Postprandial Lipid-Related Metabolites Are Altered in Dogs Fed Dietary Diacylglycerol and Low Glycemic Index Starch during Weight Loss\textsuperscript{1–3}

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Abstract

In this study, we investigated a combination of a low glycemic index starch (LGIS) and diacylglycerol (DAG) on lipid, lipoprotein (LP) metabolism, and weight management. Obese, intact female adult Beagle dogs were assigned to 1 of 4 starch/oil combination diets [LGIS/DAG (LD); LGIS/triacylglycerol (TAG); high glycemic index starch (HGIS)/DAG; and HGIS/TAG (HT)] and fed for 9 wk (n = 6/group) using an incomplete 4 \times 4 Latin square design. Each dog was fed 1 of 2 opposite starch/oil combination diets (e.g. LD and HT). At wk 1 and 8, postprandial blood was collected for plasma triacylglycerol (TG), \(\beta\)-hydroxybutyrate (BHB), total cholesterol (TC), and LP analyses. During the same week, dogs were overnight fed-deprived and post-heparin blood was collected for LP lipase and hepatic lipase activity determinations. At wk 1, 4, and 8, blood was drawn from overnight feed-deprived dogs for plasma TG, BHB, TC, LP, leptin, and adiponectin measurements. Feces were collected at wk 3 for digestibility calculations. The LGIS diets resulted in lower carbohydrate, protein, total tract dry matter digestibilities, and metabolizable energy compared with the HGIS diet groups (\(P < 0.05\)). Thus, the LGIS groups lost more body weight (\(P = 0.001\)), which was positively correlated with plasma leptin concentrations (\(r^2 = 0.427; P < 0.001\)). Moreover, the LGIS diet lowered TC concentrations in combination with DAG. The DAG diet groups decreased postprandial TG and increased BHB concentrations (\(P < 0.05\)). Starch/oil types did not alter lipase activities or adiponectin concentrations. In conclusion, the LGIS diet demonstrated potential as a weight management tool in dogs by decreasing postprandial TG and increasing BHB in combination with DAG. J. Nutr. 140: 1815–1823, 2010.

Introduction

 Obesity is a common nutritional disorder in both human and veterinary medicine and is associated with several metabolic diseases (1). Consequently, safe and effective strategies to decrease or prevent its incidence are needed. Common strategies for obesity management are to provide a combination of energy restriction and exercise. However, this approach is not always successful, because it often increases metabolic and behavioral stress in animals and related loss of compliance in humans (2). Thus, any appropriate dietary management of obesity must provide both efficient and healthy weight loss while minimizing metabolic or other stressors. Two nutrients that have been described in recent years to be of potential benefit in human and rodent studies are diacylglycerol (DAG)\textsuperscript{8} and low glycemic index starch (LGIS).

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\textsuperscript{3} Supplemental Tables 1 and 2 are available with the online posting of this paper at jn.nutrition.org.

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\textsuperscript{4} Abbreviations used: BCS, body condition score; BHB, \(\beta\)-hydroxybutyrate; DAG, diacylglycerol; HD, high glycemic index starch/diacylglycerol; HGIS, high glycemic index starch; HL, hepatic lipase; HT, high glycemic index starch/triacylglycerol; LD, low glycemic index starch/diacylglycerol; LGIS, low glycemic index starch; LP, lipoprotein; LP-C, lipoprotein-cholesterol; LPL, lipoprotein lipase; LT, low glycemic index starch/triacylglycerol; ME, metabolizable energy; MER, maintenance energy requirement; TAG, triacylglycerol; TC, total cholesterol; TG, triacylglycerol (in plasma).
DAG is a minor acylglycerol in edible oils in which 2 fatty acids are esterified to either sn-1 and 2 (1,2-DAG) or sn-1 and 3 (1,3-DAG) positions. The 1,3-DAG, one of the DAG isomers, has been reported to enhance fat oxidation as an adjunct to a hypolipidemic effect in both humans and rats (3–8). In contrast, LGIS (i.e. amylose) is a type of starch that provides slower hydrolysis of amylose, thereby providing a slower, yet more consistent, source of glucose to the blood stream. It is expected that this type of starch would stimulate less insulin release than high glycemic index starch (HGIS) (i.e. amylopectin) (9). Because insulin stimulates lipogenesis and inhibits lipolysis, it is theoretically possible that more weight loss may be achieved with longer term ingestion of LGIS-containing foods compared with HGIS-containing foods (10). The concept of DAG and LGIS in diets has been described in human nutrition; however, the effects of these nutrients on lipid metabolism in a canine model of obesity under controlled conditions have received little attention.

We previously investigated the postprandial effects of a single meal containing 20 g DAG oil and 25 g of either LGIS or HGIS mixed with 60 g of boiled boneless chicken breast to healthy intact female adult Beagles (11). The results indicated that the DAG-containing meals decreased plasma triacylglycerol (TG) 2 and 3 h postprandially (P < 0.05), whereas the LGIS meals resulted in a modestly increased plasma hepatic lipase (HL) activity in samples obtained 6 h postprandially compared with the HGIS-containing meals (P < 0.085) (11). An earlier study found that feeding a DAG-containing diet to normal adult Beagles for 6 wk led to a greater decrease in body weight compared with the triacylglycerol (TAG)-fed group (12). Therefore, it was of interest to evaluate a DAG/LGIS combined diet on canine obesity management and lipid metabolism. Our aim was to investigate the longer term (i.e. 9 wk) effects of DAG when combined with either LGIS or HGIS on postprandial and fasting plasma lipid and lipoprotein (LP) metabolism during canine weight loss.

Materials and Methods

This study was approved by the Texas A&M University Animal Care and Use Committee. All Beagle dogs were housed individually at the Laboratory Animal Research Resources facility, Texas A&M University according to the American Physiological Society Guidelines for Animal Research and guidelines set forth by Texas A&M University Care and Use Committee. Each kennel for dogs was 2.4 m long, 2.7 m high, and 1.2 m wide. Prior to the study, physical examinations, complete blood counts, and serum biochemical profile tests were performed on all dogs to ensure their normal clinical status.

Experimental diets. Four experimental diets were prepared containing starch (LGIS vs. HGIS) and oil (DAG vs. TAG) using a 2 × 2 factorial model as follows: LGIS/DAG (LD), LGIS/TAG (LT), HGIS/DAG (HD), and HGIS/TAG (HT). These diets were formulated in our laboratory using a mixture of 430 g/kg of chicken by-product meal (Tyson Foods), 135 g/kg of DAG or TAG-enriched dietary oil (Kao Corporation), and 430 g/kg of LGIS or HGIS (Nihon Shokuhin Kako) to provide the same amount of macronutrients in each diet (Supplemental Table 1). Also, 5 g/kg of a vitamin/mineral premix for dogs (Akey Industries) was added to the diets to meet maintenance requirements for the dogs. Gelatinized, high amylopectin corn starch, and waxy corn starch were prepared using the drum-drying method as LGIS and HGIS sources, respectively. After gelatinization, the contents of total, resistant, and digestible starch in 100 g of corn starch were 88 g, 17 g, and 71 g for LGIS and 88 g, 0 g, and 88 g for HGIS, respectively. The DAG and TAG oils in combination with the other diet ingredients contained similar fatty acid compositions (Supplemental Table 2). To eliminate composition alterations due to batch differences, all dry ingredients except oils were homogenized together using a mixer (Hobart Industries) at Texas A&M University and stored in a dark, ambient temperature-controlled storage room in our laboratory before the study started and were used throughout the study. The homogenized ingredients had a powdered texture, to which 2–3 volumes of water (~2500 g/kg homogenized powder diet) was added along with oils before feeding. After mixing these homogenized powders with oil and water, all diets had a gritty-like appearance due to the presence of the gelatinized starches.

Study design. This study used an incomplete 4 × 4 Latin square design (13) that consisted of two 4-wk diet acclimations followed by 9-wk experimental periods separated by a 17-wk washout period. Although 4 periods are typically required to complete this design, only 2 periods were used due to time length limitations of the study and avoidance of possible confounding metabolic alteration by repetitive yo-yo dieting between periods. The incomplete 4 × 4 Latin square was constructed via random assignment of 3 dogs in each treatment square at period 1. At period 2, the opposite oil and starch types from period 1 were assigned to dogs (i.e. if fed LD in period 1, they were then fed HT in period 2). Twelve adult intact female Beagles aged from 2 to 6 y at the beginning of the study were used. Because they were not initially obese, obesity was induced prior to the 2 experimental periods by feeding an extruded dry food (Science Diet Adult Original, Hill’s Pet Nutrition), a 50:50 (v:v) mixture of canola and soybean oils (40 g), and pegan shortbread cookies (Keebler) as an appetite stimulant. Dogs were stabilized at their obese body weights for an additional 6–8 wk prior to the experimental feeding period. An acclimation diet that contained a mixture of 50:50 (wt-wt) blend of LGIS and HGIS, a 50:50 (v:v) mixture of canola and soybean oils, the vitamin premix, and chicken by-product meal was fed for 4 wk prior to the study at an amount of energy designed to maintain their obese body weights [obese maintenance energy requirement (mMER) in kJ/d = 523 × obese body weight in kg0.75]. This diet provided similar macronutrient and fatty acid compositions to the experimental diets and with a similar texture. Because of lower palatability of this diet compared with the obesity induction diet, the dogs consumed only ~70% of the obese mMER amount of food offered. As such, they began to lose some body weight during this time. However, the extent of weight loss was minimal overall and did not affect the obese condition of the dogs based on percent body fat and body condition score (BCS). After the acclimation period, at wk 1, the dogs were randomly assigned to 4 diet groups (n = 3/group in each period) according to age, body weight, and BCS to minimize bias and were fed one of the experimental diets (LD, LT, HD, or HT) using the same energy calculation that was used during the acclimation period. This obese mMER amount of food was offered in excess of that needed to lose weight. However, because the acclimation diet mimicked the experimental diets, we expected that the animals would continue to consume a similarly reduced amount of experimental diets, thereby resulting in weight loss. In addition, we wanted to ensure that more food was available in the event that this was not the case. Indeed, the dogs voluntarily consumed 68 ± 4% (mean ± SEM) of the amount of food per day in grams that was offered independently of starch and oil types. Compared with the obesity induction diet, it is likely that the palatability of the experimental diets was not as high as those foods containing added flavors or other palatability enhancers such as exist in commercial pet food products or human foods. Furthermore, it should be noted that, in modern veterinary practice, 35–40% food restriction is commonly used as one of the strategies to achieve healthy weight loss (14). Therefore, the lower food consumption provided a useful way to model safe and consistent weight loss in the canine model using diets of known composition when fed for a longer term. The diets were prepared each morning during the acclimation and experimental feeding periods. All food was removed from the kennels 5 h post feeding and weighed. Body weights were monitored weekly. At 1 wk prior to the study and at wk 4 and 9, body fat was measured using a body fat analyzer (Kao Corporation) that employed bioelectrical impedance analysis (15). Dogs consumed water ad libitum and were allowed to exercise freely inside their kennels during the study. Prior to the study, the degree of obesity in dogs was analyzed using the body fat analyzer and BCS. Body weights, BCS, and body fat
estimates were 14.8 ± 0.2 kg, 8.4 ± 0.1/9, and 48.9 ± 3.3% (mean ± SEM), respectively, at this time.

**Fecal collection.** Twenty-four-hour fecal samples were collected for 5 d consecutively during wk 3 during both periods. Feces were pooled for each dog and frozen at -80°C for subsequent analysis.

**Blood collection.** At wk 1 and 8, 7–21 mL postprandial blood was collected at 0, 15, 30, 60, 120, and 180 min. After withholding food overnight, postprandial sampling meals were prepared to ensure rapid consumption. These meals were formulated using a mixture of 80 g of boiled chicken breast meat, 8 g of either TAG or DAG oil, 25 g of LGIS or HGIS, and water to obtain a similar degree of nutrient composition as the experimental diets. Because it was critical that the dogs consumed these meals quickly, ~30% of the obese MER amount of the meal was prepared (i.e., 1150 kJ). All dogs consumed these meals within 5 min. Catheters had been placed into the dogs’ jugular veins before the postprandial diets were fed. Blood was collected via the catheters into EDTA-containing tubes.

At wk 1, 4, and 8 food was withheld overnight and 7 mL of blood from feed-deprived dogs was collected via a jugular vein into EDTA tubes. A protease inhibitor (600 trypsin inhibitory units/L blood of aprotinin, Sigma-Aldrich) was added to blood samples for adiponectin and leptin analyses. During wk 1 and 8, on days when postprandial samples were not taken, dogs were fasted overnight and 3 mL of post-heparin blood was collected via a cephalic vein 10 min after 100 IU sodium heparin/kg body weight was i.v. injected into the opposite cephalic vein. Plasma was separated by low speed centrifugation for 15 min and stored at -20°C until analyses and <50 µL of plasma was stored at 4°C and used for LP fraction determinations within 2 d.

**Digestibility and metabolizable energy determinations.** The pooled fecal samples were sent to Midwest Laboratories and were analyzed for the following: moisture, dry matter, crude protein, acid hydrolyzed fat, crude fiber, and ash. Based on these data, digestibilities of protein, fat, carbohydrate, and total tract dry matter were calculated using the following formula: nutrient digestibility (%) = [(nutrient intake – nutrient in feces)/nutrient intake] × 100. Metabolizable energy (ME) was calculated based on the digestibility data as follows: ME = [gross energy of food consumed – gross energy of feces collected – (grams protein consumed – grams protein in feces)× correction factor for energy lost in urine]/amount of food consumed. The correction factor for energy lost in urine was considered to be 5.23 kJ/g (16).

**Plasma lipid-related metabolite determinations.** Plasma TG was measured by an enzymatic colorimetric assay using triacylglycerol GPO reagent (Bayer Healthcare). Total cholesterol (TC) was measured using an enzymatic colorimetric assay described by Warnick (17). LP fractions (β-, pre-β-, and α-LP) were separated by electrophoresis using 1% agarose gel (Helena Laboratories) (18). Relative amounts of each fraction were determined by densitometry (Bio-Rad Laboratories). Absolute amounts of the LP fractions were calculated based on TC concentrations and results presented as LP cholesterol in mmol/L. β-Hydroxybutyrate (BHB) was measured as a marker of possible fat mobilization and β-oxidation using a 2-point kinetic method (19). Mercodia Porcine Insulin ELISA (Mercodia AB) was used for insulin analyses according to Bennet et al. (20) and Sato et al. (21).

**Plasma LP lipase and HL activity measurement.** In vitro LP lipase (LPL) and HL activity were determined using the methods of Nilsson-Ehle et al. (22) with some modifications. The substrate mixture was sonicated (Biologics) in an ice bath for 4 min at 30-s intervals using a 40-W setting. Labeled fatty acids released were measured via scintillation counting (Packard Instrument Company).

**Plasma adiponectin and leptin.** Plasma adiponectin concentrations were measured using an adiponectin (murine/rat) ELISA kit (Otsuka Pharmaceutical) and appropriately validated ELISA methodology (23). Plasma leptin concentrations were determined by the procedure of Iwase et al. (24), which has been validated in dogs by Ishioka et al. (25,26).

**Statistical analyses.** Data were expressed as means ± SEM. Postprandial plasma lipid concentrations were converted to percent change from baseline concentrations (0 min) because of wide variability at 0 min. All data were analyzed by SPSS 15.0 for Windows using a GLM model repeated-measures ANOVA with oil, starch, and period as between-subject factors and postprandial time for postprandial samples and week for fasting samples as a within-subject factor. When main effects and/or interactions were observed, pairwise comparisons using a Bonferroni’s correction were performed at each level as follow-up tests. To assess oil, starch, or oil × starch interaction effects within each time period, 2-way ANOVA with blocking on period was conducted using oil and starch types as a fixed factor. Where significant interactions, main oil, or starch effects were observed, follow-up comparisons at each level by Bonferroni’s adjustment were performed.

Body weight decreased from baseline (P < 0.001) and the amount of body weight lost from baseline was greater when dogs consumed the LGIS diet compared with the HGIS diet (P = 0.001). Consequently, analysis of data obtained at wk 4 and 8 included percent body weight lost as a covariate in the above-mentioned statistical models. Correlations of leptin and adiponectin vs. body weight or body fat, and leptin vs. adiponectin were analyzed using linear regression. Normality of dependent variables and homogeneity of population variance were analyzed before all tests were conducted. If data were not normally distributed, appropriate nonparametric tests were performed. Where variances were not homogeneous, data were transformed as log_{10}. Differences were considered significant at P < 0.05.

**Results**

**Digestibility, ME, and food consumption.** None of the diets resulted in diarrhea, loose stools, or health issues. Feeding LGIS decreased carbohydrate, protein, and total tract dry matter digestibilities compared with HGIS feeding (P < 0.005; Table 1). However, fat digestibility remained high independently of the diets (Table 1). Because ME was calculated based on the digestibility data, ME was lower in the LGIS diet groups than the HGIS diet groups (P < 0.001; Table 1). The lowered ME in the LGIS diets resulted in less metabolically available energy intake than the HGIS diets (P = 0.036), although the weight of food consumed did not differ between the dogs that were fed the 2 starch types (Table 1).

**Body weight and body fat.** Except for 1 dog during period 1 eating the HT diet, all dogs lost body weight during the study. Moreover, their percent body weight loss from baseline (wk 1) differed between starch types. Beginning at wk 2 of the study and remaining significant thereafter (P < 0.01), dogs consuming the LGIS diets lost a higher percentage of their body weight at each time point than the HGIS diet groups lost (P = 0.001) (Fig. 1). Overall, repeated-measures ANOVA revealed all groups decreased their percent body fat with a 9.3% loss at the end of the study independently of starch and oil types (P < 0.001; Table 2).

**Plasma TG.** Regardless of diet, plasma TG concentrations in dogs that had been feed-deprived overnight were higher at wk 8 (0.46 ± 0.03 mmol/L) than at wk 1 (0.30 ± 0.02 mmol/L or wk 4 (0.33 ± 0.02 mmol/L). However, all concentrations were within the normal range as determined in the clinical pathology laboratory at the College of Veterinary Medicine, Texas A&M University. At wk 1, as expected, postprandial plasma TG concentrations increased compared with baseline (0 min, P < 0.001). The TAG-containing diet group had increased postprandial plasma TG at 180 min compared with the DAG-containing diet group in which TG concentrations remained relatively low during the 180-min postprandial period of this study (P = 0.009; Fig. 2A). At wk 8, a time × oil × starch interaction was observed
Further analyses within each postprandial time point revealed that only the LT diet resulted in increased plasma TG concentrations at 60 min postprandially compared with the other 3 diets ($P = 0.003$; Fig. 3A).

Plasma insulin. At wk 1 between 30 min and 180 min, postprandial plasma insulin increased in the LGIS diet groups compared with LGIS diet groups ($P = 0.003$). At wk 8, some blunting of the insulin response had occurred, because only dogs fed HT had elevated concentrations at 180 min postprandially ($P = 0.017$) (data not shown).

Plasma BHB. Plasma BHB concentrations of dogs that had been overnight feed-deprived did not differ among diet types (Table 2). At wk 1, neither starch nor oil type affected the postprandial plasma BHB concentrations (Fig. 2B). However, at wk 8, the DAG-containing diets showed a increase of plasma BHB concentrations at both 60 and 120 min postprandially compared with the TAG-containing diets ($P = 0.05$; Fig. 3B).

Plasma TC. Feed-deprived plasma TC concentrations were not altered by time, oil, or starch types (Table 2). Postprandially, a significant oil \times starch interaction was observed at wk 1; however, further analyses did not show any significant differences (Fig. 2C). By contrast, at wk 8, repeated-measures ANOVA resulted in a week \times oil \times starch interaction. Further analyses revealed lower plasma TC concentrations in the LGIS diets at 15 min postprandially ($P = 0.036$). Moreover, a significant oil \times starch interaction was found at 120 min in which the LD diet group had lower plasma TC concentrations than dogs fed the other diets ($P = 0.019$; Fig. 3C).

LPL and HL activities. LPL and HL activities were not affected by dietary starch, oil, or their interaction (Table 2).

Plasma adiponectin. A time \times oil interaction was observed ($P = 0.022$) due in part to large differences at the start of experimental periods. Indeed, further analyses revealed neither starch nor time effects within starch types (Table 2). In addition, there were no correlations between adiponectin and body weight or body fat.

Plasma leptin. Plasma leptin analyses showed a time \times starch interaction ($P < 0.001$) in which the LGIS diets decreased plasma leptin concentrations at wk 4 and 8 compared with wk 1, whereas the HGIS diets did not change. Further analysis using 2-way ANOVA, however, did not reveal any starch effect for either week (Table 2). At wk 1, plasma leptin concentrations of the LD diet group were modestly higher than the other 3 diets ($P = 0.019$; Fig. 3C). Further analyses within each postprandial time period revealed that only the LGI- and HGI-containing diet groups were $<0.001$ ns, respectively. Because the LGIS and HGIS diets were similar in terms of their NPD, these results suggest that the LGIS diets had a more pronounced effect on plasma leptin concentrations because they contained a greater proportion of DAGs than the HGIS diets.

Plasma LP. Because the pre-β LP fraction was not clearly separated in some of the samples, it was combined with the β LP fraction and data were presented as pre-β + β (Table 3). Fasting plasma pre-β + β, α-1 LP-cholesterol (LP-C) concentrations in dogs that were overnight feed-deprived did not differ as a function of time, oil, or starch types (data not shown). In

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**TABLE 1** Food consumed by dogs and the digestibility and ME of the diets varying in types of starch and fat$^1$

<table>
<thead>
<tr>
<th>Diet</th>
<th>LD</th>
<th>LT</th>
<th>HD</th>
<th>HT</th>
<th>P-value$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy consumed, MJ/d</td>
<td>2.07 ± 0.2</td>
<td>1.97 ± 0.3</td>
<td>2.59 ± 0.4</td>
<td>2.93 ± 0.4</td>
<td>ns$^4$</td>
</tr>
<tr>
<td>Food consumed, g DM/d</td>
<td>115.0 ± 9.4</td>
<td>111.8 ± 13.8</td>
<td>125.8 ± 21.4</td>
<td>143.1 ± 14.7</td>
<td>ns</td>
</tr>
<tr>
<td>Digestibility,$^5$ %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>64.9 ± 4.1</td>
<td>73.9 ± 0.9</td>
<td>98.2 ± 1.2</td>
<td>98.1 ± 0.9</td>
<td>ns &lt;0.001</td>
</tr>
<tr>
<td>Protein</td>
<td>83.1 ± 1.3</td>
<td>83.5 ± 1.8</td>
<td>94.4 ± 1.9</td>
<td>93.9 ± 3.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Fat</td>
<td>96.5 ± 0.6</td>
<td>96.5 ± 0.7</td>
<td>98.2 ± 0.7</td>
<td>98.1 ± 1.0</td>
<td>ns</td>
</tr>
<tr>
<td>Total tract dry matter</td>
<td>79.8 ± 2.1</td>
<td>82.1 ± 1.7</td>
<td>95.1 ± 1.2</td>
<td>93.7 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ME,$^6$ kJ/g</td>
<td>18.0 ± 0.6</td>
<td>17.6 ± 0.3</td>
<td>20.6 ± 0.3</td>
<td>20.2 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^1$ Values are mean ± SEM, n = 6. DM, dry matter.

$^2$ P-values are for ANOVA with starch and oil as fixed factors.

$^3$ O \times S represents an interaction effect of oil and starch.

$^4$ P = 0.05.

$^5$ Digestibility of protein, fat, carbohydrate, and total dry matter tract was calculated as follows based on fecal protein, fat, fiber, and ash contents, which were analyzed by Midwest Laboratories, Inc. Nutrient digestibility (%) = [nutrient intake – nutrient in feces]/nutrient intake] \times 100.

$^6$ ME was calculated using the following formula; ME = {gross energy of food consumed – gross energy of feces collected – (gram protein consumed – grams protein in feces) \times correction factor for energy lost in urine}/amount of food consumed. Correction factor for energy lost in urine is 5.23 kJ/g.

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*Plastic insulin* at wk 1 between 30 min and 180 min, postprandial plasma insulin increased in the HGIS diet groups compared with LGIS diet groups. Indeed, further analyses revealed neither starch nor time effects within starch types (Table 2). In addition, there were no correlations between adiponectin and body weight or body fat.
contrast, α-2 LP-C in dogs fed the HT diet increased at wk 1 compared with the other 3 diets (P = 0.026; LD, 1.71 ± 0.26 mmol/L; LT, 1.23 ± 0.18 mmol/L; HD, 1.59 ± 0.33 mmol/L; HT, 2.38 ± 0.29 mmol/L). However, this finding should not be related to experimental diet types, because overnight feed-deprived dog plasma data at wk 1 do not truly reflect a metabolic steady state induced by the experimental diets.

Similar to the plasma LP-C concentrations, the feed-deprived dogs had no significant treatment effects in any postprandial plasma LP fractions except α-2 LP-C. Postprandial plasma α-2 LP-C showed a time × oil × starch interaction at wk 1 in which the HT diet was decreased at 120 min compared with 0 min (P = 0.009). At wk 8, however, no oil, starch, or oil × starch interaction was observed (Table 3). Pre-β + β LP-C decreased at 120 min at both wk 1 and 8, but this was not related to the starch or oil types (Table 3).

**Discussion**

The aim of the present study was to determine the effects of DAG, LGIS, and a combination of DAG and LGIS on plasma lipid and LP metabolism in adult Beagles when fed for a 9-wk weight loss period. The source of the LGIS used in this study contained a high amount of amylose (~70%) and the HGIS contained 100% amylopectin. It should be noted that effects of diets containing gelatinized starch may vary depending on the conditions used during processing and the resultant distribution of resistant and digestible fractions obtained and have been detailed above for comparison purposes.

High amylose corn starch is a resistant starch that is poorly absorbed in the small intestine (27). It is widely recognized that both resistant starch and dietary fiber decrease apparent protein digestibility due to several mechanisms, including increased passage rate, endogenous nitrogen secretion, microbial production of organic acids, bacterial nitrogen fixation, adhesion of nutrients on fiber, and decreased ammonia production and absorption (28,29). Thus, some or all of these physiological properties may possibly result in lowered carbohydrate, protein, and therefore total tract dry matter digestibilities when LGIS diets are fed. The ME was calculated using total energy loss data via feces and urinary excretion, the latter of which is based on fecal protein loss. Thus, the lower ME of the LGIS diets was due to a combination of lower feed intake and decreased digestibility of organic matter.
to decreased protein and total tract dry matter digestibilities compared with the HGIS diets. Consequently, dogs fed the LGIS diets consumed less ME overall, resulting in greater reduction of body weights from their starting obese condition than those fed the HGIS diets.

In addition to a beneficial effect of LGIS on body weight, the DAG diet group had increased postprandial plasma BHB concentrations after 8 wk of feeding. The reasons why increased BHB concentrations occurred in this study are unknown at this time. Murase et al. (30) found that $\beta$-oxidation and lipid metabolism-related gene expressions were stimulated in the small intestine but not in liver, skeletal muscle, and brown adipose tissue after feeding 15% DAG to mice for 10 d. Murata et al. (5), on the other hand, found that feeding 9.4% DAG to rats for >14 d increased hepatic mitochondrial and peroxisomal oxidation of palmitoyl-CoA compared with 9.4% TAG feeding. Taking these results into account, increased BHB in this study may have been the result of increased $\beta$-oxidation in small intestine or liver. By contrast, an additional component of the postprandial increase in plasma BHB may have been the result of tissue mobilization given that the animals were in negative energy balance and all lost percent body fat and body weight during the experiment. However, it is not possible to determine the extent to which these phenomena may have separately contributed to the postprandial BHB alterations.

**FIGURE 2** Postprandial change in plasma TG (A), BHB (B), and TC (C) in dogs that consumed LD and HT or LT and HD diets at wk 1. Values are mean ± SEM, n = 6. *D and T (oil) differ at that time, P < 0.05.

**FIGURE 3** Postprandial change in plasma TG (A), BHB (B), and TC (C) in dogs that consumed LD and HT or LT and HD diets at wk 8. Values are mean ± SEM, n = 6. Means at a time without a common letter differ, $P < 0.05$. *D and T (oil) differ at that time, $P \leq 0.05$; #H and L (starch) differ at that time, $P < 0.05$. 

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TABLE 3 Postprandial LP-C concentrations in feed-deprived dogs that had consumed LD and HT or LT and HD diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Postprandial time</th>
<th>Chylomicrons</th>
<th>P-value</th>
<th>Repeated-measures ANOVA</th>
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<tr>
<td></td>
<td>min</td>
<td>mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>wk 1</td>
<td></td>
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<td>ns&lt;sup&gt;3&lt;/sup&gt;</td>
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<td></td>
<td>120</td>
<td>0.05 ± 0.01</td>
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<td>2.17 ± 0.28</td>
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<sup>1</sup> Values are mean ± SEM, n = 6. Means in a column within each LP-C without a common letter differ, P < 0.05. *0 min and 120 min within the same week for each LP fraction differ, P < 0.05. An asterisk denotes significant difference from 0 min within diets, P < 0.05.

<sup>2</sup> All variables, dietary starch, oil, and their interaction were not significant, P < 0.05.

<sup>3</sup> P > 0.05.

<sup>4</sup> The P-value indicates main time (minute) effect.

<sup>5</sup> The P-value indicates time × oil × starch interaction.

Postprandial plasma TG concentrations were lower at wk 1 when dogs consumed the DAG diets compared with the TAG diets. This observation has been consistently reported as a primary effect of DAG compared with TAG among various species, including dogs (4,30,31). After consuming the LGIS diets for 8 wk, however, TG elevations occurred only with the LT diet and not the HT diet. It should also be noted that postprandial plasma insulin concentrations significantly increased with the HT diet (data not shown). Because insulin is a lipogenic hormone, it is known to stimulate adipose LPL, fatty acid synthase, and DAG acyl transferase while directly inhibiting hormone sensitive lipase and indirectly inhibiting carnitine palmitoyl transferase I (32). Thus, the presence of increased amounts of insulin, by virtue of its stimulation of LPL activity in vivo, may have resulted in a relative lowering of plasma TG in the HT diet group, thereby masking any effect of a plasma TG elevation with TAG ingestion compared with DAG ingestion.

Although postprandial LPL activity may have been enhanced by the HGIS diets due to an increase of insulin concentration postprandially, the oil and starch type combination fed did not alter overnight feed-derived LPL and HL activity when measured in vitro in the present study.

Feeding DAG or LGIS diets to dogs did not affect their overnight feed-deprived plasma TC and LP-C concentrations under overnight feed-deprived conditions. By contrast, the LGIS-containing diets, especially the LD diet, lowered postprandial plasma TC concentrations but only at wk 8. High amylose corn starch, the source of LGIS in this study, reportedly has a TC-lowering effect involving an increase in the bile acid pool in the intestine and has been associated with increased bile acid secretion into feces due to its physiological property as resistant starch (28,29). DAG has also been found to have a cholesterol-lowering effect, but it is somewhat equivocal (33–36). Our finding that the LD diet lowered postprandial TC suggests that a synergistic response may have occurred with the dietary LGIS and DAG combination.

It is notable that feeding the HT diet decreased postprandial plasma α-2 LP-C (i.e. HDL cholesterol) concentrations at wk 1. Decreased HDL cholesterol is a risk factor for atherosclerosis (37). The LGIS- and DAG-containing diets did not decrease HDL cholesterol postprandially. Although the incidence of atherosclerosis in dogs is uncommon because of their unique LP metabolism (38), this finding is nonetheless important for understanding the effect of the DAG- and LGIS-containing diets on LP metabolism in dogs and may apply to other species such as humans.

Plasma adiponectin concentrations were not correlated with either body weight or body fat. In humans, adiponectin concentrations have been negatively correlated with the degree of obesity and are lower in obese individuals (39,40). In comparison, in dogs, Brunson et al. (41) reported that adiponectin gene expression and protein secretion are unaffected in a high fat-fed obese dog model and therefore concluded that plasma adiponectin concentrations in dogs are regulated differently compared with obese humans. Ishioka et al. (23) also noted that although plasma adiponectin can be an additional marker of adiposity in dogs, it is a less sensitive obesity index compared with leptin. By contrast, plasma leptin showed positive correlation with body weight, but its correlation with body fat was weak. During the study, even though the dogs lost an appreciable amount of body weight and decreased their BCS from 8.4 to 7, either the relative amounts of weight lost or the short term of the present ex-
periment may help explain the lack of correlation between leptin with body fat loss.

In summary, DAG, LGIS, and LD effects were demonstrated in adult Beagles fed a combination of starch (LGIS vs. HGIS) and oil (DAG vs. TAG) types for 9 wk during weight loss. First, although all dogs consumed the same amount of food on a dry matter weight basis, the LGIS diet groups increased body weight loss due to the lower ME, which resulted in decreased leptin concentrations (LGIS effects). Second, the DAG-containing diets lowered postprandial plasma TG and increased plasma BHb later in the study (DAG effects). The postprandial plasma TG in wk 8, however, was affected by both oil and starch types. Postprandial plasma TC was lowered with the combination of LGIS and DAG (a combined LD effect). Finally, postprandial plasma HDL cholesterol was not decreased by the LGIS- and DAG-containing diets. LPL and HL activities in the overnight feed-deprived dogs were not affected by LGIS and DAG in this study. In conclusion, although only dietary LGIS contributed to weight loss, its combination with DAG appears to have potential as a weight management tool in dogs and may be useful in humans by beneficially modifying lipid-related metabolites.

Acknowledgments
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Literature Cited


