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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1566 – 1575
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Dietary 1,3-diacylglycerol protects against diet-induced obesity and insulin resistance

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Received 21 February 2007; accepted 20 June 2007

Abstract

To investigate the effect of dietary 1,3-diacylglycerol (DAG) on the development of insulin resistance (IR) and obesity, brown adipose tissue–deficient mice, a model of high-fat diet–induced IR and obesity, were fed Western-type diets (WTD) containing either DAG oil (n = 8) or standard triacylglycerol (TAG) oil (n = 9) for 15 weeks, beginning at 8 weeks of age. Although brown adipose tissue–deficient mice became obese on both TAG- and DAG-enriched WTD (TAG-WTD and DAG-WTD), the mice eating DAG-WTD gained less weight and had less body fat accumulation. The results of glucose tolerance tests conducted after 5 weeks of each WTD were not different. However, after 10 weeks of each WTD, impaired glucose tolerance developed in the TAG-WTD group but was prevented by DAG-WTD. Exploratory analyses of gene expression suggested that consumption of DAG-WTD was associated with reduced phosphoenolpyruvate carboxykinase gene expression in liver and increased expression of the genes for peroxisome proliferator–activated receptor α, lipoprotein lipase, and uncoupling proteins 2 and 3 in skeletal muscle. There were no effects of the DAG-WTD on fasting and postprandial plasma triglyceride (TG) levels, hepatic TG content, or the rate of secretion of TG from the liver. These findings suggest that diets enriched in 1,3-DAG oil may reduce WTD-induced IR and body fat accumulation by suppressing gluconeogenesis in liver and stimulating fat oxidation in skeletal muscle.

1. Introduction

The epidemic of obesity in the developed world over the last 2 decades is driving a large increase in the incidence of type 2 diabetes mellitus [1] and consequentially setting the scene for an impending wave of cardiovascular morbidity and mortality [2,3]. Accumulation of fat in the abdomen, liver, and muscle is thought to be closely related to obesity-related insulin resistance (IR) [4,5] and to metabolic syndrome [6,7]. It has been proposed that several factors involved in the pathophysiology of the metabolic syndrome, particularly obesity and IR, are related to lifestyle [8]. Therefore, changes in lifestyle, especially dietary habits, can be one of the most effective ways to prevent development of the metabolic syndrome.

Diacylglycerol (DAG) oil is a naturally occurring oil that is present at low concentrations in vegetable oils [9]. DAG has a long history of use as a human food. DAG has been used as a food additive in small amounts; but a 1,3-specific lipase-catalyzed reverse reaction now allows for the large-scale production of DAG, which is commercially available as cooking oil or processed oil and in fat-containing products in the United States and Japan. As a result of studies in rodents and humans (vide infra), DAG is approved as a food for specific health use in Japan.

Several studies in humans indicate that DAG reduces postprandial hyperlipidemia and is effective in the prevention of obesity [10-13]. It has also been reported that DAG ameliorates glucose intolerance or prevents the development of impaired glucose tolerance in Otsuka Long-Evans Tokushima Fatty rats [14] and sucrose-fed Wistar rat [15], respectively. These studies suggest that the ingestion of DAG oil not only prevents the accumulation of body fat but also suppresses the development of abnormal carbohydrate metabolism. Mechanisms proposed to account for the changes seen in these various studies include decreased chylomicron formation [10,11,16], increased oxidation of fatty acids (FAs) in the small intestine and/or the liver [17-19], and weight loss [12,13,17]. We recently performed detailed studies of the metabolism of DAG in C57Bl/6 mice [20] and demonstrated that chylomicrons assembled after ingestion of DAG oil were better substrates for

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0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.06.024

lipoprotein lipase (LpL) than chylomicrons formed after ingestion of typical triacylglycerol (TAG) oil; we did not demonstrate reduced formation of chylomicrons after DAG ingestion.

To gain further insights into the actions of DAG, we now have compared the effects of long-term consumption of DAG and TAG oil–enriched Western-type diets (WTD) on glucose and lipid metabolism and the expression of relevant genes in several tissues in mice lacking brown adipose tissue (BATless mice) [21], which become severely obese and IR on high-fat diets [22,23].

2. Materials and methods

2.1. Test diet

The ester distributions and the proportions of the main FAs of TAG and DAG oil are presented in Table 1. The DAG oil was prepared according to the method of Watanabe et al [24] using a mixture of soybean oil and rapeseed oil in the presence of immobilized lipase. The prepared DAG oil consisted of 86% DAG with a ratio of 7:3 for 1,3-DAG to 1,2-DAG, 13.6% TAG, and 0.4% monoacylglycerol (MAG). The TAG oil was prepared by mixing safflower oil, rapeseed oil, and egoma oil to provide the same FA composition as the DAG oil. The ester distribution of TAG oil was 98.1% TAG, 1.9% DAG, and 0.0% MAG. The study diets were TAG- or DAG- enriched WTD (TAG-WTD or DAG-WTD), prepared as shown in Table 2. The DAG and TAG oils have the same energy values and are absorbed similarly from the small intestine [25]. The overall composition of each WTD was 21.0% fat, 34.1% sucrose, 19.5% casein, and 0.2% cholesterol. As the fat source, the above mentioned TAG or DAG oil, consisting of less than 10% saturated FA, was mixed with an equal amount of milk fat, consisting of 69% saturated FA. Overall, the fat in the study diets derived from 50% TAG or DAG and 50% WTD, resulting in an overall polyunsaturated to saturated (P/S) FA ratio of 0.8 (wt/wt).

2.2. Animals

The BATless mice were bred onto a mixed background of FVB/N and C57BL/6J strains as described by Siri et al [22].

Table 1
Compositions of test oils

	TAG oil	DAG oil
Ester distributions (%)		
MAG	0.0	0.4
DAG	1.9	86.0
TAG	98.1	13.6
FA compositions (%)		
C16:0	6.1	3.1
C18:0	2.1	1.1
C18:1	35.3	37.7
C18:2	47.4	49.2
C18:3	7.8	7.9
C20:0	0.4	0.2

Table 2
Compositions of TAG- and DAG-WTD

	TAG-WTD	DAG-WTD
Casein	195.00	195.00
DL-Methionine	3.00	3.00
Sucrose	340.96	340.96
Maltodextrin	75.00	75.00
Corn starch	75.00	75.00
Anhydrous milk fat	105.00	105.00
DAG oil	0.00	105.00
TAG oil	105.00	0.00
Cholesterol	2.00	2.00
Cellulose	50.00	50.00
Mineral mix	35.00	35.00
Calcium carbonate	4.00	4.00
Vitamin mix, Teklad 40060	10.00	10.00
Ethoxyquin (antioxidant)	0.04	0.04

Values are expressed as grams per kilogram diet.

All mice (n = 8-9 in each group) were maintained on a 12-hour light/dark cycle (light cycle was 7 AM to 7 PM) and were initially weaned onto a complete WTD (no. 88137; Teklad Premier Laboratory Diets, Madison, WI) containing 21.0% milk fat (with a P/S FA ratio of 0.07), 34.1% sucrose, 19.5% casein, and 0.2% cholesterol (wt/wt). Mice were switched to the TAG-WTD or DAG-WTD, described above, at 8 weeks of age and fed until they were 23 weeks old. The amount of diet intake was measured once a week.

2.3. Lipid, glucose, and insulin determinations

Blood samples were obtained from the retroorbital plexus, plasma was isolated at 4°C, and samples were immediately frozen at −70°C. Total plasma triglycerides (TGs), free fatty acids (FFAs), and glucose levels were measured on a Hitachi automated spectrophotometer (model 704, Hitachi Ltd, Tokyo, Japan) using commercial kits obtained from Wako Chemicals (Richmond, VA). Plasma insulin concentrations were measured by radioimmunoassay using a commercial kit (no. SRI-13K) obtained from Linco Research (St Charles, MO).

2.4. Glucose and fat tolerance test

Glucose tolerance tests (GTTs) were conducted after an overnight fast at 13 and 18 weeks of age as reported previously [22]. After a baseline blood collection, mice (n = 8-9 in each group) were injected intraperitoneally with 15% glucose in a 0.9% NaCl solution (1 g of glucose per kilogram of body weight). Subsequent blood samples were collected at 30, 60, 120, and 180 minutes for the determination of plasma glucose and insulin levels.

Fat tolerance tests (FTTs) were conducted after an overnight fast at 14 and 19 weeks of age. After a baseline blood collection, mice (n = 8-9 in each group) were given 0.4 mL emulsion containing 20% TAG oil by gavage. Subsequent blood samples were collected at 60, 120, and 240 minutes for the determination of plasma TG levels.

Table 3
Primer sequences for real-time PCR

18s F	5′-GGA GAA CTC ACG GAG GAC GA-3′
18s R	5′-CCA GTG GTC TTG GTG TGC TG-3′
PPAR-α F	5′-GGA TGT CAC ACA ATG CAA TTC GCT-3′
PPAR-α R	5′-TCA CAG AAC GGC TTC CTC AGG TT-3′
PPAR-γ1 F	5′-GAG TGT GAC AAG ATT TG-3′
PPAR-γ1 and 2 R	5′-GGT GGG CCA GAA TGG CAT CT-3′
PPAR-γ2 F	5′-TCT GGG AGA TTC TCC TGT TG-3′
SREBP-1c F	5′-GGC ACT AAG TGC CCT CAA CCT-3′
SREBP-1c R	5′-GCC ACA TAG ATC TCT GCC AGT GT-3′
AOX F	5′-CCA ACA TGA GGA CTA TAA CTT CCT-3′
AOX R	5′-TAC ATA CGT GCC GTC AGG CTT CAC-3′
ACC F	5′-GGA GGA CCG CAT TTA TCG A-3′
ACC R	5′-TGA CCA GAT CAG AGT GCC T-3′
FAS F	5′-CCT GGA TAG CAT TCC GAA CCT-3′
FAS R	5′-AGC ACA TCT CGA AGG CTA CAC A-3′
DGAT2 F	5′-AGT GGC AAT GCT ATC ATC ATC GT-3′
DGAT2 R	5′-AAG GAA TAA GTG GGA ACC AGA TCA-3′
ACS F	5′-ATC ATG GAC TCC TAG GGA A-3′
ACS R	5′-CTT TGG GGT TGC CTG TAG TT-3′
PEPCK F	5′-ATC TTT GGT GGC CGT AGA CCT-3′
PEPCK R	5′-GCC AGT GGG CCA GGT ATT T-3′
G6P F	5′-TCC TCT TTC CCA TCT GGT TC-3′
G6P R	5′-TAT ACA CCT GCT GCG CCC AT-3′
GLUT2 F	5′-GGC TAA TTT CAG TGG TT-3′
GLUT2 R	5′-TTT CTT TGC CCT GAC TTC CT-3′
GLUT4 F	5′-AAC ACT GGT CCT AGC TGT AT-3′
GLUT4 R	5′-CGT CAG ACA CAT CAG CCC AG-3′
ATGL F	5′-CGC CTT GCT GAG AAT CAC CAT-3′
ATGL R	5′-AGT GAG TGG CTG GTG AAA GGT-3′
HSL F	5′-CTG CTG ACC ATC AAC CGA C-3′
HSL R	5′-CGA TGG AGA GAG TCT GCA-3′
LpL F	5′-GTA CCT GAA GAC TCG CTC TC-3′
LpL R	5′-AGG GTG AAG GGA ATG TTC TC-3′
UCP-2 F	5′-CAT TCT GAC CAT GGT GCG TAC TGA-3′
UCP-2 R	5′-GTT CAT GTA TCT CGT CTT GAC CAC-3′
UCP-3 F	5′-AAC CTT GGC TAG ACG CAC AG-3′
UCP-3 R	5′-CAC CAT CTT CAG CAT ACA GTG-3′
TNF-α F	5′-TTC TGT CTA CTG AAC TTC GGG GTG ATC GGT CC-3′
TNF-α R	5′-GTA TGA GAT AGC AAA TCG GCT GAC GGT GTG GG-3′

F indicates forward; R, reverse; SREBP, sterol regulatory element–binding protein; ACC, acetyl–coenzyme A carboxylase; FAS, fatty acid synthase; DGAT, diacylglycerol acyltransferase; ACS, acyl–coenzyme A synthetase; GLUT, glucose transporter; ATGL, adipose triglyceride lipase.

Twenty percent TAG emulsions by weight were prepared using ultrasonication as reported previously [26]. In brief, the oil phase of the emulsion, composed of 12 mg of egg yolk phosphatidylcholine (Sigma-Aldrich, St Louis, MO) and 2 g of TAG oil shown in Table 1, was dispersed in the water phase of the emulsion, composed of 1 g of bovine serum albumin (MP Biomedicals, Irvine, CA), 250 mg of glycerin (Sigma-Aldrich), and 6.6 g of doubly distilled water, by means of the handy homogenizer (model 398; Biospec Products, Bartlesville, CA). Subsequently, the dispersion was homogenized with an ultrasound sonicator (type 853973/1, Braun-Sonic U; Braun, Los Angeles, CA) for 10 minutes at a power setting of 200 W.

2.5. Determination of in vivo TG synthesis rates

To determine the rates of hepatic TG secretion, animals were injected with Triton WR 1339 via femoral vein after a 4-hour fast [22]. Plasma samples were collected preinjection and at 30, 60, 90, and 120 minutes postinjection.

2.6. Liver TG

To measure liver stores of TG, total liver lipids were extracted by a modification of the method of Folch et al [27]. Briefly, snap frozen liver tissues (~150 mg) were homogenized and extracted twice with a chloroform-methanol (2:1 vol/vol) solution. The organic layer was dried under nitrogen gas and resolubilized in chloroform. This Folch extraction was resuspended in an aqueous solution containing 2% Triton X-100 [28] for determination of TG mass. [14C]-Triolein was added to each sample before lipid extraction to account for recovery, and final TG concentrations were adjusted accordingly.

2.7. Real-time polymerase chain reaction

Total RNA was isolated from tissues using the TRIzol reagent according to the manufacturer's protocol (Invitrogen,

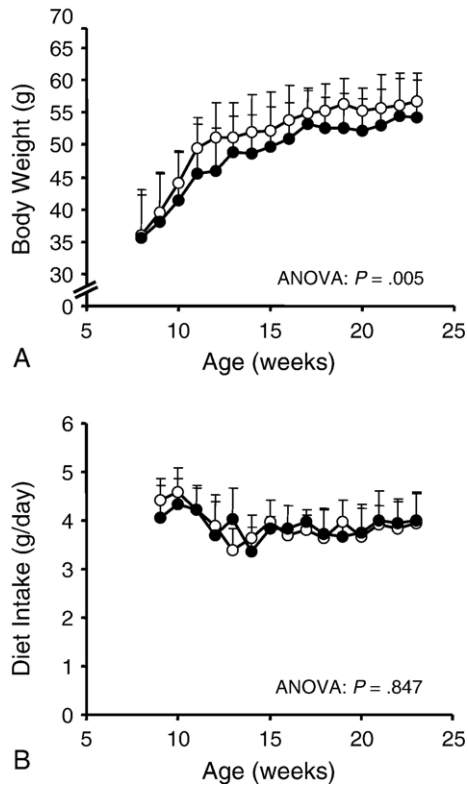

Fig. 1. Consumption of DAG-WTD was associated with lower body weights despite equal food intake. Changes in body weight (A) and amount of food consumed (B) during the study in the TAG (open circles, n = 9) and DAG (closed circles, n = 8) groups. Values are expressed as mean ± SD. Repeated 2-way ANOVA was performed on body weight and food intake over the duration of the study.

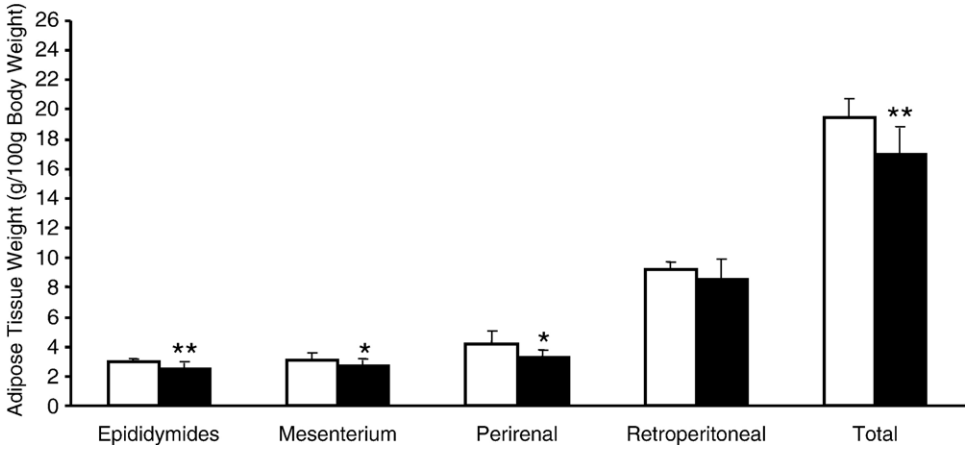

Fig. 2. Consumption of DAG-WTD was associated with lower body fat mass. Adipose tissue weight at 23 weeks of age in the TAG (open bars, n = 9) and DAG (closed bars, n = 8) groups. Values are expressed as mean ± SD. The asterisks denote statistically significant differences between the TAG and DAG groups in the fat mass at each depot site (assessed by Student *t* test; $^{**}P < .01$, $^{*}P < .05$).

Carlsbad, CA). Total RNA samples were used for complementary DNA synthesis with oligo d(T) primers using a commercial kit from Invitrogen. The resulting complementary DNA samples were then quantified for each test gene using target gene–specific primers. Quantitative real-time polymerase chain reaction (RT-PCR) was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) according to the protocols provided by the manufacturer. Detection of specific products was performed in triplicate using the Mx4000 Multiplex Quantitative PCR system (Stratagene, La Jolla, CA). Using the standard curve method, the relative quantitation of specific PCR products for each primer set was generated. For normalization, ribosomal RNA 18s was amplified from each

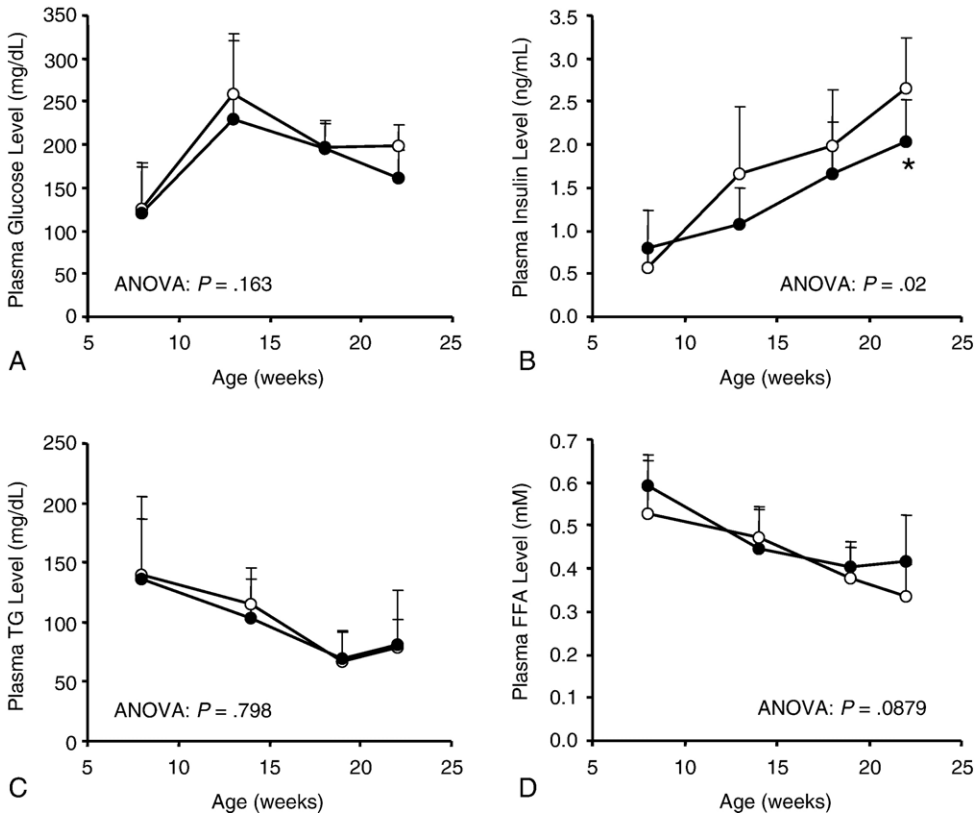

Fig. 3. Consumption of DAG-WTD was associated with less increase in fasting plasma insulin levels. Changes in plasma glucose (A), insulin (B), TG (C), and FFA (D) levels after an overnight fast (15-16 hours) in the TAG (open circles, n = 9) and DAG (closed circles, n = 8) groups. Repeated 2-way ANOVA was performed on the slope of change for each variable. The asterisk denotes statistically significant differences between the TAG and DAG groups in the individual time point for plasma insulin (assessed by Student *t* test; $^{*}P < .05$).

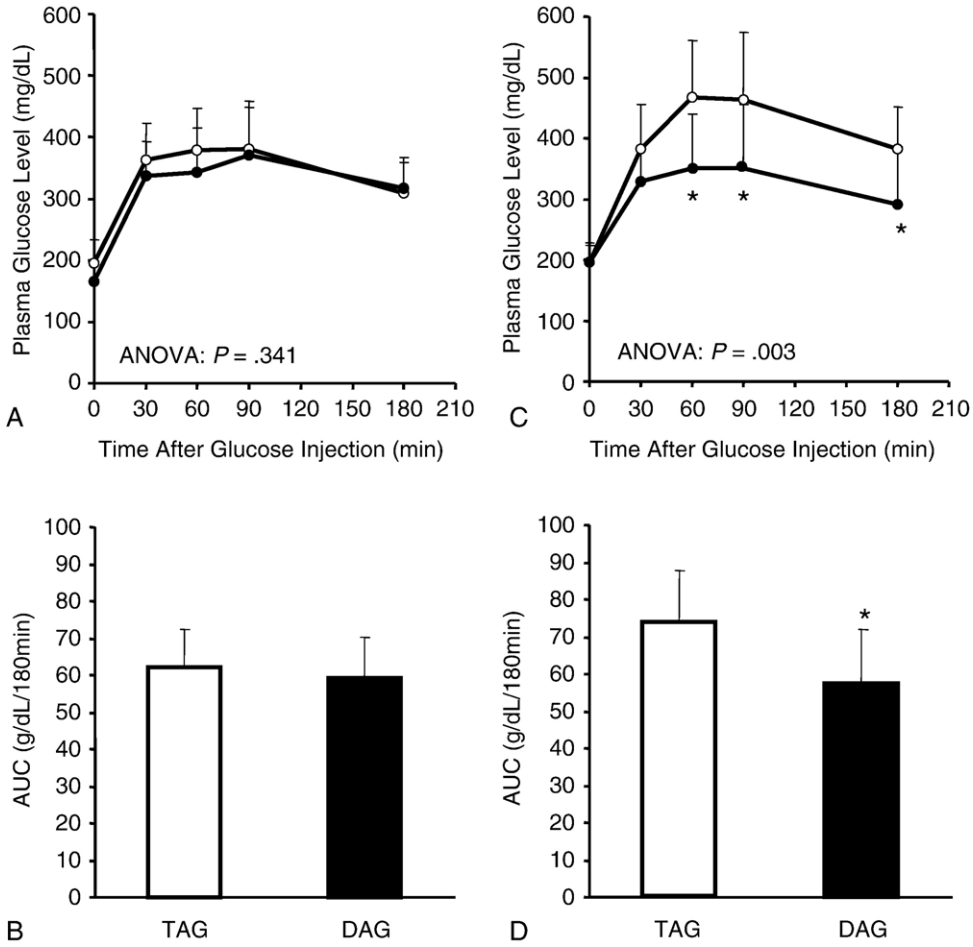

Fig. 4. Consumption of DAG-WTD was associated with better glucose tolerance. Changes in plasma glucose levels and AUC during a GTT conducted at 13 (A and B) and 18 (C and D) weeks of age in the TAG (open circles and bars, n = 9) and DAG (closed circles and bars, n = 8) groups. Values are expressed as mean ± SD. Repeated 2-way ANOVA was performed. The asterisks denote statistically significant differences between the TAG and DAG groups at individual time points and for the AUC (assessed by Student *t* test; **P* < .05).

sample. The primers used for the RT-PCR are shown in Table 3.

2.8. Statistical analysis

The means and standard deviations (SDs) are presented. Statistically significant differences (ie, $P = .05$, 2-tailed) in mean values between 2 groups were tested by Student *t* test and repeated 2-way analysis of variance (ANOVA). The response to GTT was measured by determining the areas under the curve above baseline (AUC). All of the gene expression studies were exploratory in nature; as such, we did not correct for multiple tests.

3. Results

Consumption of the DAG-WTD was associated with less weight gain during the study ($P = .005$ by ANOVA) (Fig. 1A), although food intake did not differ between the TAG-WTD and DAG-WTD groups (Fig. 1B). The reduced rise in body weight was associated with reductions in body fat in several fat depots in the mice consuming DAG-WTD (Fig. 2).

The BATless mice become IR while consuming high-fat diets [21-23]. Although there were no significant differences between the 2 diets in terms of their effects on fasting glucose (Fig. 3A), consumption of DAG-WTD was associated with less of an increase in the levels of fasting plasma insulin compared with the levels seen after consumption of the TAG-WTD for 15 weeks (Fig. 3B). Fasting plasma TG levels tended to drift lower during the study in both groups; but this was not significant, and there were no differences between the TAG-WTD and DAG-WTD groups (Fig. 3C). The trend toward lower TG levels in both groups may have been due to the switch from a full WTD, onto which they were weaned, to either the DAG-WTD or TAG-WTD at 8 weeks of age; the DAG-WTD and TAG-WTD were 50% vegetable oil–based and therefore contained much lower contents of saturated FA compared with the full WTD. Plasma fasting FFA levels did not change during the study, nor were there any differences between the diet groups (Fig. 3D).

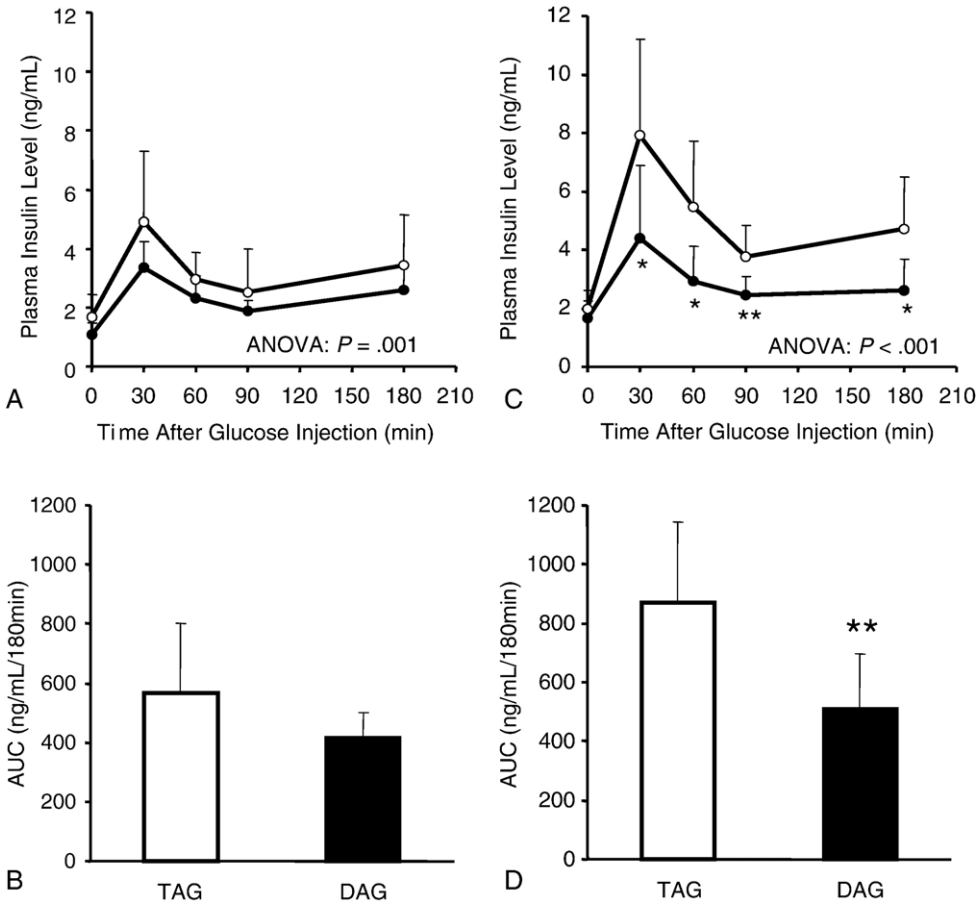

Fig. 5. Consumption of DAG-WTD was associated with better insulin sensitivity. Changes in plasma insulin levels and AUC during a GTT conducted at 13 (A and B) and 18 (C and D) weeks of age in the TAG (open circles and bars, n = 9) and DAG (closed circles and bars, n = 8) groups. Values are expressed as mean ± SD. Repeated 2-way ANOVA was performed. The asterisks denote statistically significant differences between the TAG and DAG groups at individual time points and for the AUC (assessed by Student *t* test; $^{**}P < .01$, $^{*}P < .05$).

To examine the effects of each diet on insulin sensitivity in more detail, GTTs were performed with measures of plasma glucose and insulin at 13 and 18 weeks of age (after 5 and 10 weeks of either the TAG-WTD or DAG-WTD). Although there was no significant difference in GTT glucose levels between groups of mice at 13 weeks of age (Fig. 4A, B), impairment of glucose tolerance was observed in the 18-week-old mice that continued to consume TAG-WTD (Fig. 4C, D); thus, glucose intolerance was prevented by consumption of DAG-WTD. Plasma insulin levels during the GTT in 13-week-old mice tended to be higher in the TAG-WTD group compared with the DAG-WTD group (Fig. 5A, B). However, after an additional 5 weeks of diet, insulin secretion during the GTT was clearly increased in the 18-week-old TAG-WTD group of mice compared with their earlier results; but this increase was not observed in the group of mice consuming the DAG-WTD (Fig. 5C, D). Indeed, in 18-week-old mice (after 10 weeks of either diet), the plasma insulin excursion during the GTT was significantly reduced in the DAG-WTD vs TAG-WTD mice. Taken together, the glucose and insulin data indicate that consumption of DAG oil within the background of a WTD prevented the development of IR that was seen in BATless mice consuming the TAG-WTD.

1,3-DAG oil has been observed to have effects on postprandial TG levels. Therefore, we conducted FTTs at 2 points during the study. There were no significant differences in plasma TG levels during FTTs conducted at either 14 or 19 weeks in the DAG- and TAG-WTD groups (after 6 or 11 weeks of either diet) (Fig. 6A, B). To assess the effect of DAG- or TAG-WTD on the secretion of TG from the liver, we injected Triton WR-1339 (which inhibits clearance of any circulating lipoproteins; Materials and methods) into the femoral veins of 23-week-old mice (after 15 weeks of each type of WTD) and measured TG levels in blood over the next 2 hours. The mice were then killed, and liver TG was measured. Secretion of TG from the liver was the same in the DAG-WTD and TAG-WTD groups (Fig. 7A). In addition, neither liver weight nor liver TG was different in the DAG-WTD and TAG-WTD groups (Fig. 7B).

To gain insight into the molecular basis for the effects of DAG, we conducted exploratory studies of gene expression in liver, small intestine, skeletal muscle, and white adipose

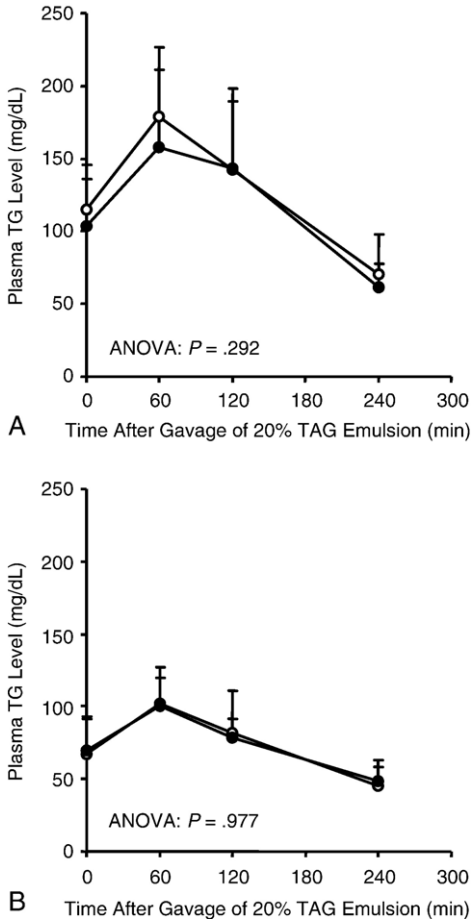

Fig. 6. Consumption of DAG-WTD did not affect postprandial TG metabolism. Changes in plasma TG levels in FTT conducted at 14 (A) and 19 (B) weeks of age in the TAG (open circles, n = 9) and DAG (closed circles, n = 8) groups. Values are expressed as mean ± SD. Repeated 2-way ANOVA was performed.

tissue (WAT) by RT-PCR. In liver, β-oxidation–related gene expression, such as peroxisome proliferator–activated receptor (PPAR) α and acyl–coenzyme A oxidase (AOX) messenger RNA (mRNA) levels, tended to be higher in the DAG group than in the TAG group ($P < .1$, Table 4). The PPAR-γ2, normally present mainly in adipose tissue and linked to lipogenesis, also tended to be higher in livers of DAG-WTD mice compared with TAG-WTD group ($P < .1$, Table 4). Messenger RNA levels of phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenesis-related gene, was lower in livers of mice consuming the DAG-WTD (Table 4). Hepatic glucose-6-phosphatase (G6P) also tended to be lower ($P < .1$). In the small intestine, AOX mRNA level tended to be higher in the DAG than in the TAG group ($P < .1$, Table 5). In skeletal muscle, levels of PPAR-α, uncoupling protein (UCP) 2, UCP-3, and LpL gene expression were increased in the DAG group (Table 6). Finally, in WAT, the level of hormone-sensitive lipase (HSL) mRNA was higher and tumor necrosis factor (TNF) α mRNA was lower in the DAG-WTD compared with the TAG-WTD group (Table 7).

4. Discussion

The major finding in this study was that DAG prevented the development of high-fat diet–induced IR and glucose tolerance in the BATless mouse, a model of decreased thermogenic capacity that is particularly sensitive to high-fat diets [21-23]. Thus, although glucose intolerance and IR developed in the BATless mice between 5 and 10 weeks of the TAG-WTD, this diet effect was absent in the BATless mice consuming DAG-WTD for the same period. Our data are consistent with those in previous studies with Otsuka Long-Evans Tokushima Fatty rats [14] and sucrose-fed Wistar rat [15]. The absence of WTD-induced IR in BATless mice consuming the DAG-WTD was associated with a reduction in hepatic expression of PEPCK. There was also a suggestion that G6P was lowered. Reduced expression of these genes, which correlates well with their protein mass and enzymatic activity, would tend to maintain hepatic insulin responsiveness during consumption of a high-fat diet by reducing gluconeogenesis. Insulin is the key repressor of both PEPCK and G6P gene expression, apparently, at least for PEPCK, via disruption of stimulatory

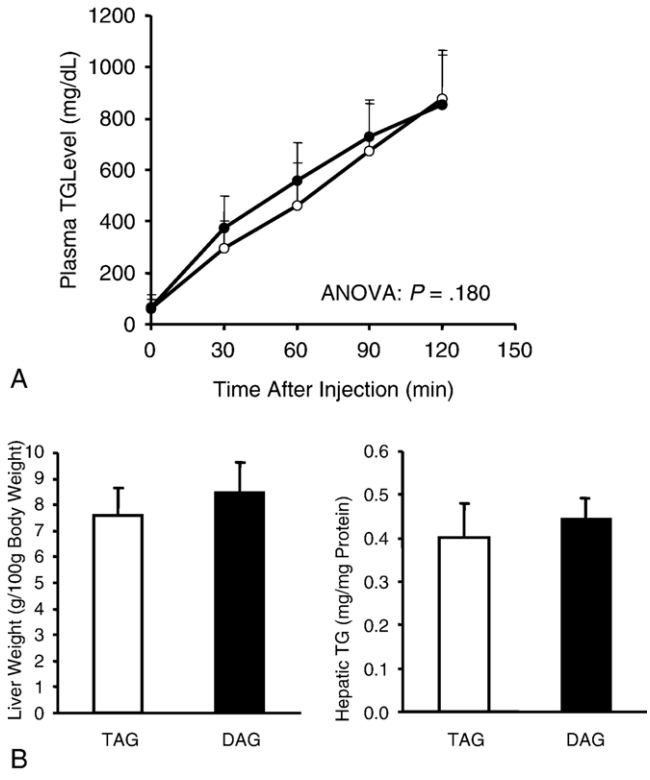

Fig. 7. Consumption of DAG-WTD did not affect either hepatic TG secretion or hepatic TG mass. A, Changes in plasma TG levels after Triton WR-1339 injection at 23 weeks of age in the TAG (open circles, n = 9) and DAG (closed circles, n = 8) groups. Values are expressed as mean ± SD. Repeated 2-way ANOVA was performed. B, Liver weight and hepatic TG levels at 23 weeks of age in the TAG (open bars, n = 9) and DAG (closed bars, n = 8) groups. Values are expressed as mean ± SD. Analysis was by Student *t* test.

Table 4
Relative mRNA expressions in liver

	TAG (n = 9)	DAG (n = 8)	*P* (*t* test)
PPAR-α	1.00 ± 0.25	1.20 ± 0.18	.08
AOX	1.00 ± 0.30	1.32 ± 0.36	.07
PPAR-γ1	1.00 ± 0.52	1.21 ± 0.55	.45
PPAR-γ2	1.00 ± 0.29	1.21 ± 0.19	.09
SREBP-1c	1.00 ± 0.31	1.32 ± 0.46	.11
ACC	1.00 ± 0.26	1.20 ± 0.59	.36
FAS	1.00 ± 0.34	1.01 ± 0.24	.96
DGAT2	1.00 ± 0.19	1.17 ± 0.28	.17
UCP-2	1.00 ± 0.42	1.21 ± 0.29	.25
ACS	1.00 ± 0.38	1.40 ± 0.42	.06
PEPCK	1.00 ± 0.41	0.60 ± 0.21	.03
G6P	1.00 ± 0.37	0.68 ± 0.30	.07
GLUT2	1.00 ± 0.31	1.21 ± 0.35	.20

Values are mean ± SD.

Table 6
Relative mRNA expressions in skeletal muscle

	TAG (n = 9)	DAG (n = 8)	*P* (*t* test)
PPAR-α	1.00 ± 0.19	1.30 ± 0.27	.02
AOX	1.00 ± 0.31	1.18 ± 0.33	.28
PPAR-γ1	1.00 ± 0.47	0.88 ± 0.27	.53
PPAR-γ2	1.00 ± 0.31	1.04 ± 0.18	.72
SREBP-1c	1.00 ± 0.32	1.04 ± 0.36	.81
FAS	1.00 ± 0.34	1.03 ± 0.29	.83
ACC	1.00 ± 0.29	1.13 ± 0.15	.27
ACS	1.00 ± 0.23	1.24 ± 0.46	.19
UCP-2	1.00 ± 0.36	1.45 ± 0.39	.03
UCP-3	1.00 ± 0.24	1.46 ± 0.54	.04
LpL	1.00 ± 0.27	1.40 ± 0.46	.04
PEPCK	1.00 ± 0.36	0.82 ± 0.22	.24
GLUT4	1.00 ± 0.23	0.99 ± 0.27	.96

Values are mean ± SD.

transcriptional complexes [29,30]. Whether improved hepatic insulin sensitivity with increased suppression of PEPCK and G6P is a direct result of DAG-WTD feeding or is related to less weight gain (see below) is unclear and will require further studies.

We also observed a reduction in WAT expression of TNF-α; adipose tissue TNF-α expression has been closely linked to obesity-induced IR [31,32]. Recent studies indicate that production of adipose tissue TNF-α may derive predominantly from macrophages attracted to adipose tissue in obese rodents and people [33,34]; adipocyte-derived monocyte chemoattractant protein 1 may be a key signal for macrophage recruitment to adipose tissue [35], and interaction of FAs with macrophage toll-like receptor 4 may be the signal for TNF-α production [36,37]. Because weight loss is associated with decreases in TNF-α [38,39], we are not able to determine whether the lower TNF-α expression in WAT that we observed in the DAG-WTD group was a direct effect of DAG or the result of DAG-induced weight loss.

The BATless mice on the DAG-WTD also gained about 10% less weight than BATless mice consuming TAG-WTD. This difference in body weight was associated with reduced accumulation of fat in essentially all depots in the DAG-WTD mice despite equal food intake in the DAG-WTD and TAG-WTD groups. DAG has been reported to increase postprandial fat oxidation and energy expenditure in humans [40,41], and Murase et al [17,18] reported increased expression of genes associated with FA oxidation in small intestine [18] and liver [17] in mice fed high-DAG diets. In our exploratory studies of gene expression, we found a trend toward increased expression of PPAR-α and AOX in liver and of AOX in small intestine of DAG-WTD–fed BATless mice. In addition, PPAR-α, LpL, UCP-2, and UCP-3 were all increased in skeletal muscle in DAG-WTD–fed mice; the roles of UCP-2 and UCP-3 in muscle energy expenditure are still poorly defined [42]. Overall, however, these differences in gene expression support some increase in FA delivery, oxidation, and energy expenditure in skeletal muscle of DAG-WTD–fed BATless mice. In addition, the modest increase in WAT expression of HSL could have contributed to the reduced adipose mass observed on the DAG-WTD; increased WAT lipolysis and release of FA, combined with increased FA oxidation in muscle, could explain the combination of reduced body fat and increased insulin sensitivity in the DAG-WTD mice. However, we did not correct for multiple tests of gene expression; and so these findings must be viewed as only suggestive. Confirmation of these findings with greater numbers of mice and appropriate

Table 5
Relative mRNA expressions in small intestine

	TAG (n = 9)	DAG (n = 8)	*P* (*t* test)
PPAR-α	1.00 ± 0.27	1.17 ± 0.10	.12
AOX	1.00 ± 0.25	1.30 ± 0.36	.07
PPAR-γ1	1.00 ± 0.32	0.81 ± 0.17	.17
PPAR-γ2	1.00 ± 0.52	1.05 ± 0.48	.85
SREBP-1c	1.00 ± 0.47	1.10 ± 0.45	.67
ACC	1.00 ± 0.44	1.11 ± 0.62	.69
FAS	1.00 ± 0.38	1.05 ± 0.33	.76
DGAT1	1.00 ± 0.25	1.07 ± 0.19	.54
UCP-2	1.00 ± 0.35	1.00 ± 0.42	.98
ACS	1.00 ± 0.47	1.05 ± 0.35	.82
PEPCK	1.00 ± 0.54	1.19 ± 0.44	.45
G6P	1.00 ± 0.49	0.90 ± 0.32	.64

Values are mean ± SD.

Table 7
Relative mRNA expressions in WAT

	TAG (n = 9)	DAG (n = 8)	*P* (*t* test)
PPAR-α	1.00 ± 0.32	1.07 ± 0.32	.64
PPAR-γ1	1.00 ± 0.30	0.84 ± 0.49	.42
PPAR-γ2	1.00 ± 0.42	0.85 ± 0.21	.39
DGAT2	1.00 ± 0.45	0.76 ± 0.28	.21
ATGL	1.00 ± 0.32	1.03 ± 0.30	.86
HSL	1.00 ± 0.22	1.23 ± 0.21	.05
LpL	1.00 ± 0.24	0.89 ± 0.37	.46
GLUT4	1.00 ± 0.48	1.08 ± 0.44	.72
TNF-α	1.00 ± 0.36	0.61 ± 0.25	.02

Values are mean ± SD.

statistical considerations will be necessary. In addition, identification of the molecular basis for these differences in gene expression, if confirmed, will require further study. It should be noted that the mice were consuming diets with almost identical FA compositions, so the effects that we have seen must be related to differences in the tissue distribution and metabolism of those FAs rather than any FA-specific effects on metabolism. Finally, as noted earlier, whether these changes are causally related to the reduced weight gain observed in the DAG-WTD fed mice or the response to reduced weight gain will require further study.

Some of the most striking effects of DAG oil diets in humans have been on postprandial lipid metabolism [10,11]. Similar benefits of a DAG-enriched diet have been observed in rodents [14,15]. By contrast, in the present study, we did not observe differences in either fasting levels of TG or FA or in postprandial responses during the TAG-FTTs between the DAG-WTD and TAG-WTD groups of BATless mice. The latter fat load studies were done at both 14 and 19 weeks of age, after each diet had been consumed for either 6 or 11 weeks. One possible reason that we did not see beneficial effects of the DAG-WTD on postprandial lipemia was that all mice consumed a complete WTD for 8 weeks before being randomized to either DAG-WTD or TAG-WTD. Indeed, fasting plasma TG levels fell from their values at 8 weeks of age, when the mice switched from full WTD to the less saturated DAG or TAG diets; the change from a diet much higher in saturated FA (P/S = 0.07) to the 2 experimental diets, which both contained much less saturated FA (P/S = 0.8), may have diminished some of the differential effects of DAG. It is possible that a longer duration of consumption of the DAG-WTD and TAG-WTD would be necessary to eliminate residual effects of the prior full WTD.

In summary, consumption of DAG oil on the background of a WTD reduced weight gain and the accumulation of body fat that was seen with a TAG oil–enriched WTD; and these effects of DAG were associated with maintenance of normal insulin sensitivity. Stimulation of fatty oxidation in skeletal muscle (and possibly liver and intestine), together with suppression of hepatic gluconeogenesis, appears to be the molecular changes underlying these favorable effects of dietary DAG.

Acknowledgment

Investigator-initiated grant from KAO, Tokyo, Japan.

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