Diacylglycerol Oil Reduces Body Fat but Does Not Alter Energy or Lipid Metabolism in Overweight, Hypertriglyceridemic Women¹–³

Quangeng Yuan,⁴,⁵ Venu R. Ramprasath,⁴ Scott V. Harding,⁴ Todd C. Rideout,⁴ Yen-Ming Chan,⁴ and Peter J. H. Jones⁴,³*²

⁴Richardson Centre for Functional Foods and Nutraceuticals and ³Department of Human Nutritional Sciences, Faculty of Human Ecology, University of Manitoba, Winnipeg R3T 2N2, Manitoba, Canada

Abstract
Diacylglycerol (DAG) may undergo differential metabolism compared with triacylglycerol (TAG) in humans, possibly resulting in decreased serum TAG concentration and TAG synthesis and increased energy expenditure (EE), thus reducing fat accumulation. Our objective was to examine the efficacy of DAG oil consumption on serum lipid profiles, hepatic lipogenesis, EE, and body weight and composition compared with a control oil-blend composed of sunflower, safflower, and rapeseed oils at a 1:1:1 ratio. Twenty-six overweight (78.3 ± 3.6 kg body weight and BMI 30.0 ± 0.7 kg/m²) mildly hypertriglyceridemic (1.81 ± 0.66 mmol/L) women underwent 2 treatment phases of 28 d separated by a 4-wk washout period using a randomized crossover design. They consumed 40 g/d of either DAG or control oil during treatment phases. The baseline, EE, fat oxidation, body composition, and lipid profiles did not differ between the DAG and control oil intervention periods. Relative to control oil, DAG oil did not alter endpoint postprandial EE, fat oxidation, serum lipid profiles, or hepatic lipogenesis. However, DAG oil consumption reduced (P < 0.05) accumulation of body fat within trunk, android, and gynoid regions at the endpoint compared with control oil, although neither DAG nor control oil altered any of these variables during the 4-wk intervention period compared with their respective baseline levels. We conclude that although DAG oil is not effective in lowering serum lipids over a 4-wk intervention, it may be useful for reducing adiposity. J. Nutr. 140: 1122–1126, 2010.

Introduction
Diacylglycerol (DAG)⁶ oil naturally occurs in several edible oils ranging from 0.8% in rapeseed oil to 9.5% in cottonseed oil (1). DAG, a glycerol derivative, possesses 2 hydroxyl groups substituted by fatty acids through ester bond formation. DAG exists either as 1, 2-DAG or 1, 3-DAG and as a digestive intermediate of lipid digestion where dietary triacylglycerols (TAG) are broken down by lipase to produce 1, 2-DAG (2). It has been hypothesized that DAG oil may be metabolized differently than TAG. Whereas TAG are hydrolyzed, reassembled, and packaged as chylomicrons in the enterocytes and transported via the lymphatic system through the peripheral tissues to be removed from circulation as a chylomicron remnant, DAG may be absorbed directly into the blood and enter hepatocytes via the portal vein for rapid oxidation (3–5). Unlike TAG, DAG are not efficiently hydrolyzed and reassembled as TAG (6); therefore, high dietary intakes may modify blood lipids (7–15) and possibly reduce the amount of lipid stored in adipose tissue (16). The speculation that DAG oil may lower blood lipids and control body adiposity has led to it being marketed as a functional food in the United States and Japan. Human trials have yet to determine effects of long-term DAG oil consumption on energy metabolism. Furthermore, the effect of DAG oil consumption on energy expenditure (EE), body composition, lipid profiles, and hepatic lipogenesis have not been assessed together in a single study. Therefore, the objective of the present work was to assess the effects of DAG oil supplementation for 4 wk on resting EE (REE), postprandial EE, fat oxidation, lipid profiles, hepatic lipogenesis, body weight, and body composition. We tested the hypothesis that DAG oil consumption would increase total EE, postprandial EE, fat oxidation, and hepatic lipogenesis and favorably modify lipid profile, body weight, and body composition in overweight, hypertriglyceridemic women.

Methods
Participants. Twenty-nine nonsmoking females who did not take lipid-lowering medication, aged between 18–65 y, with a BMI 24.5–36 kg/m²,
and serum TAG concentrations >1.0 mmol/L (1.81 ± 0.66 mmol/L) were
recruited by radio advertising. Participants were excluded if they were
diagnosed with diabetes mellitus or kidney or liver disease. Exclusion
criteria also included alcohol consumption >2 drinks/d (34.12 g alcohol)
or use of laxatives, concentrated fiber, fish oil, or plant sterols. Fasting
blood samples were collected to screen for normal biochemical and
hematological characteristics and a physical examination was carried
out by a physician. The study protocol was reviewed and approved by
the Human Ethical Review Committee of the University of Manitoba.
All participants received explanations about the protocol and provided
written consent.

Sample size. Group size (n = 26) was calculated to provide an 80%
probability at P < 0.05 of detecting an anticipated difference of 2 kg of
body fat mass as measured by dual X-ray absorptiometry (DEXA). A sample size of 14 was calculated as being sufficient for this 2
C.G.

Study design. The study was a randomized, single-blind, crossover
study consisting of 2 independent phases of 4 wk during which participants consumed either DAG oil or a control oil blend comprised
of sunflower, safflower, and rapeseed oil at a ratio of 1:1:1. A washout
period of at least 4 wk separated the 2 study phases; in our experience,
this period is sufficient. To reduce the error term associated with diet
sequencing, the women were randomly assigned to 1 of 2 predetermined
Latin squares, each of which possessed 2 sequenced phases and 2
subjects. In this manner, we ensured that the crossover design was
balanced. During each intervention, a typical North American breakfast
was provided each day by the metabolic kitchen at the Richardson
Centre for Functional Foods and Nutraceuticals (RCFFN) of the
University of Manitoba. Participants came every day during the
intervention period and consumed the breakfast containing one-half
the portion of the DAG or control oil. A total of 40 g/d of test oil was
consumed by study participants during each intervention. On each day,
20 g of test oil assigned to that phase was incorporated into participants’
breakfasts, which were consumed under supervision, while 20 g was
given to the participants to be consumed with their other meals of the
day. Food energy intake was not controlled during the study period but
was controlled only for breakfast on the day of EE measurement. Basal
energy requirements of the women were estimated using the Mifflin
equation (17). Maintaining a consistent physical activity level during the
2 interventions was strongly recommended.

Energy expenditure. EE was measured using indirect calorimetry
(Viisay Vmax Encore 29N, Summit Technology). Substrate oxidation
was determined from the respiratory quotients on a per-minute basis.
Protein oxidation was calculated and assumed to be constant for each
measurement (18). All lean mass data were obtained from the DEXA
data. The trapezoid method was used to calculate the thermic effect
of food. Metabolic rates were plotted against time under the 6-h curve
minus the REE value obtained at 0 h.

Body composition. Whole-body DEXA scans (GE Lunar BX-1 L-
8743, GE Healthcare) were performed on participants at the RCFFN at
the beginning and end of each experimental phase. The DEXA unit used
is a GE Lunar BX-1 L-8743 scanner. Software used to analyze body
composition was Encore 2005, produced by GE Healthcare. Total (fat
and lean) tissue mass and tissue masses at trunk, android, and gynoid
areas were analyzed individually.

Serum analyses. Blood was collected after a 12-h fast on d 1 and 29 of
each phase. Blood samples were centrifuged at 1500 × g at 4°C for
20 min. Serum was immediately separated and stored at −80°C for
future analysis. Total cholesterol, HDL cholesterol, and TAG concen-
trations were measured by automated methods (Ortho-Vitros 350). LDL
cholesterol concentrations were calculated using the Friedewald equa-
tion (19), because all participants had TAG concentrations <4.5 mmol/L.
All samples were analyzed in duplicate and d1 and 29 samples were used
as initial and endpoint data, respectively.

Hepatic lipogenesis. Participants received an oral dose of 0.7 g
deuterium oxide/kg estimated body water on d 28 of each study phase.
Blood was collected on d 28 (h 0) and 29 (h 24). Plasma was separated
from RBC and stored at −80°C until further analysis. Human TAG fatty
acid (TAG-FA) synthesis was measured as the rate of incorporation of
deuterium from the plasma water pool into newly synthesized fatty acids
over 24 h. The fraction of newly synthesized TAG-FA was taken as the
enrichment of this phase (d 28) pool relative to the peak level of
achievable enrichment on d 29. Lipid fractions of plasma were extracted
(20) and samples were analyzed for deuterium enrichment using GC
(Agilent 6890N) isotope ratio MS (Delta V plus, ThermoFinnigan)
(21,22). Separation was achieved using a 6890 N Agilent GC fused
capillary column. Isolated TAG was directed to the pyrolysis reactor to
release hydrogen gas into the MS analyzed for deuterium abundance by
isotope ratio MS. The isotope ratio mass spectrometer was calibrated
each time before use using 3 water standards, including Standard Mean
Ocean Water. Samples for each woman were analyzed using the same set
of standards.

Lean mass was determined from data of DEXA and whole-body
water pool size was estimated by multiplying lean body mass by a factor
of 0.73 (23). Deuterium enrichment of the plasma TAG was estimated by
the following equation:

\[
\text{Fractional synthesis rate (FSR)} = \frac{\Delta \text{TAGFA}}{\Delta \text{plasma}} \times 0.477
\]

where \(\Delta \text{TAGFA}\) is the change in deuterium enrichment over the
initial linear period of incorporation and \(\Delta \text{plasma}\) is the mean circulatory pool
deuterium enrichment over the 24 h.

The factor 0.477 was derived from the value given by Jungs of 0.87
g-atm \(H_2\) formed/g-atm carbon \(C\) \(C\) \(24\) incorporated into adipose tissue fatty
acids and a correction calculation to account for the glycerol moiety in a
hypothetical TAG containing 3 monounsaturated 17-C fatty acids as
previously described (25).

Statistical analysis. All data were expressed as means and SE. Baseline,
endpoint, and percent change from baseline data were compared using
paired Student’s t tests. Spearman rank correlation was used to test the
association between FSR and serum TAG concentrations. Differences
were considered significant at \(P < 0.05\). JMP statistical software, student
dition (SAS Institute) was utilized to carry out the analyses.

Results

Participants. Twenty-nine predominantly overweight and mild
hypertriglyceridemic females were recruited, of whom 26 (20
premenopausal and 6 postmenopausal; 34.3 ± 2.6 y, 78.3 ± 3.6 kg
body weight, BMI 30.0 ± 0.7 kg/m\(^2\)) completed the study. Reasons
for dropping out included moving away from the city (\(n = 1\)),
failure to commit to daily attendance at the RCFFN for meals (\(n = 1\)),
and no reason given (\(n = 1\)). All participants tolerated the
treatment and test oils well. Only 1 participant reported a case of flu
during the study, which was not considered to be linked to the test
oils. The sequence of treatments did not affect any of the variables
analyzed.

Energy expenditure. RPE was similar at the commencement and
endpoint of each phase (Supplemental Table 1). DAG oil
consumption did not increase EE or fat oxidation during the 6-h interval
after meal consumption compared with baseline or control oil. Similarly, total EE, fat oxidation, thermic effect
of food, carbohydrate oxidation rates, and percentage changes in

Diacetylglycerol and obesity 1123

Downloaded from jn.nutrition.org by guest on October 14, 2017
energy variables did not differ between the study periods. DAG oil consumption did not result in a different rate of thermogenesis compared with the control oil.

**Body composition.** Baseline body weights did not differ between DAG (81.2 ± 2.5 kg) and control oil (81.9 ± 2.5 kg) phases. Although endpoint body weight was higher after control oil intake (82.4 ± 2.5 kg) compared with DAG oil (81.4 ± 2.5 kg) (P < 0.05), the percent changes in body weight did not differ between the DAG oil (0.2 ± 0.3%) and control oil phases (0.6 ± 0.3%). At endpoint, DAG oil reduced total body fat and fat in trunk, android, and gynoid areas compared with control oil (Table 1) (P < 0.05) without changing lean mass (Supplemental Table 2). However, the percent changes in body composition did not differ between the dietary interventions.

**Serum lipids.** Serum lipid concentrations did not change after consumption of DAG or control oil and there were no differences between the 2 oil interventions (Supplemental Fig. 1; Table 2).

**Hepatic lipogenesis.** Body water was enriched similarly in phases (13.8 ± 6.5) differences between the 2 oil interventions (Supplemental Fig. 1). Consumption of DAG or control oil and there were no serum lipids. There were no differences between the dietary interventions. DAG consumption for 4 wk on multiple components of DAG and control oil periods.

### TABLE 1 Adipose tissue weights in overweight, hypertriglyceridemic women before and after 4-wk DAG and control oil periods

<table>
<thead>
<tr>
<th>Fat region</th>
<th>DAG oil</th>
<th>Control oil</th>
<th>Change during phase</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Endpoint</td>
<td>Baseline</td>
<td>Endpoint</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35.1 ± 1.9</td>
<td>34.8 ± 1.6</td>
<td>35.0 ± 1.8</td>
<td>35.7 ± 1.7</td>
</tr>
<tr>
<td>Trunk</td>
<td>17.4 ± 1.1</td>
<td>17.3 ± 1.0</td>
<td>16.92 ± 1.1</td>
<td>18.01 ± 1.0</td>
</tr>
<tr>
<td>Android</td>
<td>3.03 ± 0.2</td>
<td>3.05 ± 0.2</td>
<td>2.96 ± 0.2</td>
<td>3.30 ± 0.2</td>
</tr>
<tr>
<td>Gynoid</td>
<td>6.46 ± 0.3</td>
<td>6.46 ± 0.3</td>
<td>6.38 ± 0.3</td>
<td>6.67 ± 0.4</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 26.

The present results suggest that DAG oil consumption for 4 wk reduces body fat in the trunk, android, and gynoid areas in females compared with control oil consumption. The observed body weight and composition changes in response to DAG oil consumption appear to be modulated by factors other than changes in EE, fat oxidation, or lipogenesis. The exact mechanisms of action of DAG oil in modulating body fat were not identified in the present investigation.

The importance of energy balance to body weight and composition has been well established. To our knowledge, the present study is the first to examine the effects of controlled DAG oil consumption for 4 wk on multiple components of energy balance, including postprandial EE and fat oxidation, in women. The absence of change in acute and medium-term postprandial EE and fat oxidation with the consumption of DAG oil is consistent with the results reported by Hibi et al. (26). Other studies have shown that DAG oil consumption increases short-term total EE (27) and fat oxidation (27–29); in those trials (28,29), participants maintained an inactive state during the energy measurement interval, as was the case in the present protocol. Therefore, body weight and composition modifications in our study may have been due to DAG oil consumption with increased total EE and fat oxidation. More research is required to reconcile the impact of DAG oil consumption on total EE and fat oxidation with restricted energy intake and/or increased physical activity. Hibi et al. (26) suggested that DAG oil consumption may increase fat oxidation in people who are overweight or obese. However, this conclusion was based on a small sample size (n = 11). A growing body of evidence suggests that hepatic lipid oxidation may influence appetite (3,4,30,31). Inhibitors of hepatic fat oxidation do not reduce food intake and it is possible that stimulation of hepatic fat oxidation by DAG might therefore reduce appetite (3,4). However, DAG oil consumption did not affect fat oxidation in the present study, even though our participants had higher BMI values than reported in other studies (26–28). Therefore, the absence of response of postprandial total EE and fat oxidation in the present work may have been due to our participants being less sensitive to DAG oil than were individuals with higher lean mass, as overweight or obese individuals are more prone to store energy as adipose tissue fat.

We observed that the lower body weight associated with DAG oil feeding was also associated with lower total body fat as well as fat in trunk, android, and gynoid areas, consistent with previous findings (31–35). Contradictory data have shown that DAG oil exerted no effect on body weight and composition (7,36). However, these latter studies did not use a crossover design, so their ability to measure the impact of DAG oil on body weight/composition may have been compromised by individual variation. Nagao et al. (33) demonstrated that DAG oil consumption reduced body weight by 1.5 kg over 4 mo. Our results suggest that DAG oil consumption resulted in a 0.3-kg greater weight reduction than the conventional oil over the 4-wk period, which is similar to the results of Nagao et al. (33). Kamphuis et al. (28) suggested that DAG oil consumption causes 4 g/d more fat to be shunted to oxidation than when control oil with similar side chain fatty acids are consumed, resulting in

### TABLE 2 Percent changes in serum lipid concentrations in overweight, hypertriglyceridemic women after 4-wk DAG and control oil periods

<table>
<thead>
<tr>
<th>Serum lipids</th>
<th>DAG oil</th>
<th>Control oil</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>−1.5 ± 1.7</td>
<td>−1.8 ± 2.0</td>
<td>0.46</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>2.1 ± 3.0</td>
<td>−1.3 ± 3.0</td>
<td>0.49</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−3.2 ± 2.0</td>
<td>2.6 ± 3.0</td>
<td>0.63</td>
</tr>
<tr>
<td>TAG</td>
<td>−3.0 ± 7.0</td>
<td>−8.7 ± 6.0</td>
<td>0.64</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 26.
0.112 kg of fat loss in 4 wk. However, the absence of a detectable change in EE and lipogenesis data in our study does not support the weight reduction and body composition modification effect. Therefore, DAG oil may be acting as an effective appetite control agent (28). Other factors, including use of a different population, lack of control of energy intakes, men compared with women, lean compared with overweight, and different doses of test oils might have contributed to the diversity between our results and those of previous reports.

In the present study, consumption of DAG oil for a period of 4 wk showed that moderate body weight and composition changes do not alter fasting lipid concentrations, which was also suggested by Santosa et al. (37). Our results are consistent with previous studies examining the effects of DAG on lipid profiles (7,9,33,35,36,38,39). However, contradictory studies suggest that DAG oil consumption does modify lipid profiles (6,8,34). Reyes et al. (39–41) concluded that differences in dietary fat intake between Japanese and North American populations may account for the lack of response to the DAG oil consumption in the latter group. Similarly, a background high-fat diet may be one of the reasons for the lack of response in serum lipid concentrations to DAG oil consumption in the present study, in which lipid concentrations were measured once at baseline and once at the endpoint of each phase. Failure to measure concentrations for at least 2 consecutive days and averaging them could also contribute to biologic variability in the lipid measurements. However, the sample size used in the current study was higher than sample size calculated based on previous studies (7). Tomonobu et al. (42) have suggested that DAG oil consumption modifies serum TAG in healthy participants with lower baseline TAG concentrations. Therefore, baseline fasting TAG (1.81 ± 0.66 mmol/L) in the present study may not be a reason for participants not responding to DAG oil consumption.

To our knowledge, this is the first study in women examining the effects of DAG oil consumption on TAG-FA synthesis using deuterium incorporation. DAG oil consumption for a period of 4 wk did not alter hepatic lipogenesis in the present study. Our observations are consistent with the results of Saiito et al. (38). However, Taguchi et al. (16) observed a decreased hepatic TAG concentration with DAG oil consumption, but this study only assessed short term DAG oil consumption, which may explain the differences across studies. In addition, we observed a positive correlation between FSR and fasting serum TAG with control oil consumption, and a tendency for an association with DAG oil consumption. However, it is difficult to provide an explanation for these observations.

In conclusion, although similar EE, fat oxidation, blood lipid profiles, and hepatic lipogenesis rates occurred after the women consumed DAG and control oils in the present study, incorporation of DAG oil in daily diets for 4 wk resulted in lower body weight and adiposity compared with conventional oil consumption. We conclude that DAG oil may be useful for weight loss among overweight women and further research in other groups is warranted.

Acknowledgments

We thank Khatima Khalloufi for assisting in lipid extraction. P.J.H.J. and Y-M.C. designed the clinical study; Q.Y. and Y-M.C. conducted the clinical study; Q.Y. and V.R.R. analyzed data; V.R.R. and Q.Y. wrote the paper. V.R.R., Q.Y., and P.J.H.J. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited

38. Saito S, Hernandez-Ono A, Ginsberg HN. Dietary 1,3-diacylglycerol protects against diet-induced obesity and insulin resistance. Metabolism. 2007;56:1566–75.