Glucose Tolerance and Lipid Profiles in Dogs Fed Different Fiber Diets*

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**ABSTRACT**

Increased dietary fiber is thought to have beneficial effects on health in humans. In diabetic dogs, a beneficial effect of fiber on glycemia has been suggested, while its effect on lipid profiles in dogs has not been described. The effects of different amounts and types of fiber on glucose tolerance and lipid concentrations were evaluated and compared with those of a standard maintenance ration in 30 healthy dogs. It was concluded that increased fiber intake had no influence on glucose in healthy dogs but it may have modulated lipid homeostasis.

**INTRODUCTION**

Increased fiber in the diet is the goal of many health promotion efforts, and fiber diets may decrease cancer risk and cardiovascular disease.1 Diets that decrease hyperglycemia and hyperlipidemia are important for controlling obesity and diabetes. High-fiber diets have been suggested to play an important role in modulating postprandial glycemia in humans.2–7 The evidence for such a role has been controversial,8,9 however, and it is now believed that dietary fiber actually has negligible effects on glucose tolerance in humans unless large amounts are ingested.10 Evidence for a role of dietary fiber in glucose regulation in dogs is also controversial. Dietary insoluble fiber (cellulose) in large amounts (12–15 g/100 g dry matter) as well as soluble fiber (pectin) in large amounts (15 g/100 g dry matter) have been shown to improve glycemic control in a small number of alloxan-diabetic and spontaneously diabetic dogs when compared with a low-fiber diet.11,12 Recently, insoluble, but not soluble, fiber has been shown to decrease postprandial blood glucose concentrations in a small group of diabetic dogs.13 However, dogs with diabetes uncomplicated by growth hormone or glucocorticoid excess are usually of normal or low body weight and are insulin deficient (type 1 diabetes). Therefore, an energy-restricted diet, as is typical of most high-fiber diets, may be contraindicated. In addition, high fiber con-
tent often leads to a marked reduction in palatability as well as an increase in flatulence, vomiting, and fecal output, which may be objectionable to owners and complicate maintenance of regular food intake. It also has been shown that the haircoat of dogs on a high-fiber diet may become dull and lusterless, possibly due to the fact that dietary fiber influences the bioavailability of both macronutrients and micronutrients. There is evidence in some studies, but not others, that high-fiber diets reduce cholesterol and triglyceride levels and decrease the low-density lipoprotein (LDL):high-density lipoprotein (HDL) ratio in humans. Different fiber sources seem to have different effects. Triglycerides, nonesterified fatty acids, and very low-density lipoproteins (VLDLs) have been indicated as factors responsible for the detrimental effects of obesity, including decreased insulin secretion and cardiovascular changes. This has not been examined in dogs; however, one study found that obese dogs have significantly higher cholesterol and triglyceride concentrations than lean dogs. Thus, high-fiber diets might be beneficial for obese dogs as well.

Dietary fiber can be characterized based on solubility or fermentability. For this study, guar gum (GG) represents a highly soluble, highly fermentable fiber source, while cellulose (CEL) is a poorly soluble, poorly fermentable fiber source. Fiber such as CEL, with its low solubility and fermentability, is often used for the purpose of weight reduction because it contributes bulk and leads to a feeling of satiety. Sugar beet fiber used in the study represents a more typical ingredient, containing both soluble and insoluble fiber with moderate fermentability and the ability to influence lipid metabolism and small intestinal glucose transport. The purpose of this study was to revisit the question of putative beneficial glucoregulatory effects of dietary fiber. The study was designed to address this question in normal dogs, thereby eliminating fluctuations in insulin sensitivity and permitting the investigation of a larger population in a highly controlled environment.

To determine if any changes due to fiber content might be caused by changes in gastrointestinal prokinetic movement, the gastrointestinal transit times of the different diets were examined, using changes in breath hydrogen concentrations as markers. The effects of different diets on defecation frequency and fecal wet and dry weight also were examined because these are important factors for pet owners.

**MATERIALS AND METHODS**

**Animals**

Thirty healthy adult male mongrel dogs began the study weighing an average of 21.0 kg each. Their weight did not fluctuate greatly throughout the study; average weight was 21.8 kg at the end of the study. The dogs were maintained at the University of Georgia College of Veterinary Medicine Animal Care Facility under standard colony conditions, housed in individual runs with free access to water. Animal studies were approved by the University of Georgia Animal Care and Use Committee.

**Diets**

Three fiber types (beet pulp [BP], GG, and CEL) were investigated, each fed at three different dietary concentrations (low [L], medium [M], high [H]). The 30 dogs were evenly and randomly allocated to the three fiber types. The diets in each fiber-type group were manipulated in five treatment cycles in a Latin-square rotation. During each treatment cycle, dogs were randomly allocated to one of the three concentrations of its assigned dietary fiber, a dry control (DRY), or a canned control (CAN) treatment. This random assignment was restricted so that each dog received each of
the fiber concentrations and both control treatments over the five treatment cycles and so that during each treatment cycle two dogs in each group were assigned to each of these five treatments.

Fiber-supplemented diets were created by replacing a portion of the cornstarch in the CAN diet with the respective fiber sources (Table 1). All diets were formulated to be nutritionally complete and, except for fiber levels, to be similar in nutrient composition (Table 2). The initial standard food allowance was 146.4 kJ/kg twice daily and was adjusted to maintain the body weight of the dogs within a margin of approximately 5%. The dogs had been previously trained to eat their food within a 10-minute period.

**Evaluations**

After 4 and 8 weeks of consuming the same diet, the following tests were performed for all dogs: complete blood count and serum chemistry profile as routine measures of the dogs’ health; glucose tolerance, including glucose and insulin measurements; lipid profiles, including measurements of triglycerides and cholesterol (total, LDL, VLDL, and HDL); breath hydrogen; and 48-hour fecal collection for measurement of wet and dry fecal weight.

Blood samples were obtained through repeat- ed venipuncture. Blood for glucose and insulin measurements was allowed to clot and was centrifuged within 60 minutes of collection. The serum was frozen at –20°C until assay. The blood for lipid profiles was centrifuged within 30 minutes and frozen until spectrophotometric analysis by a commercial laboratory (Antec, Atlanta, GA). Enzymatic methods were used to measure total cholesterol triglycerides, and HDL. From these measurements LDL and VLDL concentrations were calculated.

On Day 3, blood samples were collected for lipid profiles, glucose, and insulin measure-


Serum glucose concentrations were determined spectrophotometrically by a glucose oxidase method, which had an intraassay coefficient of variation of 3.3% and an interassay coefficient of variation of 3.5%. Glucose concentrations were calculated as area under the curve (AUC) from time 0 to 240 minutes.

Serum insulin concentrations were measured by validated radioimmunoassay. The intra-assay coefficient of variation for the insulin assay was 2.6%, and the interassay coefficient of variation was 3.4%. The detection range was 20 to 1300 pmol/L. Total insulin secretion was calculated as AUC from time 0 to 240 minutes.

Breath hydrogen measurements were taken at time 0 and hourly for 7 hours on Day 3. Breath hydrogen was measured by gas chromatography, as described in detail by Washabau and coworkers. The instrument was calibrated each day with a standard calibration gas mixture. Briefly, exhaled breath samples were collected through a close-fitting facemask connected to a one-way valve and 1-L reservoir bag. The dead space in the system was minimized by allowing the dogs to breathe through the mask for 1 minute before collection of breath samples. The dogs were then allowed to inflate the anesthetic reservoir bag until it was full. Samples of the expired air were drawn through a sampling valve into 20-mL plastic syringes and analyzed immediately for hydrogen concentration. Total hydrogen excretion was calculated as AUC from time 0 to 7 hours.

On Days 1 and 2, defecation frequency was monitored every 2 hours for 12 hours after the morning feeding at 8:00 AM. Feces were collected at 8:00 AM and 8:00 PM and weighed on those days. The frequency of any nighttime

| TABLE 2. Nutrient Composition of Experimental Diets on a Dry-Matter Basis |
|---------------------|-----------------|----------------|----------|-------------|-----------------|--------------------|
| Diet      | Protein (%) | Fat (%) | Ash (%) | ME (kJ) | Crude Fiber (%) | Total Dietary Fiber (%) | Insoluble Fiber (%) | Soluble Fiber (%) |
| BP-L     | 26.43   | 21.27  | 5.78   | 18.03  | 1.39            | 4.88                | 4.88                | 0.00              |
| BP-M     | 27.46   | 19.48  | 6.57   | 17.45  | 2.09            | 7.58                | 5.12                | 2.46              |
| BP-H     | 26.42   | 21.42  | 7.20   | 17.49  | 3.96            | 10.57               | 7.32                | 3.25              |
| GG-L     | 26.49   | 20.70  | 5.74   | 18.11  | 0.00            | 5.54                | 1.65                | 3.88              |
| GG-M     | 26.34   | 20.78  | 6.09   | 17.82  | 0.95            | 8.81                | 4.06                | 4.77              |
| GG-H     | 25.71   | 19.84  | 7.17   | 17.74  | 0.00            | 12.02               | 2.63                | 9.43              |
| CEL-L    | 24.88   | 20.82  | 5.64   | 17.36  | 5.41            | 7.05                | 7.05                | 0.00              |
| CEL-M    | 26.21   | 20.82  | 5.64   | 16.86  | 8.85            | 12.35               | 11.15               | 1.19              |
| CEL-H    | 25.18   | 20.04  | 5.55   | 16.07  | 13.40           | 17.21               | 17.21               | 0.00              |
| CAN      | 24.77   | 20.59  | 5.65   | 18.11  | 0.00            | 3.51                | 3.51                | 0.00              |
| DRY      | 24.75   | 19.04  | 11.02  | 16.86  | 1.16            | 4.41                | 3.67                | 0.74              |

*Data shown as 0.00 if crude fiber was below detection limits (<0.20% as fed, approximate equivalent of 0.80%, dry basis).
†Data shown as 0.00 if soluble fiber was below detection limits (<0.25% as fed, approximate equivalent of 1.00%, dry basis).
BP = beet pulp; CAN = canned control; CEL = cellulose; DRY = dry control; GG = guar gum; H = high concentration; L = low concentration; M = medium concentration; ME = metabolizable energy.
defecation was determined by counting piles of feces in the runs before the 8:00 AM feeding.

**Analysis**

The data of interest were initially subjected to an analysis that investigated the effects of fiber type and the concentrations of each on the response variables, using a single linear model to incorporate the complexity of the trial design. Data were subsequently reanalyzed using a simpler approach (described below). While it is acknowledged that the simpler analysis may increase the likelihood of type II errors, no substantive differences in findings were detected between the two methods.

To test for differences between fiber types, a one-way analysis of variance (ANOVA) was performed on the mean response over all concentrations for each dog, 10 observations for each fiber type. To test for differences between concentrations within each fiber type, a separate one-way ANOVA was performed using the observations for each dog. Each dog received each concentration over the course of the experiment, resulting in treatment and error degrees of freedom of 2 and 27, respectively.

To test for the effect of fiber versus a control, a *t*-test was performed on the mean response over all concentrations for each dog after subtracting the control. This resulted in 10 observations per fiber type and a critical value from a *t*-distribution with 9 degrees of freedom. The procedure was repeated for each control within each fiber type. All data are expressed as means ± SD, unless stated otherwise.

**RESULTS**

**General Observations**

There were no changes over time or between groups in physical examination findings, complete blood count, or chemical profiles, which were normal throughout the study period (data not shown). The investigators noted that the dogs’ haircoats seemed to become dull and lusterless when they consumed fiber diets, with increasing severity as the fiber concentration increased, but the condition was most severe with the CEL-H diet.

**Food Intake**

The dogs ate all diets well and there were no significant differences in intake levels among the different diets. Intake per meal ranged from 104.2 ± 31.4 kJ/kg for CEL-L to 135.1 ± 22.2 kJ/kg for both CEL-H and BP-L.

**Glucose and Insulin Concentrations**

Baseline glucose concentrations did not differ after 4 weeks (data not shown) or 8 weeks (range = 4.7–5.6 mmol/L). When comparisons were made within fiber type after intake of food, the area under the curve for blood glucose (AUCBG) was significantly higher (*P* = .0104) for CEL (5.69 ± 0.83 mmol/L/min) and GG (5.73 ± 0.71 mmol/L/min) diets than for BP (5.12 ± 0.46 mmol/L/min; Table 3). Differences were not observed in AUCBG when fiber diets were individually compared with CAN or DRY diets, for which AUCBG ranged from 5.3 to 5.8 mmol/L/min. When food and glucose were administered, there were no differences among fiber types, among concentrations within a fiber type, or between fiber and control diets. The AUCBG ranged from 5.3 to 6.2 mmol/L/min for all diets in the study. There were no differences in AUC for insulin among any of the diets (data not shown).

**Lipid Analysis**

There were no significant differences in triglyceride concentrations (0.41–0.52 mmol/L), total cholesterol (4.8–5.9 mmol/L), LDL (1.5–2.7 mmol/L), VLDL (0.17–0.23 mmol/L), or HDL concentration (2.5–3.4 mmol/L) among different fiber types or concentrations. There was a difference, however, between some
fiber diets and control diets in that total cholesterol concentrations were lower in dogs fed BP (5.5 ± 2.0 mmol/L) than in dogs fed CAN (6.3 ± 1.0 mmol/L; $P < .003$) and higher in dogs fed CEL (5.8 ± 1.4 mmol/L) than in dogs fed DRY (5.1 ± 1.0 mmol/L; $P < .05$).

**Breath Hydrogen**

Breath hydrogen concentrations were increased in all dogs after feeding. The time course of increases in breath hydrogen concentrations after ingestion of the test diet was similar, regardless of the type of diet that was fed; the mean peak time was 3.7 ± 0.1 hours. Breath hydrogen AUC in the 7-hour period was significantly higher ($P < .05$) for GG (2.3 ± 0.7 g/kg) than for other diets (range = 2.1 ± 0.3 g/kg; $P = .0007$) or DRY (2.0 ± 0.5 g/kg; $P < .0008$) diets. Fecal dry weight was significantly less for dogs fed BP (2.3 ± 0.7 g/kg) than for dogs fed CAN (2.6 ± 0.4 g/kg; $P = .0496$) and was significantly greater ($P < .05$) for dogs fed BP (5.2 ± 0.7 g/kg) than for dogs fed CAN (5.1 ± 0.6 g/kg) or CEL (6.2 ± 1.8 g/kg) for 8 weeks (Table 4). Mean fecal wet weight was also significantly greater for dogs fed BP than for dogs fed CAN (6.5 ± 1.0 g/kg; $P = .0027$) or DRY (5.3 ± 1.1 g/kg; $P < .0027$) diets.

**DISCUSSION**

The main focus of this study was to examine the effect of different amounts and types of fiber on glucose tolerance and lipid profiles. Dietary fiber has been said to have multiple effects, including a delay in gastric emptying, binding of bile acids, increased stool weight, delay in glucose absorption, and flattening of glucose tolerance. The addition of dietary fiber to the diet of human diabetics was recommended in the 1980s; however, subsequent studies found that dietary fiber fed at ac-

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**TABLE 3. Area Under the Curve for Blood Glucose (AUCBG) in Dogs on Different Diets**

<table>
<thead>
<tr>
<th>Diet</th>
<th>AUCBG (mmol/L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beet pulp</td>
<td>5.12 ± 0.46</td>
</tr>
<tr>
<td>Dry control</td>
<td>5.35 ± 0.30</td>
</tr>
<tr>
<td>Canned control</td>
<td>5.34 ± 0.71</td>
</tr>
<tr>
<td>Guar gum</td>
<td>5.73 ± 0.71</td>
</tr>
<tr>
<td>Dry control</td>
<td>5.48 ± 0.44</td>
</tr>
<tr>
<td>Canned control</td>
<td>5.70 ± 0.56</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.69 ± 0.83</td>
</tr>
<tr>
<td>Dry control</td>
<td>5.42 ± 0.56</td>
</tr>
<tr>
<td>Canned control</td>
<td>5.80 ± 0.84</td>
</tr>
</tbody>
</table>

$\text{n} = 10$ per diet.

Values within the column with different superscripts are significantly different ($P < .01$). There were no significant differences between individual fiber types and their corresponding control diets.

**TABLE 4. Comparison of Fecal Wet Weight for Dogs on Different Diets**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Fecal Wet Weight (g/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beet pulp</td>
<td>9.2 ± 3.1</td>
</tr>
<tr>
<td>Dry control</td>
<td>5.8 ± 1.7</td>
</tr>
<tr>
<td>Canned control</td>
<td>5.8 ± 1.8</td>
</tr>
<tr>
<td>Guar gum</td>
<td>7.4 ± 1.9</td>
</tr>
<tr>
<td>Dry control</td>
<td>6.8 ± 3.0</td>
</tr>
<tr>
<td>Canned control</td>
<td>5.5 ± 2.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>6.6 ± 2.0</td>
</tr>
<tr>
<td>Dry control</td>
<td>6.2 ± 2.0</td>
</tr>
<tr>
<td>Canned control</td>
<td>6.4 ± 1.0</td>
</tr>
</tbody>
</table>

$\text{n} = 10$ per diet.

$^a$Significantly less than for beet pulp diet ($P < .05$).

$^b$Significantly less than for beet pulp diet ($P < .001$).
acceptable amounts played only a minor role, if any, in the regulation of carbohydrate metabolism. An energy-restricted diet, (e.g., containing fiber) may be beneficial for obese patients, however.

The concept of a beneficial effect of fiber on glucose tolerance has been introduced to veterinary medicine as well, and fiber has been recommended as an adjunct treatment for diabetic dogs. However, while most diet studies in humans have been conducted in non–insulin-dependent type 2 diabetics who were obese and therefore benefited from an energy-restrictive diet, studies in dogs have been performed in type 1 diabetics needing insulin for survival. Few spontaneously diabetic dogs have been studied thus far, with variable results. At best, the changes seem to be small when considering longer-term glucose control. Because most studies only examined one or two types of fiber at one concentration, the question as to whether or not dietary fiber alters glucose tolerance and affects lipid metabolism was revisited. Further, questions regarding which concentration and which fiber type might be best to achieve these effects were addressed.

Three commonly used fiber types having different solubility and fermentability characteristics were studied: 1) CEL, a fiber of low solubility and low fermentability, 2) BP, low in solubility but moderate in fermentability, and 3) GG, high in both solubility and fermentability. Solubility and fermentability have been shown to play a role in the different physiologic effects of fiber. For example, GG leads to a much greater production of short-chain fatty acids than does CEL. Certain short-chain fatty acids may be important in the maintenance of colonocyte health. Fermentability has also been shown to have an effect on the structure of the intestinal mucosa. Increased surface areas and hypertrophy of the mucosa have been observed with fermentable dietary fiber compared with CEL in dogs, and influenced glucose uptake was also noted. Recently, it was shown that feeding BP resulted in increased glucagon-like-peptide-1 (GLP-1) and insulin secretion and reduced AUCBG after ingestion of glucose. In the study reported here, the highest concentration of GG caused the greatest increase in breath hydrogen, confirming that its fermentability is greater than that of the other two fiber types; however, the AUCBG with GG was not different from that observed with the other diets.

The positive physiologic aspects of the increase in fermentability may be countered by an increase in fecal wet weight, which has been documented by Fahey and coworkers but was not observed in the present study. Bulky and wet feces may be objectionable to owners and may influence their choice of diet. An increase in defecation frequency with the fermentable diets was not observed in the present study. Defecation frequency might have an even greater influence on the diet choice.

In addition to evaluation of postprandial changes in glucose after intake of a particular diet, glucose added to the diets at levels used for oral glucose tolerance testing was examined in this study to determine whether fiber actually might acutely affect its absorption, as has been suggested. The effect of some fiber types on postprandial glycemia has been evaluated, whereas oral glucose tolerance tests, combined with food intake, have not been performed in dogs on high-fiber diets. This test was performed to assess possible interactions between fiber types and concentrations and glucose regarding transit time and absorption. In the present study, the lowest glucose concentrations after intake of food in the presence or absence of glucose occurred with BP; however, there was no significant difference compared with control diets. This confirms results reported by Massimino and coworkers, who...
attributed the lower blood glucose to a higher GLP-1 secretion, suggesting that fiber in normal dogs does not influence the absorption of glucose in the gastrointestinal tract. The authors also observed that fiber does not influence gastrointestinal transit time, as measured by an increase in breath hydrogen concentration. The mean orocolonic transit time, measured as the time between food intake and peak breath hydrogen concentration, was less than 4 hours in the present study. Since hydrogen concentration was measured for only 7 hours, it is possible that there was a later second peak when all the food was present in the large intestine. The present findings with breath hydrogen testing confirm results of a preliminary study conducted by the authors, in which the different diets were mixed with a small amount of barium sulfate solution and the transit time was determined radiographically. Arrival of the ingesta in the colon in that study was observed 3 to 4 hours after food intake. The addition of barium-impregnated markers to the food for measurement of gastrointestinal transit time was attempted, but it did not appear to be useful because the markers separated from the food in the stomach.

Present findings are in agreement with those of Nguyen and coworkers, who showed that crude fiber content had no effect on postprandial glycemia. However, other investigators found that postprandial deviation from fasting glucose was highest in dogs fed a control diet and lowest when dogs were fed a diet containing granules of soluble fiber. In alloxan-treated diabetic dogs, low-fiber diets, high-soluble-fiber (pectin) diets, or high-insoluble-fiber (cellulose) diets all led to a decrease in fasting blood glucose compared with control food. However, the fiber content of the control diet had almost twice the fiber content of the low-fiber diet and was similar in content to the high-soluble-fiber diet. A greater rise in postprandial blood glucose concentrations was noted in dogs fed the low-fiber diet than for any other diet. These researchers concluded that a high-fiber diet was beneficial for diabetic dogs; however, it appears that the effects on the glucose concentrations resulted from factors other than the fiber content. These results also differ from a later study in spontaneously diabetic dogs by the same investigators. In that study, the only change observed in 9 of 11 dogs was a significantly higher fasting blood glucose concentration for the low-fiber diet compared with that in the high-fiber diet, whereas the slope of the postprandial glucose curve was similar for both diets. In two dogs, a high-insoluble-fiber diet exacerbated glucose control compared with a low-fiber diet. It is difficult to explain these apparently contradictory results without further studies, including in vivo analysis of digestible and metabolizable energy of the diets for each dog and the measurement of glucose tolerance and insulin sensitivity. A recent study showed that three of seven insulin-dependent diabetic dogs had adverse reactions to a CEL diet, which was the only diet investigated that affected glycemic control. The changes in glycemic control were small with the CEL diet, such that fructosamine levels were reduced from a mean of 493 ± 64 µmol/L to 471 ± 70 µmol/L (reference range = 250–344 µmol/L). Lipids were not measured.

Regardless of the type of fiber fed in the present study, dogs given the highest amount of fiber developed a dull and lusterless haircoat. This is in agreement with findings of Nelson and coworkers, who also reported a change in haircoat in dogs on high-fiber diets. This may be due to an effect of fiber on the absorption of minerals and vitamins. It would be prudent to investigate this further, because diabetic dogs, in particular, should have a diet well balanced in both macronutri-
ents and micronutrients.

Consumption of fiber has been shown to result in reduced serum lipid concentrations in some studies. It is thought that the lipid-altering capacity of fiber decreases the risk of cancer, heart disease, obesity, and diabetes mellitus. In most studies, a decrease in total and LDL cholesterol concentrations appeared to be the primary consequence of feeding dietary fiber. Similar to findings in the present study, cholesterol concentrations in rats were reduced when BP was fed but were increased with CEL; however, effects of fiber type have generally been variable. While the health benefit of such changes in the lipid profile has not been examined in dogs, it seems that a decrease in cholesterol would be beneficial in obese dogs, which have been shown to have hypercholesterolemia. The effects of different fiber types on lipid profiles for obese dogs, and dogs with other diseases need to be examined.

CONCLUSION

High-fiber diet did not alter glucose tolerance in healthy dogs compared with normal dog maintenance food. However, fiber modulated cholesterol, an effect that may be important in obese animals.

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REFERENCES