NUTRITIONAL MODULATION OF CARBOHYDRATE METABOLISM IN DOMESTIC CATS (*FELIS CATUS*)

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NUTRITIONAL MODULATION
OF CARBOHYDRATE METABOLISM
IN DOMESTIC CATS (*Felis catus*)

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Thesis submitted in fulfilment of the requirements for the degree of Doctor in Veterinary Science (PhD),
Faculty of Veterinary Medicine, Ghent University, 2009
Chapter 1: Nutritional modulation of insulin resistance in the true carnivorous cat: A review

Abstract

2.1. Introduction

2.2. Strict carnivores
  2.2.1. Carbohydrate metabolism
  2.2.2. Protein metabolism

2.3. Dietary prevention and management of insulin resistance (Type II diabetes and obesity)
  2.3.1. Dietary macronutrients
  2.3.2. Source of carbohydrates
  2.3.3. Dietary fibre

Chapter 2: Scientific aims

Chapter 3: The glucose and insulin response to iso-energetic reduction of dietary energy sources in a true carnivore: the domestic cat (Felis catus)

Abstract

3.1. Introduction

3.2. Material and methods
  3.2.1. Animals and housing
  3.2.2. Diets and feeding
  3.2.3. Experimental design
  3.2.4. Analytical methods
  3.2.5. Statistical analyses

3.3. Results
  3.3.1. Diet composition, food intake and body weight
  3.3.2. Glucose tolerance test
  3.3.3. Metabolic parameters

3.4. Discussion

3.5. Conclusion

Chapter 4: Oligofructose and inulin modulate glucose and amino acid metabolism through propionate production in normal weight and obese cats

Abstract

4.1. Introduction

4.2. Materials and methods
  4.2.1. Animals and housing
  4.2.2. Diets and feeding
  4.2.3. Experimental design
CHAPTER 5: Colonic propionate might act as gluconeogenic substrate in normal weight and obese cats, without affecting glucose tolerance ................................................................. 65

Abstract.................................................................................................................................. 67
5.1. Introduction..................................................................................................................... 68
5.2. Materials and methods ............................................................................................... 69
  5.2.1. Animals and housing ............................................................................................ 69
  5.2.2. Diets and feeding .................................................................................................. 69
  5.2.3. Experimental design ............................................................................................. 70
  5.2.4. Colonic infusion .................................................................................................. 71
  5.2.5. Jugular catheter placement and blood sampling ................................................... 72
  5.2.6. General anaesthesia ............................................................................................ 73
  5.2.7. Analytical methods ............................................................................................... 73
  5.2.8. Statistical analyses ................................................................................................ 74
5.3. Results............................................................................................................................. 74
  5.3.1. Effect of body condition ....................................................................................... 74
  5.3.2. Effect of colonic propionate infusion ................................................................... 75
5.4. Discussion....................................................................................................................... 80
5.5. Conclusion ...................................................................................................................... 83

CHAPTER 6: Intestinal fermentation diminishes postprandial amino acid-induced gluconeogenesis in a true carnivore: the domestic cat (Felis catus) ......................................................... 85

Abstract.................................................................................................................................. 87
6.1. Introduction..................................................................................................................... 88
6.2. Materials and methods ............................................................................................... 89
  6.2.1. Animals and housing ............................................................................................ 89
  6.2.2. Diets and feeding .................................................................................................. 89
  6.2.3. Experimental design ............................................................................................. 91
  6.2.4. Jugular catheter placement and blood sampling ................................................... 91
  6.2.5. Faecal collection ................................................................................................... 92
  6.2.6. Analytical methods ............................................................................................... 92
  6.2.7. Statistical analyses ................................................................................................ 93
6.3. Results............................................................................................................................. 93
  6.3.1. Faecal characteristics ............................................................................................ 93
  6.3.2. Metabolic parameters ......................................................................................... 94
6.4. Discussion....................................................................................................................... 98
6.5. Conclusion .................................................................................................................... 100
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Ad libitum test</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;gluc&lt;/sub&gt;</td>
<td>Area under the glucose curve</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;ins&lt;/sub&gt;</td>
<td>Area under the insulin curve</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;ins/AUC&lt;sub&gt;gluc&lt;/sub&gt;</td>
<td>Ratio of area under the insulin to glucose curve</td>
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<tr>
<td>BCS</td>
<td>Body condition score</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
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<tr>
<td>DAPA</td>
<td>Diaminopimelic acid</td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of polymerisation</td>
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<tr>
<td>DEGG</td>
<td>Denaturing gradient gel electrophoresis</td>
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<tr>
<td>EHC</td>
<td>Euglycaemic hyperinsulinaemic clamp</td>
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<td>FOS</td>
<td>Fructo-oligosaccharides</td>
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<tr>
<td>Girth</td>
<td>Girth circumference</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon like peptide 1</td>
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<tr>
<td>Glucose t&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>Half-life for glucose disappearance</td>
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<tr>
<td>HC</td>
<td>High carbohydrate content</td>
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<td>HF</td>
<td>High fat content</td>
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<td>HFF</td>
<td>High fermentable fibre</td>
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<tr>
<td>HGC</td>
<td>Hyperglycaemic glucose clamp</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostasis model assessment</td>
</tr>
<tr>
<td>HP</td>
<td>High protein content</td>
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<tr>
<td>IRMA</td>
<td>Immunoradiometric assay</td>
</tr>
<tr>
<td>idBW</td>
<td>Ideal body weight</td>
</tr>
</tbody>
</table>
List of Abbreviations

IVGTT  Intravenous glucose tolerance test
$K_{glucose}$  Glucose disappearance coefficient
LC  Low carbohydrate content
LF  Low fat content
LFF  Low fermentable fibre
LP  Low protein content
MC  Moderate carbohydrate content
ME  Metabolisable energy
MP  Medium protein content
MR  Meal response test
NaCl  Sodium chloride
NEFA  Non-esterified fatty acids
NDF  Neutral detergent fibre
NFE  Nitrogen free extract
NIDDM  Non-insulin dependent diabetes mellitus
NSP  Non-starch polysaccharides
OMD  Organic matter disappearance
qPCR  Quantitative polymerase chain reaction
QUICKI  Quantitative insulin sensitivity check index
RIA  Radio-immunoassay
SCFA  Short chain fatty acids
scFOS  Short chain fructo-oligosaccharides
SI  Insulin to glucose ratio
SPSS  Superior Performing Software Systems
$T_3$  Total triiodothyronine
$T_4$  Total thyroxine
TDF  Total dietary fibre
Chapter 1

Nutritional modulation of insulin resistance in the true carnivorous cat: A review
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Submitted, Critical Reviews in Food Science and Nutrition
Abstract

Cats are strict carnivores that rely on nutrients in animal tissues to meet their specific and unique nutritional requirements. In their natural habitat, cats consume prey high in protein with moderate amounts of fat and minimal carbohydrates in contrast to commercial diets, which are sometimes moderate to high in carbohydrates. This change in diet has been accompanied by a shift from an outdoor environment to an indoor lifestyle and decreased physical activity, because cats no longer need to hunt to obtain food. This transformation of the cats’ lifestyle is thought to be responsible for the recent increase in incidence of obesity, insulin resistance and diabetes mellitus in domestic cats.

At first, an overview of the evolutionary physiological adaptations of carbohydrate digestion in the feline digestive tract and of the hepatic carbohydrate and protein metabolism reflecting the cats’ true carnivorous nature is given. Secondly, this literature review deals with nutritional modulation of insulin sensitivity, focusing on dietary macronutrients, carbohydrate sources and dietary fibre for prevention and treatment of insulin resistance.
2.1. Introduction

Domestication of feral cats have led to a considerable transformation of the cat’s lifestyle. Shift from an outdoor environment to an indoor setting occurred, accompanied by decreased physical activity, because cats no longer need to hunt to obtain food. Also the cat’s diet changed from low carbohydrate (LC), high protein (HP) prey typical for feral cats (e.g. domestic mouse: 55.8% crude protein, 23.6% crude fat, 8.8% nitrogen free extract and 11.8% ash on dry matter basis (Dierenfeld et al., 2002)) to a high carbohydrate (HC) commercial diet. These changes are held responsible for the recent increase in incidence of obesity, obesity-induced insulin resistance and type II diabetes mellitus in domestic cats (Rand et al., 2004; Slingerland et al., 2009). Figure 2.1 shows the causes and consequences of insulin resistance in domestic cats.

![Figure 2.1: Causes and consequences of insulin resistance in domestic cats.](image-url)
Nowadays, obesity is recognized as the most common nutritional disorder in cats, as the prevalence has been increased dramatically during the past decades. In 1973, a study in British veterinary practices found that 6 to 12.5% of 429 cats were overweight or obese (Anderson, 1973). Twenty years later, Danish investigators found about 40% of 223 cats to be overweight or obese (Sloth, 1992). Also 29% of 2000 cats presented to private veterinary hospitals in the North-Eastern United States (Scarlett et al., 1994), and 35% of more than 8000 adult cats presented to private United States veterinary practices were observed to be overweight or obese (Lund et al., 2005). Moreover, Prahl et al. (2007) reported an increased incidence of feline diabetes mellitus over the past 30 years from 1 in 1250 (0.08%) in 1970 to 1 in 81 (1.2%) cats affected by the disease in 1999, most probably due to an increase in the frequency of predisposing factors such as obesity and physical inactivity (Slingerland et al., 2009). Type II diabetes mellitus, being the most common form of diabetes that occurs in domestic cats, accounts for 80-95% of feline diabetes and closely resembles to human type II diabetes, based on histological findings, such as pancreatic islet amyloidosis and clinical characteristics, including impaired β-cell function and abnormal insulin secretion in response to a glucose load. In both species, commonly distinguished risk factors are increasing age, masculine gender and obesity (Lutz & Rand, 1995; O'Brien, 2002; Rand et al., 2004).

Despite the increasing incidence of insulin resistance (feline obesity and diabetes mellitus) in cats, the dietary treatment originates from extrapolation of the results from human and canine studies until recently. Traditionally, human diets recommended for treatment and prevention of obesity and diabetes are rich in highly digestible carbohydrates and fibre and low in fat (Franz et al., 2002). Yet, cats are true carnivores that rely on nutrients in animal tissues to meet their specific and unique nutritional requirements and are therefore metabolically adapted to lower glucose utilization and higher protein metabolism (MacDonald et al., 1984).

### 2.2. Strict carnivores

#### 2.2.1. Carbohydrate metabolism

Due to evolutionary pressures, feral cats developed several physiological adaptations of carbohydrate digestion and metabolism that reflect their true carnivorous nature, including their expected low ability to utilize dietary carbohydrates (Morris, 2002).
Morris et al. (1977) observed that adult cats efficiently digested all carbohydrates added to a meat-based diet, with the exception of wood cellulose. However, starch was digested significantly less than glucose, sucrose, lactose and dextrin. The total apparent digestibility of starch is reported to be between 40 and 100%, depending on source and treatment (Morris et al., 1977; Dewilde & Huysentruyt, 1983; Kienzle, 1993a; De-Oliveira et al., 2008), which proves that cats can use carbohydrates. However, when the digestive capacity of the small intestine is exceeded due to excessive carbohydrate intake, digestive disorders, such as diarrhoea, flatulence, and bloating, might occur (Kienzle, 1993a).

Several enzymatic and anatomic adaptations in the feline digestive tract (Figure 2.2) reflect the cat’s expected low carbohydrate utilization. Cats possess only small capacity for starch digestion by endogenous enzymes. The concentrations of amylase responsible for initiation of starch digestion in the feline saliva and salivary glands are very small (Mcgeachin & Akin, 1979). Intestinal amylase activity is also rather low and mainly of pancreatic origin, despite the low concentration of amylase in the feline pancreas in comparison with other animals (Kienzle, 1993b). In addition, pancreatic tissue showed a low maltase activity, only traces of isomaltase and no sucrase and lactase activity. The disaccharidase activity (maltase, isomaltase and sucrase) in the intestinal brush border of cats is low as well (Kienzle, 1993c). Not only the intestinal enzymes, but also the sugar transport systems in the feline brush border are non-adaptive to varying concentrations of dietary carbohydrate (Buddington et al., 1991). Yet, D-glucose transport across the intestinal brush border membrane appears to have a considerably higher capacity in cats than in cattle and rabbits, despite the similar amounts of glucose reaching the small intestine when fed a natural diet. This relatively high transport capacity could at least partially compensate for the relative deficit in absorptive surface, due to the limited length of the small intestine (Wolffram et al., 1989).

Furthermore, anatomical adaptation to the strictly carnivorous nature of the feline intestine are not only characterized by the short small intestine, but also by a rudimentary caecum and a very small colon, assuming limited ability to use poorly digestible starch and fibre by microbial fermentation in the feline hindgut (Sunvold et al., 1995a); yet, compared to dogs and human beings, healthy cats carry higher numbers of bacteria in the proximal part of the small intestine, indicating the existence of microbial fermentation in the small intestine: regardless of diet, total bacterial count was approaching $10^6$ colony-forming units/ml, and aerobes were more numerous present than anaerobes (Johnston et al., 1993; Papasouliotis et al., 1998; Sparkes et al., 1998a). The low pH values in the feline hindgut and faeces and the higher amount of short
chain fatty acids (SCFA) in caudally versus cranially sampled intestinal contents when fed low digestible starch and disaccharides also indicates incomplete digestion and occurrence of microbial fermentation in the feline colon (Kienzle, 1993a; Kienzle, 1994a).

Secondly, carbohydrate metabolism (Figure 2.3) is altered by some major evolutionary adaptations in the enzyme activity of the feline liver. In omnivores, two enzymes, hexokinase and glucokinase are responsible for the phosphorylation of glucose to glucose-6-phosphate. Hexokinase is present in the liver in only small amounts and is active at very low concentrations of glucose, whereas glucokinase has a maximal rate of glucose phosphorylation but operates only when the liver receives a large load of glucose from the portal vein and rapid glycogen synthesis is necessary (Vinuela et al., 1963). In cats, several authors reported minimal to absent

**Figure 2.2**: Enzymatic and anatomic adaptations in the feline digestive tract, reflecting the cat’s carnivorous nature and the expected low carbohydrate utilization.

hepatic glucokinase activity (Ballard, 1965; Washizu et al., 1999; Tanaka et al., 2005). According to Tanaka et al. (2005) no glucokinase gene expression was observed in the feline liver as well. Furthermore D-glucose transport activities in feline hepatocytes were considerably low (Arai et al., 1992). Ballard (1965) reported low rates of incorporation of C\textsuperscript{14} labelled glucose in glycogen which might indicate minimal activity of hepatic glycogen synthetase. On the other hand, Washizu et al. (1999) and Tanaka et al. (2005) investigated the activity of rate limiting enzymes of gluconeogenesis such as pyruvate carboxylase, fructose-1,6-biphosphatase and glucose-6-phosphatase and concluded both that these enzyme activities were significantly higher in feline livers than those in canine livers.

\textbf{Figure 2.3:} The cat’s carnivorous nature is reflected in the activity of enzymes in the feline liver: down-regulation of glycolysis and glycogen synthesis: 1) glucokinase, 2) glycogen synthetase; up-regulation of fructose utilization and gluconeogenesis: 3) fructokinase, 4) pyruvate carboxylase, 5) fructose-1,6-biphosphatase, 6) glucose-6-phosphatase; assuming that the cat’s liver is most likely to produce glucose by gluconeogenesis, rather than to remove glucose rapidly from the blood.
These findings suggest that the feline liver can produce more glucose than canine and omnivorous livers, even though cats have minimal glucokinase and glucose transport activities. This assumes that the function of the feline liver would be most likely to produce glucose by gluconeogenesis rather than to remove glucose rapidly from the blood. Some authors suggest that feline livers lack fructokinase as well. According to Kienzle (1994b), feeding cats disaccharides, such as sucrose, which is hydrolysed yielding glucose and fructose, resulted in postprandial hyperglycaemia and fructosuria, assuming lack of fructokinase, an enzyme responsible for the entry of fructose into glycolysis. However, Tanaka et al. (2005) reported higher hepatic activities of fructokinase in cats compared to dogs, indicating a higher utilization of fructose as an energy source than canine liver.

At last, most cats do not prefer foods with a sweet taste, but favour foods flavoured with animal products such as fat and protein (MacDonald et al., 1984).

2.2.2. Protein metabolism

Cats, being true carnivores, have a higher dietary protein requirement compared to dogs and omnivores, which has been attributed to the cat’s preferential use of protein as energy source to maintain blood glucose concentrations, as well as for structural purposes and to the limited ability to spare protein utilization by using carbohydrates instead (MacDonald et al., 1984; Zoran, 2002). According to Rogers et al. (1977), cats lack the capacity to regulate hepatic enzyme activity in nitrogen metabolism, gluconeogenesis and lipogenesis in response to altered dietary protein content, which may indicate that cats cannot conserve nitrogen and have a high obligatory nitrogen loss. Even so, other investigators questioned this matter. Adaptation to dietary protein intake by altering the rate of amino acids catabolism was demonstrated first by Silva & Mercer (1985). Later, also Russell et al. (2002) and Green et al. (2008) concluded that cats can adapt to a wide range of protein intake by increasing protein oxidation when fed a HP diet, but are unable to fully adapt to low concentrations of dietary protein. Therefore, Russell et al. (2002) doubted this reason for the high dietary protein requirement. Silva & Mercer (1985) suggested that regulation of the demand for gluconeogenesis is of little significance since the rate of glucose production from amino acids was not affected by the cat’s protein intake. Unlike Silva & Mercer (1985), Hoenig et al. (2006) demonstrated by using tracer glucose in combination with euglycaemic hyperinsulinaemic clamp (EHC) that cats are not always in a gluconeogenic mode. Glycolysis was increased in basal state but glycogen disposal was increased when insulin concentrations were high. These findings were supported by combining EHC with indirect
calorimetry, demonstrating increased glycolysis, glycogen production and lipogenesis under clamp conditions (Hoenig et al., 2007a).

On the other hand, the greater dietary protein intake in cats does not only reflect a higher basal requirement for nitrogen but also an increased need for specific essential amino acids in the cat’s diet namely taurine, arginine, methionine and cysteine (MacDonald et al., 1984). The importance of the amount of protein and specific amino acids in cat foods is also stressed by the presence of highly adaptable amino acid transporters in the feline small intestine, in contrast to sugar transport systems (Buddington et al., 1991).

At last, considering the high protein content of the cat’s diet, not only glucose, but also amino acids serve as very important modulators of pancreatic hormone release, especially being potent stimulators of insulin secretion (Curry et al., 1982).

2.3. Dietary prevention and management of insulin resistance

(Type II diabetes and obesity)

2.3.1. Dietary macronutrients

With the increased understanding of the feline unique protein and carbohydrate metabolism, large anecdotal evidence that HC diets may lead to diabetes mellitus through exhaustion and loss of insulin-producing β-cells (Rand et al., 2004) and that HP, LC diets affect insulin sensitivity by improving glucose homeostasis and lowering insulin requirements (Hoenig et al., 2007b), has come to light. Therefore, several investigators evaluated HC and high fat (HF) diets as risk factors for the development of feline obesity and diabetes mellitus; others examined the use of HP, LC diets for the prevention and treatment of these metabolic diseases.

In healthy cats, Farrow et al. (2002) demonstrated that feeding a HC diet (caloric distribution: protein 25% of metabolisable energy (ME), fat 26%, carbohydrate 47%) resulted in a higher postprandial area under the glucose curve when compared to a HP (protein 46% ME, fat 26%, carbohydrate 27%) and a HF diet (protein 26% ME, fat 47%, carbohydrate 26%). Area under the insulin curve (AUCins) tended to be increased as well when fed a HC diet, although the difference was not significant. These findings suggest that HC diets impair glucose tolerance and therefore contribute to the development of insulin resistance and feline diabetes mellitus. However, Slingerland et al. (2009) could not produce evidence that the proportion of dry food in the cat’s diet is a risk factor for the development of feline diabetes, despite of the higher
carbohydrate content in commercially available dry cat foods (carbohydrate 40% ME) when compared to canned foods (carbohydrate <10% ME). The results of a more recent study, also contradict the hypothesis that HC diets lead to β-cell exhaustion, since feeding a HC diet (protein 25% ME, fat 24%, carbohydrate 51%) resulted in an increased glucose-induced insulin secretion during a hyperglycaemic glucose clamp (HGC), yet, this increase was less clear than with consumption of a HF diet (protein 27% ME, fat 45%, carbohydrate 28%) (Slingerland et al., 2008) and since long-term consequences of these diets were not investigated, this increase in insulin secretion could be the first step towards β-cell exhaustion.

Nevertheless, Slingerland et al. (2009) do not disclaim that HC diets may be an indirect risk factor for the development of diabetes by promoting the development of obesity. Yet, mechanisms are speculative. Excessive carbohydrate intake might lead to fatty acid synthesis and storage in consequence of the cat’s high capability to convert amino acids into glucose and the low capacity for glucose disposal (Zoran, 2002), but might also extend insulin release, up-regulating storage of dietary fat in adipose tissue (Backus et al., 2007). This latter mechanism seems more likely since feline liver and adipose tissue appear to poorly utilize glucose for fatty acid synthesis (Richard et al., 1989). Hoenig et al. (2007b) suggest that over time, cats with the same caloric intake are more prone towards obesity and insulin resistance when fed a low protein (LP), HC diet (protein 25% ME, fat 34%, carbohydrate 34%), due to lower heat production in comparison with a HP, LC diet (protein 40% ME, fat 34%, carbohydrate 22%). In addition, it has been shown that cats eating a commercial high quality dry cat food are more likely to be overweight (Scarlett et al., 1994; Robertson, 1999; Lund et al., 2005). Still, Scarlett et al. (1994) presumed that these diets contained high amounts of fat, predisposing cats to obesity, by increasing energy intake due to high energy density and improved palatability. In fact, Nguyen et al. (2004a) confirmed increased weight gain and expansion of fat mass when fed a HF diet (protein 27% ME, fat 40%, carbohydrate 28%) in comparison to a low fat (LF) diet (protein 34% ME, fat 27%, carbohydrate 42%). Furthermore, HF, LC diets (protein 33% ME, fat 64%, carbohydrate 3%) were evidenced being more likely than LF, HC diets (protein 33% ME, fat 9%, carbohydrate 57%) to induce weight gain and a corresponding elevation in insulin (Backus et al., 2007). Likewise, in comparison with HC diets (protein 32% ME, fat 33%, carbohydrate 33%), HF diets (protein 36% ME, fat 51%, carbohydrate 10%) are expected to impair glucose tolerance as demonstrated from the slightly elongated glucose clearance and reduced acute insulin response following intravenous glucose administration in intact and neutered male cats. Moreover, plasma fatty acids were elevated, most likely causing insulin resistance (Thiess et al., 2004), by promoting fatty acid oxidation and inhibiting glucose uptake and oxidation, but
### Table 2.1: Effect of energy sources on onset, prevention and treatment of insulin resistance: a literature overview.

<table>
<thead>
<tr>
<th>Diet</th>
<th>P (%)</th>
<th>F (%)</th>
<th>C (%)</th>
<th>Diet</th>
<th>P (%)</th>
<th>F (%)</th>
<th>C (%)</th>
<th>Diet</th>
<th>P (%)</th>
<th>F (%)</th>
<th>C (%)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy Cats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Farrow et al., 2002</td>
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<td>27</td>
<td>46</td>
<td>26</td>
<td>27</td>
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<td>AL and MR: Diet A: ↑ AUC&lt;sub&gt;gluc&lt;/sub&gt;, trend for ↑ AUC&lt;sub&gt;ins&lt;/sub&gt;; Diet B: trend ↓ postprandial glucose</td>
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<td>Thiess et al., 2004</td>
<td>32</td>
<td>33</td>
<td>33</td>
<td>36</td>
<td>51</td>
<td>10</td>
<td>36</td>
<td>51</td>
<td>10</td>
<td>26</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>Nguyen et al., 2004a</td>
<td>27</td>
<td>40</td>
<td>28</td>
<td>34</td>
<td>27</td>
<td>42</td>
<td>34</td>
<td>27</td>
<td>42</td>
<td>26</td>
<td>47</td>
<td>26</td>
</tr>
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<td>Nguyen et al., 2004b</td>
<td>28</td>
<td>25</td>
<td>47</td>
<td>48</td>
<td>31</td>
<td>21</td>
<td>48</td>
<td>31</td>
<td>21</td>
<td>26</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>Laflamme et al., 2005</td>
<td>38</td>
<td>27</td>
<td>35</td>
<td>48</td>
<td>26</td>
<td>26</td>
<td>48</td>
<td>26</td>
<td>26</td>
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<td>47</td>
<td>26</td>
</tr>
<tr>
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<td>28</td>
<td>25</td>
<td>47</td>
<td>48</td>
<td>31</td>
<td>21</td>
<td>48</td>
<td>31</td>
<td>21</td>
<td>26</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>Backus et al., 2007</td>
<td>33</td>
<td>9</td>
<td>57</td>
<td>33</td>
<td>64</td>
<td>3</td>
<td>33</td>
<td>64</td>
<td>3</td>
<td>26</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>Hoenig et al., 2007b</td>
<td>25</td>
<td>34</td>
<td>34</td>
<td>40</td>
<td>34</td>
<td>22</td>
<td>40</td>
<td>34</td>
<td>22</td>
<td>26</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>Slingerland et al., 2008</td>
<td>25</td>
<td>24</td>
<td>51</td>
<td>27</td>
<td>45</td>
<td>28</td>
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<td>28</td>
<td>25</td>
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<td>47</td>
<td>26</td>
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<tr>
<td><strong>Feline Diabetes Mellitus</strong></td>
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<td>Low body fat: improved glycaemic parameters, continued to require insulin but at much lower dosage</td>
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%ME, caloric distribution, % of metabolisable energy; P, protein; F, fat; C, carbohydrate; AL, ad libitum test; MR, meal response test; AUC<sub>gluc</sub>, area under the glucose curve; AUC<sub>ins</sub>, area under insulin curve; IVGTT, intravenous glucose tolerance test; EHC, euglycaemic hyperinsulinaemic clamp; HGC, hyperglycaemic clamp; NIDDM, non-insulin dependent diabetes mellitus.
stimulating hepatic glucose production (Delarue & Magnan, 2007). Furthermore, Slingerland et al. (2008) observed augmented second phase insulin secretion and faster glucose disposal during HGC with consumption of a HF (protein 27% ME, fat 45%, carbohydrate 28%) and a HC diet (protein 25% ME, fat 24%, carbohydrate 51%) in contrast to a HP diet (protein 47% ME, fat 28%, carbohydrate 25%). However, the increase in glucose disposal was small in relation to the improvement in glucose-induced insulin secretion, suggesting diminished insulin sensitivity, but the insulin sensitivity index calculated from the HGC did not differ significantly among diets, as did measures of β-cell function and insulin sensitivity calculated from fasting plasma glucose and insulin concentrations (HOMA, homeostasis model assessment) (Slingerland et al., 2008). According to Leray et al. (2006) variation in dietary protein and carbohydrate (HP, LC diet: protein 48%ME, fat 31%, carbohydrate 21% versus medium protein (MP), HC diet: protein 28%, fat 25%, carbohydrate 47%) intake did not affect glucose tolerance or insulin sensitivity in healthy normal weight cats. No impact of dietary protein and carbohydrate content on insulin sensitivity was observed by Hoenig et al. (2007b) as well (HP, LC diet: protein 40%ME, fat 34%, carbohydrate 22% versus LP, HC diet: protein 25%, fat 34%, carbohydrate 34%). Still, dietary protein might be beneficial during weight loss, not only by stimulating postprandial thermogenesis, but also by conserving lean body mass, which is important to support protein turnover and for maintaining resting energy requirement in order to prevent weight rebound (Laflamme & Hannah, 2005). When fed a HP, LC diet (protein 48%ME, fat 31%, carbohydrate 21%), non-obese neutered cats gained lean body mass without change in body weight, whereas cats lost body weight with no change of lean body mass when fed a MP, HC diet (protein 28%ME, fat 25%, carbohydrate 47%), yet, loss of fat tissue was similar on both diets (Nguyen et al., 2004b). In obese cats during calorie restriction, a significantly greater proportion of weight loss was fat when fed a HP diet (protein 48%ME, fat 26%, carbohydrate 26%), yet, these cats lost a smaller proportion of lean body mass in contrast to cats fed a MP diet (protein 38%ME, fat 27%, carbohydrate 35%) (Laflamme & Hannah, 2005) and feeding a HP, LC diet (protein 40%ME, fat 34%, carbohydrate 22%) led to more fat loss when compared to a LP, HC diet (protein 25%ME, fat 34%, carbohydrate 34%), most probably because it maintains suppression of non-esterified fatty acids (NEFA) during EHC at the same level as is seen in lean cats (Hoenig et al., 2007b).

Beneficial effects of feeding HP, LC diets in the management of feline diabetes mellitus are reported in several clinical studies. Frank et al. (2001) evaluated the response to a HP, LC diet (protein 47%ME, fat 47%, carbohydrate 7%), of nine diabetic cats, initially fed a high fibre,
Chapter 1

Insulin resistance and nutrition in cats

moderate fat diet (protein 15%ME, fat 53%, carbohydrate 32%). Three months after changing the cat’s diet, the daily insulin dose was reduced by over 50% in eight of the nine cats, without loss of glucose control (Frank et al., 2001). Evaluating the effect of an α-glucosidase inhibitor (acarbose), combined with a LC diet (protein 34% ME, fat 61%, carbohydrate 5%) in 18 diabetic cats, Mazzaferro et al. (2003) also concluded a decreased exogenous insulin dependence and improved glycaemic control when fed the LC diet. In 11 cats which were initially obese, exogenous insulin therapy could be discontinued. Similar results were reported by Bennett et al. (2006) as well. Sixty-three diabetic cats were randomly assigned to a moderate carbohydrate (MC), high fibre diet (protein 40%, fat 41%, carbohydrate 26%) or a LC, low fibre diet (protein 37%, fat 51%, carbohydrate 12%). By week 16, cats fed the LC, low fibre diet were more likely to be well-regulated (81%) or to revert to a non-insulin dependent state (68%) when compared to the MC, high fibre diet (56% and 41% respectively) (Bennett et al., 2006). However, Hall et al. (2008) reported no differences in clinical signs, insulin doses and peak and nadir blood glucose concentrations in cats fed a HP, LC diet (dry: protein 49%ME, fat 40%, carbohydrate 11%; canned: protein 44%ME, fat 47%, carbohydrate 9%) when compared to cats fed a maintenance diet (dry: protein 32%ME, fat 41%, carbohydrate 28%; canned: protein 38%ME, fat 59%, carbohydrate 4%). Furthermore, six of the ten cats achieved complete remission by the end of the study, with no statistical differences between diets (Hall et al., 2008). The absence of diet effects on glucose control in this study is most likely due to the small number of cats, especially since the cats were divided into two diet groups. Nevertheless, the low carbohydrate content of the canned maintenance diet is thought to interfere with the results.

2.3.2. Source of carbohydrates

Not only the amount of carbohydrates consumed, but also the dietary source of carbohydrates might modulate the glycaemic response. Originally complex carbohydrates were believed to result in lower postprandial glucose concentrations, because of their slower digestion than simple carbohydrates (Bouchard & Sunvold, 2000). However, a number of studies have doubted this consideration, since several additional factors including, chemical properties of the carbohydrates; mainly amylose:amylopectine ratio (Goddard et al., 1984; Behall et al., 1989; Denardin et al., 2007), presence of protein, fat (Nguyen et al., 1994) and dietary fibre (Nishimune et al., 1991) in the diet and physical properties of the food and type of food processing (Holste et al., 1989), might modulate the glycaemic response as well. Still, the impact of the source of starch on postprandial glucose concentration remains very important.
In humans, carbohydrate-containing foods have been classified according to their glycaemic responses. Therefore, portions of test foods and glucose or white bread were fed to normal or diabetic subjects in order to calculate the area under the two-hour glucose curve, expressed as a percentage of the mean response to the standard food (glucose or white bread) taken by the same subject, to obtain the “glycaemic index” for a food (Jenkins et al., 1981; Wolever et al., 1991a). For animals no such glycaemic index exists to date.

Bouchard & Sunvold (2000) evidenced that the source of starch acts upon the postprandial glucose and insulin response in healthy cats. Rice- and sorghum-based diets resulted in an accelerated time of glucose peak and higher postprandial glucose concentrations. Wheat- and barley-based diets resulted in a prolonged postprandial glucose increase, a delayed glucose peak, but a rapid decline after the peak. Corn had an intermediate glucose response to the other diets.

In healthy cats, higher postprandial insulin responses were observed when fed rice- and wheat-based diets. Barley, corn and sorghum produced relatively low postprandial insulin responses and are therefore claimed to be the most useful starch sources in diets of cats with impaired glucose control (Bouchard & Sunvold, 2000). Similar, feeding a rice-based diet to healthy dogs revealed the greatest glucose and insulin response, contraindicating rice as a starch source for dogs with impaired glucose control. However, in contrast to cats, dogs showed the lowest glucose response when fed sorghum-based diets where as barley-based diets resulted in the lowest insulin response. Therefore both barley and sorghum are recommended to improve glucose control in dogs (Sunvold & Bouchard, 1998). As Bouchard & Sunvold (2000), Appleton et al. (2004) concluded that sorghum and corn may play a protective role in the development of feline obesity, insulin resistance and diabetes, since feeding a rice-based diet to overweight cats with impaired glucose tolerance and insulin resistance resulted in higher energy consumption and more weight gain in response to free-access feeding and tended to increase glucose concentration and insulin secretion in response to a glucose load or a test meal, when compared to a sorghum/corn-based diet (Appleton et al., 2004). However, according to De-Oliveira et al. (2008) feeding healthy cats a rice-based and a corn-based diet revealed similar results for glucose and insulin measurements during a postprandial glucose tolerance test. Only corn provoked a rise in glycaemia in relation to baseline concentrations, but for insulin, both corn and rice caused an increase in blood concentration in relation to baseline values. This recent study also mentioned a lower glucose response for sorghum compared to corn and a similar insulin response for both (De-Oliveira et al., 2008), whereas Bouchard & Sunvold (2000) did not come across differences in glucose and insulin responses when comparing sorghum and corn. Moreover, the effect of starch on the feline postprandial glucose and insulin response is of lesser
importance than on those of dogs and humans (De-Oliveira et al., 2008) and the long postprandial period of glucose and insulin elevation is a striking difference between cats and other monogastric species (Bouchard & Sunvold, 2000), suggesting the ability of cats to physiologically control their rate of absorption of carbohydrate, probably due to the evolutionary carnivorous adaptations in the feline digestive tract, retarding starch digestion and absorption and causing delayed, less pronounced glycaemic responses.

Considering the source of carbohydrate, the presence of resistant starch has also to be taken into account. Resistant starch consists of a fraction of the ingested starch as well as products of starch degradation, which escapes digestion, is not absorbed in the small intestine and passes undigested to the large bowel, where resistant starch undergoes fermentation, resulting in the production and uptake of SCFA, namely propionate, acetate and butyrate (Raben et al., 1994; Higgins, 2004). Through mechanisms similar to those exerted by dietary soluble fibre (See 2.3.3. Dietary fibre), resistant starch may influence the amount and rate of glucose absorption (Raben et al., 1994; Behall & Hallfrisch, 2002; Higgins, 2004), hepatic glucose metabolism as well as insulin sensitivity (Robertson et al., 2003; Higgins, 2004) as observed in humans. The amount of resistant starch present in starch-rich foods depends not only on the source, but also on ripeness, processing, preparation, and storage of the foods (Raben et al., 1994). To date, the use of resistant starch in the treatment of insulin resistance in cats is still to be investigated.

At last, fermentation of indigestible polysaccharides is regulated by protein source. The indigestible portion of dietary protein that escapes from the small intestine and passes into the large intestine, referred to as resistant protein, may play a role in correcting an imbalance in the ratio of carbohydrate and nitrogen as fermentative substrates for colonic microflora and is likely to have unexpected nutritional significance through the production of SCFA (Morita et al., 1998).

### 2.3.3. Dietary fibre

Dietary fibre represents a heterogeneous mixture of substances, especially non-starch polysaccharides and lignin derived from the plant cell wall, that resist hydrolysis by alimentary enzymes in the small intestine (Nelson, 1989; Blaxter et al., 1990; Nuttall, 1993; Kimmel et al., 2000) and can be categorized according to chemical structure, rate of fermentation, digestible and indigestible fractions, solubility or ability to disperse in water, water-holding capacity and viscosity (Hesta et al., 2006). However, from a metabolic point of view, it is most useful to
classify dietary fibre in two groups, water-soluble and water-insoluble (Nelson, 1989; Blaxter et al., 1990; Nuttall, 1993; Kimmel et al., 2000). Soluble fibres including pectin, gums, certain hemicelluloses and indigestible polysaccharides, are found particularly in fruits, oats, barley and legumes; whereas vegetables, wheat and most cereal fibres contain more insoluble fibres, enclosing cellulose, lignin, and most hemicelluloses (Nelson, 1989).

In humans, rodents and dogs, both fibre types have been shown to possess typical physical and chemical properties resulting in different physiologic effects, with potential benefits on glycaemic control, such as delayed carbohydrate absorption from the intestinal tract, dampened postprandial glycaemic effects and enhanced sensitivity to insulin in liver and peripheral tissues (Nelson et al., 1991; Kimmel et al., 2000). These beneficial effects may be related to one or more of the following mechanisms.

The effect of soluble fibres is associated with their ability to hydrate rapidly and form viscous colloidal dispersions and thus impair transfer of water and glucose to the absorptive surface of the small intestine as well as delay gastric emptying and shorten small intestinal transit time; resulting in a reduction of the rate of starch hydrolysis and intestinal carbohydrate absorption (Anderson & Chen, 1979; Blaxter et al., 1990; Nelson et al., 1998; Kimmel et al., 2000). Reduction of carbohydrate absorption also occurs with consumption of insoluble fibre, most probably due to bulkiness, “insulation” of available carbohydrate from digestive enzymes and resistance to carbohydrate diffusion in the intestinal lumen (Anderson & Chen, 1979; Blaxter et al., 1990). In addition, dietary soluble fibre may modify hepatic glucose metabolism through the production of SCFA, especially propionate, by bacterial fermentation in the hindgut (Roberfroid, 1993; Roberfroid & Delzenne, 1998). Following in vitro studies using perfused rat liver, propionate has been shown to inhibit hepatic gluconeogenesis from pyruvate via depletion of acetyl CoA which is an allosteric activator of pyruvate carboxylase and via its metabolic intermediaries, methylmalonyl coenzyme A (CoA) and succinyl CoA, which are specific inhibitors of pyruvate carboxylase (Blair et al., 1973; Anderson & Bridges, 1984). Propionyl CoA has also been shown to be a potent inhibitor of the same enzyme (Chan & Freedland, 1972). In addition, propionate enhances hepatic glycolysis, probably by depleting hepatic citrate which is an important metabolic inhibitor of phosphofructokinase (Blair et al., 1973; Anderson & Bridges, 1984). Consumption of soluble fibre might also alter hepatic glucose metabolism indirectly by enhancing peripheral insulin sensitivity, through production of propionate, causing a reduction of plasma fatty acid concentration; since increased plasma fatty acid availability may
Finally, other factors, such as alternations in gut hormones or pancreatic glucagon secretion may enhance hepatic glucose metabolism (Anderson & Chen, 1979).

Besides these beneficial effects, consumption of dietary soluble fibre might also evoke side effects such as gastric and intestinal discomfort, vomiting, diarrhoea, excessive flatulence, poor acceptance (Blaxter et al., 1990; Kimmel et al., 2000). Nelson et al. (1991) noted alternations in the dog’s hair coat (brittle, dry and lustreless) and reported that a diet high in soluble fibre might have a sticky consistence and tend to stick to the hard palate, hampering the dog’s ability to swallow. Supplementation of insoluble fibre might be associated with constipation and reduced palatability of the diet (Nelson et al., 1998).

In consequence of the beneficial effects of both soluble and insoluble dietary fibre, supplementation of dietary fibre is recommended for prevention and treatment of diabetes mellitus and obesity-induced insulin resistance in humans, but also in dogs and cats. Nevertheless, to our knowledge, only few data are available on the effect of both soluble and insoluble dietary fibre on glycaemic control in a true carnivorous species such as the cat.

Nelson et al. (2000) evaluated the effect of insoluble fibre on glycaemic control in diabetic cats, by feeding two test diets, a diet high in insoluble fibre, containing 12% cellulose on dry matter basis and a diet low in insoluble fibre, in a cross-over design with intervals of 24 weeks. Feeding the high fibre diet resulted in lower pre- and postprandial glucose concentrations and lower mean 12-hour glucose concentrations. The daily insulin dose decreased as well when fed the high fibre diet, yet this difference was not significant. These results support the use of diets containing high amounts of insoluble fibre for the treatment of feline diabetes mellitus. However, the carbohydrate content was higher in the low fibre diet, compared to the high fibre diet and might have contributed to differences in glycaemic control between diets.

To date, the use of soluble dietary fibre for the treatment of feline insulin resistance has not been evaluated yet. Still, the fermentability of fibrous substrates by cat faecal microflora was investigated in vitro. At first, in comparison with canine, equine, porcine and human faecal inoculum, the greatest total SCFA production resulted when substrates were fermented by cat faecal inoculum (Sunvold et al., 1995b), suggesting high fermentation of soluble fibre in the small intestine as well as in the relatively short large intestine, with the possibility to alter glucose and insulin metabolism. Secondly, soluble fibres, such as guar gum, locust bean gum,
citrus pectin and fructo-oligosaccharides (FOS) result in the highest organic matter disappearance (OMD), total SCFA production, and propionate and acetate production, whereas cellulose, an insoluble fibre, was the least fermentable fibre source (Sunvold et al., 1994; Sunvold et al., 1995a; Sunvold et al., 1995c). In vivo, increased faecal excretion of SCFA and decreasing faecal pH were reported with increasing amount of soluble fibre (FOS and inulin) added to the cat’s diet (Hesta et al., 2001a). Yet, the impact of soluble fibre on feline glucose metabolism and insulin sensitivity is still to be investigated.

**Acknowledgements:** Professor Paul Simoens, PhD Veterinary Science, from the Department of Morphology at the Faculty of Veterinary Medicine, Ghent University is gratefully acknowledged for providing the anatomical drawing of the feline digestive system.
Chapter 2

Scientific aims
Domestication of feral cats have led to a considerable transformation of the cat’s lifestyle. A shift from an outdoor environment to an indoor setting occurred, accompanied by decreased physical activity, because cats no longer need to hunt to obtain food. Also the cat’s diet changed, without taking into account the cat’s specific and unique nutritional requirements. In their natural habitat, strict carnivores, such as cats, consume prey high in protein with moderate amounts of fat and minimal digestible carbohydrates, which contrasts with commercial diets, often containing moderate to high amounts of carbohydrates. These changes in lifestyle are held responsible for the recent increase in incidence of obesity, obesity-induced insulin resistance and type II diabetes mellitus in domestic cats (Rand et al., 2004; Slingerland et al., 2009).

Gaining a better insight into the feline carbohydrate metabolism might allow preventive as well as curative dietary strategies to be developed against obesity, insulin resistance and diabetes mellitus.

Modulation of feline carbohydrate metabolism can be achieved by manipulating dietary carbohydrates in the broadest sense, by changing the concentration of energy sources, but also by adding dietary fermentable fibre. It has been suggested that the concentration of energy sources, especially the reduction of carbohydrate, improves glucose tolerance and insulin sensitivity. Yet, in earlier studies the effect of increasing one energy source was often confounded with the decrease of other energy sources (Thiess et al., 2004; Backus et al., 2007) or often meant an increase on top of an already high concentration of this energy source (Farrow et al., 2002; Leray et al., 2006; Backus et al., 2007; Slingerland et al., 2008). Despite all efforts made in these studies, they do not allow proper determination of the effect of carbohydrate reduction. Also soluble fibres such as oligofructose and inulin, have been shown to modulate glycaemia and insulinaemia in humans, rodents and dogs (Roberfroid & Delzenne, 1998). In 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 (Nelson, 1989; Blaxter et al., 1990; Nelson, 1992). Secondly, short chain fatty acids (SCFA), especially propionate, generated in the hindgut through fermentation of soluble fibres, might modify hepatic glucose metabolism by reducing hepatic gluconeogenesis and/or enhancing glycolysis (Roberfroid & Delzenne, 1998). Hepatic gluconeogenesis might also be altered indirectly by lowering the plasma fatty acid concentration, resulting in a better insulin sensitivity (Delarue & Magnan, 2007). Propionate itself is also known to enter the citric acid cycle at the level of succinyl
coenzyme A (CoA) and to generate oxaloacetate, which may enter the gluconeogenic pathway (Rémésy et al., 2004). Therefore, it is reasonable that whenever this pathway is active, propionate, when available, may interfere with the utilization of various gluconeogenic substrates, such as lactate and amino acids (Blair et al., 1973; Anderson & Bridges, 1984; Petitet et al., 1998).

The aim of this PhD thesis was to determine the effect of dietary carbohydrates in the broadest sense on feline glucose metabolism. Therefore, two objectives were pursued:

- The effect of the concentration of dietary energy sources (protein, fat and carbohydrate) on glucose and insulin response was investigated, in order to seek the combination of energy sources with the lowest glycaemic effect in cats.
- As a fourth source of energy, SCFA can originate from intestinal fermentation when fed sufficient prebiotic substrate, such as fermentable fibre. The impact of these fermentation products on glucose metabolism was investigated for the first time in cats.

Specific aims were:
- To evaluate the effect of fermentable fibre and fermentation products on glucose tolerance and insulin sensitivity in healthy normal weight as well as obese cats and to determine the metabolic differences between healthy normal weight and obese cats.
- To evaluate the amino acid sparing potential of fermentable fibre and fermentation products.
- To evaluate the plasma acylcarnitine profile, being a reflection of metabolites available for citric acid cycle (Bremer, 1983), as a new, accurate tool to investigate fermentation in the feline gastro-intestinal tract.
Chapter 3

The glucose and insulin response to iso-energetic reduction of dietary energy sources in a true carnivore: the domestic cat (*Felis catus*)
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Submitted, British Journal of Nutrition
Abstract

This study assessed the effect of separate reduction of each energy delivering nutrient - protein, fat, carbohydrate - on glucose tolerance and insulin response a strict carnivore: the domestic cat (*Felis catus*). Three iso-energetic, home-made diets with following caloric distribution; low protein (LP): protein 28% of metabolisable energy, fat 43%, nitrogen-free extract 29%; low fat (LF): 47%, 27%, 25%; low carbohydrate (LC): 45%, 48%, 7%; were tested in a 3×3 Latin square design. Nine healthy normal weight cats were randomly assigned to each of the diets in random order at intervals of three 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 higher and the second insulin peak tended to be delayed when fed the LC diet. In contrast to other studies, in which energy sources were elevated instead of reduced, the present trial contradicts the often suggested negative impact of carbohydrates on insulin sensitivity in carnivores. It even points to a negative effect of highly digestible protein on insulin sensitivity, a finding that corresponds with the highly gluconeogenic nature of amino acids in strict carnivores.
3.1. Introduction

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 (Colagiuri & Miller, 2002). 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 (Zimmet et al., 1999; Colagiuri & Miller, 2002). In individuals that 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 (Colagiuri & Miller, 2002; Rand et al., 2004). A critical role for the quantity and quality of dietary carbohydrate in the pathogenesis of this disorder has been postulated by the ‘carnivore connection’ theory (Miller & Colagiuri, 1994; Colagiuri & Miller, 2002; Rand et al., 2004). During the Ice Ages our ancestors consumed high protein (HP), low carbohydrate (LC) diets, and since the brain, foetus 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 agricultural revolution augmented the amount of digestible carbohydrates and industrial revolution was responsible for changing the quality of carbohydrates. These evolutionary changes in carbohydrate, meaning the introduction of high glycaemic index foods, can worsen insulin resistance and can be linked to the development of type 2 diabetes (Miller & Colagiuri, 1994; Colagiuri & Miller, 2002).

Over the last decades, as in humans, the diet of strictly carnivorous domestic cats changed from HP, LC prey (Zoran, 2002) 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 (Rand et al., 2004; Slingerland et al., 2009).

Several human studies (Nuttall et al., 1985; Gannon et al., 2003; Gannon & Nuttall, 2004; McAuley et al., 2005; Boden et al., 2005) as well as rodent studies (rats: Belobrajdic et al., 2004; mice: Klaus, 2005) 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 (Farrow et al., 2002). However, the hypothesis that HC diets lead to β-cell exhaustion was contradicted by Slingerland et al. (2008), since feeding a HC diet resulted in 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. (2007b) suggested that cats with the same caloric 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 (Thiess et al., 2004; Backus et al., 2007) as well as to induce weight gain (Backus et al., 2007).

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; 2) or often meant an increase on top of an already high concentration of this energy source.

The present study deals with these aspects by applying a pairwise reduction of one energy source and therefore enables to identify the separate effect of each energy source through looking at the effect of their reduction to minimal concentrations. This method has been shown effective in demonstrating single energy source effects on metabolism in poultry (Malheiros et al., 2003).

### 3.2. Material and methods

#### 3.2.1. Animals and housing

Six mixed-breed and three European short-hair cats, of which five intact females, one intact and three castrated males, were employed in this study. All cats aged between 3 and 10 year and had a mean body weight (BW) of 3.67 kg (range 2.50 - 5.24 kg). Body condition score (BCS) was determined using a five-point body condition scoring system (National Research Council, 2006). Non-obese cats with a BCS 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 gender and body weight. During the trial, cats were housed individually in separated indoor cages. Two hours a day, 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.
3.2.2. Diets and feeding

Three iso-energetic home-made diets were tested; a low protein (LP), a low fat (LF) and a low carbohydrate (LC) diet. To produce the test diets, cooked and ground chicken breast was mixed with liquid chicken lard and corn starch (Cerestar, Sas van Gent, The Netherlands). The same ingredients were used in different quantities in order to create pair-wise changes in macronutrient content (Table 3.1). The LP diet differed from LF and LC diet only by iso-energetic substitution of protein for fat and protein for carbohydrate, respectively. The LF diet differed from the LC diet by iso-energetic substitution of fat for carbohydrate. Diets contained no dietary fibre and had similar mineral concentrations and physical structure. A tailor-made vitamin and mineral premix (Institut für Physiologie, Physiologische Chemie und Tierernährung der Ludwig-Maximilians-Universität München, Lehrstuhl für Tierernährung und Diätetik, Germany) was added to balance the diet. The three diets were analyzed for proximate components (Table 3.1) and caloric distributions were: LP diet: protein 28%, fat 43%, nitrogen-free extract (NFE) 29%, LF diet: protein 47%, fat 27%, NFE 25%, LC diet: protein 45%, fat 48%, NFE 7% (Figure 3.1).

![Figure 3.1: Caloric distribution of the iso-energetic test diets: LP, LF and LC diet.](image)

LP, low protein; LF, low fat; LC, low carbohydrate; ME, metabolisable energy; NFE, nitrogen-free extract.
Cats were offered only one meal daily. The amount of food corresponded with the cat’s individual maintenance energy requirement (375 kJ/kg$^{0.75}$) (Debraekeleer et al., 2000) and was adjusted to maintain body weight. The food was available all day, except for the two hours playtime. All cats had free access to water at all times. Fresh water was supplied daily.

**Table 3.1: Composition of the test diets.**

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>LP</th>
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<th>LC</th>
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<tr>
<td>Chicken fillet</td>
<td>66.3</td>
<td>81.0</td>
<td>86.1</td>
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<td>Chicken lard</td>
<td>11.4</td>
<td>4.3</td>
<td>8.3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>20.7</td>
<td>14.1</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin/Mineral premix$^1$</td>
<td>1.5</td>
<td>0.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Nutrients on DM (%) (by Weende-analysis)**

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>LP</th>
<th>LF</th>
<th>LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>36.1</td>
<td>54.1</td>
<td>60.2</td>
</tr>
<tr>
<td>Ether extract</td>
<td>23.2</td>
<td>13.0</td>
<td>26.8</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Crude ash</td>
<td>4.05</td>
<td>4.32</td>
<td>4.05</td>
</tr>
<tr>
<td>NFE$^2$</td>
<td>36.7</td>
<td>28.6</td>
<td>9.0</td>
</tr>
<tr>
<td>Starch</td>
<td>26.0</td>
<td>27.1</td>
<td>3.3</td>
</tr>
<tr>
<td>ME (kJ/100g DM)$^3$</td>
<td>1926</td>
<td>1709</td>
<td>2001</td>
</tr>
</tbody>
</table>

**Nutrients on energy basis (g/MJ ME)**

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>LP</th>
<th>LF</th>
<th>LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>18.7</td>
<td>31.7</td>
<td>30.1</td>
</tr>
<tr>
<td>Ether extract</td>
<td>12.0</td>
<td>7.6</td>
<td>13.4</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Crude ash</td>
<td>2.1</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>NFE</td>
<td>19.0</td>
<td>16.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Starch</td>
<td>13.5</td>
<td>15.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

LP, low protein; LF, low fat; LC, low carbohydrate; DM, dry matter; NFE, nitrogen-free extract; ME, metabolisable energy.

$^1$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%, I 0.006%, vitamin A 150 IE/g, vitamin D$_3$ 12.5 IE/g, vitamin E 3.25 mg/g, vitamin B1 0.3 mg/g, vitamin B2 0.2 mg/g, taurin 75 mg/g; LF and LC diet: Ca 27.70%, Fe 0.44%, Zn 0.56%, Mn 0.057%, I 0.013%, vitamin A 225 IE/g, vitamin D$_3$ 22.5 IE/g, vitamin E 5.65 mg/g, vitamin B1 0.53 mg/g, vitamin B2 0.28 mg/g, taurin 140 mg/g.

$^2$Derived by substracting crude protein, ether extract, crude ash and crude fibre from the DM content.

$^3$Calculated: $15 \times$ crude protein + $36 \times$ ether extract + $15 \times$ nitrogen-free extract.
3.2.3. Experimental design

Prior to being entered into the study, each cat was physically examined; BW and BCS were recorded and a blood sample was drawn from the jugular vein after a 12-hour fast for complete blood count and serum biochemistry. For four weeks preceding the trial, cats were fed a standard commercial maintenance diet (Bento Kronen Torka chicken-turkey, Versele-Laga, Deinze, Belgium), prior to be randomized to one of three groups. Each group of cats was assigned on each of three diets in a random order at intervals of three weeks. This way, test diets were examined in a 3×3 Latin square design.

Absolute food intake was measured each day throughout the study and relative food intake (% of food intake relative to energy requirement) and daily energy consumption were calculated. BW 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 hours prior to the IVGTT, cats were anesthetized with buprenorphine (Temgesic, Schering-Plough n.v., Heist-Op-Den-Berg, Belgium) 10 µg/kg intravenously, followed by propofol (Propovet, Abbott Lab, Leuven, Belgium), 6-7 mg/kg to effect, intravenously, and a 20 G, 8 cm intravenous catheter (Leaderflex, Vygon n.v., Brussels, Belgium) was placed in a jugular vein (Figure 3.2), for glucose administration and blood sampling. Catheters were flushed twice daily with 1 ml heparinized saline (50 IU of heparin/ml in 0.9% NaCl solution) to maintain patency. Amoxicillin (Clamoxyl LA, GlaxoSmithKline n.v., Genval, Belgium) 15 mg/kg subcutaneously, was administered once at the time of catheter placement.

The IVGTT was performed between 9.00 a.m. and 13.00 p.m. after a 12-hour 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 to 45 seconds, followed immediately by 1 ml of saline solution to flush the catheter (Appleton et al., 2001a). Blood samples were collected from the jugular catheter (Martin & Rand, 1999) (Figure 3.3), prior to (0 minutes) and 2, 5, 10, 15, 30, 45, 60, 90 and 120 minutes after glucose administration (Appleton et al., 2001a).
Figure 3.2: Jugular catheter placement in cats using a modified Seldinger technique, as described by Martin & Rand (1999).
In addition, effects on lipid as well as protein metabolism were evaluated by the analysis of total cholesterol, triglycerides and non-esterified fatty acids (NEFA) and 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 (Fruhbeck & Salvador, 2000).

At time zero, blood samples were collected in tubes containing lithium heparin for determination of plasma leptin, and NEFA concentrations and serum tubes were used to determine basal serum total cholesterol, triglycerides, urea and creatinine concentrations. At each time interval, blood samples were collected in tubes containing sodium fluoride for determination of plasma glucose and in serum tubes for determination of serum insulin concentrations. Plasma and serum were removed 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 2006/029) and was in accordance with institutional and national guidelines for the care and use of animals.
3.2.4. Analytical methods

Plasma glucose, total cholesterol, triglycerides, 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 the WAKO NEFA C test kit (Wako Chemicals GmbH, Neuss, Germany), modified for use in the Monarch Chemistry System (Instrumentation Laboratory Belgium N.V./S.A, Zaventem, Belgium). Serum insulin concentrations were measured by a commercially available immunoradiometric assay (IRMA) test kit (insulin IRMA Ref 5251, Biosource Europe S.A., Nivelles, Belgium) as described by Slingerland et al. (2007). Plasma leptin concentrations were determined by using a commercially available radio-immunoassay (RIA) test kit (Multi-Species Leptin RIA Kit, Catalogue number XL-85K, Linco Research Inc., St.Charles, Missouri, USA). This kit was developed to quantify leptin in plasma from several species and has been validated for use in cats (Backus et al., 2000).

The glucose disappearance coefficient (K_{glucose}) and the half-life for glucose disappearance (glucose \( t_{1/2} \)) between 15-90 minutes after glucose administration (Link & Rand, 1998) as well as the basal insulin to glucose ratio (SI) and the ratio of area under the insulin to glucose curve (AUC_{ins}/AUC_{gluc}) were calculated (Appleton et al., 2005). Area under the glucose (AUC_{gluc}) and insulin (AUC_{ins}) curve were calculated according to the trapezoidal method (baseline equal to zero).

3.2.5. Statistical analyses

Statistical analyses were performed using Superior Performing Software Systems (SPSS) version 16 (SPSS Inc., Chicago, Illinois, USA). The effect of the three diets on the selected blood parameters was investigated using repeated measures ANOVA, with diet as within subject factor. A paired sample T-test was used as a post hoc test. Statistical significance was accepted at \( P<0.05 \). All data are expressed as mean ± SE.


3.3. Results

3.3.1. Diet composition, food intake and body weight

Nutrient compositions of the three test diets were determined by proximate analysis (Table 3.1). 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 and relative food intake showed no differences among the test diets. BW remained stable in all cats during the study and was not affected by test diets (Table 3.2).

Table 3.2: Mean daily energy consumption, absolute and relative food intake, body weight and hematocrit (mean ± SE) in nine healthy cats fed a low protein, a low fat and a low carbohydrate diet.

<table>
<thead>
<tr>
<th></th>
<th>LP</th>
<th></th>
<th></th>
<th>LF</th>
<th></th>
<th></th>
<th>LC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
</tbody>
</table>
| Mean daily energy consumption (kJ/d) | 773  | 55.6  | 790   | 74.0 | 782   | 36.5  |       | 0.969
| Relative food intake (%)1 | 85.8 | 5.3   | 86.8  | 6.6  | 87.3  | 4.8   |       | 0.982
| BW (kg)       | 3.2  | 0.3   | 3.3   | 0.3  | 3.2   | 0.3   |       | 0.207
| Hematocrit (%)| 34.3 | 0.8   | 32.6  | 1.0  | 32.6  | 1.5   |       | 0.388

LP, low protein; LF, low fat; LC, low carbohydrate; BW, body weight.

1Relative food intake (%) = % of food intake relative to energy requirement

P-values result from repeated measures ANOVA. Significant differences (P<0.05) resulting from post hoc analysis, are marked by different superscripts within one row. N<9 is caused by missing values.

3.3.2. Glucose tolerance test

Glucose (Table 3.3; Figure 3.4a) - Basal plasma glucose, glucose two minutes after glucose administration (glucose peak), and glucose concentrations at each other time point of the IVGTT showed no differences between test diets. In all cats, plasma glucose concentrations returned to baseline at 120 minutes after glucose administration. The AUCgluc did not differ among test diets. Kglucose as well as glucose t1/2 showed no differences.
**Table 3.3:** Plasma glucose concentrations during IVGTT (mean ± SE) in healthy cats fed a low protein, a low fat and a low carbohydrate diet.

<table>
<thead>
<tr>
<th></th>
<th>LP Mean</th>
<th>SE</th>
<th>N</th>
<th>LF Mean</th>
<th>SE</th>
<th>N</th>
<th>LC Mean</th>
<th>SE</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal glucose (mmol/l)</td>
<td>3.7 0.2 9</td>
<td></td>
<td></td>
<td>3.8 0.1 9</td>
<td></td>
<td></td>
<td>3.9 0.2 9</td>
<td></td>
<td></td>
<td>0.349</td>
</tr>
<tr>
<td>Glucose after 2 min (mmol/l)</td>
<td>33.1 4.2 9</td>
<td></td>
<td></td>
<td>38.6 10.7 7</td>
<td></td>
<td></td>
<td>32.6 2.9 9</td>
<td></td>
<td></td>
<td>0.675</td>
</tr>
<tr>
<td>Glucose after 5 min (mmol/l)</td>
<td>17.9 1.3 9</td>
<td></td>
<td></td>
<td>17.9 1.4 8</td>
<td></td>
<td></td>
<td>18.2 1.1 7</td>
<td></td>
<td></td>
<td>0.988</td>
</tr>
<tr>
<td>Glucose after 10 min (mmol/l)</td>
<td>15.1 0.8 8</td>
<td></td>
<td></td>
<td>14.8 0.7 9</td>
<td></td>
<td></td>
<td>15.4 0.4 9</td>
<td></td>
<td></td>
<td>0.438</td>
</tr>
<tr>
<td>Glucose after 15 min (mmol/l)</td>
<td>14.4 1.2 8</td>
<td></td>
<td></td>
<td>14.4 0.9 9</td>
<td></td>
<td></td>
<td>14.7 0.7 7</td>
<td></td>
<td></td>
<td>0.592</td>
</tr>
<tr>
<td>Glucose after 20 min (mmol/l)</td>
<td>10.9 0.9 9</td>
<td></td>
<td></td>
<td>11.2 0.9 9</td>
<td></td>
<td></td>
<td>12.1 0.8 9</td>
<td></td>
<td></td>
<td>0.197</td>
</tr>
<tr>
<td>Glucose after 25 min (mmol/l)</td>
<td>9.1 1.3 9</td>
<td></td>
<td></td>
<td>8.6 0.9 9</td>
<td></td>
<td></td>
<td>10.0 0.9 9</td>
<td></td>
<td></td>
<td>0.151</td>
</tr>
<tr>
<td>Glucose after 30 min (mmol/l)</td>
<td>7.4 1.2 9</td>
<td></td>
<td></td>
<td>6.5 0.8 9</td>
<td></td>
<td></td>
<td>8.0 0.9 9</td>
<td></td>
<td></td>
<td>0.205</td>
</tr>
<tr>
<td>Glucose after 35 min (mmol/l)</td>
<td>4.6 0.8 9</td>
<td></td>
<td></td>
<td>3.5 0.2 9</td>
<td></td>
<td></td>
<td>4.5 0.5 9</td>
<td></td>
<td></td>
<td>0.291</td>
</tr>
<tr>
<td>Glucose after 40 min (mmol/l)</td>
<td>3.4 0.2 9</td>
<td></td>
<td></td>
<td>3.4 0.0 9</td>
<td></td>
<td></td>
<td>3.4 0.1 9</td>
<td></td>
<td></td>
<td>0.383</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;gluc&lt;/sub&gt; (mmol/l/120min)</td>
<td>1055 110 9</td>
<td></td>
<td></td>
<td>1005 69.2 8</td>
<td></td>
<td></td>
<td>1108 127 9</td>
<td></td>
<td></td>
<td>0.324</td>
</tr>
<tr>
<td>K&lt;sub&gt;gluc&lt;/sub&gt;</td>
<td>1.62 0.16 9</td>
<td></td>
<td></td>
<td>1.77 0.15 9</td>
<td></td>
<td></td>
<td>1.50 0.14 9</td>
<td></td>
<td></td>
<td>0.501</td>
</tr>
<tr>
<td>Glucose t&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>47.7 6.7 9</td>
<td></td>
<td></td>
<td>42.5 5.0 9</td>
<td></td>
<td></td>
<td>52.0 7.9 9</td>
<td></td>
<td></td>
<td>0.470</td>
</tr>
</tbody>
</table>

LP, low protein; LF, low fat; LC, low carbohydrate; AUC<sub>gluc</sub>, area under the curve for glucose; K<sub>gluc</sub>, glucose disappearance coefficient; Glucose t<sub>1/2</sub>, half-life for glucose disappearance between 15-90 minutes after glucose administration.

P-values result from repeated measures ANOVA. N<9 is caused by missing values.
Insulin (Table 3.4; Figure 3.4b) - No differences between test diets were observed for basal serum insulin concentrations. As shown in Figure 3.3b, insulin secretion was biphasic in response to glucose administration. During the IVGTT, serum insulin concentrations were higher at 60 minutes after glucose administration, when cats were fed the LC diet, compared to the LP diet ($P=0.034$). The LF diet was intermediate. At all other time points, no differences were found among test diets, except for a tendency towards lower serum insulin concentrations at 45 minutes.
Table 3.4: Serum insulin concentrations during IVGTT (mean ± SE) in healthy cats fed a low protein, a low fat and a low carbohydrate diet.

<table>
<thead>
<tr>
<th></th>
<th>LP</th>
<th>LF</th>
<th>LC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>Basal insulin (mU/l)</td>
<td>10.4</td>
<td>0.9</td>
<td>9</td>
<td>10.0</td>
</tr>
<tr>
<td>Insulin after 2 min (mU/l)</td>
<td>35.7</td>
<td>6.4</td>
<td>8</td>
<td>31.2</td>
</tr>
<tr>
<td>Insulin after 5 min (mU/l)</td>
<td>32.5</td>
<td>4.4</td>
<td>8</td>
<td>31.5</td>
</tr>
<tr>
<td>Insulin after 10 min (mU/l)</td>
<td>43.8</td>
<td>6.6</td>
<td>9</td>
<td>42.3</td>
</tr>
<tr>
<td>Insulin after 15 min (mU/l)</td>
<td>38.6</td>
<td>6.4</td>
<td>8</td>
<td>43.6</td>
</tr>
<tr>
<td>Insulin after 30 min (mU/l)</td>
<td>32.6</td>
<td>5.7</td>
<td>8</td>
<td>31.6</td>
</tr>
<tr>
<td>Insulin after 45 min (mU/l)</td>
<td>27.6</td>
<td>8.2</td>
<td>8</td>
<td>39.4</td>
</tr>
<tr>
<td>Insulin after 60 min (mU/l)</td>
<td>21.4^a</td>
<td>8.7</td>
<td>8</td>
<td>32.0^b</td>
</tr>
<tr>
<td>Insulin after 90 min (mU/l)</td>
<td>12.8</td>
<td>12.6</td>
<td>8</td>
<td>12.9</td>
</tr>
<tr>
<td>Insulin after 120 min (mU/l)</td>
<td>7.3</td>
<td>13.6</td>
<td>7</td>
<td>10.1</td>
</tr>
<tr>
<td>AUCins (mU/l/min 120min)</td>
<td>2667^a</td>
<td>322</td>
<td>8</td>
<td>3339^a</td>
</tr>
<tr>
<td>Time 1st insulin peak (min)</td>
<td>5.6</td>
<td>1.9</td>
<td>8</td>
<td>7.6</td>
</tr>
<tr>
<td>Time 2nd insulin peak (min)</td>
<td>36.9</td>
<td>6.7</td>
<td>8</td>
<td>44.4</td>
</tr>
<tr>
<td>Height 1st insulin peak (mU/l)</td>
<td>45.8</td>
<td>7.9</td>
<td>9</td>
<td>41.2</td>
</tr>
<tr>
<td>Height 2nd insulin peak (mU/l)</td>
<td>39.5</td>
<td>6.8</td>
<td>8</td>
<td>45.3</td>
</tr>
<tr>
<td>Basal SI</td>
<td>0.15</td>
<td>0.01</td>
<td>9</td>
<td>0.14</td>
</tr>
<tr>
<td>AUCins/AUCgluc</td>
<td>0.16</td>
<td>0.03</td>
<td>8</td>
<td>0.18</td>
</tr>
</tbody>
</table>

LP, low protein; LF, low fat; LC, low carbohydrate; AUCins, area under the curve for insulin; SI, insulin to glucose ratio; AUCins/AUCgluc, ratio of area under the insulin to glucose curve.

P-values result from repeated measures ANOVA. Significant differences (P<0.05) resulting from post hoc analysis, are marked by different superscripts within one row. N<9 is caused by missing values.
after glucose administration in cats fed the LP diet. When fed the LC diet, a tendency towards later occurrence of the second insulin peak was noticed. In contrast, when fed the LP diet, there was a tendency towards earlier occurrence of the second insulin peak ($P=0.061$). This second insulin peak was also numerically higher for the LC diet compared to the LP and LF diet, but the difference was not significant. In all cats, except one individual when fed the LP diet, the serum insulin concentrations returned to baseline concentration at 120 minutes after glucose administration. The $AUC_{ins}$ was increased in cats fed the LC diet compared to the LF and LP diet ($P=0.025$). Nevertheless, fasting SI and $AUC_{ins}/AUC_{gluc}$ did not differ among test diets.

3.3.3. **Metabolic parameters**

As shown in Table 3.5, basal serum urea concentrations were decreased in cats fed the LP-diet compared to the LF diet. The LC diet was intermediate ($P=0.037$). Basal serum creatinine concentrations did not differ among test diets. Basal serum total cholesterol concentrations also showed differences between the three test diets. Feeding the LP and LF diet resulted in decreased serum total cholesterol concentrations when compared to consumption of the LC diet ($P=0.005$). No differences were observed among test diets for basal serum triglyceride and plasma NEFA concentrations as well as for basal plasma leptin concentrations.

**Table 3.5: Plasma urea, creatinine, cholesterol, triglycerides, NEFA and leptin concentrations (mean ± SE) in nine healthy cats fed a low protein, a low fat and a low carbohydrate diet.**

<table>
<thead>
<tr>
<th></th>
<th>LP Mean ± SE</th>
<th>LF Mean ± SE</th>
<th>LC Mean ± SE</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mmol/l)</td>
<td>7.8a 7.1</td>
<td>9.4b 8.7</td>
<td>8.7ab 7.7</td>
<td>0.037</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>136.5 8.8</td>
<td>130.6 8.0</td>
<td>123.8 4.4</td>
<td>0.057</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>3.2a 0.2</td>
<td>3.1a 0.3</td>
<td>3.5b 0.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.37 0.04</td>
<td>0.39 0.14</td>
<td>0.32 0.04</td>
<td>0.711</td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td>0.87 0.15</td>
<td>0.92 0.05</td>
<td>0.99 0.06</td>
<td>0.782</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>3.38 0.29</td>
<td>3.09 0.18</td>
<td>2.87 0.13</td>
<td>0.148</td>
</tr>
</tbody>
</table>

LP, low protein; LF, low fat; LC, low carbohydrate; NEFA, non-esterified fatty acids.

$P$-values result from repeated measures ANOVA. Significant differences ($P<0.05$) resulting from post hoc analysis, are marked by different superscripts within one row. N<9 is caused by missing values.
3.4. Discussion

The present study showed no differences between test diets concerning the glucose response and revealed that the LC diet induced a higher \( \text{AUC}_{\text{ins}} \) than the LP diet, with the LF diet being intermediate. Not only the increased \( \text{AUC}_{\text{ins}} \), but also the tendency towards a delayed second insulin peak suggests decreased insulin sensitivity in healthy normal weight cats when fed the LC diet.

These findings seem to contradict earlier performed studies which showed that reducing dietary carbohydrate content improved insulin sensitivity. For example, Farrow et al. (2002) 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 concentration of this energy source, as did Leray et al. (2006), Backus et al. (2007) and Slingerland et al. (2008), resulting in extremely high concentrations of carbohydrates, much higher than the currently available commercial cat foods. Therefore, investigating the effect of lowering the carbohydrate content and to compare this to higher, but commercially available concentrations of carbohydrate, is much more useful. In healthy cats, only two studies investigated the effect of reducing carbohydrates. Thiess et al. (2004) allocated impaired glucose tolerance when fed HF, LC diet to increased fat intake. However, the HF, LC diet also contained low concentrations of carbohydrates. Since, the two test diets differed by substituting fat for carbohydrates, confounding effects might have occurred. Backus et al. (2007) noted similar results, but once more, confounding effects might have arisen. In order to prevent confounding in the present trial, energy sources were substituted iso-calorically, resulting in a reduction of only one energy source, enabling to identify the effect of this energy source on glucose and insulin response. The results also seem contradictory to several earlier studies suggesting HP, LC diets to be beneficial for the treatment of type 2 feline diabetes mellitus (Frank et al., 2001; Mazzaferro et al., 2003; Bennett et al., 2006). 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 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, foetus and mammary gland, evoked certain metabolic adaptations in cats to accommodate the low glucose intake (Miller & Colagiuri, 1994; Colagiuri & Miller, 2002). 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. Both the ability of insulin to inhibit hepatic glucose production and to augment tissue glucose disposal are impaired (Miller & Colagiuri, 1994). Meaning that gluconeogenesis is more or less permanently ‘switched on’ in true carnivores (Kettelhut et al., 1980; MacDonald et al., 1984). 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 (Curry et al., 1982). 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).

In the present study, the influence of the energy delivering nutrients on metabolic parameters as well as possible modifications of plasma leptin concentrations were also investigated. The basal serum urea concentrations were decreased in cats fed the LP diet compared to the LF diet, which can be explained by the lower protein intake (Adams et al., 1993). The basal serum cholesterol concentrations were expected to be decreased in cats fed the LF diet, because of the lower fat intake (Thiess et al., 2004). In contrast with the expectations, the serum cholesterol concentrations were lower in cats fed the LP and LF diet compared to 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 parameters for lipid metabolism, basal serum triglycerides as well as plasma NEFA concentrations, did not differ among test diets. The similar basal plasma leptin concentrations after feeding the test diets are consistent with the normal and stable body weight of the cats, as increased leptin concentrations are associated positively with the amount of body fat (Martin et al., 2001). Leptin is also reversely related with insulin sensitivity, independent of adiposity, suggesting a possible role for leptin in the link between obesity and insulin resistance (Appleton et al., 2002).

3.5. Conclusion

During this study in healthy normal weight cats, reducing the dietary carbohydrate concentration below common concentrations 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.

**Acknowledgements:** The Institute of Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen) is gratefully acknowledged for financial support. We are also grateful to Versele-Laga (Deinze, Belgium) for providing the pet food during the adaptation and like to thank Herman Derycke for performing the food analyses and Steven Galle for technical assistance.
Chapter 4

Oligofructose and inulin modulate glucose and amino acid metabolism through propionate production in normal weight and obese cats
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British Journal of Nutrition 102: 694-702
Abstract

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 cross-over design; a control diet high in protein (46% on dry matter (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 four 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 (AUC_{ins}) 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 methylmalonyl-carnitine ($P=0.072$) and aspartate aminotransferase (AST) 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.
4.1. Introduction

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. (2007) and might be due to a rise in the frequency of the major predisposing factors such as obesity and physical inactivity (Rand et al., 2004). 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 (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 versus postprandial) and pathological (diabetes mellitus, obesity) conditions (Roberfroid & Delzenne, 1998). 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 (Nelson, 1989; Blaxter et al., 1990; Nelson, 1992). Secondly, the production of short chain fatty acids (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 (Roberfroid & Delzenne, 1998). 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 (Delarue & Magnan, 2007).

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 (Diez et al., 1997; Diez et al., 1998; Massimino et al., 1998; Strickling et al., 2000; Hesta et al., 2001b). 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, triglycerides, non-esterified fatty acids (NEFA) concentrations) and protein metabolism (plasma urea, aspartate aminotransferase (AST), alanine amino-transferase
(ALT) and methylmalonylcarnitine concentration) of the true carnivorous cat were scrutinized in the present study. Acylcarnitine profile was studied as a reflection of metabolites available for citric acid cycle (Bremer, 1983). 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 (Appleton et al., 2000) and insulin resistance is associated with increased leptin concentrations in both normal weight and obese cats (Appleton et al., 2002). Given that thyroid hormones are involved in the regulation of metabolism, and regulate resting metabolic rate, thermogenesis and lipolysis, thyroid function might be altered by developing obesity (Ferguson et al., 2007). Hence, total fasting triiodothyronine (T₃) and total thyroxine (T₄) 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.

### 4.2. Materials and methods

#### 4.2.1. 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. Two hours a day, 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.

#### 4.2.2. Diets and feeding

The control diet (C-diet) was a non-commercial extruded dry cat food, containing high concentrations of crude protein (46% on dry matter (DM) basis), moderate amounts of crude fat (15% DM) and low concentrations of carbohydrates (Nitrogen free extract (NFE) 27% DM), in
order to trigger the cats towards insulin resistance (Chapter 3). The food also contained moderate concentrations of crude fibre (4.6% DM) and crude ash (6.7% DM) (Table 4.1) 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-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 (6-10 % DS). The mean total number of fructose or glucose units (degree of polymerisation; DP) was 25 for the inulin; mean DP 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 4.1. Total dietary fibre (TDF) was determined by acid and enzymatic digestion using enzymes from a commercial test kit (Bioquant Total Dietary Fiber, Merck, Darmstadt, Germany), followed by correction for protein and ash and is also shown in Table 4.1.

**Table 4.1: Composition of the control and prebiotic diet (C-diet + 2.5% of a mixture of oligofructose and inulin).**

<table>
<thead>
<tr>
<th>Nutrients on DM (%) (analyzed)</th>
<th>C-diet</th>
<th>P-diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>46.4</td>
<td>44.1</td>
</tr>
<tr>
<td>Ether extract</td>
<td>15.2</td>
<td>14.9</td>
</tr>
<tr>
<td>Crude ash</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>4.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Starch</td>
<td>23.1</td>
<td>22.3</td>
</tr>
<tr>
<td>Sugars</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>NFE</td>
<td>27.3</td>
<td>29.8</td>
</tr>
<tr>
<td>NPS ¹</td>
<td>1.8</td>
<td>5.2</td>
</tr>
<tr>
<td>TDF</td>
<td>9.3</td>
<td>11.8</td>
</tr>
<tr>
<td>ME (kJ/100g as fed) ²</td>
<td>1453</td>
<td>1433</td>
</tr>
</tbody>
</table>

C-diet, control diet; P-diet, prebiotic diet; DM, dry matter; NFE, nitrogen-free extract, NSP, non-starch polysaccharides; TDF, total dietary fibre; ME, metabolisable energy.

**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, monosodiumglutamate, salt, DL-methionine, ironoxide black, choline chloride 75%

¹Derived by subtracting starch and sugars content from NFE content.

²Calculated: 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$^{0.4}$) (National Research Council, 2006) and was adapted in order to maintain a constant body weight (BW). The food was available all day, except for the two hours playtime. Cats were allowed free access to water at all times.

4.2.3. 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-hour fast for complete blood count and serum biochemistry and BW, body condition score (BCS), body mass index (BMI) and girth circumference (girth) were recorded. The BCS was determined using a five-point body condition scoring system (National Research Council, 2006). Non-obese cats with a BCS of 3/5 (mean BW: 4.3 kg; range: 3.7 to 5.3 kg) and obese cats with a BCS of 5/5 (mean BW: 6.8 kg; range: 5.3 to 9.8 kg) were used in the study (Figure 4.1). The BMI was calculated as described by Hoenig et al. (2003). Girth was measured directly behind the last rib (Hoenig, personal communication). All measurements were performed under general anaesthesia by the same person to minimize variability (Table 4.2).

Figure 4.1: (a) Normal weight cat, body weight (BW): 4.2 kg, body condition score (BCS): 3/5 according to the five-point body condition scoring system (National Research Council, 2006); (b) obese cat, BW: 9.8 kg, BCS: 5/5.
Table 4.2: Body condition score, body weight, body mass index and girth circumference in eight normal weight and seven obese neutered adult cats expressed as mean ± SE.

<table>
<thead>
<tr>
<th></th>
<th>Normal Weight</th>
<th>Obese</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>BCS</td>
<td>3.1</td>
<td>0.1</td>
<td>5.0</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>4.3</td>
<td>0.2</td>
<td>6.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>37.7</td>
<td>1.3</td>
<td>54.4</td>
</tr>
<tr>
<td>Girth (cm)</td>
<td>39.6</td>
<td>0.9</td>
<td>50.1</td>
</tr>
</tbody>
</table>

BCS, body condition score; BW, body weight; BMI, body mass index; Girth, girth circumference.  
*P-value of statistical difference between normal weight and obese cats, results from one way ANOVA.

For four weeks preceding the trial (adaptation period), all cats were fed the C-diet prior to be randomized 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 four weeks. This way, diets were examined in a cross-over design. Absolute food intake was measured daily throughout the study and relative food intake (% of ME intake relative to maintenance energy requirement) was calculated. BW 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 hours prior to the IVGTT, cats were anesthetized with buprenorphine (Temgesic®, Schering-Plough n.v., Heist-Op-Den-Berg, Belgium) 10 µg/kg intravenously, followed by propofol (Propovet®, Abbott Lab, Leuven, Belgium), 6-7 mg/kg to effect, intravenously, 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 heparinized saline (50 IU of heparin/ml in saline (0.9% NaCl) solution) to maintain patency. Amoxicillin (Clamoxyl LA®, GlaxoSmithKline n.v., Genval, Belgium) 15 mg/kg subcutaneously, was administered once at the time of catheter placement. The IVGTT was performed between 9.00 and 12.00 hours after a 12-hour fast. Glucose 0.5 g/kg (Glucose Sterop 500 mg/ml, Laboratoria Sterop n.v., Brussels, Belgium), was administered through the jugular vein catheter over 30 to 45 seconds, followed immediately by 1 ml of normal saline to flush the catheter (Appleton et al., 2001a). Blood samples were collected from the jugular catheter as described by (Martin & Rand, 1999), prior to (0 minutes) and 2, 5, 10, 15, 30, 45, 60, 90 and 120 minutes after glucose administration (Appleton et al., 2001a). At time zero, blood samples were collected in tubes containing lithium.
heparin for determination of AST, ALT, leptin, T₃ and T₄ concentrations, free carnitine and acylcarnitine profile. Serum tubes were used to determine fasting serum total cholesterol, triglycerides, 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).

4.2.4. Analytical methods

Plasma glucose, serum total cholesterol, triglycerides, urea and creatinine concentrations were determined spectrophotometrically by using the Roche/Hitachi Modular Analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Likewise, activities of AST and ALT were analyzed spectrophotometrically by using the Roche/Hitachi Modular Analyzer (Roche Diagnostics GmbH, Mannheim, Germany) 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, UK). Serum insulin concentrations were measured by using a commercially available immunoradiometric assay (IRMA) test kit (insulin IRMA Ref 5251, Biosource Europe S.A. Nivelles, Belgium) as described by Slingerland et al. (2007). Plasma leptin concentrations were determined by using a commercially avialable radio-immunoassay (RIA) test kit (Multi-Species Leptin RIA Kit, Catalogue number XL-85K, Linco Research Inc., St.Charles, Missouri, USA). This kit was developed to quantify leptin in plasma from several species and has been validated for use in cats (Backus et al., 2000). Fasting plasma T₃ and T₄ concentrations were determined using a specific radioimmunoassay as described by Darras et al. (1992). Quantitative electrospray tandem-mass spectrometry was used for free carnitine and acylcarnitine analysis, as described by Vreken et al. (1999) and Rizzo et al. (2003).

The glucose disappearance coefficient (K_{glucose}) and the half-life for glucose disappearance (glucose t_{1/2}) between 15-90 minutes after glucose administration were calculated as described by Link & Rand (1998). Area under the glucose (AUC_{gluc}) and insulin (AUC_{ins}) curve were calculated according to the trapezoidal method (baseline equal to zero). The basal insulin to
glucose ratio (SI) 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. (2005).

### 4.2.5. Statistical analyses

Statistical analyses were performed using Superior Performing Software Systems (SPSS) version 16 (SPSS Inc., Chicago, Illinois, USA). For BW, BCS, BMI and girth 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 analyzed 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 mean ± SE.

### 4.3. Results

All 16 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.

#### 4.3.1. Effect of body condition

At the start of the trial, BW, BCS, BMI and girth (\( P<0.001 \) for all) differed among normal weight and obese cats (Table 4.2). 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 4.3. Obese cats ate more compared with normal weight cats (\( P=0.019 \)), 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 \((P=0.011)\) and serum triglycerides concentrations tended \((P=0.094)\) to be higher in obese cats when compared to normal weight cats. Plasma ALT activity was increased in obese cats \((P=0.043)\), 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 \((P=0.003\) and \(P=0.026,\) respectively). Fasting plasma T\(_4\) concentrations did not differ in relation to BCS.

Table 4.3: Effect of body condition score on feed intake, fasting metabolic and endocrine characteristics, regardless of diet, expressed as mean ± SE.

<table>
<thead>
<tr>
<th>Body condition</th>
<th>Normal weight</th>
<th></th>
<th>Obese</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>N</td>
<td>Mean</td>
<td>SE</td>
<td>N</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute food intake (g/d)</td>
<td>48.0(^a)</td>
<td>2.0</td>
<td>16</td>
<td>61.9(^b)</td>
<td>3.1</td>
<td>14</td>
<td>0.019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative food intake (%)</td>
<td>94.6</td>
<td>1.8</td>
<td>16</td>
<td>93.9</td>
<td>1.1</td>
<td>14</td>
<td>0.822</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>9.2(^a)</td>
<td>0.5</td>
<td>16</td>
<td>6.7(^b)</td>
<td>0.4</td>
<td>14</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>131</td>
<td>6.5</td>
<td>16</td>
<td>117</td>
<td>5.6</td>
<td>14</td>
<td>0.252</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>36.0</td>
<td>6.0</td>
<td>16</td>
<td>28.8</td>
<td>3.2</td>
<td>14</td>
<td>0.309</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>28.8(^a)</td>
<td>3.3</td>
<td>16</td>
<td>49.7(^b)</td>
<td>9.3</td>
<td>14</td>
<td>0.043</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>3.1</td>
<td>0.2</td>
<td>16</td>
<td>3.3</td>
<td>0.2</td>
<td>14</td>
<td>0.574</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (μmol/l)</td>
<td>0.42</td>
<td>0.03</td>
<td>15</td>
<td>0.51</td>
<td>0.04</td>
<td>12</td>
<td>0.094</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td>1.24</td>
<td>0.1</td>
<td>15</td>
<td>1.28</td>
<td>0.2</td>
<td>12</td>
<td>0.783</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>7.4(^a)</td>
<td>0.7</td>
<td>16</td>
<td>20.2(^b)</td>
<td>3.2</td>
<td>12</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T(_3) (ng/ml)</td>
<td>0.26(^a)</td>
<td>0.02</td>
<td>16</td>
<td>0.34(^b)</td>
<td>0.02</td>
<td>13</td>
<td>0.026</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T(_4) (ng/ml)</td>
<td>4.4</td>
<td>0.2</td>
<td>16</td>
<td>5.1</td>
<td>0.7</td>
<td>14</td>
<td>0.472</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; ALT, alanine aminotransferase; NEFA, non-esterified fatty acids; T\(_3\), tri-iodothyronine; T\(_4\), thyroxine.

Significant differences \((P<0.05)\) between normal weight and obese cats, resulting from repeated measures ANOVA, are indicated by different superscripts within one row. \(N<16\) caused by missing values.

The effect of BCS on glucose- and insulin metabolism, regardless of diet is shown in Table 4.4. Obese cats had higher fasting plasma glucose \((P=0.030)\), higher glucose concentration at any time during IVGTT, except for 5 and 120 minutes after glucose administration (also shown in Figure 4.2a; main effect: \(P=0.013\); time \(\times\) BCS: \(P=0.043\), and higher AUC\(_{gluc}\) \((P=0.022)\). The \(K_{gluc}\) and glucose \(t_{1/2}\) were not affected by body condition (Table 4.4). As shown in Figure 4.2b, 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 \((P=0.009)\). AUC\textsubscript{ins}, fasting SI and AUC\textsubscript{ins}/AUC\textsubscript{gluc} as well as HOMA, QUICKI and Bennett index were not affected by body condition (Table 4.4).

**Table 4.4:** Effect of body condition score on glucose- and insulin metabolism, regardless of diet, expressed as mean ± SE.

<table>
<thead>
<tr>
<th>Body condition</th>
<th>Normal weight</th>
<th></th>
<th></th>
<th>Obese</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose nadir (mmol/l)</td>
<td>4.5\textsuperscript{a}</td>
<td>0.13</td>
<td>16</td>
<td>5.1\textsuperscript{b}</td>
<td>0.13</td>
<td>14</td>
<td>0.030</td>
</tr>
<tr>
<td>AUC\textsubscript{gluc} (mmol/l/120min)</td>
<td>1247\textsuperscript{a}</td>
<td>57</td>
<td>14</td>
<td>1533\textsuperscript{b}</td>
<td>49</td>
<td>12</td>
<td>0.022</td>
</tr>
<tr>
<td>(K\textsubscript{gluc})</td>
<td>1.24</td>
<td>0.09</td>
<td>14</td>
<td>1.22</td>
<td>0.07</td>
<td>12</td>
<td>0.967</td>
</tr>
<tr>
<td>Glucose t\textsubscript{1/2} (min)</td>
<td>61.3</td>
<td>6.3</td>
<td>14</td>
<td>59.5</td>
<td>4.3</td>
<td>12</td>
<td>0.798</td>
</tr>
<tr>
<td>Insulin nadir (mU/l)</td>
<td>16.5</td>
<td>1.4</td>
<td>16</td>
<td>17.2</td>
<td>1.9</td>
<td>14</td>
<td>0.820</td>
</tr>
<tr>
<td>AUC\textsubscript{ins} (mU/L/120min)</td>
<td>4467</td>
<td>557</td>
<td>14</td>
<td>5186</td>
<td>473</td>
<td>12</td>
<td>0.745</td>
</tr>
<tr>
<td>Height 1\textsuperscript{st} insulin peak (mU/l)</td>
<td>35.4</td>
<td>3.8</td>
<td>14</td>
<td>30.5</td>
<td>4.1</td>
<td>12</td>
<td>0.239</td>
</tr>
<tr>
<td>Height 2\textsuperscript{nd} insulin peak (mU/l)</td>
<td>57.6</td>
<td>7.5</td>
<td>14</td>
<td>65.8</td>
<td>7.4</td>
<td>12</td>
<td>0.777</td>
</tr>
<tr>
<td>Time 1\textsuperscript{st} insulin peak (min)</td>
<td>8.9</td>
<td>1.3</td>
<td>14</td>
<td>11.3</td>
<td>1.1</td>
<td>12</td>
<td>0.430</td>
</tr>
<tr>
<td>Time 2\textsuperscript{nd} insulin peak (min)</td>
<td>57.9\textsuperscript{a}</td>
<td>4.1</td>
<td>14</td>
<td>78.8\textsuperscript{b}</td>
<td>6.2</td>
<td>12</td>
<td>0.009</td>
</tr>
<tr>
<td>Fasting SI (mU/mmol)</td>
<td>3.7</td>
<td>0.3</td>
<td>16</td>
<td>3.3</td>
<td>0.3</td>
<td>12</td>
<td>0.469</td>
</tr>
<tr>
<td>AUC\textsubscript{ins}/AUC\textsubscript{gluc} (mU/mmol)</td>
<td>3.6</td>
<td>0.4</td>
<td>14</td>
<td>3.4</td>
<td>0.3</td>
<td>14</td>
<td>0.683</td>
</tr>
<tr>
<td>HOMA</td>
<td>3.3</td>
<td>0.3</td>
<td>16</td>
<td>4.1</td>
<td>0.5</td>
<td>14</td>
<td>0.380</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.56</td>
<td>0.02</td>
<td>16</td>
<td>0.53</td>
<td>0.02</td>
<td>14</td>
<td>0.481</td>
</tr>
<tr>
<td>Bennett index</td>
<td>1.4</td>
<td>0.1</td>
<td>16</td>
<td>1.2</td>
<td>0.08</td>
<td>14</td>
<td>0.370</td>
</tr>
</tbody>
</table>

AUC\textsubscript{gluc}, area under the curve for glucose; \(K\textsubscript{gluc}\), glucose disappearance coefficient; Glucose t\textsubscript{1/2}, half-life for glucose disappearance between 15-90 minutes after glucose administration; AUC\textsubscript{ins}, area under the curve for insulin; SI, insulin to glucose ratio; AUC\textsubscript{ins}/AUC\textsubscript{gluc}, ratio of area under the insulin to glucose curve; HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index.

Significant differences \((P<0.05)\) between normal weight and obese cats, resulting from repeated measures ANOVA, are marked by different superscripts within one row. N<16 caused by missing values.

### 4.3.2. 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 Figure 4.2a, fasting plasma glucose concentration and plasma glucose concentrations at each other time point after glucose administration were comparable. Also the AUC\textsubscript{gluc}, K\textsubscript{gluc} and glucose t\textsubscript{1/2} remained unaffected. Similarly, fasting serum insulin concentration, AUC\textsubscript{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 (Figure 4.2b).

**Figure 4.2**: Mean plasma glucose (a) and serum insulin (b) concentrations ± SE, during IVGTT 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). Significant differences (P<0.05) between normal weight and obese cats, regardless of dietary treatment at each different time points, resulting from repeated measures ANOVA, are marked by *. 
All endocrine (leptin, T₃, T₄) and metabolic (cholesterol, NEFA, urea, creatinine, ALT) characteristics remained stable, except for serum triglycerides, which tended to be increased \((P=0.065)\) and plasma AST activity which was decreased \((P=0.025)\) when fed the P-diet compared to the C-diet. As shown in Table 4.5, plasma free carnitine concentrations did not differ among diets, as did plasma acetylcarnitine concentrations. Yet, propionyl- \((P=0.03)\) and butyrylcarnitine \((P=0.002)\) were higher when fed the P-diet. Methylmalonylcarnitine tended to be decreased \((P=0.072)\) when fed the P-diet.

### Table 4.5: Effect of adding 2.5% of a mixture of oligofructose and inulin to the control diet on carnitine metabolism (free carnitine, acetyl-, propionyl-, butyryl- and methylmalonylcarnitine), regardless of body condition, expressed as mean ± SE.

<table>
<thead>
<tr>
<th></th>
<th>C-diet</th>
<th>P-diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Free carnitine (µmol/l)</td>
<td>32.15</td>
<td>2.1</td>
</tr>
<tr>
<td>Acetylcarnitine (µmol/l)</td>
<td>5.545</td>
<td>0.29</td>
</tr>
<tr>
<td>Propionylcarnitine (µmol/l)</td>
<td>0.134ᵃ</td>
<td>0.03</td>
</tr>
<tr>
<td>Butyrylcarnitine (µmol/l)</td>
<td>0.283ᵃ</td>
<td>0.02</td>
</tr>
<tr>
<td>Methylmalonylcarnitine (µmol/l)</td>
<td>0.054</td>
<td>0.01</td>
</tr>
</tbody>
</table>

C-diet, control diet; P-diet, prebiotic diet (C-diet + 2.5% of a mixture of oligofructose and inulin).

Significant differences \((P<0.05)\) between test diets, resulting from repeated measures ANOVA are marked by different superscripts within one row.

### 4.4. 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\(_{\text{gluc}}\), 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. These findings were also concluded from previous studies in cats (Nelson et al., 1990; Hoenig et al., 2006). 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. (2002) have demonstrated a strong positively relationship between leptin and insulin resistance. The significantly higher fasting plasma T₃ concentrations in obese cats during the present trial corresponds to the significant and positive correlation of T₃ with BW, girth and BMI as observed by Ferguson et al. (2007). T₄ also correlated positively with all indices of obesity and with leptin, but not with NEFA (Ferguson et al., 2007). Still, fasting T₄ concentrations remained unchanged in the present trial. Ferguson et al. (2007) also 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 humans, hyperleptinemia was also observed to be strongly correlated with elevated serum ALT activity (Ruhl & Everhart, 2003), most probably due to the high association between elevated liver enzymes, most notably high serum ALT activity and obesity (percent body fat; obesity class) and insulin resistance (measured by hyperinsulinaemic euglycaemic clamp technique or HOMA) as observed by Vozarova et al. (2002) and Marchesini et al. (2005). Therefore, the occurrence of insulin resistance might explain the rise in plasma ALT activity observed in obese cats. In addition serum triglycerides also tended to rise in obese cats, in accordance with the results found by Hoenig et al. (2003), who studied the effect of obesity on lipid profiles in neutered cats. Hoenig et al. (2003) 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. (1985) 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 (Center et al., 1993). 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.
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 triglyceride concentrations, the activity of AST and several acylcarnitines. Serum triglyceride concentrations tended to be increased in cats supplemented with oligofructose and inulin. This finding contrasts with the results from earlier trials in rodents (Levrat et al., 1991; Delzenne et al., 1993; Fiordaliso et al., 1995; Agheli et al., 1998; Busserolles et al., 2003) and dogs (Diez et al., 1997) which revealed lower triglyceride concentrations following soluble fibre supplementation. Since BW remained unchanged during the trial, an increase in BW could not explain this observation. Nevertheless, serum triglyceride concentrations remained within the references range. When compared to other trials conducted in rats (Kok et al., 1996; Agheli et al., 1998; Busserolles et al., 2003) and dogs (Diez et al., 1997; Diez et al., 1998; Massimino et al., 1998; Hesta et al., 2001b), the amount of soluble fibre used in the present trial (0.5g/kg BW) seems rather low. Nevertheless, in human studies (Luo et al., 1996; Alles et al., 1999; Luo et al., 2000), doses of oligofructose were similar to the dose used in the present trial. In healthy human subjects, ingesting 20 g fructo-oligosaccharides (FOS)/d (0.3g/kg BW) for four weeks, FOS did not modify fasting glucose and insulin concentrations, but did lower basal hepatic glucose production (Luo et al., 1996). According to Yamashita et al. (1984) 8 g FOS/d (0.1g/kgBW) for two 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. (2001a) showed that faeces became formless and apparent digestibility of protein and fat were reduced when cats ingested more than 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. (1997) observed significantly decreased postprandial glucose concentrations after incorporation of both FOS 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 (Diez et al., 1998). 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 DP 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 humans (Jenkins et al., 1980) and dogs (Nelson et al., 1991), 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 (ME) as carbohydrates, whereas our basic diet only contained 26% ME as carbohydrates. Secondly, insulin resistance might have been triggered better by using a diet containing high amounts of fat, as demonstrated in humans and rodents (Storlien et al., 1996; Lichtenstein & Schwab, 2000) as well as in cats (Thiess et al., 2004), 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 (Chapter 3). 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 metabolism of these SCFA were observed by the higher propionyl- and butyrylcarnitine concentrations in cats when fed the P-diet. Acylcarnitine was not altered among diets. Acetyl coenzyme A (CoA) is not only generate from acetate, but also from amino acids and other fatty acids. Moreover, propionate might inhibit the rise in acylcarnitine concentrations caused by butyrate, as demonstrated by Brass & Beyerinck (1988). 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 (Wolever et al., 1989). In ruminants (Judson et al., 1968; Yost et al., 1977) and horses (Simmons & Ford, 1991), 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 humans 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 (Blair et al., 1973; Anderson & Bridges, 1984).

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.
4.5. Conclusion

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.

Acknowledgments: This study was funded by the Institute for Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). Expenses for the laboratory analyses were covered by the Beneo-Group (Tienen, Belgium), who also provided the oligofructose and inulin mixture (Beneo™ Synergy 1®). The pet food was provided by Versele-Laga (Deinze, Belgium). The authors also express their gratitude to Rebekka Hollebosch and Steven Galle for taking care of the experimental animals, Stephanie Van Weyenberg and Georgios Papadopoulos for technical assistance, Herman De Rycke for performing the food analyses, and Inge Vaesen for the plasma analyses.
Colonic propionate might act as gluconeogenic substrate in normal weight and obese cats, without affecting glucose tolerance
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\textsuperscript{3}Department of Biomedical Sciences (previously: Medical Physiology), University of Copenhagen, the Panum Institute, Copenhagen, Denmark.

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\textit{Submitted, British Journal of Nutrition}
Abstract

The effect of colonic propionate on metabolism in normal weight and obese cats was assessed. Six normal weight and six obese adult cats were fed a commercial low carbohydrate diet during the study. Following general anaesthesia, two colonic infusions were tested in a cross-over design with two adaptation periods of four weeks. The test solution contained 4 mmol sodium propionate per kg ideal body weight in a 0.2% NaCl solution. Normal saline was given as control solution. Solutions were infused into the hindgut over a 30-minute period. Central venous blood samples were obtained prior to and 5, 10, 15, 30, 45, 60 and 90 minutes after starting the colonic infusion. As the cat’s body condition did not affect evaluated parameters, all data were grouped across body condition score. Serum insulin and plasma glucose concentrations showed no differences between treatments. Insulin to glucose ratio tended to be lower ($P=0.087$) when given propionate, yet numerical differences were clinically irrelevant, suggesting no substantial effect on insulin sensitivity. Plasma propionylcarnitine concentrations rose markedly towards the end of the propionate infusion and decreased afterwards (treatment: $P<0.001$, time: $P<0.001$, treatment x time: $P<0.001$); but no simultaneous increase of methylmalonylcarnitine was detected, suggesting inhibition of gluconeogenesis from amino acids and initiation of propionate-induced gluconeogenesis. Also the tendency towards lower 3-methylglutaryl carnitine ($P=0.060$) when given propionate proposes reduced amino acid utilization as substrate for gluconeogenesis. In conclusion, colonic propionate does not affect insulin sensitivity, yet is suggested to act as gluconeogenic substrate, regardless of the cat’s body condition.
5.1. Introduction

In cats, diabetes mellitus has become a major endocrine disorder. Prahl et al. (2007) observed a tendency towards a higher incidence of this disease, most probably due to a rise in the frequency of the major predisposing factors such as obesity and physical inactivity which may induce insulin resistance (Rand et al., 2004; Slingerland et al., 2009). However, the traditional dietary treatment for feline insulin resistance (diabetes mellitus and obesity), especially the use of soluble dietary fibres, originates from extrapolation of the results from human and canine studies. This dietary intervention also disregards the fact that cats are true carnivores relying on nutrients in animal tissues to meet their specific nutritional requirements, and that cats are metabolically adapted to lower glucose utilization and a higher protein metabolism (Zoran, 2002).

In several species (humans (Yamashita et al., 1984; Luo et al., 1996; Alles et al., 1999; Luo et al., 2000); rodents (Kok et al., 1996; Agheli et al., 1998; Busserolles et al., 2003); dogs (Diez et al., 1997; Diez et al., 1998; Massimino et al., 1998; Strickling et al., 2000; Hesta et al., 2001b; Respondek et al., 2008)), dietary fermentable fibre results in a better glucose tolerance and improved insulin sensitivity. However, our previous study could not demonstrate an impact of 2.5% of a mixture of oligofructose and inulin on glucose and insulin response during intravenous glucose tolerance tests in both normal weight and obese cats (Chapter 4). Therefore, we hypothesize that typical features of carnivore metabolism reduce or inhibit this effect on glucose tolerance. Besides the absence of effects on glucose tolerance, our study also revealed increased plasma propionylcarnitine concentrations, suggesting colonic fermentation and propionate absorption (Chapter 4). It is known from studies in isolated rat hepatocytes that several short chain fatty acids (SCFA), especially propionate, produced by bacterial fermentation of non-digestible carbohydrates in the hindgut, inhibit hepatic gluconeogenesis from pyruvate (Chan & Freedland, 1972; Blair et al., 1973; Anderson & Bridges, 1984) and enhance hepatic glycolysis (Blair et al., 1973; Anderson & Bridges, 1984). In cats, fermentable fibre supplementation did not only increase propionylcarnitine concentrations, but also decreased methylmalonylcarnitine concentrations and reduced plasma aspartate aminotransferase activity, all indicating reduced utilization of amino acids as substrate for gluconeogenesis by using propionate instead (Chapter 4).
In the present trial, colonic infusion of propionate was used in order to state the absence of effect of fermentable fibres on glucose tolerance in cats, being true carnivores, under controlled conditions and to clarify differences between normal weight and obese cats. The aim was also to elucidate metabolic effects of fermentation products, especially propionate, in healthy cats. Therefore, the acylcarnitine profile, being a reflection of metabolites available for citric acid cycle (Bremer, 1983) and selected parameters of carbohydrate (plasma glucose, glucagon-like peptide-1 (GLP-1) and serum insulin concentrations), protein (plasma urea and amino acid concentrations) and lipid metabolism (plasma non-esterified fatty acids (NEFA), cholesterol, and triglyceride concentrations) were scrutinized in the present study.

**5.2. Materials and methods**

**5.2.1. Animals and housing**

Twelve domestic short-hair cats, six females and six males, entered the study. All male and female cats were spayed. All cats were adults, aged between 3.5 and 6 years. All cats were healthy apart from obesity in three male and three female cats, and were not given any medication at the time of the study; none had prior medical problems. During the trial, cats were housed in their usual group cages.

**5.2.2. Diets and feeding**

For four weeks preceding the trial and during the trial, all cats were fed the same commercial diet (Hill’s Prescription Diet® Feline m/d®, Hill’s Pet Nutrition Inc., Topeka, Kansas, USA). The composition of the diet is shown in Table 5.1.

Cats were fed in group and had free access to food at all times. The amount of food calculated for group feeding corresponded with the maintenance energy requirement (lean cats: 418 kJ/kg$^{0.67}$, obese cats: 544 kJ/kg$^{0.4}$) (National Research Council, 2006). Body weight (BW) was determined weekly in order to check adequate individual food intake. Fresh water was available at all times.
Table 5.1: Composition of the commercial diet, Hill’s Prescription Diet® Feline m/d® dry food.

<table>
<thead>
<tr>
<th>Nutrients on DM (%)</th>
<th>Hill’s feline m/d®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>51.1</td>
</tr>
<tr>
<td>Crude fat</td>
<td>22.0</td>
</tr>
<tr>
<td>NFE</td>
<td>15.0</td>
</tr>
<tr>
<td>Crude ash</td>
<td>5.9</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>6.0</td>
</tr>
<tr>
<td>TDF</td>
<td>6.8</td>
</tr>
<tr>
<td>Soluble fibre</td>
<td>0.2</td>
</tr>
<tr>
<td>Insoluble fibre</td>
<td>6.6</td>
</tr>
<tr>
<td>Starch</td>
<td>8.3</td>
</tr>
<tr>
<td>Sugars</td>
<td>0.2</td>
</tr>
<tr>
<td>ME (kJ/kg as fed)</td>
<td>1644</td>
</tr>
</tbody>
</table>

DM, dry matter; NFE, nitrogen-free extract; TDF, total dietary fibre; ME, metabolisable energy.

Ingredients: dehydrated chicken and turkey protein, maize gluten meal, animal fat, dehydrated beef and pork greaves, cellulose, rice protein concentrate, digest, L-lysine hydrochloride, potassium chloride, calcium sulfate, L-carnitine, taurine, ground rice, salt, vitamins and trace elements. Naturally preserved with mixed tocoferols, citric acid and rosemary extract.

1Derived by subtracting crude protein, crude fat, NFE and crude fibre from the DM content.

5.2.3. Experimental design

Prior to being included into the study, the cats were subjected to a physical examination, after an overnight unfed period, a blood sample was drawn from the jugular vein for complete blood count and serum biochemistry. Measurements of BW, body condition score (BCS), body mass index (BMI, expressed in kg/m²) and girth circumference (Girth) were performed. BCS was determined using a five-point body condition scoring system (National Research Council, 2006). Six non-obese cats with a score of 3/5 to 3.5/5 (mean BW: 4.8 kg; range: 3.9 to 5.5 kg) and six obese cats with a score of 5/5 (mean BW: 7.4 kg; range: 5.9 kg to 9.9 kg) were used in the study (Table 5.2). The BMI was calculated as described by Hoenig et al. (2003) and girth was measured right behind the last rib (Hoenig, personal communication). All measurements were performed under general anaesthesia by the same person to minimize variability. During the trial, BW and BCS were recorded once a week.
Cats were randomly allotted to two groups, given that each group contained three obese and three normal weight cats. Each group of cats was randomly assigned to one of two treatments (control (C-infusion) and propionate infusion (P-infusion)). Treatments were tested in a cross-over design, at intervals of four weeks.

Table 5.2: Body condition score, body weight, body mass index and girth circumference in six normal weight and six obese neutered adult cats expressed as mean ± SE.

<table>
<thead>
<tr>
<th></th>
<th>Normal Weight</th>
<th>Obese</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>BCS</td>
<td>3.3</td>
<td>0.1</td>
<td>5.0</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>4.8</td>
<td>0.3</td>
<td>7.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>42.4</td>
<td>2.1</td>
<td>62.8</td>
</tr>
<tr>
<td>Girth (cm)</td>
<td>40.5</td>
<td>1.2</td>
<td>51.1</td>
</tr>
</tbody>
</table>

BCS, body condition score; BW, body weight; BMI, body mass index; Girth, girth circumference.

*P-values of statistical difference between normal weight and obese cats, resulting from one way ANOVA.

5.2.4. Colonic infusion

In order to evacuate the hind gut, food was withheld for 24 hours before the study; cats were given a laxative (Microlax®, Pfizer, Brussels, Belgium) rectally twice daily at the day before the treatment and a copious warm water enema was performed just before the treatment. On the day of the study, all cats were given a colonic infusion (10 ml/kg ideal bodyweight (idBW)). As P-infusion, cats were given 0.4 g sodium propionate (Fagron n.v., Waregem, Belgium) per kg idBW (4 mmol/kg idBW) in a 0.2% sodium chloride (NaCl)-solution (911 mOsmol/l). The C-infusion consisted of normal saline (0.9% NaCl; 310 mOsmol/l).

The solutions were prepared on the morning of the experiment and were heated to body temperature before infusion. To infuse the solutions into the colon, a 35-cm plastic tube was inserted into the rectum and colon. The other end was connected to a fluid-dispensing system and test solutions were injected over 30 min (Figure 5.1).
Chapter 5
Colonic propionate & feline metabolism

Figure 5.1: During the colonic infusion, cats were generally anesthetized. To infuse the solutions into the colon, a 35-cm plastic tube was inserted into the rectum and colon. The other end was connected to a fluid-dispensing system and test solutions were injected over 30 min.

5.2.5. Jugular catheter placement and blood sampling

On the morning of the study, just before the colonic infusion, an intravenous catheter (20 G, 8 cm, Leaderflex®, Vygon n.v., Brussels, Belgium) was placed in a jugular vein to allow repeated blood sampling. Catheters were flushed with 1 ml heparinized saline (50 IU of heparin/ml in saline solution) to maintain patency (Appleton et al., 2001a). Amoxicillin (Clamoxyl LA®, GlaxoSmithKline n.v., Genval, Belgium) 15 mg/kg BW subcutaneously, was administered once, at the time of catheter placement. Blood samples were collected from the jugular vein as described by Martin and Rand (1999), prior to (0 minutes) and 5, 15, 30, 45, 60 and 90 minutes after starting the infusions.

At each time point, blood samples were collected in vacutainer tubes containing lithium heparin for determination of plasma glucose, cholesterol, triglycerides, urea concentrations, amino acid and acylcarnitine profile and in serum vacutainer tubes to determine serum insulin and NEFA concentrations. Lithium heparin tubes were stored immediately at 4°C until centrifugation. Plasma and serum were obtained after centrifugation and stored at -20°C until assayed. For determination of GLP-1, chilled tubes containing EDTA and 20 µl dipeptidylpeptidase 4 inhibitor (Biotrend GmbH, Cologne, Germany) were used. Samples were stored at 4°C until centrifugation within 30 min after collection.
5.2.6. General anaesthesia

During the whole procedure; namely the warm water enema, jugular catheter placement, colonic infusion and blood sampling, cats were anaesthetized with isoflurane in 100% oxygen using a paediatric circle system (Spiromat 656, Dräger, Zoetermeer, The Netherlands). Premedication consisted of buprenorphin (Temgesic®, Schering-Plough n.v., Heist-Op-Den-Berg, Belgium) 10 μg/kg intravenously to supply analgesia. Propofol (Propovet®, Abbott Lab, Leuven, Belgium) 4-8 mg/kg BW intravenously given to effect was used for induction. During anaesthesia, an infusion with NaCl 0.9% at a dose rate of 10 ml/kg/h was administered to compensate for blood loss and anaesthetic-induced hypotension. Standard monitoring of vital signs was applied throughout anaesthesia (pulse-oximetry, capnography, electrocardiogram, non-invasive blood pressure).

The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2007/110) and was in accordance with institutional and national guidelines for the care and use of animals.

5.2.7. Analytical methods

Plasma glucose (VetTest 9820369), triglyceride (VetTest 9820377), total cholesterol (VetTest 982065) and urea (VetTest 982062) concentrations were determined using the VetTest 8008 analyser (Idexx Laboratories Europe B.V., Hoofddorp, the Netherlands). The apparatus is based on dry chemical technology and colorimetric reaction. Sample analysis is carried out on selective testing discs (Idexx Laboratories Europe B.V., Hoofddorp, the Netherlands) by means of laser reading the bar codes. Serum NEFA concentrations were determined enzymatically using a commercially available method (NEFA, Randox Laboratories Ltd., Crumlin, UK) on RX Daytona (Randox, UK). Quantitative electrospray tandem mass spectrometry was used to determine free carnitine, acylcarnitine (acetyl-, propionyl-, methylmalonyl-, 3-methylglutaryl-carnitine) and amino acid (valine, leucine, methionine, phenylalanine, tyrosine, glycine, alanine) profile as described by Vreken et al. (1999) and Rizzo et al. (2003). GLP-1 immunoreactivity was measured as described by Ørskov et al. (1994) using synthetic proglucagon (78-107) amide (GLP-1 (7-36)amide) for standards and 125I-labeled tracers and antiserum (code no. 89390) which was raised in rabbits against synthetic proglucagon (97-107) amide (GLP-1 (26-36)
amide), coupled to bovine serum albumin with carbodiimide. Serum insulin concentrations were measured by a commercially available immunoradiometric assay (IRMA) test kit (insulin IRMA Ref 5251, Biosource Europe S.A. Nivelles, Belgium) as described by Slingerland et al. (2007). To estimate insulin sensitivity, the insulin to glucose ratio (SI) was calculated prior to and at each time point after starting the colonic infusion (Appleton et al., 2005).

5.2.8. Statistical analyses

Statistical analyses were performed using Superior Performing Software Systems (SPSS) version 16 (SPSS Inc., Chicago, Illinois, USA). At the beginning of the trial, the effect of body condition was tested for BW, BCS, BMI and girth by one way ANOVA. Data were statistically analyzed by repeated measures ANOVA with treatment and time as within subject factors and BCS as between subject factor. Interactions between BCS and treatment were also evaluated. Since BCS did not affect the evaluated parameters except for plasma triglyceride and urea concentrations as well as no interactions between treatment and BCS were observed, all data were grouped across BCS and statistics were performed by repeated measures ANOVA with treatment and time as within subject factors, regardless of BCS. A paired sampled T-test was performed at each different time point as post hoc test whenever the treatment effect was significant. Statistical significance was accepted at $P<0.05$. All data are expressed as mean ± SE.

5.3. Results

5.3.1. Effect of body condition

At the start of the trial, BCS, BMI, Girth ($P<0.001$ for all three) and BW ($P=0.003$) differed significantly among normal weight and obese cats (Table 5.2). During the trial, BW and BCS remained stable in all cats (data not shown). BCS did not affect plasma glucose, total cholesterol, NEFA, GLP-1 and serum insulin concentrations prior to and after starting the infusions. Plasma amino acid (valine, leucine, methionine, phenylalanine, tyrosine, glycine, alanine) and acylcarnitine (free carnitine, acetyl-, propionyl-, methylmalonyl- and 3-methylglutarylcarmitine) concentrations also remained the same. Nevertheless, serum triglycerides concentrations were significantly decreased ($P=0.007$) and serum urea concentrations tended ($P=0.054$) to be higher.
in obese cats when compared to normal weight cats, regardless of treatment (data not shown). Fasting SI did not differ significantly among normal weight and obese cats.

### 5.3.2. Effect of colonic propionate infusion

Serum insulin concentrations varied in time ($P=0.008$) for both infusions, but no differences were observed among treatments nor interactions between treatment and time, as shown in Figure 5.2a. Plasma glucose concentrations showed neither differences over time, nor during or after infusion with propionate or control, and no differences were noted between treatments (Figure 5.2b). SI also tended to change over time ($P=0.081$) and tended to decrease ($P=0.087$) in cats given the P-infusion (Figure 5.2c).

Serum NEFA concentrations fell over time in all cats, regardless of treatment ($P<0.001$), showed no differences among treatments, but a tendency towards a treatment-time interaction could be observed ($P=0.079$). Yet, serum NEFA concentrations at all specific time points did not differ significantly (Figure 5.3a). Similarly plasma cholesterol (Figure 5.3b) and triglyceride (Figure 5.3c) concentrations decreased over time ($P<0.001$ and $P=0.010$, respectively), but again no treatment effect was observed. Plasma urea concentrations tended to change over time ($P=0.086$), but no treatment effect was noted (data not shown). Plasma amino acid concentrations (valine, leucine, methionine, phenylalanine, tyrosine, glycine, alanine) rose over time ($P<0.001$), but were similar for both infusions (data not shown).

Yet, acylcarnitine profile demonstrated some clear differences between treatments, as shown in Figure 5.4. Plasma free carnitine concentrations (Figure 5.4a) did not change over time, but tended to be higher ($P=0.082$) in cats given colonic propionate. A treatment-time interaction was also observed ($P=0.050$). Plasma acetylcarnitine concentrations (Figure 5.4b) declined over time ($P<0.001$) and showed no overall effect of treatment, yet an interaction between treatment and time was detected ($P=0.005$). Numerically lower plasma acetylcarnitine concentrations were observed, especially 60 and 90 minutes after starting the P-infusion. As shown in Figure 5.4c, plasma propionylcarnitine concentrations increased during P-infusion, reaching the highest concentration 30 minutes after starting the infusion. After ending the P-infusion, plasma propionylcarnitine concentrations fell, but did not reach basal values 90 minutes after starting the infusion (time: $P<0.001$, treatment: $P<0.001$, treatment $\times$ time: $P<0.001$). During and following the C-infusion, plasma propionylcarnitine concentrations remained stable at baseline.
Figure 5.2: Mean serum insulin (a) and plasma glucose (b) concentrations and mean insulin to glucose ratio (SI) (c) ± SE in 12 cats before and after starting a colonic control (C-) and propionate (P-) infusion. Arrows represent start and end of colonic infusion. P-values resulting from repeated measures ANOVA: Insulin: treatment effect: $P=0.127$, time effect: $P=0.008$, treatment × time: $P=0.907$; glucose: $P=0.883$, $P=0.796$ and $P=0.266$; SI: $P=0.087$, $P=0.081$ and $P=0.344$ respectively.
Figure 5.3: Mean serum non-esterified fatty acids (NEFA) (a), plasma total cholesterol (b) and triglycerides (c) concentrations ± SE in 12 cats before and after starting a colonic control (C-) and propionate (P-)infusion. Arrows represent start and end of colonic infusion. P-values resulting from repeated measures ANOVA: NEFA: treatment effect: $P=0.624$, time effect: $P<0.001$, treatment $\times$ time: $P=0.079$; cholesterol: $P=0.705$, $P<0.001$ and $P=0.396$; triglycerides: $P=0.685$, $P=0.010$ and $P=0.818$ respectively.
Chapter 5
Colonic propionate & feline metabolism

(a) Free carnitine (µmol/l)

(b) Acetyl carnitine (µmol/l)

(c) Propionyl carnitine (µmol/l)

(d) Methyl malonyl carnitine (µmol/l)

- C-infusion
- P-infusion
concentrations. Figure 5.4d shows plasma methylmalonylcarnitine concentrations during and after P- and C-infusion, demonstrating no main time or treatment effect, yet an interaction between treatment and time tended to exist (P=0.057). Especially 30 minutes after starting the P-infusion, plasma methylmalonylcarnitine concentrations were numerically higher. Plasma 3-methylglutaryl-carnitine concentrations (Figure 5.4e) did not change over time, but tended to be lower when given propionate (P=0.060). A relevant numerical depletion could be detected 60 and 90 minutes after starting the colonic P-infusion in comparison to the control. At last, plasma GLP-1 concentrations showed differences neither over time nor among treatments (data not shown).
5.4. Discussion

From a previous trial, it was suggested that in true carnivorous species, such as cats, soluble dietary fibre did not influence glucose tolerance and insulin sensitivity, but did alter glucose and amino acid metabolism through colonic fermentation (Chapter 4). Therefore, it was hypothesized that fermentation products, especially propionate do not have a major influence on glucose tolerance and insulin sensitivity, but rather act upon glucose metabolism through metabolic changes, resulting in amino acid sparing conditions.

In the present study, colonic infusion of propionate had as expected no major impact on plasma glucose and serum insulin concentrations in normal weight and obese cats. This absence of a plasma glucose reduction can be due to endogenous glucose production from propionate. Propionate is shown to act as primary gluconeogenic substrate in ruminants (Judson et al., 1968; Yost et al., 1977) and horses (Simmons & Ford, 1991), but also in cats utilization of propionate for gluconeogenesis was recently demonstrated by Kley et al. (2009). The increasing plasma propionylcarnitine concentrations during propionate infusion indicate that propionate was indeed absorbed from the feline colon. It can however be speculated that propionate entered the citric acid cycle at the level of succinyl CoA, being converted into glucose via oxaloacetate as observed in other less carnivorous species (Wolever et al., 1989; Rémésy et al., 2004). This assumes increasing glucose concentrations which were not observed. Reduction of amino acid utilization, as propionate is used as gluconeogenic substrate itself, might be a possible reason, yet, enhanced insulin sensitivity might also explain this finding. Despite the absence of effects on glucose and insulin concentrations, a tendency towards decreased SI was observed, suggesting enhanced insulin sensitivity when given a colonic propionate infusion, similarly in normal weight and obese cats. This can be caused by a direct effect on hepatic glucose metabolism (inhibiting gluconeogenesis from pyruvate and stimulating glycolysis) (Chan & Freedland, 1972; Blair et al., 1973; Anderson & Bridges, 1984) and/or an indirect effect via lowering plasma fatty acid concentrations (Roberfroid & Delzenne, 1998; Delarue & Magnan, 2007). The lack of a propionate-induced decrease in NEFA during the present trial does not support the latter hypothesis. Yet, numerical differences were clinically irrelevant, suggesting no substantial effect on insulin resistance. Some authors also suggest that the beneficial effects on glucose and insulin metabolism caused by dietary fermentable fibre and SCFA might be mediated via production of GLP-1 by enteroendocrine L-cells (Drucker, 2001; Drucker, 2002; Delzenne et al., 2005; Delzenne et al., 2007). The absence of differences in plasma GLP-1
concentrations among treatments in normal weight and obese cats in the present study, can also corroborate the absence of effect on insulin resistance.

It is known from studies in isolated rat hepatocytes that propionate affects metabolic pathways. Propionate decreases hepatic gluconeogenesis from pyruvate; directly by inhibition of pyruvate carboxylase via its specific intermediaries, propionyl coenzyme A (CoA) and methyl-malonyl CoA (Blair et al., 1973; Anderson & Bridges, 1984). Propionyl CoA was indeed increased during propionate infusion in the present trial, since a rise of its concurrent acylcarnitine, namely propionylcarnitine, was observed. Methylmalonylcarnitine and concurrent methylmalonyl CoA were expected to raise during and following the propionate infusion. Yet, no increase could be detected, except for a relevant numerical increase at the end of the propionate infusion, when the plasma propionylcarnitine concentrations were at most. The fact that methylmalonylcarnitine is also generated through amino acid catabolism as a metabolite of valine, methionine and isoleucine, might explain the absence of increased plasma methylmalonyl-carnitine concentrations at all times during and following the propionate infusion, suggesting amino acid sparing conditions. Inhibition of pyruvate carboxylase also occurs indirectly by depleting acetyl CoA, a specific allosteric activator of this enzyme (Blair et al., 1973; Anderson & Bridges, 1984). In the present trial, an interaction between treatment and time existed for acetylcarnitine, yet plasma acetylcarnitine concentrations were only numerically lower 30 and 60 minutes after ending the propionate infusion, indicating depletion of acetyl CoA, diminution of pyruvate carboxylase activity and inhibited gluconeogenesis from pyruvate and thus from amino acids. The tendency towards lower 3-methylglutarylarnitine when given propionate and the numerically decreased plasma 3-methylglutarylcarnitine concentrations 30 and 60 minutes after ending the propionate infusion, also proposed diminished amino acid induced gluconeogenesis, since this acylcarnitine is known to be a specific metabolite of branched chain amino acid catabolism, especially leucine (Roe et al., 1986). However, decreased plasma urea and increased amino acid concentrations were not noted during or following propionate administration when compared to the control infusion. Yet, all investigated plasma amino acid concentrations (valine, leucine, methionine, phenylalanine, tyrosine, glycine, alanine) rose over time regardless of treatment, most probably due to increased release from protein break down because cats were fasted for 24 hours and no glucose challenge was performed. Hence, no dietary source of glucose was available, and thus gluconeogenesis had to originate from tissue protein mobilization. In presence of propionate, gluconeogenesis is suggested to originate from propionate and amino
acids are assumed to be spared and used in other metabolic processes such as tissue preservation, immunity etc. by inhibition of protein catabolism.

Since obese cats are at higher risk for insulin resistance, a more pronounced effect of colonic propionate infusion was expected. Nevertheless, no effects of body condition nor interactions between treatment and BCS were observed. The absence of significant differences of colonic propionate infusion among different BCS might be explained by the absence of insulin resistance according to Appleton et al. (2005) in obese cats during the present study. Insulin resistance was estimated by fasting serum insulin concentrations and fasting SI, since Appleton et al. (2005) demonstrated a high correlation between the results obtained by the frequently sampled intravenous glucose tolerance test with minimal model analysis and fasting insulin as well as fasting SI, especially in obese cats. Neither parameter was significantly increased in obese cats during the present trial. Though, neither glucose tolerance tests, nor insulin sensitivity tests were performed. On the other hand, the same cats were used in a previous trial (Chapter 4), having the same BCS and a slightly higher BW. At that time, fasting serum insulin concentrations were also not significantly higher, but fasting plasma glucose concentrations and area under the glucose curve were significantly increased and the second insulin peak during intravenous glucose tolerance test was delayed, which may indicate impaired glucose tolerance and higher insulin resistance in obese cats. As BW was slightly increased compared to the previous trial (Chapter 4), insulin sensitivity should be increased accordingly (Appleton et al., 2001b). Yet, basal insulin and fasting SI1 showed no differences among normal weight and obese cats in the present as well as the previous trial (Chapter 4). Therefore, the use of these indicators for insulin sensitivity has to be questioned. Plasma NEFA concentrations were not elevated in obese cats, in contrast to the observations of Ferguson et al. (2007). In addition, plasma triglyceride concentrations tended to be higher in obese cats when compared to normal weight cats. Both the data on NEFA and on triglycerides are in accordance with an earlier trial on prebiotics in obese and lean cats (Chapter 4). Hoenig et al. (2003) noted a significant rise in plasma triglycerides and plasma cholesterol concentrations in obese neutered cats. Yet, elevated plasma cholesterol concentrations could not be demonstrated from the present trial. The reduced serum urea concentrations in obese cats compared to normal weight cats most probably indicates compromised urea cycle function as observed in obese rats (Barber et al., 1985) and are again in accordance with the results from the previous trial (Chapter 4).
5.5. Conclusion

In conclusion, single colonic infusion of propionate has no direct effect on glucose and insulin metabolism in healthy normal weight and obese cats, yet propionate is suggested to act as a gluconeogenic substrate itself, regardless of the cat’s body condition. These findings confirm the earlier observation that dietary prebiotics fail to affect insulin sensitivity, in healthy normal weight and obese cats, but have an indirect impact on glucose metabolism via propionate (Chapter 4). As propionate is suggested to act as gluconeogenic substrate, inhibition of gluconeogenesis from pyruvate and provocation of amino acid sparing conditions are assumed. Nevertheless, further research using stable isotope techniques is warranted to confirm these effects.

Acknowledgments: This study was funded by the Institute for Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). The authors gratefully acknowledge Rebekka Hollebosch and Steven Galle for animal care taking, Jo-Ann Vandermeiren and Lies Van Cleemput for technical assistance, Marleen Van paemel for sample handling and Inge Vaesen for the plasma analyses.
Chapter 6

Intestinal fermentation diminishes postprandial amino acid induced gluconeogenesis in a true carnivore: the domestic cat (*Felis catus*)
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\textit{Submitted, British Journal of Nutrition}
Abstract

The postprandial protein sparing effect of adding oligofructose and inulin to a high protein diet in healthy cats was assessed. Two diets were tested in a cross-over design: a high fermentable fibre (HFF) diet, being a commercial high protein cat food supplemented with 4% oligofructose and inulin on dry matter (DM) basis and a low fermentable fibre (LFF) diet, which was the same commercial diet supplemented with 4% cellulose on dry matter basis. Eight healthy adult cats were randomly allotted to each of two diets at intervals of 4 weeks. At the end of each testing period, meal response tests were performed. Blood samples were obtained before and 60, 90, 120, 150, 180, 210, 240, 270 and 330 minutes after starting the meal. Propionylcarnitine concentrations were increased at almost every time point ($P=0.015$), and its area under the curve (AUC) was elevated when fed the HFF diet ($P=0.013$), revealing a more pronounced production and absorption of propionate and suggesting increased metabolization of propionate. Despite the increased propionate metabolism, methylmalonylcarnitine concentrations and concurrent AUC were not increased, indicating reduced amino acid catabolism, when fed the HFF diet. Feeding the HFF diet also reduced 3-methylglutaryl carnitine concentrations ($P=0.026$) and AUC ($P=0.028$), thus confirming diminished amino acid-induced gluconeogenesis as well. In conclusion, in healthy cats, oligofructose and inulin added to a high protein diet were suggested to reduce postprandial amino acid-induced gluconeogenesis, by substitution with propionate.
6.1. Introduction

In all monogastric mammalian species so far studied, dietary soluble fibres escape digestion in the small intestine and are broken down in the large bowel by anaerobic bacteria. Yet, compared to dogs and human beings, healthy cats, being strict carnivorous, carry higher numbers of bacteria in the proximal part of the small intestine, indicating the existence of microbial fermentation in the small intestine as well (Johnston et al., 1993; Papasouliotis et al., 1998; Sparkes et al., 1998a). This fermentation, leads to production short chain fatty acids (SCFA), mainly acetate, propionate, butyrate and lactate, along with gases (Cummings et al., 1987; Roberfroid & Delzenne, 1998). SCFA are promptly absorbed from the colonic lumen. Whereas butyrate is to a large extent metabolized by colonocytes, propionate and acetate reach the liver via the portal vein (Engelhardt, 2004; Rémésy et al., 2004). Hepatic metabolism of propionate contributes, after entering the citric acid cycle at the level of succinyl coenzyme A (CoA), to the synthesis of oxaloacetate, which may enter the gluconeogenic pathway (Rémésy et al., 2004). Therefore, it is reasonable that whenever this pathway is active in liver cells, propionate, when available, may interfere with the utilization of various gluconeogenic substrates, such as lactate and amino acids (Blair et al., 1973; Anderson & Bridges, 1984; Petitet et al., 1998). Although the gluconeogenic pathway is only poorly active in fed monogastric species such as dogs and omnivores, the feline liver, is most likely to produce glucose by gluconeogenesis rather than to remove glucose rapidly from the blood, as shown by the high activity of rate limiting enzymes of gluconeogenesis such as pyruvate carboxylase, fructose-1,6-biphosphatase and glucose-6-phosphatase (Washizu et al., 1999; Tanaka et al., 2005) and minimal to absent hepatic glucokinase activity (Ballard, 1965; Washizu et al., 1999; Tanaka et al., 2005), as well as considerably low D-glucose transport activities in feline hepatocytes (Arai et al., 1992) and minimal activity of hepatic glycogen synthetase (Ballard, 1965).

Two previous trials suggested that soluble dietary fibres and propionate evoke amino acids sparing conditions in domestic cats as oligofructose and inulin supplementation did not only increase fasting plasma propionylcarnitine concentrations, but also reduced fasting plasma methylmalonylcarnitine concentrations and aspartate aminotransferase activity (Chapter 4). Plasma propionylcarnitine concentrations were also raised, still, plasma acetylcarnitine and 3-methylglutarylcaritinie concentrations were reduced following colonic propionate infusion (Chapter 5). Therefore, soluble dietary fibre and especially fermentation products such as propionate were postulated to reduce utilization of amino acids as substrate for gluconeogenesis.
by using propionate instead. This might reduce protein mobilization and stimulate use of amino acids in other metabolic processes such as tissue preservation and immunity. Nevertheless, in both earlier studies, cats were fasted and no dietary energy source was available, and thus gluconeogenesis had to originate from tissue protein mobilization.

Therefore, the aim of the present trial was to evaluate postprandial metabolic effects and especially a possible protein sparing effect of adding soluble dietary fibre to a high protein diet in healthy cats. Hence, the acylcarnitine profile, being a reflection of metabolites available for citric acid cycle (Bremer, 1983) and selected parameters of carbohydrate (plasma glucose concentrations) and protein metabolism (plasma urea and amino acid concentrations) were scrutinized before and after feeding.

### 6.2. Materials and methods

#### 6.2.1. Animals and housing

Eight domestic short-hair cats, four females and four males, entered the study. All male and female cats were spayed. All cats were adults and aged between 2 and 7 years. All cats were healthy and were not given any medication at the time of the study; none had prior medical problems. During the trial, cats were housed in their usual group cages, had no access to food, but water was available ad libitum. Two hours a day, at 7.00 a.m. and 7.00 p.m., cats were allowed to eat in individual indoor cages. In order to collect blood and faeces samples, cats were housed individually for ten days at the end of each testing period.

#### 6.2.2. Diets and feeding

A commercial canned cat food (Hill’s Prescription Diet® a/d®, Hill’s Pet Nutrition Inc., Topeka, Kansas, USA), containing high concentrations of crude protein and fat, but low concentrations of carbohydrates and no prebiotics and other soluble or insoluble fibres added, was used as basis to formulate both test diets. To make the high fermentable fibre (HFF) diet, the commercial diet was supplemented with 4 % oligofructose and inulin on dry matter (DM) basis. Therefore Orafti HSI® (Beneo-Group, Tienen, Belgium) containing 86% of oligofructose and inulin and 14% of a mixture of fructose, glucose and sucrose, was used. The mean total number of fructose or
glucose units (degree of polymerisation; DP) of this soluble fibre mixture was less than ten. To
make the low fermentable fibre (LFF) diet, a mixture of 86% cellulose (Arbocel© BWW 40, J.
Rettenmaier & Söhne, Rosenberg, Germany) and 14% of a mixture of fructose, glucose and
sucrose was added, resulting in a 4% supplementation of cellulose on DM basis. The proximate
analysis of the two test diets is shown in Table 6.1. Neutral detergent fibre (NDF) was
determined as described by Van Soest (1991).

Table 6.1: Nutrient composition of the low fermentable fibre and high fermentable
fibre diet.

<table>
<thead>
<tr>
<th>Nutrients on DM (%) (analyzed)</th>
<th>LFF</th>
<th>HFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>38.9</td>
<td>39.0</td>
</tr>
<tr>
<td>Ether extract</td>
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<td>28.3</td>
</tr>
<tr>
<td>Crude ash</td>
<td>6.7</td>
<td>6.6</td>
</tr>
<tr>
<td>Crude fibre</td>
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</tr>
<tr>
<td>Starch</td>
<td>12.4</td>
<td>12.7</td>
</tr>
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<td>Sugars</td>
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</tr>
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<td>NFE1</td>
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</tr>
<tr>
<td>NSP2</td>
<td>10.1</td>
<td>11.0</td>
</tr>
<tr>
<td>NDF</td>
<td>5.0</td>
<td>1.2</td>
</tr>
<tr>
<td>ME (kJ/100 g as fed)³</td>
<td>439</td>
<td>448</td>
</tr>
</tbody>
</table>

LFF, low fermentable fibre; HFF, high fermentable fibre; DM, dry matter; NFE, nitrogen-free extract; NSP, non-starch polysaccharides; NDF, neutral detergent fibre; ME, metabolisable energy.

Ingredients: Hill’s Prescription Diet® a/d®: pork liver, chicken, corn flour, pork
meat, fish oil, hydrolyzed protein, potassium chloride, taurine, magnesium oxide,
vitamins and trace elements; LFF diet: Hill’s a/d® + cellulose mixture, consisting of
86% Arbocel® BWW 40 and 14% mixture of glucose, fructose and sucrose, in
order to add 4% cellulose on DM; HFF diet: Hill’s a/d® + Orafti® HSI, consisting
of 86% oligofructose and inulin and 14% mixture of glucose, fructose and sucrose,
in order to add 4% oligofructose and inulin on DM.

1Derived by subtracting crude protein, ether extract, crude ash and crude fibre
content from the DM content.

2Derived by subtracting starch and sugars content from the NFE content.

3Calculated: 15 × crude protein + 36 × ether extract + 15 × (starch + sugars).

Cats were offered two meals daily (7.00 a.m. and 7.00 p.m.). The daily amount of food given
corresponded with the maintenance energy requirement (418 kJ/kg^{0.67}) (National Research
Council, 2006) and was adapted to maintain a constant body weight. Fresh water was available at
all times.
6.2.3. Experimental design

Prior to entering the study, the cats were subjected to a physical examination; after an overnight fast, a blood sample was drawn from the jugular vein for complete blood count and serum biochemistry. Measurements of body weight (BW) and body condition score (BCS) were performed. BCS was determined using a five-point body condition scoring system (National Research Council, 2006). Non-obese cats with a mean BW of 4.5 ± 0.3 kg and a BCS 3/5 to 3.5/5 were used in the study.

Cats were randomly divided into two groups. Each group of cats was randomly assigned to each of two diets (LFF and HFF diet) in a random order at intervals of four weeks. This way, diets were examined in a cross-over design. Food intake was measured daily throughout the study. BW and BCS were recorded twice weekly.

6.2.4. Jugular catheter placement and blood sampling

To determine the postprandial metabolic effects of dietary soluble fibre, a meal response test was performed in each cat at the end of each testing period. Hence, to allow blood sampling, a central venous catheter was placed into a jugular vein. At least 20 hours prior to the blood sampling procedure, after an overnight fast, cats were anesthetized with buprenorphine (Temgesic®, Schering-Plough n.v., Heist-Op-Den-Berg, Belgium) 10 µg/kg intravenously, followed by propofol (Propovet®, Abbott Lab, Leuven, Belgium), 6-7 mg/kg to effect, intravenously, 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. Amoxicillin (Clamoxyl LA®, GlaxoSmithKline n.v., Genval, Belgium) 15 mg/kg subcutaneously, was administered once at the time of catheter placement. The meal response test was performed between 6.30 a.m. and 1.00 p.m. During the meal response test, cats were allowed to eat for one hour (7.00 until 8.00 a.m.). Blood samples were collected from the jugular catheter as described by Martin and Rand (1999), prior to the meal (6.30 a.m.), and 60, 90, 120, 150, 180, 210 240, 270 and 330 minutes after starting the meal. Whole blood was collected in vacutainer tubes containing lithium heparin for determination of plasma glucose, and urea concentrations and for determination of the acylcarnitine and amino acid profile. Serum vacutainer tubes were used to determine serum
insulin concentrations. Plasma and serum were obtained after centrifugation and stored at -20°C until assayed.

6.2.5. Faecal collection

At the end of each testing period, cats were housed individually in metabolic cages to enable collection of all faeces over a five-day period, in order to measure total faecal production. In each collection period, fresh faeces were collected once from each cat for immediate determination of faecal pH and stored at -80°C for determination of faecal SCFA concentrations and moisture content.

The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2008/114) and was in accordance with institutional and national guidelines for the care and use of animals.

6.2.6. Analytical methods

Faecal pH was measured with a glass electrode, immediately after collection and after diluting fresh faeces with 10% ionised water. For SCFA analysis, frozen faeces samples were thawed and 1 g of fresh material was transferred into a centrifuge tube together with 5 ml of water/phosphoric acid/formic acid (550/10/1, v/v/v). Samples were shaken manually before centrifugation, after which the supernatant was filtered through glass wool prior to gas chromatography analysis according to Van Nevel and Demeyer (1977). Quantitative electrospray tandem mass spectrometry was used to determine free carnitine, acylcarnitine and amino acid profile as described by Vreken et al. (1999) and Rizo et al. (2003). Plasma glucose (VetTest 9820369) and urea (VetTest 982062) concentrations were determined using the VetTest 8008 analyzer (Idexx Laboratories Europe B.V., Hoofddorp, the Netherlands). The apparatus is based on dry chemical technology and colorimetric reaction. Sample analysis is carried out on selective testing discs (Idexx Laboratories Europe B.V., Hoofddorp, the Netherlands) by means of laser reading the bar codes. Areas under the curves (AUC) for all data during the meal response test were calculated according to the trapezoidal method.
6.2.7. Statistical analyses

Statistical analyses were performed using Superior Performing Software Systems (SPSS) version 16 (SPSS Inc., Chicago, Illinois, USA). Data during the meal response test were statistically analyzed by repeated measures ANOVA with diet and time as within subject factors. A paired sampled T-test was performed at each different time point during the meal response test as post hoc test and for statistical analyses of the AUC as well as for body weight, food intake and faecal parameters. Statistical significance was accepted at $P<0.05$. All data are expressed as mean ± SE.

6.3. Results

All eight cats completed the trial, yet, catheter placement failed in one cat and no blood samples could be obtained. Both test diets were well tolerated. None of the cats refused to eat any of the diets and none showed signs of illness or maldigestion. Food intake showed no differences among test diets. Body weight remained stable in all cats during the study and was not affected by test diets.

6.3.1. Faecal characteristics

As shown in Table 6.2, total amount of faeces produced over a five-day collection period, percentage of faecal moisture and faecal pH were comparable among diets. Also the total excretion of SCFA, expressed as µmol per day as well as the amount of individual SCFA (acetic, propionic, butyric, valeric and isovaleric acid), expressed as percentage of the total excretion and as absolute amounts (µmol per day) did not reveal any diet effects.
Table 6.2: Faecal characteristics and faecal excretion of SCFA in eight healthy cats fed a low fermentable fibre and high fermentable fibre diet, expressed as mean ± SE.

<table>
<thead>
<tr>
<th></th>
<th>LFF</th>
<th>HFF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Total faecal production (g)</td>
<td>104.0</td>
<td>20.8</td>
<td>87.9</td>
</tr>
<tr>
<td>Faecal moisture content (%)</td>
<td>71.6</td>
<td>2.6</td>
<td>70.5</td>
</tr>
<tr>
<td>Faecal pH&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6.7</td>
<td>0.08</td>
<td>6.6</td>
</tr>
<tr>
<td>Acetic acid (%)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>57.2</td>
<td>3.9</td>
<td>56.8</td>
</tr>
<tr>
<td>Propionic acid (%)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>24.0</td>
<td>2.5</td>
<td>26.4</td>
</tr>
<tr>
<td>Butyric acid (%)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>14.4</td>
<td>1.8</td>
<td>14.0</td>
</tr>
<tr>
<td>Valeric acid (%)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.6</td>
<td>0.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Isovaleric acid (%)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.8</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Total SCFA (µmol/day)</td>
<td>1505</td>
<td>327</td>
<td>1237</td>
</tr>
</tbody>
</table>

LFF, low fermentable fibre; HFF, high fermentable fibre; SCFA, short chain fatty acids.

<sup>1</sup>Collected over a five-day period; <sup>2</sup>faecal moisture content and pH were determined on fresh faecal samples; <sup>3</sup>% of total SCFA.

P-values result from a paired sampled T-test.

6.3.2. Metabolic parameters

Plasma free carnitine, acetyl-, butyryl- and methylmalonylcarnitine concentrations did not change among diets. These characteristics also revealed no time effects except for acetylcarnitine (P=0.006), nor diet-time interactions and AUC did not differ among diets. As shown in Figure 6.1a, plasma propionylcarnitine concentrations changed over time (P=0.004) and were increased at almost every time point after starting the meal in cats fed the HFF diet when compared to cats fed the LFF diet (P=0.015), yet this increase was only significant 120 and 330 minutes after starting the meal (P=0.041 and P=0.036 respectively). The AUC for propionylcarnitine was also elevated in cats fed the HFF diet (P=0.013) (Figure 6.2a). In contrast, plasma 3-methylglutarylcarnitine concentrations (Figure 6.1b) were decreased in cats fed the HFF diet (P=0.026), yet no time effect nor diet-time interaction was observed. The AUC for 3-methylglutarylcarnitine was also reduced when fed the HFF diet (P=0.028) (Figure 6.2b).

Plasma amino acid concentrations (leucine, methionine, phenylalanine, tyrosine, ornithine, citruline, glycine, and alanine) showed no diet effects, nor diet-time interactions, except for methionine (P=0.014), yet time effects were observed. Plasma leucine, methionine, ornithine, citruline (P<0.001 for all), and glycine (P=0.006) concentrations rose following the meal, in contrast to plasma phenylalanine and tyrosine concentrations which fell over time (P<0.001 for
both). AUC’s for these amino acid concentrations did also not change among diets (data not shown). Plasma urea concentrations (Figure 6.3a) showed no diet effect, no diet-time interaction and the AUC for urea remained similar among diets, yet plasma urea concentrations rose over time, regardless of diet ($P<0.001$).

![Graph of propionylcarnitine and 3-methylglutaryl-carnitine](image)

**Figure 6.1:** Mean plasma acylcarnitine concentrations ± SE in seven healthy cats before and after starting the meal consisting of a low fermentable fibre (LFF) and a high fermentable fibre (HFF) diet. $P$-values resulting from repeated measures ANOVA: A. propionylcarnitine: diet effect: $P=0.015$; time effect: $P=0.004$, diet × time: $P=0.433$; B. 3-methylglutaryl-carnitine: $P=0.026$, $P=0.291$ and $P=0.833$ respectively. Significant differences ($P<0.05$) between test diets at specific time points, resulting from post hoc analysis, are represented with *. 
Plasma glucose concentrations (Figure 6.3b) did not differ among diets and showed no time effect, yet an interaction between diet and time tended to exist \((P=0.077)\). The postprandial glucose peak tended to be delayed in cats fed the LFF diet, when compared to the HFF diet \((P=0.062)\), as the glucose peak occurred \(94 \pm 14\) minutes after starting the LFF meal, whereas the glucose peak occurred \(64 \pm 4\) minutes after starting the HFF. Yet, the height of the postprandial glucose peak and the AUC for glucose remained equal among diets.

**Figure 6.2:** Mean area under the curves (AUC) ± SE for propionyl- (A) and 3-methylglutaryl carnitine (B) in seven healthy cats following a meal response test using a low fermentable fibre (LFF) and a high fermentable fibre (HFF) diet. \(P\)-values result from a paired sampled T-test.
Figure 6.3: Mean plasma urea and glucose concentrations ± SE in seven healthy cats before and after starting the meal consisting of a low fermentable fibre (LFF) and a high fermentable fibre (HFF) diet. P-values resulting from repeated measures ANOVA: A. Urea: diet effect: \( P=0.429 \), time effect: \( P<0.001 \), diet × time: \( P=0.218 \); B. Glucose: \( P=0.221 \), \( P=0.133 \) and \( P=0.077 \) respectively.
6.4. Discussion

Soluble dietary fibres and their fermentation products are suggested to reduce protein catabolism and spare amino acids in true carnivorous species, such as cats (Chapter 4, Chapter 5). However, in both earlier studies, cats were fasted and no dietary energy source was available, and thus energy supply had to originate from tissue protein mobilization by using the gluconeogenic pathway. Therefore, the present trial evaluated amino acid sparing conditions in response to a high protein, low carbohydrate meal. These postprandial curves also demonstrate the time of onset of amino acid sparing conditions, regardless of the source of protein, dietary or muscular protein.

At first, total faecal production and faecal characteristics were scrutinized in order to evaluate the occurrence of fermentation in the feline intestine. Total faecal production, faecal moisture content and faecal pH did not change among diets, which was consistent with earlier observations. Hesta et al. (2001a) observed no differences for the amount of fresh faeces and faecal pH was only reduced following ingestion of 6% oligofructose, whereas lower concentrations did not change faecal pH. Faecal moisture content increased with 6%, but not with 3% oligofructose (Hesta et al., 2001a). In addition, the faecal excretion of SCFA, in absolute amounts as well as in percentage of the total amount, were comparable among diets. However, as up to 95% of the produced SCFA are rapidly absorbed from the colon, faecal amounts are only a very small proportion of the total SCFA production (Roberfroid & Delzenne, 1998; Hesta et al., 2006). The use of a mixture of oligofructose and inulin with a DP less than ten, promotes proximal fermentation, stimulating colonic SCFA absorption and therefore minimizing faecal SCFA excretion. The absence of effect on SCFA excretion was also once more in line with Hesta et al. (2001a). Consistent with Verbrugghe et al. (Chapter 4), the higher plasma propionylcarnitine concentrations during the meal response test and the higher AUC for propionylcarnitine when fed the HFF diet, compared to the LFF diet, provide evidence that oligofructose and inulin were indeed fermented in the feline intestine, leading to production and absorption of propionate. Also the absence of effect on plasma acetylcarnitine concentrations was in accordance with this earlier study (Chapter 4). Fasting plasma butyrylcarnitine concentrations were higher when fed the HFF diet, but this difference was not significant, most probably due to the extensive utilization of butyrate as energy source for colonocytes, resulting in only small concentrations of butyrate reaching the liver (Engelhardt, 2004; Rémésy et al., 2004). Hence, faecal characteristics and faecal SCFA excretion have only limited reliability to
produce evidence of SCFA production, whereas the analysis of the acylcarnitine profile, especially propionylcarnitine concentrations, is of much greater use to demonstrate formation of absorbable SCFA.

Secondly, acylcarnitine profiles were scrutinized to evaluated amino acid sparing conditions. Metabolization of propionate generates propionyl CoA (Rémésy et al., 2004). Since a rise of its concurrent acylcarnitine, i.e. propionylcarnitine, was observed during the meal response test and also the AUC for propionylcarnitine was increased, increased propionyl CoA concentrations in cats fed the HFF diet were confirmed. Although, propionyl CoA concentration generates methylmalonyl CoA (Rémésy et al., 2004). A concurrent rise of methylmalonylcarnitine was not observed during the meal response test in cats fed the HFF diet when compared to the LFF diet. Since catabolism of several amino acids, such as valine, methionine and isoleucine also generate methylmalonyl CoA, the absence of increased methylmalonyl CoA concentrations might be due to a decreased catabolism of these amino acids. Both propionyl- and methylmalonyl CoA are specific inhibitors of pyruvate carboxylase, implying inhibited gluconeogenesis from pyruvate (Blair et al., 1973; Anderson & Bridges, 1984). Inhibition of pyruvate carboxylase also occurs indirectly by depleting acetyl CoA, a specific allosteric activator of this enzyme (Blair et al., 1973; Anderson & Bridges, 1984). Yet, a decrease of acetylcarnitine, the corresponding acylcarnitine, in cats fed the HFF diet was not observed, most probably because acetyl CoA is generated from multiple sources, i.e. amino acids, lactate and fatty acids and differences are thus not easy to discern. The lower plasma 3-methylglutarylcarnitine concentrations before and following the meal and decreased AUC for 3-methylglutarylcarnitine in cats fed the HFF diet, corroborate the diminished amino acid-induced gluconeogenesis, since this acylcarnitine is known to be a metabolite of branched chain amino acid catabolism, especially leucine (Roe et al., 1986). Therefore, in presence of propionate, generated from colonic fermentation of oligofructose and inulin, gluconeogenesis most likely originates from propionate, and might result in the inhibition of protein mobilization and sparing amino acids. However, plasma urea and amino acid concentrations remained equal among diets before and following the meal, suggesting the use of amino acids in other metabolic processes such as tissue preservation and immunity. At last, pre- and postprandial plasma glucose concentrations remained comparable among diets, before and following the meal, which is evident since the activity of the gluconeogenesis is suggested to be stable in cats, only the substrate for gluconeogenesis is altered. The tendency towards an interaction between diet and time is most probably due to differences between diets in glucose absorption in the small intestine. Feeding the LFF diet
probably resulted in delayed glucose absorption, yet, the height of the glucose peak and the AUC for glucose did not differ among diets. This is in contrast with previous human and canine studies, which suggest that postprandial hyperglycaemia is reduced with diets high in both types of fibres, but particularly with the soluble fraction (Blaxter et al., 1990). Nevertheless, from this trial, the absence of effect of oligofructose and inulin on postprandial glycaemic response cannot be concluded, since no low fibre diet was used as control diet.

6.5. Conclusion

In conclusion, adding 4% of a mixture of oligofructose and inulin to a high protein diet is suggested to diminish postprandial amino acid-induced gluconeogenesis, by using propionate instead, generated from colonic fermentation already two hours after starting the meal. This way, amino acids might be spared and used in other metabolic processes such as tissue preservation and immunity. However, confirmation of these effects by using stable isotope techniques is warranted. Furthermore, it remains unclear which particular source of protein, dietary or muscular protein, is spared at most and if these conditions remain the same when diets with different protein content or diets deficient in specific amino acids are fed, as well as when cats have higher protein and amino acid requirement.

Acknowledgments: This study was funded by the Institute for Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). Expenses for the laboratory analyses were covered by the Beneo-group (Tienen, Belgium), who also provided the oligofructose and inulin mixture (ORAFTI HSI®). The authors also gratefully acknowledge Herman De Rycke for performing the proximate analyses of the foods, Inge Vaesen for the plasma analyses, Sarah Depauw, Rebekka Hollebosch, Jantina Devylder and Steven Galle for animal care taking and technical assistance.
Chapter 7

General discussion
The increased incidence of obesity, obesity-induced insulin resistance and diabetes mellitus has evoked a major interest in understanding and modulating the feline carbohydrate metabolism.

In Chapter 3 the effect of primary energy sources, especially reduction of the amount of energy derived from carbohydrates, on insulin sensitivity using intravenous glucose tolerance tests (IVGTT) was evaluated in healthy normal weight cats. In Chapter 4, 5 and 6 the effect of secondary energy sources such as fermentable fibre and fermentation products, e.g. propionate, on glucose metabolism was investigated. At first, different methods to confirm the occurrence of fermentation in the feline gastro-intestinal tract were assessed. Secondly, the effect of fermentable fibre, e.g. oligofructose and inulin, and colonic propionate on insulin sensitivity was determined in healthy normal weight and obese cats, using IVGTT or simplified measures of insulin sensitivity as proposed by Appleton et al. (2005). At last, amino acid sparing effects of oligofructose and inulin, as well as colonic propionate were identified.

7.1. Effect of energy sources on insulin sensitivity

7.1.1. Overview

Decreased insulin sensitivity was suggested in healthy normal weight cats fed a low carbohydrate (LC) diet, as the LC diet induced a higher area under the insulin curve ($AUC_{ins}$) than the LP diet, with the LF diet being intermediate and a tendency towards a delayed second insulin peak existed when fed the LC diet. These findings seem to contradict earlier studies in normal weight, obese and diabetic cats, which suggested that reducing dietary carbohydrate content improved insulin sensitivity. Yet, most studies investigated the effect of increasing the amount of carbohydrates on top of already high concentrations of this energy source, resulting in extremely high carbohydrate content, much higher than the ones in commercial cat foods (Figure 7.1) (Farrow et al., 2002; Leray et al., 2006; Backus et al., 2007; Slingerland et al., 2008). Secondly, the increase of one energy source was often confounded with the decrease of other energy sources (Figure 7.2) (Thiess et al., 2004; Leray et al., 2006; Backus et al., 2007).
Consequently, the effect of carbohydrate reduction could not be determined properly. The present study was therefore developed to overcome these drawbacks and to look at the isolated effects of energy sources. The amount of carbohydrates was reduced to lower concentrations, comparable to the cat’s natural diet consisting of prey and compared to higher commercially used concentrations of carbohydrate. Furthermore, to prevent confounding effects, energy sources were substituted iso-calorically, resulting in a reduction of only one energy source, enabling to identify the effect of this single energy source on glucose and inulin response during IVGTT.

A possible explanation for the decreased insulin sensitivity when fed the LC diet, which also contained high concentration of proteins, might be the true carnivorous nature of the cat. Cats appear to be genetically insulin resistant and have therefore evolved and reproduced well on a LC diet (Miller & Colagiuri, 1994). In addition, the cat pancreas is less sensitive to glucose and very responsive to amino acid modulation of hormone release (Curry et al., 1982).
Figure 7.2: Confounding effect of changing energy sources occurring in three studies: Leray et al. (2006); Backus et al. (2007); Thiess et al. (2004); caloric distribution (% of metabolisable energy (ME)) of test diets.

7.1.2. Future research

As healthy normal weight cats were included in the study, the effect of energy sources on the onset of insulin resistance was investigated. The results seem contradictory to several earlier studies suggesting high protein, LC diets to be beneficial for the treatment of type 2 feline diabetes mellitus. Nevertheless, it should be noted that managing and preventing feline insulin resistance does not necessarily require the same dietary strategy. Therefore, this study should be repeated in obese and diabetic cats, using the same dietary approach in order to prevent confounding effects and to reduce the amount of carbohydrates below commercially used concentrations instead of increasing them, as this was not performed before. Furthermore, as amino acids might be more important to stimulate insulin secretion than glucose in cats, further research is warranted to determine the effect of various amino acids.
Chapter 7
General discussion

7.2. Effect of fermentable fibre and fermentation products on glucose metabolism

7.2.1. Confirmation of fermentation in the feline gastro-intestinal tract

7.2.1.1. Overview

Due to the true carnivorous nature of the cat’s diet in the wild and anatomical adaptations of the feline gastro-intestinal tract, characterized by a rudimentary caecum and a very small, unsacculated colon, fermentative capacity of the feline is generally thought to be limited. Yet, compared to dogs and human beings, healthy cats carry high numbers of bacteria in the proximal part of the small intestine, indicating the existence of microbial fermentation in the small intestine (Papasouliotis et al., 1998). Occurrence of microbial fermentation in the feline colon was also indicated by the higher amounts of short chain fatty acids (SCFA) in caudally versus cranially sampled intestinal contents and low pH values in the feline hindgut and faeces when fed low digestible starch and disaccharides (Kienzle, 1993a; Kienzle, 1994a). Other research, using an in vitro fermentation technique, found evidence that cats can use fibre to some extent (Sunvold et al., 1995c). 12-hour in vitro fermentation of fructo-oligosaccharides (FOS) by cat faecal inoculum revealed highest organic matter disappearance (OMD), propionate and total SCFA production, whereas powdered cellulose generated the lowest OMD and least acetate, propionate, butyrate and total SCFA production (Sunvold et al., 1995c). Sparkes et al. (1998b) also noted changes in faecal flora following dietary FOS supplementation. Therefore, oligofructose and short chain inulin (mean degree of polymerization (DP) <10) were used as high fermentable fibre, where as cellulose was used as low fermentable fibre (Chapter 6).

In Chapter 6, the occurrence of fermentation in the cat’s intestine was evaluated by examination of total faecal production and faecal characteristics. Supplementation of 4% oligofructose and inulin did not change total faecal production, faecal moisture content and faecal pH (Table 7.1). According to Hesta et al. (2001a) administration of oligofructose or inulin did not alter the amount of fresh faeces as well. Faecal moisture content was only higher when fed 6% or more oligofructose or inulin, whereas faecal pH was only declined when fed 6% or more oligofructose.
Table 7.1: Effect of oligofructose and/or inulin on faecal production, moisture and pH in different feline studies.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Dose</th>
<th>Faecal production (g/d)</th>
<th>Faecal moisture (%)</th>
<th>Faecal pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hesta et al. 2001a,</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligofructose</td>
<td>0%</td>
<td>22.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>69.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>22.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.21&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>6%</td>
<td>28.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>73.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.99&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>9%</td>
<td>31.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Hesta et al. 2001a,</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>0%</td>
<td>NM</td>
<td>70.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.86</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>NM</td>
<td>71.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.81</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>NM</td>
<td>74.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.94</td>
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<tr>
<td>Oligofructose</td>
<td>3%</td>
<td>NM</td>
<td>71.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.72</td>
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<td><strong>Chapter 6</strong></td>
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<tr>
<td>Oligofructose + short chain</td>
<td>4%</td>
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<td>70.5</td>
<td>6.57</td>
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<tr>
<td>inulin</td>
<td>Cellulose</td>
<td>4%</td>
<td>20.8</td>
<td>71.6</td>
</tr>
</tbody>
</table>

<sup>abc</sup>Different superscripts indicate significant differences (P<0.05); NM, not measured.

Moreover, supplementation of 4% oligofructose and inulin in Chapter 6, did also not modify the excretion of SCFA. This absence of effect was once more similar to Hesta et al. (2001a). Yet, it has to be stated that faecal amounts are only a very small proportion of the total SCFA production, as up to 95% of the produced SCFA are quickly absorbed from the large bowel (Roberfroid & Delzenne, 1998). Moreover, proximal fermentation is promoted by the use of a fermentable fibre mixture with a DP less than ten, facilitating colonic SCFA absorption. Hence, faecal characteristics and faecal SCFA excretion have only limited reliability to produce evidence of the occurrence of fermentation and SCFA production in vivo.

Also determination of SCFA concentrations in peripheral blood is not a reliable parameter. Whereas propionate and acetate reach the liver via the portal vein, butyrate is virtually completely extracted from the colon, with very little appearing in the portal blood for the delivery to the liver, as it is extensively used as energy source by the intestinal mucosa. Acetate is the only SCFA that appears in peripheral blood to a significant extent (Cummings et al., 1987; Rémésy et al., 2004). As in humans, the total amount of SCFA in peripheral blood represents only 21% of the concentrations in portal blood and only 5.5% for propionate in particular (Cummings et al., 1987), determination of peripheral SCFA concentrations is not an appropriate method to find prove of fermentation.
In the present study, the acylcarnitine profile, being a reflection of metabolites available for citric acid cycle (Bremer, 1983) was used as an alternative method to provide evidence of fermentation and metabolization of SCFA in vivo (Table 7.2).

**Table 7.2: Acylcarnitine profile in response to fermentable fibre in the different experiments.**

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Mean DP</th>
<th>Dose</th>
<th>Acetyl-carnitine (µmol/l)</th>
<th>Propionyl-carnitine (µmol/l)</th>
<th>Butyryl-carnitine (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapt. 4 Oligofructose + inulin</td>
<td>4</td>
<td>0%</td>
<td>Fasting</td>
<td>5.55</td>
<td>0.13**</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2.5%</td>
<td>Fasting</td>
<td>6.32</td>
<td>0.28**</td>
</tr>
<tr>
<td>Chapt. 6 Oligofructose + short chain inulin Cellulose</td>
<td>&lt;10</td>
<td>4%</td>
<td>Fasting</td>
<td>5.90</td>
<td>0.41†</td>
</tr>
<tr>
<td>Oligofructose + short chain inulin Cellulose</td>
<td>4%</td>
<td></td>
<td>Fasting</td>
<td>5.08</td>
<td>0.27†</td>
</tr>
<tr>
<td></td>
<td>&lt;10</td>
<td>4%</td>
<td>AUC postprandial (330min)</td>
<td>1676.9</td>
<td>86.0**</td>
</tr>
<tr>
<td>Cellulose</td>
<td>4%</td>
<td></td>
<td>AUC postprandial (330min)</td>
<td>1643.8</td>
<td>57.4**</td>
</tr>
</tbody>
</table>

DP, degree of polymerisation; AUC, area under the curve.

Significant differences and tendencies between test diets are indicated by superscripts: *0.05<P<0.1; **P<0.05.

In Chapter 4, fasting propionylcarnitine concentrations were increased in cats fed an oligofructose and inulin supplemented diet, when compared to a low fibre diet. In Chapter 6, fasting and postprandial propionylcarnitine concentrations were also increased in response to a meal containing oligofructose and inulin compared to a diet containing cellulose. These findings confirm production and absorption of propionate following fermentation of oligofructose and inulin in the feline gastro-intestinal tract. A higher production of butyrate was also observed in Chapter 4 by the higher fasting butyrylcarnitine concentrations when fed an oligofructose and inulin supplemented diet. Yet, in Chapter 6 the increase of butyrylcarnitine concentrations when fed the oligofructose and inulin diet was not significant, most likely because only small amounts of butyrate reach the liver as this SCFA is extensively used as energy source by the intestinal mucosa (Cummings *et al.*, 1987; Rémésy *et al.*, 2004). In both studies, acetylcarnitine concentrations were not altered among diets, as acetyl coenzyme A (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 (Brass & Beyerinck, 1988).
Therefore, the analyses of the acylcarnitine profile is of much greater use than faecal characteristics, faecal and peripheral SCFA to demonstrate formation and metabolization of absorbable SCFA \textit{in vivo}.

7.2.1.2. Future investigations

In the future, the acylcarnitine profile should be related to other techniques estimating bacterial mass, and to the classical method of bacterial culturing but also to innovative molecular techniques which has been used as golden standard.

The adapted Mason method, described in sheep (Mason, 1969) and adapted for diets rich in animal protein (Hesta \textit{et al.}, 2003), is one of the methods assessing bacterial mass. In Chapter 6, this method was also applied, yet, no differences were noted among diets (data not shown).

Another indicator for bacterial mass is the faecal concentration of diaminopimelic acid (DAPA), an amino acid only found in bacteria. This method was utilized earlier in cats for the investigation of microbial degradation of taurine (Backus \textit{et al.}, 1994). Also quantification of purines might be useful as a marker to assess microbial protein (Zinn & Owens, 1986) and was used before in dogs (Howard \textit{et al.}, 2000), but not in cats. It has to be emphasized that these techniques only provide estimations of bacterial mass, whereas the acylcarnitine profile provides proof of production, absorption and metabolism of SCFA as a result of fermentation.

Traditional cultivation methods have been used extensively in the past. However, total viable counts are typically lower than total microscopic counts, not only due to the number of dead cells, but also because many species cannot be cultured (Zoetendal \textit{et al.}, 2004). Therefore, culture-independent molecular techniques such as quantitative polymerase chain reaction (qPCR) which allows the quantification of microbial populations as well as denaturing gradient gel electrophoresis (DGGE) and 16S rDNA sequencing which enable the measurement of gut diversity and species identification respectively, show great promise and have been used in cats before to determine the effect of dietary protein on intestinal microflora (Lubbs \textit{et al.}, 2009). Moreover, \textit{in vivo} screening of different fermentable fibre sources at different doses using qPCR, DGGE and 16S rDNA sequencing should be of great interest.
7.2.2. Effect on insulin sensitivity

7.2.2.1. Obese cat model

In both studies (Chapter 4 and 5), an obese cat model was used as obesity is known to be related to insulin resistance. A higher risk for insulin resistance and impaired glucose tolerance was confirmed in obese cats in Chapter 4. Obese cats had no higher fasting serum insulin concentrations, but the increased area under the glucose curve, the higher fasting plasma glucose concentration and the later appearance of the second insulin peak during IVGTT resulted in an impaired glucose tolerance and decreased insulin sensitivity in obese cats. These findings were also concluded from earlier feline studies (Nelson et al., 1990; Hoenig et al., 2006). In contrast, no prove of insulin resistance in obese cats was found in Chapter 5. Insulin resistance was estimated by fasting serum insulin concentrations and fasting insulin to glucose ratio (SI), since Appleton et al. (2005) demonstrated a high correlation between these parameters and the results obtained by the frequently sampled IVGTT with minimal model analysis, especially in obese cats. Both characteristics were not significantly increased in obese cats during the present study. Though, neither glucose tolerance tests, nor insulin sensitivity tests were performed. On the other hand, the same cats were used in the previous study (Chapter 4), having the same body condition score (BCS) and a slightly higher body weight (BW) (Table 7.3).

| Table 7.3: Differences between normal weight and obese cats in different experiments. |
|---------------------------------|-----|-----|-----|-----|
|                                | Chapt. 4 |       | Chapt. 5 |
|                                | Normal weight | Obese | Normal weight | Obese |
| BCS                            | 3.1*** | 5.0*** | 3.3*** | 5.0*** |
| BW (kg)                        | 4.3*** | 6.8*** | 4.8*** | 7.4*** |
| BMI (kg/m²)                    | 37.7*** | 54.4*** | 42.4*** | 62.8*** |
| Glucose nadir (mmol/l)         | 4.5*** | 5.1**  | 5.4      | 5.0    |
| Insulin nadir (mU/l)           | 16.5   | 17.2   | 15.9     | 12.2   |
| Fasting SI (mU/mmol)           | 3.7    | 3.3    | 2.8      | 2.4    |
| AUCgluc (mmol/l/120min)        | 1247   | 1533   | NM       | NM     |
| AUCins (mU/l/120min)           | 4467   | 5183   | NM       | NM     |
| Leptin (ng/dl)                 | 7.4*** | 20.2***| NM       | NM     |

BCS, body condition score; BW, body weight; BMI, body mass index; SI, insulin to glucose ratio; AUCgluc, area under the glucose curve; AUCins, area under the insulin curve; NM, not measured.

Significant differences and tendencies between test diets are indicated by superscripts:
**P<0.05; ***P<0.01.
The higher plasma leptin concentrations in obese cats (Chapter 4; Table 7.3), also predicts the occurrence of insulin resistance, as a strong positive relationship between leptin and body weight (Appleton et al., 2000) but also between leptin and insulin resistance (Appleton et al., 2002) were demonstrated. On the one hand, increased leptin concentrations may contribute to the diminished insulin sensitivity, seen in obese cats. Alternatively, the compensatory hyperinsulinaemia found with insulin resistance in obese cats, could stimulate leptine production (Appleton et al., 2002). In humans, Ruhl & Everhart (2003) also observed that the prevalence of elevated alanine aminotransferase (ALT) activity is positively associated with increasing body mass index (BMI) and waist-to-hip circumference ratio. Hyperleptinemia and hyperinsulinemia were found to be the major determinants of this association (Ruhl & Everhart, 2003). Also Vozarova et al. (2002) and Marchesini et al. (2005) observed high association between elevated liver enzymes, especially ALT activity and obesity as well as insulin resistance. Therefore, the rise in ALT activity in obese cats in Chapter 4, also predicts the occurrence of insulin resistance.

7.2.2.2. Overview

In contrast to earlier studies in omnivores and less strict carnivores, dietary oligofructose and inulin did not alter glucose and insulin response during IVGTT in healthy normal weight and obese cats (Chapter 4).

Yet, 2.5% of oligofructose and inulin on dry matter (DM) basis (0.5 g/kg BW) seems rather low when compared to earlier studies in rats (Kok et al., 1996; Agheli et al., 1998; Busserolles et al., 2003) and dogs (Diez et al., 1997; Diez et al., 1998; Massimino et al., 1998; Hesta et al., 2001b). However, according to Respondek et al. (2008), 0.2 g short chain fructo-oligosaccharide (scFOS)/kg BW improved insulin sensitivity in obese dogs as observed by the increased rate of glucose infusion during euglycaemic hyperinsulinaemic clamp (EHC). In healthy human subjects, a 4-week administration of 0.3 g fructo-oligosaccharides (FOS)/kg BW did not alter fasting glucose and insulin concentrations, but did reduce in vitro basal hepatic glucose production (Luo et al., 1996). Whereas, in type 2 diabetic patients, a 2-week ingestion of 0.1 g FOS/kg BW resulted in diminished fasting glycaemia (Yamashita et al., 1984). In cats however, no data are available on the effect of fermentable fibre on glucose tolerance and insulin sensitivity. Yet, higher doses are not recommended for practical use in cats, as Hesta et al. (2001a) noted formless faeces and reduced apparent digestibility of protein and fat following ingestion of more than 3% oligofructose and inulin.
Nonetheless, also the type of fibre and more specific the DP are of great importance, as the rate of degradation of oligomers with a DP<10 is approximately twice that of molecules with a DP>10 (Roberfroid et al., 1998); meaning that reducing the total number of fructose or glucose units will lead to a more extensive proximal fermentation, whereas a higher DP will result in a slower and more distal fermentation. The soluble fibre supplement used in Chapter 4, consisted of both oligofructose and inulin. Yet, the proportion of long chain inulin (mean DP: 25) was rather high, suggesting a slower fermentation in the cat’s relative short intestinal tract.

The absence of effect of oligofructose and inulin on insulin sensitivity is confirmed as colonic infusion of propionate did not also alter insulin sensitivity, as observed by the absence of effect on SI, plasma non-esterified fatty acid concentration and glucagon-like peptide-1 (GLP-1) (Chapter 5).

However, the single 30-minute infusion of propionate does not fully correspond with the more continuous propionate production from hindgut fermentation. In both human studies performed by Wolever et al. (1989; 1991b) single dosing of SCFA was administered and a lack of reduction in blood glucose was observed. In contrast, Berggren et al. (1996) opted for a repeated dosing and administered a 30-minute infusion of sodium propionate rectally in obese, hyperinsulinaemic rats on a daily basis during a 19-day period, resulting in decreased fasting glucose concentrations. On the other hand dietary supplementation as assessed in humans (Venter, 1990; Todesco et al., 1991) and rats (Cameron-Smith et al., 1994; Boillot et al., 1995; Berggren et al., 1996), leading to a more continuous dosing of propionate, enhanced glucose tolerance and insulin sensitivity, with the exception of the study performed by Cameron-Smith et al. (1994). Yet, in these studies no certainty exists that propionate was absorbed from the colon as occurs under physiological conditions, but most probably propionate absorption took place in the small intestine. However, in the present trial, absorption of propionate from the colon is a certainty as demonstrated by the higher plasma propionylcarnitine concentrations during and following propionate infusion. Furthermore, the administered dose must be questioned. The dose used in the present study (4 mmol propionate /kg ideal body weight (idBW)) was similar to the one used in rats (4 mmol/kg BW) (Berggren et al., 1996) and was higher than the ones used in humans (1.3 and 2.5 mmol/kg BW respectively) (Wolever et al., 1989; Wolever et al., 1991b). It also corresponds to a 12-hour in vitro fermentation of 4 g FOS/kg idBW with feline faecal microflora (Sunvold et al., 1995b). The dosage of propionate administered in the present trial was much higher than the amount of propionate generated through fermentation of oligofructose and inulin.
ingested during the earlier trial (0.5 g/kg BW), yet, in contrast to omnivores, no modulation of insulin sensitivity was observed in normal weight and obese cats.

The strict carnivorous nature of the cat and more specific the high activity of gluconeogenic enzymes and low activity of glucokinase and glucose-transport activities, might explain the absence of effect on glucose and insulin metabolism, in contrast to omnivores and less strict carnivores such as dogs. In humans and rodents, propionate was shown to inhibit gluconeogenesis from pyruvate and to stimulate glycolysis, resulting in hypoglycaemic effects. Nevertheless, in cats, propionate also inhibits gluconeogenesis from pyruvate (See 7.2.3. Amino acid sparing effect), but glycolysis is limited at all times. As propionate acts as gluconeogenic substrate itself (See 7.2.3. Amino acid sparing effect), hypoglycaemic effects will occur in cats to a lesser extent when compared to omnivores and less strict carnivores.

### 7.2.3. Amino acid sparing effect

#### 7.2.3.1. Overview

Amino acid sparing effects of fermentable fibre and fermentation products, as shown by the differences in acylcarnitine profile in different experiments, are summarized in Figure 7.3. and Table 7.4.

Adding oligofructose and inulin to the cat’s diet, resulted in a higher production and metabolization of propionate as observed by the increased fasting (Chapter 4) and postprandial (Chapter 6) plasma propionylcarnitine concentrations. In Chapter 5, colonic infusion of propionate also resulted in increased plasma propionylcarnitine concentrations, confirming propionate absorption. Metabolism of propionate in the liver, as shown in Figure 7.3, enters the citric acid cycle at the level of succinyl CoA, and leads to the synthesis of oxaloacetate, which may enter the gluconeogenic pathway (Rémésy et al., 2004). Therefore, interference of propionate with the use of other gluconeogenic substrates, such as amino acids is acceptable, whenever the gluconeogenesis is active (Blair et al., 1973; Anderson & Bridges, 1984; Petitet et al., 1998). Although propionyl CoA generates methylmalonyl CoA, a concurrent rise of methylmalonylcarnitine was only observed in Chapter 5 at the end of the propionate infusion, when the plasma propionylcarnitine concentrations were highest. At all other time points during and following propionate infusion as well as with dietary oligofructose and inulin, no increase of methylmalonylcarnitine was detected. This absence of effect can be explained by the fact that
methylmalonylcarnitine is also a metabolite of valine, isoleucine and methionine catabolism, and results in amino acid sparing conditions.

Propionate also reduces hepatic gluconeogenesis from pyruvate; directly by inhibition of pyruvate carboxylase (Figure 7.3. (1)) through its specific intermediaries, propionyl CoA and methylmalonyl CoA, but also indirectly by depleting acetyl CoA, a specific activator of the same enzyme (Blair et al., 1973; Anderson & Bridges, 1984). Yet, following oligofructose and inulin supplementation, a decrease of acetylcarnitine was not observed, most probably because differences are not easy to distinguish as acetyl CoA is generated from multiple sources, i.e. amino acids, lactate and fatty acids. However, acetylcarnitine concentrations were numerically lowerl 60 and 90 minutes after starting the colonic propionate infusion, suggesting inhibited gluconeogenesis from pyruvate and reduced amino acid utilization.

Figure 7.3: Propionate inhibits amino acid-induced gluconeogenesis, and acts as gluconeogenic substrate itself. (1): inhibition of pyruvate carboxylase; (2): inhibition of aspartate aminotransferase.
Table 7.4: Amino acid sparing effects of fermentable fibre and fermentation products, as shown by the differences in acylcarnitine profile in different experiments.

<table>
<thead>
<tr>
<th>Administration</th>
<th>Supplement</th>
<th>Mean DP</th>
<th>Dose</th>
<th>Acetyl-carnitine (µmol/l)</th>
<th>Propionyl-carnitine (µmol/l)</th>
<th>Methylmalonyl-carnitine (µmol/l)</th>
<th>3-Methylglutaryl-carnitine (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapt. 4 Dietary</td>
<td>Oligofructose + inulin</td>
<td>4</td>
<td>0%</td>
<td>Fasting</td>
<td>5.55</td>
<td>0.13***</td>
<td>0.054**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>2.5%</td>
<td>Fasting</td>
<td>6.32</td>
<td>0.28**</td>
<td>0.036*</td>
</tr>
<tr>
<td>Chapt. 5 Colonic</td>
<td>Propionate</td>
<td>0 mmol/kgBW</td>
<td>At 30 minutes</td>
<td>3.81</td>
<td>0.08***</td>
<td>0.036**</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 mmol/kgBW</td>
<td>At 30 minutes</td>
<td>3.77</td>
<td>2.38***</td>
<td>0.048**</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Propionate</td>
<td>0 mmol/kgBW</td>
<td>At 90 minutes</td>
<td>4.02*</td>
<td>0.11***</td>
<td>0.042**</td>
<td>0.016***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 mmol/kgBW</td>
<td>At 90 minutes</td>
<td>3.47*</td>
<td>1.54***</td>
<td>0.033**</td>
<td>0.008***</td>
</tr>
<tr>
<td></td>
<td>Propionate</td>
<td>0 mmol/kgBW</td>
<td>AUC (90min)</td>
<td>369.35</td>
<td>9.72***</td>
<td>3.83</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 mmol/kgBW</td>
<td>AUC (90min)</td>
<td>344.71</td>
<td>150.04***</td>
<td>3.84</td>
<td>1.11</td>
</tr>
<tr>
<td>Chapt. 6 Dietary</td>
<td>Oligofructose + short chain inulin</td>
<td>&lt;10</td>
<td>4%</td>
<td>Fasting</td>
<td>5.90</td>
<td>0.41*</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Cellulose</td>
<td></td>
<td>4%</td>
<td>Fasting</td>
<td>5.08</td>
<td>0.27*</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Oligofructose + short chain inulin</td>
<td>&lt;10</td>
<td>4%</td>
<td>AUC postprandial (330min)</td>
<td>1676.9</td>
<td>86.0*</td>
<td>196.9</td>
</tr>
<tr>
<td></td>
<td>Cellulose</td>
<td></td>
<td>4%</td>
<td>AUC postprandial (330min)</td>
<td>1643.8</td>
<td>57.4**</td>
<td>178.2</td>
</tr>
</tbody>
</table>

**DP, degree of polymerisation; BW, body weight; AUC, area under the curve.**

**Significant differences and tendencies between test diets are indicated by superscripts:** *0.05<P<0.1; **P<0.05; ***P<0.01.
Dietary oligofructose and inulin also resulted in decreased postprandial 3-methylglutaryl carnitine concentrations (Chapter 6), as did colonic infusion of propionate (Chapter 5). 3-methylglutaryl CoA is known to be a metabolite of branched chain amino acid metabolism, especially leucine (Roe et al., 1986) and therefore, reduction of its concurrent acylcarnitine corroborates to diminished amino acid-induced gluconeogenesis.

At last, the reduction of plasma aspartate aminotransferase (AST) activity (Figure 7.3. (2)) in cats supplemented with oligofructose and inulin, as observed in Chapter 4, indicates inhibited gluconeogenesis from aspartate as well.

Hence, in presence of propionate, generated from colonic fermentation of oligofructose and inulin or colonic propionate infusion, gluconeogenesis most likely originates from propionate, diminishing amino acid-induced glucose production. Eventually this may lead to inhibition of protein mobilization and amino acid sparing conditions. However, plasma urea and amino acid concentrations remained equal at all times, suggesting use of amino acids in other metabolic processes such as tissue preservation and immunity.

7.2.3.2. Potential applications

These amino acids sparing conditions can be of great importance at all times in strict carnivorous species such as cats, because of the high activity of the gluconeogenic pathway. Also in humans and less carnivorous species such as dogs, these conditions may be useful in whatever situation, resulting in activation of the gluconeogenic pathway. In intensive care patients, patients that are injured, sick, septic or anorectic, patients during starvation, patients with cancer, cachexia, muscle atrophy and particularly cats with hepatic lipidosis, the gluconeogenic pathway is permanently activated in liver and muscle tissue, due to hypermetabolism, resulting in the use of endogenous energy, especially the use of skeletal muscles protein (alanine and branched chain amino acids) (Chan & Freeman, 2006). Also patients with renal or liver disease, in which protein intake has to be reduced to minimize clinical symptoms related to nitrogen waste, might benefit from this prebiotic effect, since the small protein amount provided will not be spoiled for energy production, but utilized for tissue preservation and immunity. At last, also athletes and sporting dogs may possibly benefit of this amino acid sparing prebiotic effect, as their protein demand is increased due to combined increases in the rates of tissue protein synthesis and branched chain amino acid catabolism (leucine, isoleucine, valine) (Toll & Reynolds, 2000).
7.2.3.3. Future investigation

In the future, it is definitely necessary to look more in depth into the mechanism of this amino acid sparing effect, as it still remains unclear which particular source of protein, muscular or dietary protein, is spared at most. It also stays uncertain if these conditions remain the same when diets with different protein content or diets deficient in specific amino acids are fed, as well as when cats have higher protein and amino acid requirement for example due to stimulation of immunity.

7.3. Conclusions

In contrast to omnivores, reducing the dietary carbohydrate content below common concentrations decreased insulin sensitivity in normal weight cats, most probably because amino acids are more important stimulators of insulin secretion than glucose. Yet, further research is needed to determine the effect of various amino acids. Since prevention and management of insulin resistance do not necessarily require the same dietary strategy, the effect of reducing the dietary carbohydrate content should be investigated in obese and diabetic cats, using the same dietary approach.

Concerning the effect of dietary fibre, fermentation of oligofructose and inulin in the feline intestinal tract was confirmed by investigating the acylcarnitine profile, which is a more accurate method to evaluate fermentation in vivo than faecal characteristics, faecal and peripheral SCFA. Secondly, oligofructose and inulin as well as propionate did not improve glucose tolerance and insulin sensitivity in normal weight and obese cats, in spite of clear signs of insulin resistance in obese cats. Since no diabetic cats were studied, future research should focus on the effect of fermentable fibre and fermentation products in feline diabetes mellitus. At last, oligofructose and inulin diminished amino acid-induced gluconeogenesis by substitution with propionate generated from fermentation in the feline intestinal tract. This way, amino acids are spared and used in other metabolic processes. Investigation of these amino acid sparing conditions in cats with protein or amino acid deficiency and in cats with higher protein requirements would be of great interest.
Summary

Cats are strict carnivores in contrast to dogs and have a unique protein and carbohydrate metabolism. Carbohydrates are not dietary essential. The capacity of the feline gastro-intestinal tract to digest and absorb carbohydrates and of the feline liver to process glucose is limited. An overview of the evolutionary anatomical and physiological adaptations of carbohydrate digestion and absorption and of hepatic glucose and protein metabolism to the cat’s strictly carnivorous nature is reported in Chapter 2, Paragraph 2.2.

In their natural habitat, cats consume prey, which are high in protein, moderate in fat and contain minimal carbohydrates. However, many commercial cat foods are high in carbohydrates. This change from a low carbohydrate, high protein diet typical for feral cats to a high carbohydrate diet in domestic cats, has been accompanied by a considerable transformation of the cat’s lifestyle. Many cats no longer live outdoors, but have a sedentary lifestyle. In addition, cats no longer need to hunt for food, leading to a decreased physical activity. These changes of the cat’s lifestyle have only become widespread over the last decades and are sometimes held responsible for the recent increase in incidence of obesity, obesity-induced insulin resistance and diabetes mellitus.

A better insight in the feline carbohydrate metabolism allows to act preventive as well as curative with dietary strategies against the development of these disorders. Modulation of the feline carbohydrate metabolism can be achieved by manipulating dietary carbohydrates in the broadest sense. Chapter 2, Paragraph 2.3 gives an overview of the present literature concerning this nutritional modulation and focuses especially on dietary macronutrients, carbohydrate sources and dietary fibre.

Hereabout, only little research has been performed in strict carnivores such as cats. Therefore, this PhD thesis looked into the effect of dietary carbohydrates in the broadest sense on feline glucose metabolism, on the one hand by varying the amount of primary energy sources – protein, fat and carbohydrate – (Chapter 3) and on the other hand by generating short chain fatty acids (SCFA) through intestinal fermentation of prebiotics (Chapter 4 & 6) or through supplementation of propionate (Chapter 5).
In Chapter 3 the effect of dietary primary energy sources – protein, fat and carbohydrate – on glucose tolerance and insulin sensitivity was investigated, in order to seek for the combination of energy sources with the lowest glycaemic effect.

Carbohydrate reduction is increasingly proposed to improve insulin sensitivity. Yet, in earlier studies the effect of increasing one energy source was often confounded with the decrease of another energy source or was often conceived as an increase on top of an already high concentration of this energy source. This has led to doubt about the effect of carbohydrate reduction. The study in Chapter 3 was therefore developed to overcome these drawbacks and to look at the isolated effects of energy sources. The amount of carbohydrates was reduced to lower concentrations, comparable to the cat’s natural diet and compared to higher commercially used concentrations of carbohydrate. Furthermore, to prevent confounding effects, energy sources were substituted isocalorically, resulting in a reduction of only one energy source. The effect of this single energy source on glucose tolerance and insulin sensitivity was identified using intravenous glucose tolerance tests (IVGTT). This study showed that, in contrast to omnivores, reducing dietary carbohydrate below commercially available concentrations diminished insulin sensitivity in cats with a normal body weight, as shown by the higher area under the insulin curve when fed the low carbohydrate diet, compared to the low protein diet, with the low fat diet being intermediate and by the tendency towards a delayed second insulin peak when fed the low carbohydrate diet. A possible explanation for these findings might be the true carnivorous nature of the cat. On the one hand, feline pancreatic β-cells are much less sensitive to glucose than those from omnivorous, but on the other hand, amino acids are proven to be important modulators of pancreatic insulin release. Further research is warranted to determine the effect of various amino acids.

Fermentable fibre such as oligofructose and inulin modulate glycaemia and insulinaemia in humans, rodents and dogs, by regulation of the hepatic glucose metabolism via SCFA, especially propionate, generated through intestinal fermentation. This results in inhibition of gluconeogenesis and/or stimulation of glycolysis. Hepatic gluconeogenesis might also be influenced indirectly through reduction of plasma fatty acid concentration, enhancing insulin sensitivity. Besides, propionate itself can, after conversion to oxaloacetate via succinyl coenzyme A (CoA), enter the gluconeogenesis as well. It is therefore reasonable that whenever this pathway is active, propionate, when available, may interfere with the utilization of various gluconeogenic substrates, such as lactate and amino acids.
Since only little is known about the metabolic effects of fermentation in cats, three studies were conducted: In Chapter 4 the effect of fermentable fibres, namely oligofructose and inulin (2.5% on dry matter (DM) basis), on glucose tolerance and insulin sensitivity was compared to a low fibre diet, by using IVGTT. Metabolic differences between normal weight and obese cats were also scrutinized. The study discussed in Chapter 5, focused on the metabolic effect of fermentation products, through colonic administration of propionate in normal weight and obese cats. Insulin sensitivity was assessed by simplified measurements such as fasting insulin concentration and the insulin to glucose ratio (SI). The last study (Chapter 6) examined the postprandial metabolic effects of oligofructose and inulin (4% on DM), compared to a diet supplemented with cellulose (4% on DM). In this study, but also in both earlier studies, metabolites available for citric acid cycle were reflected by the analysis of the acylcarnitine profile. This way the occurrence of fermentation was evidenced in peripheral blood and an amino acid sparing effect was reported.

In contrast with earlier studies in omnivores and less strict carnivores, oligofructose and inulin did not affect glucose and insulin response during IVGTT in normal weight and obese cats (Chapter 4). The absence of effect of fermentable fibre on insulin sensitivity was supported in Chapter 5, since colonic administration of propionate did also not modify insulin sensitivity, as observed by the unaltered SI, plasma non-esterified fatty acid (NEFA) and glucagon-like peptide-1 (GLP-1) concentrations during and following the infusion. Yet, both studies clearly confirmed that obesity is associated with a higher risk for insulin resistance, derived from higher fasting glucose concentrations, higher area under the glucose curve during IVGTT, but also elevated fasting leptin concentrations and alanine aminotransferase activity.

Fermentation in the feline gastro-intestinal tract was proven by the analysis of the acylcarnitine profile, as in Chapter 4 and 6 clearly increased propionylcarnitine concentrations were observed, affirming production and absorption of propionate when fed a diet supplemented with fermentable fibre. In addition, butyrylcarnitine concentrations were increased in Chapter 4, evidencing production of butyrate and occurrence of fermentation. Chapter 6 also revealed that the acylcarnitine profile is a more accurate method to demonstrate fermentation than faecal characteristics and faecal SCFA, since the latter parameters were not influenced by feeding a diet containing 4% fermentable fibre.
Moreover, the acylcarnitine profile also reflects metabolization of absorbable SCFA. Addition of oligofructose and inulin to the cat’s diet resulted in a higher production and metabolization of propionate as shown by the increased fasting (Chapter 4) and postprandial (Chapter 6) propionylcarnitine concentrations. Also in Chapter 5 higher propionylcarnitine concentrations were observed during and following administration of propionate in the colon. Metabolization of propionate in the liver leads to the synthesis of oxaloacetate, which may enter the gluconeogenesis. As mentioned before, it is acceptable that whenever this pathway is active and propionate is available, propionate will interfere with the utilization of other gluconeogenic substrates, such as amino acids. Despite that propionyl CoA generates methylmalonyl CoA, a rise of methylmalonylcarnitine was not detected at all times. Methylmalonylcarnitine being a metabolite of valine, isoleucine and methionine, may explain these findings, as an amino acid sparing effect prevents increased methylmalonylcarnitine. Additionally, inhibition of gluconeogenesis from pyruvate and therefore reduced use of amino acids is demonstrated, since pyruvate carboxylase is inhibited directly by propionyl CoA and methylmalonyl CoA, but also indirectly by depletion of acetyl CoA. However, decreased acetyl carnitine concentrations were only noticed following colonic infusion of propionate. Most probably because acetyl CoA is generated from various sources and therefore, changes are not easy to discern.

Furthermore, oligofructose and inulin (Chapter 6), as well as administration of propionate in the colon (Chapter 5), evoked a diminution of 3-methylglutaryl carnitine, a metabolite of leucine, again contributing to a reduced utilization of amino acids as gluconeogenic substrate. At last, the lower aspartate aminotransferase (AST) activity in cats supplemented with oligofructose and inulin also means a diminished amino acid-induced gluconeogenesis, specifically decreased use of aspartate (Chapter 4).

As neither fermentable fibre (Chapter 4 & 6), nor administration of colonic propionate (Chapter 5) affected plasma urea and glucose concentrations, it can be assumed that amino acids, spared by restraining amino acid-induced gluconeogenesis, are applied in other vital functions such as tissue preservation and immunity.

In conclusion, reduction of dietary carbohydrates, below commercially available concentrations, diminish insulin sensitivity in non-obese cats, in contrast to omnivores. Amino acids, as important stimulators of insulin secretion are a possible cause of these findings. Yet, further research, investigating the effect of various amino acids is warranted.

Concerning the effect of fermentable fibre, fermentation of oligofructose and inulin in the feline gastro-intestinal tract was confirmed by investigating the acylcarnitine profile, which is a more
accurate method to evaluate *in vivo* fermentation than faecal characteristics and faecal SCFA. Secondly, fermentation products, such as propionate, were shown not to improve glucose tolerance and insulin sensitivity in both non-obese and obese cats, despite clear signs of insulin resistance in obese cats. At last, oligofructose and inulin diminish amino acid-induced gluconeogenesis by substitution with propionate, generated from fermentation in the feline intestinal tract. This way amino acids are spared and can be used in other metabolic processes. Investigation of these amino acid sparing conditions in cats with protein or amino acid deficiency or in cats with higher protein requirements is recommended in the future.
Samenvatting

Katten zijn, in tegenstelling tot honden, strikte carnivoren met een uniek eiwit- en koolhydraatmetabolisme. Koolhydraten zijn niet diëtisch essentieel. De capaciteit van het gastro-intestinaal stelsel van de kat om koolhydraten te verteren en te absorberen en van de feliene lever om glucose te verwerken is bovendien beperkt. Een overzicht van de evolutionaire anatomische en fysiologische aanpassingen van de koolhydaraatvertering en koolhydraatabsorptie en van het hepatisch glucose- en eiwitmetabolisme aan de strikt carnivore natuur van de kat, wordt weergegeven in Hoofdstuk 2, Paragraaf 2.2.

In hun natuurlijke habitat consumeren katten vooral prooien, die veel eiwit, matig vet en slechts minimale hoeveelheden koolhydraten bevatten. Heel wat commerciële kattenvoeders bevatten echter hoge gehalten aan koolhydraten. Deze verandering van een koolhydraatarm en eiwitrijk dieet typisch voor wilde katten naar een koolhydraatrijk dieet bij de huiskat, ging tevens gepaard met een verandering van de levensstijl van de kat. Veel katten leven niet langer buitenshuis, maar houden er een sedentaire levensstijl op na. Katten zijn niet meer genoodzaakt te jagen, wat een daling van de lichaamsactiviteit teweegbrengt. Deze veranderingen van de feliene levensstijl traden pas op gedurende de laatste decennia en zouden aan de basis liggen van de recent toegenomen incidentie van obesitas, obesitas-geïnduceerde insulineresistentie en diabetes mellitus.

Een beter inzicht in het feliene koolhydraatmetabolisme laat toe om met gerichte voederstrategieën preventief en curatief op te treden tegen de ontwikkeling van deze aandoeningen. Door manipulatie van diëtaire koolhydraten in ruime zin kan het feliene koolhydraatmetabolisme worden gemoduleerd. Hoofdstuk 2, Paragraaf 2.3 geeft dan ook een overzicht van de huidige literatuur betreffende deze nutritionele modulatie en focust hierbij vooral op diëtaire macronutriënten, koolhydraatbronnen en voedingsvezels.

Hieromtrent werd bij strikte carnivoren zoals de kat nog maar weinig onderzoek verricht. Daarom beoogde dit doctoraatsproefschrift het effect na te gaan van de diëtaire koolhydraten-fractie in ruime zin op het feliene glucosemetabolisme, door enerzijds het gehalte aan primaire energiebronnen eiwit, vet en koolhydraten te variëren (Hoofdstuk 3) en anderzijds kortketen vetzuren, als alternatieve energiebron, te genereren door intestinale fermentatie van prebiotica (Hoofdstuk 4 & 6) of suppletie van propionzuur (Hoofdstuk 5).
In Hoofdstuk 3 werd het effect onderzocht van primaire energiebronnen – eiwit, vet en koolhydraten – in het voeder op glucosetolerantie en insulinengevoeligheid, om zo op zoek te gaan naar de minst glycemische combinatie van energiebronnen. Steeds vaker wordt koolhydraatreductie voorgesteld ter verbetering van de insulinengevoeligheid. In vroegere studies werd het effect van stijging van één energiebron echter vaak verward met het dalen van een andere energiebron of werd een stijging beoogd bovenop een reeds hoog gehalte van deze energiebron. Dit heeft er toe geleid dat tot nu toe het effect van koolhydraatreductie niet met zekerheid kon worden gedetermineerd. De studie besproken in Hoofdstuk 3 werd dan ook zo danig ontwikkeld dat deze nadelen werden vermeden en dat het geïsoleerd effect van energiebronnen kon worden nagegaan. De hoeveelheid koolhydraten werd gereduceerd tot lagere gehalten, vergelijkbaar met het natuurlijke dieet van katten en vergeleken met hogere commercieel beschikbare koolhydraatgehaltes. En om verwarringseffecten te vermijden werden energiebronnen isocalorisch uitgewisseld, zodat telkens slechts één energiebron verlaagd werd. Het effect van reductie van deze ene energiebron op glucosetolerantie en insulinengevoeligheid werd geïdentificeerd met behulp van intraveneuze glucosetolerantietesten (IVGTT). Deze studie toonde echter aan dat, in contrast met omnivoren, bij katten met een normaal lichaamsgewicht, het reduceren van het diëtaire koolhydraatgehalte onder commercieel beschikbare gehalten de insulinengevoeligheid verlaagde. Dit werd aangetoond door de grotere oppervlakte onder de insulinecurve bij voederen van het koolhydraatarm dieet in vergelijking met het eiwitarm dieet; met het vetarm dieet als intermediair, en ook door de trend naar een uitgestelde tweede insulinepiek bij het voederen van het koolhydraatarme voer. De oorzaak hiervoor is hoogstwaarschijnlijk te vinden in de strikt carnivore natuur van de kat. De feliene pancreas is enerzijds minder gevoelig voor glucose dan die van omnivoren, maar anderzijds zijn ook aminozuren bij de kat belangrijke stimulatoren van de insulinesecretie. Verder onderzoek naar het effect van verschillende aminozuren is echter noodzakelijk.

Fermenteerbare vezels zoals oligofructose and inuline moduleren de glycemie en insulinenemie bij mensen, knaagdieren en honden, onder andere via regulatie van het hepatisch glucose-metabolisme door kortketen vetzuren, vooral propionzuur, gevormd bij intestinale fermentatie. Dit resulteert in inhibitie van de gluconeogenese en/of stimulatie van de glycolyse. De hepatische gluconeogenese kan ook indirect worden beïnvloed door het verlagen van de plasma vrije-vetzuurconcentratie, resulterend in een betere insulinengevoeligheid. Daarnaast kan propionzuur zelf, na omzetting tot oxaloacetaat, via succinyl coenzyme A (CoA) ook de gluconeogenese intreden. Het is dan ook aanvaardbaar dat wanneer deze pathway actief is,
propionzuur, wanneer aanwezig, kan interfereren met het gebruik van verschillende gluconeogene substraten, zoals lactaat en aminozuren.

Aangezien slechts weinig gekend is omtrent de metabole effecten van fermentatie bij de kat, werden drie studies uitgevoerd: In **Hoofdstuk 4** werd het effect van fermenteerbare vezels, namelijk oligofructose en inuline (2.5% op droge stof (DS)) op glucosetolerantie en insulinegevoeligheid, in vergelijking met een vezelarm voer geëvalueerd. Dit door gebruik van IVGTT. Ook werden de metabole verschillen tussen obese en niet-obese katten gedetermineerd. De studie in **Hoofdstuk 5** focuste op het metabole effect van fermentatieproducten, via toediening van propionzuur in het colon bij zowel obese als niet-obese katten. De insulinegevoeligheid werd in deze studie beoordeeld via vereenvoudigde metingen zoals nuchtere insulinconcentratie en de insuline tot glucose ratio (SI). De laatste studie (**Hoofdstuk 6**) onderzocht de postprandiale metabole effecten van oligofructose en inuline (4% op DS), door vergelijking met een voeder gesuppleerd met cellulose (4% op DS). Bij deze studie, maar ook bij de twee voorgaande studies werd door bepaling van het acylcarnitineprofiel een reflectie van de metabolieten beschikbaar voor de citroenzuurcylcus weergegeven. Op die manier kon een bewijs voor het optreden van fermentatie in perifeer bloed worden geleverd en werd een aminozuursparend effect aangetoond.

In contrast met vroegere studies bij omnivoren en minder strikte carnivoren, werd aangetoond dat oligofructose en inuline geen invloed hebben op de glucose- en insulinerespons tijdens IVGTT bij zowel katten met een normaal lichaamsgewicht als dikke katten (Hoofdstuk 4). De afwezigheid van effect van fermenteerbare vezel op insulinegevoeligheid werd tevens bekrachtigd in Hoofdstuk 5, aangezien ook toediening van propionzuur rechtstreeks in het colon de insulinegevoeligheid niet moduleerde, zoals aangetoond door de ongewijzigde SI, plasma vrije-vetzuurconcentratie en glucagon-like peptide-1 (GLP-1) concentratie tijdens en na het infuus. Toch werd bij beide studies duidelijk bevestigd dat obesitas gepaard gaat met een verhoogd risico voor insulineresistentie, uitgaande van de hogere nuchtere glucoseconcentratie, de verhoogde oppervlakte onder de glucosecurve en het later optreden van de tweede insulinepiek tijdens IVGTT, maar ook de hogere nuchtere leptineconcentratie en alanine aminotransferase activiteit.

Door de bepaling van het acylcarnitineprofiel kon het optreden van fermentatie in het gastro-intestinum van de kat worden geconfirmeerd, omdat zowel in Hoofdstuk 4 als in Hoofdstuk 6
een duidelijke stijging van propionylcarnitine werd geconstateerd. Dit bevestigt de vorming en absorptie van propionzuur bij voederen van een dieet gesupplement met fermenteerbare vezels. In Hoofdstuk 4 werd tevens ook een verhoogde butyrylcarnitineconcentratie waargenomen, wat ook de vorming van boterzuur en het optreden van fermentatie bekrachtigt. Uit Hoofdstuk 6 bleek verder ook dat het acylcarnitineprofiel een meer accurate methode is om het optreden van fermentatie aan te tonen dan faecale karakteristieken en faecale kortketen vetzuren, omdat deze laatste niet werden beïnvloed door het voederen van een dieet gesupplement met 4% fermenteerbare vezels.

Daarenboven is het acylcarnitineprofiel ook een reflectie van de metabolisatie van absorbeerbare kortketen vetzuren. Toevoegen van oligofructose en inuline aan het dieet van de kat resulteerde in een hogere productie en metabolisatie van propionzuur, zoals aangetoond door de hogere nuchtere (Hoofdstuk 4) en postprandiale (Hoofdstuk 6) propionylcarnitine concentraties. Ook in Hoofdstuk 5 werd een verhoogde propionylcarnitineconcentratie waargenomen tijdens en na toediening van propionzuur in het colon. Metabolisatie van propionzuur in de lever leidt tot de synthese van oxaloacetaat dat de gluconeogenese kan intreden. Zoals reeds eerder aangehaald, is het dan ook aanvaardbaar dat wanneer deze pathway actief is en propionzuur voorhanden is, propionzuur zal interfereren met het gebruik van andere gluconeogene substraten zoals aminozuren. Ondanks het feit dat propionyl CoA, methylmalonyl CoA genereert, werd niet steeds een stijging van methylmalonylcarnitine gezien. Dat methylmalonyl CoA ook een afbraakproduct is van valine, isoleucine en methionine kan hiervoor een verklaring zijn, wat maakt dat een aminozuursparend effect de stijging in methylmalonylcarnitine verhindert. Ook inhibetie van de gluconeogenese uit pyruvaat en dus verminderd gebruik van aminozuren is een feit, omdat pyruvatcarboxylase direct geïnhibeerd wordt door propionyl CoA en methylmalonyl CoA, maar ook indirect door depletie van acetyl CoA. Een daling van acetylcarnitine werd echter enkel opgemerkt na toedienen van propionzuur in het colon, vermoedelijk omdat acetyl CoA uit meerdere bronnen gegenereerd wordt en veranderingen dus moeilijk vast te stellen zijn. Ook resulteerden oligofructose en inuline (Hoofdstuk 6), net als de toediening van propionzuur in het colon (Hoofdstuk 5), in een daling van 3-methylglutaryl-carnitine, een metaboliet van leucine, wat weerson bijdraagt tot een gereduceerd gebruik van aminozuren als gluconeogeen substraat. Ten laatste betekent ook de verminderde aspartaataminotransferase (AST) activiteit bij katten gesuppleerd met oligofructose and inulin, een gereduceerde aminozuurgeïnduceerde gluconeogenesis, uitgaande van aspartaat in dit geval (Hoofdstuk 4).
Het feit dat noch fermenteerbare vezels (Hoofdstuk 4 & 6), noch toediening van propionzuur rechtstreeks in het colon (Hoofdstuk 5) de plasma ureum- en glucoseconcentraties beïnvloedden, veronderstelt dat de aminozuren gespaard door inhibtie van aminozuurgeïnduceerde gluconeogenese, aangewend worden voor andere vitale functies, zoals weefselbehoud en immuniteit.

Tot slot kan worden geconcludeerd dat in contrast met omnivoren, reductie van de hoeveelheid koolhydraten in de voeding, onder commercieel beschikbare gehalten, de insulinegevoeligheid bij niet-obese katten vermindert. Aminozuren, zijnde belangrijke stimulatoren van de insuline-secretie zijn mogelijk de oorzaak hiervan. Verder onderzoek naar het effect van verschillende aminozuren is echter noodzakelijk.


Curriculum Vitae


Op 1 oktober 2005 trad zij in dienst als doctoraatsbursaal bij de vakgroep Voeding, Genetica en Ethologie aan de Faculteit Diergeneeskunde, Universiteit Gent en werd gedurende vier jaar gefinancierd door het IWT-Vlaanderen (Instituut voor de Aanmoediging van Innovatie door Wetenschap en Technologie in Vlaanderen), met als doel haar doctoraatsonderzoek met als titel “Nutitionele modulatie van het koolhydraatmetabolisme bij de kat” te voltooien. Gedurende deze vier jaar groeide de interesse in voeding en diëtetiek van de kleine huisdieren en werd zij ook resident bij het ECVCN (European College of Veterinary and Comparative Nutrition), begeleid door Prof. Dr. M. Hesta, met als doel het examen voor het behalen van een diplomacy bij het ECVCN af te leggen in 2010. Binnen het kader van deze residency, nam zij deel aan verschillende residency classes en stond zij ook in voor de consultaties en dienstverlenging betreffende voeding voor kleine huisdieren, in samenwerking met de Kliniek Kleine Huisdieren aan de Faculteit Diergeneeskunde. Daarnaast verzorgde zij ook de practica diëtietiek van de kleine huisdieren voor laatstejaarsstudenten optie Kleine Huisdieren. Ook gaf zij meerdere postuniversitaire bijscholingen voor practici en stond als promoter en co-promoter in voor de begeleiding van meerdere studenten bij het voltooien van hun scriptie of masterproef. In 2007 behaalde ze het certificaat van proefleider na het volgen van de Basic Course in Laboratory Animal Science (FELASA categorie C).

Adronie Verbrugghe is auteur en mede-auteur van meerdere wetenschappelijke publicaties in internationale tijdschriften. Ze nam ook op regelmatige basis actief deel aan verschillende internationale congressen, waarvan één op uitnodiging.
Bibliography

1. Publications in refereed scientific journals


2. Abstracts presented at international scientific meetings

Verbrugghe A, Hesta M & Janssens GPJ ‘Differences in palatability between dry foods with 1% addition of either chicken lard, rapid-harvest salmon oil or non-rapid-harvest salmon oil in dogs’. Proceedings 31ste Studiedag Nederlandstalige Voedingsonderzoekers (NVO), April 7th 2006, Rotterdam, The Netherlands, p.36.

*Oral presentation*


*Poster presentation*
*Poster presentation*

*Oral presentation*

*Oral presentation*

*Poster presentation, award winning poster.*

*Oral presentation*

*Oral presentation*

Verbrugghe A, Hesta M, Daminet S, Polis I & Janssens GPJ ‘Effect of colonic propionate on glucose and insulin metabolism in normal weight and obese cats’. In proceedings of the 18th Congress of the European College of Veterinary Internal Medicine – Companion Animals (ECVIM-CA), September 4th-6th 2007, Ghent, Belgium, p.214.
*Oral presentation*

Oral presentation


Poster presentation


Oral presentation


Oral presentation


Oral presentation
Dankwoord
Toen mijn grootvader gediagnosticeerd werd met type II diabetes, groeide mijn interesse voor deze aandoening en in het bijzonder voor de diëtaire behandeling. Ik ben dan ook vereerd dat ik de kans kreeg dit onderwerp dieper uit te spitten. Aangezien ook bij de kat type II diabetes, obesitas en insuline-resistentie veel voorkomen, wordt de kat vaak als model voor de mens gebruikt. Toch stootten we tijdens dit doctoraatsonderzoek op een aantal opmerkelijke bevindingen en bleek de kat door haar strikt carnivore natuur uniek te zijn, wat dit proefschrift alleen maar interessanter heeft gemaakt.

Aan het einde van dit boeiend doctoraatsonderzoek, rest mij alleen nog iedereen te bedanken die hebben bijdragen tot dit doctoraat.


Ik bedank ook Prof. Dr. Ir. Wouter Hendriks als lid van de begeleidingscommissie voor de feedback en kritische ideeën. Verder ook een welgemeende uiting van dank aan alle leden van de examencommissie, Prof. Dr. Christian Burvenich, Prof. Dr. Richard Ducatelle, Prof. Dr. George Fahey en Dr. Marianne Diez.

Onderzoek bij katten is natuurlijk niet evident zonder de katten zelf. Zonder deze prachtige beestjes met een ongelofelijk karakter was mijn doctoraat niet mogelijk geweest. Daarom bedank ik graag de R&D afdeling van Janssens Animal Health voor het ter beschikking stellen van hun populatie proefkatten. Ook bedankt aan Rebekka om zo goed voor onze schatjes te zorgen. De katten mogen van geluk spreken dat zij er bent, steeds in de weer om het zo goed mogelijk naar hun zin te maken, nieuwe kooiverrijking, borstelen en op tijd en stond vele knuffels. Rebekka, merci! Ook Steven, Bart en Herman bedankt, want ook op zon- en feestdagen hebben onze poezen verzorging nodig.

Naast de dagdagelijkse verzorging, gingen de proeven ook gepaard met intensieve staalnames: katheters steken met alle bijhorende moeilijkheden en frustraties, van ’s morgens vroeg, vele uren bloednemen, dag en nacht waken bij de katten om verse mest te verzamelen, soms dagen aan één stuk… Helemaal alleen had ik dit nooit gekund. Vaak kon ik beroep doen op studenten. Vooral Lies, Eva en Jantina, bedankt dat ik op jullie kon rekenen. Ook bedankt aan Kris, Tim, Koen en Prof. Dr. Ingeborgh Polis voor het vele
geduld bij het plaatsen van de centraal veneuze katheters. De katten waren hiervoor niet het beste voorbeeld en maar al te vaak lokten ze diepe frustraties uit. Toch bedankt om steeds vol te houden en niet op te geven. Bovendien bedankt aan alle andere collega’s van de Kliniek Kleine Huisdieren. Als jullie mij met mijn katten door de gangen van de kliniek zagen lopen, wisten jullie al snel hoe laat het was. Bedankt voor jullie steun en de leuke samenwerking. Ook bedankt voor het doorsturen van patiënten, zodat ik naast theoretische kennis ook heel wat praktische kennis inzake klinische voeding heb verworven.


Vervolgens nog een woord van dank voor mijn familie. In het bijzonder vake en moeke, mijn schoonouders, broer en zus, alsook mijn schoonboers en schoonzussen, bedankt voor jullie interesse, begrip, steun en vertrouwen. Bedankt voor jullie luisterend oor, maar ook voor de vele gezellige momenten die voor ontspanning zorgden tijdens de voorbije drukke tijden. Daarenboven wens ik graag mijn broer, Valentijn extra bedanken voor het op punt stellen van de lay-out en de grafische vormgeving van dit doctoraat.

Tenslotte wil ik ook Koen bedanken. Jou liefde, steun en begrip waren onmisbaar voor mij. Je stond altijd open om mij te helpen, ook al was mijn doctoraatsonderzoek vaak Chinees voor jou. Als ik ’s avonds thuis kwam kon ik steeds bij jou terecht, ook wanneer het wat minder ging. Jou optimisme zorgde er steeds voor dat ik bleef doorzetten. Bedankt Koen, om er altijd te zijn voor mij!!

Adronie