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REVIEW

Lipid-associated metabolic signalling networks in pancreatic beta cell function

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Abstract

Significant advances have been made in deciphering the mechanisms underlying fuel-stimulated insulin secretion by pancreatic beta cells. The contribution of the triggering/ATP-sensitive potassium (K_{ATP})-dependent Ca^{2+} signalling and K_{ATP}-independent amplification pathways, that include anaplerosis and lipid signalling of glucose-stimulated insulin secretion (GSIS), are well established. A proposed model included a key role for a metabolic partitioning 'switch', the acetyl-CoA carboxylase (ACC)/malonyl-CoA/carnitine palmitoyltransferase-1 (CPT-1) axis, in beta cell glucose and fatty acid signalling for insulin secretion. This model has gained overwhelming support from a number of studies in recent years and is now refined through its link to the glycerolipid/NEFA cycle that provides lipid signals through its lipolysis arm. Furthermore, acetyl-CoA carboxylase may also control beta cell growth. Here we review the evidence supporting a role for the ACC/malonyl-CoA/CPT-1 axis in the control of GSIS and its particular importance under conditions of elevated fatty acids (e.g. fasting, excess nutrients, hyperlipidaemia and diabetes). We also document how it is linked to a more global lipid signalling system that includes the glycerolipid/NEFA cycle.

Keywords Beta cell · CPT-1 · Glycerolipid/NEFA cycle · Insulin secretion · Lipid signalling · Malonyl-CoA · Metabolic coupling factor · Monoacylglycerol · Pancreatic islets · Review

Abbreviations

ABHD6	α/β-Hydrolase domain 6
ACC	Acetyl-CoA carboxylase
ACL	ATP citrate lyase
ACOT7	Acyl-CoA thioesterase-7
AMPK	AMP-activated protein kinase
CACT	Carnitine acylcarnitine translocase
CPT-1	Carnitine palmitoyltransferase-1
DAG	Diacylglycerol
FA-CoA	Fatty acyl-CoA
FOXO	Forkhead box protein O
G3PP	Glycerol 3-phosphate phosphatase
GSIS	Glucose-stimulated insulin secretion
HADHSC	Short-chain hydroxy acyl-CoA dehydrogenase
K_{ATP}	ATP-sensitive potassium
MAG	Monoacylglycerol
MCD	Malonyl-CoA decarboxylase
MCF	Metabolic coupling factor
NEFA	Non-esterified fatty acid
PPAR	Peroxisome proliferator-activated receptor
TOFA	5-(Tetradecyloxy)-2-furoic acid

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Introduction

Insulin secretion in response to various fuel stimuli, such as glucose, some amino acids and fatty acids, involves transduction systems that require metabolism of the fuel stimulus in the

pancreatic beta cell. Although much progress has been made in recent years, we still have not entirely elucidated the pathways and signalling molecules involved. A glucose-induced rise in the cytosolic ATP/ADP ratio leads to inhibition of ATP-sensitive potassium (K_{ATP}) channels and depolarisation of the beta cell, followed by an increase in cytosolic Ca^{2+}, which promotes insulin granule exocytosis [1, 2]. The K_{ATP} channel-independent actions of glucose in beta cell signalling, also known as the amplification pathways, involve several metabolic coupling factors (MCFs) that link fuel metabolism to insulin exocytosis [3, 4].

Of the numerous amplification pathways that contribute to glucose-stimulated insulin secretion (GSIS), the following are thought to be important players: anaplerosis/cataplerosis, the ATP citrate lyase (ACL)/acetyl-CoA carboxylase (ACC)/malonyl-CoA/carnitine palmitoyltransferase-1 (CPT-1) axis, the glycerolipid/NEFA cycle [5], post-translational attachment of small ubiquitin-like modifier to target lysine residues (SUMOylation) [6], NADPH [7], reactive oxygen species [8] and the redox control of exocytosis proteins [5, 9] (Figs 1, 2). The idea that anaplerosis/cataplerosis and pyruvate cycling provide some of the essential MCFs is well accepted and this has been reviewed extensively [5, 9–13].

Lipid signalling is essential for GSIS. Thus, a fatty acid-dependent step is critically important for both GSIS and non-glucose-stimulated insulin secretion in vivo [14] and ex vivo [15] and if islets are deprived of NEFA their response to GSIS is compromised [16]. Lipid signalling of GSIS was proposed to involve three mechanisms. Extracellular lipid signalling is mediated by NEFA activation of free fatty acid-activated receptor-1 (FFAR1, also known as GPR40), leading to generation of intracellular diacylglycerol (DAG) and inositol trisphosphate [17]. The intracellular pathways involve the ACC/malonyl-CoA/CPT-1 network and generation of lipid molecules via the glycerolipid/NEFA cycle [18, 19] (Fig. 2). Our laboratory identified two important enzymes of the glycerolipid/NEFA cycle: glycerol 3-phosphate phosphatase (G3PP) and α/β-hydrolase domain 6 (ABHD6). G3PP hydrolyses glucose-derived glycerol 3-phosphate, the precursor for lipogenesis [20], whereas ABHD6 controls the last step of lipolysis by hydrolysing 1-monoacylglycerol (MAG) [21]. Importantly, 1-MAG is an MCF of GSIS by activating Munc13-1, an exocytosis-facilitating protein [21] (Figs. 1, 2).

This review focuses on intracellular lipid signalling for glucose- and NEFA-induced insulin secretion, highlighting the role of the ACC/malonyl-CoA/CPT-1 network.

What is the ACC/malonyl-CoA/CPT-1 metabolic signalling network?

The hypothesis proposing an intracellular lipid amplification arm for GSIS was originally laid out by us [22–25]. We initially proposed that glucose-metabolism-derived malonyl-CoA, by inhibiting CPT-1, diverts long-chain fatty acyl-CoA (FA-CoA) from mitochondrial β-oxidation towards the synthesis of complex lipids, such as DAGs, that can act as signals for insulin secretion. The subsequent realisation that lipolysis plays a key role in GSIS led us to refine the model by linking the ACC/malonyl-CoA/CPT-1 network to the glycerolipid/NEFA cycle (Fig. 1). In this revised model malonyl-CoA acts as a 'metabolic switch' signal by modulating fuel partitioning (the relative rates of glucose and NEFA oxidation) and is a regulatory MCF in insulin secretion, whereas the lipid signals generated via lipolysis in the glycerolipid/NEFA cycle act as effector signals [9]. Thus, inhibition of CPT-1 and fat oxidation allows continuous operation of the glycerolipid/NEFA cycle.

Here, we review the evidence for and against the ACC/malonyl-CoA/CPT-1 hypothesis and present a consensus view that emerges (Figs. 1, 2).

In vitro evidence for the role of the ACC/malonyl-CoA/CPT-1 metabolic signalling network in metabolic signalling

Biochemical evidence

It was initially noticed using HITβ cells and rat islets, that glucose stimulation causes marked alterations in the acyl-CoA profile, with early change occurring in malonyl-CoA levels [22–25]. The rise in malonyl-CoA that preceded insulin release and correlated with the dose dependency of GSIS [22–25] was confirmed in several studies using metabolomics approaches in INS-1(832/13) cells [26, 27]. As predicted by the hypothesis, glucose caused a decrease in NEFA oxidation in association with a rise in citrate and lipogenesis in rodent islets and beta cell lines, and both correlated with the glucose dose dependence of insulin secretion [9, 10, 26]. Finally, glucose decreased ACC phosphorylation and increased its activity in a beta cell line and this was closely related to insulin secretion [28].

Pharmacological evidence

CPT-1 inhibition In early studies, inhibition of pancreatic islet fatty acid oxidation by 2-bromostearate was shown to restore GSIS in fasted islets [29]. In addition, inhibition of CPT-1 by 2-bromopalmitate in isolated rat islets promoted GSIS while blocking β-oxidation [30]. Similarly, the CPT-1 inhibitor etomoxir reduced β-oxidation in isolated rat islets and this was associated with enhanced GSIS [30]. The reduced GSIS in *db/db* mouse islets could be restored to near normal levels by incubating the islets with etomoxir [31]. Evidence

suggesting a role for CPT-1 in regulating GSIS was also obtained in studies using CPT-1-overexpressing INS1E cells; these cells showed a reduced GSIS response, which could be restored by etomoxir [32]. Acute addition of etomoxir to the INS1E cells also partially reversed the decreased GSIS in cells chronically exposed to NEFA, a condition wherein NEFA oxidation is enhanced [31].

ATP citrate lyase inhibition Besides its participation in the Krebs cycle, citrate exits mitochondria via the dicarboxylate/tricarboxylate carrier. In the cytoplasm, citrate is cleaved by ACL to oxaloacetate and acetyl-CoA. ACC converts cytosolic acetyl-CoA to malonyl-CoA, which regulates β-oxidation by inhibiting CPT-1, the rate-limiting enzyme involved in the transport of fatty acyl groups into mitochondria (Fig. 2). Further evidence to support the ACC/malonyl-CoA/ CPT-1 hypothesis was garnered in a rat pancreas perfusion study, which showed that inhibition of ACL by hydroxycitrate caused a profound decline in GSIS [30]. The functional importance of cataplerosis (the exit of Krebs cycle intermediates into the cytoplasm) via citrate for GSIS is further supported by the observation that radicicol, another ACL inhibitor, partially blocked GSIS in purified rat beta cells [33] and INS832/13 cells [34].

Pyruvate carboxylase inhibition The Krebs cycle generates citrate via the condensation of acetyl-CoA and oxaloacetate formed by pyruvate carboxylase. Malonyl-CoA is derived from cytosolic citrate. GSIS in beta cells was found to be reduced by the inhibition of oxaloacetate formation by pyruvate carboxylase using phenylacetate [10, 11].

Inhibition of ACC It was noticed that ACC1 is the predominant isoform expressed in pancreatic islets and INS-1(832/13) beta cells and that inhibitors of ACC1, CP-640186 and 5-(tetradecyloxy)-2-furoic acid (TOFA), which curtailed lipogenesis, inhibited GSIS [35].

FA-CoA synthase inhibition Triacsin C, a FA-CoA synthase inhibitor, curtailed GSIS in INS832/13 cells only in the presence of added NEFA, emphasising the importance of incubation conditions in addressing the role of lipid signalling for GSIS [15]. This important point, which is at the root of the controversy in the field, will be further discussed below. In fact, depletion of NEFA by exhaustive washing with bovine serum albumin in MIN6 beta cells was found to lower GSIS, which could be restored by the addition of NEFA [16].

Evidence from altered expression of relevant genes

ACL and mitochondrial citrate carrier Higher expression levels of ACL in the islets (a non-lipogenic tissue) in comparison with liver, both in rodents and in humans [36], supports a role for this enzyme in GSIS. The expression of ACL was found to be reduced by 60% in islets from individuals with vs without type 2 diabetes, suggesting that a reduction in malonyl-CoA formation in diabetic beta cells could contribute to compromised GSIS [37]. Indeed, RNAi-knockout of *Acl* (also known as *Acly*) in INS-1(832/13) cells inhibited GSIS and the K_{ATP}-independent pathway of insulin secretion [34]. In a recent study, the relative increase in GSIS response in pancreatic islets from the juvenile stage to adulthood was attributed to

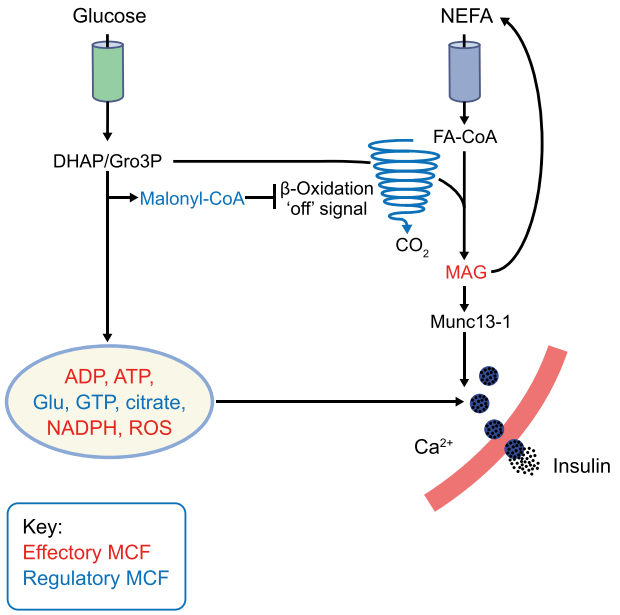

Fig. 1 Metabolic signal transduction of the beta cell in response to glucose and NEFA. Glucose metabolism gives rise to various regulatory (glutamate, GTP, citrate) and effectory (ADP, ATP, NADPH, ROS) MCFs. The signalling actions of these key metabolites contribute to the facilitation of insulin granule exocytosis at different steps, including Ca^{2+} influx following the closure of K_{ATP} channels. Malonyl-CoA formed from glucose metabolism in beta cells controls the flux of NEFA through β-oxidation, an 'off' pathway of GSIS, by inhibiting the rate-limiting step of β-oxidation (catalysed by CPT-1). The accumulating FA-CoA thus is diverted towards the lipogenesis arm of the glycerolipid/NEFA cycle (see Fig. 2), leading to the formation of the lipolysis-derived MAG, which is an effectory MCF. 1-MAG directly binds and activates Munc13–1, an insulin granule exocytosis-facilitating protein. 1-MAG may be hydrolysed by the 1-MAG hydrolase ABHD6 (see Fig. 2) to generate NEFA for subsequent oxidation, thereby negatively affecting the secretion of insulin. Importantly, the inhibition of NEFA oxidation by malonyl-CoA prevents the catabolism of lipid signalling molecules, such as MAG. Figure 1 summarises the lipid signalling pathways that are shown in more detail in Fig. 2. We previously proposed that two types of MCF can be defined: (1) regulatory MCFs that modulate key metabolic pathways and networks involved in fuel-induced insulin secretion; and (2) effectory MCFs that are directly involved in the triggering and amplification arms of fuel-induced insulin secretion at late steps of their signalling cascade (e.g. exocytosis or membrane ionic events) [9]. ROS are produced in mitochondria, during electron transport, when there is excess supply of electron donors, such as glycerol 3-phosphate. DHAP, dihydroxyacetone phosphate; Glu, glutamate; Gro3P, glycerol 3-phosphate; ROS, reactive oxygen species. This figure is available as part of a downloadable slideset

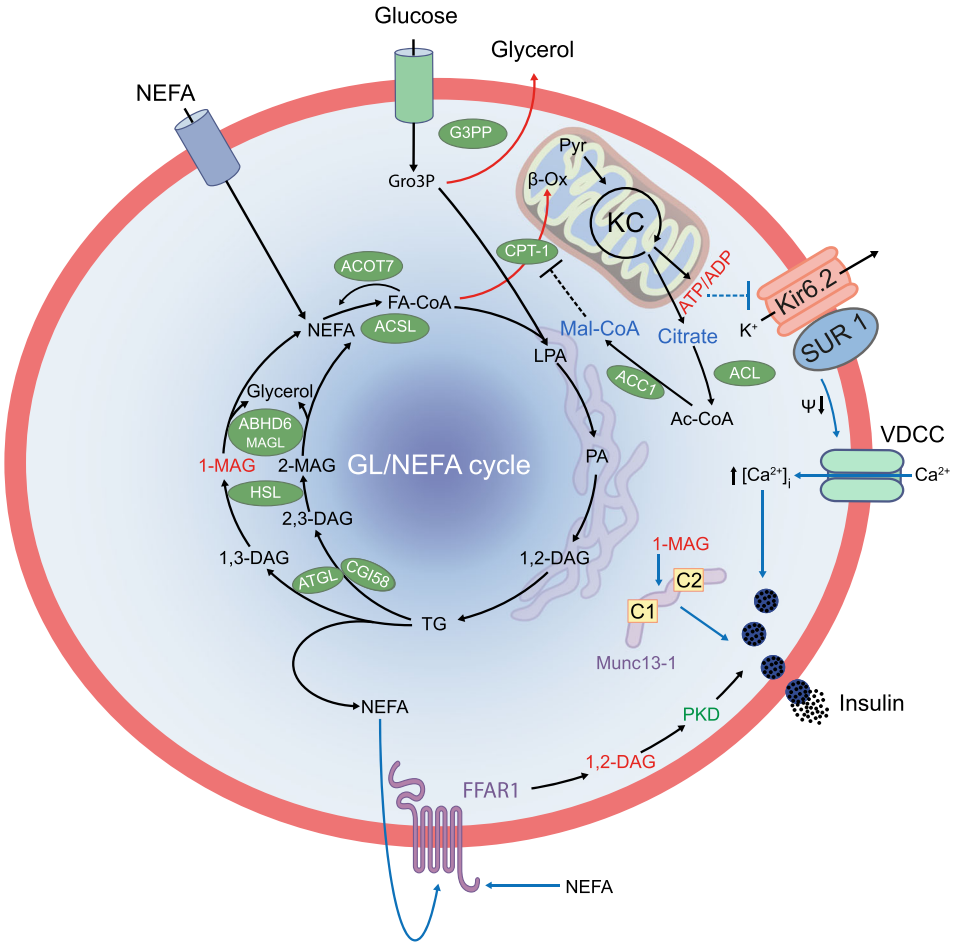

Fig. 2 Lipid signalling and the ACC/malonyl-CoA/CPT-1 metabolic signalling network in beta cell function. Pyruvate produced from glucose in the beta cells enters the mitochondria, where it is metabolised via the Krebs cycle to generate citrate. Under conditions of high glucose availability, a significant amount of citrate exits the mitochondria (cataplerosis) and is used by cytosolic ACL to form acetyl-CoA, which in turn is carboxylated by ACC1 to generate malonyl-CoA. Elevated glucose levels result in increased production of malonyl-CoA, which acts as a metabolic switch by inhibiting CPT-1, the rate-limiting enzyme that controls the entry of fatty acyl groups into mitochondria for β-oxidation. Inhibition of CPT-1 results in diversion of FA-CoA towards the glycerolipid/NEFA cycle, which produces signals that act as MCFs for insulin secretion. The entry of FA-CoA into the lipogenic arm of the glycerolipid/NEFA cycle is dependent on the availability of glycolysis-derived glycerol 3-phosphate, the cytosolic levels of which are controlled by G3PP, which negatively regulates the amplification of GSIS. As it is important to maintain FA-CoA levels, normal beta cells do not express ACOT7, which hydrolyses FA-CoA. The lipolysis arm of the glycerolipid/NEFA cycle generates 1-MAG, which activates Munc13–1 (insulin exocytosis facilitator), and ABHD6 hydrolyses 1-MAG to negatively control GSIS (not illustrated). ABHD6 is a more predominant MAG hydrolase than the classical monoacylglycerol lipase (MAGL) in pancreatic beta cells. NEFA generated via the lipolysis arm of the glycerolipid/NEFA cycle can exit the cell and activate free fatty acid-activated receptor-1 (FFAR-1), resulting in a signalling cascade of 1,2-DAG/ protein kinase D (PKD), stimulating insulin exocytosis. Thus, malonyl-CoA, by controlling fat oxidation and the glycerolipid/NEFA cycle flux, can regulate the lipid amplification of GSIS at several steps. Blue arrows indicate an activating effect on insulin exocytosis. Red arrows indicate pathways that negatively regulate GSIS. Dotted lines indicate inhibitory effect on the corresponding target molecules. Molecules in blue are key regulatory MCF signalling molecules, while those in brick-red colour are effectory MCF molecules. Metabolic enzymes are shown in green ovals. ψ, membrane potential; Ac-CoA, acetyl-CoA; ACSL, acyl-CoA synthetase, long-chain; ATGL, adipose triglyceride lipase; C1, phorbol esters/diacylglycerol binding domain of Munc13–1; C2, calcium-dependent phospholipid binding domain of Munc13–1; $[Ca^{2+}]_i$, intracellular calcium concentration; CGI58, comparative gene identification-58; GL, glycerolipid; Gro3P, glycerol 3-phosphate; HSL, hormone-sensitive lipase; KC, Krebs cycle; Kir6.2, a major subunit of the ATP-sensitive K^+ channel, an inward-rectifier potassium ion channel; LPA, lysophosphatidic acid; Mal-CoA, malonyl-CoA; β-Ox, β-oxidation; PA, phosphatidic acid; Pyr, pyruvate; SUR1, sulfonylurea receptor-1; TG, triacylglycerol; VDCC, voltage-dependent calcium channel. This figure is available as part of a downloadable slideset

elevated pyruvate–citrate cycling, ACL expression and malonyl-CoA production [38]. RNAi-knockdown of the mitochondrial citrate carrier [39] in INS-1(832/13) cells resulted in a decline in GSIS, which is compatible with a role for malonyl-CoA signalling.

The possibility of cytosolic acetyl-CoA being generated from glucose-derived pyruvate by alternative routes was suggested by the observation that inhibition of ACL still allows glucose carbon incorporation into lipids without compromising GSIS, probably via the acetoacetate pathway [40]. Thus,

redundant pathways exist that can support malonyl-CoA formation and the generation of lipid MCFs for GSIS.

ACC-1 Employing INS-1 cells stably expressing antisense *Acc* mRNA, it was noticed that a decline in ACC protein expression was associated with reduced malonyl-CoA formation, elevated β-oxidation and decreased GSIS response [41].

Malonyl-CoA decarboxylase Overexpression of cytosolically targeted malonyl-CoA decarboxylase (MCD) in INS-1(832/13) cells and rat islets resulted in lowered malonyl-CoA levels in association with elevated fatty acid oxidation and reduced GSIS in the presence of added NEFA, but not in their absence [15]. This underscores the importance of added NEFA in ascertaining the significance of lipid signalling for GSIS, a key point that will be discussed below in the section related to evidence against the hypothesis.

CPT-1 Studies in which wild-type CPT-1 was overexpressed in INS1E cells, showed reduced GSIS, restorable by etomoxir or supply of NEFA [32]. This strongly supports a role for CPT-1 in the negative control of GSIS. In addition, overexpression of malonyl-CoA-insensitive mutant CPT-1 (M593S) in INS-1(832/13) cells and rat islets curtailed GSIS and enhanced fatty acid oxidation [42].

Long-chain acyl-CoA synthase The significance of FA-CoA in GSIS was demonstrated in a study showing that RNAi-knockdown of long-chain acyl-CoA synthases (either ACSL3 or ACSL4), two enzymes concentrated in the insulin granules and synthesising FA-CoA, led to a decline in GSIS in human islets and INS-1(832/13) cells [43]. In addition, FA-CoA directly promotes exocytosis of insulin granules in beta cells [44].

Acyl-CoA thioesterase-7 About 60 genes expressed ubiquitously are relatively silenced (disallowed) in beta cells. One such gene encodes acyl-CoA thioesterase-7 (ACOT7), which hydrolyses FA-CoA. *ACOT7* downregulation is needed if FA-CoA signalling is central to insulin secretion. *Acot7*-overexpressing INS-1(832/13) cells showed impaired GSIS, supporting a role for FA-CoA or its derivatives in GSIS [45]. Of note, all *Acot* family members are poorly expressed in mouse islets [45].

Carnitine acylcarnitine translocase An important step in fatty acid oxidation is the transport of fatty acylcarnitines into mitochondria by carnitine acylcarnitine translocase (CACT) [46]. Deficiency of the CACT-encoding gene (*Slc25a20*) is associated with hypoketotic hypoglycaemia, though insulin levels were not reported. Downregulation of CACT by miR-132 or miR-212 in beta cells led to elevated fatty acylcarnitines and increased insulin secretion [47]. Although the authors of this study proposed that acylcarnitines may directly modulate exocytosis, the possibility of a build-up of FA-CoA for lipid signalling still remains.

In vivo studies in support of ACC/malonyl-CoA/CPT-1 signalling for insulin secretion

Pharmacological studies

CPT-1 inhibition Supranormal GSIS response was noticed when 24 h-fasted rats under hyperglycaemic clamp were maintained at high plasma levels of NEFA, and infused with etomoxir to block fatty acid oxidation [48].

Suppression of ACC1 activity Support for the role of ACC1 in insulin secretion has been reported in studies using the ACC inhibitor ND-630, which lowered malonyl-CoA levels in tissues and reduced GSIS in Sprague Dawley rats fed a high-sucrose high-fat diet, and also in ZDF rats [49].

Inhibition of ACL activity Inhibition of ACL in vivo by bempedoic acid in high-fat-diet-fed mice curtailed hyperinsulinaemia and improved glucose tolerance [50]. Interestingly, a crossover study involving 3 days of administration of the ACL inhibitor hydroxycitrate revealed a consistent decrease in plasma insulin levels, though the results were not significant possibly due to the low number of participants ($n = 10$) [51].

Genetic studies

Fatty acid oxidation enzymes Many genetic defects in fatty acid oxidation, including those of CPT-1, CPT-2, CACT, long-chain acyl-CoA dehydrogenase and short-chain hydroxy acyl-CoA dehydrogenase (HADHSC), are associated with hypoketotic hypoglycaemia, although the precise causes are unknown [52]. At least in the case of the HADHSC defect, it was found to be associated with hyperinsulinism [53, 54]. In many other cases, insulin levels were not reported. Thus, it would be interesting to know whether the hypoglycaemia is partly due to elevated plasma insulin levels, besides other contributions such as reduced gluconeogenesis.

***Acot7*-overexpressing mice** Transgenic expression of mitochondrial *Acot7* specifically in the beta cells of mice led to glucose intolerance and reduced GSIS [45]. The changes in GSIS were related to reduced islet FA-CoA and ATP/ADP. Of interest, islet levels of DAG and MAG, which are thought to be MCFs for insulin secretion, were reduced by 45% and 60%, respectively, although they did not reach significance possibly due to the large SD.

Modulation of transcription factors controlling lipid metabolism genes An inverse relationship between fatty acid β-oxidation and GSIS was noticed in studies wherein the transcription factors peroxisome proliferator-activated receptor (PPAR)δ [55] or PPARα [56, 57], which control the expression of β-oxidation genes, including *Cpt-1* (also known as *Cpt1A*), were genetically deleted. *Pparα* (also known as *Ppara*)-knockout mice develop hyperinsulinaemic hypoglycaemia in the fasting state [56]. Similarly, using a mouse model in which three isoforms of forkhead box protein O (FOXO) were knocked down specifically in beta cells, we observed that there is a preferential utilisation of lipids by the beta cells, associated with elevated β-oxidation and curtailed GSIS [58]. Also, a knockin mouse model expressing deacetylated FOXO1 (activated form) specifically in beta cells displayed reduced mitochondrial β-oxidation and enhanced GSIS response in vivo [59].

***Acc1*-knockout and -knockin mice** A recent study employing beta cell-specific *Acc1* (also known as *Acaca*)-knockout mice demonstrated that ACC1 is critical for GSIS [60]. Interestingly, ex vivo islet studies showed that the reduced secretion occurs at low and intermediate glucose concentrations and that the inhibitory effect was overridden at high (20 mmol/l) glucose. A simple explanation for why high glucose overrides the inhibition of GSIS in the beta cell *Acc1*-knockout mice may be that other amplification pathways take over in order to ensure secretion. In our view, this paper provides direct key support for the role of malonyl-CoA in GSIS. The authors mentioned that *Acc1*-knockout mouse islets did not show changes in fatty acid oxidation or lipid signalling molecules. Unfortunately, they measured these variables at a concentration of glucose where secretion was unchanged (20 mmol/l) and they did not show under their experimental condition that high glucose itself was able to inhibit fat oxidation, as anticipated. Interestingly, this study reported a novel role of ACC1 related to the control of beta cell mass prior to adulthood [60].

Regulation of ACC1 by AMP-activated protein kinase (AMPK) is well established [61]. It was found that mice with serine-to-alanine knockin mutations in both *Acc1* (Ser79) and *Acc2* (also known as *Acacb*) (Ser212), which render ACC constitutively active and not susceptible to inhibitory phosphorylation by AMPK, display elevated fasting plasma insulin levels [62]. Thus, it appears that in vivo, when ACC activity is reduced and there is less malonyl-CoA production in the beta cell, insulin secretion is reduced; conversely, when ACC is constitutively active, beta cells secrete more insulin.

Liver kinase B1-knockout mice Beta cell-specific *Lkb1*-knockout mice show enhanced insulin secretion by an undefined mechanism [63–65]. They display impaired mitochondrial metabolism and lower ATP levels following glucose stimulation, yet compensate for this by upregulating the production of citrate. It was found that at low-glucose $Lkb1^{-/-}$ beta cells failed to inhibit ACC1 and consequently accumulated malonyl-CoA derived fatty acids [65]. Thus, this study also supports a role for ACC/malonyl-CoA signalling in insulin secretion.

Evidence against a role for ACC/malonyl-CoA/CPT1 signalling for insulin secretion

In vitro pharmacological approaches

Despite overwhelming support for the role of malonyl-CoA/CPT-1/fatty acyl-CoA metabolic signalling in the regulation of GSIS, a few studies have raised doubts about this model. These discrepant results can be explained by the methodology that was employed.

Inhibition of fatty acid oxidation by bromopalmitate In a study using normal mouse islets incubated in the presence of 30 mmol/l KCl plus the K_{ATP} opener diazoxide to examine K_{ATP}-independent signalling, the fatty acid oxidation inhibitor bromopalmitate failed to modify basal (3 mmol/l) and high-glucose (20 mmol/l)-stimulated insulin secretion, which would be against a role for the malonyl-CoA switch [66]. However, at supramaximal concentrations of glucose, fat oxidation is maximally inhibited such that bromopalmitate cannot further change fat oxidation. Conversely, at 3 mmol/l glucose, cytosolic Ca^{2+} is low and because a rise in Ca^{2+} is a prerequisite for the amplification pathways of secretion, even if bromopalmitate reduced fat oxidation, it should not change secretion. In addition, fat oxidation was not measured, and this study examined pathways under interesting but totally unphysiological conditions (very high KCl and cytosolic Ca^{2+} plus diazoxide) so its relevance to a normal situation is unclear.

Inhibition of ACC, ACL and fatty acyl-CoA synthase An inhibitor of ACC1, TOFA, was found to have no effect on GSIS in INS-1 cells [67]. Similarly, the ACL inhibitor hydroxycitrate lacked effect on GSIS in INS1(832/13) cells and rat islets [68]. In ^{13}C isotopomer flux measurements, inhibiting the flux of glucose carbons into malonyl-CoA by hydroxycitrate had no effect on GSIS in INS1 cells [40]. Finally, the use of the FA-CoA synthase inhibitor, triascin C, did not reveal a modification of GSIS in INS-1 cells [69]

or INS 832/13 cells [70]. However, in all these studies, no fatty acid was present during incubations. The premise for the lipid signalling hypothesis is that substantial endogenous triacylglycerol stores or exogenous NEFA are required because the first step of this process is the synthesis of FA-CoA. These experiments were in fact performed in fatty-acid-depleted cells due to the presence of 0.2% fatty-acid-free BSA, which traps cellular fatty acids. At the time these studies were done, scientists were not aware of the potential caveats related to depleting beta cells of NEFA in examining lipid signalling for GSIS. Thus, the experimental conditions were not appropriate for testing the hypothesis.

In vitro gene expression studies

MCD overexpression One of the arguments against the role of malonyl-CoA as a regulator of GSIS is that overexpression of MCD in INS-1 cells had no effect on GSIS in one study [69]. However, this study suffers from drawbacks: (1) the MCD construct used was not directed to cytosol and may not have lowered the cytosolic pool of malonyl-CoA and (2) the INS-1 cell line used does not elicit the K_{ATP} channel-independent pathway of GSIS, as discovered subsequently [71]. To address this caveat, a later study by the same group employed an MCD construct that lacked both mitochondrial and peroxisomal targeting sequences for overexpression in INS-1(832/13) cells and reported a lack of effect on GSIS at 15 mmol/l glucose, both in the presence and absence of NEFA [70]. If one closely examines the results in cells cultured in the presence of NEFA, they do indicate a substantial (40%) reduction of GSIS in MCD-overexpressing cells, compared with controls, although the difference was deemed not significant [70]. Additionally, 15 mmol/l glucose is a supramaximal concentration for INS832/13 cells; no intermediate glucose concentration was tested and very high glucose concentrations override the inhibition of GSIS, as discussed above. Thus, the inhibition of GSIS in *Acc1*-knockout mouse islets is observed at basal and intermediate but not maximal glucose concentrations [60]. In a subsequent study, we repeated the MCD overexpression experiments and found similar results, observing that GSIS is markedly reduced in MCD-overexpressing INS832/13 cells but only if the medium is supplemented with exogenous fatty acids to permit intracellular lipid signalling [15].

Suppression of ACL ACL knockdown was reported to have no effect on GSIS in INS-1(832/13) cells when measured only at maximal 16.7 mmol/l glucose, possibly due to the absence of NEFA in the incubation medium for insulin secretion as well as GSIS not being tested at submaximal glucose [68]. The lack of effect could also be due to the presence of alternate routes that provide cytosolic acetyl-CoA, as mentioned above [40]. We reported that ACL knockdown in the same cell line under similar experimental conditions does result in reduced GSIS but that the inhibitory effect is most prominent in the presence of exogenous NEFA or at intermediate glucose [34].

Where does the balance tip?

Collectively, the above summarises the comprehensive information accumulated over the years and provides overwhelming evidence in favour of an ACC1/malonyl-CoA/CPT-1/fatty acyl-CoA network playing a key role in the regulation of beta cell glucose and fatty acid signalling for insulin secretion. This network appears to be particularly involved in GSIS regulation under conditions where fatty acids are elevated (fasting, cells chronically exposed to NEFA, obesity and diabetes) (see Textbox). In vitro and in vivo studies, as well as data from human islets, addressing various components of this network employing biochemical, pharmacological, molecular and genetic tools directly support this view. In contrast, the evidence against the hypothesis is not strong and is based on questionable experimental approaches. Furthermore, these experiments have been exclusively in vitro without support from in vivo experiments.

Conclusion and perspective

The consensus that emerges places malonyl-CoA as a signal that acts as a ‘metabolic switch’, playing a critical role in regulating insulin secretion promoted by glucose and other fuel stimuli, particularly when islet fat oxidation is elevated as under conditions of fasting, obesity and diabetes, via modulation of β-oxidation flux (see Textbox). Thus, the Textbox shows many situations where the ACC/malonyl-CoA/CPT1 network was shown to be involved in GSIS. The role of this network as a metabolic on/off switch is also in line with the significance of the glycerolipid/NEFA cycle and its lipolysis arm in GSIS (Fig. 1). Nevertheless, unlike MAG, which acts as an ‘effectory’ MCF [9] at the late event of insulin exocytosis by activating Munc13-1, malonyl-CoA is a ‘regulatory’ MCF, controlling insulin secretion at earlier steps in the amplification pathway. Besides functioning as a switch for redirecting fatty acid flux, malonyl-CoA has also been ascribed a central fuel-sensing role in the hypothalamus and as an anorectic mediator in the central control of feeding [72, 73]. Interestingly, these actions may in part be independent of fatty acid oxidation regulation. Thus, future studies should be directed towards delineating the underlying mechanisms that encompass the various regulatory roles of malonyl-CoA in

Evidence in favour of a role of an ACC/malonyl-CoA/CPT-1 signalling network in glucose and fatty acid stimulation of insulin secretion

In vitro beta cell studies

Biochemical studies

Citrate and malonyl-CoA levels correlate with GSIS [9, 10, 22–27]

Glucose decreases beta cell fat oxidation and promotes lipogenesis and lipolysis [9, 10, 26]

Glucose reduces beta cell ACC phosphorylation and increases its activity [28]

Only the nutrients or combination of nutrients increasing malonyl-CoA cause secretion [25]

Pharmacological studies

Bromopalmitate inhibits fat oxidation and restores GSIS in fasted rat islets [30]

Etomoxir inhibits fat oxidation and increase GSIS in rat islets [30]

Etomoxir restores GSIS in *db/db* diabetic mouse islets [31]

Etomoxir restores GSIS in INS1E beta cells chronically exposed to NEFA [32]

ACL inhibitors hydroxycitrate and radicicol reduce GSIS [30, 33, 34, 40]

Pyruvate carboxylase and ACC inhibitors reduce GSIS [10, 11, 35]

FA-CoA synthase inhibitor triascin C reduces GSIS only in the presence of NEFA [15]

Depletion of beta cell NEFA in MIN6 beta cells with high albumin lowers GSIS [16]

Evidence from altered expression of relevant genes

ACL expression is high in normal islets and is decreased in islets from individuals with type 2 diabetes [36, 37]

RNAi suppression of ACL and mitochondrial citrate carrier lowers GSIS [34, 39]

Antisense mRNA against *Acc1* in INS-1 cells lowers malonyl-CoA levels and GSIS [41]

Overexpression of MCD reduces GSIS only in the presence of NEFA in INS-1(832/13) cells [15]

Overexpression of CPT-1 in INS-1 cells reduces GSIS, which is restored by etomoxir or NEFA [32]

Overexpression of malonyl-CoA-insensitive M593S mutant CPT-1 in islets lowers GSIS [42]

RNAi-knockdown of acyl-CoA synthetase long-chain in INS-1(832/13) cells lowers GSIS [43]

ACOT7 overexpression decreases GSIS in INS-1(832/13) cells [45]

Downregulation of beta cell CACT by miR-132 or miR-212 elevates GSIS [47]

Knockdown of the fatty acid oxidation enzyme HADHSC enhances fuel-induced insulin secretion [54]

In vivo studies

Pharmacological studies

CPT-1 inhibition by etomoxir in fasted rats enhances GSIS at high levels of plasma glucose and NEFA [48]

ND-630 suppression of ACC1 in vivo lowers malonyl-CoA levels and GSIS in the Zucker Diabetic Fatty rat [49]

Inhibition of ACL by bempedoic acid in high-fat-diet-fed mice reduces hyperinsulinaemia [50]

ACL inhibitor hydroxycitrate decreases plasma insulin levels in human [51]

Genetic studies

Genetic defects in NEFA oxidation enzymes are accompanied with hypoketotic hypoglycaemia, and hyperinsulinism in the case of HADHSC [52, 53, 54]

Beta cell-specific overexpression of *Acot7* in mice causes reduced GSIS [45]

PPARδ or PPARα knockdown in mice lowers CPT-1 and NEFA oxidation genes and elevates GSIS [55-57]

PPARα knockdown in mice results in hyperinsulinaemic hypoglycaemia in the fasting state [56]

Beta cell-specific triple FOXO knockdown in mice results in elevated beta cell NEFA oxidation and reduced GSIS [58]

Mice with beta cell-specific activation of *Foxo1* show reduced beta cell NEFA oxidation and high GSIS [59]

Acc-1-knockout mice show reduced GSIS at low and intermediate glucose levels [60]

Constitutively active *Acc-1*-knockin mice have fasting hyperinsulinaemia [62]

Beta cell-specific *Lkb1*-knockout mice have elevated ACC1 activity at low glucose and enhanced GSIS [63–65]

beta cell function, some independent of CPT-1, possibly involving protein malonylation [74]. Studies of these processes may also be relevant to fuel signalling in other tissues.

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