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Oligofructose and inulin modulate glucose and amino acid metabolism through propionate production in normal-weight and obese cats

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The effect of dietary oligofructose and inulin supplementation on glucose metabolism in obese and non-obese cats was assessed. Two diets were tested in a crossover design; a control diet high in protein (46 % on DM basis), moderate in fat (15 %), low in carbohydrates (27 %), but no soluble fibres added; and a prebiotic diet, with 2·5 % of a mixture of oligofructose and inulin added to the control diet. Eight non-obese and eight obese cats were allotted to each of two diets in random order at intervals of 4 weeks. At the end of each testing period, intravenous glucose tolerance tests were performed. Area under the glucose curve (AUC_{gluc}) was increased ($P=0·022$) and the second insulin peak was delayed ($P=0·009$) in obese compared to non-obese cats. Diets did not affect fasting plasma glucose concentrations, blood glucose response at each glucose time-point after glucose administration, AUC_{gluc}, fasting serum insulin concentrations, area under the insulin curve, and height and appearance time of insulin response. Yet, analysis of acylcarnitines revealed higher propionylcarnitine concentrations ($P=0·03$) when fed the prebiotic diet, suggesting colonic fermentation and propionate absorption. Prebiotic supplementation reduced methylmalonylcarnitine ($P=0·072$) and aspartate aminotransferase concentrations ($P=0·025$), both indicating reduced gluconeogenesis from amino acids. This trial evidenced impaired glucose tolerance and altered insulin response to glucose administration in obese compared to non-obese cats, regardless of dietary intervention; yet modulation of glucose metabolism by enhancing gluconeogenesis from propionate and inhibition of amino acid catabolism can be suggested.

Carbohydrate metabolism: Cats: Dietary fibre: Obesity

Diabetes mellitus has become one of the major endocrine disorders in cats. Recently a tendency towards a higher incidence of this disease was reported by Prahl *et al.* [(1)] and might be due to a rise in the frequency of the major predisposing factors such as obesity and physical inactivity[(2)]. However, the current dietary treatments for feline diabetes mellitus, especially the use of soluble fibres, originates from extrapolation of the results from human and canine studies. To our knowledge, the effect of soluble fibres on carbohydrate metabolism in both healthy and insulin-resistant cats (obese or diabetic cats) has not been studied.

Soluble fibres such as oligofructose and inulin have been shown to modulate glycaemia and insulinaemia, although effects may depend on nutritional (fasting *v.* postprandial) and pathological (diabetes mellitus, obesity) conditions[(3)]. In the literature, two hypotheses are proposed. At first, soluble fibres might impair digestion of macronutrients by delaying gastric emptying and/or by reducing small intestinal transit time[(4–6)]. Secondly, the production of SCFA in the hindgut is stimulated by offering soluble fibres as energy source for colonic microbial flora. After being absorbed, the SCFA, especially propionate, might modify the hepatic glucose metabolism by reducing hepatic gluconeogenesis and/or enhancing glycolysis; consequently, blood glucose concentrations will be decreased[(3)]. Hepatic gluconeogenesis might also be influenced indirectly by lowering the plasma fatty acid concentration, since increased plasma fatty acid availability may induce impaired insulin sensitivity by promoting fatty acid oxidation and inhibiting glucose uptake and oxidation but stimulating hepatic glucose production[(7)].

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC_{gluc}, area under the glucose curve; AUC_{ins}, area under the insulin curve; BCS, body condition score; C-diet, control diet; glucose $t_{1/2}$, half-life for glucose disappearance; IVGTT, intravenous glucose tolerance test; HOMA, homeostasis model assessment; K_{gluc}, glucose disappearance coefficient; P-diet, prebiotic diet; QUICKI, quantitative insulin sensitivity check index; T_3, tri-iodothyronine; T_4, thyroxine.

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In other less strict carnivores than cats, such as dogs, few studies investigating the effect of soluble dietary fibre have been performed, resulting in a decreased postprandial glycaemia and/or insulinaemia[8–12]. Yet, to date, no data are available on the effect of prebiotics on the carbohydrate metabolism in more strict carnivorous species such as the cat.

Not only carbohydrate metabolism, but also acylcarnitine profile and selected characteristics of lipid (plasma cholesterol, TAG, NEFA concentrations) and protein metabolism (plasma urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and methylmalonylcarnitine concentration) of the true carnivorous cat were scrutinised in the present study. Acylcarnitine profile was studied as a reflection of metabolites available for the citric acid cycle[13]. Endocrine characteristics such as leptin and thyroid function were investigated as well, since these characteristics are identified to be related to obesity and insulin resistance. Leptin is distinguished to be strongly and positively correlated with adiposity in obese cats[14] and insulin resistance is associated with increased leptin concentrations in both normal-weight and obese cats[15]. Given that thyroid hormones are involved in the regulation of metabolism, and regulate RMR, thermogenesis and lipolysis, thyroid function might be altered by developing obesity[16]. Hence, total fasting triiodothyronine (T_3) and total thyroxine (T_4) concentrations were also obtained in the present trial.

Seeing that very little is known about the metabolic effects of prebiotics in cats, the purpose of the present trial was to evaluate the effect of adding oligofructose and inulin to a basic diet on feline carbohydrate, lipid and protein metabolism as well as to determine the metabolic differences between healthy normal-weight and obese cats.

Materials and methods

Animals and housing

Sixteen domestic short-hair cats, six males and ten females, were included in the study. All male and female cats were neutered. All cats were adult and aged between 3·5 and 6 years. All cats were healthy apart from chronic obesity in eight cats and were not given any medication at the time of the study; none had prior medical problems. During the trial, the cats were housed in individual indoor cages. For 2 h/d, cats were allowed to play in their usual group cages. At that time, cats had no access to the food, but water was available *ad libitum*.

Diets and feeding

The control diet (C-diet) was a non-commercial extruded dry cat food, containing high concentrations of crude protein (46 % on DM basis), moderate amounts of crude fat (15 % DM) and low concentrations of carbohydrates (N-free extract 27 % DM), in order to trigger the cats towards insulin resistance (A Verbrugghe *et al.*, unpublished results). The food also contained moderate concentrations of crude fibre (4·6 % DM) and crude ash (6·7 % DM) (Table 1) and was coated with palm oil on the outside of the kibble. No prebiotics and other soluble fibres were added. To make the prebiotic diet (P-diet), 2·5 % mixture of oligofructose and inulin (Beneo™ Synergy 1®; Beneo-Orafti, Beneo-Group, Tienen, Belgium) was added to the C-diet. This soluble fibre mixture is a co-spray dried 1:1 mixture of long-chain chicory inulin molecules, enriched with short-chain oligofructose obtained by partial enzymatic hydrolysis of chicory inulin and containing low concentrations of fructose, glucose and sucrose as well. The mean total number of fructose or glucose units (degree of polymerisation) was 25 for the inulin; mean degree of polymerisation of oligofructose was 4. The soluble fibre was not mixed with the ingredients, but was added to the palm oil coating. The proximate analysis of the diets is shown in Table 1. Total dietary fibre was determined by acid and enzymatic digestion using enzymes from a commercial test kit (Bioquant Total Dietary Fiber, Merck, USA), followed by correction for protein and ash and is also shown in Table 1.

Table 1. Composition of the control diet (C-diet) and prebiotic diet (P-diet; C-diet +2·5 % of a mixture of oligofructose and inulin)*

Nutrients on DM basis (%) (analysed)	C-diet	P-diet
Crude protein	46·4	44·1
Ether extract	15·2	14·9
Crude ash	6·7	6·7
Crude fibre	4·6	4·5
Starch	23·1	22·3
Sugars	2·4	2·4
N-free extract	27·3	29·8
NSP†	1·8	5·2
Total dietary fibre	9·3	11·8
Metabolisable energy (kJ/100 g as fed)‡	1453	1433

* Ingredients: greaves meal, wheat flour, chicken meal, wheat, bovine chicken fat, linseed, meat and bone meal, brewer's yeast, premium cat digest liquid, fish meal, premix, monosodium glutamate, salt, DL-methionine, iron oxide black, choline chloride 75 %.
† N-free extract − starch − sugars.
‡ Calculated: 15 × crude protein + 36 × ether extract + 15 × (starch + sugars).

The amount of food calculated corresponded to the maintenance energy requirement (normal-weight cats 418 kJ/$kg^{0·67}$; obese cats 544 kJ/$kg^{0·4}$)[17] and was adapted in order to maintain a constant body weight. The food was available all day, except for the 2 h playtime. Cats were allowed free access to water at all times.

Experimental design

Prior to being entered into the study, the cats underwent a physical examination, a blood sample was drawn from the jugular vein after a 12 h fast for complete blood count and serum biochemistry and body weight, body condition score (BCS), BMI and girth circumference were recorded. The BCS was determined using a five-point body condition scoring system[18]. Non-obese cats with a score of 3/5 (mean body weight 4·3 kg; range 3·7–5·3 kg) and obese cats with a score of 5/5 (mean body weight 6·8 kg; range 5·3–9·8 kg) were used in the study. The BMI was calculated as described by Hoenig *et al.* [19]. Girth circumference was measured directly behind the last rib[19]. All measurements were performed under general anaesthesia by the same person to minimise variability (Table 2). For 4 weeks preceding the trial (adaptation period), all cats were fed the C-diet prior to being

Table 2. Body condition score (BCS), body weight, BMI and girth circumference in eight normal-weight and seven obese neutered adult cats

(Mean values and standard deviations)

	Normal weight		Obese		
	Mean	SD	Mean	SD	*P*
BCS	3·1	0·2	5·0	0·0	$<0·001$
Body weight (kg)	4·3	0·6	6·8	1·4	$<0·001$
BMI (kg/cm^2)	37·7	3·7	54·4	9·2	$<0·001$
Girth (cm)	39·6	2·5	50·1	5·0	$<0·001$

randomised to one of two groups, each containing four normal-weight and four obese cats. Each group of cats was assigned to each of two diets (C-diet and P-diet) in a random order at intervals of 4 weeks. This way, diets were examined in a crossover design. Absolute food intake was measured daily throughout the study and relative food intake (% of metabolisable energy intake relative to maintenance energy requirement) was calculated. Body weight was recorded twice weekly.

To determine the effect on glucose and insulin metabolism, an intravenous glucose tolerance test (IVGTT) was performed in each cat at the end of each testing period. Hence, a central venous catheter was placed into a jugular vein to allow glucose administration and blood sampling. At least 20 h prior to the IVGTT, cats were anaesthetised with buprenorphine (10 μg/kg intravenous; Temgesic®; Schering-Plough n.v., Heist-Op-Den-Berg, Belgium), followed by propofol (6–7 mg/kg to effect, intravenous; Propovet®; Abbott Lab, Leuven, Belgium), and a 20 G, 8 cm intravenous catheter (Leaderflex®, Vygon n.v., Brussels, Belgium) was placed in a jugular vein. Catheters were flushed twice daily with 1 ml heparinised saline (50 IU of heparin/ml in saline (0·9 % NaCl) solution) to maintain patency. Amoxycilline (15 mg/kg; Clamoxyl LA®; GlaxoSmithKline n.v., Genval, Belgium) was administered subcutaneously once at the time of catheter placement. The IVGTT was performed between 9.00 and 12.00 hours after a 12 h fast. Glucose (Glucose Sterop 500 mg/ml; Laboratoria Sterop n.v., Brussels, Belgium) was administered (0·5 g/kg), through the jugular vein catheter over 30–45 s, followed immediately by 1 ml normal saline to flush the catheter[20]. Blood samples were collected from the jugular catheter as described by Martin & Rand[21], prior to (0 min) and 2, 5, 10, 15, 30, 45, 60, 90 and 120 min after glucose administration[20]. At time zero, blood samples were collected in tubes containing lithium heparin for determination of AST, ALT, leptin, T_3 and T_4 concentrations, free carnitine and acylcarnitine profile. Serum tubes were used to determine fasting serum total cholesterol, TAG, NEFA, urea and creatinine concentrations, at time zero. At each time interval, blood samples were collected in tubes containing sodium fluoride for determination of plasma glucose concentrations and in serum tubes for determination of serum insulin concentrations. Plasma and serum were obtained by centrifugation and stored at −20°C until assayed.

The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2007/013).

Analytical methods

Plasma glucose, serum total cholesterol, TAG, urea and creatinine concentrations were determined spectrophotometrically using the Roche/Hitachi Modular Analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Likewise, activities of AST and ALT were analysed spectrophotometrically using the Roche/Hitachi Modular Analyzer with pyridoxal phosphate activation. Plasma NEFA concentrations were determined enzymatically using a commercially available method (NEFA; Randox Laboratories Ltd, Crumlin, UK) on RX Daytona (Randox). Serum insulin concentrations were measured using a commercially available immunoradiometric assay test kit (insulin immunoradiometric assay Ref 5251; Biosource Europe S.A., Nivelles, Belgium) as used by Slingerland *et al.* [22]. Plasma leptin concentrations were determined using a commercially available RIA test kit (Multi-Species Leptin RIA Kit, catalogue number XL-85K; Linco Research Inc., St. Charles, MO, USA). This kit was developed to quantify leptin in plasma from several species and has been validated for use in cats[23]. Fasting plasma T_3 and T_4 concentrations were determined using a specific RIA as described by Darras *et al.* [24]. Quantitative electrospray tandem-MS was used for free carnitine and acylcarnitine analysis, as described by Vreken *et al.* [25] and Rizzo *et al.* [26].

The glucose disappearance coefficient (K_{gluc}) and the half-life for glucose disappearance (glucose $t_{1/2}$) between 15 and 90 min after glucose administration were calculated as described by Link & Rand[27]. Area under the glucose curve (AUC_{gluc}) and area under the insulin curve (AUC_{ins}) were calculated according to the trapezoidal method (baseline equal to zero). The basal insulin to glucose ratio and the ratio of area under the insulin to glucose curve (AUC_{ins}/AUC_{gluc}) as well as the homeostasis model assessment (HOMA), the quantitative insulin sensitivity check index (QUICKI) and the Bennett index were calculated as described by Appleton *et al.* [28].

Statistical analysis

Statistical analysis were performed using Superior Performing Software Systems version 16 (SPSS Inc., Chicago, IL, USA). For body weight, BCS, BMI and girth circumference at the beginning of the trial one-way ANOVA was performed. For plasma glucose and serum insulin concentrations during IVGTT, repeated-measures ANOVA was used, with BCS as between-subject factor and diet and time as within-subject factor. All remaining data, including the different glucose time-points during IVGTT, were statistically analysed by repeated-measures ANOVA with BCS as between-subject factor and diet as within-subject factor. Interactions between BCS and diet were also evaluated, but were not present. Statistical significance was accepted at $P<0·05$. All data are expressed as means and standard deviations.

Results

All sixteen cats except one completed the trial. One obese cat died as a consequence of an unrelated cause during general anaesthesia. During the first sampling period, the IVGTT

failed in four cats (one of each group) due to technical problems. In these cats only fasting blood samples could be obtained.

Effect of body condition

At the start of the trial, body weight, BCS, BMI and girth circumference ($P<0{\cdot}001$ for all) differed among normal-weight and obese cats (Table 2). During the trial, body weight remained stable in all cats (data not shown). The effect of BCS on feed intake, fasting metabolic and endocrine parameters, regardless of diet, is shown in Table 3. Obese cats ate more compared with normal-weight cats ($P=0{\cdot}019$), but no differences were noted in relative food intake. Fasting serum creatinine, NEFA and cholesterol concentrations were comparable between normal-weight and obese cats. However, fasting serum urea concentrations were lower ($P=0{\cdot}011$) and serum TAG concentrations tended ($P=0{\cdot}094$) to be higher in obese cats when compared to normal-weight cats. Plasma ALT activity was increased in obese cats ($P=0{\cdot}043$), but plasma AST activity did not change among obese and normal-weight cats. Fasting plasma leptin and T_3 concentrations were also increased in obese cats in contrast to normal-weight cats ($P=0{\cdot}003$ and $P=0{\cdot}026$, respectively). Fasting plasma T_4 concentrations did not differ in relation to BCS. The effect of BCS on glucose and insulin metabolism, regardless of diet, is shown in Table 4. Obese cats had higher fasting plasma glucose ($P=0{\cdot}030$), higher glucose concentration at any time during IVGTT, except for 5 and 120 min after glucose administration (also shown in Fig. 1; main effect: $P=0{\cdot}013$; time × BCS: $P=0{\cdot}043$), and higher AUC_{gluc} ($P=0{\cdot}022$). The K_{gluc} and glucose $t_{1/2}$ were not affected by body condition (Table 4). As shown in Fig. 2, body condition did not influence serum insulin concentrations at any time-point, as well as the height of the first and second insulin peak and the appearance time of the first insulin peak. Yet, the second insulin peak was delayed in obese cats when compared to normal-weight cats ($P=0{\cdot}009$). AUC_{ins}, fasting basal insulin to glucose ratio and AUC_{ins}/AUC_{gluc} as well as HOMA, QUICKI and Bennett index were not affected by body condition (Table 4).

Effect of adding 2·5 % mixture of oligofructose and inulin to a control diet

Both diets were well tolerated. During the trial, none of the cats refused to eat either of the diets and none showed signs of illness or maldigestion. Absolute and relative food intake showed no significant differences between diets. Adding 2·5 % mixture of oligofructose and inulin to the C-diet did not alter characteristics related to glucose and insulin metabolism in healthy normal-weight or obese cats. As shown in Fig. 1, fasting plasma glucose concentration and plasma glucose concentrations at each other time-point after glucose administration were comparable. Also the AUC_{gluc}, K_{gluc} and glucose $t_{1/2}$ remained unaffected. Similarly, fasting insulin, AUC_{ins} as well as height and appearance time of the insulin peaks were similar with both diets with slightly, though not significantly, higher release with fructan intervention (Fig. 2). All endocrine (leptin, T_3, T_4) and metabolic (cholesterol, NEFA, urea, creatinine, ALT) characteristics remained stable, except for serum TAG, which tended to be increased ($P=0{\cdot}065$) and plasma AST activity which was decreased ($P=0{\cdot}025$) when fed the P-diet compared to the C-diet. As shown in Table 5, plasma free carnitine concentrations did not differ among diets, as did plasma acetylcarnitine concentrations. Yet, propionylcarnitine ($P=0{\cdot}03$) and butyrylcarnitine ($P=0{\cdot}002$) were higher when fed the P-diet. Methylmalonylcarnitine tended to be decreased ($P=0{\cdot}072$) when fed the P-diet.

Table 3. Effect of body condition score on feed intake, fasting metabolic and endocrinologic characteristics, regardless of diet*

(Mean values and standard deviations)

	Body condition						
	Normal weight			Obese			
	Mean	SD	*n*†	Mean	SD	*n*†	*P*
Absolute food intake (g/d)	48·0[a]	7·9	16	61·9[b]	11·4	14	0·019
Relative food intake (%)	94·6	7·2	16	93·9	4·0	14	0·822
Urea (mmol/l)	9·2[a]	2·1	16	6·7[b]	1·3	14	0·011
Creatinine (μmol/l)	131	26	16	117	21	14	0·252
AST (U/l)	36·0	24·1	16	28·8	11·8	14	0·309
ALT (U/l)	28·8[a]	13·0	16	49·7[b]	34·9	14	0·043
Cholesterol (mmol/l)	3·1	0·6	16	3·3	0·9	14	0·574
TAG (μmol/l)	0·42	0·2	15	0·51	0·4	12	0·094
NEFA (mmol/l)	1·24	0·4	15	1·28	0·6	12	0·783
Leptin (ng/ml)	7·4[a]	2·8	16	20·2[b]	11·2	12	0·003
T_3 (ng/ml)	0·26[a]	0·08	16	0·34[b]	0·06	13	0·026
T_4 (ng/ml)	4·4	0·8	16	5·1	2·7	14	0·472

ALT, alanine aminotransferase; AST, aspartate aminotransferase; T_3, tri-iodothyronine; T_4, thyroxine.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).
* For details of procedures, see Materials and methods.
† $n<16$ caused by missing values.

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Table 4. Effect of body condition score on glucose and insulin metabolism, regardless of diet*
(Mean values and standard deviations)

	Body condition						
	Normal weight			Obese			
	Mean	SD	*n*†	Mean	SD	*n*†	*P*
Glucose nadir (mmol/l)	4·5[a]	0·5	16	5·1[b]	0·7	14	0·030
AUC_{gluc} (mmol/l per 120 min)	1247[a]	214	14	1533[b]	170	12	0·022
K_{gluc}	1·24	0·34	14	1·22	0·26	12	0·967
Glucose $t_{1/2}$ (min)	61·3	23·5	14	59·5	14·9	12	0·798
Insulin nadir (mU/l)	16·5	5·7	16	17·2	7·0	14	0·820
AUC_{ins} (mU/l per 120 min)	4467	2083	14	5186	1640	12	0·745
Height 1st insulin peak (mU/l)	35·4	14·1	14	30·5	14·0	12	0·239
Height 2nd insulin peak (mU/l)	57·6	27·9	14	65·8	25·5	12	0·777
Time 1st insulin peak (min)	8·9	4·8	14	11·3	3·8	12	0·430
Time 2nd insulin peak (min)	57·9[a]	15·4	14	78·8[b]	21·3	12	0·009
Fasting SI_1 (mU/mmol)	3·7	1·2	16	3·3	1·1	12	0·469
AUC_{ins}/AUC_{gluc} (mU/mmol)	3·6	1·6	14	3·4	1·1	14	0·683
HOMA‡	3·3	1·3	16	4·1	2·0	14	0·380
QUICKI‡	0·56	0·09	16	0·53	0·07	14	0·481
Bennett index‡	1·4	0·4	16	1·2	0·3	14	0·370

AUC_{gluc}, area under the curve for glucose; AUC_{ins}, area under the curve for insulin; fasting SI_1, basal insulin to glucose ratio; glucose $t_{1/2}$, half-life for glucose disappearance between 15 and 90 min after glucose administration; HOMA, homeostasis model assessment; K_{gluc}, glucose disappearance coefficient; QUICKI, quantitative insulin sensitivity check index.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of procedures, see Materials and methods.
† $n < 16$ caused by missing values.
‡ Calculated according to Appleton *et al.* [28].

Discussion

In the current study, obese cats confirmed a higher risk for insulin resistance and impaired glucose tolerance than normal-weight cats. Under the test conditions, differences in main blood glucose control characteristics were not observed. Obese cats, taking part in the present trial, had no significantly higher fasting serum insulin concentrations, but the increased AUC_{gluc}, the higher fasting plasma glucose concentration and the later appearance of the second insulin peak during IVGTT may have resulted in an impaired glucose tolerance and a higher insulin resistance in obese cats regardless of diet. The present findings were also concluded from previous studies in cats[29,30]. The significantly higher fasting plasma leptin concentrations in obese cats might also predict the occurrence of insulin resistance, since leptin is postulated to mediate some of the metabolic consequences of obesity. In cats, Appleton *et al.* [15] have demonstrated a strong positively relationship between leptin and insulin resistance. The significantly higher fasting plasma T_3 concentrations in obese cats during the present trial corresponds to the significant and positive correlation of T_3 with body weight, girth circumference and BMI as observed by Ferguson *et al.* [16]. T_4 also correlated positively with all indices of obesity and with leptin, but not with NEFA[16]. Still, fasting T_4 concentrations remained unchanged in the present trial. Ferguson *et al.* [16] also

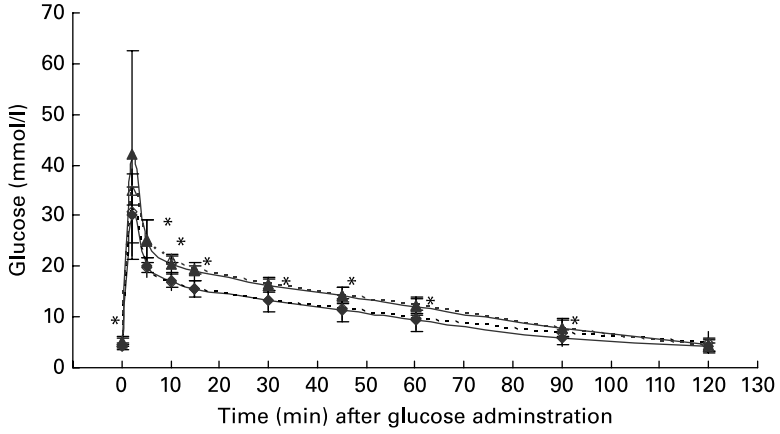

Fig. 1. Plasma glucose concentrations during the intravenous glucose tolerance test in healthy normal-weight (body condition score (BCS) 3/5) and obese (BCS 5/5) cats fed the control diet (C-diet) and prebiotic diet (P-diet; C-diet +2·5 % of a mixture of oligofructose and inulin). ◆, BCS 3, C-diet; ◇, BCS 3, P-diet; ▲, BCS 5, C-diet; △, BCS 5, P-diet. Values are means with their standard deviations depicted by vertical bars. Mean values were significantly different at each different time-point between normal-weight and obese cats, regardless of dietary treatment: *$P<0·05$.

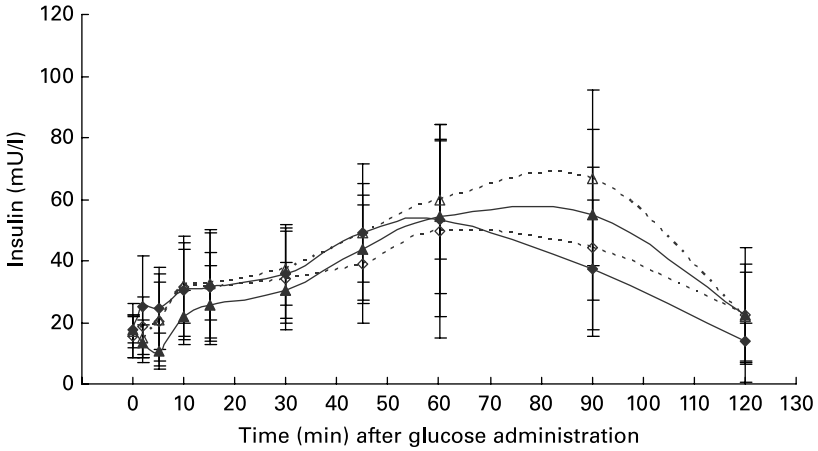

Fig. 2. Serum insulin concentrations during the intravenous glucose tolerance test in healthy normal-weight (BCS 3/5) and obese (BCS 5/5) cats fed the control diet (C-diet) and prebiotic diet (P-diet; C-diet +2·5 % of a mixture of oligofructose and inulin). ◆, BCS 3, C-diet; ◇, BCS 3, P-diet; ▲, BCS 5, C-diet; △, BCS 5, P-diet. Values are means with their standard deviations depicted by vertical bars.

demonstrated increased leptin and NEFA after weight gain and proposed an obesity-induced relative state of thyroid hormone resistance, either caused by leptin or by increased NEFA concentrations, or both. In the present trial, fasting leptin concentrations were indeed elevated in obese cats, but no significant differences were noted for serum NEFA concentrations. This supports that the relative state of thyroid hormone resistance might be most probably explained by the rise in fasting plasma leptin concentrations. In overweight man, hyperleptinaemia was also observed to be strongly correlated with elevated serum ALT activity[31], most probably due to the high association between elevated liver enzymes, most notably high serum ALT activity and obesity (percentage body fat[32]; obesity class[33]) and insulin resistance (measured by hyperinsulinaemic euglycaemic clamp technique[32] or HOMA[33]) as observed by Vozarova *et al.* [32] and Marchesini *et al.* [33]. Therefore, the occurrence of insulin resistance might explain the rise in plasma ALT activity observed in obese cats. In addition, serum TAG also tended to rise in obese cats, in accordance with the results found by Hoenig *et al.* [19], who studied the effect of obesity on lipid profiles in neutered cats. Hoenig *et al.* [19] also noted a significant rise in plasma total cholesterol concentrations in obese cats; yet, this could not be demonstrated from the present trial. At last, fasting serum urea concentrations were reduced in obese cats compared to normal-weight cats. In rats made obese by feeding a cafeteria-diet, similar results were demonstrated. According to Barber *et al.* [34], a decreased serum urea concentration in obese rats might be due to a decrease in the activities of all enzymes of the urea cycle and a lower rate of synthesis of urea from precursors in hepatocytes. To date, no data are available on the impact of feline obesity on urea concentrations and the activity of urea cycle enzymes, yet in obese cats suffering from severe hepatic lipidosis it is observed that blood urea nitrogen might be subnormal caused by chronic anorexia or presuming impaired urea cycle function[35]. However, during the present trial cats were not anorectic, did not lose any weight and did not show any other clinical signs reflecting hepatic lipidosis, which probably indicates reduced serum urea concentrations due to compromised urea cycle function as observed in obese rats.

Table 5. Effect of adding 2·5 % of a mixture of oligofructose and inulin to the control diet on carnitine metabolism, regardless of body condition*

(Mean values and standard deviations)

	Diet						
	C-diet			P-diet			
	Mean	SD	*n* †	Mean	SD	*n*†	*P*
Free carnitine	32·15	6·07	15	41·42	21·03	15	0·579
Acetylcarnitine	5·545	0·823	15	6·321	2·500	15	0·352
Propionylcarnitine	0·134[a]	0·082	15	0·275[b]	0·109	15	0·030
Butyrylcarnitine	0·283[a]	0·069	15	0·474[b]	0·186	15	0·002
Methylmalonylcarnitine	0·054	0·029	15	0·036	0·012	15	0·072

C-diet, control diet; P-diet, prebiotic diet (C-diet +2·5 % of a mixture of oligofructose and inulin).
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of procedures and diets, see the Materials and methods section and Table 1.
† $n < 16$ caused by missing values.

Adding 2·5 % mixture of oligofructose and inulin to a basic diet did not alter the investigated standard characteristics for glucose and insulin metabolism, or other metabolic and endocrine characteristics, in healthy normal-weight and obese cats; except for serum TAG concentrations, the activity of AST and several acylcarnitines. Serum TAG concentrations tended to be increased in cats supplemented with oligofructose and inulin. The present finding contrasts with the results from earlier trials in rodents[36–40] and dogs[8] which revealed lower TAG concentrations following soluble fibre supplementation. Since body weight remained unchanged during the trial, an increase in body weight could not explain the present observation. Nevertheless, serum TAG concentrations remained within the references range. When compared to other trials conducted in rats[39–41] and dogs[8–10,12], the amount of soluble fibre used in the present trial (0·5 g/kg body weight) seems rather low. Nevertheless, in human studies[42–44] doses of oligofructose were similar to the dose used in the present trial. In healthy human subjects ingesting 20 g fructooligosaccharide/d (0·3 g/kg body weight) for 4 weeks, fructooligosaccharides did not modify fasting glucose and insulin concentrations, but did lower basal hepatic glucose production[42]. According to Yamashita *et al.*[45], 8 g fructooligosaccharide/d (0·1 g/kg body weight) for 2 weeks resulted in a reduced fasting glycaemia in type 2 diabetic patients. For the results of the current study, species differences including insulin sensitivity must be taken into account. For higher doses, Hesta *et al.*[46] showed that faeces became formless and apparent digestibility of protein and fat were reduced when cats ingested more then 3 % oligofructose or inulin, suggesting a limitation of more than 3 % oligofructose or inulin for practical use in cats. Concerning the type of soluble fibre, Diez *et al.*[8] observed significantly decreased postprandial glucose concentrations after incorporation of both fructooligosaccharides and sugar beet fibre in healthy dogs, in spite of non-significant effects on fasting glucose concentration or postprandial glucose curve after supplementation of inulin or sugar-beet fibre[9]. In the present trial the diet was supplemented with a mixture of both oligofructose and inulin, containing a higher proportion of inulin which has a higher degree of polymerisation compared to oligofructose. This suggests a slower fermentation in the relative short large bowel of the cat. Another reason for the absence of effect on standard characteristics for glucose and insulin metabolism might be the control diet. At first, it has been demonstrated in man[47] and dogs[48] that supplementing soluble as well as insoluble fibre to a basic diet only results in a better glucose and insulin response when the diet contains more than 40 % of the metabolisable energy as carbohydrates, whereas our basic diet only contained 26 % of metabolisable energy as carbohydrates. Secondly, insulin resistance might have been triggered better by using a diet containing high amounts of fat, as demonstrated in man and rodents[49,50] as well as in cats[51], instead of a high-protein, low-carbohydrate diet as used in the present trial. Nevertheless, a previous trial showed an increased insulin resistance in healthy non-obese cats fed a low-carbohydrate diet when compared to a low-fat and a low-protein diet (A Verbrugghe *et al.*, unpublished results). Mechanisms responsible for enhancing glucose tolerance and insulin sensitivity due to soluble fibre as described in other species might be less pronounced in cats.

Despite the absence of a direct effect on glucose tolerance and insulin sensitivity, an effect on colonic fermentation and the production of SCFA could be demonstrated by acylcarnitine analysis. Higher production of propionate and butyrate and metabolisation of these SCFA were observed by the higher propionyl- and butyrylcarnitine concentrations in cats when fed the P-diet. Acetylcarnitine was not altered among diets. Acetyl-CoA is not only generated from acetate, but also from amino acids and other fatty acids. Moreover, propionate might inhibit the rise in acetylcarnitine concentrations caused by butyrate, as demonstrated by Brass & Beyerinck[52].

In the present trial, oligofructose and inulin might have contributed to the citric acid cycle through propionate. Theoretically, propionate, being a gluconeogenic SCFA, can be converted to glucose via succinyl-CoA and oxaloacetate[53]. In ruminants[54,55] and horses[56], propionate is known to be the primary gluconeogenic substrate. In strictly carnivorous species, such as cats, no data are available yet. It is also known from studies in man as well as experimental animals that propionate decreases gluconeogenesis from pyruvate. This occurs directly through inhibition of pyruvate carboxylase via its specific intermediaries, methylmalonyl CoA and propionyl CoA and indirectly through depletion of acetyl CoA, a specific allosteric activator of this enzyme[57,58]. The present trial revealed increased propionylcarnitine concentrations, suggesting inhibited gluconeogenesis from pyruvate, resulting in sparing amino acids. Methylmalonylcarnitine tended to decrease among diets, which supports the hypothesis of reduced amino acid catabolism, since methylmalonylcarnitine is known to be a metabolite of valine, methionine and isoleucine catabolism. Moreover, the significantly decreased plasma AST activity in cats supplemented with oligofructose and inulin indicates inhibited gluconeogenesis from aspartate.

In conclusion, impaired glucose tolerance and increased insulin resistance were observed in obese cats compared to normal-weight cats, regardless of diet. Adding 2·5 % of a mixture of oligofructose and inulin to a basic diet did not affect glucose tolerance in healthy normal-weight or in obese cats. However, modulation of glucose metabolism by enhancing gluconeogenesis from propionate and therefore inhibition of amino acid catabolism can be suggested, and can be beneficial in the treatment of feline insulin resistance and diabetes. Yet, further research investigating the postprandial effects of prebiotics on acylcarnitine profile can be of interest.

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