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# The glucose and insulin response to isoenergetic reduction of dietary energy sources in a true carnivore: the domestic cat (*Felis catus*)

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The present study assessed the effect of separate reduction of each energy-delivering nutrient – protein, fat and carbohydrate – on glucose tolerance and insulin response in a strict carnivore: the domestic cat (*Felis catus*). Three isoenergetic, home-made diets with the following energetic distribution, low protein (LP): protein 28 % of metabolisable energy; fat 43 %; nitrogen-free extract 29 %; low fat: 47, 27 and 25 %; low carbohydrate (LC): 45, 48 and 7 %, were tested in a 3 × 3 Latin square design. Nine healthy normal-weight cats were randomly assigned to each of the diets in a random order at intervals of 3 weeks. At the end of each testing period, intravenous glucose tolerance tests were performed. Plasma glucose concentrations and area under the glucose curve showed no differences. Area under the insulin curve was lower when cats were fed the LP diet, and the second insulin peak tended to be delayed when the LC diet was fed. In contrast to other studies, in which energy sources were elevated instead of being reduced, the present trial contradicts the often suggested negative impact of carbohydrates on insulin sensitivity in carnivores, and shows that reducing the dietary carbohydrate content below common amounts for commercial foods evokes an insulin-resistant state, which can be explained by the cats' strict carnivorous nature. It even points to a negative effect of protein on insulin sensitivity, a finding that corresponds with the highly gluconeogenic nature of amino acids in strict carnivores.

**Carbohydrates: Carnivores: Cats: Energy sources: Insulin resistance**

Insulin resistance is a state in which greater than normal insulin concentrations are required to elicit a quantitatively normal glucose response in the body, tissues and cells[1]. Insulin resistance and consequent hyperinsulinaemia are associated with a cluster of abnormalities, such as hypertension, dyslipidaemia and central obesity, which increase cardiovascular risk. This cluster is referred to as the metabolic syndrome[1,2]. In individuals who are unable to compensate for reduced insulin sensitivity, impaired glucose tolerance and overt diabetes might occur because of the prolonged and increased demand on β-cells to secrete insulin[1,3].

A critical role for the quantity and quality of dietary carbohydrates in the pathogenesis of this disorder has been postulated by the 'carnivore connection' theory[1,3,4]. During the Ice Ages, our ancestors consumed high-protein (HP), low-carbohydrate (LC) diets, and since the brain, fetus and mammary gland all have specific needs for glucose, metabolic adaptations were necessary to adapt to low glucose intake. Therefore, resistance to the glucose-lowering effects of insulin offered survival and reproductive advantages. The event of the agricultural revolution augmented the amount of digestible carbohydrates, and the industrial revolution was responsible for changing the quality of carbohydrates. These evolutionary changes in carbohydrates, meaning the introduction of high-glycaemic index foods, can worsen insulin resistance and can be linked to the development of type 2 diabetes[1,4].

Over the last decades, as in humans, the diet of strictly carnivorous domestic cats changed from HP, LC prey[5] to commercial diets, often containing moderate to high amounts of highly digestible carbohydrates. As in humans, these dietary changes are held responsible for the recent increase in incidence of feline insulin resistance and diabetes mellitus[3,6].

Several human studies[7–10] as well as rodent studies (rats[11] and mice[12]) demonstrated that glucose tolerance and insulin sensitivity could benefit from HP, LC diets. In healthy cats, high-carbohydrate (HC) diets are suggested to impair glucose tolerance[13]. However, the hypothesis that HC diets lead to β-cell exhaustion was contradicted by Slingerland *et al.*[14], since feeding a HC diet resulted in

**Abbreviations:** AUC, area under the curve; HC, high carbohydrate; HF, high fat; HP, high protein; IVGTT, intravenous glucose tolerance tests; LC, low carbohydrate; LF, low fat; LP, low protein.

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increased glucose-induced insulin secretion during hyperglycaemic glucose clamps. Yet, this could be the first step towards β-cell exhaustion, but long-term consequences were not investigated. It can, however, not be disclaimed that HC diets might be an indirect risk factor for diabetes by promoting obesity. Hoenig *et al.* [15] suggested that cats with the same energetic intake are more prone towards obesity and insulin resistance when fed a low-protein (LP), HC diet in comparison with a HP, LC diet. In contrast, high-fat (HF), LC diets were also shown to impair glucose tolerance[16,17] as well as to induce weight gain[16].

A plausible explanation for the lack of agreement among these studies is that the effect of increasing one energy source (1) was often confounded with the decrease of other energy sources; or (2) often meant an increase on top of an already high level of this energy source.

The present study deals with these aspects by applying a pairwise reduction of one energy source, and therefore enables the identification of the separate effect of each energy source by looking at the effect of its reduction to minimal amounts. This method has been shown to be effective in demonstrating single energy source effects on metabolism in poultry[18].

## Material and methods

### *Animals and housing*

Six mixed-breed and three European shorthair cats, five intact females and one intact and three neutered males, were employed in the present study. All cats were aged between 3 and 10 years, and had a mean body weight of 3·67 kg (range 2·50–5·24 kg). Body condition score was determined using a five-point body condition scoring system[19]. Non-obese cats with a body condition score of 2·5/5 to 3·5/5 were used. All cats were healthy and were not given any medication at the time of the study; none had prior medical problems. Cats were divided into three groups based on sex and body weight and were housed individually in separated indoor cages during the trial. For 2 h a day, the cats were allowed to play in their usual group cages. At that time, the cats had no access to the food, but water was available *ad libitum*.

### *Diets and feeding*

Three isoenergetic home-made diets were tested: a LP, a low-fat (LF) and a LC diet. To produce the test diets, cooked and ground chicken breast was mixed with liquid chicken lard and maize starch (CstarGel 03 401, native maize starch; Cargill, Sas van Gent, The Netherlands). The same ingredients were used in different quantities in order to create pairwise changes in macronutrient content (Table 1). The LP diet differed from LF and LC diets only by isoenergetic substitution of protein for fat and protein for carbohydrate, respectively. The LF diet differed from the LC diet by isoenergetic substitution of fat for carbohydrate. The three test diets contained no dietary fibre, and had similar mineral concentrations and physical structure. A tailor-made vitamin and mineral premix (Institute for Physiology, Physiological Chemistry and Nutrition, Ludwig-Maximilians-University München, Oberschleißheim, Germany) was added to balance the diet. The three diets were analysed for proximate components (Table 1), and energetic distributions were as follows: LP diet: protein 28 %, fat 43 % and nitrogen-free extract 29 %; LF diet: protein 47 %, fat 27 % and nitrogen-free extract 25 %; LC diet: protein 45 %, fat 48 % and nitrogen-free extract 7 %.

**Table 1.** Composition of the test diets

| | LP | LF | LC |
|---|---|---|---|
| Ingredients (%) | | | |
| Chicken fillet | 66·3 | 81·0 | 86·1 |
| Chicken lard | 11·4 | 4·3 | 8·3 |
| Maize starch* | 20·7 | 14·1 | 5·0 |
| Vitamin/mineral premix† | 1·5 | 0·6 | 0·6 |
| Nutrients on DM (%; by Weende analysis) | | | |
| Crude protein | 36·1 | 54·5 | 60·2 |
| Diethyl ether extract | 23·2 | 13·1 | 26·6 |
| Crude fibre | 0·4 | 0·4 | 0·6 |
| Crude ash | 3·1 | 4·3 | 4·1 |
| NFE‡ | 37·2 | 27·7 | 8·5 |
| Starch | 26·0 | 27·1 | 3·3 |
| TDF | 1·9 | 1·8 | 3·7 |
| ME (kJ/100 g DM)§ | 1938 | 1762 | 2025 |
| Nutrients on energy basis (g/MJ ME) | | | |
| Crude protein | 18·7 | 32·0 | 30·3 |
| Diethyl ether extract | 12·0 | 7·7 | 13·4 |
| Crude fibre | 0·2 | 0·2 | 0·3 |
| Crude ash | 1·6 | 2·5 | 2·1 |
| NFE | 19·2 | 16·2 | 4·3 |
| Starch | 13·4 | 15·9 | 1·7 |
| Amino acid content on DM (%) | | | |
| Asp | 2·8 | 3·8 | 3·9 |
| Thr | 1·4 | 2·2 | 2·3 |
| Ser | 1·1 | 1·7 | 1·9 |
| Glu | 4·4 | 6·3 | 6·5 |
| Gly | 1·4 | 2·2 | 2·2 |
| Ala | 2·0 | 3·0 | 3·1 |
| Val | 1·7 | 2·5 | 2·6 |
| Ile | 1·8 | 2·6 | 2·6 |
| Leu | 2·7 | 4·0 | 4·1 |
| Tyr | 1·5 | 2·2 | 2·4 |
| Phe | 1·4 | 2·0 | 2·1 |
| His | 1·1 | 1·7 | 1·8 |
| Lys | 3·0 | 4·4 | 4·7 |
| Arg | 2·0 | 3·0 | 3·0 |

LP, low protein; LF, low fat; LC, low carbohydrate; NFE, nitrogen-free extract; TDF, total dietary fibre; ME, metabolisable energy.

* Native maize starch (CstarGel 03 401, Cargill).

† Provides: LP diet: Ca 14·10 %, P 3·41 %, K 8·4 %, Mg 0·3 %, Na 0·34 %, Fe 0·31 %, Zn 0·29 %, Mn 0·038 %, Cu 0·014 %, iodine 0·006 %, vitamin A 45 μg/g, vitamin $D_3$ 0·31 μg/g, vitamin E 3·25 mg/g, vitamin $B_1$ 0·3 mg/g, vitamin $B_2$ 0·2 mg/g, taurine 75 mg/g; LF and LC diets: Ca 27·70 %, Fe 0·44 %, Zn 0·56 %, Mn 0·057 %, iodine 0·013 %, vitamin A 67·5 μg/g, vitamin $D_3$ 0·56 μg/g, vitamin E 5·65 mg/g, vitamin $B_1$ 0·53 mg/g, vitamin $B_2$ 0·28 mg/g, taurine 140 mg/g.

‡ Derived by subtracting crude protein, diethyl ether extract, crude fibre and crude ash from the DM content.

§ Estimated by using a four-step calculation[27].

Cats were offered only one meal daily. The amount of food corresponded with the cats' individual maintenance energy requirement ($375\ kJ/kg^{0·75}$)[20], and was adjusted to maintain body weight. The food was available all day, except for the 2 h playtime. All cats had free access to water at all times. Fresh water was supplied daily.

### *Experimental design*

Before being entered into the study, each cat was physically examined; body weight and body condition score were recorded and a blood sample was drawn from the jugular

vein after a 12 h fast for complete blood count and serum biochemistry. For 4 weeks preceding the trial, all cats were fed a standard commercial maintenance diet (Bento Kronen Torka chicken-turkey; Versele-Laga, Deinze, Belgium), before being randomised to one of the three groups. Each group of cats was assigned to each of the three test diets in a random order at intervals of 3 weeks. This way, the test diets were examined in a 3 × 3 Latin square design.

Absolute food intake was measured each day throughout the study, and daily energy consumption was calculated. Body weight was recorded weekly.

To determine the effect on glucose and insulin metabolism, intravenous glucose tolerance tests (IVGTT) were performed at the end of each testing period. Hence, at least 20 h before the IVGTT, cats were anaesthetised with buprenorphine (Temgesic, Schering-Plough n.v., Heist-Op-Den-Berg, Belgium), 10 μg/kg intravenous, followed by propofol (Propovet, Abbott Lab, Leuven, Belgium), 6–7 mg/kg to effect, intravenous, and a 20G, 8 cm intra venous catheter (Leaderflex, Vygon, Écouen, France) was placed in a jugular vein for glucose administration and blood sampling. The catheter was flushed twice daily with 1 ml heparinised saline (50 IU heparin/ml in 0·9 % NaCl solution) to maintain patency. Amoxicillin (Clamoxyl LA, GlaxoSmithKline n.v., Genval, Belgium), 15 mg/kg subcutaneous, was administered once at the time of catheter placement. The IVGTT was performed between 09.00 and 13.00 hours after a 12 h fast. Glucose, 0·5 g/kg (Glucose Sterop 500 mg/ml, Laboratoria Sterop n.v., Brussels, Belgium), was administered via the jugular vein catheter over 30–45 s, followed immediately by 1 ml of saline solution to flush the catheter[21]. Blood samples were collected from the jugular catheter[22] before (0 min) and 2, 5, 10, 15, 30, 45, 60, 90 and 120 min after glucose administration[21].

In addition, effects on lipid as well as protein metabolism were evaluated by the analysis of total cholesterol, TAG, NEFA, urea and creatinine, respectively. The effect of energy-delivering nutrients on plasma leptin concentrations was also investigated, since leptin also influences carbohydrate and fat metabolism[23].

At time zero, blood samples were collected in tubes containing lithium heparin for the determination of plasma leptin and NEFA concentrations, and serum tubes were used to determine basal serum total cholesterol, TAG, urea and creatinine concentrations. At each time interval, blood samples were collected in tubes containing NaF for the determination of plasma glucose and in serum tubes for the determination of serum insulin concentrations. Plasma and serum were removed by centrifugation and stored at −20°C until assayed. The experimental protocol of the present study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2006/029), and was in accordance with the institutional and national guidelines for the care and use of animals.

### *Analytical methods*

The test diets were analysed for DM, ash, crude protein, diethyl ether extract, starch, crude fibre, total dietary fibre and amino acid profile. DM and ash content were determined by drying to a constant weight at 103°C and combusting at 550°C, respectively. Crude protein (6·25 × N) was determined using the Kjeldahl method (ISO 2005), and diethyl ether extract was analysed with the Soxhlet method (ISO 1973). Starch, crude fibre and total dietary fibre were determined using the Association of Official Analytical Chemists methods[24–26]. Amino acids (aspartate, threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine) were analysed according to the directive 98/64/CE. Metabolisable energy was estimated by using predictive equations according to a four-step calculation based on the calculation of gross energy and digestibility of energy[27].

Plasma glucose, total cholesterol, TAG, urea and creatinine concentrations were determined spectrophotometrically by using the Roche/Hitachi Modular Analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Plasma NEFA concentrations were determined spectrophotometrically by using the WAKO NEFA C test kit (Wako Chemicals GmbH, Neuss, Germany) modified for use in the Monarch Chemistry System (Instrumentation Laboratories n.v./S.A., Zaventem, Belgium). Serum insulin concentrations were measured by using a commercially available immunoradiometric assay test kit (insulin IRMA Ref 5251, Biosource Europe S.A., Nivelles, Belgium) as used by Slingerland *et al.*[28]. Plasma leptin concentrations were determined by 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[29].

The glucose disappearance coefficient ($K_{glucose}$) and the half-life for glucose disappearance ($t_{1/2}$) were calculated by linear regression of $\log_{10}$ of glucose concentrations between 15 and 90 min after glucose administration, according to Link & Rand[30], using the following equations:

$$K_{glucose} = (|b| \times 100)/\log_{10}e \text{ and glucose } t_{1/2}$$

$$= (\log_e 2 \times 100)/K_{glucose}$$

respectively, where $|b|$ is the absolute value of the slope and e is the natural logarithm base.

The basal insulin to glucose ratio (fasting basal insulin to glucose ratio), the ratio of area under the insulin to glucose curve ($AUC_{ins}/AUC_{gluc}$), the homoeostasis model assessment, the quantitative insulin sensitivity index and the Bennett index were also calculated[31]. $AUC_{gluc}$ and $AUC_{ins}$ were calculated according to the trapezoidal method (baseline equal to zero).

### *Statistical analyses*

Statistical analyses were performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). Data were statistically analysed by univariate general linear model analysis with diet and period as fixed factors and animal as a random factor. Whenever significant differences were observed, a Bonferroni test was performed as *post hoc* test. Statistical significance was accepted at $P<0·05$. All data are expressed as mean values with their standard errors.

**Table 2.** Plasma glucose concentrations during intravenous glucose tolerance tests in healthy cats fed a low-protein (LP), a low-fat (LF) and a low-carbohydrate (LC) diet

(Mean values with their standard errors)

| | LP | | | LF | | | LC | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | *n* | Mean | SE | *n* | Mean | SE | *n* | *P* |
| Basal glucose (mmol/l) | 3·6 | 0·2 | 9 | 3·8 | 0·1 | 9 | 3·9 | 0·2 | 9 | 0·395 |
| Glucose after 2 min (mmol/l) | 32·8 | 4·2 | 9 | 38·2 | 10·6 | 7 | 32·3 | 2·9 | 9 | 0·627 |
| Glucose after 5 min (mmol/l) | 17·8 | 1·2 | 9 | 17·8 | 1·4 | 8 | 18·0 | 1·1 | 7 | 0·703 |
| Glucose after 10 min (mmol/l) | 15·0 | 0·8 | 8 | 15·5 | 1·0 | 9 | 15·8 | 0·6 | 9 | 0·568 |
| Glucose after 15 min (mmol/l) | 14·2 | 1·2 | 8 | 14·3 | 0·9 | 9 | 14·6 | 0·7 | 9 | 0·615 |
| Glucose after 30 min (mmol/l) | 10·8 | 0·9 | 9 | 11·1 | 0·9 | 9 | 12·0 | 0·8 | 9 | 0·102 |
| Glucose after 45 min (mmol/l) | 9·0 | 1·3 | 9 | 8·6 | 0·9 | 9 | 9·9 | 0·9 | 9 | 0·176 |
| Glucose after 60 min (mmol/l) | 7·4 | 1·2 | 9 | 6·5 | 0·8 | 9 | 7·9 | 0·9 | 9 | 0·245 |
| Glucose after 90 min (mmol/l) | 4·6 | 0·8 | 9 | 4·0 | 0·5 | 9 | 5·0 | 0·7 | 9 | 0·332 |
| Glucose after 120 min (mmol/l) | 3·6 | 0·3 | 9 | 3·6 | 0·3 | 9 | 4·1 | 0·5 | 9 | 0·349 |
| $AUC_{gluc}$ (mmol/l × 120 min) | 976 | 85·5 | 8 | 1005 | 69·2 | 8 | 1108 | 86·2 | 9 | 0·186 |
| $K_{gluc}$ | 1·62 | 0·16 | 9 | 1·77 | 0·15 | 9 | 1·50 | 0·14 | 9 | 0·334 |
| Glucose $t_{1/2}$ (min) | 47·7 | 6·7 | 9 | 42·5 | 5·0 | 9 | 52·0 | 7·9 | 9 | 0·463 |

$AUC_{gluc}$, area under the curve for glucose; $K_{gluc}$, glucose disappearance coefficient; $t_{1/2}$, half-life for glucose disappearance between 15 and 90 min after glucose administration.

## Results

### *Diet composition, food intake and body weight*

Nutrient compositions and amino acid profiles of the three test diets are given in Table 1. The amino acid supply was lower when cats were fed the LP diet compared with the LF and LC diets, but all amino acids remained within the recommendations of the National Research Council[32]. All test diets were well tolerated. None of the cats refused to eat any of the diets, and none showed signs of illness or maldigestion. Mean daily energy consumption (LP: 778 kJ/d; LF: 814 kJ/d and LC: 791 kJ/d; $P=0·692$) showed no differences among test diets. Body weight (LP: 3·23 kg; LF: 3·82 kg and LC: 3·19 kg; $P=0·177$) remained stable in all cats during the study, and was not affected by test diets.

### *Glucose tolerance test*

*Glucose*. Basal plasma glucose, glucose 2 min after glucose administration (glucose peak) and glucose concentrations at each other time point of the IVGTT showed no differences among test diets. In all cats, plasma glucose concentrations returned to baseline at 120 min after glucose administration. The $AUC_{gluc}$ did not differ among test diets. $K_{glucose}$ as well as glucose $t_{1/2}$ showed no differences (Table 2).

*Insulin*. No differences among test diets were observed for basal serum insulin concentrations. As shown in Fig. 1, insulin secretion was biphasic in response to glucose administration. During the IVGTT, serum insulin concentrations tended to be lower at 45 min after glucose administration when cats were fed the LP diet compared with the LF and LC diets ($P=0·069$). At 60 min after glucose administration, serum insulin concentrations also tended to be lower when cats were fed the LP diet, compared with the LC diet, with the LF diet being intermediate ($P=0·051$). At all other time points, no differences were found among test diets. When cats were fed the LC diet, a tendency towards later occurrence of the second insulin peak was noticed. In contrast, when the LP diet was fed, there was a tendency towards earlier occurrence of the second insulin peak ($P=0·060$). This second insulin peak was also numerically higher for the LC diet compared with the LP and LF diets, but the difference was NS. In all cats, except one individual when fed the LP diet, the serum insulin concentrations returned to baseline level at 120 min after glucose administration. The $AUC_{ins}$ was decreased in cats fed the LP diet compared with the LF and LC diets. Nevertheless, fasting basal insulin to glucose ratio, $AUC_{ins}/AUC_{gluc}$, homoeostasis model assessment, quantitative insulin sensitivity index and Bennett index did not differ among test diets (Table 3 and Fig. 1).

### *Metabolic parameters*

Basal serum urea concentrations were decreased in cats fed the LP diet compared with the LF diet, with the LC diet being intermediate ($P=0·039$). Basal serum creatinine

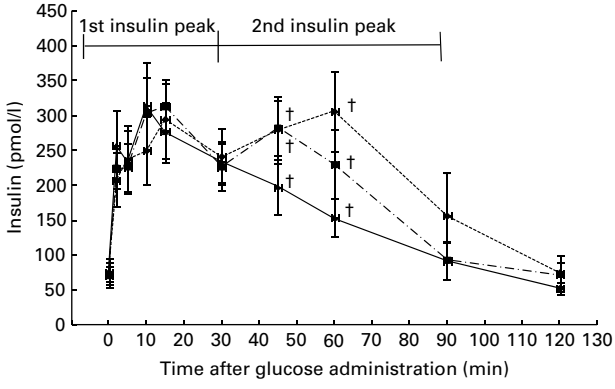


**Fig. 1.** Insulin curve during intravenous glucose tolerance tests in healthy cats fed a low-protein (LP), a low-fat (LF) and a low-carbohydrate (LC) diet. Values were expressed as expressed as mean values with their standard errors. † Mean values tended to be different among test diets ($0·05 < P < 0·1$). - -◆- -, LC diet; - .■- ., LF diet; —▲—, LP diet.

**Table 3.** Serum insulin concentrations during intravenous glucose tolerance tests in healthy cats fed a low-protein (LP), a low-fat (LF) and a low-carbohydrate (LC) diet

(Mean values with their standard errors)

| | LP | | | LF | | | LC | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | *n** | Mean | SE | *n** | Mean | SE | *n** | *P* |
| Basal insulin (pmol/l) | 74·6 | 21·7 | 9 | 71·4 | 13·0 | 9 | 75·3 | 13·4 | 9 | 0·971 |
| Insulin after 2 min (pmol/l) | 256·1 | 50·9 | 8 | 224·0 | 28·6 | 7 | 208·3 | 38·0 | 9 | 0·510 |
| Insulin after 5 min (pmol/l) | 233·5 | 45·3 | 8 | 225·7 | 34·4 | 8 | 236·7 | 48·1 | 7 | 0·533 |
| Insulin after 10 min (pmol/l) | 314·0 | 63·2 | 8 | 303·3 | 51·1 | 9 | 249·9 | 49·7 | 9 | 0·239 |
| Insulin after 15 min (pmol/l) | 276·7 | 43·1 | 6 | 313·1 | 34·1 | 9 | 294·7 | 57·1 | 9 | 0·901 |
| Insulin after 30 min (pmol/l) | 233·9 | 29·8 | 8 | 226·5 | 33·5 | 9 | 241·3 | 40·3 | 9 | 0·339 |
| Insulin after 45 min (pmol/l) | 198·0 | 38·9 | 8 | 282·6 | 39·2 | 9 | 280·2 | 48·1 | 9 | 0·069 |
| Insulin after 60 min (pmol/l) | 153·1 | 26·9 | 8 | 229·8 | 50·0 | 9 | 306·0 | 57·5 | 9 | 0·051 |
| Insulin after 90 min (pmol/l) | 91·7 | 26·1 | 8 | 92·6 | 27·7 | 9 | 156·3 | 61·6 | 9 | 0·251 |
| Insulin after 120 min (pmol/l) | 52·3 | 8·3 | 7 | 72·2 | 16·4 | 9 | 74·8 | 25·4 | 8 | 0·538 |
| $AUC_{ins}$ (pmol/l × 120 min) | 19139[a] | 2380 | 8 | 23955[b] | 3325 | 8 | 26356[b] | 4022 | 9 | 0·019 |
| Time 1st insulin peak (min) | 5·6 | 1·9 | 8 | 7·6 | 1·9 | 9 | 9·6 | 2·2 | 9 | 0·091 |
| Time 2nd insulin peak (min) | 36·9 | 6·7 | 8 | 44·4 | 4·8 | 9 | 52·5 | 7·5 | 8 | 0·060 |
| Height 1st insulin peak (pmol/l) | 328·6 | 56·7 | 8 | 295·6 | 38·0 | 9 | 301·4 | 54·5 | 9 | 0·845 |
| Height 2nd insulin peak (pmol/l) | 283·4 | 48·8 | 8 | 324·3 | 50·2 | 9 | 364·5 | 62·4 | 9 | 0·436 |
| Basal SI | 19·9 | 5·4 | 9 | 18·9 | 3·2 | 9 | 19·0 | 3·0 | 9 | 0·967 |
| $AUC_{ins}/AUC_{gluc}$ | 20·9 | 3·3 | 8 | 24·0 | 3·0 | 8 | 24·4 | 3·4 | 9 | 0·386 |
| HOMA | 12·6 | 3·9 | 9 | 12·1 | 2·4 | 9 | 13·5 | 2·7 | 9 | 0·905 |
| QUICKI | 0·45 | 0·03 | 9 | 0·43 | 0·01 | 9 | 0·42 | 0·02 | 9 | 0·386 |
| Bennett index | 1·13 | 0·14 | 9 | 0·99 | 0·05 | 9 | 0·99 | 0·09 | 9 | 0·349 |

$AUC_{ins}$, area under the curve for insulin; $AUC_{ins}/AUC_{gluc}$, ratio of area under the insulin to glucose curve; HOMA, homoeostasis model assessment; QUICKI, quantitative insulin sensitivity index.

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

* $n < 9$ is caused by missing values.

concentrations were increased in cats fed the LP diet compared with the LC diet, with the LF diet being intermediate ($P=0·034$). Feeding the LP and LF diets resulted in decreased serum total cholesterol concentrations when compared with the consumption of the LC diet ($P=0·005$). No differences were observed among test diets for basal serum TAG and plasma NEFA concentrations as well as for basal plasma leptin concentrations (Table 4).

## Discussion

The present study showed no differences among test diets concerning the glucose response, and revealed that the LP diet induced a lower $AUC_{ins}$ than the LF and LC diets. Not only the higher $AUC_{ins}$, but also the tendency towards a delayed second insulin peak suggests decreased insulin sensitivity in healthy normal weight cats when cats were fed the LC diet.

These findings seem to contradict earlier performed studies that showed reducing dietary carbohydrate content to improve insulin sensitivity. For example, Farrow *et al.* [13] suggested impaired glucose tolerance when fed a HC diet in comparison with a HF and a HP diet. Yet, the amount of carbohydrates was increased on top of an already high level of this energy source, as also conducted by Slingerland *et al.* [14], Backus *et al.* [16] and Leray *et al.* [33], resulting in extremely high amounts of carbohydrates, much higher than the currently available commercial cat foods. Therefore, investigating the effect of lowering the carbohydrate content and comparing this to higher, but commercially available, concentrations of carbohydrate are much more useful. However, more than contradictory, the findings of the present study might be

**Table 4.** Results for biochemical indices and leptin in healthy cats fed a low-protein (LP), a low-fat (LF) and a low-carbohydrate (LC) diet

(Mean values with their standard errors, *n* 9)

| | LP | | LF | | LC | | |
|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | *P* |
| Urea (mmol/l) | 7·8[a] | 0·7 | 9·4[b] | 0·9 | 8·7[a,b] | 0·8 | 0·039 |
| Creatinine (μmol/l) | 136·5[a] | 9·0 | 130·6[a,b] | 8·3 | 123·8[b] | 10·0 | 0·034 |
| Cholesterol (mmol/l) | 3·2[a] | 0·2 | 3·1[a] | 0·3 | 3·5[b] | 0·3 | 0·006 |
| TAG (mmol/l) | 0·37 | 0·05 | 0·39 | 0·14 | 0·32 | 0·04 | 0·718 |
| NEFA (mmol/l) | 0·87 | 0·15 | 0·92 | 0·10 | 0·99 | 0·15 | 0·804 |
| Leptin (ng/ml) | 3·38 | 0·29 | 3·09 | 0·28 | 2·87 | 0·18 | 0·182 |

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

complementary to earlier studies, suggesting that the effect of carbohydrates on insulin sensitivity might be a U-shaped response, and that both extremely low and extremely high amounts of carbohydrates evoke diminished insulin sensitivity. In healthy cats, the effect of reducing carbohydrate content was investigated earlier in two other studies. According to Thiess *et al.* [17], the impaired glucose tolerance when feeding a HF, LC diet was due to increased fat intake. However, the HF, LC diet also contained low concentrations of carbohydrates. Since the two test diets differed only by substituting fat for carbohydrates, confounding effects might have occurred. Backus *et al.* [16] noted similar results, but once more, confounding effects might have arisen. In order to prevent confounding in the present trial, energy sources were substituted isoenergetically, resulting in a reduction of only one energy source, enabling identification of the effect of this energy source on glucose and insulin response. The results also seem to be contradictory to several earlier studies suggesting HP, LC diets to be beneficial for the treatment of type 2 feline diabetes mellitus[34–36]. Nevertheless, as healthy normal-weight cats were included in the study, the effect of energy sources on the onset of insulin resistance was investigated, and it should be noted that managing and preventing insulin resistance do not necessarily require the same dietary strategy.

The present study applied IVGTT as a method to determine glucose tolerance and insulin response. This method does not require as much blood samples as do minimal model analysis of the frequently sampled IVGTT and euglycaemic–hyperinsulinaemic clamps, which is considered the 'golden standard' in human subjects. Also, insulin injection and sophisticated mathematical computer software are not necessary. Moreover, results of IVGTT ($AUC_{ins}$ and $AUC_{ins}/AUC_{gluc}$) were well correlated with results obtained by the frequently sampled IVGTT as did basal insulin concentrations, fasting basal insulin to glucose ratio, homoeostasis model assessment, quantitative insulin sensitivity index and Bennett index[31]. Yet, these simplified measures did not differ among diets. However, the use of these indicators of insulin sensitivity has been questioned as no differences between normal weight and obese cats could be demonstrated[37,38]. Basal plasma leptin concentrations were similar among test diets as well, and were consistent with the normal and stable body weight of cats, as increased leptin concentrations are associated positively with the amount of body fat[39,40]. In cats, leptin is also reversely related with insulin sensitivity, independent of adiposity. Yet, it still remains unclear whether leptin is causally involved in this strong relationship[41]. Chronic hyperinsulinaemia, associated with insulin resistance, may stimulate leptin production and secretion, whereas the influence of leptin on insulin secretion is less clear[41]. Therefore, the changes in insulin sensitivity demonstrated in the present study do not necessarily require differences in basal leptin concentrations.

The cat's strictly carnivorous nature is most likely the explanation for the present findings. As in humans during the Ice Ages, chronic ingestion of a HP, LC diet and specific requirements for glucose of the brain, fetus and mammary gland evoked certain metabolic adaptations in cats to accommodate the low glucose intake[1,4]. True carnivorous animals like the cat have evolved and reproduced well on a LC diet, since they appear genetically insulin resistant in liver and peripheral tissues. The ability of insulin to inhibit hepatic glucose production as well as to augment tissue glucose disposal is impaired[4]. Therefore, gluconeogenesis is more or less permanently 'switched on' in these true carnivores[42,43]. In addition, feline pancreatic β-cells are much less sensitive to glucose than those of omnivores, and amino acids are proven to be important modulators of pancreatic insulin release[44]. Hence, cats fed the LC diet relied even more on dietary amino acids to stimulate their pancreatic insulin secretion and to maintain their blood glucose concentrations, first-phase insulin secretion was unaltered, but more insulin was needed to clear the blood glucose (second-phase insulin). Whereas feeding the LP diet resulted in a lower $AUC_{ins}$ and therefore enhanced insulin sensitivity, most probably due to the lower dietary intake of gluconeogenic amino acids and diminished amino acid-induced stimulation of pancreatic β-cells.

In the present study, the influence of the energy-delivering nutrients on metabolic indices as well as possible modifications of plasma leptin concentrations was also investigated. The basal serum urea concentrations were decreased in cats fed the LP diet compared with the LF diet, which can be explained by the lower protein intake[45]. The basal serum cholesterol concentrations were expected to be decreased in cats fed the LF diet, because of the lower fat intake[17]. In contrast with the expectations, the serum cholesterol concentrations were lower in cats fed the LP and LF diets compared with the LC diet, although the serum cholesterol concentrations remained within the reference range and numerical differences were very small, suggesting clinical irrelevance of this finding. Other indices for lipid metabolism and basal serum TAG as well as plasma NEFA concentrations did not differ among test diets.

During the present study in healthy normal-weight cats, reducing the dietary carbohydrate level below common amounts for commercial foods evoked an insulin-resistant state, which can be explained by the carnivore connection theory. In addition, amino acids might have been more important to stimulate insulin secretion than carbohydrates, but further research is needed to determine the effect of various amino acids.

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