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Modulation of Fatty Acid Metabolism as a Potential Approach to the Treatment of Obesity and the Metabolic Syndrome

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Increased *de novo* lipogenesis and reduced fatty acid oxidation are probable contributors to adipose accretion in obesity. Moreover, these perturbations have a role in leading to non-alcoholic steatohepatitis, dyslipidemia, and insulin resistance—via "lipotoxicity"-related mechanisms. Research in this area has prompted an effort to evaluate several discrete enzymes in these pathways as targets for future therapeutic intervention. Acetyl-CoA carboxylase 1 (ACC1) and ACC2 regulate fatty acid synthesis and indirectly control fatty acid oxidation via a key product, malonyl CoA. Based on mouse genetic and preclinical pharmacologic evidence, inhibition of ACC1 and/or ACC2 may be a useful approach to treat obesity and metabolic syndrome. Similarly, available data suggest that inhibition of other enzymes in this pathway, including fatty acid synthase, stearoyl CoA desaturase, and diacylglycerol acytransferase 1, will have beneficial effects. AMP-activated protein kinase is a master regulator of nutrient metabolism, which controls several aspects of lipid metabolism. Activation of AMPK in selected tissues is also a potential therapeutic approach. Inhibition of hormone-sensitive lipase is another possible approach. The rationale for modulating the activity of these enzymes and their relative merits (and downsides) as possible therapeutic targets are further discussed.

Key Words: Fatty acid synthesis; lipogenesis; fatty acid oxidation; insulin resistance; obesity; atherosclerosis; dyslipidemia.

Introduction—Role of Altered Fatty Acid Metabolism in the Pathogenesis of Obesity and Metabolic Syndrome

The obesity pandemic continues unabated; in fact, the number of obese (BMI ≥30 kg/m^2) adults in the world is now estimated to exceed 300 million. Because of shortcomings of the existing marketed weight loss drugs, a large "treatment gap" exists between those in need and those effectively treated; thus, new therapies targeted to key pathogenic pathways, which can be employed in concert with dietary restriction and exercise, are desperately needed. Obesity is associated with significant morbidity and mortality. Related diseases include sleep apnea, impaired fertility, certain cancers, osteoarthritis, and gallbladder disease. Most importantly, obesity is a critical component of—and a major underlying driver for—"Metabolic Syndrome." This prevalent cluster of interrelated common clinical disorders includes obesity, insulin resistance, glucose intolerance, hypertension, and dyslipidemia (hypertriglyceridemia and low HDL cholesterol levels). According to recent criteria, the metabolic syndrome is associated with a greater risk of atherosclerotic cardiovascular disease than any of its individual components *(1)*.

Our increasing understanding of the pathogenesis of obesity and its co-morbidities has revealed a strong link to underlying alterations in the biochemical pathways that regulate fatty acid metabolism—both the synthesis of fatty acids (lipogenic pathways) and catabolic pathways that mediate fatty acid oxidation. As noted in Fig. 1, the regulation of lipogenesis and fatty acid oxidation are controlled in a reciprocal fashion via a key intermediate, malonyl CoA, which contributes to subsequent fatty acid synthesis and coincidentally inhibits fatty oxidation, which is facilitated by carnitine palmitoyl transferase (CPT) 1. In addition, components of these pathways are also regulated by a master nutrient sensor, AMP-activated protein kinase (AMPK).

The liver is the principal site for *de novo* lipogenesis (DNL)—synthesis of fatty acids from carbohydrate sources. Lipogenic pathways are also engaged in the synthesis of triglycerides within adipose tissue. In contrast, fatty acid oxidation occurs in multiple tissues including liver, cardiac, and skeletal muscle, which are largely dependent on fatty acids as a fuel source. Recent evidence has also implicated a critical role for central (hypothalamic) regulation of food intake and energy expenditure (and peripheral glucose metabolism) via the presence and activity of the fatty acid oxidizing pathway and AMPK *(2)*. Thus, components of the fatty acid metabolism pathways may serve as an

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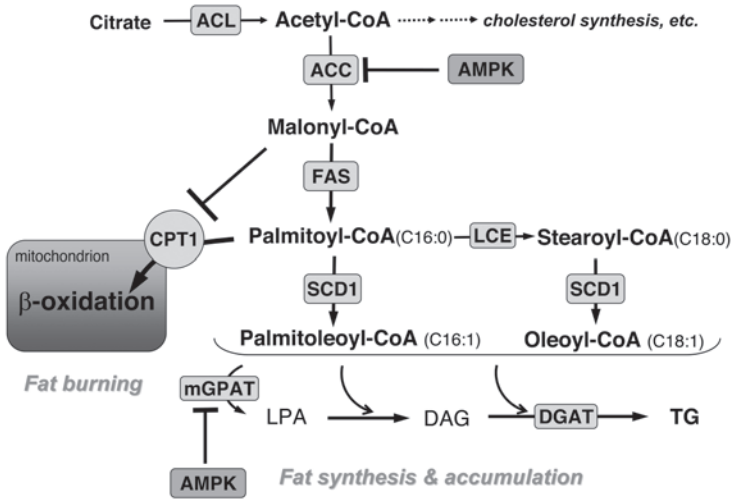

Fig. 1. Pathways mediating lipid synthesis and oxidation. Key biochemical pathways which mediate fatty acid and subsequent triglyceride (TG) synthesis as well as mitochondrial fatty acid oxidation are depicted. The following enzymes have important roles in these pathways: AMP-activated protein kinase (AMPK); long-chain elongase (LCE), ATP-citrate-lyase (ACL), stearoyl CoA desaturase 1 (SCD1), fatty acid synthase (FAS), diacylglycerol acyltransferase 1 (DGAT1), carnitine pamitoyl transferase 1 (CPT1), mitochondrial glycerol-3-phospate acyltransferase (mtGPAT).

important central sensing system, which can respond to states of peripheral nutrient excess or deficiency.

The contribution of hepatic DNL to net synthesis of circulating and stored triacylglyercols in humans is estimated to be relatively low (approx 10%). However, recent observations indicate that hepatic DNL is actually increased in obese and hyperinsulinemic humans in both the fasting state *(3)* and in response to carbohydrate feeding *(4)*. In obese humans, DNL is likely to account for as much as 24–26% of circulating VLDL and hepatic triacylglyerol content *(3)*. Thus, increased DNL may contribute to the accretion of adipose stores and to the pathogenesis of dyslipidemia and hepatic steatosis—an important aspect of obesity-associated insulin resistance and type 2 diabetes (see below).

Obesity obviously is the consequence of an imbalance in caloric intake vs energy expenditure (EE). In addition to physical activity, which is often reduced in obesity, adaptive thermogenesis (principally the thermic effect of food) and resting metabolic rate (RMR) are major components of net EE *(5)*. Pathways that mediate adaptive thermogenesis (and contribute to setting RMR) include thyroid hormone and β3 adrenergic receptor signaling and uncoupling proteins. These could potentially be harnessed as a means of preventing or treating obesity. With increasing body mass, RMR is also increased. However, RMR is more closely related to fat-free mass than to total body mass *(6)*. In addition, a variety of lines of evidence have implicated small differences in RMR as a component of the pathogenesis of obesity *(7)*. Thus, RMR is genetically determined and lower RMR has been shown to predict weight gain in several prospective studies *(6,7)*. Moreover, reduced rates of fatty acid oxidation (vs carbohydrate oxidation) have also been implicated as a cause of increased weight gain. A higher respiratory quotient (RQ), indicating lower fatty acid vs higher carbohydrate oxidation, has been found to predict subsequent weight gain in prospective studies *(7)*. In addition, obese patients have higher postprandial RQ values *(4)* and have higher RQ after diet-mediated weight loss vs weight-matched controls *(7)*.

A prominent role for altered fatty acid metabolism in the pathogenesis of obesity-associated co-morbidities is broadly accepted. As noted above, increased rates of DNL are a likely contributor to hypertriglyceridemia. In addition, reduced lipid catabolism can be envisaged to contribute to dyslipidemia *(8)*. Mechanisms of insulin resistance and subsequent type 2 diabetes are likely to include the following aspects relating to altered fatty acid metabolism: (1) increased circulating free fatty acids; (2) accumulation of muscle (intramyocellular) triglycerides and long-chain fatty acyl-CoA esters; and (3) triacylglyercerol accumulation in liver (hepatic steatosis) *(1)*. Collectively, these processes have been referred to as "lipotoxicity." Notably, increased lipolysis—the release of fatty acids from adipose triglyceride stores—appears to also contribute to increased circulating fatty acids and the "ectopic" deposition of lipids in other tissues *(1)*. Several lines of evidence also suggest that impaired pancreatic beta cell function and survival may be related to lipotoxicity mechanisms that lead to impaired insulin secretion and the development of overt diabetes *(9)*.

Given that altered fatty acid metabolism has an important role in the pathogenesis of obesity and its co-morbidities, therapeutic attempts to modulate fatty acid metabolism can now be envisaged. Indeed, several available drugs used to treat dyslipidemia or diabetes appear to act via these pathways. Prominent examples include fibrates, which augment fatty acid oxidation via activation of PPARα *(10)*, and met-

formin, an antidiabetic agent that acts, in part, via AMPK activation *(11)*. As discussed in the remainder of this review, targeted approaches to inhibit (or activate) discrete enzymes in the DNL and fatty acid oxidizing pathways have recently been suggested. Based on favorable results obtained using animal models of obesity and the metabolic syndrome, the development of specific small molecule drugs affecting these enzymes is now worthy of consideration.

Novel Therapeutic Approaches

ACC Inhibitors

Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, which is a key molecule controlling intracellular fatty acid metabolism (Fig. 1). There are two major isozymes that have different physiological roles due to distinct subcellular distributions *(12)*.

A cytoso1ic enzyme, ACC1, supplies malonyl-CoA to fatty acid synthase and is committed to DNL in many tissues via further nutritional and hormonal regulation. ACC1 is ubiquitously expressed in many tissues, but higher levels occur in lipogenic tissues including liver and adipose *(13)*. In fact, ACC1 gene expression and activity are markedly induced either by high carbohydrate feeding or hyperinsulinemia in animals, resulting in increased adiposity and lipoprotein secretion *(14)*. It is predicted that ACC1 blockade will reduce flux through the DNL pathway in lipogenic tissues, causing reduced adiposity and lipoprotein secretion. It is therefore plausible that ACC1-specific inhibitors have therapeutic potential for treatment of obesity and hyperlipidemia. However, validation of this concept by studying the consequences of genetic disruption of the ACC1 gene has not been possible owing to the fact that homozygous deficiency of ACC1 causes fetal lethality *(15)*.

In contrast to ACC1, ACC2 is anchored to the mitochondrial surface via a unique N-terminal domain consisting of 20 hydrophobic amino acids, a so-called "mito-sequence" *(12)*. ACC2 produces malonyl-CoA on the mitochondrial surface; insulin and citrate can modulate this process depending on nutritional status *(14)*. As depicted in Fig. 1, malonyl-CoA is the potent endogenous inhibitor of CPT1 (also located on mitochondrial surface). Thus, ACC2 indirectly prevents influx of fatty acids into mitochondria and subsequent beta oxidation. In response to fasting (or exercise), ACC2 activity and its product, malonyl-CoA, are decreased *(16,17)*. These conditions allow for greater mitochondrial fatty acid beta oxidation. ACC2 expression is consistently high in energetic organs such as liver, skeletal muscle, and heart *(18)*. Therefore, it can be expected that specific blockade of ACC2 could increase whole body fatty acid oxidation. To the extent that fatty acid oxidation may also mediate a net increase in energy expenditure, negative energy balance may also ensue.

The beneficial phenotypes of the ACC2 null mouse strongly support the above concepts. The ACC2 knockout mouse has reduced tissue malonyl-CoA levels and increased fatty acid oxidation in skeletal muscle *(19)*. Higher levels of plasma ketones in fasted mice indicate an increase in hepatic fatty acid oxidation as well. Although this mouse is hyperphagic, reduced body fat and attenuated hepatic triglyceride accumulation occur *(19)*. Importantly, the mouse is resistant to diet-induced body weight gain and insulin resistance *(20)*. It is interesting that not only fatty acid oxidation, but glucose oxidation and insulin sensitivity as well are also increased in white adipose tissue from ACC2 null mice *(21)*. These results strongly suggest that ACC2 specific inhibitors could increase fatty acid oxidation in liver, skeletal muscle, and maybe adipose tissue, leading to reduced lipotoxicity and increased whole body energy expenditure. Therefore, obesity, type 2 diabetes, and hepatic steatosis may be improved by ACC2 specific blockade. In addition, ACC2 null mice are fertile and have a normal life span, suggesting that ACC2 inhibition may be safer than inhibiting ACC1.

Although isozyme selective ACC inhibitors have yet to be identified, favorable effects of isozyme non-selective ACC inhibitors on multiple aspects of metabolic syndrome in animals have been reported. The efficacy of ACC 1/2 dual inhibition is predicted to be greater than efficacy derived from inhibiting either isozyme alone because dual inhibition could deplete multiple intracellular malonyl-CoA pools, simultaneously reducing DNL and increasing fat oxidation.

5-(Tetradecyloxyl)-2-furancarboxylic acid (TOFA), after conversion to its CoA ester, can inhibit ACC 1/2 activity *(22)*. Reduced DNL and triglyceride secretion have been observed in TOFA-treated cultured hepatocytes *(22,23)*. It is also reported that TOFA decreased plasma cholesterol and triglycerides in rats, hamsters, and rhesus monkeys *(23–25)*. Similarly, MEDICA 16 (b,b,b',b'-tetramethylhexadecanoic acid) is converted to MEDICA 16-CoA, which inhibits ACC activity in a competitive manner with acetyl-CoA *(26)*. MEDICA 16 treatment of diabetic rats improves hyperlipidemia and hyperglycemia *(27)*; however, this agent is also known as an ATP citrate lyase inhibitor *(28)*, an activity that could contribute to efficacy (see below). Recently, a bipiperidylcarboxamide compound class, CP-640186, was reported to include potent inhibitors of both ACC1 and ACC2 *(29)*. After chronic administration to high sucrose diet fed rats, CP-640186 reduced liver, skeletal muscle, and adipose tissue triglycerides and decreased body weight and fat mass. In addition, reduced plasma leptin levels and improved insulin sensitivity were observed in the rats treated with CP-640186 *(30)*. Similar favorable effects of this compound were also observed in diet-induced obese mice. These results indicate that pharmacological blockade of both ACC1 and 2 can favorably affect multiple aspects of metabolic syndrome in rodents. In addition to CP640186, several distinct compound classes have been claimed as mammalian ACC 1/2 dual inhibitors for potential treatment for metabolic syndrome, from Fujirebio, Ajinomoto, and Roche *(30)*. How-

ever, the current status of preclinical or clinical development for these agents is unknown. In addition, potential mechanism-based liabilities related to both ACC1 and 2 inhibition will require further elucidation.

FAS Inhibitors

Fatty acid synthase (FAS) catalyzes the *de novo* synthesis of long-chain fatty acids (LCFA) (Fig. 1) *(31,32)*. As a crucial biosynthetic enzyme, FAS has effects in addition to affecting energy homeostasis. This is underscored by the finding of *in utero* lethality in FAS knockout mice; in fact, even heterozygotes have reduced viability at several developmental stages *(33)*. In lipogenic tissues, FAS inhibition could theoretically suppress *de novo* LCFA synthesis with a consequent reduction of fat stores. However, FAS inhibition might also evoke an increase in the concentration of cellular malonyl-CoA; this could inhibit CPT-1, preventing fatty acid oxidation (Fig. 1) thereby enhancing fat accumulation in selected tissues. As discussed above, LCFA accumulation in liver and muscle is viewed as a major aspect of the pathogenesis of insulin resistance *(34)*. Therefore, the potential of FAS inhibition as a viable therapeutic approach is still controversial.

FAS inhibition has received substantial attention based on the anti-obesity efficacy of a known FAS inhibitor, C75. C75 strongly suppressed appetite after intraperitoneal (IP) and intracerebroventicular (ICV) injections in mice *(35)*. Moreover, ICV co-administration of C75 with the ACC inhibitor, TOFA, significantly ameliorated the anorectic effects of C75, suggesting that C75 regulated appetite through the central regulation of malonyl-CoA concentrations *(35)*. At least in part, the anorectic effects of C75 may be evoked by changes in neuropeptide signals. Thus, C75 decreased mRNA expression of orexigenic peptides, neuropeptide Y (NPY) and agouti-related peptide (AGRP), and increased mRNA expression of anorectic peptides, proopiomelanocortin (encoding αMSH) and cocaine-amphetamine-related transcript *(36)*. Presumably, the FAS system can impinge on leptin signals to regulate feeding behavior. As discussed elsewhere, a number of other lines of evidence suggest that central (hypothalamic) regulation of fatty acid synthesis and/or oxidation can modulate feeding and energy expenditure via cross-talk with leptin signaling and other hypothalamic mediators.

Taken together, the above findings suggest that the FAS system could be a pivotal point for intervention via the regulation of both feeding and lipogenesis. However, recent evidence indicates that C75 can directly activate CPT-1 and thereby enhance fatty oxidation via a FAS-independent effect *(37)*. Although these data suggest yet another approach to obesity therapy involving lipid metabolizing enzymes (CPT-1 activation), the extent to which this effect (vs FAS inhibition) contributes to the potent anti-obesity effect of C75 in rodents remains to be established. Therefore, to address the potential of FAS inhibition more precisely, an assessment of more specific pharmacologic inhibitors is of paramount importance.

AMP Kinase Activation

AMPK is a heterotrimeric serine/threonine protein kinase complex consisting of one catalytic subunit (α) and two non-catalytic regulatory subunits (β and γ). The three subunits are encoded by two (α and β) or three (γ) distinct genes leading to the production of 12 potential enzyme complexes *(38)*. AMPK is a critical sensor of cellular energy charge that is activated via multiple mechanisms—direct allosteric and indirect—in response to several stimuli including 5'-AMP and increasing the AMP/ATP ratio (Fig. 2). Numerous physiologic and pathophysiologic stimuli lead to AMPK activation including cellular hypoxia, muscle contraction, and drugs such as metformin and rosiglitazone *(39)*. Interestingly, an important effect of leptin—to induce fatty acid oxidation in skeletal muscle—appears to be mediated by AMPK activation (and subsequent ACC inactivation) *(40)*. Moreover, several lines of investigation have implicated AMPK activation as a component of signaling pathways mediating the effects of a key adipose-derived "adipokine," adiponectin *(41)*. These findings suggest that several of the probable beneficial effects of adiponectin (insulin sensitization, glucose lowering, anti-atherogenesis) might involve AMPK activation *(41)*.

AMPK activation leads to direct and indirect modulation of several key metabolic pathways, which may affect obesity and obesity-associated co-morbidities (Figs. 1 and 2). ACC1 and ACC2 are both direct substrates of AMPK. ACC phosphorylation leads to its inactivation; thus, AMPK activation can promote both a decrease in lipogenesis and an increase in fatty acid oxidation (see ACC section above). Indeed, activation of AMPK in liver or muscle-derived cells with known pharmacologic AMPK modulators such as metformin or AICA-riboside (AICAR, an AMP analog) can attenuate fatty acid synthesis and enhance fatty acid oxidation *(11,39)*. Mitochondrial glycerol-3-phospate acyltransferase (mtGPAT), the first committed step in *de novo* triglyceride synthesis (Fig. 1), is also negatively controlled by AMPK *(42)*. In addition, hormone-sensitive lipase (HSL) is a target of AMPK in adipose tissue *(39)*. As described further below, inhibition of HSL would be expected to have favorable effects to ameliorate obesity and type 2 diabetes. Phosphorylation and inactivation of hepatic HMG-CoA reductase by AMPK is predicted to reduce cholesterol synthesis analogous to the effect of statins, which are direct HMG-CoA reductase inhibitors. Additional beneficial effects on lipid metabolism are likely to be mediated via AMPK's effect to suppress the expression of lipogenic genes by inhibiting the expression or activation of a key transcription factor, sterol regulatory element binding protein 1c (SREBP1c) *(11)*. AMPK's effects in skeletal muscle include stimulat-

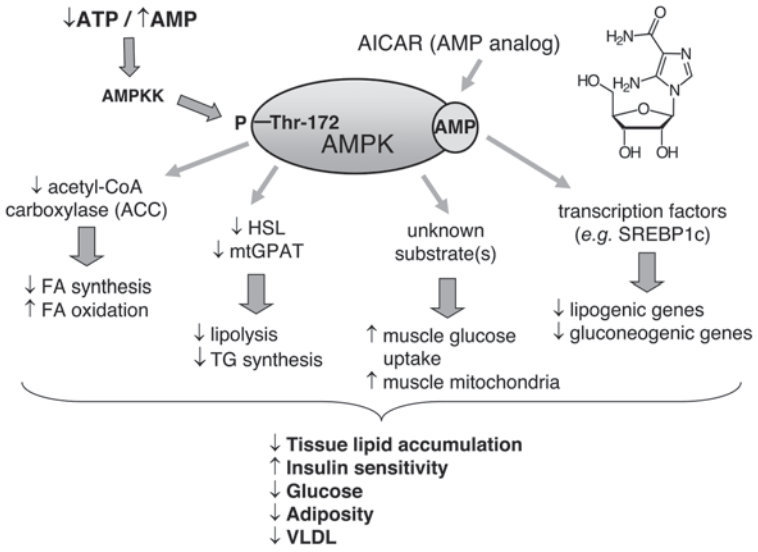

Fig. 2. The AMP-activated protein kinase system. Mechanisms of AMPK activation—allosteric direct activation and activation via phosphorylation by an upstream kinase (AMPKK)—are shown along with selected downstream effectors of AMPK action that can be impacted in peripheral tissues such as liver, muscle and adipose. Those AMPK linked pathways which regulate lipid and glucose metabolism are highlighted.

ing glucose uptake via the insulin-responsive transporter (GLUT4) system *(39)*. Hepatic AMPK activation also attenuates glucose production (gluconeogenesis and glycogenolysis) via poorly defined mechanisms, which may include modulation of selected gene transcription. The latter two effects may contribute to the glucose-lowering action of metformin, which is widely used in the treatment of type 2 diabetes *(11)*.

Numerous in vivo studies conducted using AICAR have attempted to further define the physiologic roles of AMPK and the potential utility of pharmacologic AMPK activation. In several chronic rodent efficacy studies, AICAR was shown to modestly reduce adipose tissue mass (reviewed in ref. *42*). More impressive insulin-sensitizing and glucose-lowering actions of AICAR have been observed in several rodent models of obesity and type 2 diabetes *(39,42)*. Mechanisms underlying the anti-obesity and anti-diabetic effects of AMPK activation may include reduced ectopic fat accumulation in muscle and liver, which in turn could be mediated by increased fatty acid oxidation and reduced lipogenesis via specific pathways noted above. In addition, AMPK activation in muscle appears to enhance mitochondrial biogenesis and function, which could also contribute to metabolic benefits *(43)*. Given these promising effects and the known benefits of other agents (metformin, rosiglitazone) and stimuli (e.g., exercise) that lead to AMPK activation, a search for more potent and specific AMPK activators and characterization of their effects in animal models of obesity and metabolic syndrome is highly warranted.

An intriguing twist to the AMPK "story" involves the recent discovery that AMPK and other components of the fatty acid oxidizing pathway are present and operative in the hypothalamus. Importantly, it now appears that AMPK *inhibition* and net *increases* in ACC activity within the hypothalamus form part of a central nutrient sensing system which can result in decreased food intake and may also be linked to the central effects of leptin and other hormonal signals regulating energy homeostasis at the level of the hypothalamus *(44,45)*. These recent findings present a paradox for those considering AMPK and ACC as drug targets—opposite effects on these enzymes may be desired in central vs peripheral tissue compartments.

Stearoyl-CoA Desaturase Inhibitors

Stearoyl-CoA desaturase (SCD) catalyzes *de novo* synthesis of monounsaturated fatty acids (MUFA) from saturated fatty acids by introduction of *cis*-double bonds at the Δ9 position *(46)*. In humans, two isoforms, SCD1 and SCD5, have been reported and both isoforms have similar Δ9 desaturase activity. hSCD1 mRNA expression is highest in adipose and liver tissues, whereas hSCD5 mRNA is highest in brain and pancreas. SCD5 is unique to primates and there is only 58% similarity between the isoforms based on the predicted amino acid sequence *(47)*. The mouse orthologue of hSCD1, mSCD1 is abundantly expressed in liver and adipose as well. In contrast to human, other minor isoforms, mSCD2, 3, and 4 are expressed in rodent brain, skin and heart, respectively *(48,49)*.

Palmitoyl (16:0)- and stearoyl (18:0)-CoA are known as preferred substrates for SCDs and are converted into palmitoleoyl (16:1)- and oleoyl (18:1)-CoA, respectively (Fig. 1) *(46)*. Oleic acid (18:1) is the most abundant fatty acid found

in triglycerides, phospholipids, wax esters, and cholesterol esters. Therefore, SCD1 (and other SCDs) is thought to play an important role in lipid metabolism. Earlier findings using the *asebia* mouse, which has a natural defect in SCD1, revealed marked reductions in hepatic triglyceride and cholesterol ester content in homozygous mutant mice *(50)*. Although a high carbohydrate diet readily induces hepatic triglyceride synthesis and steatosis in heterozygous mutant mice, these effects were not observed in homozygotes *(51)*. Plasma VLDL and LDL levels are also very low in the *asebia* mouse compared with wild-type controls. These results clearly indicate that SCD1-mediated biosynthesis of MUFA is a critical step for triglyceride synthesis, storage, and secretion (as lipoproteins).

Cross breeding of the *asebia* mouse with the leptin-deficient *ob/ob* mouse provided striking results *(52)*. In contrast to *ob/ob* littermate mice, body weight and fat mass were significantly lower in *ob/ob* mice bearing the SCD1 mutation despite even greater hyperphagia. An observed increase in whole body energy expenditure was considered to be a major cause for the anti-obese phenotype of SCD1 deficiency. Recent data derived from a "synthetic" SCD1 knockout mouse model are consistent with findings from the *asebia* mouse and have provided more detailed information *(53)*. In addition to an anti-obese phenotype and increased whole body energy expenditure, improved glucose tolerance and insulin sensitivity were noted in SCD1 null mice *(54)*. Also, plasma ketone levels were elevated in fasted knockout mice more than in wild-type controls, indicating that hepatic fatty acid oxidation is increased by SCD1 disruption *(54)*. Reduced hepatic malonyl CoA and an increase in the expression of genes encoding enzymes which regulate CPT1 activity and mitochondrial ^{14}C-palmitoyl-CoA oxidation may underlie this effect *(55)*.

Other mechanism(s) by which SCD1 deficiency leads to increased fatty acid oxidation and energy expenditure remain unknown. Activation of AMPK has been proposed as one potential cause *(55)*; however, there are no clear links between SCD1 disruption and AMPK activation. Another possibility involves increasing palmitoyl-CoA levels (in response to the absence of SCD1), which could in turn directly activate mitochondrial CPT-1 (see ACC inhibitor section). Because similar beneficial phenotypes including increased hepatic fatty acid oxidation were also observed in PPARα and SCD1 double knockout mice *(56)*, the PPARα pathway can apparently be excluded as a potential mechanism.

The above noted findings in *asebia* and SCD1 null mice suggest that SCD1 is a promising drug target for potential treatment of diabetes, obesity, and non-alcoholic hepatic steatosis. Recently, pharmacological intervention in diet-induced obese mice using SCD1-specific antisense oligonucleotides was also shown to exert anti-obesity and insulin-sensitizing efficacy *(57)*. According to recent patent publications, piperadine derivatives and related compounds have been claimed as small molecule SCD1 inhibitors *(58)*. However, the current status of preclinical or clinical development is unknown.

As a cautionary note, it should be noted that SCD1 null mice also develop skin abnormalities, alopecia, and atrophic changes in sebaceous and meibomian glands due to a local lack of triglycerides, cholesterol, and wax esters *(56)*. Therefore, inhibition of SCD1 might lead to side effects that could preclude the development of systemically active pharmacological agents.

Hormone-Sensitive Lipase Inhibitors

Hormone-sensitive lipase (HSL) is a cytosolic enzyme that catalyzes the hydrolysis of triacylglycerol, diacylglycerol, and cholesteryl esters to liberate free fatty acids. HSL is predominantly expressed in adipose tissue and is believed to have a crucial role in the regulation of lipid homeostasis by release of lipid from adipocytes. It is well-known that HSL-mediated lipolysis is activated through beta-adrenergic signaling. In contrast, insulin blocks the signaling pathway by activating phosphodiesterase 3B, leading to anti-lipolytic activity *(59,60)*. Given the important role of excessive FFAs and ectopic lipid deposition in promoting "lipotoxicity" as described above, HSL blockade may have a therapeutic impact on the metabolic consequences of obesity by reducing FFA release from the adipose tissues.

In vivo genetic validation of the concept of HSL blockade has been reported by several groups. In the HSL KO mice, lipolysis induced by beta-adrenergic agonism was significantly attenuated in isolated adipocytes and white adipose tissue *(61,62)*. Consequently, plasma TG, plasma FFA, and liver TG levels were significantly lower in fasted HSL KO mice, indicating that the lipid mobilization from adipose in response to fasting is defective. Importantly, improved hepatic insulin sensitivity, decreased hepatic TG production, and accelerated VLDL clearance were observed in fasted KO mice *(63–65)*. These favorable findings support the concept of HSL blockade for potential treatment of type 2 diabetes and dyslipidemia associated with obesity.

Interestingly, it also appears that genetic HSL disruption can affect the nature of adipose tissue: (1) heterogeneity in cell size and preadipocyte accumulation in white fat *(66)*; (2) marked enlargement in adipocytes from brown adipose tissue with increased UCP-2 levels (predictive of an increase in energy expenditure) *(67)*; and (3) not only lipolysis, but also lipogenesis and glucose uptake-metabolism were suppressed in white adipose tissue *(68)*. Although these observations remain unexplained, an accumulation of diacylglycerol, which was evident in both white and brown adipose from knockout mice, might have a role. Presumably, such effects may have contributed to the resistance to diet-induced obesity, which was also evident in HSL null mice *(67,69)*.

As noted above, pharmacological HSL blockade has the potential to impact obesity, or more specifically its metabolic consequences (including hepatic steatosis). As potential issues, male sterility and existence of other lipases involved

in the regulation of lipolysis, such as adipose triglyceride lipase, should be noted *(61,70)*.

Diacylglycerol Acyltransferase 1 (DGAT1) Inhibitors

Acyl-CoA:diacylglycerol acyltransferase (DGAT) 1 is one of two known DGAT enzymes that are solely committed to the final step in mammalian triglyceride synthesis. DGAT1 is highly homologous with acyl-CoA:cholesterol acyltransferease (ACAT) 1, but not homologous with DGAT2, which belongs to an independent gene family including acyl-CoA: monoacylglycerol acyltransferase (MGAT) *(71,72)*. DGAT1 is integrated in ER membrane as a homo tetrameric protein *(73)* and is widely expressed in many tissues with the highest expression levels in small intestine *(74)*.

A DGAT1 null mouse was created to investigate its pathophysiological roles *(75)*. Although body weight was almost the same in knockout vs wild-type mice on a regular chow diet, significant decreases in fat depot weight were noted in knockouts. Surprisingly, these mice still retained some amount of DGAT activity and the ability to sustain triglyceride synthesis, probably due to the compensatory activity of DGAT2. Further interest in DGAT1 is based on the fact that DGAT1 null mice are resistant to diet-induced body weight and fat mass gain as well as insulin resistance *(75, 76)*. Importantly, although insulin resistance and obesity attributable to leptin-resistance (agouti-yellow mice) are ameliorated by DGAT1 disruption, the metabolic consequences of leptin deficiency (*ob/ob* mice) are not improved by the lack of DGAT1 *(76)*. This discrepancy has been explained by a difference in expression of DGAT2, which is downregulated by leptin. Quite strikingly, transplantation of white adipose tissue from DGAT1 null into wild-type mice decreases adiposity and increases insulin sensitivity, either on chow or high-fat diet *(77,78)*. One potential mechanism could be an increase in the secretion of adiponectin from DGAT1 deficient adipose; adiponectin is known to promote fatty acid oxidation and to enhance insulin sensitivity (see AMPK section above). A local accumulation of diacylglycerol resulting from cellular DGAT1 deficiency could conceivably serve as an important signal driving further effects. Additional features of DGAT1 deficient adipose tissue include smaller-sized adipocytes and altered gene expression profiles which suggest increased local energy expenditure *(76,77)*. Favorable changes in DGAT-deficient adipocytes may be similar to the effects of HSL deficiency (see Hormone-Sensitive Lipase Inhibitors section).

Taken together, the above observations suggest that pharmacological blockade of DGAT1 could yield anti-diabetic and anti-obesity effects. Although DGAT1 null mice are viable and fertile, some degree of alopecia is evident and females mouse lack adequate milk production *(75,79)*. Recently, it has also been reported that liver-specific knockdown of DGAT activity—achieved using DGAT2-specific antisense oligonucleotides—resulted in marked improvement of hepatic steatosis in either diet-induced obese or *ob/ob* mice *(80)*. However, no improvement in adiposity and insulin resistance was noted. It should also be noted that DGAT2 null mice are lipopenic and die soon after the birth, probably due to profound reductions in substrates for energy metabolism and an impairment of skin barrier function *(81)*. Therefore, from a safety perspective, DGAT1 selective inhibition would be preferable.

Other Potential "Drug Targets" Affecting Fatty Acid Metabolism

Because ATP citrate lyase (ACL) plays an important role in supplying acetyl-CoA for both *de novo* synthesis of cholesterol and fatty acids (Fig. 1), ACL has been considered as a drug target for potential treatment of hyperlipidemia. In fact, a potent ACL inhibitor, SB-204990, significantly decreased plasma levels of cholesterol and triglycerides in either rat or dog in a dose-dependent manner *(82)*. However, it should be noted that ACL homozygous knockout mice are embryonic lethal *(83)*.

Mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) is committed to the initial and rate-limiting step of triglyceride synthesis in the so-called "glycerol phosphate pathway" (Fig. 1) *(84)*. It is known that this enzyme is located on the mitochondrial outer membrane *(85)* and that it shares its substrate, palmitoyl-CoA, with CPT-1 in this location *(86)*. Because CPT-1 has a critical role as the first step toward mitochondrial fat oxidation, mtGPAT may serve to dispatch palmitoyl-CoA for triglyceride synthesis vs fat oxidation. As noted above, mtGPAT is also regulated (inhibited) by AMPK. Favorable phenotypes with respect to body weight, plasma triglycerides, insulin resistance, and hepatic steatosis have been reported in mtGPAT null mice *(87,88)*. These observations imply that mtGPAT may also be a potential drug target for metabolic syndrome.

Acyl-CoA:monoacyltransferase (MGAT) is believed to be a key enzyme in the "monoacylglycerol pathway," which accounts for approx 80% of total triglyceride re-synthesis in the small intestine leading to net triglyceride absorption and chylomicron generation *(89)*. In agreement with its purported physiological role, MGAT activity is extremely high in the small intestine relative to other organs *(90,91)*. In addition, a pathological connection of intestinal MGAT to diabetes was suggested in a rat model *(92)*. Therefore, it can be expected that MGAT blockade could inhibit net fat absorption via the gut and improve metabolic abnormalities resulting from excess dietary fat intake. However, to date, there has been no direct evidence indicating favorable effects of genetic MGAT ablation or pharmacologic inhibition.

Summary and Future Directions

A clear recognition of the importance of obesity and its consequences to human health have emerged. Most recently, revised criteria for the metabolic syndrome also stipulated that central obesity (as measured by waist circumference)

Table 1
Rationale for Selected Drug "Targets" Which May Modulate FA Synthesis and/or Oxidation

Molecular Target [Approach]	Rationale	References
Acetyl CoA carboxylase (ACC) [inhibitor]	ACC2 null mice are lean; ACC inhibitors ameliorate aspects of metabolic syndrome in rodents.	*19,95*
Fatty Acid synthase (FAS) [inhibitor]	FAS is a key rate controlling step in lipogenesis. C75 (a FAS inhibitor) produces marked decreases in body weight in treated rodents.	*31,35,37*
AMP kinase, [activator—peripheral; inhibitor—central]	AMPK contributes to metformin efficacy; AMPK activation inactivates ACC, HSL, mtGPAT (see below); peripheral AMPK activators induce favorable metabolic effects.	*11,39,42*
Stearoyl CoA desaturase (SCD) 1, [inhibitor]	SCD1 deficient mice are lean; SCD1 deficiency ameliorates features of metabolic syndrome in *ob/ob* mice. SCD1 antisense oligonucleotide treatment of obese mice lowers body weight; improves insulin sensitivity.	*52,54,57*
Hormone sensitive lipase (HSL) [inhibitor]	HSL deficient mice are resistant to diet induced obesity and leptin deficiency; HSL deficiency ameriolates hepatic steatosis and its associated insulin resistance and dyslipidemia.	*65,67,69*
Diacylglycerol acyltransferase 1 (DGAT1) [inhibitor]	DGAT1 null mice are resistant to diet induced obesity and insulin resistance. Adipose tissue from DGAT1 null mice transplanted into normal mice confers metabolic benefits.	*75–77*

should now be included as an obligate feature *(93)*. Along with increasing recognition of the public health issues at hand, it is now abundantly clear that the pathogenesis of obesity is related to abnormalities in the metabolism of fatty acids—both with respect to increased *de novo* lipogenesis (DNL) and reduced fatty acid oxidation. Moreover, aspects of the metabolic syndrome that include insulin resistance, type 2 diabetes, and dyslipidemia as well as a related morbid condition—nonalcoholic steatohepatitis *(94)*—can be linked to abnormal fatty acid metabolism and to the ectopic deposition of lipids in tissues other than adipose ("lipotoxicity"). Given the importance of the pathways of fatty acid metabolism to the pathogenesis of obesity and its complications, an assessment of potential therapeutic approaches focused on modulation of these pathways is now warranted.

In this review, we have discussed several recently implicated "drug targets" that represent key enzymatic checkpoints in the regulation of fatty acid synthesis and oxidation. As summarized in Table 1, recent results, principally derived from the study of mouse genetic models, have provided a rationale to further consider several of these enzymes as candidates for therapeutic intervention. Unfortunately, no clinical data are yet available to indicate which, if any, of these approaches are truly viable. Moreover, only scant preclinical results have been obtained using highly selective and potent pharmacologic tools directed toward these targets at present. Therefore, it is certain that more intensive research focused on the discovery of better pharmacologic agents which can be thoroughly characterized in animal models is needed.

There are several critical issues that may pertain more broadly to these approaches and must be addressed in the course of further research in this area. First, it is apparent that DNL may represent a quantitatively more important contributor to accretion of adipose and circulating lipids in rodents than in humans. Second, the extent to which intervening with approaches that augment fatty acid oxidation can actually prevent or treat obesity remains to be established. Third, there is the potential for significant adverse effects associated with interventions that modulate fat synthesis or catabolism. Evidence for this includes neonatal lethality with several knockout mouse models, altered skin morphology and function in some such models, and other lines of evidence implicating adverse effects on energy metabolism in selected cell types including muscle. Finally, it now appears that, at least in certain cases, opposite regulation of a given pathway may be desirable in central (hypothalamic) vs peripheral (e.g., liver) tissue compartments as discussed above (see AMPK section).

Having cited several notes of caution, we also wish to point out that each of the individual approaches discussed in this report merits further investigation. In addition, there are substantial differences between each such that the inability to progress toward a viable therapy with one should not preclude further pursuit of the others. Over the coming few years, it is expected that remarkable progress toward further assessing the therapeutic utility and potential downsides of these approaches will occur. In addition, the expansion of basic research in this field to include a much broader "functional genomic" survey of pathways and individual regulatory molecules is very likely to yield additional, and

perhaps more compelling, candidate therapeutic approaches that involve modulating of fatty acid metabolism.

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