Abstract—Because the ability to make triglycerides is essential for the accumulation of adipose tissue, inhibition of triglyceride synthesis may ameliorate obesity and its related medical consequences. Acyl coenzyme A (CoA):diacylglycerol acyltransferase 1 (DGAT1) is 1 of 2 DGAT enzymes that catalyze the final reaction in the known pathways of mammalian triglyceride synthesis. Mice lacking DGAT1 are resistant to obesity and have increased sensitivity to insulin and leptin. DGAT1-deficient mice are also resistant to diet-induced hepatic steatosis. The effects of DGAT1 deficiency on energy and glucose metabolism result in part from the altered secretion of adipocyte-derived factors. Although complete DGAT1 deficiency causes alopecia and impairs development of the mammary gland, these abnormalities are not observed in mice with partial DGAT1 deficiency. These findings suggest that pharmacological inhibition of DGAT1 may be a feasible therapeutic strategy for human obesity and type 2 diabetes. (Arterioscler Thromb Vasc Biol. 2005;25:482-486.)

Key Words: acyl CoA:diacylglycerol acyltransferase • energy • glucose • metabolism • insulin resistance • type 2 diabetes

Obesity can be viewed as an energy balance disorder, arising when energy input exceeds energy output, with most of the excess calories converted into triglycerides and stored in the adipose tissue. Medications currently approved for the treatment of obesity attempt to restore energy balance primarily by decreasing energy input—by either suppressing appetite or interfering with lipid absorption in the small intestine. Because of the rapid increase in the prevalence of obesity worldwide and the lack of efficacy of current medical therapies,1 efforts to develop novel pharmacological therapies for obesity have intensified.

One potential therapeutic strategy involves inhibiting triglyceride synthesis. Although triglycerides are essential for normal physiology, excess triglyceride accumulation results in obesity and, particularly when it occurs in nonadipose tissues, is associated with insulin resistance. However, until recently it had been unclear whether disrupting triglyceride synthesis would be feasible or would instead cause significant adverse consequences. In the past several years, pharmacological inhibition of acyl coenzyme A (CoA):diacylglycerol acyltransferase 1 (DGAT1) has emerged as a potential strategy for inhibiting triglyceride synthesis. Here, we review recent findings from studies of mice lacking DGAT1 that are pertinent to the prospects of DGAT1 as a pharmaceutical target.

DGAT Enzymes and Triglyceride Synthesis
The cloning of genes encoding DGAT enzymes provided important molecular tools to examine the relationship between triglyceride synthesis and obesity. The DGAT1 gene was identified from its homology to acyl CoA:cholesterol acyltransferase genes2,3 and was shown to encode an enzyme with DGAT activity.2 The DGAT2 gene was identified by...
DGAT2-deficient (Dgat2−/−) mice are smaller than wild-type controls and die within hours after birth. Carcass triglyceride content is severely reduced (~90%), and the mice therefore lack substrates for oxidative metabolism. They also lack essential fatty acids, which results in abnormalities in skin lipids and impaired epidermal barrier function. As a result, newborn Dgat2−/− mice rapidly become dehydrated, which hastens their demise. These findings indicate an essential role for DGAT2 in triglyceride metabolism. In a mixed genetic background, Dgat2−/− mice are not protected from diet-induced obesity (S. Stone and R. Farese, unpublished observations), suggesting that a 50% reduction in DGAT2 expression is not limiting for triglyceride synthesis. Thus, based on the available data in mice, a strategy to inhibit DGAT2 as an anti-obesity therapy may face the obstacle of a narrow therapeutic window, with too little inhibition being ineffective and too much causing severe side effects. Further studies are required to clarify the role of DGAT2 in triglyceride metabolism in specific tissues.

Obesity Resistance and Increased Insulin Sensitivity in DGAT1-Deficient Mice

In contrast to Dgat2−/− mice, DGAT1-deficient (Dgat1−/−) mice are viable and have more modest reductions in tissue triglycerides. Adult Dgat1−/− mice have ~50% less adipose mass and smaller adipocytes than wild-type mice on a chow diet. Although tissue triglyceride levels are reduced by ~50% in Dgat1−/− mice, levels of diacylglycerol and fatty acyl CoA, substrates of the DGAT reaction, are not significantly elevated and, in fact, tended to be lower in skeletal muscle and livers of Dgat1−/− mice (and unpublished data). Despite the absence of DGAT1, serum triglyceride levels are normal in Dgat1−/− mice of a mixed genetic background. This suggests that DGAT1 does not play a rate-limiting role in hepatic secretion of triglycerides. Alternatively, it is possible that compensatory mechanisms offset a reduction in DGAT1-mediated hepatic triglyceride secretion and are able to maintain normal serum triglyceride levels.

When fed a high-fat diet, Dgat1−/− mice are resistant to weight gain, and inbred DGAT1-heterozygous (Dgat1+/−) mice have an intermediate phenotype (Figure 2). Dgat1+/− mice are also protected from diet-induced hepatic steatosis. Reductions in fat pad mass and tissue triglyceride levels most likely account for the differences in body weight, because DGAT1 deficiency does not affect lean body mass.

Correlating with the decrease in adiposity, insulin sensitivity is increased in Dgat1−/− mice, as shown by an increase in glucose infusion rate during hyperinsulinemic–euglycemic clamp studies. Early Postnatal Lethality in DGAT2-Deficient Mice

To better understand the physiological functions of the enzymes, we inactivated the DGAT genes in mice. Newborn Dgat2−/− mice have an intermediate phenotype (Figure 2). Adult Dgat1−/− mice have ~50% less adipose mass and smaller adipocytes than wild-type mice on a chow diet.

The absence of DGAT1, serum triglyceride levels are normal in Dgat1−/− mice of a mixed genetic background. This suggests that DGAT1 does not play a rate-limiting role in hepatic secretion of triglycerides. Alternatively, it is possible that compensatory mechanisms offset a reduction in DGAT1-mediated hepatic triglyceride secretion and are able to maintain normal serum triglyceride levels.

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**Figure 1.** Triglycerides synthesis and DGAT enzymes. Triglycerides (triacylglycerols) are the end product of a multistep pathway. The final reaction is catalyzed by 1 of 2 known DGAT enzymes, DGAT1 and DGAT2. GPAT indicates glycerol-3-phosphate acyltransferase; AGPAT, acylglycerol-1-phosphate acyltransferase; PPH-1, phosphatidic acid phosphohydrolase-1; MGAT, acyl CoA:monoacylglycerol acyltransferase.

**Figure 2.** Effects of partial DGAT1 deficiency on body weight and adipose tissue in male mice fed a high-fat diet (21% fat and 0.15% cholesterol). A, Growth curves (n=5 to 7 per genotype). P<0.05 for Dgat1+/− vs Dgat1−/−, Dgat1+/− vs Dgat1−/−, and Dgat1−/− vs Dgat1+/−. B, Total fat pad content after 18 weeks of high-fat diet (n=4 to 5 per genotype). C, Adipocyte size (n=2 per genotype). Adipocyte size is determined by computer imaging analysis as described. Values are means±SD.
clamp studies. Insulin-stimulated glucose transport is increased in the skeletal muscle and white adipose tissue (WAT) of Dgat1⁻/₋ mice, and insulin-stimulated activities of phosphatidylinositol-3 kinase, protein kinase B, and protein kinase C, 3 key molecules in the insulin signaling pathway, are also increased in the skeletal muscle of Dgat1⁻/₋ mice (Figure 3). Decreased levels of serine-phosphorylated insulin receptor substrate-1, a molecule implicated in insulin resistance, were observed in the skeletal muscle and WAT.

Partial DGAT1 deficiency appears to increase insulin sensitivity as well, as demonstrated by decreased blood glucose levels after intraperitoneal injections of insulin in Dgat1⁻/₋ mice (Figure 4). Although it is not surprising that the leanness in DGAT1-deficient mice correlates with increased insulin sensitivity, the precise molecular mechanisms that promote insulin sensitivity in lean conditions remain unclear.

Increased Energy Expenditure and Food Intake in DGAT1-Deficient Mice

The increased adiposity and resistance to diet-induced obesity in Dgat1⁻/₋ mice result from an increase in total energy expenditure. The increased energy expenditure is attributable to at least 2 mechanisms—increased ambulatory physical activity on a chow (unpublished observations) or high-fat diet and an increase in the expression of uncoupling protein 1, a major mediator of nonshivering thermogenesis in rodents. Interestingly, Dgat1⁻/₋ mice eat more than wild-type mice, and the hyperphagia is more pronounced during exposure to cold. These results suggest that the increased food intake in Dgat1⁻/₋ mice is a secondary phenomenon and compensates for the increased energy expenditure.

Increased Leptin Sensitivity in DGAT1-Deficient Mice

Another possible contributing mechanism to the increased energy expenditure in Dgat1⁻/₋ mice is increased leptin sensitivity. Dgat1⁻/₋ mice lose more weight than wild-type mice in response to subcutaneous leptin infusion, consistent with increased leptin sensitivity. Although leptin acts primarily in the central nervous system, we did not observe differences in the expression of leptin-regulated genes in the hypothalamus of Dgat1⁻/₋ mice, either at baseline or after leptin treatment. These observations suggest an alternative mechanism of enhanced leptin sensitivity in Dgat1⁻/₋ mice. Interestingly, DGAT1 deficiency protects against obesity and insulin resistance in Agouti yellow mice, a model characterized by leptin resistance, but has no apparent effect in leptin-deficient ob/ob mice. Thus, the effects of DGAT1 deficiency on energy and glucose metabolism appear to require an intact leptin pathway.

Altered Endocrine Function of DGAT1-Deficient WAT

Although how DGAT1 deficiency increases energy expenditure remains incompletely understood, one contributing mechanism is the altered endocrine function of WAT in Dgat1⁻/₋ mice. Changes in Dgat1 expression alter adipocyte size in mice, and alterations in adipocyte size are known to correlate with changes in the endocrine function of WAT. DGAT1 deficiency affects the expression and secretion of several adipocyte-derived factors that modulate energy and glucose metabolism.

To test the hypothesis that DGAT1 deficiency in WAT affects its endocrine function, we transplanted WAT from Dgat1⁻/₋ mice into wild-type recipient mice and assessed its effects on glucose disposal and the response to a high-fat diet.
DGAT1 deficiency alters the endocrine function of the WAT.16,17 In some conditions, adiponectin expression is increased in Dgat1−/− mice.15,16,17 One candidate factor is adiponectin, a WAT-derived hormone that alters the levels of secreted factors, specifically increasing the levels of a factor or factors that promote energy expenditure and enhance insulin sensitivity. An intact leptin pathway appears to be required for these effects. This model does not exclude the possibility that DGAT1 deficiency in other tissues may also contribute to the obesity resistance and insulin sensitivity in Dgat1−/− mice.

Remarkably, transplantation of Dgat1−/− WAT conferred partial obesity resistance, increased insulin-stimulated glucose disposal, and enhanced activation of the insulin signaling pathway in the recipient mice.10,15 In contrast, transplantation of Dgat1+/− WAT into Dgat1−/− mice did not adversely affect their resistance to diet-induced obesity or impair their enhanced response to insulin.15 These results provide strong evidence that the altered endocrine function of Dgat1−/− WAT has beneficial effects on systemic energy and glucose metabolism. Moreover, they suggest that these effects result from increased secretion of adipocyte-derived factors by Dgat1−/− WAT (Figure 5).

It will be of considerable interest to better understand how DGAT1 deficiency alters the endocrine function of the WAT and to determine which factors contribute to the increased energy expenditure and insulin sensitivity in Dgat1−/− mice. One candidate factor is adiponectin, a WAT-derived hormone that enhances insulin sensitivity and increases fatty acid oxidation.16,17 In some conditions, adiponectin expression is increased in Dgat1−/− WAT. For example, adiponectin mRNA expression in WAT is increased 2-fold in Dgat1−/− mice fed a high-fat diet,15 and in obese models, Dgat1−/− WAT produces more adiponectin than an equivalent amount of Dgat1+/− WAT.15 However, adiponectin expression is not increased in chow-fed Dgat1−/− mice (unpublished observations). Studies to determine the contribution of adiponectin to the DGAT1 deficiency phenotype are needed.

Additional Metabolic Effects of DGAT1 Deficiency

Consistent with the broad expression pattern of the enzyme, DGAT1 deficiency affects the biology of several other organs, including the small intestine, skin, and mammary gland. Because DGAT1 is expressed highly in the small intestine, DGAT1 deficiency affects the handling of fat when dietary fat content is high. Although DGAT1 deficiency does not cause steatorrhea, and although the quantitative absorption of dietary fat is normal in Dgat1−/− mice,8 fat absorption appears to be delayed in the enterocytes of Dgat1−/− mice after either acute or chronic feeding of fat-rich food.18 The physiological consequences of delayed lipid absorption in the Dgat1−/− small intestine remain to be elucidated.

Complete DGAT1 deficiency also results in several skin abnormalities. Dgat1−/− mice have dry fur and hair loss develops after puberty.19 These findings, more prominent in male mice, are associated with atrophic sebaceous glands and the absence of wax diesters, a major species of fur lipids in rodents. As a result, water repulsion and thermoregulation after water immersion are impaired in Dgat1−/− mice.19 These abnormalities are not apparent in Dgat1+/− mice, suggesting that the effects of DGAT1 deficiency on the skin require a complete absence of the enzyme.

Complete DGAT1 deficiency also interferes with mammary gland physiology. Postpartum Dgat1−/− mice are unable to nurse their offspring because of an absence of milk production,8 and this defect is associated with impaired development of the mammary epithelium in the mammary glands.20 Although the precise mechanism for the impaired mammary gland development remains unknown, the absence of DGAT1 in the stromal tissues (possibly adipocytes) appears to contribute to this aspect of the DGAT1 deficiency phenotype.20 Female Dgat1−/− mice, in contrast, have no apparent defects in lactation.

From Mice to Humans: Clinical and Pharmaceutical Implications

Because the function and expression patterns of DGAT1 are similar in mice and humans, findings related to its physiological functions in mice should be pertinent to humans. However, this remains formally untested because humans with DGAT1 mutations that result in a nonfunctional protein have not been identified. A common polymorphism in the DGAT1 promoter region that modestly affects promoter activity has been identified,21 and variation at this polymorphic site correlates with alterations in body mass index in a population of Turkish women.21 However, no such effect was apparent in Turkish men22 or in a French population.23 Thus, it remains unclear whether alterations in DGAT1 expression caused by genetic variation affect body weight in humans.

Studies in Dgat1−/− mice, however, suggest that pharmacological inhibition of DGAT1 may be a useful strategy for treating human obesity and type 2 diabetes. As an enzyme, DGAT1 is a suitable target for a small-molecule inhibitor. In addition, findings in Dgat1−/− mice suggest that it may be possible to adequately inhibit the enzyme for the treatment of obesity and diabetes without causing untoward side effects. Although there are no available synthetic inhibitors of DGAT1, several naturally occurring compounds have been reported to inhibit DGAT activity in vitro.23–28 To date, no studies to our knowledge have reported the effects of these DGAT inhibitors on energy and glucose metabolism in animal models. It is also unclear whether these compounds specifically inhibit DGAT1 or also antagonize the activities of DGAT2. It should be noted that numerous synthetic inhibitors have been generated for acyl CoA:cholesterol acyltransferase enzymes,29 which are DGAT1 homologues.
DGAT1 inhibition could be considered for treating other disorders. Although serum triglyceride levels are not decreased in Dgat1−/− mice, it remains possible that DGAT1 inhibitors could lower serum triglyceride levels in humans, particularly because DGAT1 appears to be expressed at higher levels in human liver than in mouse liver. Alternatively, DGAT1 inhibitors may limit hepatic triglyceride accumulation and may be used to treat nonalcoholic hepatic steatosis. In addition, the effects of DGAT1 deficiency on sebaceous glands in mice suggest that topical DGAT1 inhibition may be beneficial for acne vulgaris.20

Despite the promising findings in Dgat1−/− mice, enthusiasm for the potential benefits of DGAT1 inhibition should be tempered by the possibility that pharmacological inhibition of an enzyme may not produce the same metabolic effects as its genetic inactivation. More importantly, what works in rodents might not necessarily work in humans. This is especially relevant to DGAT1 inhibition, because the mechanism of obesity resistance in Dgat1−/− mice involves increased expression of uncoupling protein 1 in brown adipose tissue. Because nonshivering thermogenesis through uncoupling plays a relatively minor role in the energy expenditure of adult humans, it remains to be seen whether inhibition of DGAT1 would produce robust decreases in adiposity or body weight clinically. Nonetheless, results in Dgat1−/− mice appear to justify the identification and development of DGAT1 inhibitors. Additionally, further study of the mechanisms underlying the increased energy expenditure and increased sensitivity to insulin and leptin in Dgat1−/− mice may identify other novel therapeutic targets.

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References
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