Protein Intake Does Not Affect Insulin Sensitivity in Normal Weight Cats

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EXPANDED ABSTRACT

KEY WORDS: • protein intake • insulin sensitivity • cat • leptin • IGF-1

Type 2 diabetes is the most common form of diabetes in cats, and genetic and environmental factors play a role in the progression of this disease (1). Obesity is one of the predominant factors. Indeed, obesity is related to insulin resistance and impaired glucose tolerance in many species. Thus, in obese insulin-resistant subjects, glucose tolerance is reduced, i.e., to control hyperglycemia, the insulin secretion must be higher than in normal-weight and insulin-sensitive subjects and the insulin response to stimuli, i.e., the β cell response to glucose (2) or arginine is impaired (3). Prevention of diabetes involves minimizing the rise in postprandial glucose concentration and insulin secretion by dietary manipulations, thus reducing the demand on the β cells to produce insulin. Several studies demonstrated that insulin sensitivity could benefit from high-protein, high-fat, and low-carbohydrate diets in mice (4) or in genetically type 2 diabetic mice (5). In humans, low-carbohydrate diets improved diabetes (6), whereas some studies showed no effect of high-protein diets (7). The results of some studies suggest that a high-carbohydrate intake could increase glucose tolerance in humans (8). In diabetic cats, a high protein diet might reduce their insulin requirement, but clear evidence is lacking (9).

The mechanism by which obesity leads to insulin resistance is not well established, but may be mediated by hormonal factors. One factor is the hepatic insulin-like growth factor-1 (IGF-1). The IGF-1 receptor has immunological, structural, and functional analogies with the insulin receptor, which could explain how IGF-1 plays an insulin-like role, especially on glucose metabolism (10) and is involved in insulin resistance. The relation between insulin sensitivity and IGF-1 levels is not clear. We and others showed that insulin resistance is accompanied by increased IGF-1 levels, resulting from higher synthesis rate of IGF-1 and lower production of IGF-1 binding proteins, leading to increased IGF-1 plasma levels (11,12). Nevertheless, others showed an inverse relation with lower IGF-1 plasma levels in insulin-resistant subjects (13).

Another hormonal factor is leptin, produced by expression of the ob gene. This hormone is secreted mainly by white adipose tissue (14) as a 16-kDa peptide and binds to receptors in the hypothalamus. It plays an active role in the regulation of food intake, energy expenditure, and reproductive function. The circulating level is directly proportional to the total amount of fat in the body (15). We showed previously that cats fed a high-protein diet for 6 mo gained fat-free mass without a change in body weight (BW) (16). The effect of such a diet on plasma leptin levels is not known. The present study was conducted to compare insulin sensitivity, insulin secretion, and plasma leptin and IGF-1 in cats fed diets differing in protein and carbohydrate content.

MATERIALS AND METHODS

Animals

Normal-weight cats (n = 16; 11 female and 5 male, aged 4.5 ± 0.1 y, all neutered, Domestic Shorthair) were used in this study. For the 6 mo before the study, all cats had free access to a commercially available extruded food [340 g/kg crude protein and 14.64 MJ of metabolizable energy (ME)/kg as fed]. The study had a crossover design, with 2 consecutive 6-mo periods. Mean initial body weight (BW) was 4.83 ± 0.13 kg, and body condition score was (1–5 possible) 3.4/5 ± 0.1. Cats were allocated to 1 of 2 groups, and fed 1 of 2 test diets for 6 mo, before being transferred (without a washout period) to the second diet for 6 mo. The extruded diets were isocaloric, either high-protein (HP, 528 g/kg crude protein) or moderate-protein (MP, 297 g/kg crude protein) (Table 1).

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Abbreviations used: AUC, area under the curve; BW, body weight; HP, high-protein; IGF-1, insulin-like growth factor 1; IVGTT, intravenous glucose tolerance test; ME, metabolizable energy; MP, moderate-protein.
Cats were housed in groups of 3, but fed individually through electronically controlled cat flaps. Each cat was offered throughout the study a mean energy allowance of 209 kJ ME/(kg BW•d) (according to BW at the beginning of each period). This energy allowance is considered sufficient to maintain optimal weight under relatively inactive conditions (17). The actual value for each cat was calculated at the end of each study period according to the mean BW during that period. All cats had access to water. Fresh water was supplied daily and was continuously available.

All experimental protocols were approved by the animal Use and Care Advisory Committee of the Nantes Veterinary School, and adhered to European Union guidelines.

**Insulin sensitivity and insulin secretion**

The studies were performed in the late morning after removal of food from the cages 12 h earlier. The animals were anesthetized with an i.p. injection of tiletamin-zolazepam (7 mg/kg BW, Zoletil, Virbac). Blood samples were taken from the jugular vein into heparinized tubes.

Insulin sensitivity was assessed by measuring insulin secretion after an i.v. glucose tolerance test (IVGTT). Glucose solution (i.v.; G30; 0.5 g/kg BW, Aguetant) was rapidly injected. At specific time points after injection, blood samples were collected (0, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min) into heparinized tubes and immediately centrifuged at 3000 × g for 10 min; plasma was separated and then stored at −20°C until analysis.

Insulin secretion was measured after arginine injection. Arginine (i.v.; 0.1 g/kg BW, Sigma) dissolved in saline water was rapidly injected. At specific time points after injection, blood samples were collected (0, 2, 4, 6, 8, 10, 20, and 30 min) into heparinized tubes and immediately centrifuged at 3000 × g for 10 min; plasma was separated and then stored at −20°C until analysis.

**Assays**

Insulin concentration was determined radioimmunochemically using a guinea pig anti-porcine insulin antibody and 125I-labeled human insulin as tracer and standard (Linco Research). Plasma glucose concentrations were determined with a blood glucose monitor (Accu-Check®, Roche Diagnostics). Plasma leptin concentration was determined radioimmunochemically according to the manufacturer's instructions (Multispecies Leptin RIA kit, Linco Research). The IGF-1 concentration was determined radioimmunochemically according to the manufacturer's instructions (Nichols Institute Diagnostics).

**Statistics**

The data are presented as means ± SEM. The effect of diet was investigated using Student's t test for paired values. Differences with P < 0.05 was considered significant. All calculations were performed using Statview software (Abacus Concepts).

**RESULTS**

**Insulin sensitivity and insulin secretion**

The insulin secretion after i.v. injection of glucose did not differ in cats fed either diet (Table 2). Insulin sensitivity, calculated as the area under the curve (AUC) of insulin secretion during the 2 h after i.v. injection of glucose, did not differ after consumption of the MP or HP diet.

Insulin secretion after the arginine test, expressed as the AUC of insulin secretion after i.v. injection of arginine, did not differ after consumption of either diet.

**Plasma leptin level**

Plasma leptin concentrations did not differ after consumption of the MP or HP diet (Table 2).

**Plasma IGF-1 level**

Plasma IGF-1 concentration was lower for 6 mo in cats fed the HP diet than in the same cats fed the MP diet for 6 mo (Table 2).

**DISCUSSION**

In this study, we assessed the effect of increasing protein intake on the insulin response to the ingestion of glucose and insulin sensitivity, as well as possible modifications of leptin and IGF-1 plasma levels in normal-weight cats. Cats are strict

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**TABLE 2**

**Insulin responses after i.v. glucose and arginine administration, plasma leptin and plasma IGF-1 levels in cats before and after 6 mo of consuming the MP or HP diet**

<table>
<thead>
<tr>
<th></th>
<th>Glucose tolerance test. Insulin secretion after glucose injection</th>
<th>Pancreatic function. Insulin secretion after arginine injection</th>
<th>Leptin plasma</th>
<th>IGF-1 plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IU/mL 1st 5 min</td>
<td>h 1</td>
<td>h 2</td>
<td>Total (2 h)</td>
</tr>
<tr>
<td>MP T0</td>
<td>15 ± 4</td>
<td>431 ± 92</td>
<td>435 ± 96</td>
<td>880 ± 174</td>
</tr>
<tr>
<td>MP T6 mo</td>
<td>24 ± 6</td>
<td>440 ± 70</td>
<td>313 ± 73</td>
<td>753 ± 130</td>
</tr>
<tr>
<td>HP T0</td>
<td>13 ± 4</td>
<td>331 ± 75</td>
<td>318 ± 93</td>
<td>613 ± 143</td>
</tr>
<tr>
<td>HP T6 mo</td>
<td>22 ± 4</td>
<td>424 ± 79</td>
<td>385 ± 78</td>
<td>809 ± 141</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 16 in each group. *Different from IGF-1 plasma concentration in cats before they were fed the HP diet for 6 mo, P < 0.05 (Student’s t test for paired values).
carnivores; as such, their natural diet consists mainly of protein and fat, but very little carbohydrate.

Our results suggest that the HP diet would not affect insulin sensitivity or insulin secretion in response to glucose in normal-weight cats. This seems to be inconsistent with previous studies conducted in other species and under other conditions. Indeed, previous studies showed an increase in insulin secretion in response to glucose after a high-protein diet and thus a decrease in insulin resistance in rats made insulin resistant by a high-fat diet (18) and in genetically obese Zucker rats (19). An increase in the protein-carbohydrate ratio in a high-fat diet improved glucose homeostasis in mice (4), or in genetically type 2 diabetic mice (5), but had no effect on insulin sensitivity in humans (7); however, some authors showed inverse results (20). In diabetic cats, replacement of a moderate carbohydrate, high-fiber diet with a low-carbohydrate, high-protein diet improved insulin sensitivity (21). In our study, cats were normal weight and had insulin sensitivity. The secretion of insulin in response to a glucose test or an arginine challenge was normal before the study began. Any improvement in these responses would therefore be small and possibly masked by individual variability.

The discrepancy among all results from previous studies could also be explained by the different sources of protein used. Some proteins may be more efficient than others in normalizing glucose homeostasis and insulin secretion. Indeed, in rats made insulin resistant by a high-fat diet, whey protein was more effective than red meat in improving insulin sensitivity and reducing body weight gain (18). Another study showed that serum insulin concentration was lower after consumption of a soy protein diet than after a casein diet (22).

Possible explanations for the beneficial effects of high-protein diets were postulated. Such a diet could enhance the diet-induced thermogenesis due to the high protein content; it could increase the sense of fullness and satiety, decrease muscle protein loss, and enhance glycemic control. Moreover, a high-protein diet could increase brain response to the appetite-regulating hormones (23). Part of the beneficial effects could also be mediated by peroxisome proliferator-activated receptors (19).

The results of this study show a lower IGF-1 plasma level in cats after consumption of a HP diet for 6 mo. Previous studies showed that elevated plasma IGF-1 concentrations have an effect on insulin secretion, which could, over time, lead to insulin resistance (24,10). This is consistent with results previously obtained in our laboratory. Plasma IGF-1 concentrations were higher in obese insulin-resistant dogs compared with the same dogs when they were lean and insulin sensitive (12), and weight loss normalized these IGF-1 levels (23). The lower plasma IGF-1 level induced by a high-protein diet could be advantageous for the prevention of obesity, impaired glucose tolerance, and diabetes in cats.

The similar plasma leptin level after the 2 diets is consistent with the normal weight of the cats. Several previous studies showed that leptin concentrations are higher in obese than in normal-weight individuals and are correlated with body fat in humans (15) and in overweight cats (26). Another study in cats demonstrated a reverse relation between plasma leptin levels and insulin sensitivity, independently of adiposity, and suggested the involvement of insulin in increasing leptin secretion, and the physiological role of leptin in the link between obesity and insulin resistance (27).

In conclusion, we did not show any effect of a high vs. medium protein diet on glucose tolerance or insulin sensitivity, but found that prolonged feeding of a high protein diet decreased plasma IGF-1 in normal-weight cats. Further studies, with proteins from different sources in obese cats, would be necessary to allow more firm conclusions about the effects of these diets on glucose homeostasis.

LITERATURE CITED


