Acyl-CoA:Diacylglycerol Acyltransferase: A Key Player in the Regulation of Fatty Acid and Glucose Metabolism in Mammalian Systems

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ABSTRACT

Acyl-CoA:diacylglycerol acyltransferase (DGAT) is a critical enzyme involved in the last committed step of triglyceride synthesis. DGAT has two known isoforms that are expressed in various tissues being crucial to energy homeostasis in mammals. DGAT inhibits the accumulation of diacylglycerol, a lipid thought to promote insulin resistance, by incorporating them into triglyceride. However, both deletion and overexpression studies targeting DGAT have resulted in decreased diacylglycerol content and caused increased insulin sensitivity. Studies analyzing obese models though have noticed a positive correlation between DGAT and obesity. Thus, it remains elucidated whether DGAT plays a causal role in the development of diet-related diseases or provides protection by preventing accumulation of diacylglycerol. The purpose of this review is to present an overview of DGAT, state factors that may influence its expression, explain the potential consequences of DGAT overexpression or deficiency, and discuss its relative importance to glucose metabolism and energy storage.

Keywords: Athlete’s paradox; de novo lipogenesis; hepatic steatosis; intramyocellular triglycerides; lipotoxicity; stearoyl-CoA desaturase.

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1. INTRODUCTION

Acyl-CoA:diacylglycerol acyltransferase (DGAT) is known as a transmembrane enzyme in eukaryotes that is involved in the last step towards triglyceride (TG) synthesis. There are currently two known isoforms of DGAT (DGAT1 and DGAT2) which are considered nearly ubiquitous in eukaryotic species [1]. DGAT1 and DGAT2 are actually structurally unrelated enzymes with two different genes coding for their respective DGAT isozyme [2,3]. DGAT2 is encoded by the diacylglycerol acyltransferase gene family while DGAT1 is coded by the acyl-CoA:cholesterol acyltransferase (ACAT) gene family [4]. High expression of DGAT1 is present in skin, mammary glands, small intestines, testes, and adipose tissue [5]. DGAT2 has shown to be highly expressed in liver and adipose tissue [3].

It appears that while at least one DGAT isoform is required for TG synthesis and lipid droplet formation in adipocytes, a single deficiency in either DGAT1 or DGAT2 does not inhibit lipid droplet formation or TG synthesis in these cells. Additionally, it is not mandatory for all mammalian cell types to need either DGAT isozyme function for lipid droplet formation [6]. However, while knocking out DGAT2 in vivo results in fatality [7], DGAT1 knockout (KO) mice display a lean phenotype with an increase in insulin sensitivity and leptin in addition to resistance to diet induced obesity and hepatic steatosis [8]. Authors from the latter reference indicate these traits in DGAT1 null mice may arise from TG synthesis being inhibited and alterations in adipocyte derived factors. This implies that manipulating DGAT1 may result in potential therapeutic benefits for obesity driven diseases.

2. VARIOUS EFFECTS FROM EXPRESSION OF DGAT ISOFORMS

Expression patterns of DGAT1 in mice and humans are similar in adipose tissue and skeletal muscle, but lower in the livers of mice [5]. Dissimilarities in metabolic regulation occur when TG accumulates in muscle when compared to adipose tissue. It is speculated that TG formation in adipocytes is a benign process [9] that may offer protection against metabolic syndrome [10]. Evidence has demonstrated that TG accumulation provides protection against lipotoxicity due to inhibiting saturated fatty acids to enter pathways that may lead to apoptosis and instead promoting saturated fats to be used for TG formation [11], as TG are considered an inert energy substrate [12]. However, TG accumulation in skeletal muscle is thought to promote insulin resistance [13, 14]. Some data has shown high levels of TG in muscle to be positively correlated with insulin sensitivity though [15].

DGAT activity is important to TG formation in skeletal muscle [2]. Both known isoforms appear to play a major role in the accumulation of intramyocellular triglyceride (IMTG) [6]. DGAT1 appears to be a more suitable isoform to study in transgenic mice though, as deleting DGAT2 in mice leads to early mortality [7]. There are currently two explanations why a DGAT2 deficiency is lethal. One, DGAT2 deficiency results in skin anomalies, causing severe dehydration via loss of water through the skin. Second, deletion of DGAT2 leads to impairments in metabolism from a marked decrease in energy substrates [7]. Interesting to note is that DGAT2 null mice are strikingly similar to edematous (ed) mutant mice and Stearoyl-CoA desaturase-2 (SCD-2) KO mice in some regards. SCD is a critical enzyme to fatty acid metabolism and biosynthesis, as it converts saturated fatty acids to monounsaturated fats [16]. Both DGAT2 KO mice and SCD-2 KO mice display analogous skin disturbances. Neonate mice with a SCD-2 deletion demonstrate a skin permeability barrier flaw which results in a high mortality rate soon after parturition. These results have made scientists conjecture that SCD-2 may provide DGAT2 with monounsaturated fatty
acids needed to maintain appropriate epidermal permeability and biosynthesis of lipids when skin is at the early developmental stage [17]. The oed mutant mice, a spontaneous mouse line now considered extinct [2], presented itself with pleiotropic effects that including altered lipid metabolism, hepatic TG deficiencies, brittle and shiny skin, hemorrhaging, edema, leukocytosis, and expired a few hours following birth [18]. Unfortunately, it was never determined if the mutation in oed mice was due to DGAT2 or a comparable protein [2].

Advances in biotechnology have resulted in developing DGAT1 KO and overexpression rodent models that have significantly contributed to better understanding lipid synthesis and TG formation. The DGAT1 KO and SCD-1 KO mouse models display similar characteristics. These include protection from diet induced obesity, increased energy expenditure and insulin sensitivity, hair loss, sebaceous gland abnormalities, and cold temperature intolerance, with cold environmental conditions resulting in glycogen depletion, hypoglycemia, and hypothermia [2, 19-23]. Such striking similarities between these two KO mice may make it plausible to consider a potential interaction between these two enzymes, but unlike the colocalization present between SCD-1 and DGAT2, no interaction has yet to be discovered for DGAT1 and SCD-1 [2].

A study using DGAT1 KO mice showed that they have a reduced content of ectopic fat after high fat feeding, including liver and skeletal muscle, when compared to wild type (WT) mice. Additionally, diacylglycerol (DAG) content was not increased in liver, adipose tissue, or skeletal muscle of DGAT1 deficient animals when fed either chow or a high fat diet [24]. A decrease in ceramides in cardiomyocytes has also been observed in DGAT1 null mice as well [25]. This decrease in ceramides and DAG is in contrast to what is assumed to occur following DGAT1 deficiency, as increased formation of DAG and ceramides would instead be expected, triggering lipotoxicity [26]. Since DGAT1 deletion increases energy expenditure [22], this may at least partially attribute to DAG and ceramides not being elevated in DGAT1 KO mice.

Cell culture experiments employing myocytes have given researchers a better understanding of DGAT activity in skeletal muscle under isolated conditions. Overexpression of DGAT1 in C2C12 myocytes resulted in increases in DGAT activity and IMTG corresponding with lowered DAG levels [27]. In the same study, ex vivo experimentations demonstrated that insulin stimulation resulted in the highest glucose uptake in muscle creatine kinase (MCK)-DGAT1 soleus muscle (a transgenic mouse model that exclusively overexpresses DGAT1 in myocytes), followed by WT, then DGAT1 KO. Moreover, two hours of 0.375 mM palmitic acid and 0.375 mM oleic acid preincubation caused insulin resistance in all these groups, but was least profound in the MCK-DGAT1 group. Additionally, MCK-DGAT1 soleus muscle displayed the most favorable alterations in TG, DAG, and ceramide concentrations under preincubated fatty acid conditions [27]. Another study analyzed neonatal rat cardiomyocytes after a transient transfection of a CMV-FLAG-tagged DGAT1 expression vector. DGAT1 overexpression in these cells stimulated an increase in lipid droplets after exposure to palmitic acid [28].

A study utilizing both skeletal muscle and cardiac myocytes reveal interesting data when inhibiting DGAT1. C2C12 myocytes and AC16 human cardiomyocytes were allowed to become confluent and formed into myotubes for experiments involving a DGAT1 inhibitor and palmitic acid exposure [25]. Both cell culture models were analyzed after palmitic acid treatment, DGAT inhibitor treatment, and DGAT inhibitor plus palmitic acid treatment, along with control myocytes. Inhibiting DGAT1 led to decreased expression of PPARs and their target genes involved in fatty acid oxidation of both types of cells. Furthermore, exposure to
the DGAT1 inhibitor prevented increased expression of PPARs and their respective target genes in both cell types when treated with 0.5 mM of palmitic acid [25].

As stated previously, DGAT1 deletion protects mice against obesity. There are likely multiple mechanisms that result in obesity resistance observed in DGAT1 KO mice. A DGAT1 deficiency leads to lean mice that have reductions in TG accumulation and adiposity corresponding with increases in activity and energy expenditure. These effects appear independent to changes in dietary caloric intake [29]. DGAT1 null mice also display insulin and leptin sensitivity. This happens despite DGAT1 KO mice being fed a high fat diet [24]. It has been suggested that leptin function is mandatory for the positive effects upon metabolism to occur in DGAT1 KO mice [30], as DGAT1 KOleptin resistant mice are still protected against obesity and insulin resistance, while ob/ob mice are not [24]. A positive response to peripheral leptin infusions does occur however when given to ob/ob DGAT1 KO mice [30]. Another possible explanation of leptin resistant DGAT1 KO mice being protected against insulin resistance and obesity is that leptin lowers DGAT2 expression, as increased expression of DGAT2 has been observed in DGAT1 deficient ob/ob mice [24].

It is not entirely understood why a DGAT1 deletion increases insulin sensitivity. Conclusions from a study involving fat pad transplantation led researchers to speculate that a marked increase in adiponectin expression from adipose tissue of DGAT1 deficient mice may contribute to insulin sensitivity [31]. However, a more recent study has questioned if adiponectin is required for insulin sensitivity to occur during DGAT1 deletion, as mice lacking both adiponectin and DGAT1 do not develop hepatic steatosis, obesity, and glucose intolerance from high fat feedings [32]. Another plausible mechanism is positive alterations in insulin signaling. A study revealed an increase of insulin stimulated glucose transport in adipose and muscle tissue of DGAT 1KO mice. Increases in PKC-λ, Akt, and phosphatidylinositol 3-kinase corresponding with a decrease in serine insulin receptor substrate-1 (IRS-1) were associated with insulin stimulated glucose transport in these KO mice [33].

The positive health attributes connected to DGAT1 deletion in mice have resulted in scientists suggesting the use of DGAT1 inhibitors for the treatment of insulin and leptin resistance in humans that is associated with obesity [24]. Interestingly, both natural and synthetic inhibitors of DGAT have been described [34]. Data from studies employing DGAT1 inhibitors in mice and human in vitro appear promising [35, 36]. Human in vivo published research is scarce though and unfortunately nausea and vomiting were common side effects when given a DGAT1 inhibitor orally [37]. There are currently clinical trials being conducted in humans using DGAT1 inhibitors though [38]. Scientists speculate that DGAT1 inhibitors may become beneficial for people suffering from non-alcoholic fatty liver disease [36].

Results with DGAT1 overexpression specifically in muscle have been particularly intriguing. Data with MCK-DGAT1 mice have shown a higher rate of fatty acid oxidation coinciding with increased TG production and storage compared to WT mice. Moreover, MCK-DGAT1 mice exhibit higher energy expenditures and lower bodyweights ingesting a high fat diet compared to WT littermates while ingesting the same amount of food. However, while MCK-DGAT1 mice were protected from lipotoxicity and insulin resistance within skeletal muscle on a high fat diet, insulin resistance and lipotoxicity still occurred in hepatic tissue. MCK-DGAT1 mice however still displayed better systemic insulin sensitivity after high fat feedings compared to WT controls; even with hepatic insulin resistance and lipotoxicity present [39].
MCK-DGAT1 mice appear to be protected from skeletal muscle insulin resistance even though of the increased lipogenic effect displayed in this tissue. Observations with MCK-DGAT1 mice have presented evidence that these animals may mimic the athlete's paradox [27]. MCK-DGAT1 mice demonstrate the unique ability to be protected against insulin resistance that results from high fat feedings. This protection may be due to diminishing the activation of the stress inflammatory c-Jun N-terminal kinase 1 (JNK1) pathway and mitigating stimulation of PKCs, which are known to be activated by DAG [27]. Positively influencing insulin signaling may allow better insulin sensitivity in these mice as well. Increased GLUT4 membrane translocation and Akt activation concomitant with decreases in IRS-1 serine phosphorylation have been noted in these transgenic mice [27].

Skeletal muscle is universally recognized as the largest pool for stored glycogen in the human body [40], making it a valuable tissue to glucose metabolism. While the maximum amount of glycogen content is tightly controlled based on amount of muscle mass, the capacity of skeletal muscle to store TG is highly variable [41]. Skeletal muscle lipids are generally associated with insulin resistance [42, 43]. However, metabolic derangements that can arise from increased lipids in skeletal muscle may not be due to a high IMTG content. Instead, high levels of DAG and free fatty acids (FFA) may contribute to certain defects in insulin signaling which may result in insulin resistance [44-46]. Therefore, DGAT may provide protection against insulin resistance by assisting in the esterification of DAG into TG. DGAT may also protect against insulin resistance by preventing the formation of deleterious fatty acid derivatives, such as ceramides, by shuttling palmitic acid into TG esterification [27].

While DGAT1 overexpression specifically in skeletal muscle may appear to benefit that tissue, overexpressing DGAT1 in cardiac muscle seems more controversial. Evidence has revealed that in transgenic mice selectively overexpressing DGAT1 in cardiac myocytes, cardiomyopathy, diastolic then systolic dysfunction, increased TG content, and cardiac fibrosis occurs [28]. Conversely, another study demonstrated that DGAT1 overexpression solely in cardiac tissue resulted in decreases in heart ceramides, DAG, and FFA with double the amount of TG, while cardiac function remained unchanged. Moreover, cardiac function improved when DGAT1 was overexpressed in mice that are prone to lipotoxic cardiomyopathy. Interestingly, researchers from this study demonstrated DGAT1 overexpression in the heart is comparable to what occurs in endurance athletes, as similarities in FVB mice undergoing an intense two week swimming protocol were observed when compared to cardiac DGAT1 overexpression mice [47]. It is also possible that DGAT1 deficiency may be able to benefit the heart. DGAT1 KO mice have increased glucose uptake, lower ceramides and DAG, reduced fat oxidation, and normal cardiac function [25]. An intriguing point to note is that overexpression and deleting DGAT1 in the heart have both resulted in a larger heart size compared to controls [25, 28].

3. FACTORS INFLUENCING DGAT

DGAT may be influenced by a variety of factors in different tissues. In adipocytes, insulin increases DGAT2 expression and glucose increases DGAT1 expression, while treatment with both agents display an additive effective upon DGAT [48]. DGAT activity in hepatocytes increases after a 72 hour incubation period with a 0.5 mM concentration of saturated fatty acids, monounsaturated fatty acids, or polyunsaturated fatty acids [49]. Increases in DGAT also occur in the liver during fasting-refeeding [50]. When specifically analyzing DGAT isoforms in C57BL/6 mice however, DGAT2 mRNA hepatic expression measured by northern blotting decreased with fasting and increased with refeeding, while DGAT1 failed to respond to fasting-refeeding. The adipose tissue in these mice followed a similar pattern as
the liver in response to fasting-refeeding [48]. Another distinguishing attribute to the two known DGAT isoforms are contrasting effects in the adipose tissue of obese and diabetic mouse models. In \( ob/ob \) and insulin receptor substrate-2 (IRS-2) KO mice, a decrease in DGAT1 concomitant with an increase in DGAT2 within the adipose tissue has been observed when fed standard chow. Additionally, high fat feedings caused the same result in C57BL/6 mice [51]. Counterregulatory hormones appear to lower DGAT enzymatic activity. Glucagon reduces DGAT levels in isolated rat hepatocytes [52] while epinephrine reduces the activity of DGAT in isolated rat adipocytes [53] and isolated rat cardiomyocytes [54]. The DGAT2 isoform appears to be influenced by leptin, as increased DGAT2 expression has been observed in the small intestines, adipose tissue, and skeletal muscle in \( db/db \) mice [55]. This may imply DGAT2 expression being suppressed by leptin [2].

DGAT appears to be modulated by covalent modification. High levels of ATP have been shown to result in DGAT inactivation via phosphorylation [56]. Unfortunately, there is no conclusive evidence which DGAT isoform(s) are controlled by covalent modification and which exact DGAT residues may be dephosphorylated or phosphorylated to modify activation or inactivation. DGAT may also be regulated allosterically, too. A corresponding increase with DGAT activity from fatty acid supplementation in hepatocytes during incubation has been observed when an equivalent amount of oleic acid and palmitic acid were incorporated into the cell media [57].

Physical activity can have a dramatic impact upon increasing DGAT in skeletal muscle. Exercise is alleged to promote increases in DGAT1, which results in higher levels of TG coinciding with decreases in lipotoxic substrates, preventing insulin resistance [27]. A single bout of endurance exercise has shown to significantly increase both IMTG and protein DGAT1 levels, while decreasing DAG and ceramides, plus prevent fatty acid induced insulin resistance in humans [58]. Mice performing an intensive one week swimming protocol displayed increases in muscle DGAT activity, DGAT1 expression, and IMTG with a consequent reduction in DAG and ceramide concentrations in the soleus when compared to sedentary controls [27]. A study that assessed exercise trained mice to sedentary MCK-DGAT1 mice noted many similarities that included increased TG, fatty acid oxidation, and insulin sensitivity [39]. Researchers have speculated that overexpression of DGAT1 in muscle mimics the athlete’s paradox and indicate exercise induced DGAT activation may be a factor to the development of this condition [27].

Physical activity may increase DGAT in specific tissues only. In particular, exercise may only increase DGAT in type I oxidative muscle fibers. Male Wistar rats performing a progressive six week running protocol had increases in both DGAT1 and DGAT2 mRNA in the soleus, but not in the extensor digitorum longus (EDL) [59]. Overexpression of DGAT1 via biotechnological methods however may not distinguish between amplification of DGAT1 in various fiber types, as in vivo DNA electroporation of DGAT1 in the tibialis anterior of male Wistar rats resulted in DGAT1 upregulation and an increase of IMTG in both oxidative and glycolytic fibers [60].

4. DGAT: HELPFUL AND HARMFUL?

Scientists have studied whether increasing TG solely in adipocytes is actually innoxious. A study conducted with transgenic mice overexpressing DGAT1 exclusively in adipose tissue noted larger fat pads and adipocytes compared to WT mice when both mice were fed chow. When these transgenic mice were fed a high fat diet though, obesity occurred in the absence of insulin resistance, as determined by insulin and glucose tolerance tests. This led
researchers from the study to assume that the accretion of TG in insulin sensitive tissues other than adipose results in insulin resistance [61]. In contrast, FVB mice overexpressing DGAT1 in adipocytes do not become obese after chronic high fat feeding, but instead develop several health abnormalities, including insulin and leptin resistance, hyperglycemia, hyperinsulinemia, hepatic steatosis, and glucose intolerance. Scientists from this study speculated that DGAT1 overexpression in the adipose tissue of FVB mice fed a high fat diet results in an excessive circulation of FFA coming from adipocytes via increased lipolysis which causes a large FFA flux to be delivered to the liver that triggers hepatic dysfunction [62]. Conflicting results of these two studies may be due to the fact that there is evidence to support FVB mice being resistant to diet induced obesity [63].

While studies directly assessing potential factors that may influence DGAT specifically in skeletal muscle are scarce, evidence demonstrates DGAT1 gene expression [27] and protein content [58] can be increased by exercise. Since DGAT1 expression in muscle appears to be important to TG formation [60], identifying other factors that may influence its expression in this particular tissue may allow a better understanding of the importance of lipogenesis in skeletal myocytes. Lipogenesis in ectopic tissues is believed to be associated with health abnormalities [64-66]. Furthermore, high levels of IMTG are linked to insulin resistance in rats [67] and humans [68, 69]. However, the mechanisms of action relating to IMTG being positively correlated with insulin resistance are not completely understood, as this correlation also appears related to obesity [70] and a sedentary lifestyle [71]. Conversely, a high IMTG resulting from exercise has been associated with insulin sensitivity [72].

Raising IMTG specifically in glycolytic muscle may be deleterious [73]. A transgenic mouse created to overexpress DGAT2 at the MCK promoter has produced interesting results. As expected, mRNA DGAT2 levels in MCK-DGAT2 mice were markedly higher in both the soleus and gastrocnemius when compared to controls. Surprisingly though, when the skeletal muscles of transgenic and controls were separated by isolating the oxidative and glycolytic fibers, DGAT2 mRNA was only significantly higher in type II tissues of MCK-DGAT2 mice. WT mice had much higher mRNA expression in oxidative muscle though in relation to its mRNA levels within glycolytic tissue. MCK-DGAT2 mice presented with high accumulation of TG and ceramides. Glucose homeostasis in these mice was dysregulated as well. Impaired glucose uptake and insulin signaling was observed in type II muscle of MCK-DGAT2 mice. Additionally, insulin resistance and impaired glucose tolerance was observed in these transgenic mice [73]. This data is much different in comparison to the beneficial effects detected when DGAT1 is overexpressed at the MCK promoter. Therefore, it may be plausible to assume that overexpression of different DGAT isoforms in certain tissues may produce contrasting results.

Endurance athletes exhibit a high content of IMTG in type I muscle, which they use very efficiently during aerobic events, whereas IMTG in glycolytic muscle remains unchanged [74]. Obese individuals have also shown to carry an appreciable amount of IMTG in slow-twitch muscle, and when compared to lean populations, present with higher IMTG in both fibers types [75]. Oxidative muscle appears to be more insulin sensitive compared to glycolytic muscle [76]. Furthermore, diabetic and obese subjects seem to have a higher proportion of type II skeletal muscle than non-obese individuals [77, 78]. Diabetics and obese individuals have also demonstrated lowered fatty acid oxidative capabilities combined with increases in glycolytic enzymatic activities [79, 80]. There has been an inverse correlation observed in pertinence to an increase in IMTG from individuals who are obese
and/or suffering from type 2 diabetes mellitus that follows reduced oxidative capacity in all skeletal muscle fiber types [81].

Type I fibers prefer to utilize beta-oxidation for energy, while type II fibers rely more on glucose. It is plausible to assume that a large, local reservoir of TG located in the type I oxidative muscle of endurance athletes may be beneficial, while fatty acids contained in type II glycolytic muscles may hinder appropriate metabolic activity, such as using glycolysis effectively. Therefore, high activity of DGAT in oxidative skeletal muscle may be advantageous while high activity in glycolytic tissue may be considered detrimental. Future studies to examine this should be conducted to determine whether increased DGAT activity is beneficial only in specific skeletal muscle fibers, and if so, why and which isoforms are beneficial or harmful. Additionally, experiments performed utilizing dietary components and other hormonal factors to better understand what influences skeletal muscle DGAT activity, gene, and protein expression in vitro and in vivo need to be carried out. This type of information will give researchers more specific knowledge in regards to the overall importance of DGAT in skeletal muscle and how it may influence lipid metabolism and glucose homeostasis in myocytes and systemically.

5. CONCLUDING REMARKS

DGAT is a unique enzyme that plays an essential role in lipid metabolism. Research has also shown that DGAT largely influences glucose homeostasis and energy expenditure in mammals. At present time, it is difficult to discern if overexpression or inhibition of DGAT is ultimately beneficial or deleterious, but it is likely that different cells respond in various (and possibly contrasting) ways when DGAT activity is changed, either tissue-specifically or systemically. Further, it is highly probable that the condition of the organism (i.e. lean vs obese; sedentary vs athlete) is decisive in whether DGAT expression is advantageous in certain tissues or not. Therefore, future studies should be conducted to analyze the tissue-specific mechanistic action of DGAT in different diabetic and obese models and determine which tissues are the most appropriate to target via DGAT manipulation for the treatment of diabetes and obesity.

COMPETING INTERESTS

The author has declared that no competing interests exist.

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