcarnitines, including LCACs, are probably substantially higher than the levels found in the bloodstream given the large dilution of metabolites in the circulation. In rodent models of obesity, using reported values for muscle C14-carnitine species, LCAC abundance per mg tissue (assuming 1 g is equivalent to 1 mL), could be at least ~5–10 μM.[61, 69]

Cellular stress responses

Inflammation—Lipid-associated inflammation is believed to be one cause of insulin resistance. Inflammation promotes signalling cascades, such as the transcription factor NF-κB and the c-Jun N-terminal kinase (JNK; mitogen-activated protein kinase [MAPK] group of proteins), that lead to increased expression of cytokines, serine phosphorylation of insulin receptor substrate 1 (IRS-1), and subsequent blunting of the insulin response. [70]

Ceramides act through both the JNK and NF-κB axes to increase insulin resistance, [71] whereas diacylglycerol affects phosphorylation of IRS-1 through activation of specific protein kinase C (PKC) subtypes in muscle (PKC θ) and liver (PKC ε).[72] Saturated LCFAs activate some pattern recognition receptors, such as toll-like receptor 2 (TLR2) and TLR4[73-76]; therefore it was postulated that acylcarnitines have the potential to mediate inflammation.[57] In a pilot study using a murine macrophage cell line, D,L-C12-carnitine and C14-carnitine activated a reporter gene for the critical inflammatory signalling node, NF-κB, indicating the potential of LCAC to activate NF-κB transcription and hence inflammation-associated pathways.[57] In follow-up studies using the same cell line, the naturally-occurring L-forms of LCACs activated reactive oxygen species and prostaglandin G/H synthase 2 (cyclooxygenase-2), an inflammation-mediating enzyme and NF-κB target gene, in a chain-length dependent manner.[31] Furthermore, L-C14-carnitine stimulated dose-dependent mRNA expression and secretion of pro-inflammatory cytokines, and stimulated phosphorylation of JNK and extracellular-signal-regulated kinases (ERK), common mediators of the inflammatory response. All of these responses were found to be MyD88-dependent, and MyD88 is a critical adaptor molecule for some receptor-mediated inflammatory signalling, indicating that they were probably receptor-mediated or membrane-mediated responses.[31] A study using mouse bone marrow-derived macrophages also showed that L-C12-carnitine activated pro-inflammatory cytokines. [77]

Together, these intriguing results support a potential pro-inflammatory role for some medium-chain acylcarnitines (i.e., C12-carnitine) and LCACs, triggered at concentrations an order of magnitude below that of saturated long-chain fatty acids.[73, 74] However, LCACs—although displaying MyD88-dependency for inflammation effects—did not activate any of the TLR family members in *in vitro* cell based receptor–reconstituted reporter systems[31], designed to overexpress target receptor proteins linked to activation of transcriptional proteins. Thus, it remains to be determined whether LCACs serve as ligands for as yet unidentified receptors, or if they modulate cellular functions through other mechanisms.

Lipotoxicity—Given the pro-inflammatory properties of LCACs in immune cells maintained in culture, it seems reasonable to suggest that these metabolites might also alter cellular stress outcomes in other cell types.

Skeletal muscle is not generally considered an inflammatory organ and it is a primary source of LCAC generation owing to its large concentration of carnitine and mitochondria, which enables high oxidative capacity. Therefore, under physiological conditions, LCACs would not be expected to elicit myocyte stress responses that lead to compromised cellular integrity. However, in certain disease states, such as FAODs or cardiac ischemia, excessive LCAC accumulation could in theory promote myocyte lipotoxicity. Myopathy and rhabdomyolysis are common (albeit episodic) clinical manifestations of FAODs associated with LCFAs that emerge under catabolic states that challenge the oxidative machinery and lead to accumulation of high levels of LCACs.[2]

In studies of mouse myotubes treated with varying extracellular concentrations of LC14-carnitine and L-C16-carnitine (≥5 μM) that might mimic episodic plasma concentrations among patients with FAODs,[23, 24] increases in interleukin 6 (IL-6) secretion (~10-fold) and gene expression (~2.5-fold) were detected.[30] Further studies using the same mouse myotube model revealed that high concentrations of LCACs can also elicit dose-dependent phosphorylation of JNK, ERK and p38 MAPK, and modestly increase the caspase-3 activity. L-C16-carnitine can induce the activity of recombinant caspase-3 at concentrations as low as 1 μM, and was speculated to be involved in Fas-mediated cell death.[78] In the mouse myocyte studies outlined above, the IL-6 inflammatory response was concurrent with a rise in markers of cell death and loss of membrane integrity (measured by adenylate kinase leakage), with a blunting of mitochondrial function, but no apparent apoptosis. Treatment with LCACs at concentrations as low as 5–10 μM increased mouse myotube intracellular Ca^{2+} levels, which in turn mediated the LCAC-associated production of IL-6 but not cell death and/or permeability[32]. As discussed in detail below, L-C16-carnitine can bind to,[33] and inhibit the ability of, the lipid-sensitive and Ca^{2+}-sensitive conventional PKCs(α, β_I, β_{II} and γ)) to phosphorylate substrates.[79] The findings of these studies, therefore, indicate that low concentrations of LCACs can increase myocyte intracellular Ca^{2+} and at high concentrations can trigger a Ca-dependent IL-6 response. LCACs might also activate caspases under some conditions. Thus, at the current time, excessive LCAC accumulation within or around skeletal (or heart) muscle cannot be ruled out as a potential contributing factor to the pathophenotypes observed among patients with long-chain FAODs.

A working model of LCAC functions

Along a normal-to-pathological spectrum of tissue accumulation, LCACs could theoretically regulate physiological processes and at high concentrations trigger cellular stress outcomes. As naturally-occurring acyl-group-containing zwitterions (neutral molecules with both positive and negative charge), LCACs could affect membrane-associated systems within the cell (Figure 2).

Mouse myotubes responded to LCACs in a similar manner and magnitude to the zwitterionic detergent molecule amidosulfobetaine-16 with respect to increased secretion of IL-6, increases in intracellular Ca^{2+} levels and cell permeability and/or death.[32] The amphipathic nature of LCACs promotes micelle formation, with C16-carnitine having a proposed critical micelle concentration of 75–100 μM, depending on the test system used.[80] Thus, our working model (Figure 2) predicts that under pathological conditions, LCACs alter plasma membrane fluidity and/or function, leading to cell stress, and compromised membrane integrity. At its most extreme, this situation might also contribute to the development of rhabdomyolysis and myopathy in some patients with FAODs. Under conditions that promote modest elevations in cellular LCAC concentrations (intense exercise or progression to type 2 diabetes mellitus), the model proposes that the natural LCAC zwitterions serve as modulators of cellular function by influencing membrane receptor-mediated signal transduction or specific ion channels. In other words, LCACs might participate in physiological regulation of membrane-based systems but also play a part in the pathological response when their concentrations become abnormally high.

This plasma membrane-focused model does not rule out the probability that LCACs interact with specific receptors or proteins, as demonstrated by the ability of C16-carnitine to activate recombinant caspase-3[78] and possibly alter the activity individual ion channels.[41, 49, 50, 52] Reports published in the 1980s provided evidence that palmitoylcarnitine inhibits or modulates PKC activity.[79, 81, 82]. A variety of cell biological effects of LCACs (at least for the most-studied metabolite, palmitoylcarnitine) have subsequently been attributed to modulation of PKC. For example, administration of low concentrations of palmitoylcarnitine (≤10 μM) altered PKC activity in a variety of cell types. In addition, this treatment also partially blocked the ability of some tumour-promoting agents to inhibit gap-junction intercellular communication in a rat liver epithelial cell line;[83] reduced Fcγ receptor-mediated endocytosis in human neutrophils;[84] and triggered terminal differentiation and reduced proliferation of leukaemia cells[85] and neuroblastoma cells. [86]

Furthermore, our working model does not negate the possibility that short-chain acylcarnitines exhibit bioactivity through mechanisms other than altering membrane fluidity or dynamics. Mouse myotubes treated with C4-carnitine display blunted insulin-stimulated phosphorylation of Akt, similar to the effects of C14-carnitine and C16-carnitine;[30] however, C4-carnitine does not elicit myocyte permeability and/or death, probably owing to short length of the acyl chain and hence a more limited membrane association than is observed with LCACs.[32] Studies on carnitine O-acetyltransferase , a major producer of acetylcarnitine, have shown the importance of this highly-abundant metabolite as a trafficking agent for carbon atoms and for mitochondrial protein acetylation.[87]

The exciting possibility also exists that, in addition to plasma and sarcoplasmic reticulum membranes, the interaction of LCACs with mitochondrial membranes influences mitochondrial function. Mitochondrial membranes are composed of both lipids and proteins, arranged as an outer membrane and an inner membrane. Key metabolic proteins are located within each type of membrane, including CPT1 (outer membrane), CPT2, uncoupling proteins, and proteins of the electron transport chain (largely the inner membrane).[88, 89] If the proposed LCAC—mitochondrial membrane interaction model is true, then the normal ebb and flow of LCACs, or alterations in the efficiency or completeness of β-oxidation, would be predicted to regulate a variety energetic processes and metabolite shuttles in the mitochondria. Under conditions of extreme LCAC accumulation, such as in FAODs, mitochondrial dysfunction and myopathy could ensue, even at LCAC concentrations well below those needed to permeabilize cell membranes. The concept that LCACs affect mitochondrial membrane-associated systems to influence energetics or other aspects of mitochondrial function remains speculative at this time, but forms the foundation for hypothesis-testing in future studies.

Future research prospects

A limitation of studies that use exogenously-administered LCACs is that the interaction between these metabolites and individual cells does not depend solely on the concentration of the exogenous LCAC. Indeed, the LCAC concentration relative to the number of cells and/or the amount of membrane in the experimental system will also influence the observed outcomes. This situation is further complicated by any potential binding of LCACs to cellular or extracellular proteins. Thus, additional research is warranted to understand the relative incorporation of exogenous and endogenous LCACs into cellular membranes and binding of LCACs to proteins internal or external to the cell. The difficulty encountered in measuring concentrations of ‘free’ (unbound) LCACs in and around cells to correlate changes in LCAC accumulation with physiological and pathophysiological outcomes should also be addressed.

The potential role of LCAC—membrane associations in PKC function is also an area of interest, considering the importance of membrane localization and membrane-derived factors in PKC biology.[90] These knowledge gaps remain to be addressed in future work.

Conclusions

This Review highlights the potential cellular effects of LCAC accumulation in both physiological and pathophysiological conditions. Although a direct link between acylcarnitine accumulation and human disease has yet to be confirmed, cell culture studies raise the possibility that some FAOD or diabetic phenotypes involve increased plasma and tissue levels of LCACs that influence cellular membrane-associated systems, including ion channels and specific proteins involved in intracellular cell signalling, such as caspases and PKC. These studies underscore the need for further clinical and *in vivo* research regarding LCACs and their cellular effects. The body of evidence presented in this Review strongly suggests that, regardless of the mechanisms involved, LCACs at concentrations encountered

in vivo do not just mark metabolic status, but rather play an active role in regulating metabolic physiology.

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Sean H. Adams has a Bachelors degree in Biology from California State University, Fresno, CA, USA; a Masters degree in Marine Sciences from the University of California, Santa Cruz, CA, USA; and a PhD in Nutritional Sciences from the University of Illinois, Urbana-Champaign, IL, USA. Dr. Adams is currently Director of the Arkansas Children's Nutrition Center, and Professor and Section Chief, Developmental Nutrition, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA. His research examines metabolic physiology and pathophysiology by interpreting analyte patterns emerging from metabolomics studies, in a range of systems from organelles to cells, rodent models and clinical experiments. His ultimate aims are to characterize the underlying factors that differentiate metabolic health from disease; to determine the origins of systemic metabolite patterns, including xeno-metabolites; and to identify clinically useful biomarkers of metabolic disease risk.

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Key points

- Long-chain acylcarnitines (LCACs) are lipid derivatives generated from carnitine by mitochondrial carnitine palmitoyltransferase 1 or through the reversible activity of carnitine palmitoyltransferase 2 on long-chain acyl-coenzyme A metabolites
- Long-chain acyl-coenzyme A metabolites are impermeable to mitochondrial membranes; therefore, LCACs act as the fatty acid transport moieties for oxidative catabolism
- Mutations in key enzymes increase mitochondrial and cytosolic pools of long-chain acylcoenzyme A, promoting an accumulation of LCACs that is diagnostic of fatty acid oxidation disorders
- LCACs can modulate inflammation, insulin sensitivity, myocyte stress, protein kinase C signalling and ion balance, suggesting that they contribute to both physiological and pathophysiological processes beyond fuel trafficking
- A model is proposed to explain how LCACs affect disparate cell systems on the basis of their zwitterion biochemical structure and published evidence that they interact with plasma membranes
- In this model, accumulation of LCACs is predicted to alter the activities of key receptors, transporters, channels and enzymes that associate with (or involve) plasma, mitochondrial or other membranes

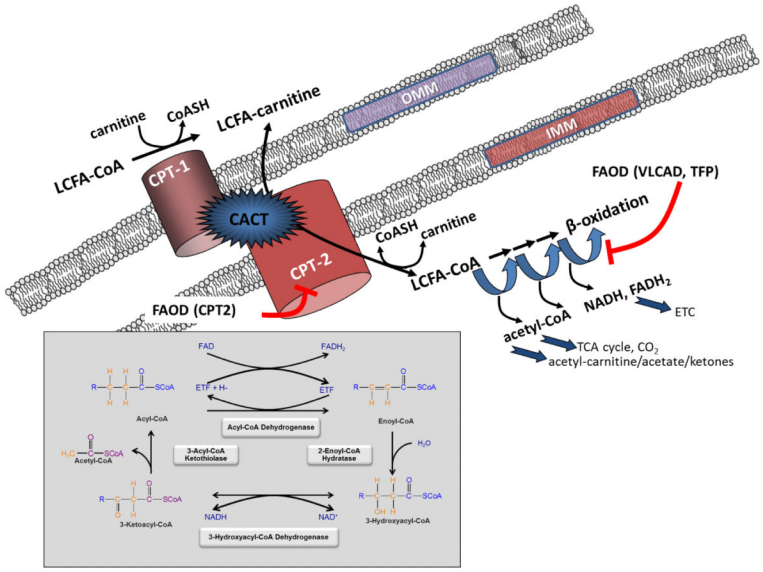

Figure 1.

Generation and transport of LCACs under physiological and pathophysiological conditions. CPT1 facilitates transesterification of a cytosolic carnitine molecule to LCFA-CoA, yielding LCAC plus free cytosolic CoASH. LCACs are transported across the mitochondrial membranes via CAC, where CPT2 catalyzes re-esterification to generate LCFA-CoA plus free carnitine. LCFA-CoA undergoes β-oxidation to generate reducing equivalents (acetyl-CoA, NADH and $FADH_2$) required for energy generation via the TCA cycle and the ETC. Upstream acyl-CoA intermediates accumulate if fuel delivery exceeds energy generation capacity. Under these conditions, intermediates are converted to ketones, acetyl-carnitine or acetate. LCFA-CoA is retroconverted to LCAC and transported out of mitochondria and ultimately, the cell. FAODs arising from defects in key enzymes (e.g. CPT2, TFP and VLCAD) are characterized by the accumulation of LCACs and reduced ketogenesis. Inset: Primary enzymes involved in long-chain fatty acid metabolism. Abbreviations: CAC, carnitine–acylcarnitine translocase; CoA, coenzyme A; CoASH, coenzyme A with sulfhydryl functional group; CPT, carnitine 0-palmitoyltransferase; ETC, electron transport chain; FAOD, fatty acid oxidation disorder; LCAC, long-chain acylcarnitine; LCFA, long-chain fatty acid; TCA, tricarboxylic acid; TFP, trifunctional protein; VLCAD, very long-chain acyl-CoA dehydrogenase. Inset pathway figure, modified, courtesy of J. Vockley (University of Pittsburgh, PA, USA).

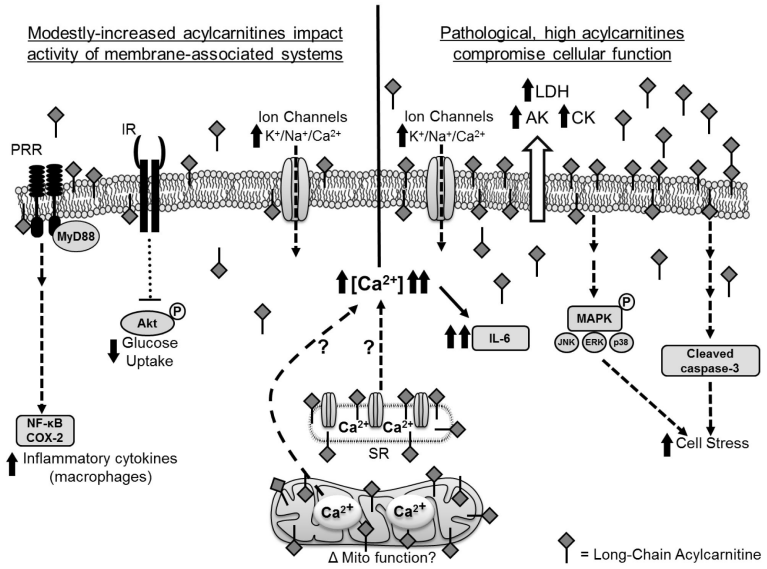

Figure 2.

Working model of LCAC modulatory activity. At modest concentrations (<10μM), zwitterionic LCACs interact with cell membranes to alter membrane proteins and membrane-associated systems, such as MyD88-associated PRR complexes, IR complexes and ion channels. Increased LCAC concentrations (10-25μM) could modulate cellular membrane ion channels to increase intracellular Ca^{2+} levels, potentially through effects on the sarcoplasmic reticulum and mitochondria. These effects might explain LCAC-associated increases in cellular inflammation (immune cells) and decreases in insulin-stimulated phosphorylation of Akt and glucose uptake (myocytes). At LCAC concentrations that mimic severe symptomatic fatty acid oxidation disorders (>25μM), cellular permeability could occur, leading to release of the intracellular proteins and subsequent cell death (i.e. rhabdomyolysis or cardiac-cell death). LCACs might increase release of IL-6 through Ca^{2+}-mediated pathways. Furthermore, high concentrations of LCACs are linked to activation of cellular stress pathways (MAPK, JNK, ERK and p38) and cleavage of caspase-3. LCACs could also regulate mitochondria to alter energy metabolism. Abbreviations: AK, adenylate kinase; CK, creatine kinase; ERK, extracellular-signal-regulated kinase; IL-6, interleukin 6; IR, insulin receptor; JNK, c-Jun N-terminal kinase; LCAC, long-chain acylcarnitine; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; PRR, pattern recognition receptor.