REVIEW ARTICLE

The malonyl-CoA–long-chain acyl-CoA axis in the maintenance of mammalian cell function

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Long-chain acyl-CoA esters have potent specific actions (e.g. on gene transcription, membrane trafficking) as well as non-specific ones (e.g. on phospholipid bilayers). They are synthesized on the cytosolic aspects of several intracellular membranes, to give rise to (a) cytosolic pool(s) to which a variety of enzymes and processes have access, including some localized in the nucleus. Their concentration in cells is highly regulated, interconversion with corresponding acylcarnitines being the most important mechanism involved. This reaction is catalysed by cytosol-accessible carnitine long-chain acyl (palmitoyl) transferase

activities that are themselves located on multiple membrane systems. Regulation of these activities is through the inhibitory action of malonyl-CoA. Hence the existence of a potent malonyl-CoA–acyl-CoA axis through which many processes involved in the maintenance of mammalian cell function are regulated. The molecular, topographical and physiological interactions that make this possible are described and discussed.

Key words: carnitine palmitoyltransferase, diacylglycerol, fatty acids, liver, secretion.

INTRODUCTION

There has been heightened interest in recent years in the role of malonyl-CoA as a regulator of metabolism, as it is increasingly realised that its functions are not restricted to the reciprocal regulation of fatty acid oxidation and synthesis. Relevant recent reviews will be highlighted. The emerging role of malonyl-CoA as a metabolite that is intimately concerned with the maintenance of cell function illustrates that metabolic processes do not fit neatly within the confines fashioned for them in textbook ‘pathways’ or in metabolic charts. It demonstrates that a given metabolite, acting as an inhibitor of a specific enzyme, can exert a very high degree of control over a wide range of processes because of the amplification that results from modulation of the concentrations of substrates and/or products of the enzyme. Besides being an intermediate for fatty acid synthesis and elongation, malonyl-CoA acquires its significant regulatory role by virtue of its inhibition of a class of carnitine acyltransferases (collectively termed palmitoyltransferases or CPTo, so termed because of the overt or cytosol-facing active site; see [1] for a recent classification of these enzymes) that catalytically have access to the cytosolic pool(s) of long-chain acyl-CoA esters. CPTo enzymes catalyse the transesterification between long-chain acyl-CoA and the corresponding acylcarnitine esters. As a consequence of this reaction, the availability of the long-chain acyl-CoA and carnitine esters for various metabolic processes is modulated.

MALONYL-CoA SYNTHESIS AND DEGRADATION

Malonyl-CoA is the product of acetyl-CoA carboxylase (ACC), the activity of which is acutely modulated by reversible phosphorylation and by allosteric effectors (see [2–4] for reviews),

notably citrate (activation) and long-chain acyl-CoA esters (inhibition). Reversible phosphorylation at several serine residues plays a major role in the regulation of ACC activity [2,5]. The kinases that are thought to be most important *in vivo* are cAMP-dependent protein kinase (PKA) and AMP-activated protein kinase (AMPK) [6]. PKA and AMPK phosphorylate rat ACC₂₆₅ isoform (see below) at Ser⁷⁷ and Ser¹²⁰⁰, and at Ser⁷⁹, Ser¹²⁰⁰ and Ser¹²¹⁵ respectively. Inactivation appears to result from phosphorylation at Ser⁷⁹ and Ser¹²⁰⁰ [7]. It is now apparent that kinase action on ACC is dependent on the exact ACC isoform [7], and this may explain the different tissue distribution of the two isoforms: one of molecular size 265 kDa (ACC- α) and another of 280 kDa (ACC- β). The former occurs in tissues that have high rates of *de novo* fatty acid synthesis and, therefore, in which the flux through ACC is considerable. Skeletal and cardiac muscles also express ACC- β , although the possibility has been raised that cardiac ACC- β is distinct from that expressed in skeletal muscle [7]. Therefore, ACC dimers (and presumably higher polymers) can exist as α , β -heteromers [8]. The two forms are separate gene products and differ primarily in the 200-residue extension in ACC- β N-terminus, the most significant structural feature of which is its high degree of hydrophobicity, which has been suggested to play a role in channelling malonyl-CoA to CPTo in the mitochondrial outer membrane by insertion of the hydrophobic domain into the membrane [9]. However, as will be discussed below, CPTo is found abundantly in other intracellular membranes too, making specific outer-membrane targeting solely because of hydrophobic interactions unlikely. Phosphorylation of ACC by PKA or AMPK renders the enzyme inactive and insensitive to activation by citrate. Interestingly, in some tissues (e.g. liver), even when PKA is specifically activated through elevation of cyclic AMP levels (e.g. by exposure to glucagon), the

Abbreviations used: CPT(o), (overt) malonyl-CoA-sensitive carnitine palmitoyltransferase; ACC, acetyl-CoA carboxylase; PKA, cAMP-dependent protein kinase; AMPK(K), AMP-activated protein kinase (kinase); FAS, fatty acid synthase; ER, endoplasmic reticulum; CPT-I, mitochondrial-outer-membrane CPTo; CPT-II, mitochondrial-inner-membrane CPT; TM, transmembrane; ACBP, acyl-CoA binding protein; IMS, intermembrane space; NEFA, non-esterified fatty acid; PDH, pyruvate dehydrogenase; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; TAG, triacylglycerol; VLDL, very-low-density lipoprotein; GPAT, glycerol-3-phosphate acyltransferase.

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pattern of serine phosphorylation of ACC- α corresponds to that mediated by AMPK [10–12]. Detailed discussion of the mechanism for such cross-talk between the two kinase pathways leading to ACC inhibition is beyond the scope of this Review, but it is evident that modulation of AMPK activity is highly important in the regulation of ACC activity both in lipogenic and non-lipogenic tissues (see below).

Insulin is able to activate ACC by lowering tissue cAMP, by activating the phosphatase that inhibits AMPK [13–15] and possibly by activating a kinase that specifically phosphorylates an 'I' site within ACC [16]. Hormones that elevate tissue cAMP (β -adrenergic agents, glucagon) inhibit ACC activity, as do conditions that elevate AMP (e.g. activation of the rate of ATP utilization beyond the capacity of the tissue to synthesize ATP). Owing to the sensitivity of ACC to extra- and intra-cellular stimuli, the tissue content of malonyl-CoA is altered accordingly, in parallel with changes in ACC activity. This occurs if the processes through which intracellular malonyl-CoA is utilized are highly active relative to ACC but not saturated by malonyl-CoA at intracellular concentrations of the metabolite [3]. Fatty acid synthase (FAS) may partly fulfil this role, even in tissues that are not highly lipogenic, as the concentration of malonyl-CoA depends on the relative activities of ACC and FAS, rather than on the absolute flux through the fatty acid-synthetic pathway. However, it is possible that other malonyl-CoA-utilizing enzymes may be involved in terminating the malonyl-CoA signal to the cell in non-lipogenic tissues. Such a role has been suggested for the fatty acid chain-elongation system [17] and for malonyl-CoA decarboxylase [4,18]. However, malonyl-CoA decarboxylase is not thought to have a cytosolic distribution in mammalian tissues [19].

The potential for specialized functions of the two isoforms of ACC is currently highly speculative. In particular, suggestions have been made that ACC- α may be specialized for the regulation of *de novo* fatty acid synthesis, whereas ACC- β may be specialized for mitochondrial fatty acid oxidation [20,21]. The main kinetic difference between ACC- α and ACC- β is the higher K_m of the latter for acetyl-CoA [4]. However, it is more likely that the structural features of the β -form may enable the enzyme to respond to a range of intra- and/or extra-cellular stimuli different from those to which ACC- α responds. For example, there is recent evidence that ACC- α and muscle ACC- β may be differentially phosphorylated by the respective AMPK isoforms and by PKA in liver and muscle. In tissues that express significant activities of both isoforms, α , β -heterodimers/polymers may exist [7], making distinctive responses to external effectors a much more likely rationale for differential regulation of the two isoforms. Moreover, a clear dichotomy of function of the two isoforms is difficult to reconcile with the pattern of expression of ACC in tissues such as rat liver, in which mitochondrial fatty acid oxidation is controlled primarily by ACC α -mediated modulation of intracellular malonyl-CoA levels (but see below).

THE TARGET OF MALONYL-CoA: CPTo

Malonyl-CoA-sensitive CPT activity has been generally considered in relation to mitochondrial function. Hence the terminology of CPT-I (for mitochondrial-outer-membrane CPTo) and CPT-II, for the inner-membrane CPT (extensively reviewed elsewhere [22,23]). However, malonyl-CoA-sensitive CPT activity also occurs on the cytosol-facing aspects of the endoplasmic reticulum and of peroxisomes [24]. An activity has also been described in the nucleus [25] [or associated endoplasmic reticulum (ER)] and in the plasma membrane of human erythrocytes [26]. Therefore it has been suggested that malonyl-CoA-sensitive CPT

activities should be generically termed CPTo [23]. This is especially relevant in view of the strong evidence that the ER and peroxisomal CPTo are very similar to, if not identical with, the mitochondrial CPTo (CPT-I). In the liver, CPTo activity in each of these membranes is positively and quantitatively related to the labelling of an 88 kDa protein by dinitrophenyletomoxir [24], a substrate analogue inhibitor that binds covalently to the protein. In addition, microsomal and peroxisomal CPTo cross-react with several antibodies raised against linear epitopes of the mitochondrial outer membrane CPTo [24]. The mitochondrial and peroxisomal proteins have extremely similar kinetic properties, although the microsomal CPTo has a lower IC_{50} value for malonyl-CoA [27]. This property coincides with distinctive immunoreactive properties of the N-terminal domain of the microsomal protein [24].

The multiple location of identical or highly similar CPTo in different cell membranes raises important questions. First, are acylcarnitines effectively compartmented into separate functional pools according to the membrane on which they are synthesized?, e.g. are acylcarnitines generated at the mitochondrial outer membrane exclusively used by the mitochondria, or are they available to other organelles? The traffic of carnitine esters of chain-shortened very-long-chain fatty acids between peroxisomes and the ER and mitochondria [28] would appear to suggest that this is not the case, but does not explain the requirement for expression of the protein(s) at all three sites. It is possible that CPTo in membranes of different lipid composition or subtle differences in structure (such as those suggested by the observations of [24] on the N-terminal domain of CPTo) may affect the kinetic properties of the enzyme in different locations. Evidence supporting this has emerged from the work of Ramsay [27], who showed that the malonyl-CoA-sensitivity of microsomal CPTo is about 2-fold higher than that of the peroxisomal and mitochondrial enzyme, and that it does not show such a large change in kinetic characteristics as the others on fasting of the rats.

Two isoforms of malonyl-CoA-sensitive CPT have been identified: liver-type (L- or α) and muscle-type (M- or β); details of their tissue distribution have been given elsewhere [29]. There is an interesting correlation between the tissue distribution of L- and M-isoforms of CPTo and the two isoforms of ACC. Thus, in the rat, ACC- α predominates in those tissues (e.g. liver, pancreatic β -cell, kidney) in which CPTo- α (L- or liver form) predominates, whereas ACC- β is present in tissues (e.g. heart, skeletal muscle) that express primarily CPTo- β (M- or muscle form). L- and M-CPTo isoforms will be discussed in detail below, but the two most prominent kinetic differences between them are the 10–100-fold difference in their sensitivity to malonyl-CoA (M-type being more sensitive) and the several-fold difference in their K_m for carnitine (M-type having the higher K_m) [30,31]. It is noteworthy, however, that the total tissue content of malonyl-CoA is of the same order of magnitude (low micromolar) in tissues as metabolically disparate as cardiac muscle and liver [32], whereas the content of carnitine is markedly higher in aerobic muscles (e.g. heart) than in liver. Although the significance of this correlation in ACC and CPTo isoform distribution can only be speculative at present, it would appear that the expression of the respective isoforms is closely linked and may be related to the requirements of different cell types for specific concentrations of metabolites (e.g. acetyl-CoA, carnitine) in the cytosolic compartment. Therefore it would be appropriate, when considering the roles of the two ACC isoforms, to take into account that they function against a background of the different kinetic properties of the two isoforms of the enzyme (CPTo) upon which their product, malonyl-CoA, acts. The possibility that overall

tissue malonyl-CoA content is compartmentalized within micro-environments (e.g. in the vicinity of CPTo-containing membranes) cannot be ruled out at present.

MALONYL-CoA ACTION ON CPTO

Malonyl-CoA-sensitive CPTs are integral proteins of the membranes in which they reside and, at least in the mitochondrial outer membrane and the ER, appear to have the same membrane topology elucidated in detail for the CPTo of the mitochondrial outer membrane [33,34]. The protein has two transmembrane (TM) segments within the N-terminal 15% of its primary amino acid sequence, and both the N- and C-termini protrude into the cytosolic compartment, with only a short loop between the two TM segments on the non-cytosolic side of the membrane [in mitochondria this is the intermembrane space (IMS)]. Corroborative evidence suggests that this loop would protrude into the lumina of the ER and peroxisomes [24]. Functionally, this topology results in both the catalytic and regulatory (malonyl-CoA-binding) sites being exposed on the cytosolic aspect of the respective membranes [24,33,34]. The possible teleological rationale for this topology is twofold. First, long-chain acyl-CoA esters and malonyl-CoA are impermeant through phospholipid bilayers, although permeant through the mitochondrial outer membrane (by virtue of the presence of porin in the latter). Therefore, this impermeability necessitates that the binding sites for CoA esters should be expressed on the cytosolic aspect of the ER and peroxisomal membranes. Secondly, even though the mitochondrial outer membrane is permeable to molecular species smaller than approx. 5 kDa in mass [35], the tight binding of long-chain acyl-CoA by acyl-CoA binding protein (ACBP; $K_D < 10^{-10}$ M [36]) coupled to the equimolar abundance of ACBP and acyl-CoA [37] makes it likely that the effective substrate for CPTo (and other long-chain acyl-CoA-utilizing processes) is the ACBP-long-chain acyl-CoA complex (molecular mass approx. 11 kDa) [34]. This is larger than the effective cut-off of mitochondrial-outer-membrane porin [35]. Moreover, having the acyl-CoA binding site on the cytosolic aspect of the outer membrane obviates the need to maintain a relatively high concentration of long-chain acyl-CoA esters within the IMS, i.e. in the proximity of the inner membrane, the energy conservation function of which is known to be deleteriously affected by the general detergent properties of these molecules, as well as by their specific interactions with inner-membrane proteins, e.g. the adenine nucleotide translocator [38]. Movement of acyl moieties into the IMS of the mitochondria is presumed to occur as acylcarnitine esters. These too are membrane-active, but their concentration within the IMS is suggested to be kept extremely low through the action of the carnitine/acylcarnitine carrier expressed on the inner membrane, which has a very high affinity for long-chain acylcarnitines [39].

The hairpin topology of CPTo within the mitochondrial outer membrane was also shown to be important for the expression of the kinetic characteristics of the enzyme [33,34,40]. It was shown that the extreme N-terminus is essential for the expression of the malonyl-CoA sensitivity of CPTo and of optimal catalytic activity. As the catalytic site is thought to reside within the C-terminal domain of the enzyme, and as malonyl-CoA and long-chain acyl-CoA binding to the protein appear to mutually interfere at a common site or close-set sites [41], it was suggested that N-to-C interdomain interactions are crucial for the maintenance of the C-domain in a conformation that is optimal for expression of catalytic activity and malonyl-CoA sensitivity. These conclusions have now been confirmed through the expression of mutants of the protein having N-terminal truncations

or specific amino acid substitutions [42–44]. In particular, the importance of Glu³ and His⁵ for the expression of malonyl-CoA sensitivity has been demonstrated [43,44]. In addition, the observation that a CPTo protein with a Glu³ → Ala substitution also has a markedly lower specific activity [43] has confirmed the role of the extreme N-terminus in the maintenance of full catalytic activity [33,34]. However, it has not been ascertained yet whether the N-terminus is important because it is directly involved in contributing residues to the malonyl-CoA-binding and catalytic sites or because its interaction with the C-domain maintains the latter in an optimal conformation for the acquisition of the required three-dimensional conformation of these sites. It is noteworthy that the mutant proteins with an altered N-terminus retain the same kinetic characteristics, with respect to carnitine and palmitoyl-CoA, as the native protein, in spite of losing their malonyl-CoA sensitivity [43]. Similarly, studies on chimaeras in which the entire C-terminal domain [plus the TM segment (TM2) proximal to it] of the M- and L-type CPTo were exchanged, the malonyl-CoA sensitivity and other kinetic characteristics of the expressed chimaeric proteins were very similar to those of the respective native proteins ([44]; see also below).

It is to be emphasized that the sensitivity of L-CPTo to malonyl-CoA, as well as the K_m for palmitoyl-CoA, are both modulated by physiological state [45–48] (but not in cardiac muscle [49]) and that these changes (i) can be mimicked by experimentally induced changes in the fluidity of the mitochondrial outer membrane *in vitro* [50] and (ii) are correlated with changes in the molecular order of the lipids of outer membranes prepared from mitochondria isolated from livers of rats maintained under different physiological conditions [51]. It is inferred that protein-membrane interactions are likely to be important in determining the malonyl-CoA sensitivity of CPTo, and that they are likely to be due to interaction between the TM segments as well as between them and the annular membrane lipids [34]. Such interactions are also anticipated to modulate the degree of interaction between the N- and C-domains and may determine the kinetic characteristics of the protein under different physiological conditions. Therefore, it may be relevant that there are important structural differences between the predicted TM segments of the L- and M- isoforms of CPTo that may contribute towards their distinctive kinetic characteristics and their responses to membrane-fluidity changes *in vivo* [51] and *in vitro* [50,52].

It is sometimes stated that malonyl-CoA is an allosteric inhibitor of CPTo (see, e.g., [4]). In fact the mechanism of action of malonyl-CoA on CPTo is not known. Blocking of the active site abolishes high-affinity binding of malonyl-CoA to mitochondria [41], but it is not known whether this is due to allosteric or steric effects. Malonyl-CoA does not act as an orthodox competitive inhibitor of CPTo with respect to palmitoyl-CoA, as it induces sigmoidicity in plots of activity against increasing palmitoyl-CoA concentrations *in vitro* [53] (further complicated by palmitoyl-CoA-albumin interactions [54]). However, malonyl-CoA inhibition is always attenuated at higher palmitoyl-CoA concentrations, implying a competitive-type mechanism. Both catalytic activity (through effects on the K_m for carnitine) and malonyl-CoA sensitivity are greatly affected by pH in the region 7.1–6.5 [31,55], giving rise to the possibility that critical histidine residues exist that determine the kinetic parameters of the protein. His⁵ and His¹³⁹ in the rat liver and muscle CPTo isoforms have been suggested to fulfil this role for malonyl-CoA sensitivity [43,44]. However, the same residues do not appear to be involved in determining the K_m for carnitine [44]. These combined observations suggest that there are other, as yet

Scheme 1 Metabolic consequences of the accumulation of the reactants of CPT₀, the activity of which is inhibited by malonyl-CoA

Further abbreviations: TCA, tricarboxylic acid cycle; PKC, protein kinase C; PL, phospholipid. Broken lines indicate an effector action of long-chain acyl-CoA.

undescribed, factors that determine the kinetic characteristics of CPT₀, especially since an inverse relationship between the affinity of the two isoforms for malonyl-CoA and carnitine exists which has not been reflected in the mutants studied so far [31].

CONSEQUENCES OF INHIBITION OF CPT₀ BY MALONYL-CoA

CPT₀ catalyses a freely reversible transesterification reaction between long-chain acyl-CoA, carnitine, CoA and acylcarnitine. Therefore, theoretically its activity is able to equilibrate the concentrations of its reactants in the cytosolic compartment. However, in practice the acylcarnitine esters are rapidly removed into the respective organelles, e.g. into the mitochondrial and peroxisomal matrices, because of the high expression of a carnitine/acylcarnitine translocase in the matrix-bounding membranes of these organelles [24]. Such rapid removal of the long-chain acylcarnitines gives the CPT₀-catalysed reaction a high degree of unidirectionality. Moreover, the effective activity of the enzyme *in vivo* is expected to be considerably below its potential maximum *in vitro*, owing to the action of malonyl-CoA. Therefore, modulation of CPT₀ activity has two major consequences: increased activity results in increased provision of long-chain acylcarnitines for utilization within the respective compartments (ER lumen, mitochondrial and peroxisomal matrices), and (ii) decreased activity will result in increased concentrations of long-chain acyl-CoA esters within the cytosolic compartment. Some possible functional consequences of changes in CPT₀ activity are summarized in Scheme 1.

FUNCTIONS OF LONG-CHAIN ACYLCARNITINES

Movement of acyl moieties across membranes for β -oxidation

The formation of acylcarnitines enables acyl moieties to cross intracellular membranes, which are otherwise impermeable to acyl-CoA esters. This occurs through the action either of a specific carnitine/acylcarnitine carrier [39,56], highly expressed in both mitochondrial inner membrane and peroxisomal membrane [24], or by a channel mechanism within the ER membrane [57]. In all three membrane systems, acyl-CoA is regenerated on the luminal/matrix side of the membrane through the action of a second, latent (i.e. non-cytosol-facing), CPT. These latent CPT activities are products of three distinct genes [58–60], that for the mitochondrial protein (usually termed CPT-II) being the best characterized (see [23,29] for reviews). The possible fates of the

acyl-CoA generated by these latent CPT activities are shown in Scheme 2. In mitochondria, complete β -oxidation of matrix long-chain acyl-CoA generates acetyl-CoA, which is used to generate reducing equivalents and thence ATP. In the liver, neonatal small intestine, astrocytes and lymphocytes, acetyl-CoA is also used in the formation of ketone bodies. In hepatic peroxisomes only limited β -oxidation of very-long-chain acyl-CoA occurs and results in the formation of chain-shortened acyl moieties that can be transported (presumably as acylcarnitines) either for complete β -oxidation in mitochondria or for glycerolipid synthesis in the ER, depending on the identity of the acyl moiety [28].

Role of malonyl-CoA–acyl-CoA axis in fuel selection by muscle

In aerobic muscle (including heart and diaphragm) the formation of acylcarnitines is an important route for the generation of ATP through mitochondrial β -oxidation. In these tissues the malonyl-CoA–long-chain acyl-CoA axis forms part of the array of mechanisms used in fuel selection in response to changes in the relative availability of substrates and hormones. Moreover, the effect of malonyl-CoA on mitochondrial CPT₀ enables the tissue to respond instantaneously to a deficit between the rate of formation of ATP and that of its utilization. This involvement arises directly from the formation of malonyl-CoA from glucose (see Scheme 3). Under conditions characterized by adequate availability of glucose and insulin, ACC exists in the active state (see above) which favours malonyl-CoA formation and inhibition of fatty acid oxidation. Conversely, under conditions characterized by high concentrations of plasma non-esterified fatty acids (NEFA) the formation of malonyl-CoA from glucose is impaired through the inhibition of glycolysis, pyruvate dehydrogenase and ACC [61]. Consequently, malonyl-CoA levels are diminished and oxidation of fatty acids by the tissue is favoured at the expense of glucose oxidation. Indeed, under these conditions most of the pyruvate from glucose is not oxidized, but converted into lactate. Such mechanisms are expected to operate in skeletal muscle at the onset of exercise. Inhibition of ACC, with consequent lowering of tissue malonyl-CoA, occurs as a result of the increased deficit between the rate of ATP production and ATP utilization that leads to increased formation of AMP and activation of AMPK. The ensuing fall in tissue malonyl-CoA content activates fatty acid oxidation [4,62,63]. Moreover, in untrained rats and humans, graded exercise results in a fall in

Scheme 2 Possible metabolic fates of acylcarnitine esters after gaining access to the matrices or lumina of different subcellular compartments in the liver

Further abbreviations: ERM, PM, IM, endoplasmic-reticular, peroxisomal and mitochondrial inner membranes respectively; CAC, carnitine acylcarnitine carrier; LC, long-chain; VLC, very-long-chain.

Scheme 3 Concerted action of insulin at the adipose and muscle levels to co-ordinate the utilization of fatty acids and glucose as substrates

Abbreviation: LC, long-chain. Broken lines indicate actions of insulin and malonyl-CoA; dotted lines indicate multi-step pathways.

the peripheral insulin concentration accompanied by increased blood glucose; concomitantly, the concentrations of glucagon and catecholamines increase [64–66]. These changes are expected to lead to the inactivation of ACC and the lowering of malonyl-CoA, as indeed observed experimentally [62,63]. As insulin has an anti-lipolytic effect on adipose tissue, the exercise-induced direct enhancement of the ability of muscle to oxidize fatty acids coincides with increased delivery of NEFA to the tissue through the circulation.

Ruderman and colleagues (see [4] for a review) have emphasised the role that citrate may play in linking glucose and fatty acid oxidation (in parallel rather than reciprocally) through its effect on malonyl-CoA concentrations. Citrate has long been

known to inhibit glycolysis at the reaction catalysed by fructose 6-kinase (see [61] for a recent review) and is also the immediate precursor of cytosolic acetyl-CoA, and an allosteric activator of ACC (see above). Although the activity of ATP citrate-lyase is very low in muscle [67], the absolute magnitude of the flux leading from mitochondrial acetyl-CoA to cytosolic malonyl-CoA is immaterial for the maintenance of muscle malonyl-CoA concentrations, as rate of malonyl-CoA utilization is also low (see above). Interestingly, a stronger correlation is found between malonyl-CoA content of muscle and the combined total tissue concentrations of citrate plus malate rather than citrate alone [68,69]. In muscle, both these metabolites arise mainly from mitochondrial oxaloacetate due to the low activity of ATP-citrate lyase [67]. Therefore, their combined concentrations reflect (i) the rate of formation of intramitochondrial acetyl-CoA (from pyruvate, fatty acids, ketone bodies) and (ii) the redox state of the mitochondria – a higher NADH/NAD⁺ ratio resulting in the malate ↔ oxaloacetate equilibrium being shifted towards malate. This may explain the inability of acetoacetate to raise citrate and malonyl-CoA concentrations when presented as the sole substrate to the perfused heart [68], as, although acetoacetate forms intramitochondrial acetyl-CoA, in the short-term it also oxidizes the NADH/NAD⁺ couple through equilibration with 3-hydroxybutyrate. This oxidation of the NADH/NAD⁺ couple not only favours oxaloacetate formation from malate (a counter-ion for citrate efflux from mitochondria), but also increases tricarboxylic-acid-cycle flux [70], with consequent diversion of citrate away from efflux (the citrate transporter is very poorly expressed in muscle mitochondria [71]). As expected from these considerations, acetoacetate does not prevent glucose from raising cellular malonyl-CoA content [68], because the net effect of acetoacetate would be to favour flux through pyruvate dehydrogenase (PDH) by lowering the intramitochondrial concentrations of both NADH and acetyl-CoA (both inhibitors of PDH). Therefore, the relationship between muscle citrate plus malate content and malonyl-CoA concentrations arises simply from the combined ability of a fuel (e.g. glucose, fatty acids, 3-

hydroxybutyrate) to raise tissue NADH and to generate intramitochondrial acetyl-CoA. Acetoacetate only fulfils the latter requirement.

Cardiac muscle

The role of the malonyl-CoA–long-chain acyl-CoA axis in fuel selection is also evident in cardiac muscle (see [4,72] for reviews). The heart depends on fatty acid oxidation for a large proportion (70 %) of its energy requirements, even under normoinsulinaemic conditions. In hypoinsulinaemic or insulin-resistant states this proportion is even higher, owing primarily to the chronic inhibition of PDH [73]. The latter is achieved acutely through the generation of mitochondrial acetyl-CoA as a product of fatty acid oxidation, and chronically through the fatty acid-mediated increase in the expression of PDH kinase [74,75]. Carnitine and its short-chain derivatives are able to overcome this inhibition, primarily by shifting the mitochondrial carnitine acetyltransferase-catalysed equilibrium away from acetyl-CoA and towards acetylcarnitine (see [76]). Propionyl-L-carnitine is particularly effective in promoting glucose oxidation at the expense of that of fatty acids, possibly because, in addition of the above effect of the carnitine moiety, the propionyl moiety generates oxaloacetate in the mitochondria, thus promoting the further utilization of intramitochondrial acetyl-CoA and increasing the rate of citrate formation (see [76]). The possibility that acetylcarnitine formation could lead to inhibition of CPTo through the formation of cytosolic malonyl-CoA [77] appears to have receded in view of the demonstration that cardiac muscle does not express a cytosolic carnitine acetyltransferase activity [78]. However, previous reports had indicated that an overt activity of the enzyme exists in the endoplasmic reticulum of the liver [79]; it is not known whether an analogous overt activity exists in the sarcoplasmic reticulum. The debate as to whether cells are able to interconvert acetyl-CoA and acetylcarnitine within the cytosolic compartment is a very important one (see [23]) as it has implications for the fate of acetylcarnitine released from both mitochondria and peroxisomes, as well as for the ability of the intramitochondrial and cytosolic pools of acetyl-CoA to communicate at all through acetylcarnitine.

The AMPK-mediated link between cellular energy state and the malonyl-CoA–long chain acyl-CoA axis is particularly relevant to ischaemic cardiac muscle. By analogy with skeletal muscle, the rapid rise in AMP content due to oxygen deficiency results in the inhibition of ACC, leading to lowered malonyl-CoA content and the further activation of CPTo [72,80]. This is a paradoxical situation for cardiac muscle as, under these conditions, long-chain acyl-CoA esters continue to be generated within the mitochondrial matrix in the absence of a sufficiently high rate of oxygen delivery through the circulation to oxidize them. This has deleterious consequences for the integrity of the mitochondrial inner membrane, even though an obvious relationship between gross overall tissue long-chain acyl-CoA levels and tissue damage may not be able to be discerned experimentally [81,82]. The consequences of this sequence of events is well illustrated in experimental low-flow ischaemia [83]. Importantly, upon reperfusion of ischaemic rat hearts, AMP content and AMPK activity remain elevated for a considerable time [72], such that heart mitochondrial CPTo would be anticipated to continue to generate acylcarnitines and thus intramitochondrial long-chain acyl-CoA [84]. It is widely accepted that such reperfusion coincides with irreversible injury to the cardiomyocytes and the opening of the mitochondrial transition pore [85], with consequent poor recovery of the tissue. It is paradoxical that cardiac muscle responds to ischaemia by in-

creasing the activity of the first reaction committed to the utilization of a substrate that can only be metabolized aerobically, jeopardizing its own recovery in the process. The malonyl-CoA–long-chain acyl-CoA axis which, in the fully functional heart enables rapid responses to transient, modest deficits between the rates of energy formation and utilization, may be ultimately incompatible with survival when ischaemia arises.

The importance of AMPK-induced inhibition of ACC extends beyond tissues that are subject to rapid changes in ATP demand or deficit. In pancreatic- β -cell-derived INS-1 cells, the activity of AMPK varies inversely with the availability of glucose and insulin secretion [86], raising the prospect that modulation of kinase activity through glucose-induced changes in cellular AMP content may provide a mechanism through which glucose availability is signalled (through malonyl-CoA) to long-chain acyl-CoA concentration, and thence to insulin secretion. Such an acutely acting mechanism would complement the longer-term stimulation by glucose of ACC gene expression (see below).

SYNTHESIS OF GLYCEROLIPIDS

Liver

Long-chain acyl-CoA esters are formed from fatty acids through the action of long-chain acyl-CoA synthase which, like the CPTo activities, is found in multiple subcellular sites. The long-chain acyl-CoA synthase activities in the mitochondrial outer membrane, peroxisomal and ER membranes (as distinct from the very-long-chain acyl-CoA synthases) are thought to be the products of a single gene [87,88].

In the ER of the liver, most of the enzyme reactions involved in glycerolipid synthesis occurs on the cytosolic aspect of the membrane [89]. However, experiments on rat liver microsomes [90,91] have suggested that the final step in the synthesis of triacylglycerols (TAGs) destined for secretion occurs from diacylglycerol (DAG), which is membrane-permeant, and acyl moieties delivered into the lumen as acylcarnitine esters [57] (see Schemes 2 and 4). DAGs used for TAG synthesis (as opposed to those used in signalling) are formed, through the action of a cytosol-facing DAG acyltransferase (DGAT 1) on the cytosolic aspect of the ER, either directly through the phosphatidate pathway or after hydrolysis of cytosolic droplet TAG [91]. An ER lumen-facing DGAT 2 that would be able to catalyse the synthesis of luminal TAG [destined for secretion within very-low-density lipoproteins (VLDL)] has been described [90]. The importance of the partitioning of DAG in determining the rate of hepatic TAG secretion has also been demonstrated [91]. Involvement of ER CPTo activity in hepatic TAG secretion would imply that conditions that lead to decreased ACC activity and, therefore, decreased hepatic malonyl-CoA concentration, would tend not only to increase the rate of fatty acid oxidation, but also enable a greater proportion of DAG to be diverted towards the synthesis of secretory TAG within the ER. Such a mechanism may be required for the maintenance of a substantial rate of TAG secretion under conditions of low hepatic malonyl-CoA levels, in spite of the diminished overall rate of TAG synthesis. In support of this proposition is the observation that, *in vivo*, in spite of the much lower proportion of hepatic fatty acids utilized for overall TAG synthesis in insulin-deficient states, the fractional secretion rate of fatty acids newly incorporated into TAG remains high [92–96]. Whether raised malonyl-CoA concentrations inhibit TAG secretion by limiting acylcarnitine formation is difficult to ascertain at present, especially as a strong relationship between the rate of *de novo* fatty acid synthesis and VLDL TAG secretion is invariably observed [97]. The rate of TAG synthesis can vary independently from that of TAG

Scheme 4 Reciprocal regulation of the activities of CPTo and GPAT by the action of insulin on AMPK

AMPK is phosphorylated and activated by AMPKK, and dephosphorylated by a phosphatase that is activated by insulin (see the text). The products of the acyl-CoA synthase-catalysed reaction [long-chain (LC) acyl-CoA and AMP] are activators of AMPKK, and AMP also activates AMPK directly. AMPK inhibits the activity of ACC (thus activating CPTo) and inhibits the activity of mitochondrial GPAT, while leaving microsomal (ER) GPAT unaffected (see the text). Abbreviation: PA, phosphatidic acid.

secretion both *in vivo* [93,96] and in cultured hepatocytes [98–100], but conflicting observations have been made on the consequences of the irreversible inhibition of CPTo activity on VLDL TAG secretion by isolated perfused livers (in which secretion is increased [101]) and cultured rat hepatocytes (in which it is inhibited [102]).

It is noteworthy that the refeeding of previously fasted rats only raises the malonyl-CoA content of their livers to less than half of that observed in livers of non-fasted animals for several hours after the start of refeeding [94]. It has been suggested that this permits mitochondrial CPTo to continue to compete successfully for the cytosolic acyl-CoA and thus enable the maintenance of a sufficiently high intramitochondrial acetyl-CoA to be maintained for the continued activity of pyruvate carboxylase [103]. In this respect, CPTo in the ER has a lower IC_{50} for malonyl-CoA [27] and may thus be inhibited to a greater extent than the mitochondrial enzyme during the early stages of refeeding. As discussed above, this may be relevant to the possible role of ER CPTo in the synthesis of secreted TAG. Utilization of fatty acids for the synthesis of secreted TAG as a proportion of total TAG synthesis (i.e. the fractional secretion rate) is acutely inhibited for 3–4 h after the start of refeeding of fasted rats, whereas the potential for mitochondrial oxidation of acyl moieties remains high during this period [94]. The differential sensitivity to malonyl-CoA of mitochondrial and ER CPTo [27] may make this possible. It is noteworthy that, in meal-trained rats, diurnal feeding is associated with a much higher and more rapid rise in hepatic malonyl-CoA concentration, accompanied by rapid inhibition of the utilization of fatty acids, not only for oxidation, but also for TAG secretion [93].

Most glycerolipid synthesis is initiated through the action of glycerol-3-phosphate acyltransferase (GPAT), which competes with CPTo for cytosolic acyl-CoA. In the liver, GPAT activity, too, is found in multiple locations: in the mitochondrial outer

membrane and in the ER. However, the two forms appear to be different gene products, as their kinetic and other properties are markedly different [104,105]. The phosphatidic acid pathway results in the formation of DAG, TAG and phospholipids, and as such is very important in the maintenance of cell structure and function. However, synthesis of TAG from DAG is much more variable between tissues. The liver can both store (within cytosolic droplets) and secrete TAG within VLDL particles. In this tissue the utilization of acyl moieties for TAG synthesis is substantial, and the reciprocal regulation of CPTo and GPAT activities is an important determinant of the relative rates of fatty acid oxidation and TAG synthesis. In particular, it is the activity of mitochondrial GPAT that undergoes variation with physiological state, e.g. it is diminished in the liver of diabetic or starved rats [106,107] and is acutely activated by insulin in the perfused liver [108]. By contrast, insulin increases the sensitivity of mitochondrial CPTo to malonyl-CoA inhibition [109] and decreases transcription of the CPTo gene in H4IIE cells [109]. Moreover, the recent observation that AMPK may phosphorylate and inhibit mitochondrial GPAT [110] suggests that insulin may be able to co-ordinate the regulation of CPTo and mitochondrial GPAT activities acutely through its additional ability to activate AMPK phosphatase activity [13]. As mentioned above, AMPK, which is itself phosphorylated and inhibited by a AMPK kinase (AMPKK), phosphorylates and inhibits ACC. Therefore, inhibition of AMPK by insulin [13] would simultaneously activate ACC, inhibit CPTo (through the ensuing raising of malonyl-CoA concentrations) and activate mitochondrial GPAT through the inhibition of AMPK activity. It is noteworthy that AMPKK is itself activated by long-chain acyl-CoA and AMP, and that these are both products of the AMP-linked acyl-CoA synthase reaction. These interactions are depicted in Scheme 4, from which it is evident that the long-chain acyl-CoA–malonyl-CoA axis is central to their co-ordination.

Scheme 5 Possible interaction between chronically elevated blood glucose and plasma NEFA concentrations to enhance the formation of ceramide in pancreatic β -cells in insulin-resistant animals

It is intriguing that AMPK appears to inhibit more readily the mitochondrial isoform of GPAT [110]. If preferential utilization of acyl-CoA esters occurs at their site of synthesis (see above), this would suggest that AMPK activation, by inhibiting mitochondrial GPAT, would more specifically tend to favour the activation of mitochondrial (rather than ER or peroxisomal) CPTo by decreasing the competition of mitochondrial GPAT for long-chain acyl-CoA at the mitochondrial-outer-membrane surface. Therefore AMPK inhibition of mitochondrial GPAT may be a mechanism through which specificity is imparted to the effects of AMPK on the various CPTo activities located in different intracellular membranes (Scheme 4). The smaller effect (if any) of AMPK on ER GPAT [110] may reflect the requirement for continued phospholipid synthesis at the cytosolic surface of the ER even under conditions characterized by high rates of fatty acid oxidation. Reciprocal regulation of mitochondrial GPAT and CPTo by AMPK would target the effect of changes in kinase activity at the mitochondrial synthesis of lysophosphatidate and acylcarnitine, while sparing lysophosphatidate synthesis in the ER.

Synthesis of glycerolipids in muscle and pancreatic β -cells

The utilization of long-chain acyl-CoA for phospholipid synthesis is important for all cell types, whereas that for TAG synthesis is also important in tissues not normally considered to be lipogenic. In cardiac muscle, TAG synthesis is very active [111] and mobilization of intracellular TAG stores enables the tissue to continue to synthesize acylcarnitines and intramitochondrial acyl-CoA even during ischaemia (see above). In insulin-resistant states the TAG content of muscle is increased [112], presumably due to the combination of the chronic elevation of the rate of delivery of plasma NEFA and glucose to the tissue (see Scheme 5). It has been pointed out that muscle malonyl-CoA content is also elevated in several models of insulin resistance [4,113]. This is expected to predispose muscle cells to accumulate intracellular TAG when plasma NEFA are elevated, especially when blood glucose is also elevated. However, such intracellular TAG accumulation can be achieved without a prior increase in tissue malonyl-CoA if the supply of NEFA is high enough to exceed the maximal rate at which muscle can oxidize fatty acids [114]. Therefore elevated muscle malonyl-CoA levels are neither necessary nor sufficient to induce accumulation of TAG in insulin resistance, but are facilitative.

Another illustration of this point is provided by the pancreatic β -cell. The provision of acyl-CoA, or a complex lipid synthesized therefrom, appears to be essential for the insulin secretory mechanism [115–118]. Fatty acids acutely stimulate insulin secretion and the effect is mimicked by fatty acid analogues that generate non-metabolizable acyl-CoA esters within the cells [119]. The malonyl-CoA–long-chain acyl-CoA axis is implicated by observations that inhibition of CPTo with etomoxir results in the elevation of cellular acyl-CoA and activation of insulin secretion; several insulin secretagogues raise the concentration of malonyl-CoA prior to stimulation of secretion [120]. In pancreata isolated from fasted animals, supplementation of fatty acids in the medium is essential for glucose-stimulated insulin secretion [121], raising the prospect that the depletion of β -cell TAG during 18–24 h of fasting eliminates a source of acyl-CoA that is required for secretion. In the fasted animal, plasma NEFA concentrations are high, thus enabling the pancreas to respond to refeeding with the appropriate insulin-secretory response. This interpretation is supported by the observation that leptin-induced depletion of TAG from β -cells makes them incapable of secreting insulin in response to glucose alone; perfusion with fatty acids is required to restore the secretory response [122]. However, the involvement of malonyl-CoA in this mechanism has been questioned by work in which adenovirus-mediated expression of malonyl-CoA decarboxylase in the cytosol of pancreatic β -cells was shown to attenuate the changes in intracellular malonyl-CoA induced by secretagogues, without markedly affecting the intensity of the insulin response [123,124]. Whether these data are sufficiently definitive to negate the large amount of evidence accumulated in favour of the involvement of the acyl-CoA–malonyl-CoA axis in insulin secretion requires clarification, but some of the considerations mentioned above about compartmentation of enzyme activities and/or metabolites may be relevant to interpretation of data obtained on overall cellular concentrations.

Many possible mechanisms (discussed in detail elsewhere; see, e.g., [118]) have been suggested in the literature through which elevated intracellular acyl-CoA concentrations could affect insulin secretion both in the short and the long term (see Scheme 2). Briefly, it is possible that, in the short term, provision of acyl-CoA esters as substrates for phospholipid synthesis could help meet the additional membrane turnover requirements imposed by the stimulated secretory process, and that DAG synthesis could result in the activation of specific isoforms of protein kinase C involved in stimulation–secretion coupling. Acyl-CoA esters are also required for budding and fusion of membrane vesicles and for protein membrane trafficking [125], suggesting that acyl-CoA esters may directly affect the insulin-secretory machinery. In addition, fatty acids are modulators of gene transcription [126]; long-chain acyl-CoA esters bind to nuclear factors [127] and affect transcription-factor binding to DNA [128].

Chronically elevated plasma NEFA concentrations may act on the β -cell not solely through provision of substrate, but also through effects on gene expression. Thus chronic exposure of β -cells *in vitro* to NEFA results in the induction of the expression of the CPT-I gene [129] and a decrease that of ACC [130]. The latter effect is the opposite of the effect of glucose [131]. Fatty acids also decrease the expression of Bcl-2, an effect that is prevented by leptin-induced activation of fatty acid oxidation and depletion of cellular TAGs [132]. In Zucker *fa/fa* rats, β -cell GPAT expression is induced severalfold, and they accumulate TAG to a much greater extent than normal [133]. By analogy with other tissues, conditions characterized by increased circulating NEFA are also expected to result in the

increased expression of PDH kinase [74,75], thus contributing to the impairment of the pathway leading from (glucose-derived) pyruvate to malonyl-CoA [134].

Utilization of long-chain acyl-CoA for ceramide synthesis

Chronically elevated plasma NEFA concentrations and/or inhibition of CPTo activity can result in the diversion of long-chain acyl-CoA into ceramide synthesis. Pancreata from Zucker *fa/fa* rats have a higher rate of synthesis of ceramide, which is associated with the induction of apoptosis in a much greater proportion of their β -cells [133,135,136]. If these observations can be extrapolated to pancreatic function in humans, they may provide a link between obesity, insulin resistance and the development of type 2 diabetes [133,135,136]. The initial, modest, but chronic, elevation of plasma NEFA in the prediabetic state may be pro-apoptotic for β -cells, especially if this is exacerbated by elevated intracellular malonyl-CoA content due to raised blood glucose concentrations. This may induce a deleterious spiral of metabolic events leading to loss of β -cell function and number.

Ceramide has recently been shown to release cytochrome *c* from mitochondria [137], suggesting that it causes disruption of their outer membrane. In view of the dependence of CPTo activity on the physical state of the membrane [50,51], this may explain why ceramide appears to have a direct stimulatory effect on the activity of CPTo [138]. Such activation may, in turn, provide a feedback mechanism limiting the availability of acyl-CoA for further ceramide synthesis. In view of the current interest of the pharmaceutical industry in using oral L-CPTo inhibitors to modulate fatty acid oxidation in the liver [139,140], it may be important to ensure that such inhibitors do not concomitantly activate ceramide synthesis in the pancreatic β -cell. An experimental demonstration of the ability of pharmacological inhibition of CPTo to induce apoptosis was made by Paumen et al. [141], who showed that inhibition of CPTo by etomoxir addition to two murine haematopoietic cell lines increased diversion of long-chain acyl moieties into ceramide synthesis and the induction of apoptosis when the cells were given either palmitate or stearate as substrates. Exposure of these cells – in which expression of CPTo is low – to the fatty acids alone also resulted in apoptosis, but etomoxir enhanced this effect. Whether the interaction between CPTo and Bcl-2, suggested by the yeast-two-hybrid experiments performed by the same authors [142], is involved in modulating the effects of increased long-chain acyl-CoA levels is not known at present.

Integration of the rates of hepatic gluconeogenesis and fatty acid oxidation

The rate of hepatic gluconeogenesis is a major contributor towards increased hepatic glucose output in hypoinsulinaemic states. The malonyl-CoA–long-chain acyl-CoA axis is intimately involved in the regulation of gluconeogenesis. Fatty acid oxidation provides ATP and reducing equivalents required for gluconeogenesis. β -Oxidation also produces intramitochondrial acetyl-CoA, which is essential for activation of pyruvate carboxylase [143], a key regulatory enzyme of gluconeogenesis [144]. There are many studies which confirm the important role that increased fatty acid mobilization from adipose tissue is linked to increased gluconeogenesis [145], e.g. during diurnal surges of cortisol, growth hormone or catecholamines. This mechanism is also thought to play an important role in sustaining gluconeogenesis (required for rapid replenishment of hepatic glycogen stores) during the absorptive period following refeeding of fasted rats, in spite of the antilipolytic action of insulin [94].

Malonyl-CoA, long-chain acylcarnitine esters and membrane phospholipid turnover

An indirect role in the control of phospholipid turnover and membrane repair has been suggested for a malonyl-CoA-sensitive CPT that is found in the plasma membrane of human erythrocytes [26]. Inhibition of the enzyme with etomoxir results in a decrease in the rate of phospholipid acyl-chain turnover, as judged by the incorporation of exogenously added fatty acids into membrane phospholipids [146]. Therefore, these authors have suggested that this CPTo performs the function of coupling acyl-CoA synthesis with the (re-)acylation of phospholipids; acylcarnitines are suggested as acting as a reservoir of acyl moieties [147]. Alternatively, it is possible that an acylcarnitine-derived pool of acyl-CoA is preferentially channelled towards phospholipid synthesis within the erythrocyte plasma membrane. This would imply that this specialized acyl-CoA pool would not equilibrate with the (ACBP-complexed) acyl-CoA pool derived directly through acyl-CoA synthase action on fatty acids taken up by the cells. One could speculate that the plasma-membrane location of this CPTo may enable it to generate and channel acyl-CoA directly to the site of phospholipid turnover. It is noteworthy that, in the study by Fyrst et al. [148], it was found that a membrane pool of long-chain acyl-CoA was used for phospholipid synthesis from lysophospholipids with an affinity even greater than that shown by ACBP for acyl-CoA esters (see above). Evidence for acyl-CoA pool(s) dedicated to lysophospholipid acylation exists for other cell types too (e.g. hepatocytes [149]). The identity of the CPTo isoform expressed in human erythrocytes is not known, nor is it known whether its malonyl-CoA sensitivity is involved in its regulation *in vivo*. However, the discovery of a possible involvement of CPTo in phospholipid turnover of erythrocytes raises the prospect that CPTo activity in other cell types may also be involved in phospholipid turnover.

FUTURE DIRECTIONS

The question that invariably arises when an individual, narrow aspect of metabolism is reviewed (and promoted) is: how important is it really in determining the outcome of particular physiological situations *in vivo*? Attempts have been made to address these questions through Metabolic Control Analysis, which is necessarily restricted in the information it provides because of the strictly defined experimental conditions under which it has to be performed. In the present context, the experiments of [123,124], in which the effects of imposing limits on the magnitude of variation of intracellular malonyl-CoA content of INS-1 cells concomitantly with studying the effects of secretagogues on insulin release were studied, can be considered to be attempts at performing Metabolic Control Analysis of the role of the malonyl-CoA–long-chain acyl-CoA axis within a more physiological context. Similar approaches are likely to emerge in trying to solve another paradoxical situation, namely the large discrepancy between the sensitivity of M-type CPTo to malonyl-CoA and the overall malonyl-CoA content of cardiac and skeletal muscle. Such studies, in spite of their limitations, are necessary to verify whether malonyl-CoA is really the mighty metabolite it currently appears to be.

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