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Glycerolipid Metabolism and Signaling in Health and Disease

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Maintenance of body temperature is achieved partly by modulating lipolysis by a network of complex regulatory mechanisms. Lipolysis is an integral part of the glycerolipid/free fatty acid (GL/FFA) cycle, which is the focus of this review, and we discuss the significance of this pathway in the regulation of many physiological processes besides thermogenesis.

GL/FFA cycle is referred to as a "futile" cycle because it involves continuous formation and hydrolysis of GL with the release of heat, at the expense of ATP. However, we present evidence underscoring the "vital" cellular signaling roles of the GL/FFA cycle for many biological processes. Probably because of its importance in many cellular functions, GL/FFA cycling is under stringent control and is organized as several composite short substrate/product cycles where forward and backward reactions are catalyzed by separate enzymes. We believe that the renaissance of the GL/FFA cycle is timely, considering the emerging view that many of the neutral lipids are in fact key signaling molecules whose production is closely linked to GL/FFA cycling processes.

The evidence supporting the view that alterations in GL/FFA cycling are involved in the pathogenesis of "fatal" conditions such as obesity, type 2 diabetes, and cancer is discussed. We also review the different enzymatic and transport steps that encompass the GL/FFA cycle leading to the generation of several metabolic signals possibly implicated in the regulation of biological processes ranging from energy homeostasis, insulin secretion and appetite control to aging and longevity. Finally, we present a perspective of the possible therapeutic implications of targeting this cycling. (*Endocrine Reviews* 29: 647–676, 2008)

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Abbreviations: AA, Arachidonic acid; ACC, acetyl-CoA carboxylase; ACL, ATP-citrate lyase; ACSL, acyl-CoA synthase; ADRP, adipocyte differentiation-related protein; 2-AG, 2-arachidonylglycerol; AGPAT, 1-acyl-*sn*-Gly3P acyltransferase; AgRP, Agouti-related protein; AMPK, AMP-activated protein kinase; ATGL, adipose triglyceride lipase; CB, cannabinoid; CE, cholesterol ester; CGI-58, comparative gene identification-58; CIDE-A/B, cell death inducing DFF-α (DNA fragmentation factor-α)-like effector A/B; DAG, diacylglycerol; DAGK, DAG kinase; DGAT, DAG acyltransferase; EC, endocannabinoid; ER, endoplasmic reticulum; FA, fatty acid; FABP, FA binding protein; FACoA, fatty acyl-coenzyme A; FAS, FA synthetase; FAT, FA transporter; FATP, FAT proteins; FFA, free FA; GL, glycerolipid; GlyK, glycerol kinase; Gly3P, glycerol-3-phosphate; GPAT, Gly3P phosphate acyltransferase; GPCR, G protein-coupled receptor; GPR40, GPCR 40; GS2, gene sequence-2; HIF, hypoxia-inducible factor; HSL, hormone-sensitive lipase; HSP, heat shock protein; iPLA2, independent phospholipase A2; KO, knockout; LD, lipid droplets; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; MAG, monoacylglycerol; MAGK, MAG kinase; MGAT, MAG acyltransferase; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD; NFκB, nuclear factor κB; NP-Y, neuropeptide Y; PA, phosphatidic acid; PAP, PA phosphatase; PEDF, pigment epithelium-derived factor; PEPCK, phosphoenolpyruvate carboxykinase; PKA, protein kinase A; PKC, protein kinase C; PL, phospholipid; PPAR, peroxisomal proliferator-activated receptor; PPi, pyrophosphate; PPI, polyphosphoinositides; SCD, stearoyl-CoA desaturase; SIRT, silent information regulator-2 homolog; T2D, type 2 diabetes; TG, triacylglycerol; UCP, uncoupling protein; VLDL, very low density lipoprotein; ZDF, Zucker diabetic fatty (rats); ZF, Zucker fatty(rats).

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I. Introduction

ONE OF THE primordial symptoms of disease known to mankind is fever, *i.e.*, a steady maintenance of elevated body temperature above 37 C. Ancient physicians dating back to Hippocrates thought that the body uses fever as a protective tool. In fact, they induced fever with pyrogenic extracts in patients to fight against certain infections (1). What they did not know is that the basis of their strategy was at least in part if not largely centered around lipolysis. Body temperature can also be increased after exercise. Although a

complicated network of regulatory mechanisms involving, in particular, cytokines and the hypothalamus play a role in thermogenesis and energy expenditure, most of the pathways that control body temperature converge on lipolysis, *i.e.*, the breakdown of lipids (2–5). Hydrolysis of glycerolipids (GLs) (both neutral and phospholipids) has largely been viewed as a pathway unconnected with lipogenesis and lipid esterification processes. However, as detailed below, lipolysis is an integral part of an essential metabolic pathway, the GL/free fatty acid (FFA) cycle, which is the focus of this review. As it unfolds, this pathway's contribution to body temperature maintenance (6) is only the tip of the iceberg when one considers its physiological importance. Thus, the emerging evidence indicates that GL/FFA cycling produces many signaling molecules that regulate a number of biological processes. Because of the essential nature of the role of lipids, both at structural and signaling levels, any perturbation of their metabolism leads to a wide range of pathophysiological phenomena, including conditions such as obesity, type 2 diabetes (T2D), nonalcoholic fatty liver disease, and cancer.

Fatty acid (FA) is the major form by which energy is stored in complex organisms and animals. Besides being an essential component of energy metabolism, lipids are involved in both intracellular and extracellular (autocrine and paracrine) and whole animal (endocrine) signaling processes. It is increasingly becoming evident that disturbances in lipid metabolism, particularly those involving the components of GL/FFA cycling, are strongly associated with other diseases related to the metabolic syndrome, as well as inflammation and the pathogenesis of some cancers (7, 8). On the other hand, energy restriction, resulting in marked reduction in adipose tissue lipid storage in association with lipolysis (9), is known to slow aging and to obliterate the onset of aging-associated metabolic diseases (10), such that lipid metabolism is likely related to the processes of senescence.

In this review, we discuss GL/FFA cycling at the cellular level and underscore its novel role as a metabolic machine that churns out a plethora of signaling molecules controlling numerous biological processes. GL/FFA cycling refers to the cyclic process of esterification of FFA onto a glycerol backbone to synthesize GL, followed by its hydrolysis with the release of the FFA that can be reesterified (Fig. 1). GL/FFA cycling is constitutively active in all cells, allowing for continuous production of a wide array of both neutral [monoacylglycerols (MAG), diacylglycerols (DAG), and triacylglycerols (TG)] and polar [phospholipids (PLs)] GLs, in addition to participating in acyl-chain exchange between various GL molecules (11, 12). However, we emphasize that it is not necessary for the cell to operate the complete cyclic process to generate any of these intermediates because most of the involved enzymatic steps can also occur independent of each other and, as detailed below, there are many "short cycles" within the GL/FFA cycle for controlling flux through individual steps and the corresponding metabolite levels in the cell.

Lipid droplets (LD) are cytosolic lipidic organelles comprised of a monolayer of amphipathic lipids (*e.g.*, PL) surrounding (13) a core of neutral lipids [*e.g.*, DAG, TG, and cholesterol esters (CEs)] and coated by LD-associated proteins such as perilipin, adipophilin, and lipase enzymes. LD are the major intracellular source of GL for GL/FFA cycling, are central to almost all lipid-related processes in the body, and are vital for specialized functions in almost all cell types (14).

GL/FFA cycling is generally referred to as "a futile cycle"

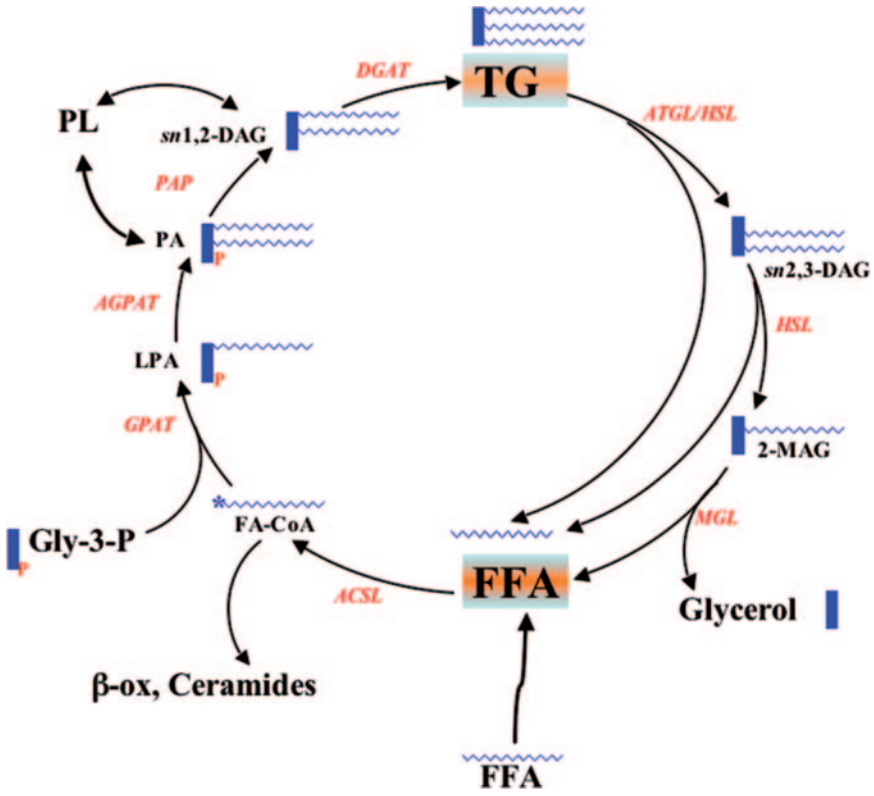

FIG. 1. Enzymes and intermediates of the TG/FFA cycle. FACoA is formed by long-chain ACSL from FFA supplied externally or produced by the hydrolysis of TG, DAG, and MAG. The accumulating FACoA is partitioned into the formation of complex lipids through the condensation with glucose-derived Gly3P by GPAT to form LPA. LPA is further converted by AGPAT to PA, which eventually gives rise to DAG by the action of PAP or PLs. DAG thus formed is acylated by DGAT to form TG. DAG derived from TG by ATGL and/or HSL is hydrolyzed to 2-MAG by HSL. 2-MAG is hydrolyzed by MAG lipase (MGL) to FFA and glycerol, which is secreted out of the cell. In some cells, the released glycerol is recycled back into TG/FFA cycling by conversion to Gly3P by glycerokinase. β-ox, Oxidation.

in that it consumes ATP with the release of heat (6). We believe that the term "futile," while being technically correct, severely understates its key importance for many biological functions essential for both the cell and whole organism. Furthermore, it is becoming increasingly clear that alterations in this cycling process are involved in the pathogenesis of multiple disease states. Thus, we propose that GL/FFA cycling should be considered as "vital," and the energy consumed for this cycle's operation is beneficial for processes such as fuel detoxification and is also the price cells pay for the production of vital signals. We believe that aberrations in this process can be "fatal" and contribute to the development of T2D and pathogenesis associated with the metabolic syndrome. The possible implication of GL/FFA cycling in cancer and aging is also briefly discussed.

II. GL/FFA Cycle and Lipid Droplets: Overview

In this review we will discuss the biological processes that are directly related to the actual cycling process of the GL/FFA cycle, as well as those that implicate its lipolytic or anabolic segments *per se*.

We will first consider GL/FFA cycling from the anabolic perspective by looking at the synthesis of the energy storage glycerolipid, TG. The anabolic phase of this cycle utilizes glycerol-3-phosphate (Gly3P) and fatty acyl-coenzyme A (FACoA) as substrates. Gly3P is derived from glycolysis, glyceroneogensis, or, in some tissues (*e.g.*, liver), from the recycling of glycerol by glycerol kinase (GlyK) (11). (Fig. 1). The FFA for FACoA synthesis are derived from cellular uptake of exogenous FFA, the recycling of FFA arising from lipolysis (GL/FFA cycling), and *de novo* synthesis (lipogenesis) of FFA from substrates such as glucose. Essentially, the energy in the thioester bond of FACoA is used to make an ester bond between the hydroxyl group of glycerol backbone and the fatty acyl moiety. During the synthesis of TG, FACoA is used as the substrate by each of the three esterification enzymes, *viz.*, glycerophosphate acyltransferase (GPAT), 1-acyl-*sn*-Gly3P acyltransferase (AGPAT), and DAG acyltransferase (DGAT). The catabolic phase occurs with the hydrolysis (lipolysis) of the ester bonds by lipase enzymes such as adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and MAG lipase. When each ester bond of TG is hydrolyzed, the energy in the bond cannot be reused for building another energy-rich bond (*e.g.*, reformation of FACoA) and is released as heat. Therefore, when cycling occurs, for every TG-ester bond that is hydrolyzed, the energy provided by two ATP molecules is lost, and during complete hydrolysis of TG to glycerol and FFA, the heat produced comes from the energy provided by seven high-energy phosphodiester bonds from ATP. Thus, to form one molecule of TG, three ATP are converted to AMP and pyrophosphate (PPi) at the FACoA synthase step, and an additional ATP is required by the GlyK reaction (Fig. 1), hence the name "futile cycle." Of note, the significance of this cycle in thermogenesis was proposed more than 30 yr ago by Newsholme and Crabtree (6).

There are several intermediate lipids in the production of TG. These include lysophosphatidic acid (LPA), phosphatidic acid (PA), and DAG. Furthermore, both PA and DAG can be partitioned into the production of PL (*e.g.*, phosphatidylcholine). Acyl-CoA can also be esterified with cholesterol, producing CEs, and once formed, the acyl-chain of CE may also be cycled. It is important to realize that GL/FFA cycling may involve shorter cycles than the full cycle of TG synthesis followed by its complete hydrolysis. Examples of shorter cycles would be the hydrolysis of TG to DAG with the reesterification of DAG back to TG and the hydrolysis of DAG to MAG, which can be reesterified to DAG (Fig. 2). These bypass loops ensure the production of different intermediates of this cycle, as and when needed, without the complete formation of TG and its lipolysis. Thus, it is important to note that GL/FFA cycling, being a composite of many shorter cycles where different enzymes catalyze the forward and reverse reactions, can be fine-tuned under rigorous control to produce the appropriate amount of various metabolic intermediates and signaling molecules according to the organism's need. DAG can also arise from the hydrolysis of PL by phospholipase-C enzymes and could contribute to the total DAG pool. Thus, although the literature traditionally refers to TG/FFA cycling, we propose to use a more general term that encompass all these GL cycling processes (Figs. 1 and 2), that is GL/FFA cycling. We have used the term GL/FFA cycling as synonymous to TG/FFA cycling, although the latter term was in some instances preferred while referring to literature. Renaming this process as GL/FFA cycling appears to us more appropriate because TG is not the necessary fate of the FFA in terms of esterification processes before hydrolysis. Thus, MAG and DAG once formed can be directly hydrolyzed without being transformed to TG first (Fig. 2). In addition, DAG released from phosphoglycerolipid hydrolysis via the action of phospholipases also participate in this cycling (Fig. 1).

GL/FFA cycling can be at the whole body level or at a cellular level. Whole body GL/FFA cycling plays an important role under conditions of nutritional deficit (starvation) and cancer cachexia and after severe burns to the body by mobilizing the much needed energy resource in the form of FFA from adipose tissue, and it also plays a role in thermogenesis (6). An example of whole body cycling would be the lipolysis of adipose tissue TG with release of both FFA and glycerol into the circulation, followed by their uptake, reesterification, and GL synthesis in another tissue (Fig. 3). Whereas some FFA may be taken up by tissues such as heart and skeletal muscle for oxidation and therefore not reesterified, FFA in addition to glycerol, can be taken up by the liver, where TG is resynthesized for its eventual export within very low-density lipoprotein (VLDL) particles. The major focus of this review, however, is at the intracellular level, with some consideration also of local extracellular production of lipid moieties.

GL synthesis occurs on the mitochondrial outer membrane and in the endoplasmic reticulum (ER) (15). There are several hypotheses regarding the formation of LD in the cell (16), although the role of ER appears to be significant (17). There is ample evidence indicating that the synthesized lipid is initially contained within the ER lipid bilayer membrane and is then packaged into cytosolic LD. LD-associated proteins from the PAT family [perilipin, adipocyte differentiation-

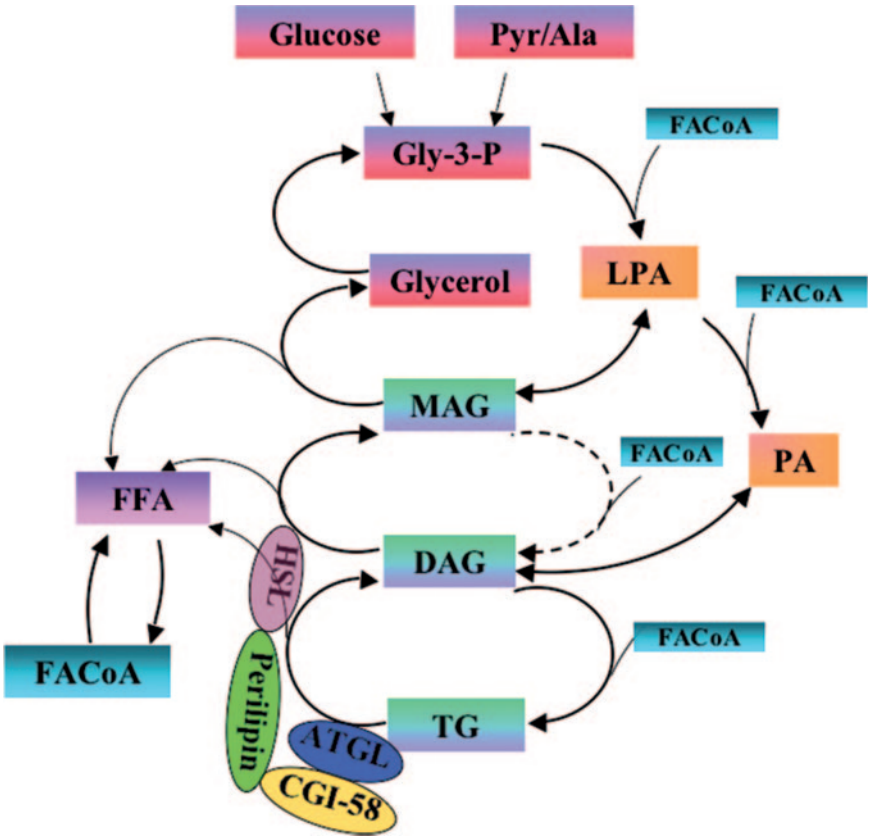

FIG. 2. Cellular GL/FFA cycling processes. The scheme depicts the various stages of the GL/FFA cycling processes. Glucose or pyruvate and alanine (Pyr/Ala) contribute to the formation of Gly3P, which can also be formed from glycerol in some tissues. LPA formed from Gly3P is either further acylated to PA or dephosphorylated to form 1-MAG. PA and MAG can be converted to 1,2-DAG by acylation and dephosphorylation, respectively. MAG acylation (*dotted line*) takes place mostly in intestinal epithelial cells. DAG acylation to TG is the final step of lipogenic segment of the GL/FFA cycling. DAG hydrolysis to 2-MAG by HSL and TG hydrolysis by ATGL is facilitated by perilipin and CGI-58 on the surface of LD. The next step in the lipolytic segment of the GL/FFA cycling is the hydrolysis of MAG by the ubiquitous MAG lipase to glycerol and FFA. In most cells, 50–70% of the FFA released in the lipolytic segment is recycled into the lipogenic segment after its activation to FACoA. It is important to note that at every acylation step the reaction product can be converted back to starting components.

related protein (ADRP), and TIP47] are essential for the normal genesis and stabilization of the droplets (18). The maturation of LD in adipose tissue and adrenal steroidogenic tissue involves the replacement of ADRP with perilipin. Perilipin is more effective at stabilizing the droplets, thus enhancing lipid storage. Perilipin also allows for regulated lipid hydrolysis according to nutrient and hormonal controls (19). ADRP is the major LD surface protein in most other tissues. Recent proteomic analyses of LD (13) suggest quite complex associations of multiple proteins with the LD that may be involved in intracellular transport of lipids, as well as storage and trafficking of the proteins themselves. LD dynamics, including GL/FFA cycling, may be key in the trafficking of lipids toward the correct subcellular sites for functions such as incorporation into cell membranes, lipid signaling, FFA oxidation, and the synthesis of lipoproteins for lipid export.

Perilipin, on the surface of the LD, binds with a protein called, comparative gene identification-58 (CGI-58; also known as ABHD5), and a defect in this protein leads to Chanarin-Dorfman syndrome, characterized by abnormal intracellular accumulation of LD in many tissues (20, 21). The mutated CGI-58 in this syndrome is unable to bind with perilipin and is not recruited to the LD surface (21). CGI-58 has been shown to bind and activate ATGL on the surface of LD (22). Phosphorylation of perilipin by protein kinase A (PKA) likely plays an important role in the CGI-58 mediated activation of lipolytic process (23).

Lipolysis is also regulated by the cell death inducing DFF-α (DNA fragmentation factor α)-like effector (CIDE) family of proteins, which share sequence homology with the proapoptotic protein DFF-α (24). It has been shown that CIDE-A, which is predominantly localized in adipose tissue (25, 26), and CIDE-B, which is expressed mostly in liver (27), regulate lipolysis, lipogenesis, fat oxidation, and energy metabolism. Mice with deletion of either of these proteins are lean and resistant to diet-induced obesity, have lower plasma TG and FFA levels, and show enhanced insulin sensitivity (24, 27). CIDE proteins are localized in mitochondria, and their overexpression leads to apoptosis (24, 27). Although the exact mechanism of action of these proteins on lipid metabolism is presently unknown, CIDE-A appears to mediate its effects via the MAPK pathway (26).

Several ER proteins are localized in LD (14, 28). It has been proposed that LD originate between the two leaflets of the ER membrane, with the deposit of the newly formed TG during the course of TG biosynthesis by DGAT, which is present in the ER (28). As the LD grows in size, it buds off from the ER, and this perhaps explains the phospholipids monolayer and the presence of ER proteins (*e.g.*, BiP) in LD. Considering that LD originate from ER and that within the cell, both ER and LD are in close proximity, it is likely that there may be a functional interaction between these two organelles (28). However, it is not clear whether ER directly influences the number and the size of the LD.

Early studies on glycerol and FFA production from adipose tissue in both rats and humans showed that most of the FFA are released into the cell and extracellular medium, followed by their uptake and reesterification to TG (29), by which nearly 40% of the FFA are rapidly recycled back to TG (30, 31). Later studies revealed that such recycling occurs not

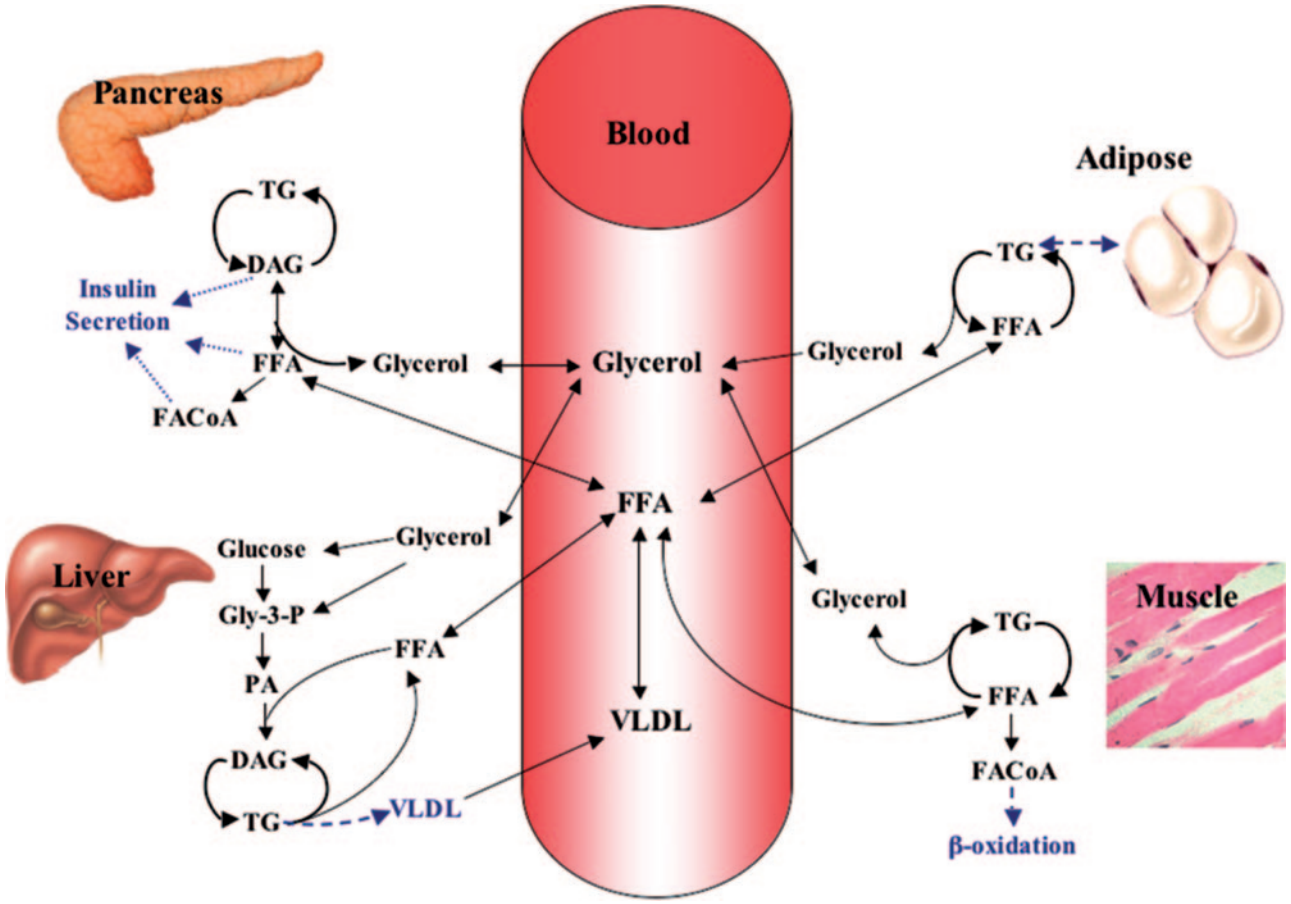

FIG. 3. Intertissue relationship in GL/FFA cycling processes. The scheme illustrates how the body tissues share the reactants of GL/FFA cycling through blood, and therefore how GL/FFA cycling processes in various tissues are interlinked for the purpose of particular organs. Adipose tissue contributes to a major portion of glycerol in blood, and other tissues that lack glycerokinase also release glycerol through GL/FFA cycling into blood. Liver and skeletal muscle can produce glycerol and also utilize it because they have glycerokinase and recycle it into the cycling. The majority of the FFA produced in most cells through the lipolytic segment of the cycling are recycled back into lipogenic reactions, and the remaining FFA enters circulation. FFA in skeletal muscle is β-oxidized for most part, whereas it is used for TG synthesis and VLDL assembly in liver. VLDL from liver is secreted into blood, where it can contribute to FFA through lipoprotein lipase activity. TG formed in adipose tissue is stored as LD. The recent evidence indicates that the operation of GL/FFA cycling is essential for glucose-induced insulin secretion in pancreatic β-cells, probably by the generation of critical signaling molecules.

only in adipose tissue but also in liver and skeletal muscle (11). Only a small fraction of the FFA derived from TG lipolysis in white adipose tissue is oxidized, whereas the major portion of the FFA are reesterified either in the adipose tissue itself or in other tissues. In a given cell, the fraction of lipolysis-released FFA that is recycled back is relatively constant, at different rates of GL/FFA cycling (turnover) under different metabolic conditions (11).

III. Metabolic Signaling Machinery

The enzymes and key proteins involved in the biosynthesis and breakdown of GL have been reviewed in several recent publications (15, 32, 33). They are discussed below only within the scope of their implication in metabolic signaling and their novel roles in various (patho)physiological processes.

A. Glycerolipid anabolism

1. Gly3P supply via glycolysis and glyceroneogenesis. The major source of Gly3P for the synthesis of GL under postprandial conditions is glycolysis via cytosolic nicotinamide adenine dinucleotide (NAD)-linked Gly3P dehydrogenase. Of significance, this reaction regenerates NAD^+, which participates in other important pathways in the cell, which are discussed in *Section VII*. Adipocytes, hepatocytes, and cancer cells, however, can also synthesize TG under fasting and nutrient-deprived conditions when glycolysis is reduced. This can be achieved via glyceroneogenesis using phosphoenolpyruvate carboxykinase (PEPCK)-derived Gly3P (11, 34, 35). Interestingly, an increase in adipose tissue glyceroneogenesis by adipose tissue-specific overexpression of PEPCK in mice has been shown to cause increased adipose tissue mass and body weight with decreased circulating FFA (36). This was probably due to enhanced activity of the esterification arm of GL/FFA cycling in adipose tissue (36). Consistent with this, the peroxisomal proliferator-activated receptor γ (PPARγ) agonist thiazolidinedione drugs up-regulate PEPCK and GlyK in adipose tissue and thus contribute to increased glyceroneogenesis and TG synthesis, thereby reducing circulating FFA (37–39). Surprisingly, recent findings of Hakimi *et al.* (40) showed that transgenic mice with skeletal muscle-specific overexpression of PEPCK-C have lower body weights,

higher TG content in skeletal muscle, increased energy output, and much higher exercise endurance, and they lived much longer than the wild-type mice. Although phosphorylation of glycerol by GlyK is important for GL synthesis in liver and adipose tissue, PEPCK-derived Gly3P seems essential under conditions of elevated FFA supply (*e.g.*, starvation) for maintaining the proper balance of lipolysis, *de novo* lipogenesis, and lipid storage (34, 41–43).

2. Aquaporins and GlyK. An important player in the regulation of GL/FFA cycling is the plasma membrane transporter of glycerol. Recent results suggest that whereas aquaporin-7 is responsible for glycerol efflux in adipocytes (44, 45), aquaporin-3 might undertake this function in other cell types (46, 47). Blocking glycerol efflux in adipose tissue by deleting aquaporin-7 results in altered TG/FFA cycling accompanied by elevated circulating FFA, obesity, insulin resistance, and abnormal glucose tolerance (48, 49). Glycerol accumulation in aquaporin-7-deleted adipocytes induces GlyK with the resultant increase in Gly3P to feed into GL/FFA cycling, leading to TG accumulation followed by FFA release. However, unlike glycerol, the FFA released during lipolysis still enters blood circulation, thus contributing to insulin resistance and abnormal glucose homeostasis (48, 49). This indicates that GL/FFA cycling in adipose tissue plays a crucial role in regulating whole-body glucose homeostasis and insulin sensitivity (44, 45). Aquaporin-7 deletion in mice was recently shown to lead to reduced β-cell mass and elevated insulin secretory response, which is associated with increased glycerol accumulation and GlyK activity in the β-cell (50). Interestingly, increased intracellular glycerol also caused elevated insulin gene transcription in these cells. The significance of glycerol-transporting aquaporins in other tissues is presently not known, but they likely play a critical role in the control of GL synthesis and associated functions of GL/FFA cycling in particular tissues.

3. FA transport and long-chain acyl-CoA synthetase (ACSL). FA transport across the plasma membrane can be due to simple diffusion through the lipid bilayer or possibly via facilitated transport (51). FA transporter (FAT)/CD36, FA binding proteins (FABP), and FAT proteins (FATP) are thought to be important players in mediating FFA transport. There is increasing evidence that FAT/CD36, in association with plasma membrane lipid rafts, facilitates the uptake of long chain FAs, a process dependent on the presence of cholesterol in the lipid rafts (52). CD36 is a broadly expressed 88-kDa (postglycosylation molecular weight) membrane glycoprotein with 471 amino acids that also acts as a receptor for various ligands including modified forms of low-density lipoprotein, thrombospondins, fibrillar β-amyloid, and apoptotic cells. Although the exact mechanism of CD36 action is not clear, its absence affects FA uptake by various tissues. Thus, in CD36 knockout (KO) mice the uptake of FA analogs is specifically reduced in muscle and adipocytes, and these mice have a fasting hypoglycemia. Similar reduction in FA uptake has been reported in the heart muscle of CD36-deficient humans (53). Besides CD36, it has been suggested that the six members of the FATP family of proteins, whose expression varies with the tissue, are also directly involved in the translocation of FFA across the plasma membrane. It has been proposed that FATP and FAT/CD36 may exist as a functional complex in the plasma membrane, and according to this hypothesis, FFAs first accumulate near the plasma membrane by binding to CD36, which then transfers them to FATP for subsequent translocation across the membrane. Once FFA cross the membrane, they are bound by FABPs for delivery to different subcellular locations. It has been observed that FATP overexpression enhances intracellular ACSL activity and that purified recombinant FATP1 possesses ACSL activity (52). Because of this, it is presently unknown whether FATP act truly as FFA transporters or act as ACSL that "trap" FFA within the cell. This is particularly relevant because so far FATP have not been documented to be able to transport FA across liposomal membranes.

After their entry into the cell, long-chain FAs need to be activated for use by the cell by the creation of a thiol-ester with coenzyme A. This reaction is catalyzed by ACSL, and the acyl-CoA produced can be used for FA oxidation, esterification, and acylation processes. Acyl-CoAs play a direct role in metabolic regulation and many signaling processes, such as the modulation of ion channel activity and gene expression (54). The ACSL reaction requires ATP with the production of AMP and PPi. Five different rat ACSL enzymes have been cloned, each from different genes with different subcellular and tissue distributions (55). ACSL1, ACSL2, and ACSL5 comprise one subfamily with about 60% homology to each other; ACSL3 and ACSL4 make another subfamily with 70% homology. There is some evidence suggesting that the different isoforms regulate intracellular partitioning of acyl-CoA toward different functions.

The possibility of the five different ACSL isoenzymes partitioning FFA for specific metabolic/signaling pathways has been examined recently. ACSL5 is localized on mitochondria and also on ER and is likely involved in FA oxidation. Results from overexpressing ACSL5 suggested that this isoenzyme partitions exogenous FFA toward TG synthesis (56). It was noticed that ACSL1, whose expression increases in liver cells under both lipogenic and oxidative conditions, is localized on the ER but not on mitochondria or plasma membrane in rat primary hepatocytes. ACSL1 overexpression in hepatocytes increased oleate incorporation into DAG and PL and decreased incorporation into CEs and secreted TG without affecting oleate incorporation into TG and β-oxidation (57). Importantly, pulse-chase experiments suggested that ACSL1 on the ER enhances the reacylation of oleate derived from TG and DAG hydrolysis and partitioned the FA toward TG and PL synthesis away from CE synthesis (57). However, others reported that ACSL1 interacts with the FA transporter FATP1 in adipocytes and constitute the first described enzyme pair involved in a vectorial acylation system in mammals (58, 59). Also, on the basis of inhibition experiments using triacsin-C, a compound that inhibits all isoforms of ACSL except ACSL5 and -6, it has been proposed that mitochondrial outer membrane must also possess ACSL1 activity (58).

4. GPAT. GPAT catalyzes the first committed step in GL (including PL) synthesis resulting in the formation of LPA from acyl-CoA and Gly3P. Four isoforms of GPAT have been identified, two in mitochondria (15) and two in the ER. The

ER/microsomal GPAT accounts for 80–90% of total activity in most tissues and 50–80% of total activity in the liver. ER-associated GPAT3 has been recently characterized and was found to have less than 15% sequence identity with mitochondrial GPAT1 (60). The acyltransferase sequence motifs, however, are present in this protein (60). Overexpression of microsomal GPAT3 in mammalian cells leads to an increase in TG formation but not PL, indicating that the LPA synthesized by GPAT3 in the ER favors TG synthesis (60). GPAT3 is dramatically up-regulated during adipocyte differentiation and is also induced by treatment with PPARγ agonists (60). Its expression is reduced in adipose tissue and increased in the livers of *ob/ob* mice (60). Recent studies demonstrated the presence in several tissues of another microsomal protein with GPAT activity (GPAT4), which was earlier thought to be an AGPAT (61, 62).

Mitochondrial GPAT1 has been characterized more thoroughly than the other isoforms. Up to 50% of total GPAT activity in the liver is due to mitochondrial GPAT1, which is elevated in obese rodents (63, 64). Knockdown of the expression of liver GPAT1 in obese *ob/ob* mice results in decreased hepatic TG, DAG, and FFA, as well as lowered plasma cholesterol and glucose (65). Regulation of mitochondrial GPAT1 occurs in response to both nutritional and hormonal changes (66). It occurs both at the gene transcription and protein modification levels. Generally, fasting reduces and refeeding increases its expression and activity. Posttranslational regulation of GPAT1 is via (de)phosphorylation. GPAT1 is activated by phosphorylation by casein kinase II and protein kinase C (PKC) in T-lymphocytes (67). However, AMP-activated protein kinase (AMPK) inhibits GPAT by phosphorylation and also reduces its expression (68). A second mitochondrial GPAT (GPAT2) was identified in livers of GPAT1 KO mice (69). Its role is less clear at this stage. The LPA product of mitochondrial GPAT needs to be transported to the ER for further GL synthesis into TG and PL because this is where the other enzymes are located.

5. AGPAT. AGPAT (also known as LPA acyltransferase) catalyzes the acylation of LPA to PA. It exists in multiple isoforms (70, 71). Only the α and β (1 and 2) isoforms show significant activity. They contain two to four predicted transmembrane domains and two highly conserved acyltransferase family motifs, H(X)4D and EGTR, which are essential for catalytic activity (72). In humans, AGPAT-1 has broad tissue distribution. AGPAT-2 is restricted to heart, liver, adipose tissue, and pancreas. AGPAT-8 is expressed mostly in heart and kidney (70, 73). An inherited form of congenital lipodystrophy, characterized by inactivating mutations in the AGPAT-2 gene and near complete loss of adipose tissue, has been noticed (74). Such lipodystrophy due to either inherited or acquired inactivating mutations of AGPAT-2 is associated with diabetes (75), possibly due to the ectopic accumulation of fat in nonadipose tissues, thus causing insulin resistance and perhaps β-cell dysfunction as well (76, 77). Like many other lipid synthesis enzymes, AGPAT-2 is overexpressed in various cancers (78), and specific inhibition of AGPAT induces apoptosis of cancer cells (79, 80). PA can also be synthesized from DAG by DAG kinase (DAGK), although this reaction may be more important in the generation of lipid-signaling molecules than for TG synthesis (81).

6. PA phosphatase (PAP). PAP dephosphorylates PA to DAG. Two types of PAP exist in mammalian tissues, with PAP-1 believed to be involved in the synthesis of TG and PL at the ER. PAP-2 produces DAG form PA released from membrane PL by phospholipase D and is involved in PL signal transduction pathways. There are three integral membrane PAP-2 isoenzymes that are able to catalyze the hydrolysis not only of PA, but also LPA, ceramide 1-P and sphingosine 1-P. Although PAP-2 can produce DAG that can be moved to the ER for TG and PL synthesis, this is thought to be quantitatively small.

The importance of PAP-1 in fat homeostasis became evident in 2001 when it was reported that lipodystrophy in the fatty liver dystrophy mouse (*fld* mouse) was due to a mutation in the *Lipin1* (lipin-1) gene (82). The family of lipin genes (lipin-1, -2, and -3) has subsequently been shown to have PAP-1 activity, and lipin-1 has been documented to be the major PAP-1 in white and brown adipose tissue and skeletal muscle (83). Hepatic PAP-1 activity is maintained in *fld* mice, most probably due to the expression of lipin-2 and -3. The lipins (unlike PAP-2 enzymes) do not have hydrolytic activity for LPA, ceramide 1-P, and sphingosine 1-P. In adipose tissue, lipin-1 is involved in adipocyte differentiation and TG accumulation (84). In skeletal muscle, lipin-1-deficient mice have increased rates of FA oxidation, and lipin-1 overexpression causes lipid accumulation (84). In addition to exhibiting PAP-1 activities, lipins interact with transcription factors from the PPAR family (85).

7. DGAT. DGAT acylates DAG to TG and plays an important role in the regulation of energy storage and metabolism. Two separate genes code for the DGAT-1 and -2 isoforms (86). DGAT-1 is highly expressed in intestine and skeletal muscle and also in other tissues, whereas DGAT-2 is mostly expressed in liver and adipose tissue (87). In liver and other tissues, DGAT-1 is present on the cytosolic aspect of the ER membrane, where it is responsible for the synthesis of cytosolic TG. Hepatic DGAT-2, on the other hand, is thought to be localized on the luminal aspect of the ER membrane and is needed for the synthesis of TG for assembly of VLDL particles (87–90). However, overexpression experiments on the topographical organization of DGAT-2 paradoxically revealed that most of this protein is distributed on the cytosolic side of the ER membrane, including its active site (91). In these overexpression experiments, the neutral lipid binding domain of DGAT-2 appeared to reside in the transmembrane domain of the protein, close to the ER lumen (91). Because the neutral lipid binding domain is essential for its activity and because this domain likely binds the reaction product TG, it is possible that DGAT-2 may deliver the TG it produces to the proteins involved in TG trafficking (91). Interestingly, it has also been recently shown that DGAT-2 is localized in LDs besides ER membrane (92). Additional work is required to establish the intracellular localization of DGAT-2 unequivocally.

DGAT-1 overexpression in isolated rat islet cells was shown to increase palmitate incorporation into TG and to

cause a modest accumulation in islet TG content. After culture of these islets for 3 d at 16 mM glucose, glucose-stimulated insulin secretion was reduced (93). The impaired secretion might be due to "glucolipotoxicity" (77, 94, 95) or alternatively reduced DAG levels and DAG signaling via activation of PKC and Munc-13, which are involved in the insulin secretion process (96–99). It appears that in mammalian tissues, TG synthesized from DAG is controlled by the stereoselectivity of DGAT enzymes. PA hydrolysis generates only *sn*1,2-DAG, which is the preferred substrate for DGAT enzymes in most tissues (100, 101), whereas, in small intestine, both 1,2 and 2,3 enantiomers of DAG could be acylated (102). Evidence for the stereoselectivity of adipose tissue DGAT enzymes also came from FA incorporation studies *in vivo* (103).

We reported that refeeding of fasted rats results in elevated activities of liver lipid synthesis enzymes including GPAT1 and DGAT, and that these changes are brought about by a reduction in AMPK activity (104). The PPARγ agonists and insulin sensitizers, pioglitazone and rosiglitazone, have been reported to elevate the activities of DGAT-1 in adipose tissue of individuals with impaired glucose tolerance (105). In contrast, whole body deletion of DGAT-1 in mice was shown to increase insulin sensitivity and enhance glucose tolerance. The DGAT-1 KO mice were also resistant to diet-induced obesity (106). Excessive lipid deposition ("lipotoxicity") in muscle and islet tissue is associated with insulin resistance and impaired insulin secretion and affects fuel metabolism in many tissues. Whether, in the face of fuel surfeit, DGAT protects from the toxicity of excess FA or contributes to lipotoxicity by promoting TG accumulation is not clear. Thus, diverting FFA and FACoA to the formation of "inert" LD-associated TG has been shown *in vitro* to be protective against the toxic action of elevated FFA (107). However, excessive TG accumulation in adipose and other tissues might eventually result in an "overflow" of fat through lipolysis, which could cause lipotoxicity (108–110). As will be discussed in more detail in *Section VI.B*, we believe that strategies aimed at enhancing the activity of GL/FFA cycling, rather than lipolysis or esterification processes only, provide an interesting avenue to combat the devastating consequences of fuel surfeit on the organism.

8. MAG acyltransferase (MGAT). The MAG pathway for TG synthesis occurs mostly in the small intestine in adults and also in the livers of neonatal rats and guinea pigs (111). Whereas liver microsomal MGAT has been observed to specifically acylate 2-MAG to 1,2-DAG, the intestinal enzyme is not specific for 2-MAG and can also use 1-MAG as a substrate (111). Soon after the appearance of 2-MAG from dietary fat digestion in the small intestine, it is acylated by MGAT to form DAG. Among the three recently cloned MGAT isoenzymes, MGAT-2 and MGAT-3 are present in small intestine (112, 113). Whether there is any regulated expression of MGAT in other adult tissues is not clear. Recently, it has been shown that MGAT-3, localized in the small intestine ER of higher mammals and humans, also possesses DGAT activity and can sequentially add two fatty acyl groups to MAG to form TG (114). This enzyme is notably absent in rodents (114).

9. MAG kinase (MAGK) and DAGK. Both MAG and DAG can be phosphorylated by specific enzymes. Either 1-MAG or 2-MAG can be phosphorylated to corresponding LPA products by MAGK, also known as acylglycerol kinase. Pieringer and Hokin (115) proposed a specific lipid kinase for LPA formation more than 40 yr ago; however, only recently was this enzyme identified at the molecular level (116), and it was found to phosphorylate both MAG and DAG to form LPA and PA, respectively, although the relative activity with MAG is higher. MAGK is expressed abundantly in heart, kidney, muscle, and brain and localized in mitochondria with little or no presence in ER (116, 117). LPA produced on mitochondrial membrane is likely transported by FABP to the ER, where it can be converted to PA by AGPAT. It was noticed that MAGK expression was significantly elevated in prostate cancers as compared with the normal prostate tissues from the same patient (116, 117). Considering that LPA formed by MAGK transactivates epidermal growth factor-receptor pathway in prostate cancer cells as an autocrine and paracrine signaling factor, MAGK may have an important role in the proliferation of prostate cancer cells (116, 117).

DAGKs catalyze the conversion of *sn*1,2-DAG to PA. They show very little or no activity toward the 2,3-DAG enantiomer, and in fact, 2,3 DAG was shown to inhibit α and ζ DAGK isoenzymes (118). Nearly 10 mammalian DAGK isozymes have been identified, and they contain two or three characteristic C1 domains besides the catalytic domain. Because of the alternative splicing in six of the DAGK genes, at present about 17 isoforms of DAGK are known, which differ in their tissue expression pattern and molecular properties (119, 120). Because both DAG and PA are important signaling molecules that regulate the function of a number of enzymes and proteins, it is necessary to maintain a fine balance between their concentrations in the cell, and DAGKs are rightly poised to achieve this regulation (120). The multiplicity of DAGK isoenzymes and their different subcellular localization ensures diverse regulation of various physiological processes such as development, neural and immune responses, cytoskeleton reorganization, and pathological phenomena including carcinogenesis, where these enzymes play an important role. In response to agonists, a number of DAGKs, including DAGK-α, DAGK-γ, DAGK-δ, DAGK-ζ, and DAGK-θ, have been found to translocate to the nucleus, where they may be involved in the control of DNA replication and cell cycle (121). Inasmuch as DAGKs are localized in different subcellular compartments, DAG generated at the level of LD may also be a potential substrate for these enzymes. This is likely to be particularly important because LD are distributed in the vicinity of plasma membrane as well.

10. Stearoyl-CoA desaturase-1 (SCD-1). SCD-1 catalyzes the synthesis of monounsaturated FA from saturated FA (122, 123). SCD-1 is localized in the ER in many tissues and specifically introduces a double bond between carbons 9 and 10 in saturated FA, with a high preference for palmitic and stearic acids (122). Oleoyl-CoA and palmitoleoyl-CoA are the preferred substrates for DGAT, and thus SCD-1 activity is important for overall TG synthesis (122). Although there are three isoforms of SCD-1 in mouse tissues, only SCD-1 has

been identified in humans (122, 124). Studies of asebia mice with a natural mutation in *Scd-1* and mice with KO of *Scd-1* showed significantly lowered hepatic TG synthesis and decreased tissue content of TG and other lipids. Adipogenesis was also hampered in these mice, indicating the important role of SCD-1 in whole body lipid metabolism (125, 126).

Hypertriglyceridemia in humans has been associated with elevated SCD-1 activity (127). Although targeted disruption of the *Scd-1* gene in lean mice increases insulin sensitivity and protects from diet-induced obesity, in leptin-deficient obese animals (ob/ob) *Scd-1* gene KO aggravated diabetes, causing β-cell dysfunction (128). Additional work is needed to determine whether SCD-1 inhibition is still a valid approach for obesity/diabetes treatment.

SCD-1 deficiency results in increased activities of metabolic pathways that promote β-oxidation and reduce lipid accumulation in liver and skeletal muscles via alterations in gene expression and also through activation of AMPK (122). Mainieri *et al.* (129) suggested that SCD-1 suppresses thermogenesis in skeletal muscle by augmenting *de novo* lipogenesis and by reducing the β-oxidation of saturated FFA, thereby inhibiting substrate recycling between *de novo* lipogenesis and lipid oxidation. Thus, SCD-1 might also contribute to GL/FFA cycling regulation by accelerating the *de novo* lipogenic pathway and inhibiting β-oxidation.

B. Glycerolipid catabolism

1. TG hydrolysis and ATGL. The initial step of lipolysis, the hydrolysis of TG to DAG, is achieved primarily by one of the two different lipases, HSL and the recently identified ATGL (also named desnutrin and patatin-like domain containing phospholipase A2, PNPLA2, or PLA2ζ), and possibly adiponutrin and gene sequence-2 (GS2) (130) as well. All four enzymes contain a "patatin-like" domain with broad lipid acyl-hydrolase activity. An additional enzyme, TG hydrolase, has been identified in rat adipose tissue (131). Although the hydrolysis of TG is likely catalyzed by all these lipases, HSL hydrolyzes DAG to MAG better than TG, whereas ATGL almost exclusively hydrolyzes TG and releases DAG (130, 132).

Studies using lipase substrate analogs with fluorescently labeled activity tags suggested that the TG hydrolysis reaction product of both HSL and ATGL is predominantly *sn*2,3-DAG (133). Earlier studies with hepatic and pancreatic lipases (134) and microbial lipases (135) showed a clear preference for *sn*1 position on the TG molecule for hydrolysis, suggesting that most TG lipases prefer to hydrolyze *sn*1 position.

ATGL is specifically activated severalfold by interaction with CGI-58 protein (136, 137). CGI-58 is mutated or truncated in individuals with Chanarin-Dorfman syndrome (also designated as neutral lipid storage disease), leading to the accumulation of TG in multiple tissues due to lowered activity of ATGL (136).

ATGL in adipocytes has been shown to be located in cytosol as well as on LD, probably in association with CGI-58 and perilipin, a protein which, when unphosphorylated, limits the access of both HSL and ATGL to the droplets for TG hydrolysis (138, 139). Recent studies demonstrated that cAMP-dependent PKA-mediated phosphorylation of serine-517 residue on perilipin-A in adipocytes is important in initiating hormone-stimulated lipolysis by ATGL (140). In nonadipose mammalian cells also, ATGL was shown to have an important lipolytic role (138). ADRP, which is found on the surface of LD, has recently been shown to negatively influence the association of ATGL with LD and thereby lower the turnover of TG (141).

The expression of ATGL and adiponutrin, which have high sequence homology, is increased during adipogenesis (142). However, nutritional signaling has opposite effects on these proteins: insulin decreases ATGL and elevates adiponutrin in adipocytes, whereas fasting has exactly opposing effects (142). It was also proposed that because adiponutrin has very little or no net TG lipase activity it might function in GL/FFA cycling as a FA reacylation enzyme rather than in net lipolysis (142). Recently, several calcium-independent phospholipase A2 (iPLA2) isoenzymes have been identified, including iPLA2ε, iPLA2η, and iPLA2ζ (143). The latter three enzymes have been previously described as the TG lipases adiponutrin, GS2 and ATGL, respectively. These proteins are classified as belonging to the iPLA2 family based on the presence of iPLA2 signature motifs (143), although their phospholipase activity is 100- to 500-fold lower than that of their TG-lipase activity, questioning the physiological relevance of their ascribed phospholipase activity (143). It was shown that these iPLA2 proteins possess transacylase activities because they could synthesize TG when incubated in the presence of *sn*1,2(2,3)-DAG and oleoyl-labeled 1-MAG (143), with iPLA2η (GS2) having maximal activity, followed by iPLA2ζ (ATGL) and iPLA2ε (adiponutrin) (143). In fact, using rat intestinal microsomes, Lehner and Kuksis (144) demonstrated earlier the presence of a transacylase that formed TG using both *sn*1,2-DAG and *sn*2,3-DAG, although the molecular identity of this enzyme is not known. Thus, it is possible that ATGL-generated *sn*2,3-DAG from TG can be reacylated to TG by a transacylase reaction.

Interestingly, it has been reported (145) that in retinal pigment epithelial cells, ATGL is glycosylated and located in the plasma membrane, where it acts as the putative receptor for pigment epithelium-derived factor (PEDF). PEDF binding was shown to enhance the PLA2 activity of plasma membrane-associated ATGL. However, because both the PEDF binding domain and active sites of the plasma membrane-associated ATGL are supposedly localized on the outer surface of the plasma membrane, the products of PL hydrolysis should also be released outside the cell (145). It will be interesting to determine whether ATGL is present in the plasma membrane besides its intracellular localization in other cell types, especially in pancreatic β-cells, because its activity can generate signaling molecules including lysophosphatidylcholine (LPC). Thus, intracellular lipolysis (12), PLA2 enzymes (146) and the products of their action, arachidonic acid (AA) (147), and LPC (148) appear to play a role in fuel-induced insulin secretion (12). The factors that determine subcellular trafficking of ATGL are not known, although glycosylation likely plays a role (145).

2. HSL. *sn*2,3-DAG produced from TG hydrolysis is located mostly in the cytosol and is preferentially hydrolyzed by HSL to 2-MAG and FFA. HSL is recruited to the LD upon hormone (*e.g.*, catecholamines) stimulation, and this translocation, unlike that of ATGL, is not dependent upon PKA-mediated phosphorylation of perilipin-A (140, 149). On the basis of the recent observations of Miyoshi *et al.* (140, 149), it can be postulated (Fig. 2) that on the LD surface, perilipin acts as a scaffold by interacting with HSL on one side and with CGI-58-ATGL complex on the other side, thus facilitating TG availability to ATGL upon serine-517 phosphorylation of perilipin by PKA. Such a hypothetical multiprotein complex would offer a fine and metabolically economical regulation of lipolytic flux. HSL was shown to colocalize with insulin secretory granules in the β-cell (150), in accordance with the view that lipolysis plays a key role in glucose-induced insulin secretion (12, 151, 152). The possibility that a transacylase reaction could effectively generate *sn*1,2-DAG by transferring an acyl group from *sn*2,3-DAG (produced by ATGL) to 2-MAG (produced by HSL) needs to be explored. Such a bypass reaction could contribute via lipolysis to the generation of *sn*1,2-DAG, which is an established lipid-signal molecule. Thus, because of the prochiral nature of the middle carbon of glycerol molecule, *sn*1,2-DAG is not equivalent to *sn*2,3-DAG, and these two forms of DAG are enantiomers and do not have the same biological effects.

3. DAG lipase. The relative contribution of other lipases to DAG hydrolysis is unclear. *sn*1,2-DAG, produced by the removal of phosphate from PA and from phosphoinositides and other PL by phospholipase-C enzymes on the inner side of cell membrane, can be further hydrolyzed by *sn*1-DAG lipase. Two *sn*1-DAG lipase isoenzymes have been identified. Their activity in brain and pancreas is much higher than in other tissues, and these enzymes show remarkable specificity toward DAG containing AA at position 2, thereby releasing 2-arachidonylglycerol (2-AG) (153). It has been reported that pancreatic β-cells have significantly high activity of DAG lipase in their plasma membrane, and its specific inhibition by RHC-80267 results in markedly lowered fuel-stimulated insulin secretion (154).

4. MAG lipase. The hydrolysis of MAG is conducted by a specific MAG lipase (155). MAG lipases are capable of hydrolyzing both 1-MAG and 2-MAG. An important role for MAG lipases has been proposed in the hydrolysis of the endocannabinoid (EC), 2-AG (155). It is of interest to note that in ductal breast cancer cells, the activity of MAG lipase is increased severalfold (5- to 27-fold), although its implication for cancer growth is not clear (156).

IV. GL Metabolism and the AMPK/ Malonyl-CoA Network

Malonyl-CoA occupies a central position at the intersection of the glucose and FA metabolic crossroads. The cellular level of malonyl-CoA is regulated by enzymes responsible for its biosynthesis [ATP-citrate lyase (ACL), acetyl-CoA carboxylase (ACC), and di-/tricarboxylate transporter], utilization [FA synthetase (FAS)], and degradation (malonyl-CoA decarboxylase). The biosynthesis of malonyl-CoA is controlled by AMPK, which is activated at high AMP/ATP ratio (*i.e.*, decreased energy state) in the cell. Conditions that accelerate the GL/FFA cycle, which needs a continuous supply of FACoA, might thus effectively cause activation of AMPK via elevated AMP levels because ACSL produces AMP (157). Activated AMPK phosphorylates and inhibits the activities of ACC (158) and GPAT (68) and also decreases the expression of ACC, GPAT, and FAS (159). Thus, the activation of AMPK results in lowered cellular malonyl-CoA levels associated with decreased *de novo* biosynthesis of FA and TG and increased FA oxidation. AMPK also brings about the activation of malonyl-CoA decarboxylase (68) to facilitate the decrease in malonyl-CoA levels. Malonyl-CoA binds to carnitine palmitoyltransferase-1 of the mitochondrial outer membrane to inhibit its activity and thereby the β-oxidation of FA (160–163). This regulatory role of malonyl-CoA is important for the diversion of FA toward the synthesis of TG.

The role of AMPK in the regulation of adipocyte lipolysis is controversial. In some studies, elevated AMPK activity was found to be associated with lowered lipolysis in the adipocyte (164, 165). However, it has also been reported that AMPK activation leads to enhanced lipolysis in adipocytes (166) but inhibits it in muscle by decreasing epinephrine-stimulated phosphorylation of HSL at Ser660 by PKA (167). This action on HSL phosphorylation was absent in 3T3-L1 adipocytes (167). This opposite effect of AMPK on both tissues is physiologically sound because during "fight or flight" stress (AMPK activated and epinephrine elevated), the organism needs to mobilize lipid stores from the adipose tissue to feed muscle tissue, which is poised to oxidize the supplied FFA to generate energy. Under such conditions, the adipose TG stores are used primarily while the muscle mobilizes its glycogen stores. It appears that in adipocytes, lipolysis and AMPK activate each other. Thus, conditions that elevate cAMP-dependent lipolysis and FA activation to FACoA enhance AMPK due to a rise in the AMP:ATP ratio. This can be abrogated by the lipase inhibitor orlistat and also by the ACS inhibitor triacsin-C (168).

AMPK was shown to phosphorylate and reduce the activity of skeletal muscle mitochondrial GPAT in response to exercise (68, 169). Also, a strong negative correlation was noticed between the activities of AMPK and DGAT in liver of fasted/re-fed rats (104), suggesting inhibitory regulation of this enzyme similar to GPAT. However, phosphatidylcholine biosynthesis in hepatocytes appears not to be regulated by AMPK (170). Thus, it is possible that AMPK-mediated regulation of complex lipid formation is directed mostly toward TG but not PL metabolism.

Nutrient status, which influences the activity of AMPK, also affects lipolysis in various tissues. Glucose reduces lipolysis in adipose and enhances it in pancreatic β-cells (171). Physiologically, this is important because under fed state, inhibition of lipolysis in adipose tissue ensures lowered circulating FFA, whereas the elevated lipolysis in β-cells is used as an amplification process of insulin secretion, which favors lipid storage in fat cells (172, 173). We described recently (171) that FACoA, by acting through interaction with acyl-

CoA binding protein or FABP, inhibits HSL in adipose tissue, whereas FACoA are stimulatory to β-cell lipolysis. The disparate effects of FACoA are likely to be due to differences in the lipases in these tissues. Thus, adipocyte HSL has a molecular weight of 84 kDa, whereas the β-cell isoform is longer with a molecular weight of 89 kDa (150). Also, the inhibitory effects of FACoA are dependent upon HSL enzyme phosphorylation status (171).

In terms of metabolic signaling, it would be of interest to determine whether AMPK controls GL/FFA cycling in pancreatic β-cells, where lipolysis is acutely activated by all fuel stimuli (glucose, some amino acids, FAs) (171) and is linked with insulin secretion (12, 174, 175). We suggest that glucose and other nutrient stimuli reduce β-cell AMPK activity (176), leading to a change in the phosphorylation state of ATGL and/or HSL, or regulatory proteins with subsequent enhanced lipolysis and the activation of the lipid amplification arm of glucose signaling for insulin secretion (12). In accordance with this hypothesis are the following observations: 1) both HSL and ATGL contain AMPK regulatory sites; 2) glucose rapidly reduces islet AMPK activity (177, 178); 3) the AMPK activator 5-aminoimidazole carboxamide riboside (AICAR) and a constitutively active AMPK mutant curtail glucose-stimulated insulin secretion in MIN6 β-cells (178).

V. Established Functions of GL/FFA Cycling

A. Energy homeostasis and thermogenesis

In animal species that maintain stable body temperature, thermogenesis is a consequence of intermediate metabolism. Physiologically, heat generation takes place through basal metabolism, postprandial thermogenesis, and thermogenesis induced by exercise and changes in the environmental temperature (179). Exergonic reactions in various metabolic pathways contribute to heat generation, and thus this process is tightly coupled to the same underlying regulatory mechanisms that govern the metabolic pathways. Many biochemical reactions, where net loss of energy from high-energy bonds (*e.g.*, ATP, CoA esters, *etc.*) occurs, and loosely coupled mitochondrial respiration both lead to heat production. Mitochondrial uncoupling proteins (UCP), UCP-1 in brown adipose and possibly UCP-2 and UCP-3 in other tissues, are involved in the uncoupling of respiration from oxidative phosphorylation and help in dissipating the respiratory energy as heat (180, 181). UCPs generally show uncoupling activity only upon activation by FFA (181) and reactive oxygen species as well (182). Although UCP-1 in brown adipose tissue is known to contribute to thermogenesis, the role of UCP-2 and UCP-3 in this process in other tissues is uncertain (181, 183, 184).

FFA are released in various cell types upon β-adrenergic stimulation due to lipolysis, and this stimulation plays a key role in heat production because blockers of β-adrenergic receptors compromise thermogenesis (185). Therefore, the lipolytic segment of GL/FFA cycling produces FFA, which in turn participate in activating UCPs in various tissues with diverse outcomes (Fig. 4). Although FFA also come from diet, it was demonstrated that inhibitors of lipolysis, including acipimox, abrogate thermogenesis by lowering plasma FFA (186), suggesting that lipolysis-derived FFA are likely to be important players in regulating thermogenesis. Interestingly, promoting intracellular FFA reesterification and lipid storage by activating PPARγ with thiazolidinediones decreases thermogenesis (187). Thus, various energy-consuming futile cycling processes such as the phosphorylation and dephosphorylation of glucose contribute to thermogenesis (188). However, the GL/FFA cycling process is particularly (as discussed in *Section II*) exergonic in this respect. It is possible that the reduced tolerance to cold of ob/ob mice (189), ATGL-KO mice (22), and Zucker fatty (ZF) rats (190) may be related to their decreased ability to mount adequate thermogenic response by enhancing lipolysis.

The formation and breakdown of FACoA is also seemingly futile and is regulated via the balance between the activities of ACSL and FACoA hydrolases. These enzyme activities maintain the cellular concentrations of FACoAs, which have several signaling and regulatory functions besides supplying activated acyl groups for various metabolic reactions (191, 192). Because the hydrolysis of FACoA yields heat energy, this step may in fact contribute to thermogenesis as well. Thus, in mouse brown adipose tissue, brown-fat-inducible thioesterase was shown to be cold-induced and likely involved in thermogenesis (193). The overall contribution of this process to thermogenesis in the body is not known.

Dorsomedial hypothalamic neurons play a key role in the regulation of thermogenesis and body temperature (194). The AMPK/malonyl-CoA/carnitine palmitoyltransferase-/FACoA network, which is intimately linked to GL/FFA cycling in various tissues, likely plays an important role in the control of food intake and thermogenesis (195–197). Although the hypothalamus is not known to be active in lipolysis, perhaps activation of UCP in this tissue also requires local availability of FFA (181). Our recent results indicate that GL/FFA cycling does take place at least *in vitro* in the GT1–7 hypothalamic neuronal cell line (our unpublished observations).

A recent study showed that the core body temperature is regulated by neurons in the medial preoptic area of the hypothalamus (198). A rise in the temperature of these neurons in mice by engineered tissue-specific UCP-2 leads to a reduction in the whole body temperature by 0.3–0.5 C and significantly increased life span (198). In this respect, it is noteworthy to observe that a reduction in leptin secretion promotes UCP-2 activation in the neuropeptide Y (NP-Y)/Agouti-related protein (AgRP) neurons of the arcuate nucleus in the hypothalamus, increases the number of mitochondria in these neurons, and leads to the production and secretion of NP-Y and AgRP, which regulate appetite and feeding (199). Leptin is known to promote the oxidative metabolism of FFA and TG depletion in various tissues (110). Thus, it would be of interest to determine whether hypothalamic lipid signaling modulates NP-Y and AgRP secretion, appetite, and the local temperature in specific brain areas in part via changes in hypothalamic GL/FFA cycling. Because HSL is shown to be present in hypothalamus (200) and ATGL is detectable in brain, although at much lower levels (201), it may be speculated that local temperature control by the operation of GL/FFA cycling in hypothalamus

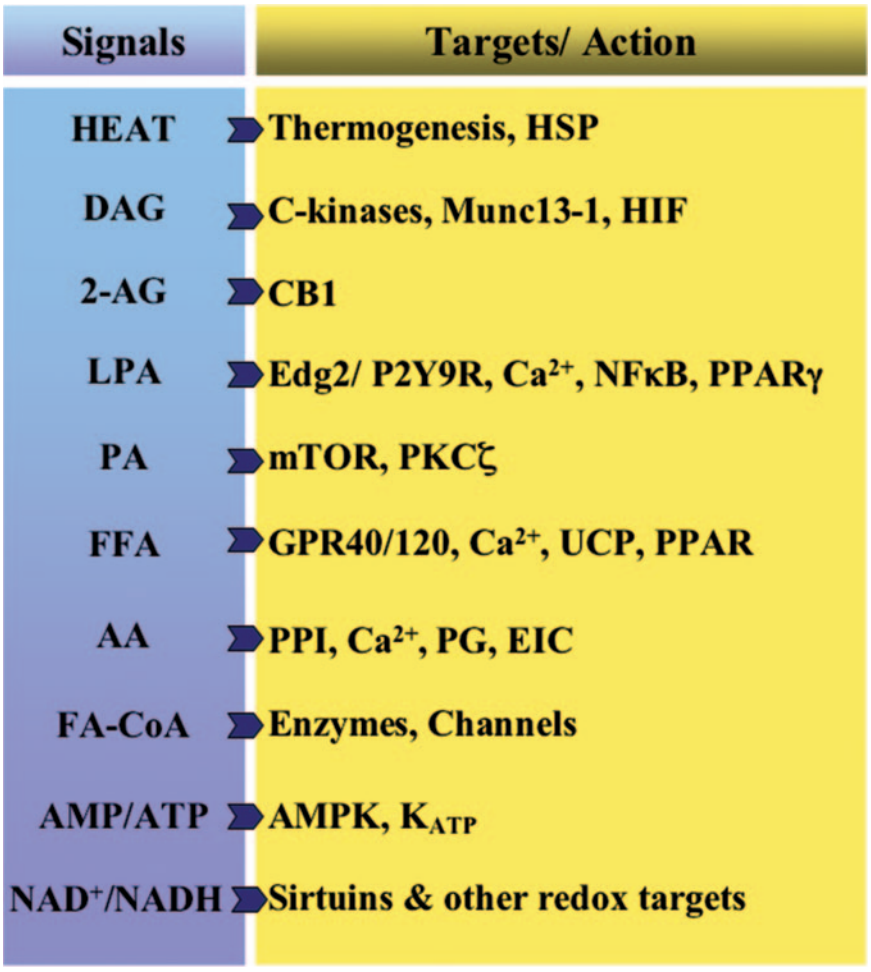

FIG. 4. GL/FFA cycle generates various signaling molecules. The operation of GL/FFA cycling generates different metabolites, which influences the activity of various enzymes, receptors, or channels in the cell and may contribute to the regulation of cytosolic redox and energy charge ratios. Many of the signaling molecules produced during GL/FFA cycling have been implicated in various biological processes such as cell survival, multiplication, motility, and secretion. The figure illustrates possible signals and their targets. The hydrolysis of ATP (seven ATP-derived high-energy phosphate bonds per TG/FFA cycle) causes heat production and may lead to the induction of HSPs. DAG is an important regulator of C-kinases, Munc13 proteins involved in exocytosis, and also HIF. GL/FFA cycling also contributes to the generation of the EC 2-AG, which activates CB1/2 receptors. LPA is a high-affinity ligand for the G protein-coupled receptors Edg2 and P2Y9, and it is involved in the activation of Ca^{2+} signaling, PPARγ, and NFκB. PA activates mTOR and PKCζ. FFA activate the membrane receptors GPR40/120 to cause intracellular Ca^{2+} mobilization. FFA are also ligands for PPARs and may also activate or induce UCP. Another signal produced by GL/FFA cycling is AA, which is important for the formation of polyphosphoinositides (PPI), prostaglandins (PG), and eicosanoids (EIC), and which regulates intracellular Ca^{2+} homeostasis. Long chain FACoA modulate the activity of various enzymes, ion channels, and transcription factors. GL/FFA cycling uses a significant amount of ATP and generates AMP at the acyl-CoA synthase step, which may result in favorable AMP/ATP and ADP/ATP ratios for AMPK activation and also for the opening of plasma membrane K_{ATP} channels, respectively. Removal of glucose carbons as Gly3P results in NAD reoxydation in cytosol, which may modulate the activity of sirtuins and other redox targets.

plays a role in the determination of the life span of the organism.

VI. Emerging Roles of GL/FFA Cycle

A. Generation of multiple signaling metabolites

A particularly interesting aspect of GL/FFA cycling, which has been mostly ignored so far, is that it likely provides a crucial link between intracellular fuel homeostasis and the modulation of a multitude of cell signaling processes. Thus, except for TG, all the metabolites of the GL/FFA cycling process are established lipid-signaling molecules. It is somewhat surprising that those have been linked so far to cell signaling almost exclusively from the angle of the PL signaling cascades or membrane receptor activation, but not in association with GL synthesis or lipolysis. A nonexhaustive list of signals/targets and biological processes that are possibly influenced by GL/FFA cycling-derived molecules is illustrated in Figs. 4 and 5. The magnitude of the effectiveness of these metabolites on a given pathway is anticipated to depend upon the context of their generation, compartmentalization (*e.g.*, cytosol, plasma membrane), and further metabolism.

One of the primary metabolites of the GL/FFA cycle is *sn*1,2-DAG, which participates in several signaling pathways (Fig. 4). AA-containing DAG, produced at the inner plasma membrane surface via phosphoinositide hydrolysis during membrane receptor signaling, is involved in the activation of PKC enzymes (96, 202, 203). PKC is activated by *sn*1,2-DAG but not by *sn*1,3- or *sn*2,3-DAG isomers (204, 205). Munc13 proteins are intracellular DAG receptors that play an important role in exocytosis and neurotransmission (206). Activated Munc13-1 has been shown to be necessary for the priming and fusion of insulin secretory granules in β-cells, and its reduced expression results in altered insulin secretion and glucose intolerance (98, 99). Thus, it has been proposed that DAG-mediated activation of Munc13-1 provides a link between glucose-induced lipolysis in the β-cell and the amplification arm of fuel-induced insulin secretion (99). Besides the activation of PKC and Munc13-1, DAG is also implicated in the expression of hypoxia-inducible factor (HIF) (see *Section VI.D*). It is important to note that DAG produced by TG hydrolysis is largely LD-associated, and PL hydrolysis-derived DAG is likely to be in the plasma membrane, whereas, *de novo* synthesized DAG from PA hydrolysis on ER is likely to be associated with or sequestered within ER. Thus, depending upon the site of production, DAG may trigger different signaling cascades.

Another signaling molecule derived by the operation of GL/FFA cycle is the EC 2-AG, a MAG. 2-AG plays a role in

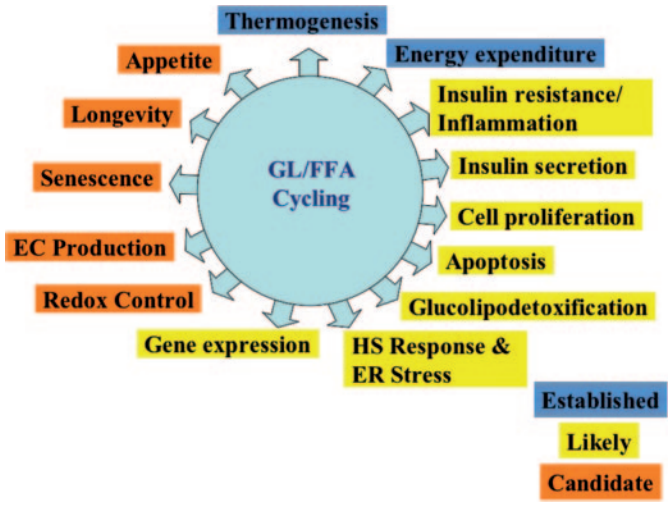

FIG. 5. Biological processes regulated by GL/FFA cycling. Operation of GL/FFA cycling generates various metabolites and signals involved in the control of multiple biological processes. The figure illustrates established and candidate processes, as well as those clearly emerging from recent studies. HS, Heat shock.

the regulation of many pathways, including lipogenesis and appetite control (see *Section VII.B*).

The esterification processes in GL/FFA cycling produce LPA and PA, which are well-studied lipid-signaling molecules. LPA activates the G protein-coupled receptors (GPCR), Edg2/LPA-R and P2Y9/GPR23 (207, 208). Because Edg2 receptors are activated by very low concentrations of LPA, it can be envisaged that even minute amounts of cellular LPA "secreted" by membrane flip/flop mechanism will be able to bind and activate its cognate receptors. Additionally, LPA receptor activation is thought to enhance nuclear factor κB (NFκB) activity via the adapter proteins Bcl10 and Malt-1 (209). Interestingly, LPA may also be directly produced on the external surface of the plasma membrane by glycosylated ATGL, which may have PLA2 activity (145) to activate LPA-receptor signaling, Ca^{2+} influx, and cAMP production (210). Plasma membrane ATGL may also produce LPC, which causes GPR119 activation (148, 211). GPR119 is predominantly expressed in the β-cell, where it may be involved in the regulation of insulin secretion (148, 212).

PA directly activates mammalian target of rapamycin (mTOR) (210, 213). PA activation of mTOR is likely to have important physiological implications because mTOR is a cellular stress and nutritional balance sensor (214). PA can be produced by different mechanisms, including the acylation of LPA, the phosphorylation of DAG, and phospholipase-D hydrolysis of phosphatidylcholine (213). The activation of mTOR is essential for cell survival. In particular, the rapidly growing and nutritionally stressed cancer cells are dependent on active mTOR, and these cells undergo apoptosis if the synthesis of PA is inhibited (213). Recently, it has been shown that active mTOR in hypothalamic neurons is essential for the appetite-suppressing activity of leptin (215). Therefore, it is not unreasonable to propose a role for PA in hypothalamic food intake regulation. Thus, PA, an intermediate of the GL/FFA cycle, appears to play a key role in the cellular response to nutritional stress. PA is also known to directly bind and activate PKC-ζ (216), and interestingly, it was proposed that high glucose leads to the stimulation of PKC-ζ/λ in adipocytes and muscle cells probably involving PA production (217).

FFA produced during lipolysis can participate in various pathways including autocrine/paracrine stimulation of the GPCRs GPR40/120 (12, 218, 219), uncoupling proteins (184), and PPARs (220). The activated form of FFA, FACoA, modulates the activity of a multitude of enzymes and ion channels, and is involved in transcriptional control as well (54). AA released during lipolysis is an established signaling molecule implicated in the modulation of ion fluxes (147) and insulin secretion (154) and is the carbon precursor in the synthesis of prostaglandins and eicosanoids.

Finally, a most attractive possibility in terms of its therapeutic implication is that GL/FFA cycling is linked to the control of the energy status of the cell, in particular the cytosolic AMP/ATP ratio and the modulation of AMPK activity. This stems from the fact that the more operational the cycle is, the more it will produce AMP because the FA-CoA synthase reaction uses one ATP to release AMP, PPi, and heat. Thus, Gauthier *et al.* (168) showed that enhanced lipolysis by adrenergic agents in adipocytes is associated with a reduction in cellular ATP:AMP ratio. Variations in the ATP/ADP ratio influence metabolically active K-ATP channels that play a key role in insulin secretion (221) and the activity of hypothalamic neurons (222).

Because the production of glucose-derived Gly3P, the glycerol backbone in GL synthesis, is linked to the cytosolic Gly3P dehydrogenase reaction and the reoxidation of reduced NAD (NADH), GL/FFA cycling is possibly implicated in the control of cytoplasmic redox, sirtuins, and glycolytic flux (see *Section VII.A* and Fig. 7).

We will now discuss in greater detail various biological processes that are possibly regulated by GL/FFA cycling (Fig. 5). Those are listed either as "emerging" and "likely" to reflect the fact that GL/FFA cycling's involvement in their regulation is supported by the recent literature or as "candidate" because the implication of GL/FFA cycling in the regulation of these processes is more hypothetical at this stage.

B. Detoxification of fuel oversupply

We recently showed that there is no accumulation of TG in the pancreatic islets of obese normoglycemic hyperlipidemic ZF rats, although their serum TG is elevated 10 times more than in control animals (174). We also reported that GL/FFA cycling is markedly enhanced in the islets of these animals with enhanced esterification of FA into DAG and TG, in association with enhanced β-oxidation and increased lipolysis and the activity of lipolytic enzymes (174). Enhanced GL/FFA cycling activity provides an attractive mechanism by which a given cell might escape the toxic action of fuel surfeit to maintain its differentiated function. Glucotoxicity arises at high concentrations of glucose, due to the inability of the cell to effectively eliminate/use the glucose carbons, resulting in the elevated production of reactive oxygen species, protein O-glycosylation, inflammation and amyloid deposits in pancreatic islets, and altered FFA me-

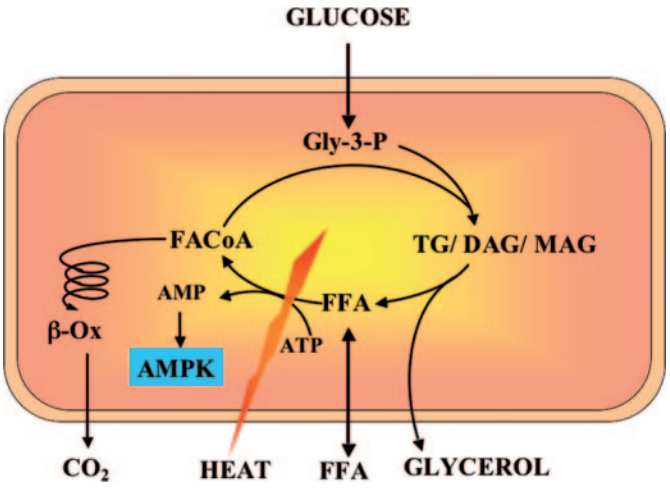

FIG. 6. Model depicting the role of GL/FFA cycling in tissue glucolipodetoxification. An elevation in glucose promotes the cycle by 1) inhibition of FA β-oxidation (β-Ox) via malonyl-CoA inhibition of carnitine palmitoyltransferase-1, which catalyzes the limiting step of this pathway such that cytosolic FACoA increases; 2) provision of Gly3P for GL synthesis; and 3) activation of lipolysis at least in some tissues such as the β-cell. Elevated exogenous FFA feed into the cycle, increasing the level of several intermediates. The cycle prevents cell steatosis in the face of hyperlipidemia and hyperglycemia because it allows for detoxification of lipids via lipolysis and β-oxidation of FFA that are released from cells as CO_2. It also allows detoxification of glucose via conversion of the glucose carbons to glycerol that escapes most cells because the majority of tissues express very low levels of glycerokinase. It allows detoxification of fuel surfeit in general because it is a very energy-consuming futile cycle that indirectly generates heat and CO_2 from calorigenic nutrients. Finally, fast cycling may be linked to AMP production and AMPK activation, a fuel-sensing enzyme whose activation enhances glucose and fat oxidation.

tabolism, leading to the accumulation of toxic FFA metabolites in the cells (94). As described in Fig. 6, this cycle allows glucodetoxification because the glucose carbons are directed to glycerol, which escapes the cell in most tissues that do not express GlyK (223). We have noticed that in INS832/13 β-cells the extent of glucose conversion to glycerol amounts to approximately 40% of the total glycolytic flux at 10 mM glucose, indicating glucodetoxification via GL/FFA cycle (our unpublished results). In addition, lipodetoxification occurs because lipolysis allows the TG-FAs to be secreted from the cell or oxidized in the mitochondria. Thus, isolated islets have been shown to secrete both saturated and unsaturated FFA into the medium (224). Because the cycle consumes much energy, enhanced GL/FFA cycling is linked to augmented fuel oxidation and heat generation, a process that contributes to reducing the cellular fuel load. Finally, as discussed in *Section IV*, the intriguing possibility should be evaluated that enhanced GL/FFA cycling activates AMPK or maintains the enzyme at low activity in the face of fuel excess, due to the fact that the FACoA synthase reaction generates AMP. Thus, the nutrient sensor AMPK (165), when activated, reduces storage of fuels and promotes their oxidation and possibly their detoxification (225). The role of AMPK in fuel detoxification has also been highlighted in the studies on ZDF rats and ob/ob mice, where treatment of these animals with AMPK activator AICAR led to a decrease in ectopic lipid accumulation and prevention of diabetes (226). It should be underscored that the "glucolipodetoxification" concept related to GL/FFA cycling, which has been originally proposed in the β-cell (94), is applicable to other tissues as well and might therefore play an essential role in preventing the toxicity of fuel surfeit in an organism. Compromising GL/FFA cycling at any of the different steps (*e.g.*, gene KO of aquaporin, GPAT, AGPAT, PAP, ATGL, HSL, or CD36) is known to cause disturbed fuel homeostasis and energy metabolism (discussed in *Section III.A*).

Glucolipodetoxification is analogous to cholesterol detoxification. Although cholesterol is needed for many cellular functions, excess free cholesterol is toxic to the cell and is esterified with FFA by acyl-CoA:cholesterol acyltransferase-1 to CEs and then transported out of the cell. CEs exist in the cytosol mainly as LD and are hydrolyzed by CE hydrolases, regenerating free cholesterol. Efflux of cholesterol is important in the regulation of cellular cholesterol homeostasis (227), and this cholesterol is directed to liver where it is packaged into lipoproteins and secreted out or processed further for excretion. Cholesterol homeostasis in liver is regulated via its *de novo* synthesis, dietary cholesterol availability from the intestinal absorption, and secretion into bile and as lipoproteins (228). In liver and intestine, cholesterol esterification seems to be predominantly catalyzed by acyl-CoA:cholesterol acyltransferase-2 (228).

In pancreatic β-cells, metabolism of CE is not clearly established. However, recent studies show that CEs contribute significantly to the neutral lipid reserve in the β-cells and that FA desaturation is important for CE formation (229). In fact, it has been shown recently that at elevated glucose concentrations as much as 15% of the total incorporated glucose label in β-cells can be in CEs, whereas the proportion of the label in total GL was found to be nearly 70% (230). This suggests that cholesterol and CE formation in β-cells is relatively small in comparison to GL and that the contribution of GL/FFA cycle-mediated glucolipodetoxification is quantitatively larger than via cholesterol/CE cycling.

ATP-binding cassette transporter-A1 (ABCA1) catalyzes the efflux of cholesterol from β-cells, and deletion of this protein results in islet accumulation of cholesterol and impaired insulin secretion and glucose homeostasis in mice (231). Although cholesterol is needed for proper assembly of secretory granules and for other cellular functions, its accumulation reduces glucose-stimulated insulin secretion in islets (231). Thus, the emerging view indicates that the formation of both glycerol and cholesterol from glucose as well as GL/FFA and cholesterol/CE cycling processes serve as important fuel detoxification mechanisms in cells.

C. Cell survival and proliferation

TG deposition and GL/FFA cycling in nonadipose cells likely provide a mechanism to handle excess exogenous and/or endogenous-derived FFA and to contain them in the form of nontoxic LD depots. Thus, high FFA levels, particularly in the presence of elevated glucose (glucolipotoxicity) (232), can interfere with many cellular functions and in particular cause mitochondrial dysfunction (233) and impaired cell energy homeostasis (234, 235). Although massive TG accumulation in nonadipose cells is toxic (109, 236), recent

studies have shown that TG accumulation is in fact employed as a defense mechanism against acute lipid toxicity in nonadipose cells (107, 237). We would like to propose that this TG build-up, when combined with TG/FFA recycling, which is energy consuming, contributes to long-term defense against glucolipoapoptosis (94) and might possibly also signal cellular proliferation. Thus, build-up of TG may protect the cell as long as the cell retains the ability to hydrolyze TG to regenerate FFA and thereby prevent steatosis and ER stress.

Apparently, survival against lipotoxicity caused by saturated FFA is directly proportional to the capacity of the cells for TG accumulation (107, 237). Thus, the novel view is that TG deposition is not very toxic to cells, but rather it acts as a buffer to handle excess FA, diverting them from various cytotoxic pathways, in particular ceramide formation (109, 238). Consistent with this view are the following studies. Interfering with the lipogenic/adipogenic pathways in 3T3-L1 adipocytes by leptin (239) or with a plant phenolic compound, esculetin (240) was shown to decrease their viability. Leptin can promote β-cell apoptosis (241). Similarly, the HIV protease inhibitor ritonavir caused lipodystrophy (depletion of adipose mass) in patients undergoing long-term highly active antiretroviral therapy. Evidence was provided that by inhibiting lipogenic enzymes, ritonavir causes apoptosis of the patients' adipocytes (242). Impairing fat synthesis in various cancer cells via targeting enzymes of the lipogenic pathway (ACL, ACC, and FAS) using the RNA interference technology was shown to induce cell death (7). Overexpressing SCD-1, which promotes TG synthesis, protected MIN6 cells from lipoapoptosis (229). Finally, we observed that oleate promotes growth factor-independent cell survival in MDA-MB231 breast cancer cells and that this effect is associated with TG deposition and enhanced GL/FFA cycling (243).

Besides controlling the fat burden and allowing glucolipodetoxification (see Section VI.B), GL/FFA cycling might contribute to cell survival and growth via the direct activation of "classical" antiapoptotic pathways and transcription factors that promote them. Thus, terminally differentiated adipocytes live long, and through the up-regulation of the antiapoptotic proteins Bcl-2 and Bcl-xl are thought to resist lipoapoptosis (244–246). Recent evidence indicates that LPA, a PL involved in the GL/FFA cycle that can also be produced on the cell surface by membrane-bound ATGL (145) and PLA2 enzymes (247), can activate NFκB via a GPCR pathway involving Bcl-10 and Malt-1 (209). NFκB is involved in the regulation of the expression of the antiapoptotic proteins Bcl-2 and Bcl-xl in a variety of cells (248, 249) and might therefore also link lipolysis and GL/FFA cycle operation to cell survival.

With respect to cell proliferation, several lipid-signaling molecules derived from the lipolytic cascade such as LPA, PA, and AA have been shown to promote cell proliferation in many cell systems (207, 213). In addition, various long-chain monounsaturated and saturated FA rapidly induce various protooncogenes such as c-*fos*, c-*myc*, and c-*jun*, and enhance ^{3}H-thymidine incorporation in several cell lines (250–253).

Thus, the attractive possibility emerges that the formation of TG and the continuous lipolysis and FA reesterification (GL/FFA cycling) is a constitutive survival/cell growth pathway built into the life cycle of all cells.

D. Regulation of gene expression

There is mounting evidence indicating that certain metabolites produced during GL/FFA cycling either activate or elevate the levels of HIF-1α. HIF is a transcription factor that regulates the expression of various glycolytic enzymes and vascular endothelial growth factor and also favors cell survival (254). Hypoxic conditions that prevail in the core region of many solid tumors lead to the induction of HIF in that area (255). Similarly, adipocytes in the center of the adipose tissue show elevated levels of HIF (256, 257). In both cases, increased levels of HIF result in the expression of vascular endothelial growth factor, which promotes angiogenesis (257).

Hypoxia in different types of cells leads to elevation of DAG and PA (258), intermediates of GL/FFA cycling processes. DAG may play a role in the expression of HIF-1α through the formation of PA (258) after the DAGK reaction or through mechanisms involving DAG-activated PKC (259). PA directly activates mTOR (213), and in various cells mTOR activation leads to HIF-1α induction (260). Recent studies revealed that LPA, another intermediate of GL synthesis and cycling processes, activates HIF-1α and promotes its translocation to the nucleus via the phosphoinositide 3-kinase/mTOR/p70S6K pathway (261). These observations are interesting because they suggest that PA and LPA might provide a link between GL metabolism and amino acid metabolism, as well as insulin signaling in which mTOR plays a key regulatory role (262).

Nonhypoxic conditions that lead to an elevation of cellular DAG levels or enhanced GL/FFA cycling, for example, elevated glucose concentration (263–265), also lead to a rise in HIF-1α protein. Similarly, HIF-1α expression is elevated by about 3-fold in white adipocytes from fasted rats (266). This could be due to fasting-mediated augmentation of the GL/FFA cycle and DAG production in adipose tissue.

The link between GL metabolism and GL/FFA cycling with the transcriptional response has been studied relatively poorly. PPARs are activated by long-chain unsaturated FFA (220) that are produced during lipolysis, and therefore their direct relationship with GL metabolism and GL/FFA cycle is evident. To give one example, during fasting lipolysis is enhanced and the released FFA activate PPARα and PPARδ, leading to a coordinated induction of fat transport and oxidation genes (220, 267). It is important to mention that LPA has also been shown to activate PPARγ by occupying the ligand binding site (268). Because the PPAR field has been covered by many excellent reviews (269–271), it will not be further discussed here.

Changes in GL metabolism and GL/FFA cycle activity are associated with variations in the production of many lipid-signaling molecules and possibly the energy state or AMPK. Thus, it is almost certain that many coordinated transcriptional responses are associated with variations in GL/FFA cycle activity-derived lipid mediators. These transcriptional responses and the identification of additional transcription

factors besides HIFs and PPAR associated with TG mobilization and GL/FFA cycling merit further investigation.

E. Insulin secretion

Intracellular GL metabolism yields DAG, MAG, glycerol, and FFA, and of these DAG and FFA are known to stimulate insulin secretion when added exogenously to β-cells (96). Inasmuch as both FA esterification and lipolysis processes are glucose-responsive in the β-cell (12), it is likely that GL/FFA cycling, and in particular its lipolytic segment, plays a role in glucose-stimulated insulin secretion (12). Thus, glucose (152, 173), FFA, and FACoA (171) increase lipolysis in rodent islets, and furthermore, we provided evidence that glucose-responsive islet FA esterification and lipolysis contribute to the hyperinsulinemia associated with sustained β-cell compensation in nondiabetic, severely insulin-resistant ZF rat (174).

The significance of GL/FFA cycling-mediated signaling for insulin secretion became evident from studies showing curtailed glucose-induced insulin secretion in rat islets upon inhibition of lipolysis by the pan-lipase inhibitors orlistat (152) and 3,5-dimethylpyrazole (272) and also by deletion of HSL (151, 173). Exocytosis of insulin granules in β-cell is a multistep process, and the lipid intermediates generated in GL/FFA cycle are likely to promote one or more of these steps. For example, *sn*1,2-DAG binds and activates the vesicle-priming protein Munc-13-1 (273), which is essential for insulin granule exocytosis (99, 274). Also, *sn*1,2-DAG activates PKC, which plays an important role in insulin secretion (96). Similarly, FACoA is used as a substrate by the enzymes that acylate synaptosomal-associated protein-25 (275) and synaptogamin (276), which are part of the vesicle-docking machinery. In addition, FACoA (277) and FFA (94) stimulate exocytosis in the β-cell, the latter in part by increasing the readily releasable pool of granules. GL/FFA cycling near cell membrane may also generate various ligands (Fig. 4), including LPC, LPA, FFA, and 2-AG, which may traverse the membrane and activate corresponding cell surface receptors (GPR119, Edg2/P2Y9R, GPR40, and CB1/2, respectively) and trigger a cascade of events that ultimately culminate in the secretion of insulin by β-cells. It is also possible that the GL/FFA cycle acts as a signal-delivery apparatus at the necessary subcellular sites within the β-cell to promote insulin secretion. In this context, it is worth noting that HSL has been shown to be localized in insulin granules (150).

Recently, it has been shown that during the formation of LD in liver and muscle cells in the presence of excess FFA, the synaptosomal associated protein receptor protein system, known for its role in exocytosis (278), plays an important role in the LD fusion process. Most of the cellular SNAP23 protein appears to be used up by LD fusion, limiting the supply of this protein for other cellular functions, including insulin sensitivity (279). From these observations, it can be envisaged that in β-cells also prolonged exposure to glucose and FFA may lead to increased formation of LDs, and this may sequester the SNAP23 in the cell leading to lowered availability of this protein for insulin granule exocytosis, ultimately resulting in β-cell failure to secrete insulin. Thus, glucose and FFA, which acutely stimulate insulin secretion, can lead to β-cell dysfunction after chronic exposure to these nutrients at high concentrations (94). It will be of interest to examine whether overexpression of SNAP23 in β-cells rescues them from glucolipotoxicity.

VII. Candidate Roles for GL/FFA Signaling

A. Cytosolic NAD reoxidation, glycolysis, anaplerosis, and biosynthetic reactions

The continuous lipogenic and lipolytic reactions of GL/FFA cycle are likely to exert a "pull" on glycolysis for a sufficient supply of Gly3P (Fig. 7). We recently proposed (243) that this pull pressure on glycolysis also leads to the cytosolic NAD-linked Gly3P dehydrogenase-catalyzed regeneration of NAD^+ from NADH produced during the glyceraldehyde-3-phosphate dehydrogenase reaction. Thus, the operation of GL/FFA cycle necessitates a constant supply of not only the glycerol carbon backbone from glycolysis but also the reducing equivalents to synthesize Gly3P. NAD^+ regeneration will in turn favor high glycolytic flux. An augmented GL/FFA cycle, therefore, is also expected to increase the entry of glucose carbons into mitochondria in the form of pyruvate, which results in enhanced Krebs cycle activity and ATP production, as well as the carboxylation of pyruvate to form oxaloacetate via the pyruvate carboxylase reaction (an anaplerotic reaction). The ample supply of Krebs cycle intermediates will result in the cataplerosis (egress of cycle intermediates from the mitochondrion) of citrate and other tricarboxylic acid cycle intermediates (Fig. 7). Citrate, in turn, contributes to lipogenesis via the formation of acetyl-CoA and malonyl-CoA, whereas other dicarboxylic acids enter into biosynthetic pathways of amino acids, porphyrin, purine, and pyrimidine nitrogen bases.

Thus, enhanced GL/FA cycling may favor the cataplerotic output of Krebs cycle intermediates, which can have direct signaling function in the cytoplasm, or indirectly through the production of metabolic coupling factors (*e.g.*, malonyl-CoA, reduced NAD phosphate). In addition, high glycolytic flux will favor biosynthetic reactions (protein, nucleic acid, porphyrin, *etc.*, synthesis) by providing the necessary intermediates (Fig. 6), which are essential for functions such as cell growth.

B. Endocannabinoid signaling and appetite

Endogenous ECs (anandamide and 2-AG) are part of the neural circuitry involved in appetite control. The orexigenic action of ECs involves activation of cannabinoid (CB)-1 receptors in the lateral hypothalamus (280). Pharmacological blockade or genetic ablation of CB1 receptors in obese rodents causes a transient reduction in food intake accompanied by sustained weight loss and reduced adiposity (281, 282). However, the CB1 and CB2 receptors are found in many peripheral tissues, including the pancreatic β-cell, where they may participate in the control of insulin secretion (283).

ECs play an important role in FA metabolism in part through the regulation of the expression of the transcription factor sterol regulatory element binding protein 1c (284, 285). Stimulation of hepatic CB1 receptors results in an increased

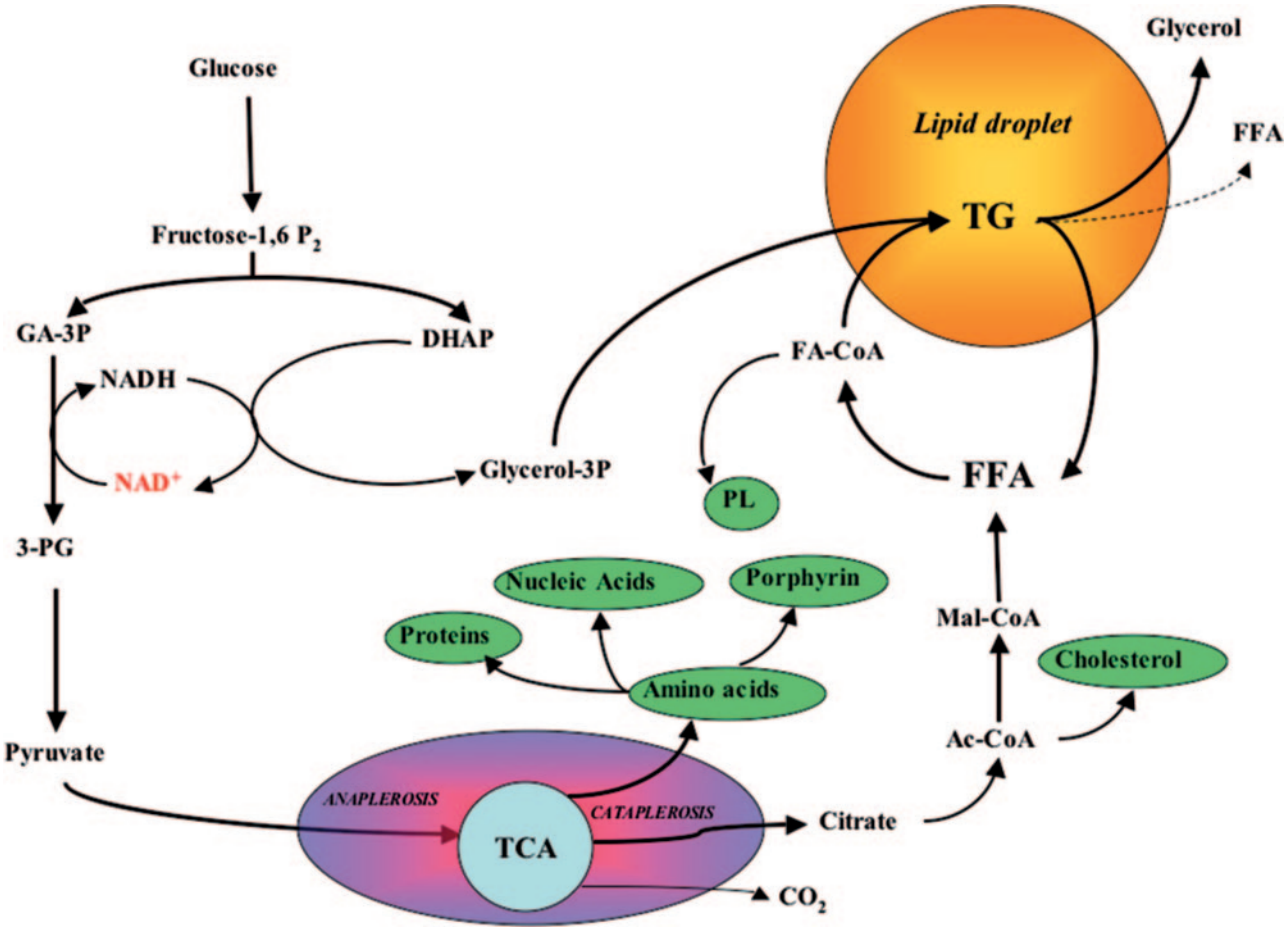

FIG. 7. Model illustrating the role of GL/FFA cycling in cytosolic NAD^+ reoxidation, glycolysis, anaplerosis/cataplerosis, and biosynthetic reactions. GL/FFA cycling needs a continuous supply of Gly3P, which is produced from glucose via dihydroxyacetone phosphate (DHAP) reduction. This process results in the reoxidation of NADH produced in glycolysis during glyceraldehyde-3-phosphate (GA-3P) oxidation to 3-phosphoglycerate (3-PG). Thus, the continuous production of Gly3P and regeneration of NAD^+ exerts a "pull" on glycolysis and thus allows fast glucose utilization. This in turn permits ample production of pyruvate and its entry into mitochondrial tricarboxylic acid (TCA) cycle with associated oxidative metabolism and ATP production. Pyruvate is also carboxylated to enhance the production of cycle intermediates (anaplerosis), followed by their egress from the cycle (cataplerosis). For example, citrate formed in TCA cycle exits mitochondria and becomes a major source for cytosolic acetyl-CoA (Ac-CoA), which is used for either cholesterol or FFA biosynthesis. Other TCA cycle intermediates (*e.g.*, 2-oxoglutarate and oxaloacetate) contribute to amino acids and subsequently to proteins, nucleic acids, and porphyrins and other complex biomolecules. During pyruvate/citrate cycling (not shown), reduced NAD phosphate is produced for biosyntheses or signaling purposes. Gly3P, produced from glucose, is esterified to form TG, which is stored as a LD or PLs.

expression of sterol regulatory element binding protein 1c, and this leads to enhanced lipogenesis in association with an induction of ACC-1 and FAS (284, 285). Although the synthesis of 2-AG is thought to occur predominantly at the cell surface from receptor-stimulated hydrolysis of the phosphoinositides and phosphatidylcholine, the contribution of TG hydrolysis has not been addressed.

One can expect a link to exist between GL/FFA cycling and 2-AG production because a significant pool of the released FA are used for the synthesis of membrane PL (286). The AA content of TG is significant and is known to increase in diabetes (by ~100%) and insulin resistance (by ~50%) (287, 288). As a result of enhanced GL/FFA cycling, the AA derived from the TG pool is incorporated into phosphoinositides and phosphatidylcholine in the membrane and these PL in turn become the source for the generation of 2-AG via DAG formation after PLC and PLD activation by various agonists. DAG thus generated is associated with the plasma membrane and is acted upon by membrane-bound *sn*1-DAG lipases to produce 2-AG. Recent studies (153) have identified two genes coding for two *sn*1-DAG-lipases, which hydrolyze the fatty acyl group specifically at position 1 in the DAG molecule, releasing 2-AG. Thus, *sn*1-DAG-lipases can be responsible for the production of 2-AG from membrane-associated DAG, containing AA in position 2. These enzymes are inhibited strongly by the lipase inhibitor orlistat and do not have significant activity toward MAG and TG. Interestingly, besides strong expression in neuronal tissues, these enzymes are expressed in significant levels in pancreas, suggesting an important role for 2-AG in pancreatic functions (153).

However, an overlooked pathway of the production of the EC 2-AG may be related to the sequential hydrolysis of TG and *sn*2,3-DAG on LD. The positional specificity of adipose HSL suggests that *sn*2,3-DAG hydrolysis by HSL will also result in the formation of 2-AG (289). This is interesting from a signal transduction standpoint because it supports the

novel concept that TG lipolysis provides not only FFA and DAG for metabolic purposes but also metabolites for cell-signaling actions. Considering that AA constitutes a significant portion of TG FAs (290, 291), the LD-associated ATGL and HSL, might generate 2-AG, in a hierarchical fashion, in various cells. Thus, it is important to realize that 2-AG might be generated in ample quantities both from plasma membrane and LD/cytosolic-associated DAG and may be able to participate in the activation of CB1/CB2 receptors after its transport out of the cell via a specific EC transporter (281).

A role for MAG lipases as an "off" signal in EC action has been proposed (155). Plasma levels of 2-AG are increased in obese individuals, and it was suggested that this might contribute to orexigenic stimuli and elevated appetite (292, 293). In the genetically obese *ob/ob* mouse, 2-AG levels are elevated in the uterus, and this is probably due to reduced MAG-lipase activity (294). Although the elevated circulating 2-AG levels in other obese animal models and humans have not been correlated with MAG lipase, it is possible that this enzyme activity is lowered in other tissues as well in obese situations. Elevated 2-AG might also contribute to the hyperphagia, which is a characteristic of leptin-deficient mice. It will be interesting to know whether the activity of this enzyme is altered in obese individuals.

In conclusion, the GL/FFA cycle may be linked to EC production at least in part via the production of plasma membrane AA-containing PL. Whether agonist-induced lipolysis plays an acute regulatory role in the production of ECs is an attractive possibility that remains to be demonstrated. If so, it might reveal important links between cellular TG stores and key physiological processes such as body weight, thermogenesis, and appetite control.

C. Heat shock response and ER stress

There might be an important and hitherto unrecognized purpose of the TG/FFA cycle and its associated thermogenesis besides contributing to body temperature and fuel detoxification. The heat produced locally in particular cells where the cycle takes place could also play a role in the induction of the heat shock response and heat shock proteins (HSPs). It has been suggested that as the adipocyte size increases with copious lipid deposition, total lipolysis also is elevated. This is accompanied by local induction of BiP, an ER stress protein that plays a role in the unfolded protein response of ER (28). Unfolded protein response signals in turn lead to augmented inflammatory cytokine (IL-8, IL-6, and TNFα) production (28). These cytokines stimulate lipolysis (2, 3), causing a feed-forward activation (vicious cycle) of this pathway.

HSPs are highly conserved proteins that protect cells against various stresses and deleterious stimuli. They function as molecular chaperones in protein folding or transport and act as antiapoptotic regulators of cell death signaling pathways. They have been implicated in many diseases, in particular inflammatory and cardiovascular diseases, diabetes, and cancer (295).

It has been proposed that lipolysis-derived FFA act on mitochondrial UCPs in muscles and lead to localized cytosolic heat production and subsequent activation of nitric oxide synthase (183). There is an increased synthesis of HSP in conditions of elevated TG/FFA cycling, for example in starvation (296) and in adipocytes conducting active lipolysis (297). It has been shown that amphetamine administration to rats enhances lipolysis and leads to elevated body temperature, and this causes induction of several HSPs in a tissue-specific manner (298). Hence, the possible link between lipolysis, GL/FFA cycling, and the heat shock response merits investigation.

D. Cell senescence and longevity

Sirtuins [silent information regulator-2 homologs (SIRTs)] have been implicated in the molecular mechanisms of aging (10). The mammalian sirtuin SIRT-1 deacetylates several proteins in an NAD^+-dependent manner. SIRT-1 substrates are involved in the regulation of cell differentiation, growth, survival, chromatin remodeling, and adaptive transcriptional responses to nutrient availability (299). Starvation or calorie restriction increases the activities of both SIRT-1 and SIRT-3 in adipocytes and in liver, and it has been proposed that this increase is instrumental in the extension of mammalian life span in response to fuel deprivation (10). SIRT-1 attenuates adipogenesis and also enhances lipolysis, probably through the repression of PPARγ in mouse adipocytes (300). Thus, it may be speculated that a link exists between SIRT and GL/FFA cycling and that the latter is an integral part of the cellular compensatory mechanisms against senescence.

There is evidence suggesting that the adipocytokine visfatin (also named pre-B-cell colony enhancing factor) provides an additional link between fuel availability on one hand and SIRT and GL/FA cycling on the other hand. Thus, recent studies have shown that visfatin enhances human vascular smooth muscle cell survival by elevating NAD^+ content and the activity of SIRT isoenzymes that use NAD^+ as a substrate (301). Interestingly, there is evidence that visfatin is in fact nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis (302). Conditions that augment GL/FFA cycling in adipocytes (*e.g.*, exercise) elevate not only SIRT activity but also visfatin expression (303). Likewise, secretion of visfatin from adipocytes appears to be abrogated by elevated FFA but enhanced by the PPARγ activator rosiglitazone (304), which enhances GL/FFA cycling (37). Finally, overfeeding, which attenuates the lipolytic segment of the GL/FFA cycle, causes a reduction in serum visfatin levels in young men, and this positively correlated with serum TG levels (305).

It is well known that leptin secretion by adipose tissue is increased in the fed state and decreased upon fasting (306). Similar to fasting, exercise causes a decrease in leptin secretion (307). It is interesting to note that visfatin and leptin levels change in opposite directions in conditions that modify GL/FFA cycling in adipocytes. In contrast, adiponectin secretion by the adipose tissue is elevated during exercise and also after pioglitazone treatment (308), indicating a relationship similar to visfatin secretion.

Thus, there is much correlative evidence that favors the

view that a link exists between the levels of some adipokines (visfatin, adiponectin, and leptin), SIRT activity, and GL/FFA cycling because these processes vary in parallel under a number of physiological and experimental conditions, including fuel surfeit or depletion, exercise, as well as treatment with thiazolinediones. The relationships at the molecular level between GL/FFA cycling and visfatin, adiponectin, and leptin production and secretion are yet to be understood. It is possible that an augmented lipolytic segment of the GL/FFA cycle leads to enhanced secretion of visfatin and adiponectin, whereas elevated lipogenic activity of the cycle results in increased secretion of leptin, a speculation that is worth testing experimentally.

Because fuel surfeit favors cell senescence and death and because GL/FFA cycling is energy consuming and likely involved in glucolipodetoxification, it is attractive to suggest that GL/FFA cycling might play a role in the longevity of organisms and thus may provide a target for countering cell senescence. The idea that GL/FFA cycling in hypothalamus potentially contributes to the regulation of whole organism longevity is discussed in *Section V.A*.

VIII. GL/FFA Cycle and Pathological Processes

A. Islet β-cell failure in type 2 diabetes

The pathogenesis of T2D in many obese individuals is preceded by the development of insulin resistance and compensating hyperinsulinemia such that normoglycemia is maintained (309, 310). To keep the glycemia normal, insulin levels rise in insulin-resistant prediabetic individuals due to β-cell compensation. However, when the β-cells fail to secrete enough insulin in the face of insulin resistance, hyperlipidemia, or increased insulin demand, diabetes develops (232). Several mechanisms have been proposed to explain β-cell failure, including altered balance between insulin biosynthesis and secretion (also named β-cell exhaustion) (311), oxidative stress (235, 312), ER stress (313), inflammation (314, 315), glucotoxicity (316), lipotoxicity (317, 318), and glucolipotoxicity proposed by us in which elevated saturated FFA synergize with mild postprandial glucose elevations to impair β-cell function or cause cell death (77, 159, 319). Consistent with the idea that altered TG/FFA cycling is involved in β-cell failure, transgenic mice fed a high-fat diet and overexpressing HSL in the β-cell show enhanced lipolysis and reduced islet TG content, in association with glucose intolerance and severely impaired glucose stimulated insulin secretion (320). Thus, it is important to note that alteration of GL/FFA cycling, either by lowering the lipolytic segment by HSL-KO (151, 173) or by enhancing it via overexpressing HSL severalfold above control (320), can lead to impaired glucose-stimulated insulin secretion in β-cells.

The mechanism of β-cell compensation is not well understood, but recent work has provided support for the view that enhanced GL/FFA cycling is implicated in this process. We have shown that in the islets of obese normoglycemic insulin resistant ZF rats, a model for β-cell compensation, enhanced glucose- and palmitate-stimulated insulin secretion coincide with elevated FFA esterification processes, increased fat oxidation, lipolysis, and expression of lipolytic enzymes (174). This is associated with a paradoxical reduction in islet TG content, in the face of marked hyperlipidemia and elevated circulating FFA. This indicates a very active GL/FFA cycling in the islet of these animals (94, 174).

Enhanced GL/FFA cycling activity provides an attractive mechanism by which the β-cell of the compensating ZF rat escapes glucolipotoxicity and can continuously supply enough insulin to the organism such that diabetes is prevented. Thus, as described in Fig. 7, this cycle allows glucodetoxification because the glucose carbons are directed to glycerol, which escapes the β-cell because of the low activity of GlyK in these cells (223). In addition, lipodetoxification occurs because lipolysis allows the TG-FAs to be oxidized in the mitochondria or released from the β-cell. As far as compensatory insulin secretion is concerned, enhanced GL/FFA cycling and lipolysis produce various lipid-signaling molecules such as DAG, FFA, AA, PA, and LPA, all of which are thought to play a role in nutrient-induced insulin secretion (12).

In conclusion, enhanced islet GL/FFA cycling might contribute to the ability of many obese individuals with insulin resistance to maintain normoglycemia by secreting elevated levels of insulin. Whether genetic susceptibility to diabetes entails, in part, a failure to enhance GL/FFA cycling in islets as well as other tissues is an interesting possibility that needs to be explored. In this respect, it would be of interest to assess GL/FFA cycling longitudinally in islet tissues of various animal models of diabetes.

B. Insulin resistance and the metabolic syndrome

Accumulation of TG, DAG, and malonyl-CoA in muscle accompanied by lowered FA oxidation is thought to contribute to insulin resistance in obesity and T2D (159, 310, 321). However, if GL/FFA cycling activity is elevated in muscles, it might help to restore insulin sensitivity (321, 322). The antidiabetic action of the thiazolidinediones is thought to be primarily related to enhanced FFA esterification and accumulation of TG in the adipose tissue, allowing a redirection of peripheral fat to this organ (270). However, their antidiabetic action might also be due to other effects, such as AMPK activation (159) and enhanced GL/FFA cycling (37). Adipocytes have very low activity of GlyK and thus normally cannot reutilize the glycerol produced during lipolysis (37). However, in response to the thiazolidinedione rosiglitazone, which induces adipocyte GlyK (37), the released glycerol can be phosphorylated, thus enhancing FA esterification and GL/FFA cycling, resulting in lowered circulating FFA, which cause insulin resistance (39). Also, rosiglitazone treatment of ob/ob mice resulted in enhanced GL/FFA cycling and fat oxidation in white adipocytes (297). Whether this action of rosiglitazone is due to PPARγ or AMPK, which is also activated by thiazolidinediones (323), is uncertain. Similarly, in transgenic mice overexpressing PEPCK in adipose tissue, there is increased FA reesterification, and these mice develop obesity without insulin resistance or diabetes (36). This dichotomy is explained by the observation that a rise in PEPCK activity contributes to the production

of Gly3P and augmentation of the lipogenic segment of GL/FFA cycle without altering adipose tissue lipolysis flux, such that there is no enhancement of FFA release and accumulation of fat in other insulin-sensitive tissues. Finally, HSL-deficient mice are insulin-resistant and glucose-intolerant (151, 324), possibly due to elevated *sn*2,3-DAG in muscle and adipose tissues (325) and reduced lipolysis in β-cells coupled to glucose-stimulated insulin secretion (151). In addition HSL-deficient mice are protected from high-fat diet-induced insulin resistance in muscle tissues, which is associated with reduced im TG and FACoA levels (326).

Thus, there is much evidence suggesting that altered GL/FFA cycling (at cellular and/or whole body level) contributes to insulin resistance. Whether it may also be implicated in other pathological conditions associated with the metabolic syndrome, such as hypertension, abdominal obesity, hepatic steatosis, cardiomyopathy, and hypercholesterolemia, remains to be assessed. In this respect, it is noteworthy that ZDF rats show altered GL/FFA cycling (they accumulate excess TG in their islets and adipose tissue at low FA concentrations, unlike the lean controls) (327), are obese and insulin resistant, and also show high blood pressure, hepatic steatosis, and cardiomyopathy (328).

C. Cancer

In nonadipose cells, TG accumulation is considered to be a defense mechanism against acute lipid toxicity, and the capacity for TG accumulation is likely proportional to their survival (11, 107). We have shown that exogenous oleate conferred serum-independent growth to MDA-MB-231 breast cancer cells, which die within 24–48 h of serum removal (329). Oleate also suppressed their apoptosis caused by serum deprivation (243). Interestingly, a short (1 h) incubation time of MDA-MB231 cells with oleate conferred long-term (up to 1 wk) survival of the cells, even in the absence of both serum and the FA. Oleate-treated MDA-MB-231 cells accumulated TG severalfold in the form of LD, and this was accompanied by increased GL/FFA cycling (243) that persisted for 1 wk in the absence of both serum and exogenous FFA (243). If these observations may be extended *in vivo*, it is possible that elevated GL/FFA cycling, due to its ability to provide survival and growth signals (Fig. 4), is an integral part of the antiapoptotic pathways that favor tumor cells' growth in environments poor in nutrients and growth factors, as occurs in poorly vascularized tumors or within the core of tumors. Consistent with this view, we showed that monounsaturated FFA, but not saturated FFA, activated the protooncogene AKT/PKB (251) as well as phosphoinositide 3-kinase (329) in breast cancer cells. Enhanced GL/FFA cycling might promote cancer cell growth through the FFA-activated GPCR GPR40 via secretion of FFA released by lipolysis. Thus, reducing the expression of GPR40 in MDA-MB-231 cells curtailed oleate-induced cell proliferation, whereas enhancing its expression in various breast cancer cell lines amplified the proliferative action of FFA (251).

Besides providing lipid-signaling molecules promoting cell growth, the significant amount of heat generated by the GL/FFA cycle itself and by the activation of mitochondrial UCPs by the released FFA could favor high glycolytic flux and cell growth. It is noteworthy that breast tumors with rapidly proliferating cells maintain elevated temperatures compared with the surrounding normal tissue (330, 331). It would be of interest to determine whether this is related to a high GL/FFA cycle activity. Additionally, because of the heat produced locally in the cytosol where the GL/FFA cycle takes place, there might be an induction of HSPs. Most cancer cells have elevated levels of HSP (332, 333), which helps protect the cell from stress and favors survival pathways (334). Besides HSPs, heat shock also leads to the nuclear translocation of heat shock transcription factor-1, which regulates various stress response genes (335) and plays an important role in cancer cell migration (336).

Lipogenic enzymes, in particular FAS, are expressed at very high levels in cancer cells and as such contribute to the overall operation of GL/FFA cycling because they generate *de novo* long-chain FFA. The knockdown of lipogenic gene expression (ACL, ACC, and FAS) in cancer cells induces their apoptosis (7), and FAS down-regulation is associated with reduced expression level of the Her2/*neu*/erbB-2 oncoprotein (337–340). So far, it has not been considered whether the elevated cancer cell apoptosis by lowered expression of lipogenic enzymes might be related to reduced GL/FFA cycling.

There is pharmacological evidence in support of a role for GL metabolism and lipolysis in cancer. Orlistat, a pan-inhibitor of lipases, induces apoptosis of cancer cells (341). Also, the DGAT inhibitor xanthohumol is toxic to cancer cells *in vitro* (342). In cancer patients with solid tumors, the continuous weight loss and depletion of tissue fat stores have been attributed in part to elevated GL/FFA cycling (343, 344). It has been suggested that the increased activity of β-adrenoreceptors in these patients contributes to the wasting of fat stores via enhanced oxidative metabolism and GL/FFA cycling (345). It has also been demonstrated in experimental animals bearing solid tumors that the elevated circulating FFA (due to increased lipolysis) induce skeletal muscle UCP-3, which participates in the energy-wasting process (346).

Thus, it is attractive to propose that GL/FFA cycling, while important and beneficial for normal cell metabolism, survival, and proliferation, works as a double-edged sword in cancer, inflicting damage to the whole organism by two processes: by promoting tumor growth and cancer cell survival and by causing weight loss and tissue wasting.

IX. Perspectives and Therapeutic Implications

Although GL/FFA cycling has been considered as a metabolically futile process that results in wastage of cellular energy, research conducted over the past few years has demonstrated that this process occurs in most tissues, and emerging evidence indicates that it plays a key role in metabolic signal generation. Thus, the GL/FFA cycle, which generates many lipid-signaling molecules and growth signals, has been linked to many processes such as cell growth and apoptosis,

insulin secretion and action, heat production, and energy expenditure and might possibly be implicated in the control of food intake and longevity as well. However, to directly link this cycle to a particular biological process, methods need to be developed to precisely quantify metabolic flux through this cycle, and we also need to better understand how it is regulated under various physiological conditions. Perhaps an estimate of the cycling activity *in vivo* may be attained by measuring various GL species, FFA, and glycerol after a pulse-chase experiment with dual-labeled glucose and FFA.

Many regulatory aspects of the enzymes involved in the GL/FFA cycle are yet to be unraveled. For example, the possibility of a multiprotein complex on the surface of LD conducting coordinated lipolysis under the fine regulation of hormones via protein kinases including PKA or AMPK needs to be explored. These lipolysis steps offer a means for pharmacological intervention through which the release and availability of FFA can be controlled. This is particularly physiologically relevant because lipid accumulation in various tissues is thought to play a critical role in the development of insulin resistance, the metabolic syndrome, and T2D. The development of rimonabant, an antagonist of CB1 EC receptor, as a drug (marketed as Accomplia) for obesity and associated T2D can be discussed within this context. Thus, considering that GL/FFA cycling likely plays a role in the synthesis of ECs, pharmacological intervention leading to precise modulation of the lipolytic steps in required tissues, in particular the brain (347), might provide alternate ways to accomplish antiobesity and antidiabetic effects. Noteworthy in this respect is that the antidiabetic agent rosiglitazone alters GL/FFA cycling in human adipose tissue by means of glyceroneogenesis and glycerol phosphorylation (39).

The role of GL/FFA cycling in cancer development has been relatively ignored, although emerging evidence suggests that inhibiting this cycle may provide a novel approach for developing anticancer therapeutics. Finally, some of the recent findings on ATGL and HSL distribution (348) suggest the possibility of the existence of different TG pools in the cytosol, each possibly under different metabolic control. This added dimension of complexity in the regulation of GL/FFA cycling may be necessary because this process generates various metabolites that are triggers for the initiation of diverse metabolic signaling cascades.

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