Improved glucose tolerance in acyl CoA:diacylglycerol acyltransferase 1-null mice is dependent on diet

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Abstract

Background: Mice that lack acyl CoA:diacylglycerol acyltransferase (Dgat1−/− mice) are reported to have a reduced body fat content and improved glucose tolerance and insulin sensitivity. Studies so far have focussed on male null mice fed a high fat diet and there are few data on heterozygotes. We compared male and female Dgat1−/−, Dgat1+/− and Dgat1+/+ C57Bl/6 mice fed on either standard chow or a high fat diet.

Results: Body fat content was lower in the Dgat1−/− than the Dgat1+/+ mice in both experiments; lean body mass was higher in male Dgat1−/− than Dgat1+/+ mice fed on the high fat diet. Energy intake and expenditure were higher in male Dgat1−/− than Dgat1+/+ mice; these differences were less marked or absent in females. The body fat content of female Dgat1+/− mice was intermediate between that of Dgat1−/− and Dgat1+/+ mice, whereas male Dgat1+/− mice were similar to or fatter than Dgat1−/− mice. Glucose tolerance was improved and plasma insulin reduced in Dgat1−/− mice fed on the high fat diet, but not on the chow diet. Both male and female Dgat1+/− mice had similar glucose tolerance to Dgat1+/+ mice.

Conclusion: These results suggest that although ablation of DGAT1 improves glucose tolerance by preventing obesity in mice fed on a high fat diet, it does not improve glucose tolerance in mice fed on a low fat diet.

Background

The final step of triglyceride synthesis in mammals is catalysed by the enzymes acyl CoA:diacylglycerol acyltransferase 1 and 2 (DGAT1 and DGAT2), which have dissimilar amino acid sequences. Both enzymes are widely expressed and are highly expressed in adipose tissue and liver [1]. Previous reports have described resistance to diet-induced obesity [2] and amelioration of obesity due to the A/+, but not the Lep+/+ or LepR+/+, mutation [3] in DGAT1-deficient (Dgat1−/−) mice. Resistance to obesity was associated with increased reductions in body weight and food intake in response to peripherally but not centrally administered leptin [3,4]. Food intake relative to body weight was either unchanged [2] or raised [3,4], and energy expenditure relative to body weight was raised [2], suggesting that the primary effect of DGAT1 deficiency is...
on the energy expenditure side of the energy balance equation. Increased locomotor activity and brown adipose tissue thermogenesis have been invoked to account for increased energy expenditure [2,3,5]. It appears, however, that fat oxidation is increased in liver as well as skeletal muscle and brown adipose tissue because the concentration of diacylglycerol was reduced in the liver of Dgat1+/− mice [3]. This finding is paradoxical since one might expect the concentration of diacylglycerol – the substrate of DGAT1 – to be raised in Dgat1+/− mice, but it can be rationalised if fat oxidation is somehow increased.

Stimulation of fatty acid oxidation is usually associated with improved insulin sensitivity and glucose tolerance, possibly because the concentrations of lipid metabolites that inhibit the insulin signalling pathway (e.g. long chain fatty acyl CoA, diacylglycerol, ceramide) are reduced [6]. Moreover, in the longer term, insulin sensitivity will improve due to the reduced plasma non-esterified fatty acid concentration and altered adipokine profile associated with decreased adipocyte lipid stores. Therefore it is not surprising that Dgat1+/− C57Bl/6 mice have been reported to show improved insulin sensitivity [3,7,8]. However, overexpression of DGAT1 in adipose tissue of C57Bl/6 mice was associated with obesity but not with impaired glucose disposal [9]. By contrast, overexpression of DGAT1 in adipose tissue of FVB mice, a strain known to be resistant to diet-induced obesity, was not associated with obesity, but was associated with insulin resistance [10]. These results support the view that an increased capacity for triacylglycerol synthesis is not detrimental to insulin sensitivity provided it is confined to adipocytes and newly formed adipocytes can accommodate any excess triacylglycerol.

We found in a pilot experiment [11] that, in contrast to a previous report [2] glucose tolerance and insulin tolerance were actually worse, and fasting plasma insulin was raised in Dgat1+/− compared to wildtype mice fed on a chow (low fat) diet. The knockout mice appeared to be raised in Dgat1+/− mice, but it could be rationalised if fat oxidation is somehow increased.

Results
When the mice were fed throughout on a chow diet, terminal body weight and lean body mass (at 20 weeks of age) were not significantly affected by genotype (Figs. 1A, C). However, the female Dgat1+/− mice had a higher body weight than either the Dgat1+/− or the Dgat1−/− mice at 9 weeks of age (21.5 ± 0.3, 20.0 ± 0.4, 19.3 ± 0.3 g respectively; n = 12; P < 0.05). When the mice were fed on the high fat diet, the male Dgat1+/− mice had a markedly higher lean body mass compared to the Dgat1+/− mice (Fig. 1D), but this was not reflected in terminal body weight (Fig. 1B) because body fat content was reduced (Fig. 1F).

This reduction in body fat content in Dgat1+/− mice when the mice were fed on a high fat diet was larger (7 g in males and 8 g in females, compared to Dgat1+/− mice; Fig. 1F) than when the mice were fed on chow (2 g in females, and 0.7 g, which was not statistically significant, in males; Fig. 1E). The Dgat1+/− mice did not have significantly altered body fat content compared to the Dgat1+/− mice on either diet, but in females their body fat content was intermediate between that of the Dgat1+/+ and Dgat1+/− mice, whereas in males the Dgat1+/− mice fed on the chow diet actually tended to have a higher body fat content than the Dgat1+/− mice (P = 0.046 by Fisher least significant difference test, but not significant after Bonferroni correction for four tests). Adiposity was reflected in terminal fat pad weights and plasma leptin levels (Table 1). The male Dgat1+/− mice had significantly higher fat pad weights and leptin levels than Dgat1+/− mice even after Bonferroni correction.

In the chow diet experiment, food intake from 9 to 20 weeks of age was higher in both male and female Dgat1+/− than in Dgat1+/+ mice (Fig. 2A). Energy expenditure at 12 weeks of age was raised in the male Dgat1+/+ mice (Fig. 2B). Energy expenditure was not significantly raised in the female Dgat1+/− mice, but there was a trend (P = 0.087 by Fisher test) to an increase that paralleled the food intake data. In the high fat diet experiment, food intake from 12 to 30 weeks of age was higher in male Dgat1+/− than Dgat1+/+ mice, but there was no difference in food intake between the female genotypes (Fig. 2C). Food intake and energy expenditure in the Dgat1+/− mice were no different from in the Dgat1+/+ mice.

An unexpected finding was that in the chow diet experiment, glucose tolerance was, by contrast with a previous report [2], very similar in mice of the three different genotypes (Fig. 3A). However, in the high fat diet experiment, the area under the glucose tolerance curve was, as
Final body weight (A, B), lean body mass (C, D) and fat mass (E, F) in Dgat1+/+ (+/+), Dgat1+/− (+/-) and Dgat1−/− (-/-) mice fed on a chow diet (A, C, E) or a high fat diet (B, D, F).

Results are means of 12 values ± S.E., except that there were 6 female Dgat1−/− mice fed on the high fat diet.

**$P < 0.01$; ***$P < 0.001$, compared to Dgat1+/+ mice of the same sex.
expected, lower in the male Dgat1-/- than in the male Dgat1+/+ or Dgat1+/- mice, and in both male and female Dgat1+/+ mice the blood glucose concentration was reduced at the later time-points (Figs. 3C, D). The fasting plasma insulin concentration was similarly unaffected by genotype in mice fed on the chow diet (Fig. 4A), but lower in the Dgat1+/- than the Dgat1+/+ or Dgat1+/-/+ mice fed on the high fat diet (Fig. 4B). The plasma insulin concentration was also lower in the female Dgat1-/- than the Dgat1+/+ or Dgat1+/-/+ mice 30 min after the glucose load (Fig. 4C).

**Discussion**

Our results are consistent with a previous report [2] in showing that Dgat1+/+ mice have a lower body fat content than Dgat1+/-/+ mice and are resistant to diet-induced obesity. They also agree that energy turnover is increased in male Dgat1+/+ mice, although this was less evident in female Dgat1+/+ mice. They differ, however, from previous reports that show [2] or imply [7] that glucose tolerance is improved in Dgat1+/+ mice fed on a chow diet.

Body fat content was higher in the Dgat1+/+/+ mice fed on the high fat diet compared with the same mice fed on the chow diet, but it was similar in Dgat1+/-/+ mice fed on the high fat and chow diets. Consequently, the effect of ablation of the gene was greater in mice fed on the high fat diet. The effect of ablation of the Dgat1 gene was as apparent in female as in male mice, which have been the focus of previous studies.

Why the absence of an enzyme involved in triacylglycerol synthesis should increase fat oxidation and energy expenditure is not known. One study suggested that a factor released in increased amounts from the adipose tissue of Dgat1+/+ mice could play a role [7], but it seems unlikely that this factor is adiponectin [1,12]. Plasma adiponectin was decreased in male Dgat1+/+ mice in our pilot experiment [11], which is also inconsistent with this hypothesis. Increased locomotor activity in Dgat1+/+ mice raises the possibility that ablation of DGAT1 in the brain raises energy expenditure in the periphery. Thus hypothalamic lipid metabolites are known to affect energy expenditure [13]. However, overexpression of DGAT1 in adipose tissue alters body composition [9,10], and overexpression of DGAT1 in rat isolated islets of Langerhans increases triglyceride synthesis [14]. Therefore, at least in adipose tissue and islets, DGAT1 can have a direct effect on triglyceride storage.

An unexpected finding that may in part account for increased energy expenditure in male Dgat1+/+ mice was that lean body mass, which has more influence on energy expenditure than fat mass [15-17], was increased in male Dgat1+/+ mice fed on the high fat diet. Lean body mass was not raised significantly in the particular group – male Dgat1+/+ mice – where energy expenditure was significantly raised in the present study, but energy expenditure was measured at 12 weeks of age and body composition at 20 weeks of age. Other workers have not observed increased lean body mass in Dgat1+/+ mice [12], although a trend to increased percentage protein content in Dgat1+/+ mice fed on a chow diet has been reported [2].

Our finding that glucose tolerance was not improved in Dgat1+/+ mice fed on a chow diet compares with studies on the overexpression of DGAT1. This did not result in impaired glucose disposal in a strain of mice that was able to respond with increased adipose tissue fat stores [18], but did cause insulin resistance in a strain that was unable to accommodate extra lipid in adipocytes [10]. In the current study, in contrast to our pilot experiment [11], ablation of DGAT1 did not impair glucose tolerance, but nevertheless, inhibition of lipid synthesis in animals that have a low capacity for adipocyte triglyceride accumulation may risk exacerbating insulin sensitivity. In this regard, it is interesting that lipodystrophic animals and humans are characterised by insulin resistance [19] and a recent report suggests that lipodystrophic humans have an increased lean body mass [20], a feature displayed by male Dgat1+/+ mice in the current work.
Figure 2
Food intake (A) and energy expenditure (B) in Dgat1<sup>+/+</sup> (+/+), Dgat1<sup>+/−</sup> (+/-) and Dgat1<sup>−−</sup> (-/-) mice fed on a chow diet, and food intake (C) in mice fed on a high fat diet. Results are means of 4 values ± S.E., except that there were two values for food intake (C) for the female Dgat1<sup>−−</sup> mice fed on the high fat diet (and hence the top of the error bar corresponds to the higher of these two values, which differed by only 1.6%). *p < 0.05; **p < 0.01; ***p < 0.001 compared to Dgat1<sup>+/+</sup> mice of the same sex.
There were no significant differences between the heterozygote ($Dgat1^{+/-}$) and $Dgat1^{+/-}$ mice, except for the surprising finding of increased fat pad weights and leptin concentration in the male $Dgat1^{+/-}$ mice fed on chow. This contrasts with a previous report that male heterozygote mice had a total fat pad weight intermediate between $Dgat1^{-/-}$ and $Dgat1^{+/-}$ mice [1]. Female heterozygote mice, by contrast, did have an intermediate phenotype in terms of fat mass (Fig. 2). Perhaps compensatory mechanisms, such as increased DGAT2 activity, were more pronounced in males than in females. Glucose tolerance was very similar in heterozygote and wildtype mice. This suggests that to treat type 2 diabetes with an inhibitor of DGAT1 it may be necessary to inhibit activity markedly. The activities of DGAT1 in the wildtype and heterozygote mice were not compared, however.

**Conclusion**

$Dgat1^{+/-}$ mice had a lower body fat content than wildtype mice and males had an increased lean body mass when they were fed on a high fat diet. Improved glucose tolerance and reduced plasma insulin levels were apparent only when the mice were fed on a high fat diet. Inhibition of triglyceride synthesis does not improve glucose tolerance if adipocyte lipid stores are already low.

**Methods**

**Animals**

Procedures were conducted in accordance with the University of Buckingham Home Office UK project licence under the Animals (Scientific Procedures) Act (1986) and as agreed by the University of Buckingham Ethical Review Board.
Plasma insulin concentrations in $Dgat^{+/+}$ (+/+), $Dgat^{+/−}$ (+/-) and $Dgat^{−−}$ (−/-) mice fed on a chow diet (A) or a high fat diet (B) immediately before administration of glucose in the glucose tolerance tests, and also 30 min after administration of glucose in the high fat-fed mice (C).

**Figure 4**

Plasma insulin concentrations in $Dgat^{+/+}$ (+/+), $Dgat^{+/−}$ (+/-) and $Dgat^{−−}$ (−/-) mice fed on a chow diet (A) or a high fat diet (B) immediately before administration of glucose in the glucose tolerance tests, and also 30 min after administration of glucose in the high fat-fed mice (C). Results are means of 12 values, except that there were 6 female $Dgat^{−−}$ mice fed on the high fat diet.

$^* P < 0.05$; $^{**} P < 0.01$; $^{***} P < 0.001$ compared to $Dgat^{+/+}$ mice of the same sex.
Three male Dgat1+/+ and three female Dgat1+/− mice back-crossed onto a C57Bl/6 background for ten generations (B6.129S4 – Dgat1tm1Far/J) were purchased from Jackson Laboratories (Bar Harbor, Maine, USA) and a colony was expanded in our facilities, breeding from male Dgat1+/+ and female Dgat1+/− mice. The method used to delete the Dgat1 gene has been reported previously [2]. The offspring from the breeding of the Dgat1+/+ and Dgat1+/− mice were genotyped between 4 to 5 weeks of age and in order to generate littersmates of all three possible genotypes, male and female Dgat1+/− mice were used for further rounds of breeding to obtain the experimental mice. Genomic DNA was extracted from tail tip samples and Dgat1 and neomycin genes detected by PCR using the primers previously described [2]. The presence of both neomycin and Dgat1 genes indicated heterozygosity.

Protocol
The mice were fed on a standard chow diet that contained 10% fat, 70% carbohydrate and 20% protein by energy (14.0 kJ/g metabolisable energy; Beekay Feed, B&K Universal Ltd., Hull, UK), or they were fed on chow until they were 12 weeks old and then on a sweetened high fat diet that contained by energy 45% fat, 35% carbohydrate (of which half was sucrose) and 20% protein (19.3 kJ/g metabolisable energy; diet code D12451; Research Diets, New Brunswick, USA). They were housed at 22 °C with lights on from 07.00 to 19.00 h.

There were twelve mice of each sex and genotype (Dgat1+/+, Dgat1+/− and Dgat1−/−) housed in threes, except that only six female Dgat1+/− mice were fed on the high fat diet. Food intake for each cage of mice was measured daily and body weight was measured weekly from the age of 9 or 13 weeks in the chow or high fat diet experiments, respectively. Energy expenditure was measured at 12 weeks of age in mice fed on the chow diet. Glucose tolerance tests were performed at 15 and 24 weeks of age in the chow and high fat diet experiments respectively. The mice were killed after a 5-hour fast aged 20 (chow diet) or 30 (high fat diet) weeks and blood was taken for the measurement of plasma leptin (Crystal Chem Inc, Chicago, IL). Body composition was determined by dual energy X-ray absorptiometry (Lunar PNI 

Glucose tolerance
Mice were fasted for 5 h from 09.00 h before administration of glucose (2 g/kg, i.p. body weight). Blood samples were taken from the tip of the tail after the topical application of a local anaesthetic (lignocaine gel) at 30-minute intervals. Glucose and insulin were measured as described previously [21].

Energy expenditure
Energy expenditure was measured over 24 h, beginning at 10.00 h in the mice’s home cage by open circuit indirect calorimetry using the equation of Weir [22]. The volume of the mouse cages was 23 litres and the flow rate was 0.8 l/min. Such a system has a calculated half-life of 23.5 min for responding to a step change in energy expenditure [17]. It is therefore not suitable for instant measurement of energy expenditure but with the mice undisturbed in their home cages, it is ideal for measurement of daily energy expenditure. The energy expenditure of all the male mice of all the different genotypes was measured in a single run over 24 h and after recalibration, the energy expenditure of all the female mice was measured on the following day.

Data analysis
Results are presented as means ± S.E. They were analysed by one-way analysis of variance followed by Bonferroni’s post-test for selected comparisons (wild-type mice with heterozygous or homozygous mutant mice of the same sex) unless stated otherwise.

Authors’ contributions and conflict of interest
JA supervised all stages of the study. SW planned and conducted the experiments with support in some experiments from CC and JO. SW analysed and interpreted the data, and supported JA in the writing of the manuscript. MC provided advice at all stages. SW is now an employee of AstraZeneca, who have an interest in DGAT-1 inhibitors.

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