The Effects of Diet Matrices on Feline Bioenergetics and Behaviour

by

Margaret A. Gooding

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Doctor of Philosophy
in
Animal and Poultry Science

Guelph, Ontario, Canada

© Margaret A. Gooding, June, 2012
ABSTRACT

The Effects of Diet Matrices on Feline Bioenergetics and Behaviour

Margaret A. Gooding Advisor:
University of Guelph, 2012 Professor J. Atkinson

Obesity is the most prevalent nutritional disorder in cats (*Felis catus*; Hoenig et al., 2011). High carbohydrate diets, prescribed for weight loss, may contribute to adiposity (Thiess et al., 2004). The effects of a high fat (HF; 30% fat, 10% carbohydrate), high carbohydrate (HC; 10% fat, 46% carbohydrate) and a moderate diet (15% fat, 30% carbohydrate) supplemented with a calorie restriction mimetic (mannoheptulose (MH); 8 mg/kg BW), fed to energy requirements, on feline metabolism and behaviour were investigated (n=20; 4 ± 2.5 kg).

An 11 week acclimation procedure was designed to adapt cats to 24-hr restriction within a chamber used for indirect calorimetry. Stress indicative behaviour (Kessler and Turner, 1997) declined with repeated exposure to increasing lengths of restriction within chambers and on week 11 stress levels were low and consistent (P<0.05).

Neither the HF nor HC diet impacted body weight (p>0.05); however, HF feeding caused an increase in body fat (0.75 kg (baseline) vs. 1 kg (86d)) after long-term feeding. Energy expenditure (EE) was not impacted by dietary fat/carbohydrate. Respiratory quotients (RQ) increased and decreased with exposure to the HC (fasted= 0.80 ± 0.008; fed= 0.87 ± 0.008), HF (fasted= 0.76 ± 0.008; fed= 0.78 ± 0.008) diet, respectively. Glucose to insulin (G:I) ratio increased with HF feeding; indicating improved insulin sensitivity. Physical activity, measured using accelerometers, declined with HF (-1.6 counts/hr) and HC (-2.8 counts/hr) feeding from baseline. T-maze performance decreased and increased with HF (-0.85 score/10) and HC (0.85 score/10) feeding from baseline (p<0.05).
MH did not impact body weight or composition (p>0.05). Area under the curve for EE increased during the 15-22 hour post feeding with MH treatment (2370.3 (-MH) vs. 3292.0 (+MH) ± 0.0002). RQ and G:I were not impacted by MH (p>0.05). MH increased play motivation, measured using obstruction tests (p<0.05).

Diets high in carbohydrate are not ideal for weight loss since they negatively impact insulin sensitivity and voluntary EE. Diets promoting elevated EE, activity and normal glucose/insulin profiles are ideal for weight control and MH offers a unique opportunity for use in weight loss regimes.

**Keywords:** Feline, indirect calorimetry, fat, carbohydrate, mannoheptulose, behaviour
Acknowledgements

I thank Dr. Anna Kate Shoveller for your unwavering support and enthusiasm throughout the completion of this degree. I sincerely appreciate the intellectually challenging academic and professional environment that you created to allow me to grow as both a scientist and young professional. I sincerely thank Dr. Jim Atkinson and Dr. Ian Duncan for allowing me the honour of being one of their graduate students and for their intellectual and technical guidance throughout my degree.

Dr. Lee Neil, thank you for your contributions to the design of the T-maze protocol.

Dr. Derek Haley and Dr. Jacquie Rand, thank you for your rigorous and insightful review of this thesis.

To Procter and Gamble for their financial support of my degree and to my colleagues and friends who made me feel like a welcome member of the P&G team. I thank Cindy Lanman and Jason Brewer for there full commitment to the success of my projects. I am grateful to Beth Flickinger, Gary Davenport, Jin Zhang, Dawn Spangler and Mike Hayek for their contributions, support and advice throughout my degree. And, of course, I thank the cats, for this degree could not be completed without their full cooperation. Their kind nature, unpredictability and ability to make me laugh everyday made my research pleasant, challenging and exciting.

I appreciate my friends outside of the university and Procter and Gamble for your friendship and genuine interest in the success of my degree. Pat Gillis, you have inspired me and provided me an opportunity to develop a love and respect for animals.

Jane and Emma Gooding, I thank you for your encouragement, kindness and love. You can truly brighten my day.

Ryan, I thank you for your unconditional support, love and acceptance of my dreams and ambitions.

Pauline and Ron Gooding I could not have completed this degree without your love, precious guidance and continual support. All that I am and hope to be I owe to you.
# Table of Contents

List of Tables ........................................................................................................................................ viii
List of Figures ......................................................................................................................................... x

Literature Review ..................................................................................................................................... 1

- Impact of CHO and Fat Dietary Inclusion on Feline Metabolism and Body Weight ...................... 1
- Mood Effects of Diets High in CHO and Fat .................................................................................... 4
- Cognitive Effects of Diets High in CHO and Fat .............................................................................. 7
- Satiety Effects of Diets High in CHO and Fat ................................................................................... 9
- Hunger and Feline Play Motivation ................................................................................................ 10
- Calorie Restriction and Calorie Restriction Mimetics ..................................................................... 11
- Methods used to Determine Energy Requirements ........................................................................... 15
- Behavioural Responses of Cats to Stressful Environments and Research Methods .................... 20
- References ...................................................................................................................................... 24

Development and validation of a behavioural acclimation protocol for cats to respiration chambers used for indirect calorimetry studies ........................... 49

- Abstract ........................................................................................................................................... 50
- Introduction ..................................................................................................................................... 51
- Material and Methods ...................................................................................................................... 52
- Results ............................................................................................................................................ 56
- Discussion ....................................................................................................................................... 64
- References ...................................................................................................................................... 69

Effects of high fat and high carbohydrate diets on fat and carbohydrate oxidation and plasma metabolites in healthy cats ........................................................................ 73

- Abstract ........................................................................................................................................... 74
- Introduction ..................................................................................................................................... 75
Conclusion .......................................................................................................................................... 181

References..................................................................................................................................... 184
**List of Tables**

Table 1: Advantages and disadvantages of the methods used to measure energy requirement in the domestic cat.

Table 2: Nutrient (%) and metabolizable energy content of washout and test diets on an as-fed basis.

Table 3: Energy, fat and carbohydrate intake of cats consuming a high fat and high carbohydrate diet during acute (Day 0), semi-chronic (Day 4) and chronic (Day 13) dietary exposure.¹

Table 4: The effects of high fat or high carbohydrate diets on macronutrient metabolism after acute (Day 0), semi-chronic (Day 4) and chronic (Day 13) exposure.¹

Table 5: The effects of high fat or high carbohydrate diets on fasted blood plasma metabolites after acute (Day 1), semi-chronic (Day 5) and chronic (Day 14) exposure.¹

Table 6: Nutrient (%) and metabolizable energy content of washout and test diets on an as-fed basis.

Table 7: Body weight, total body fat and lean body mass and bone mineral content in adult domestic short hair cats after baseline (Day -7) exposure to a control diet and after chronic (Day 76) exposure to either a HF or HC diet.¹

Table 8: Fasted and fed respiratory quotient (A), energy expenditure and energy expenditure as a percent (%) of energy intake (B) in adult domestic short hair cats after baseline (Day -7) exposure to a control diet and after acute (Day 35) and chronic (Day 76) exposure to either a HF or HC diet.¹²

Table 9: AUC for total EE (kcal/d) represented as a change from baseline as affected by diet (HF vs. HC), day and time post feeding.

Table 10: Blood plasma metabolites in adult domestic cats with baseline (Day -6) exposure to a control diet and after acute (Day 36) and chronic (Day 77) exposure to either the HF or HC diet.¹

Table 11: Change in measures of the adult domestic cats willingness to work to obtain access to a stuffed toy mouse at varying door weights (0-600 g) from baseline (Day -20) exposure to a control diet and after
chronic (Day 64) exposure to either a HC or HF diet.

Table 12: Change in performance (number of correct per ten trials) during T-maze testing in adult domestic cats from baseline (Day -1) exposure to a control diet and after acute (day 42) and chronic (Day 84) exposure to either a HF or HC diet.

Table 13: Analyzed nutrient (%) and metabolizable energy content of control (-MH) and test (+MH) diets on an as-fed basis.

Table 14: Body weight, total body fat and lean body mass in adult domestic short hair cats during the consumption of the washout/control diet (-MH, Day -2) and after chronic exposure (Day 28) to a test (+MH) or control (-MH) diet.

Table 15: Energy metabolism in adult domestic cats after acute (Day 0) and chronic (Day 21) exposure to a control (-MH) and test (+MH) diet.

Table 16: The effects of MH and length of exposure on blood plasma metabolites in adult domestic cats consuming a control (-MH) and test (+MH) diet after acute (Day 1) and chronic (Day 22) exposure.

Table 17: Nutrient (%) and metabolizable energy content of control (-MH) and test (+MH) diets on an as-fed basis.

Table 18: Descriptions of behaviour patterns recorded during play motivation tests (Hall et al., 1998)

Table 19: Mean frequency (A) and duration (B) of performance of predatory and non-predatory play behaviours of cats consuming control (-MH) and test (+MH) diets.

Table 20: Measures of the cats willingness work to obtain access to a stuffed toy mouse at varying door weights during test (+MH) and control (-MH) dietary treatments.
List of Figures

Figure 1: Least squares means of Cat-Stress-Scores (mean±SEM) of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment on day 1 of each week of the acclimation procedure at the 10%, 50% and 80% time points. Letters identify relative differences among Cat-Stress-Scores for time within day and over the 11 acclimation weeks, means not sharing a superscript letter are significantly different; “a” represents the highest value and “f” the lowest (P<0.05).

Figure 2: Least squares means of Cat-Stress-Scores (mean±SEM) of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment on each sampling day within a particular week with multiple observations per week. Letters identify relative differences among Cat-Stress-Scores within week, means not sharing a superscript letter are significantly different; “a” represents the highest value and “d” the lowest value (P<0.05). Days within week without superscripts do not differ (P>0.05). Weeks 8-11 are not represented as there were no repeated day measures within these weeks.

Figure 3: Least Squares Means of latency to approach in seconds (mean±SEM) to the novel object within a 5 cm radius of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment. Letters identify relative differences among latency to approach; means not sharing a superscript letter are significantly different; “a” represents the highest value and “d” the lowest value (P<0.05).

Figure 4: Product-limit survival estimates for latency to approach the novel object within a 5 cm radius of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment.

Figure 5: Least squares means of weekly feed refusal (ORTs) with a 60 g daily feed offering (mean +/- SEM) of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment. Letters identify relative differences among feed refusal, means not sharing a superscript letter are significantly different; “a” represents the highest value and “c” the lowest value (P<0.05).

Figure 6: Comparison of least means squares (mean±SEM) of Cat-Stress-Scores of un-acclimated (N=10) and acclimated cats at that 10%, 50% and 80% time points during a 5 hr exposure to the indirect calorimetry equipment and associated environment (N=14). Letters identify relative differences among Cat-Stress-Scores within day; “a” represents the highest value and “b” the lowest value (P<0.05). Means
not sharing a common single or double asterisk are significantly different between groups; "*" represents the highest value and "**" the lowest value (P<0.05).

Figure 7: The effects of high fat or high carbohydrate diets on mean body weight (kg) ± SEM, n=10, after acute (Day 0), semi-chronic (Day 4) and chronic (Day 13) exposure. Means without a common superscript differ due to exposure within a dietary treatment, P<0.05. NS, P≥0.05. Means not sharing a common single or double asterisk are significantly different between groups; "*" represents the highest value and "**" the lowest value (P<0.05). P-value refers to the ANOVA for diet*exposure effect of treatment.

Figure 8: Mean (N=10) fat and CHO oxidation measured in grams oxidized/hour during the fasted, fed, post-prandial and return to fasted states over a 20 hr period in cats consuming a HF (A; 30% fat) and HC (B; 10% fat) diet during chronic (Day 13) dietary exposure.

Figure 9: Mean (n=10) RQ measured half-hourly during the fasted, fed, post-prandial and return to fasted states over a 22 hr period in adult domestic cats consuming a control diet at baseline (Day -7) and either a HF or HC diet after chronic (Day 76) dietary exposure.

Figure 10: Mean ± SEM activity count after baseline (Day -14; control diet), acute (Day 28) and chronic (Day 70) dietary exposure. Values are least-square means ± SEM, n = 10. Means within an exposure with superscripts without a common symbol differ, P < 0.05. Means across exposures with superscripts without a common letter differ, P<0.05. NS, P≥0.05. P-value refers to the ANOVA for diet*exposure effect of treatment.

Figure 11: EE (A) and RQ (B) in cats consuming the control (-MH) and test (+MH) diet during chronic dietary exposure. Values are expressed as means, means within time points having different superscripts are significantly different (P<0.05).

Figure 12: Least square mean ± SEM for area under the curve (AUC) for energy expenditure during the post-prandial (0-3), fed (3-9), return to fasted (9-15) and fasted (15-22) after acute (day 0; A) and chronic (day 21; B) exposure to a control (-MH) and test (+MH) diet.

Figure 13: Plasma glucose (A), insulin (B) and MH (C) in adult domestic cats following the consumption of either a control (-MH) or test (+MH) diet during an IVGTT on day 28 with and without MH treatment1.
Figure 14: Maximum weight cats would push in order to obtain access to a toy when exposed to the test (+MH; solid) and control (-MH; checker) diets. Values are maximum door weights (grams) each cat was willing to push open to gain access to a toy during the test and control dietary treatment.
Literature Review

Metabolic adaptations suited to the consumption of carnivorous diets have caused the cat to exhibit metabolic idiosyncrasies of energy metabolism including: reduced glucose clearance times (Nelson et al., 1990), prioritized fat oxidation (Gooding et al., 2012; Tanaka et al., 2005), elevated gluconeogenesis (McDonald et al., 1984) and reduced capacity to digest carbohydrates (Zoran, 2002; CHO) as compared to more omnivorous species like the human, dog and rat. Little is known about particular diet compositions and their associated metabolic effects as risk factors contributing to adiposity and blood glucose/insulin perturbations in the cat. Understanding feline energy metabolism and associated behavioural effects of the consumption of diets with differing macronutrient content can reduce the relative risk for the development of obesity and associated diseases. The following is a review of the effects of diets high in fat and carbohydrate on energy metabolism and behaviour (mood, cognition and satiety) and novel techniques that may offer an opportunity to positively manipulate feline energy metabolism, the methods used to study feline energy metabolism and the necessity to use non-stressed cats during experiments to yield valid and reliable results.

Impact of CHO and Fat Dietary Inclusion on Feline Metabolism and Body Weight

It has been suggested that prolonged consumption of high CHO diets contribute to the development of diabetes mellitus, primarily Type 2 diabetes in cats (Rand, 1999), by promoting obesity, apoptosis, and by inducing a large postprandial glucose and insulin response that may lead to overstimulation of the pancreatic β cells to the point of exhaustion and dysfunction (Brand Miller and Colagiuri, 1994; Rand et al., 2004). Cats fed a high CHO/low protein diet (38.1 and 24.7% ME) exhibited a higher level of non-esterified fatty acid (NEFA) suppression that was hypothesized to be a consequence of increased lipogenesis contributing to fat deposition in muscle (Hoenig et al., 2007a). Hoenig et al., 2007a, concluded that the high CHO diet had no effect on insulin sensitivity or glucose effectiveness after being fed for a minimum of 4 months. On the contrary, other researchers have found that diets high in CHO and low in protein increase postprandial plasma glucose and insulin response versus diets high in protein and low in CHO (Farrow et al., 2002; Singh et al., 2006 (unpublished-cited in Coradini et al., 2011); Coradini et al., 2011; Hewson-Hughes et al., 2011). Although, Coradini et al., 2011, observed a decrease in plasma glucose/insulin with the low CHO, high protein diet (23 and 47% ME) they also found that the diet was associated with increased weight gain when fed ad libitum. Slingerland et al., 2007a, suggests that high
carbohydrate intakes on a metabolizable energy (ME) basis should not be considered a risk factor for the
development of diabetes; alternatively, indoor confinement and physical activity are more likely
independent risk factors. Further, Hoenig et al., 2007b, concluded that obesity rather than dietary
 macronutrient content was the largest risk factor for insulin resistance and reduced glucose efficiency.
Lastly, Slingerland et al., 2007b, reasoned that consumption of high CHO and high fat diets actually
improve β-cell function as demonstrated by increased glucose induced insulin secretion in response to
intake. However, the high fat diet group displayed a trend towards an increased glucose disposal rate
versus the high CHO group suggesting that increased CHO intake may lead to a decline in glucose
disappearance and insulin sensitivity.

Carbohydrate source as opposed to level of CHO inclusion has been cited as the greatest risk factor for
obesity and reduced insulin sensitivity/glucose responsiveness (Rand et al., 2004). Cats fed rice based
versus a corn and sorghum diet consumed more energy, gained more weight and displayed higher glucose
concentrations and insulin secretion with *ad libitum* feeding (Appleton et al., 2004). Glycemic index (GI)
is defined as an indicator of the potential of CHO in different types of food to raise blood glucose levels
within hours of ingestion (Jenkins et al., 1981). Low GI foods are described as being digested and
absorbed slowly and high GI foods are described as being digested and absorbed rapidly. High GI foods
can induce hormonal and metabolic responses in humans that are associated with negative health effects
such as diabetes and cardiovascular disease (Brouns et al., 2005). Alternatively, low GI diets are
implicated in the reduction of obesity in humans through the reduction of energy intake by promoting
satiety; however, these findings are uncertain and warrant further investigation (Niwano et al., 2009).
Though the effect of different CHO sources and GI has received limited attention in cats, the results from
Appleton et al., 2004, indicate that it is not only amount of CHO but type of CHO that may contribute to
feline obesity via perturbations in glucose and insulin handling.

Several studies have shown that cats fed a high fat (HF), rather than HC diets are more susceptible to
weight gain and metabolic disorders (Backus et al., 2007; Thiess et al., 2004; Slingerland et al., 2007b).
In humans and cats, HF diets have been shown to increase energy intake, decrease energy expenditure (via
a reduction in thermic effect of feeding), and increase body fat accumulation (due to impaired
autoregulation between fat intake and oxidation) (Schwartz et al., 1985; Treuth et al., 2003). Cats fed HF
diets may be more susceptible to metabolic changes that produce weight gain and perturbations in glucose
handling (Thiess et al., 2004; Backus et al., 2007). Feeding a high fat diet increased concentrations of
plasma triacylglycerides (TAG), non-esterfied fatty acids (NEFA), β-hydroxybutyrate, blood urea nitrogen
and cholesterol as measured over a 24-hr sampling period with no difference in glucose (Thiess et al., 2004). Additionally, cats fed the high fat diet displayed elongated glucose clearance times and reduced acute insulin responses to glucose during a glucose tolerance test. Exchanging dietary CHO for fat (protein: ME constant) caused an increase in body weight and plasma insulin concentration with no change in glucose concentration (Backus et al., 2007). Higher dietary fat (11 and 21% w/w) caused a greater increase in body fat mass in young cats soon after gonadectomy (Nguyen et al., 2004). These data suggest that high fat diets may be a primary contributor to the development of feline diabetes mellitus, whereas high carbohydrate presents less of a risk. Higher dietary fat inclusion in premium or specialty dry cat foods has a stronger association with weight gain than dry “grocery type” diets, which are typically higher in CHO content (Scarlett et al., 1994). However, this observation has not been consistently replicated. Fat oxidation has been shown to be linearly correlated to increasing dietary fat intake with a mean slope of 0.91 in adult cats (Lester et al., 1999). Cats also successfully maintained body weight on each diet of increasing fat content; however, the feeding period was only 8 days. In other epidemiological studies, obesity rates had a stronger association in cats fed commercially available dry type diets higher in CHO versus commercially available canned, higher fat diets (Oscai et al., 1984; West et al., 1994).

Composition of dietary fat versus total fat intake has been identified as a risk factor for the development of cardiovascular disease and diabetes. Namely, the consumption of diets high in Trans-unsaturated and saturated fats are associated with coronary heart disease (Hu et al., 1997). On the contrary, diets with a greater amount of fat from monounsaturated fatty acids (MUFA) and especially polyunsaturated fatty acids (PUFA), namely omega-3 fatty acids (Martin de Santa Olalla et al., 2009), are effective at preventing coronary heart disease, obesity and diabetes. Increasing the relative concentration of PUFAs lowers LDL cholesterol, regulates blood TAG levels and may act to regulate glucose and insulin via improvements in membrane fluidity, signal transduction and anti-inflammatory affects (Tapsell et al., 2009; reviewed in Rudkowska, 2010); however, the latter association is less clear and requires further research (Astrup et al., 2011).

In conclusion, the relative level of risk for the development obesity and associated diseases during the consumption of diets high in fat or CHO remain unclear and compounded by the types of fat and CHO included within the diets. In addition, there may be some effects of dietary fat and CHO on behaviour that may interact with the metabolic effects of dietary fat and CHO and further contribute to weight gain.
Mood Effects of Diets High in CHO and Fat

Mood, defined as an internal, subjective state or enduring emotion driven by feelings and inferred from observable behavioural responses in animals (Mendl et al., 2010), may be affected by the consumption of certain diets with differing fat and CHO content that influence glucose and insulin profiles. For instance, anecdotal evidence suggest that high levels of plasma glucose cause cats to become lethargic which is hypothesized to be a result of the rapid and persistent elevation in plasma glucose and insulin.

In humans and rats, high CHO diets, that impact plasma insulin and glucose levels, have been shown to influence mood by reducing energy level and alertness while increasing susceptibility to depression (Pivonka & Grunewald, 1990; Kapas et al., 1993; Wells et al., 1997; Nabb and Benton, 2006). However, the individual and combined effects of glucose and insulin on mood, potentially influenced by CHO intake, remain unclear and much of the research is contradicting. Rats injected with glucose and insulin, not glucose alone, exhibited an increase in sleep duration over 24 hours (Danguir and Nicolaidis, 1980). Conversely, several researchers have concluded that hyperglycemia alone causes: 1) reduced arousals during sleep, 2) increased sleep durations during both light and dark periods, 3) an increase in slow wave (deep) sleep, 4) a reduction in walking and 5) a decline in feeding in rats (Bendtson et al., 1992; Astrom and Trojaborg, 1992; De Saint Hilaire et al., 1995). Furthermore, hyperinsulinemia has been shown to influence mood by reducing the expression of brain noradrenaline transporters; thus, decreasing the release of epinephrine causing calmness (Figlewicz et al., 1993). Type versus amount of CHO has been cited as the greatest risk factor for negative moods. Diets with a high GI versus a low GI value may contribute to depressive like symptoms and higher levels of sleep due to their effects on glucose and insulin (Gold et al., 1995; Owens et al., 1996; Nabb and Bentom, 2006; Afagji et al., 2007; Cheathum et al., 2009). However, this effect was not observed in all studies (Rodin et al., 1985). GI effects on mood may also be, in part, due to the impact of diet on satiety since low GI diets contribute to gut fill and low hunger levels that cause feelings of tiredness (Kukorelli and Juhasz, 1976; Fisher et al., 2004; Holt et al., 1999). However, the contrary effect has been observed in cats as stimulation of the small intestinal mucosa induces sleepiness and sleep (Wells et al., 1995).

Some researchers have found that diets high in CHO do not promote lassitude but facilitate the converse effect and promote positive effects on mood including feelings of less fatigue and depression (De Castro, 1987; Wells et al., 1997; Brinkworth et al., 2009). For instance, subjects reported being happier and more energized several hours after the consumption of diets high in CHO (Benton, 2002). Studies based on
observational data have reported a strong inverse relationship between symptoms of depression and carbohydrate intake among free-living humans (De Castro, 1987; Pellegrin et al., 1998). Specifically, CHO intake has been shown to improve mood in humans suffering from seasonal affective disorder and pre-menstrual syndrome (Benton, 2002). Further, high CHO intake, yielding elevated blood glucose levels has been associated to elevated feelings of energy during cognitively demanding tasks that was hypothesized to be an effect of increased glucose supply to the brain (Owens et al., 1997). It has been theorized that high CHO diets and the consumption of palatable CHOs act to improve mood by promoting the production of the neurotransmitter serotonin associated to reduced sleepiness, depression and anxiety (Porter and Horne, 1981; Fernstrom, 1986). However, this association can be greatly influenced by other dietary constituents (protein and fat) thus; the effects of CHO on serotonin may not be relevant in general populations consuming mixed palatable diets (Benton, 2002).

Several research groups have concluded that high fat diets promote greater feelings of lethargy and feebleness (Wells et al., 1995, 1997; Lloyd et al., 1994, 1996). After infusion with fat, sucrose or saline; fat infusion caused subjects to have reduced EE and heart rate versus when they were infused with sucrose (Wells et al., 1998). Subjects also felt more sleepy and dreamy with fat infusion versus saline and sucrose. Newborn babies fall asleep faster after the ingestion of a meal high in fat content versus high CHO (lactose) drinks (Oberlander et al., 1992). Meals high in fat have also been cited as increasing pain thresholds (90 min post feeding) by promoting more relaxed feelings versus isoenergetic diets high in CHO (Zmarty et al., 1997). Jenkins et al., 2006, observed reduced wakefulness in mice transferred from a low fat mouse chow (16% of energy) to a high fat test diet (59% of energy); however, the mice had increased feed intake and body weight gain with HF feeding that may have influenced behavioural outcomes and confounded the effects of diet. Humans who prescribe to the HF Atkins diet (60% fat, 10% CHO of energy) have reported significant increases in daytime fatigue, sleepiness and depression that may be a consequence of hypoglycemia and ketosis often observed during the consumption of diets low in CHO/high in fat (Afaghi et al., 2007; 2008; 2009). Dietary lipids act to the greatest extent over proteins and carbohydrates to stimulate the release of cholecystokinin (CCK) and somatostatin from the duodenum (Kapas et al., 1991). CCK, a peptide hormone responsible for the stimulation of digestion of fats and proteins, has been strongly implicated in the mediation of postprandial sleepiness as infusion of CCK produces sedative like effects in humans and animals (Stacher et al., 1979). Depression sensitive rats versus depression resistant rats displayed enhanced depressive like behaviours when consuming a high fat diet (Abildgaard et al., 2011). These impairments in mood were associated to higher fasting blood glucose in the depression sensitive rats, suggesting a metabolic association to mood impairments.
Conversely, some mood disorders, like depression, have been linked through epidemiological studies to deficits in fish consumption and thus, omega-3 FA; namely, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Parker et al., 2006). The decline in omega-3 FA consumption is caused by an increase in consumption of omega 6-FA (from saturated FA and common vegetable oils) leading to an increase in the ratio of omega-6 FA to omega-3 FA from 1:1 to 10:1 (Holub, 2002). Higher omega-6 causes a higher number of inflammatory eicosanoids in cell membranes potentially contributing to high rates of depression (Parker et al., 2006). Furthermore, high levels of inflammatory eicosanoids have been measured in individuals with unipolar and bipolar depression (Lieb et al., 1983). Low fat diets contributing to low concentrations of plasma cholesterol and TAG have also been associated to increased depression and impulsivity in otherwise healthy patients (Pompili et al., 2010). Conversely, diets contributing to high plasma cholesterol and TAG levels are often cited as being associated to a greater risk for cardiovascular disease; thus, a narrow range of plasma cholesterol and TAG is necessary to promote optimal health (Troisi, 2011).

While the immediate effects of the consumption of diets of differing fat and CHO types/amounts on mood is important, the long term effects should also be considered. Certain diets, as they contribute to metabolic disorders including: cardiovascular disease, obesity and diabetes, are hypothesized to have long term effects on mood through over activation of the hypothalamic-pituitary-adrenocortical (HPA) axis leading to elevated cortisol, reactivity to stress and clinical depression (Rivenes et al., 2009). Elevated cortisol may perpetuate obesity as heightened levels of the hormone can promote weight gain, via central adiposity, and increased feed intakes (Vicennati et al., 2009). In addition, increased inflammatory markers including: proinflammatory cytokines tumor necrosis factor-α, interleukin-1, interleukin-6, and interferon-γ, often found in obese patients, contributes to the development and pathogenesis of depression (Reviewed in Markowitz et al., 2008).

The mood of the cat can be inferred from their behaviour and appears to be affected by the consumption of diets of differing macronutrient (namely fat and CHO) content. While some suggest that there is no impact of dietary fat or CHO on mood (Rosen et al., 1982 and1985; Halyburton et al., 2007), a significant amount of research supports the idea that dietary fat and CHO content impacts mood through metabolic (energy sensing) effects on the brain. Differences in results have primarily been attributable to timing of measures (Parker et al., 2006). For instance, a sucrose preload prior to the consumption of a mixed meal caused calmness in healthy, adult men and women at 120 min post dosing as compared to preloads with
oil that caused feelings of tiredness at 60 min post dosing (Reid and Hammersley, 1999). In addition to the effects of diet on mood, CHO and fat content of the meal may impact cognitive function since macronutrient content of the diet has been shown to influence opioidergic and dopaminergic neurotransmission as evaluated by behavioural observation, physiological responses, and cognitive tests (Gibson and Green, 2002).

**Cognitive Effects of Diets High in CHO and Fat**

Certain diets, obesity and diabetes (causing perturbations in glucose and insulin handling) have been shown to cause cognitive deficits as these factors modify insulin, glucose, serotonin and leptin concentrations and function (Riby 2008; Gonzales et al., 2010; Gerozissis, 2010). These compounds further interact with hormones in the central nervous system (CNS) to influence cognition as represented through memory, learning and dementia (Gerozissis, 2010; Lustman and Clouse 2005).

The relationship between glucose supply and brain function is well established and focus has turned to the intricate relationship between glucose responsiveness, insulin sensitivity and cognition (Sieber and Trastman 1992; Sunram-Lea et al., 2001; Grinberg et al., 2003). The difference in response to glucose administration in healthy individuals with normal or modified glucoregulation and those individuals with diabetes suggests that a critical range of plasma glucose exists to promote cognitive function; levels outside of this critical range have been shown to promote cognitive malfunctions (Greenwood and Winocur, 2005). In rats, Long et al., 1992, found a correlation between impaired glucose tolerance and poor performance during maze testing. Similar results have been observed in humans as impaired glucose regulation, measured during a glucose tolerance test (GTT), was associated to poor performance during a vigilance task (Donohoe and Benton, 2000). Subjects with poor glucose regulation exhibited inferior performance on all memory tests; however, this reduced neuropsychological performance was corrected through the oral ingestion of a glucose drink (Awad et al., 2002). Impaired performance during memory testing has been seen in elderly humans with Type 2 diabetes (characterized by reduced insulin/glucose handling) (Strachan et al., 1997) versus healthy, similar age matched subjects (Kalmijn et al., 1995; Vanhanen et al., 1998; Manning et al., 1990). Cognitive impairments in diabetics can be reduced with improved glucose regulation (Parson and Gold, 1992). This suggests that inefficient glucoregulation causes cognitive declines (Gradman et al., 1993; Meneilly et al., 1993; Hewer et al., 2003; Riby et al., 2008). Diets high in CHOs (especially high GI CHOs) can perpetuate cognitive dysfunction in diabetic patients since acute hyperglycaemic episodes result in enhanced transitory cognitive malfunctions.
The administration of glucose has been shown to enhance memory in rats/humans consuming high fat diets without diabetes but likely exhibiting impairments in glucose tolerance and insulin sensitivity (Clandinin et al., 1993; Messier 1997; Hughes, 2002, 2003; Hughes and Neeson, 2003). Glucose administration centrally or peripherally can enhance memory function in healthy rats and humans if there is a decline in glucose availability to brain cells which often occurs with increased cognitive demands of memory (McNay et al., 2000; Gold, 1995). The decline in glucose availability within the brain during cognitive demands is supported by the fact that there is a significant decline in forebrain glycogen stores immediately following training and again 55 minutes later indicating the glycogenolysis is necessary for short term memory recall and long term processing and consolidation (O’Dowd et al., 1994; Hutchinson et al., 2008). The effects of glucose administration in healthy individuals are sometimes minimal and not all studies have concluded that glucose administration facilitates improved cognition (Means and Edmonds, 1998; Means et al., 1996; Messier, 1998). The intimate metabolic relationship between glucose and insulin can make it difficult to separate the effect of each substance on the CNS and some researchers have also highlighted insulin as one of the primary influencers of cognitive performance.

In early studies of the brain the role of insulin on glucose uptake was hypothesized to be insulin independent; however, recent research suggests that insulin may influence glucose uptake into the brain (McNay et al., 2010; McNay and Recknagel, 2011). Insulin is now highlighted as one of the main variables of cognition and CNS neuroplasticity (Burks et al., 2000; Gerozissis, 2003, 2004, 2008; Park, 2001). The essential role of insulin in the CNS is supported by the concentrated appearance of insulin receptors in the brain (Unger et al., 1991). Specifically, insulin functions to influence the actions of the medial temporal lobe (important for memory), hypothalamus and hippocampus as evidenced by modifications in glucose uptake and metabolism within these regions of the brain during complex learning and memory tasks (Bingham et al. 2002; Reger and Craft, 2006). Several studies have demonstrated that cognition is facilitated, in the form of enhanced memory, through an acute intravenous injection of insulin in healthy human subjects (Kern et al. 2006; Park et al. 2000; Reger and Craft 2006; Watson and Craft 2004). However, chronically high levels of peripherally circulating insulin which leads to insulin insensitivity and lower circulating levels in the brain causes impairments in cognitive function (Craft, 2007). The individual or combined effects of diet, obesity and diabetes can impact insulin sensitivity and, in turn, influence cognitive function. Impairments in cognitive function can impact quality of life and these types of effects are especially important to consider in elderly populations suffering from dementia and at greater risk for perturbations in glucose and insulin handling.
Certain types of fatty acids and carbohydrates can impact cognitive function. Increasing omega-3 FA consumption facilitates neurodevelopment during pre-natal growth (Karr et al., 2011), has a protective effect on cognitive decline during aging and improves cognition in animal models of Alzheimer’s by reducing β-amyloid pathology and neuronal cell death (Hooijmans et al., 2012). DHA has positive effects on brain function and cognition by promoting synaptic plasticity, transmission and membrane homeostasis (Wu et al., 2011). The effects of omega-3 consumption on brain glucose and insulin metabolism and sensitivity remain unclear and have only recently been investigated (Nugent et al., 2011). However, some researchers conclude that DHA stimulates glucose utilization (Pifferi et al., 2005) in the brain by changing brain insulin receptor signaling (Agrawal et al., 2011). Saturated fatty acids have the converse effect on cognitive function and have been shown to promote cognitive decline in rodents and exacerbate cognitive impairment in aging humans (Reviewed in Gomez-Pinilla, 2008). While CHO load may impact cognition glyceamic index also impacts memory; specifically, the consumption of diets with low GI values appears to improve learning in healthy children and adults (Benton et al., 2003; Micha et al., 2011). However, not all studies support the finding that low GI diets, that maintain elevated blood glucose levels in the later postprandial phase, promote improved cognitive function (Nilsson et al., 2009). Rather, diets with high GI values have been shown to improve cognition by increasing glucose availability within the brain for use during cognitive challenges (Smith and Foster, 2008). Lastly, some hypothesize that it is impairments in glucose tolerance that lead to the observed cognitive impairments regardless of glycemic load or index of the diet (Lamport et al., 2009); however, since certain diets with high versus low GI diets may pose a greater risk for the development of perturbations in glucose and insulin handling; thus, further investigation is warranted.

**Satiety Effects of Diets High in CHO and Fat**

Fat and CHO are hypothesized to have differing effects on feelings of satiety and hunger (Stubbs et al., 1993). Impacts of macronutrient load and composition of the diet and associated feelings of satiety may further impact cognition (Fischer et al., 2000), mood (deCastro, 1987) and spontaneous energy intake contributing to adiposity, weight gain and associated disorders (Rolls and Hammer, 1995). High fat diets may elicit increased (van Amelsvoort et al., 1989, 1990; Rolls et al., 1994; Cotton et al., 2007), decreased (Warwick et al., 1993) and similar (Driver, 1988; Rolls et al., 1991; Caputo and Mattes, 1992; Shide et al., 1995) feelings of hunger versus diets high in CHO. Increased energy intake with high fat diets have been hypothesized to be a consequence of: 1) the high level of palatability of fat leading to overeating,
contributing to gut fill and elevated caloric intake (Rolls and Shide, 1992), 2) decreased energy expenditure because of the low TEF of fat (Crovetti et al., 1998) and 3) positive fat balance due to the lack of autoregulation between fat intake and oxidation (Schwartz et al., 1985; Treuth et al., 2003). Flatt, 1987, concluded that CHO content of the diet drives energy intake due to the human body’s reliance on glucose for energy with limited storage capacity versus fat. However, this hypothesis has been disproven (Stubbs et al., 1993) and Little, 2007, argues that fat intake favors suppression of appetite via slowed gastric emptying and release of anorexigenic gastrointestinal hormones. Alternatively, chronic high fat intake may cause attenuation in feedback signals contributing to dissociation between fat intake and energy balance (Little, 2007). It remains unclear as to whether diets high in fat, CHO and protein contributes to feelings of satiety or hunger especially in the cat. Bradshaw et al., 1996, concluded that cats do not always exhibit hyperphagia with HF diets as is often hypothesized. In addition, Backus et al., 2007, found that plasma ghrelin, an orexigenic hormone, was negatively correlated to level of dietary fat in cats suggesting that fat is more satiating than initially thought. Conversely, it may be concluded that a reduced capacity to handle high amounts of CHO may lead to altered passage rates and gut fill contributing to satiety in cats (Wells et al., 1998). Lastly, Hewson-Hughes et al., 2011, concluded that cats have a “ceiling” for carbohydrate intake where fat intake appears to be more flexible to allow for balance of nutrients (namely protein). This “ceiling” of CHO intake significantly impacted total energy intake and thus, cats may be less satiated with HC diets than initially hypothesized because intake was below predicted values (Bermingham et al., 2010). The impact of dietary macronutrient content on satiety may not only influence feed consumption and ultimately body weight in the domestic cat but may also impact activity (energy expenditure) in the form of voluntary activity like play.

Hunger and Feline Play Motivation

Unlike other mammals, when cats are on a low plane of nutrition they do not decrease play behaviour, but have been observed to increase play behaviour (Bateson et al., 1990). Kittens subjected to weaning at 5 weeks of age compared to kittens that remained with their mother, demonstrated higher frequencies of play (Bateson and Young, 1981). The elevated play performance was likely due to the energy restriction (supported by measures of food intake) that often correlates to weaning. When milk provision was restricted, kittens (6-8 wks of age) developed play behaviour earlier than control kittens not subjected to lactation interruption (Bateson et al, 1981). In addition, a CR of 20% for the first 18 days after birth also caused increased demonstrations of play of kittens 78-84 days after birth (Bateson et al., 1990).
effects have been observed in adult cats as play motivation increased with greater time since feeding (Hall and Bradshaw, 1998). Overall, feeding (hunger) and predation appear to have related but separate motivational systems (Polsky, 1975) while the motivational system underlying play and predation are likely the same for cats (Hall and Bradshaw, 1998). Physiological support for this conclusion is the existence of separate but interacting areas of the brain that control predatory/play behaviour and hunger (Panksepp, 1998; Fonberg and Serduchenko, 1980). Stimulation of this integrative point evokes the full set of patterns of predatory behaviour and feeding in cats (Flynn et al., 1970). Similar responses have been observed in rats during the killing of a mouse; however, to our knowledge, has not been investigated in other non-predatory species (Karli et al., 1969). Hunger can act to enhance play and predation (Adamec, 1976; Hall and Bradshaw, 1998); however, hunger is not necessary for the performance of these behaviours if the appropriate stimuli are available and if an animal kills it does not mean the animal will subsequently feed on the carcass (Adamec, 1976). Furthermore, extreme hunger or starvation may cause a cat, previously identified as a non killer, to kill for food (Polsky, 1975). Therefore, the combined effects of level of hunger, external characteristics of the prey and/or availability of prey will dictate whether the animal demonstrates play, predatory or feeding behaviour as these behaviours are controlled by separate but interacting regions of the brain.

Metabolic perturbations in glucose and insulin handling may be influenced by diet type (composition and total intake). Diets high in fat and CHO; namely, high GI diets and those high in saturated fatty acids are hypothesized to be the greatest risk factors for the development of obesity and diabetes. Diet and therefore, plasma glucose and insulin can also impact behaviour via mood, cognition, satiety and consequently play motivation in the cat further contributing to the development of weight gain. Since the impacts of fat/CHO content on metabolism have not been clearly identified novel techniques designed to promote ideal glucose and insulin profiles are needed. One of the most vigorous techniques to promote optimal glucose/insulin profiles is calorie restriction or calorie restriction mimetics.

Calorie Restriction and Calorie Restriction Mimetics

Calorie restriction (CR), also known as dietary or energy restriction is defined as undernutrition without malnutrition. CR often involves a reduction in total energy intake by 30-50% relative to ad libitum controls with all essential nutrients and vitamins still being provided within the diet. CR is the most vigorous and reproducible intervention to inhibit the physiological effects of aging (nephrosis, periartheritis, myocardial degeneration and sarcopenia (Berg and Simms, 1965), to delay the
commencement of most pathologies (cardiovascular disease, diabetes, cancer, brain atrophy) and to extend mean and maximum lifespan by 20 to 40% (McCay et al., 1935; Weindruch and Sohal, 1997). The precise phenomena underlying the mechanisms of CR are currently unknown; however, there are specific physiological and behavioural changes linked to the benefits of CR including: increased physical activity, reduced body weight, fat, blood glucose, insulin, triglyceride and cholesterol, and increased insulin sensitivity and glucose tolerance (Koubova and Guarente, 2003). However, there are concerns associated with the feasibility of implementing such CR regimes for long periods of time because of the difficulty to maintain the reduced caloric intake and additionally because CR is related with negative behavioural effects.

Calorie restriction mimetics (CRMs) have been studied as an alternative to CR and to avoid some of the negative effects of CR regimens (Ingram et al., 2004). The objectives of CRM strategies are to produce the same pro-longevity effects that CR provides without reducing caloric intake. Since the prolongevity strategies of CR influence systems involved in energy sensing, and regulation of metabolism, some targets of CRMs focused on metabolites that modify glucose metabolism, one of the primary pathways used for the energy production via storage or catabolism. Certain CRM’s, known to influence glucose metabolism, that have been studied include: 2-deox-D-glucose (2DG), 5-thio-D-glucose, 3-O-methylglucose, anhydrosugars including 1,5-anhydro-D-glucitol, 2,5-anhydro-D-glucitol, 2,5-anhydro-D-mannitol and mannoheptulose (MH). These anti-metabolites exert a number of physiological effects that include a reduction of body weight, decrease in plasma insulin concentrations, reduction of body temperature, reduction in the rate of tumor formation and growth and elevation of circulating glucocorticoid hormone concentrations (Roth et al., 2001). The physiological effects mimicking CR result from CRM administration over an extended period of time and act through inhibition of glycolysis and/or glucose metabolism. Of the glucose anti-metabolites MH is deemed as the most effective, “safe” and available CRM to a variety of species including rabbits, humans, monkeys, rats and dogs (Roe and Hudson 1936; Blatherwick et al., 1940; Koh and Berdanier, 1974; Issekutz et al., 1977). Intestinal absorption was demonstrated in all highlighted species since blood levels and urinary excretion were found to be related to the volume of the ingested dose (Viktora et al., 1969).

MH, a seven carbon sugar, is present in common food sources; for instance, avocados can contain up to 5% wet weight (LaForge, 1916/17). MH enters cells via the GLU2 transporter (Rasschaert et al., 2001) and acts as a hexokinase (HK) inhibitor, more specifically a glucokinase (GK) inhibitor (Malaisse et al., 1968). By impacting the function of GK, MH prevents the phosphorylation of glucose to glucose-6-
phosphate blocking flux through the glycolytic pathway in the pancreas and the liver (German et al., 1993). The blockade of the glycolytic pathway with intramuscular (~25 mg MH/kg BW), subcutaneous (~1-2 ml MH/g BW) and oral administration (~4 ml MH/g BW) of MH leads to a decline of serum insulin facilitating a hyperglycemic state similar to that observed in diabetics (Viktora et al., 1969). Lernmark and Hellman, 1970, found that insulin secretion in both the early (initial transient release) and late stage (second more persistent release) was decreased with MH administration. These findings were supported by in vitro studies where MH was shown to inhibit glucose-stimulated release of insulin and reduce levels of cyclic AMP in slices of pancreatic tissues (Coore et al., 1963; Paulsen et al., 1967); however, Suzuki et al., 1976, concluded that MH only impacted early phase insulin release since the effect was observed 5 min after MH incubation with islet cells. Furthermore, elevated plasma glucose failed to suppress the release of glucagon when rats were fed MH (Simon and Frenkel, 1972) resulting in increased plasma concentrations of glucagon and hepatic cyclic AMP, likely for higher gluconeogenesis (Klain et al., 1976). The elevated glucose often observed with MH treatment may be caused by two factors: 1) a reduction in glucose clearance and uptake by cells due to inhibited insulin release and insulin insensitivity, and 2) an increase in gluconeogenesis due to the stimulatory effects of MH on the activity of hepatic fructose-1,6-bisphosphate, phosphoenolpyruvate carboxykinase and conversion of alanine to pyruvate for gluconeogenesis in the liver (Issekutz et al., 1977; Klain et al., 1976). It is unclear if increased glycogenolysis is one of the reasons for the observed elevation in plasma glucose concentration since a decline in liver glycogen content (Boquist, 1980) and an increase in liver glycogen (Simon and Kraicer, 1957) have both been measured in rodents fed MH. Differences may be attributable to time of measure since liver glycogen serves as an immediate glucose source maintaining blood levels and becoming depleted 12-18 hours after fasting (Akram et al., 2011). However, differences may also be correlated to level of glycogenesis in the liver. Glycogenesis may be increased if blood glucose levels are high favoring glucose storage; however, with MH administration glucagon to insulin ratios are high, insulin signaling may be impaired and the action of glucokinase in the liver is blocked promoting glycogen deposition in muscle which contains only 1% glycogen versus the liver which contains 6-8% glycogen or hyperglycemia (Akram et al., 2011). Furthermore, acute treatment with MH causing a correlated suppression in insulin release leads to a decline in intestinal glucose absorption following an intragastric [U-14C] glucose administration in vivo (Argiles et al., 1992). The combined effect of MH on insulin and glucose leads to a lowered rate of whole-body glucose oxidation (Argiles et al., 1992). Several other groups have also observed that dietary MH supplementation causes a decline in glucose oxidation in isolated islet cells of the mouse pancreas (Hedekov et al., 1972; Ashcroft et al., 1970; Matschinsky et al., 1971). The inhibitory effect of MH on glucose oxidation has further been supported by Sener et al., 1998,
who noted that islet cells of the pancreas incubated with mannoheptulose at 1.0 mmol/l on decreased glucose utilization in addition to glucose oxidation. Scruel et al., 1998, noted that other organs (liver and parotid cells) were less impacted than pancreatic islet cells on functional responses to glucose.

Increased fatty acid (FA) oxidation and decreased FA synthesis are hypothesized to be the underlying metabolic adaptations to CR (Bruss et al., 2010). MH has been shown to produces similar effects. Koh and Berdanier, 1974, found that FA oxidation increased with a 20 mg dose of MH. The additional FA oxidation is likely a consequence of increased release of NEFA, due to the absence of the inhibitory affect of insulin, into plasma by adipose tissue and enhanced hepatic uptake of FA for oxidation as has been observed in MH treated rats (Simon et al., 1972; Mitzkat and Meyer, 1970). Klain et al., 1976, also demonstrated that MH suppresses hepatic fatty acid synthesis as indicated by a reduced incorporation of carbon skeletons of glucose into fatty acids and by a decreased activity of acetyl CoA carboxylase. Fatty acid synthesis was restored by exogenous insulin therefore the effect was thought to be mediated by glucagon. MH had no consequence on the action of other enzymes that support lipogenesis; further, MH did not affect adipose tissue fatty acid synthesis (Klain et al., 1976). MH causes a shift in energy metabolism from glucose metabolism to favor FA oxidation and a decline in lipogenesis beneficial for weight maintenance and regulation of blood glucose and insulin.

Overall, MH acts in a competitive manner to block D-glucose phosphorylation impacting the functional and metabolic effects of glucose in the pancreatic islet B-cells by inhibiting the activity of glucokinase and hexokinase (Volsky and Dimant, 1978; Scruel et al., 1998). Cats, as obligate carnivores, have unique metabolic processes, thus glucokinase (GK) activity and GK gene expression are minimal or absent in the feline liver (Kley et al., 2009). GK, one of the four isoenzymes of the mammalian hexokinase (HK) group, is considered to be one of the rate limiting enzymes of glycolysis as GK is responsible for catalyzing the first reaction of glycolysis. In the cat, the activities of hexokinase, fructokinase (FK), pyruvate kinase (PK), glucose-6-phosphate dehydrogenase (G6PD), fructose-1,6-bisphosphatase (FBPase), and glucose-6-phosphatase (G6Pase) are significantly higher as compared to dogs, who are thought to have normal GK activity (Tanaka et al., 2005). The higher activity of these alternative enzymes may compensate for the lack of GK activity in cats and impact the responsiveness of feline energy metabolism to MH since compensatory mechanisms already exist to adapt to limited glucose phosphorylation. However, Picton and Malaisse, 1999, demonstrated that cells with minimal GK activity (starved) were still capable of responding to MH. Therefore, there are likely additional factors influencing responsiveness to dietary MH and the minimal GK activity in the cat may not completely inhibit the
physiological effects observed in other species. Cats are also unique to many other species as they appear to prioritize fatty acid oxidation versus glucose oxidation. This is evidenced through the elevated activities of FK, PK and G6PD in the feline liver that promote higher activities of fatty acid synthesis as compared to the canine liver (Tanaka et al., 2005). Furthermore, cats appear to be in a constant state of gluconeogenesis (MacDonald et al., 1984); enzymatic support for the elevated rate of gluconeogenesis in the cat liver is the observed increase of FBPase and G6Pase activity - two key enzymes of gluconeogenesis (Rogers et al., 1977). Several researchers have shown that the effectiveness of the inhibitory action of MH on glucose metabolism can be influenced by the cellular environment namely: 1) the capacity for intracellular transport of MH and 2) the extracellular (or in vitro medium) glucose concentration (Malaisse et al., 1968; Scruel et al., 1998; Picton and Malaisse, 1999). Therefore, the unique cellular environment created by a constant state of gluconeogenesis and prioritized FA oxidation in the cat may influence MH inhibition of glucose phosphorylation and warrants further investigation. However, due to the prevalence of feline obesity a technique such as MH designed to positively impact energy metabolism favoring longevity and optimal glucose/insulin profiles is invaluable.

In conclusion, intricate relationships exist between dietary fat and CHO content, metabolism and behaviour in the cat, an obligate carnivore. Research is needed to elucidate the direct affects of each and the relative risk for the development of weight gain and associated diseases since cats, characterized by unique metabolisms, are popular house pets and the prevalence of obesity is increasing. To address these issues, identification of techniques and methods to facilitate accurate and reliable measures of energy metabolism in the cat must be identified and utilized effectively.

Methods used to Determine Energy Requirements

The techniques that have been utilized to determine the energy requirements (ER) of the domestic cat include: predictive equations, feeding experiments and weight maintenance studies, the doubly labeled water technique and indirect calorimetry (Table 1). During maintenance, a physiological steady state, energy requirements are based on the idea that there is no net change in body energy content and energy balance is zero (Baldwin, 1995). Some methods approximate the amount of energy required from food to support daily requirements; however, only assumptions can be made regarding the individual components, including resting EE (similar to BMR), of the energy budget. Methods, like indirect calorimetry, able to measure resting EE are of interest since resting EE accounts for approximately 70% of total daily EE. When resting EE is subtracted from EE in the fed state the thermic effect of feeding (TEF) can be calculated. TEF is influenced by the energetic costs of digestion and assimilation that include bond
breakage, nutrient absorption, and synthesis of digestive proteins and storage of nutrients (Baldwin, 1995). When the summation of EE and TEF are subtracted from metabolizable energy (ME) of the diet, net energy (NE) balance can be determined.

Equations predictive of ER were developed to compare basal metabolic rate (BMR) of a variety of species. Kleiber, 1932, and Brody et al., 1945, formed an equation for analysis of interspecific basal heat production based on the relationship between fasting heat production (FHP) and body weight (BW) as these relate to body surface area. The equation $FHP = 70W^{0.75}$ permits for a simple comparison across species; however, it is not appropriate for within species comparisons since age, body weight and gender significantly impact statistical fits to the equation changing coefficients and exponents drastically (Baldwin, 1995). NRC, 2006, recommends the equation $100 \text{ kcal ME/kg BW}^{0.67}$ to predict ER for normal adult lean cats and $130 \text{ kcal ME/kg BW}^{0.4}$ for adult obese cats. Though these equations offer some guidelines, the NRC, 2006, commented on variability between cats and within similar environments some cats may require 50% more or fewer calories per day than the mean estimate. More recently the equations $53.7 \text{ kcal/kg BW}^{-1.061}$, $46.8 \text{ kcal/kg BW}^{-1.115}$ and $131.8 \text{ kcal/kg BW}^{-0.366}$ have been proposed for use in light, normal and heavy cats following a recent review of the literature pertaining to ER of cats (Bermingham et al., 2010). These equations are frequently utilized by clinicians to estimate ER of cats; however, equations do not offer a means to study the effects of age, sex, breed, health, environment, neutering status and diet on ER (Baldwin, 1995); therefore, close monitoring of body condition/weight correlated to certain daily energy intakes is commonly utilized to find an ideal energy requirement. Similar practices are occasionally employed in the research environment and are referred to as feeding experiments.

Feeding experiments to measure weight maintenance are the most popular methods to determine ER of cats. Feeding studies are simple to conduct, require minimal human intervention, place few experimental demands on the animal and allow the animal to live in its natural environment. In a recent review by Bermingham et al., 2010, the mean daily ER for cats as measured during feeding experiments is equal to $230.2 \pm 6.9 \text{ kcal/d}$ or $58.0 \pm 1.6 \text{ kcal/kg BW/d}$. A feeding experiment will often last 40 days during which feed is offered to the subject *ad libitum* or at a pre-determined amount. Daily energy intake to support BW within a certain range (± 5% BW) is determined by means of measuring the caloric density of the diet and total amount consumed per day (Nguyen et al., 2004). Disadvantages associated to feeding experiments include the length of the study, the large sample sizes, the inability to measure specific metabolic factors such as: BMR, heat increment of feeding (HIF) and energetic costs of activities. Also, body weight can be
maintained in animals with too low and too high conditioning; thus, an understanding of ideal body composition is necessary so animals are not maintained at inappropriate body weights.

Mean daily ER for cats as measured using the doubly labeled methodology is equal to 234.2 ± 17 kcal ME/kg BW or 59.2 ± 2.4 kcal ME/kg BW/d (Bermingham et al., 2010). Doubly labeled water (\(^2\text{H}_2\text{O}\)) can be administered orally or intravenously. Following isotopic equilibrium between the O\(_2\) atoms of body water and CO\(_2\), the labeled hydrogen (\(^2\text{H}_2\)) is eliminated as water (\(^2\text{H}_2\text{O}\)) while the O\(_2\) isotope is eliminated as water (H\(_2\)\(^{18}\text{O}\)) and as expired carbon dioxide (C\(^{18}\text{O}_2\)). Differential elimination rates between labeled oxygen and hydrogen equals the carbon dioxide production representing carbohydrate, fat and protein oxidation and EE. A typical doubly labeled water study will last 7 to 21 days (Seale and Rumpler, 1997). The doubly labeled water method is advantageous since measurement of EE is in free living, natural conditions in a variety of animal species of different stages of life. The method is considered to be highly accurate with an error of approximately 6-8% within a group (Levine, 2005). Many assumptions are associated to technique and the primary assumption is that the body water pool is steady and isotopes are only exchanged with body water and CO\(_2\) (bicarbonate) pools; however, these simplistic exchanges are unrealistic (Schoeller, 1988). Steady state is unlikely to occur since water and CO\(_2\) fluxes are dependent on changes in physical activity and water intake; two factors that are highly variable but quantitatively unimportant. In addition, oxygen and hydrogen exchange outside of water and bicarbonate pools. For instance, oxygen may be lost during exchange with bone phosphate and carbonate and hydrogen may be lost from feces, urea, synthesis reactions (lipid or protein) and exchange with gut contents (i.e. cellulose). Together, these losses may contribute to an overestimation of water space but, have minimal effects on overall measures (Schoeller, 1988). The isotopes can be expensive and RQ of the food must be estimated to permit for the calculation of EE which may also induce errors (Seale and Rumpler, 1997). Lastly, timely collection of blood, saliva or urine, in particular, required for isotope analysis may be difficult and stressful for free living and domesticated animals since collections must be taken before the isotopes are completely eliminated.
Table 1: Advantages and disadvantages of the methods used to measure energy requirement in the domestic cat.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Predictive Equations</th>
<th>Feeding Experiments</th>
<th>Doubly Labeled Water Technique</th>
<th>Indirect Calorimetry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple to employ</td>
<td>Non-invasive</td>
<td>Free living</td>
<td>Application during a variety of activities (exercise, rest) under highly controlled conditions</td>
</tr>
<tr>
<td></td>
<td>No cost</td>
<td>Free living</td>
<td>Accurate measure of ER (6-8% error within a group)</td>
<td>Used on wide range of species in different life stages and health statuses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimal human intervention required</td>
<td>Measure ER at various life stages (including during lactation)</td>
<td>Allows for long or short term studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Can collect long term (mean=1-3 week) measures of EE</td>
<td>High level of accuracy (within a single subject or group) for a relatively low cost</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ability to measure specific elements of ER (BMR, HIF, activities)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Minimal level of invasiveness</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>No measure of the effects of age, sex, breed, health, environment, neutering status and diet on ER</td>
<td>Long term</td>
<td>Expensive</td>
<td>Not easily measured in free living animals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Large sample size</td>
<td>Relatively invasive</td>
<td>Several assumptions/correction factors used</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inability to measure specific elements of ER (BMR, HIF, activities)</td>
<td>Necessitates animal handling and re-capture for saliva, blood or urine samples</td>
<td>Machinery must be accurately maintained</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Several assumptions/correction factors used</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cannot measure 24 hr ER or elements of ER</td>
<td></td>
</tr>
</tbody>
</table>
Indirect calorimetry is used to determine EE and substrate oxidation from respiratory gas exchange (volume of oxygen consumed (VO₂) and carbon dioxide produced (VCO₂)). EE data is combined with respiratory quotient (RQ) measurements to determine absolute amounts of fat, carbohydrate and protein utilized for energy. RQ is defined as the ratio between VCO₂/VO₂ or nCO₂ produced/nO₂ consumed molecules. The general formula to determine the volumes of CO₂ and O₂ required for oxidation of a fuel is: Fuel + nO₂ \rightarrow nCO₂ + nH₂O. RQ estimates typically range between 0.7 and 1.00 as fat has an RQ value of approximately 0.7, protein has an RQ value of approximately 0.84, and carbohydrate has an RQ value of about 1.00. Fat has an RQ value of 0.7 because during the metabolism of fat more oxygen is consumed relative to the amount of carbon dioxide produced; however, during carbohydrate metabolism the amount of oxygen consumed equals the amount of carbon dioxide produced leading to an RQ value closer or equal to 1.0. Meta-analysis of the literature from 1933-2009 suggests that mean daily ER for cats, as measured during indirect calorimetry experiments, is equal to 203.7 ± 8.2 kcal ME/kg BW or 50 ± 1.5 kcal ME/kg BW/d (Bermingham et al., 2010). Indirect calorimetry measures are advantageous as they allow for the calculation of BMR, TEF and patterns/levels of substrate oxidized; however, the energy expended during activities of normal living are not included in ER estimates using indirect calorimetry. As a limitation, there are several assumptions associated with the indirect calorimetry methodology which include: 1) all oxygen that disappears from the inspired air is solely utilized for biological oxidations, 2) all carbon dioxide added to the expired air is derived exclusively from the complete combustion of substrates, and 3) the entire concentration of urinary nitrogen results only from the oxidation of amino acids. Twenty-four hour urinary nitrogen is difficult to reliably and non-invasively collect in animals; thus, a constant is commonly placed into the algorithm used to calculate EE to reflect mean daily nitrogen excretion (Weir, 1949). This estimation of nitrogen excretion results in minimal error (~4%) and is therefore considered to be clinically acceptable (Ferrannini, 1988). Lack of recent validation and calibration of the machine could lead to inaccurate results and a lack of steady state kinetics may lead to the rejection of the aforementioned assumptions and predictions (Blaxter, 1989).

In conclusion, indirect calorimetry is considered the “gold standard” in measuring EE in clinical environments since the technique provides a sensitive, relatively inexpensive and non-invasive means to monitor energy metabolism. However, the unique metabolism of cats often requires a minimum 20 hour collection period. The prolonged restriction is of concern since cats are highly sensitive to the characteristics of their external environment and a novel environment, such as a respiratory chamber, may trigger a stress response. Stress responses in the cat are not overt and pose an additional issue for
researchers to accurately monitor welfare via behavioural changes indicative of stress. To reduce variability and improve validity of results cats selected for nutritional trials should be healthy, display normal behaviours, and be well-adapted to a particular environment to represent a physiological replica of the “pet cat” characterized by a constricted range of physical, behavioural, and clinical factors (Meunier, 2006).

**Behavioural Responses of Cats to Stressful Environments and Research Methods**

The domestic cat is unusual in that many display a reduced or inhibited behavioural repertoire in situations of chronic stress (Rochlitz, 1998). This inhibition is unlike many species that often show active abnormal, stereotypical behaviours such as pacing in order to cope with stress or frustration (Shepherdson et al., 1993; Casey and Bradshaw, 2007). Several behaviours that are often inhibited in response to stress in the domestic cat, include self-maintenance, exploratory, and play behaviours (McCune 1992, Rochlitz, 1998). Play and exploration may be indicative of positive emotions and are rapidly inhibited in many species during situations of fitness threats or when environmental conditions are perceived as more challenging (Fraser and Duncan, 1998; Dawkins 2006); however, self-maintenance behaviour (feeding, elimination, and grooming) is important for survival and is less likely to be inhibited in stressful environments as is observed in the cat (Rochlitz, 2000). There is; however, some abnormal behaviour exhibited by the cat in response to confinement triggering a stress response that may include demonstration of: pica, self mutilation, increased aggression, fearfulness, and reduced reproductive success (Rochlitz, 2005). Short term stress has received much less attention than chronic stress responses in cats. Behavioural and postural changes describing extreme vigilance and escape/hiding behaviour can be indicators of acute stress as they often decline after short term exposure to a novel environment (McCune, 1992). As discussed previously, a decline in overt signs of stress may not indicate that all stressors or frustrations have been resolved. The characteristics of the environment that are most often cited as influencing cat behaviour include the quantity of space, quality of space; specifically the social, sensory, and nutritional environments (Rochlitz, 2005). Understanding that cats may act to hide their stress response warrants careful monitoring under situations that might be perceived as threatening. This is particularly important in research environments in which novel practices are often utilized since overt signs of stress may be absent.

Acclimation procedures reduce fear and discomfort improving psychological well-being of the animal (Laule and Whittaker, 2007). To minimize the effects of a captive environment acclimation procedures have been implemented. Acclimation procedures employed on a variety of captive species, including
nyala (Grandin et al., 1995), bongo (Phillips et al., 1998), snow leopards (Broder et al., 2008), baboons (O’Brien et al., 2008) and chimpanzees (Lambeth et al., 2006) have resulted in reduced serological and behavioural indicators of acute stress responses. Furthermore, white blood cell count, neutrophils, hematocrit, glucose, cortisol, escape behaviours and the need for anesthesia during routine husbandry practices can be minimized with acclimation. Acclimation procedures include desensitization, habituation, and operant conditioning. Desensitization is the process of minimizing/removing aversive stimuli associated to an event by providing a positive stimulus during the event (Chance, 2003). Habituation is repeated exposure to a particular stimulus; consequently, the response is decreased over time (Webster, 1994). Operant conditioning is the association of particular behaviours and the resultant consequences, the consequences (positive or negative reinforcement) then influence the types and frequency of behaviour displayed. It is likely that a combination of techniques practiced in a consistent manner would produce the best outcome for cats since the highest degree of environmental control would be provided through predictable and positively reinforced practices (Carlstead et al., 1993).

To reduce animal variability, cats selected for nutritional trials should be healthy, display normal behaviour, and be well-adapted to a particular environment to represent a physiological replica characterized by a constricted range of physical, behavioural, and clinical factors (Meunier, 2006). Stressful environments interfere with normal behavioural and physiological responses. As the animal attempts to cope with the environment (fight or flight response) the associated stress modifies physiological parameters via activation of the sympathetic nervous system and the hypothalamo-pituitary-adrenal axis causing release of epinephrine and glucocorticoids (Seematter et al., 2005). Cumulatively, these hormones act to modify metabolism increasing: 1) resting energy expenditure; 2) glycolysis (Seematter et al., 2000), 3) lipolysis (Wennlund et al., 1994); 4) insulin insensitivity (Selye, 1971), 5) blood pressure (Herd, 1991) and 6) ventilation rate (Seematter et al., 2005). Stressful environments can influence the validity of the experiment by introducing abnormal animals, or animals demonstrating behaviour patterns outside of their normal behavioural repertoire (Garner, 2005). The reliability and replicability can be reduced by increasing behavioural and thus physiological variation between and within individual animals due to differing environmental perceptions and techniques used to cope to stressful situations (Garner, 2005). To ensure that the biological measurements of the research cats are applicable to free living “pet” cats, behavioural tests must be completed to quantify the stress levels of the cats. The behavioural tests, often utilized to quantify stress levels, include the Cat-Stress-Score test, the novel object test, feeding, and elimination behaviour (McCobb et al., 2005). These behavioural tests have been utilized to assess an animal’s response to a novel environment and to determine when an animal is
acclimated to a particular environment to produce good quality scientific data (Boxall et al., 2004). Valid results applicable to the “pet” cat population are indicated by the performance of normal and low stress indicative behaviour (Carlstead et al., 1993, Loveridge et al., 1995). In addition to behaviour tests of stress, physiological markers of stress that can be measured include: glucocorticoids (cortisol in the cat via activation of the hypothalamic-pituitary-adrenocortical axis), metabolic and cardiovascular changes (Iki et al., 2011). However, these physiological measures may be unpredictable and not always correlated to behavioural responses of stress (Koolhaas et al., 2007). For instance, cortisol is pulsatile in nature and all physiological parameters may be influenced by emotional reactivity of the animal (coping style) age, body weight, seasonal changes, diet and measurement technique (Reviewed in Iki et al., 2011).

Acclimation procedures used to accustom cats to temporary restriction within chambers used in indirect calorimetry studies range from no acclimation (Carpenter, 1944; Peachey et al., 1999; Nguyen et al., 2004; Hoenig et al., 2006; Leray et al., 2006) to an acclimation of one week with restriction periods of 2, 4 and 6 hours, and, to our knowledge, only two studies, used observations of feeding, drinking and movement, to determine successful acclimation (Lester et al., 1999; Green et al., 2008). Lester et al., 1999, concluded that cats were acclimated to respiration chambers when normal behaviour patterns were observed; however, it is unclear what the researchers defined as normal. Since cats can be uncommunicative when stressed, further explanation of the term ‘normal’ is warranted. Green et al., 2008, allowed cats to acclimate to respiration chambers for 3 consecutive days prior to collections; “behaviour” (undefined) and feed intake were monitored to measure adaptation. Russell et al., 2002, stated that cats were “familiarized” to the respiratory chambers; however, details of this program were also omitted. Wichert et al., 2007 and 2009, allowed cats to acclimate to the chambers for one day prior to collecting data on days 2 to 5 with no comment on indices of acclimation. Based on previous research, the acclimation procedures describe for indirect calorimetry studies are insufficient to fully adapt cats to restriction within chambers. Kessler and Turner, 1997, demonstrated that two thirds (N=140) of the cats adjusted to a novel environment (boarding cattery) after two weeks of exposure after which cats were removed. Measurement of stress was completed using a detailed behavioural and postural scoring system called the Cat-Stress-Score. Furthermore, when cats were exposed to a novel quarantine situation or group/individual cage restriction, stress response (measured using behavioural scoring systems for stress) declined within the first 24 hours, continued to decline after several days of exposure and further declined with prolonged exposure periods up to five weeks when measurements ended (Smith et al., 1994; McCune, 1992; Rochlitz, 1995).
Indirect calorimetry measures are sensitive to small changes in metabolism. Since stress response can impact metabolism the necessity for complete adaptation to chambers is essential (Seematter et al., 2005). Based on the evidence presented, regarding length of adaptation to novel environments (~2 weeks) and restriction (~5 weeks), the procedures used to acclimate cats to respiration chambers are insufficient. Robust measures of metabolism via calorimetry allow researchers to study the impacts of macronutrient content on metabolism. Specifically, fat and CHO content of the diet have been cited as risk factors for the development of obesity and associated diseases; however, the data available to support these conclusions are limited (Panciera et al., 1990; Lund et al., 2005; McCann et al., 2007; Slingerland et al., 2007a). Obesity is often associated with dysregulation of metabolic pathways, particularly by changes in glucose, insulin and lipid metabolism, which can, in turn, be further influenced by diet type and other environmental factors (Lewis et al., 2002). Factors contributing to obesity, specifically the impact of fat and CHO on metabolism, are of concern since the most commonly cited disease in cats is obesity (Hoenig et al., 2011). Also, the effects of diet on behaviour (mood, cognition, feed intake and voluntary activity) need to be investigated as they may act to compound metabolic effects further contributing to weight gain. A holistic assessment of the factors contributing to feline obesity will allow for the design of optimal diets supporting superior welfare.
References


Agrawal, R.; Ying, Z.; Gomez-Pinilla, F., 2011: Dietary DHA influences cognition by modulating brain insulin receptor signaling. Society for Neuroscience Abstract Viewer and Itinerary Planner, 41


Bateson, P.; Mendl, M.; Feaver, J. 1990: Play in the domestic cat is enhanced by rationing of the mother during lactation. *Animal Behavior* 40, 514-525.


Berg, B. N.; Simms, H.S., 1965: Nutrition onset of disease and longevity in rat. *Canadian Medical*
Association Journal 93(17), 911.


Caputo, F.A.; Mattes, R.D., 1992: Human dietary responses to covert manipulations of energy, fat, and


Castre’n, E.; Rantamäki, T. 2008: Neurotrophins in depression and antidepressant effects. *Novartis Found Symposium* 289, 43-59, 87-93.


Craft, S. 2007: Insulin resistance and alzheimer's disease pathogenesis: Potential mechanisms and


Fernstrom, J.; Wurtman, R., 1971: Brain serotonin content - increase following ingestion of carbohydrate diet. *Science* 174(4013), 1023


and Molecular Neurobiology 23(1), 1-25.


Green, A.S.; Ramsey, J.J.; Villaverde C.; Asami, D.K.; Wei, A.; Fascetti, A.J., 2008 Cat are able to adapt protein oxidation to protein intake provided their requirement for dietary protein is met. Journal of Nutrition 138, 1053-1060.


Lamport, D. J.; Lawton, C. L.; Mansfield, M. W.; Dye, L., 2009: Impairments in glucose tolerance can have a negative impact on cognitive function: A systematic research review. Neuroscience and Biobehavioral Reviews 33(3), 394-413.


Lernmark, A.; Hellman, B., 1970: Effect of epinephrine and mannoheptulose on early and late phases of glucose-stimulated insulin release. Metabolism 19(8), 614


Markowitz, S.; Friedman, M. A.; Arent, S. M., 2008: Understanding the relation between obesity and depression: Causal mechanisms and implications for treatment. *Clinical Psychology-Science and Practice*


Means, L. W.; Edmonds, S. M., 1998: Glucose minimally attenuates scopolamine- but not morphine-induced deficits on a water maze alternation task. *Journal of Neural Transmission*, 105(10-12), 1171-


Messier, C., 1997: Object recognition in mice: Improvement of memory by glucose. *Neurobiology of Learning and Memory* 67(2), 172-175.


Nabb, S. L.; Benton, D. 2006: The effect of the interaction between glucose tolerance and breakfasts


Nguyen, P.G.; Duman, H.J.; Siliart, B.S.; et al. 2004: Effects of dietary fat and energy on both bodyweight and composition after gonadectomy in cats. *American Journal of Veterinary Research* 65, 1708–1713


Tragelaphus eurycerus) for veterinary and husbandry procedures at the denver zoological gardens. *Zoo Biology* 17, 25-32.


Shepherdson, D.J.; Carlstead, K.; Mellen, J.D.; Seidensticker, J., 1993: The influence of food presentation on the behaviour of small cats in confined environments. *Zoo Biology* 12, 203-216


Slingerland, L.I.; Vasilova, V.V.; Plantinga, E.A.; Kooistra, H.S.; Beynen, A.C., 2007a: Indoor confinement and physical inactivity rather than the proportion of dry food are risk factors for the development of feline type 2 diabetes mellitus. *Veterinary Journal*. In Press.


Stubbs, R. J.; Murgatroyd, P. R.; Goldberg, G. R.; Prentice, A. M., 1993: Carbohydrate balance and the
regulation of day-to-day food-intake in humans. *American Journal of Clinical Nutrition* 57(6), 897-903.


British Journal of Nutrition 61(2), 267-283.


Wells, A.; Read, N., Macdonald, I., 1998: Effects of carbohydrate and lipid on resting energy expenditure,
heart rate, sleepiness, and mood. *Physiology & Behavior* 63(4), 621-628.


CHAPTER 1: Development and validation of a behavioural acclimation protocol for cats to respiration chambers used for indirect calorimetry studies\textsuperscript{1,2}

Margaret A. Gooding\textsuperscript{1}, Ian J.H. Duncan\textsuperscript{1}, Jim L. Atkinson\textsuperscript{1}, and Anna K. Shoveller\textsuperscript{1,2}

Authors Last Name for PubMed indexing: Gooding, Duncan, Atkinson, Shoveller

Addresses:
\textsuperscript{1}University of Guelph, Animal and Poultry Science, Guelph, Ontario, Canada N1G 2W1
\textsuperscript{2}Procter and Gamble Pet Care, Mason, Ohio, USA 45040

*To whom correspondence should be addressed: shoveller.ak@pg.com

Keywords: Acclimation, Cat-Stress-Score, Novel Object Test, cat, indirect calorimetry

Funding: This work was supported by Procter and Gamble Pet Care, Mason, Ohio, USA 45040

Conflict of Interest and Funding Disclosure: I.J.H.D., and J.L.A have no conflicts of interest. A.K.S has financial and personal interest in The Procter and Gamble Co. due to employment with the funding company and M.A.G is a PhD. intern.

Authors’ Contributions: M.A.G. and A.K.S. designed research with major contributions from J.L.A. and I.J.H.D. M.A.G conducted research, analyzed data, wrote the paper and had primary responsibility for final content. All authors read and approved the final manuscript.

Abbreviations used: CSS, Cat-Stress-Score, NOT, Novel Object Test
Abstract

Cats exposed to stressful and novel environments such as, animal shelters, boarding catteries and restriction within cages, initiate stress responses as evidenced by behavioural and physiological changes which, in turn, modify energy requirement and metabolism leading to the collection of unreliable results from nutritional trials conducted on stressed cats. Fourteen cats (10 ± 2 months) were subjected to an 11 week acclimation procedure to adapt cats to restriction within a chamber used for indirect calorimetry studies. Cats were acclimated to the chambers during weeks 1-3 by means of 24 hr free access to the chambers in their home environment, to the study room during weeks 4-6 with daily periods of 20, 40 and 60 min exposure on successive weeks, and to increasing periods of restriction of 1 to 20 hrs within the chamber during weeks 7-11. Ten additional cats (11 ± 1 month), used as controls, were subjected to a single 5 hr restriction without prior chamber or study room exposure to compare responses. Stress level, feed intake, fearfulness and eliminations were recorded. Cat-Stress-Scores (CSS) and latencies to approach a novel object both peaked on weeks 4 and 7 (P<0.05). CSS declined with exposure to a novel experience and on week 11, stress levels were low and consistent (P<0.05). There was a difference in CSS between un-acclimated and acclimated cats (P<0.05). In conclusion, acclimation protocols prepare cats for repeated, temporary restriction within chambers, whereas short acclimations do not. A step up acclimation procedure with behavioural indices of stress should be utilized to prepare cats for research that necessitates restriction in calorimetry chambers.
Introduction

Training animals to different environments or tasks ensures that they are accustomed and do not experience stress while encountering these. Acclimation and training procedures employed on a variety of captive species, including nyala (Grandin et al., 1995), bongo (Phillips et al., 1998), snow leopards (Broder et al., 2008), baboons (O’Brien et al., 2008) and chimpanzees (Lambeth et al., 2006) have resulted in reduced serological and behavioural indicators of acute stress responses. Furthermore, white blood cell count, neutrophils, hematocrit, glucose, cortisol, escape behaviours and the need for anesthesia during routine husbandry practices have been minimized in response to successful training and acclimation.

Appropriate housing conditions and low stress levels are important to obtain optimal welfare for research cats. Stress related behavioural and neuroendocrine responses modify energy requirements and macronutrient metabolism potentially increasing animal variability and reducing the reliability and validity of results from nutritional trials (Meunier, 2006). Cats usually respond to novel and stressful environments such as animal shelters, boarding catteries and restriction within cages, by inhibiting normal activity patterns and/or reducing overall activity (Rochlitz, 1999). The domestic cat is very unusual in that many individuals also show a reduced or inhibited behavioural repertoire in situations of chronic stress generally showing less active stereotypical behaviours such as pacing in order to cope with stressful situations (Shepherdson et al., 1993; Casey and Bradshaw, 2007). Normal behaviour patterns inhibited in response to chronic stress, include self-maintenance, exploratory, and play behaviour (McCune 1992, Rochlitz, 1999). Acute stress has received much less attention than chronic stress responses in cats; however, there are behavioural and postural indicators of acute stress, like activity, body and tail position, which may decline after short term exposure to a novel environment (McCune, 1992).

Stress response and fearfulness of cats can be measured using a variety of behavioural observations, such as: the Cat-Stress-Score (CSS) and Novel Object Test (NOT). The CSS is a non-invasive behaviour score to test for fearfulness and has been utilized to measure acclimation of cats to novel environments (McCune, 1994, 1995; Kessler and Turner, 1997) and ranges from “fully relaxed” (score 1) to “extremely stressed” (score 7) based on posture and behavioural elements. The Novel Object Test is a test for fear response to, or the perception of threat from, novel stimuli (Boissy, 1995).

Behavioural acclimation procedures used to accustom cats (*Felis Silvestris Catus*) to temporary restriction within chambers used in indirect calorimetry studies range from no acclimation to an acclimation of one
week with restriction periods of 2, 4 and 6 hours, and, to our knowledge, only one study, used behavioural measurements of feeding, drinking and movement, to determine successful acclimation (Lester et al., 1999). Lester et al (1999) concluded that cats were acclimated to respiration chambers when normal behaviour patterns were observed; however, it is unclear what the researchers defined as normal, and since cats can be uncommunicative when stressed, further explanation and definition of the term ‘normal’ is warranted. Kessler and Turner (1997) demonstrated that two thirds of all cats (N=140, total) in their study adjusted to a novel environment (boarding cattery) after two weeks of exposure, as indicated by measurement of stress behaviour. When cats were exposed to a novel environment, such as a quarantine situation or group/individual cage restriction, stress response declined within the first 24 hours, continued to decline after several days of exposure and further declined with prolonged exposure periods up to five weeks (Smith et al., 1994; McCune, 1992; Rochlitz, 1995). Therefore, it can be hypothesized that the acclimation protocols used in previous oxidation studies were insufficient for complete acclimation to a novel environment such as a respiratory chamber. Based on the evidence presented, regarding length of adaptation to novel environments (~2 weeks) and restriction (~5 weeks), an eleven week acclimation protocol was hypothesized to be a conservative estimate of a sufficient amount of time to produce full acclimation.

The objectives of the present study were to design an acclimation procedure for cats to respiratory chambers, and to demonstrate that cats successfully adapt to the chambers. Acclimation was measured by the performance of normal behaviour, including feed intake, elimination, low fear response, and a low incidence of behaviour indicative of stress (Carlstead et al., 1993, Loveridge et al., 1995). We hypothesized that over the eleven week acclimation period, the cats would successfully acclimate to restriction within the respiratory chambers and the associated environment; acclimation would be indicated by low CSS, and fear responses, the demonstration of elimination and by normal feed intake as determined during baseline observations.

**Material and Methods**

All procedures were reviewed and approved by The Institutional Animal Care and Use Committee established at The Iams Company, Procter and Gamble - Pet Care and was in accordance with The Iams International Animal Welfare Advisory Board standards.

*Animals:* A total of 24, domestic shorthaired cats (10 months ± 2, 3 kg ± 1.5) were selected from the Pet
Health and Nutrition Center (PHNC) at Procter and Gamble - Pet Care, Lewisburg, Ohio. Standard veterinarian evaluation of overall health was completed prior to the initiation of the study and all cats entered the study healthy.

*Diet:* Cats were fed 60 g/d of Iams® Multi-cat dry Chicken diet; each cat was provided approximately 232 ME kcal/d. Cats were fed 30 g per feeding, and feed was provided at 7:30 am and 1:00 pm. Cats were fed individually and each cat was permitted 60 minutes to eat during both food offerings.

*Housing:* Cats were group housed in a free-living environment with inside/outside access during the day but kept inside at night. The room was outfitted with environmental enrichment including perches, beds, toy houses, scratching posts, toys and climbing apparatuses. All cats had daily social interaction for a minimum of 60 minutes. Socialization entailed human petting, grooming and interaction time with restricted access toys. Cats were maintained on a 12 hour lighting schedule with the lights turning on at 6:30 am and turning off at 6:30 pm. The room temperature was maintained at 22°C and relative humidity >50%. Rooms were cleaned daily, and disinfected weekly. Water was provided ad libitum from automatic waterers.

Respiration calorimetry chambers, made of Plexi-glass, were 53.3x53.3x76.2 cm. The chambers were outfitted with a shelf, feeder, water bowl, hammock, litter box, toy and a free area with a fleece bed. Water was provided ad libitum from water bowls. The chamber was designed to allow separation of feeding, sleeping and elimination areas. Chambers and water bowls were disinfected, and litter, litter boxes, toys, hammocks and fleece beds were removed, cleaned and replaced after each use. Calorimetry machinery was provided by Qubit Systems®, Kingston, Ontario, Canada.

*Acclimation Procedure:* Fourteen cats (N=14) were selected (based on age (<1 yr) and health) and subjected to an eleven week acclimation procedure to respiratory chambers. Fourteen cats were used since oxidation techniques require at least 10 to 12 cats to provide an adequate number of data points for determining nutrient requirements. The cats were allocated to one of three groups based on body weight as determined prior to the initiation of the study. Cats were acclimated to the primary researcher prior to the study; normal behaviour was observed during these social interaction periods and all cats were observed to willingly approach and interact in a friendly manner. Once the cats were determined to be acclimated to the primary researcher, baseline behavioural observations were taken.
**Weeks 1 to 3:** For the first three weeks of acclimation the cats had free access (24 hrs) to the respiratory chambers within the room in which the cats were permanently housed. One chamber was closed so cats could become acclimated to the metal door and the other chamber was open to allow free access to the chamber and materials inside. Chambers were outfitted as if prepared for temporary restriction as described above.

**Weeks 4 to 6:** During weeks four to six, cats were introduced to the study room. With each week of exposure to the study room cats were introduced to increasing amounts of calorimetry machinery as the installation process was completed. On week four, five and six of the acclimation period cats were exposed to the study room for 20 minutes, 40 minutes, and one hour, respectively. Cats were exposed to the study room five times each week; therefore, each cat was exposed to the study room 15 times prior to restriction within the chamber. The primary researcher was present to offer positive reinforcement through talking, playing with and petting the cats.

**Weeks 7-11:** During week seven, the cats were restricted within the respiratory chamber three times for one hour/restriction. Following restriction, cats were permitted 20 minutes of free access within the study room. On week eight, individual cats were restricted within the respiration chamber for 5 consecutive hours with 20 minutes of free access to the study room after restriction. On week nine, ten and eleven, cats were housed in the respiration chamber for 10 hours, 15 hours and 20 hours, respectively. Each cat was restricted only one time each week throughout weeks eight to eleven. At the end of each confinement period, cats were provided with positive reinforcement in the form of petting, playing, and talking. Food was offered to the cats if restriction coincided with regular feeding times.

**Behavioural Observations:** The Cat-Stress-Score (CSS) was utilized to rank the level of stress of cats based on postural and behavioural indicators. The CSS system used has been described at length in Kessler and Turner (1997). Briefly, a score of 1 was given to cats demonstrating fully relaxed behaviour and a score of seven was administered to cats demonstrating terrorized behaviour; if cats demonstrated behaviour split between two levels then a half score was assigned. Once acclimated to the primary researcher and prior to the introduction of novel stimuli five baseline CSS were collected over a five day period. During the three weeks of free access to the chambers (weeks one to three) five CSS observations were conducted per week for a total of 15 observations. Throughout weeks four to eleven cats were assessed at 10%, 50%, and 80% time points, for two minutes, after initiation of each acclimation period with the exception of the 20 minute study room acclimation period on week 4 in which the behavioural
measurements were completed at 5, 10 and 15 minutes. Time intervals are represented as a percent of the total time for a particular acclimation period for each study day. To calculate a particular time point for behavioural assessment the following equation was used:

\[
\text{Observation Time (min) = \% Time \times Total Duration of Exposure (min)}
\]  

[Equation 1]

Cats exhibiting abnormal behaviour or stress levels above or equal to a CSS of 6 were removed from the study. One observer was responsible for all CSS measurements.

Novel object tests were conducted to determine the cats’ latency to approach within a 5 cm radius of the novel object, as measured in seconds, to assess level of fearfulness and response to novelty. The duration of each observation was five minutes; if a particular cat did not approach during the five minute observation period they were classified as “no approach”. Cats were assessed at the 85% time point once each week on the first day of exposure (day 1) of each week for the entire acclimation period. Novel object tests completed in the study room (Weeks 4 to 6), without restriction within the chamber, were initiated upon the placement of the novel object in the center of the study room. The novel object tests completed while the cats were maintained in their feeding cages and chambers, started when the experimenter placed a novel object in the front left corner of the chamber or cage. The novel object was changed for each test to maintain novelty. Each novel object that was selected differed from those that cats had been routinely exposed to and additionally differed each week of study. Order of exposure was consistent for all cats.

After each twice-daily feeding, the remaining feed was weighed and daily feed intake was calculated for each cat. After each restriction period eliminations were recorded for each cat.

**Control Group: Un-acclimated Cats:** Two groups of five cats (N=10) of similar age, weight and genetics to the cats undergoing acclimation to the respiratory chambers were selected (base on age (<1 yr) and health) to act as a control group that had no previous experience of the study room or respiration chambers. The ten cats were restricted one time within the chambers without acclimation and for a total of 5 hours. During the five hour restriction, behaviour was monitored, including: stress level, fearfulness. Elimination was not measured in the control group of un-acclimated cats as the restriction duration was only 5 hours total. Three CSS values were measured during restriction at the 10%, 50%, and 80% time points. If cat stress scores exceeded or equaled a CSS of 6, then the cats were immediately removed from
the chambers. A NOT was performed to measure level of fearfulness of the cats. The NOT was performed at the 85% time point. The control group of cats was subjected to similar housing and daily management routines as the acclimated group of cats.

**Statistical Analysis:** CSS, feed intake and NOT data analysis for the acclimated group of cats were completed using repeated measures ANOVA. CSS data were analyzed within week, within day and between weeks while NOT and feed intakes were analyzed between week using the proc mixed function of Statistical Analysis System (SAS, version 9.1; SAS Institute Inc., 2002-2003). NOT data were further analyzed using the life-test procedure of SAS to test homogeneity of survival curves for latency to approach the NOT which yielded product-limit survival estimates for the acclimated group. Correlation between CSS and NOT during acclimation was tested using Pearson correlation coefficients and the corr procedure of SAS. CSS and NOT of acclimated versus un-acclimated cats was completed using repeated measures ANOVA and was further analyzed using the proc glm function of SAS. Differences were compared using the Wilcoxon signed-rank test and the Kruskal–Wallis one-way analysis of variance to ensure consistency of results. Differences were considered significant when \( P<0.05 \) and outliers were determined using a 95% confidence interval.

**Results**

**Cat-Stress-Score:** Cats displayed between-week, within-week and within-day variation in Cat-Stress-Scores. During weeks four (first exposure to study room) and seven (first restriction in respiration chamber) of the acclimation procedure the cats displayed significantly higher stress scores than all other weeks (\( P<0.05 \)). Between weeks 4 to 6 and 7 to 11 of the acclimation procedure, CSS decreased. On weeks 4, 5, 7 and 8, CSS at the 10% time point within each study day were significantly higher than the CSS obtained at the 50% and 80% time points (Figure 1; \( P<0.05 \)). By week 11 of the acclimation procedure CSS was consistent throughout the entire exposure period and did not change significantly within day (Figure 1; \( P<0.05 \)). Overall, CSS at the end of the acclimation procedure during week 11 were lower than the CSS obtained during baseline observations (Figure 1, \( P>0.05 \)). No cat subjected to the acclimation procedure displayed significantly higher stress scores when compared to another cat undergoing acclimation.
Figure 1: Least squares means of Cat-Stress-Scores (mean±SEM) of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment on day 1 of each week of the acclimation procedure at the 10%, 50% and 80% time points. Letters identify relative differences among Cat-Stress-Scores for time within day and over the 11 acclimation weeks, means not sharing a superscript letter are significantly different; “a” represents the highest value and “f” the lowest (P<0.05).

CSS declined with day of exposure within week for weeks 4 to 7 as there was a significant decline in CSS from Day 1 to Day 5. CSS did not decline significantly during weeks 1 to 3 and for week 3 there was a small decline on day 3; however, CSS did not differ significantly between Day 1 and Day 5 (Week 4-6) or Day 3 (Week 7) (Figure 2; P>0.05).
Figure 2: Least squares means of Cat-Stress-Scores (mean±SEM) of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment on each sampling day within a particular week with multiple observations per week. Letters identify relative differences among Cat-Stress-Scores within week, means not sharing a superscript letter are significantly different; “a” represents the highest value and “d” the lowest value (P<0.05). Days within week without superscripts do not differ (P>0.05). Weeks 8-11 are not represented as there were no repeated day measures within these weeks.

**Novel Object Test:** One cat consistently exhibited a higher latency to approach the novel object (seconds) and was therefore removed from analysis as a statistical outlier (P>0.05). One cat did not approach the novel object within the 300 second time limit on Week 5 of the acclimation procedure and was classified as a “No Approach”.

Latencies to approach the novel object were significantly different on different weeks of the acclimation procedure (Figure 3; P<0.05). Increased latency to approach the novel object appeared to be correlated to the additional introduction of a novel event (study room or restriction within the chamber) as latency to approach was high on both weeks 4 and 8 when cats were exposed to the study room and restriction within the chamber.
Figure 3: Least Squares Means of latency to approach in seconds (mean±SEM) to the novel object within a 5 cm radius of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment. Letters identify relative differences among Cat-Stress-Scores; means not sharing a superscript letter are significantly different; “a” represents the highest value and “d” the lowest value (P<0.05).

On weeks 1 to 3 and 9 to 11, the cats displayed a similar approach pattern to the novel object (Figure 4). During these weeks cats approached the novel object almost immediately and the probability that all cats approached the novel object within 15 seconds of exposure was 100%. On weeks 4 to 8, survival distribution and the pattern of approach to the novel object was variable since all cats did not approach the novel object immediately following exposure. Approximately, 20% of cats subjected to the NOT displayed longer latencies, greater than 25 seconds, to approach the novel object on weeks 4 to 8. During weeks 9 to 11, the pattern of approach was uniform, since all cats approached the novel object within 10 seconds of exposure.
**Feed Intake**: Feed intake did not significantly differ between individual cats (P>0.05). There were differences observed in average weekly feed intake (Figure 5; P<0.05); however, these differences were < 3.57 grams or 6% of total intake; therefore, we do not feel this is physiologically significant.
Figure 5: Least squares means of weekly feed refusal (ORTs) with a 60 g daily feed offering (mean +/- SEM) of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment. Letters identify relative differences among feed refusal, means not sharing a superscript letter are significantly different; “a” represents the highest value and “c” the lowest value (P<0.05).

Elimination Behaviour: All cats subjected to the acclimation protocol showed some form of elimination behaviour during restriction within the respiration chamber. Although there were no quantitative measurements of elimination; the evidence was simply that the litter box had been used.

Correlation between Cat-Stress-Score and Novel Object Test: There was no consistent correlation between CSS at the 80% time point and the latency to approach the novel object on the specific test day (P>0.05). The associated Pearson Correlation r and p-values were as follows: week 4 (r=-0.02, p=0.9); week 5 (r=-0.02; p=0.6); week 6 (r=0.18, p=0.5); week 7 (r=0.53, 0.05); week 8 (r=0.51, p=0.4); week 9 (r=0.02, p=0.5); week 10 (r=0.3, p=0.3); week 11 (r=0.03; p=0.9). CSS and latency to approach were only correlated on Week 7 of the acclimation procedure.

Comparison of Un-acclimated and Acclimated Cats: Three un-acclimated cats were removed from the chambers because these cats exhibited a CSS of at least 6. Average CSS for the un-acclimated (3 ± 0.5) cats (excluding the cats that were removed) was significantly greater (P<0.05) as compared to the average CSS for the acclimated cats (2 ± 0.3).
CSS at the 10% time point was higher than the CSS at the 50% and 80% time points demonstrating that time (10%, 50%, and 80%) within test day had a significant effect on the CSS of both un-acclimated and acclimated groups of cats. The reduction in CSS was significant for the acclimated group of cats from the 10% to the 50% and 80% time point; however, the CSS of the un-acclimated cats did not decrease significantly during the same time frame (Figure 6; P<0.05).

Figure 6: Comparison of least means squares (mean±SEM) of Cat-Stress-Scores of un-acclimated (N=7) and acclimated cats at that 10%, 50% and 80% time points during a 5 hr exposure to the indirect calorimetry equipment and associated environment (N=14). Letters identify relative differences among Cat-Stress-Scores within day; “a” represents the highest value and “b” the lowest value (P<0.05). Means not sharing a common single or double asterisk are significantly different between groups; “*” represents the highest value and “**” the lowest value (P<0.05).

Un-acclimated cats had a mean latency (± SEM) to approach the novel object as measured at the 85% time within the 5 hr restriction in seconds of 13.21 ± 13.36 and the acclimated cats exhibited a mean latency to approach of 9.89 ± 13.36. Latency to approach the novel object in seconds was not significantly different between the un-acclimated and acclimated group of cats with one or two sided t-tests used to analyze these data.
Discussion

All cats successfully acclimated to the respiration chambers when exposed to the acclimation procedure, whereas cats that were not exposed to an acclimation procedure demonstrated greater levels of stress-related behaviour during restriction. CSS increased when cats were introduced to a novel environment but declined over time (within day, week and between weeks) with exposure. During the last weeks of acclimation cats demonstrated low fear responses and rapid approaches to a novel object. All acclimated cats demonstrated some form of elimination behaviour, and feeding patterns were consistent with those observed during baseline observations.

At the start of the last week of acclimation (week 11), CSS had declined to levels below those observed during baseline observations but similar to those observed during weeks 1, 2 and 3 when cats had unrestricted access to chambers located within the group living room (Figure 1). Elevated stress levels during baseline observations were likely a consequence of a disturbed room environment because one of the cats required veterinary care disrupting the normal housing routine, which has been demonstrated, can act as a stressor for cats (Carlstead et al., 1993). Cats did not display behaviour indicative of stress when subjected to 24 hour free access to respiration chambers. This lack of disruption was likely achieved by placing the chambers in a familiar environment. This procedure provided cats with an opportunity to control the situation by facilitating behavioural choices and adoption of various flexible strategies decreasing the perceived degree of novelty (Broom and Johnson 1993; Rochlitz, 2000). The largest increase in stress response was observed during weeks 4 and 7 when cats were initially exposed to the study room and to chamber confinement. The change in stress level could be attributable to the novelty of the environments as a consequence of spatial modification, through changes in scent, auditory and visual stimuli, and sudden changes in the predictability of the environment, coupled with a degree of social isolation during chamber confinement (Carlstead et al., 1993). CSS levels at the 10% time point on week 6 (third week of exposure to the study room) and week 8 (second week of restriction) were similar to baseline levels. CSS at the 50% and 80% in week 8 declined to levels observed in weeks 1 to 3 suggesting that cats in fact adapted more rapidly to enclosure restriction than to the study room environment (Figure 1). The study room indeed contained higher levels of novel stimuli which would tend to stimulate curiosity, vigilance, and responsiveness of cats (Weipkema and Koolhass, 1992). Cats (including the control group) were acclimated to enclosure restriction as kittens and had been routinely exposed to all devices employed within the chambers in an attempt to minimize associated stress response. Research indicates that previous exposure to a cattery or shelter reduced the time required to fully
acclimate cats to the environment upon return (Kessler and Turner, 1997; McCune, 1994, 1995). Cats require between two and five weeks of acclimation when exposed to a novel environment and this depends to some extent on previous exposure (Rochlitz et al., 1998; Kessler and Turner, 1997). Indeed, in the present study, cats required approximately two weeks to fully adapt to restriction with previous experience of restriction and exposure to the chamber and associated environment, and more than three weeks to adapt without previous exposure to the study room environment. Rochlitz et al. (1998) and Smith et al. (1990), found that cats continued to show reduction in stress characteristics when exposed to a shelter or quarantine environment for greater than one month; however, we did not observe any similar reduction in CSS beyond one month of exposure. McCobb et al. (2005) observed a consistent decline in CSS with increased exposure to a shelter environment. However, others have suggested that some cats may not in fact have fully adapted to an extended period of restriction but may have instead lapsed into a state of listlessness and depression resulting in increased time spent sleeping typical of adoption of a passive defensive system commonly observed in felids and other carnivores in captivity (Carlstead et al., 1992). The CSS attempts to distinguish between stressful, feigned sleep and true, relaxed sleep; however, the difference is often difficult to determine and occasionally disturbed cats which exhibit apparently normal sleep behaviour may be incorrectly classified as having a low CSS. The CSS was selected for this study as the scale has successfully been proven and validated and classified as a non-invasive behavioural measure of stress in cats in response to novel environments (Kessler and Turner 1997, 1999; Kakuma and Bradshaw, 2001), people (Kessler and Turner, 1999) and enrichment tools (Kry and Casey, 2007). We felt that the CSS, when used in conjunction with other established behavioural measures, would facilitate accurate assessment of non-invasive behavioural characteristics fundamental to proper determination of the true status of cat acclimation.

CSS status did not significantly change during weeks 1 and 2 with changes observed during week 3 of the acclimation program. Average CSS during day 1 was not different from average CSS on day 5 for weeks 1, 2 and 3 as would be expected since no novel experience or object was introduced. Conversely, within-week CSS did significantly decrease from the first day to the last day on weeks 4, 5, 6 and 7 that cats were exposed to the room or chamber environment (Figure 2). Smith et al., 1990, found that behavioural indicators of stress in cats exposed to a novel environment declined quickly after the first four days of exposure to the novel environment. The present results are similar to those of previous studies where cats exposed to a novel environment displayed the highest stress responses during the first few days of the experience (Smith et al., 1990; McCune 1992 and 1995; Rochlitz, 1994; Kessler and Turner, 1997).
On weeks 4, 5 and 7 to 10, CSS at the 10% time point of exposure, within each study day, were observed to be significantly higher than the CSS obtained at the 50 and 80% time point. During weeks 11 and 6 CSS between percent time points was not different (Figure 1). McCune (1992) observed that the greatest reduction in stress levels occurred during the first ten minutes of exposure and between the first and second hour after restriction within a novel caged environment. Similarly, our data suggest that observed stress levels decline with increasing exposure and the largest reduction in stress response occurs between the 10% and 50% time point.

All cats subjected to the acclimation procedure successfully adapted to the respiration chambers; no cat subjected to the acclimation procedure displayed significantly higher CSS at any time point. Kessler and Turner (1997) observed some variability amongst cats, as approximately 4% of their cats did not demonstrate any behavioural signs of acclimation after two weeks in the cattery. McCobb et al. (2005), also observed some variability between cats, but as cats spent more time in the shelter environment, variability between cats declined. Behavioural variability is attributed to differences in genetics, maternal care, previous experience, and features of the current situation (Mendl and Harcourt, 2000). The similarity in genetics, maternal care and previous experience of the cats in our particular study was likely the reason for the observed similarity in their response to novelty and restriction.

During the NOT, cats demonstrated neophobic tendencies during weeks 4 to 8 as latency to approach the novel object were highest during these weeks (Figure 3). Cats in stressful environments will frequently exhibit neophobic behaviour as a consequence of their perception of threat due to the unknown controllability and predictability of the environment (Boissy, 1995). Though cats demonstrated higher levels of stress during exposure to the study room and during the first two weeks of restriction, there was no correlation between CSS at the 80% time point and the latency to approach the novel object on the specific test day. The delayed approach to the novel object on weeks 4 to 6 may have been a consequence of a lack of interest, as cats often continued interacting/playing with another object within the study room. The higher latencies to approach the novel object during exposure to the study room may have been a direct consequence of the environment and the distance which the cats had to travel, as the NOT were completed during restriction for all weeks except weeks 4 to 6. Latency to approach the NOT on weeks 7 and 8, the first two weeks of restriction, differed with the higher approach latency being associated with week 8. The delay in responsiveness to novel stimuli during restriction may have been a consequence of the feeding regime imposed upon these cats as they are individually restricted for an hour within an assigned cage once daily for feeding; therefore, the hour restriction in the chambers may not have been
entirely novel. Latency to approach the NOT on weeks 1 to 3 and 9 to 11 were rapid (less than 25 seconds) and consistent between cats (Figure 4). The low level of avoidance, fearfulness and the consistency of time to approach the object at the end of the acclimation period would suggest that cats were successfully adapted to such restriction.

All cats subjected to the acclimation demonstrated some form of elimination behaviour. Contributors to inhibition of elimination behaviour include environmental and social stress, household disruptions/changes medical problems, in particular feline urological syndrome, and a cats’ idiosyncratic inclination for specific elimination locations (Olm and Houpt, 1988). Since study cats demonstrated normal elimination patterns it can be reasonably concluded that restriction within the chamber did not in fact cause any significant stress response which may have inhibited or modified normal urination or defecation patterns.

In stressful environments, cats have been observed to inhibit feed intake, modify feed selection habits and generally demonstrate anorexia and/or neophobic tendencies when a food source is presented under stressful conditions (Mugford and Thorne, 1980). In the present study, cats maintained consistent feed consumption patterns during the entire acclimation procedure and this suggests that the cats were not stressed to a level which inhibited nor modified feed intake. The small variation in feed intake observed during weeks 1 to 3 of the study may have been attributed to the change in feeding process put in place for the study; once the feeding protocol was regimented feed intake did not change. The decrease in feed intake on week 11 was ~2 g and likely not physiologically indicative of stress as all other signs of stress were low on this week (Figure 5).

There were significant differences noted between stress levels in cats undergoing acclimation and the control cats who were subjected to a one time five hour restriction. The cats that were subject to unscheduled removal from the chambers displayed a variety of stress-related characteristics including strong escape behaviour, extreme vigilance, increased respiratory rates, fearful immobility and destructive behaviour (Casey and Bradshaw, 2007); no such characteristics were observed in the acclimated group of cats. Further, un-acclimated cats did not show a significant reduction in CSS with exposure to the respiratory chambers in contrast to the acclimated group (Figure 6). Such differences were determined to be a consequence of previous experience within the study room and with restriction within the respiration chambers causing differing perceptions of the degree of novelty of the environment and level of perceived threat to homeostasis thus, initiating a proportional stress response. Un-acclimated cats took longer to
approach the novel object, and this is associated with display of strong neophobic behaviour and further supports the conclusion that un-acclimated cats had significantly higher stress responses to restriction within respiration chambers than acclimated cats.

The eleven week acclimation procedure led to successful acclimation of young cats to the novel respiration chamber and study environment. This acclimation procedure may be used for cats being trained for indirect calorimetry studies and, with a high success rate, fewer animals may need to be selected for training since it is expected that almost all candidate cats will become fully and successfully acclimated. It should be noted that this acclimation procedure utilized young, spayed and neutered cats and the acclimation procedure may require adaptation if applied to older or intact cats. Furthermore, as there was an increase in stress score with each introduction to a novel environment it may be beneficial to increase the length of acclimation to minimize the significant increase in stress response with each new exposure. It is imperative that normal study cats, following full acclimation, demonstrate low levels of stress during confinement in the respiration chambers in order to achieve robust, accurate and repeatable data collection derived from indirect calorimetry studies. High stress responses and, presumably cortisol, typical of the un-acclimatized cat may have an adverse impact on macronutrient metabolism, energy requirements and the overall wellbeing of the research animals.
References

Boissy, A., 1995: Fear and fearfulness in animals. Quart Rev Biol. 70, 165-191


Kessler, M.R.; Turner, D.C., 1997: Stress and adaptation of cats (Felis Silvestris Catus) housed singly, in


Shepherdson DJ, Carlstead K, Mellen JD, Seidensticker J. The influence of food presentation on the


CHAPTER 2: Effects of high fat and high carbohydrate diets on fat and carbohydrate oxidation and plasma metabolites in healthy cats\textsuperscript{1,2}

Margaret A. Gooding\textsuperscript{1}, Elizabeth A. Flickinger\textsuperscript{2}, Jim L. Atkinson\textsuperscript{1}, Ian J.H. Duncan\textsuperscript{2} and Anna K. Shoveller\textsuperscript{1,2}

Authors Last Name for PubMed indexing: Gooding, Flickinger, Atkinson, Duncan, Shoveller

Addresses:
\textsuperscript{1}University of Guelph, Animal and Poultry Science, Guelph, Ontario, Canada N1G 2W1
\textsuperscript{2}Procter and Gamble Pet Care, Lewisburg, Ohio, USA 45338

*To whom correspondence should be addressed: shoveller.ak@pg.com

Running Title: Carbohydrate and fat oxidation in cats

Keywords: indirect calorimetry, carbohydrate balance, fat balance, oxidation, energy expenditure, glucose.

Funding: This work was supported by Procter and Gamble Pet Care, Lewisburg, Ohio, USA 45338

Conflict of Interest and Funding Disclosure: I.J.H.D. and J.L.A have no conflicts of interest. E.A.F. and A.K.S have financial and personal interest in The Procter and Gamble Co. due to employment with the funding company and M.A.G is a PhD. intern.

Authors’ Contributions: M.A.G. and A.K.S. designed research with major contributions from E.A.F., J.L.A. and I.J.H.D, M.A.G conducted research, analyzed data, wrote the paper and had primary responsibility for final content. All authors read and approved the final manuscript.

Abbreviations used: CHO, carbohydrate, HF, high fat, HC, high carbohydrate, EE, energy expenditure, RQ.
Abstract

High fat (HF) or high carbohydrate (HC) diets (30% fat, 18.9% carbohydrate; HF and 10% fat, 46.3% carbohydrate; HC) and lengths of adaptation were investigated in cats (Felis catus; 10 ± 2 mo, 3 ± 1 kg). Cats randomly received each treatment for 14 d in a crossover design with a 14 d washout period between each diet. Three 20-h indirect calorimetry studies were conducted after acute (d 0), semi-chronic (d 4) and chronic (d 13) dietary exposure. Blood samples collected after a 24 hr fast on d 1, 5 and 14. When cats consumed the HC and HF diet, oxidation of the restricted nutrient exceeded intake while oxidation of the nutrient in excess matched intake. Mean max energy expenditure (EE) of cats consuming the HF and HC diet were 107 and 102 kcal/kg^{0.67}/d and occurred at a mean of 245 and 712 min post feeding, respectfully. Maximal fat (0.90 g/h) and carbohydrate (CHO; 1.42 g/h) oxidation were attained at 26 and 626 min post feeding, respectfully. The changes observed in macronutrient oxidation and EE suggest that cats adapt whole-body nutrient metabolism in response to changes in dietary macronutrient content, but may require longer than 14 d to adapt to a macronutrient that is present at a lower concentration in the diet.
Introduction

Approximately 35% of cats (*Felis Silvestris Catus*) are classified as overweight or obese in the United States (Lund et al., 2005). Consequentially, diabetes mellitus is a widespread disorder in the domestic cat population with an estimated incidence of 2.45 cases/1000 cat years-of-risk (Panciera et al., 1990). Along with age, physical activity, and indoor confinement, type of diet has been identified as a risk factor for the development of weight gain and associated metabolic disorders including diabetes (Panciera et al., 1990; Lund et al., 2005; McCann et al., 2007; Slingerland et al., 2007a). Obesity, metabolic syndrome, and diabetes are associated with dysregulation of metabolic pathways, particularly by changes in glucose, insulin and lipid metabolism, which can, in turn, be further influenced by diet type and other environmental factors (Lewis et al., 2002).

It has been hypothesized that prolonged consumption of high carbohydrate (CHO) diets may contribute to the development of feline diabetes mellitus by promoting obesity and by exaggerating the postprandial glycemia and insulinemia. This overstimulation of the pancreatic β cells may lead to β cell exhaustion and ultimately dysfunction (Brand Miller and Colagiuri, 1994; Rand et al., 2004). These hypotheses are based on the theory that cats have a limited capacity to handle high CHO loads due the diminished presence of CHO-sensing and processing pathways. Cats do not express of the *TAS1r2I* gene, which is essential to taste dietary sugars (Li et al., 2005). Cats also lack salivary amylase, which aids in initiating CHO digestion (Kienzle, 1993). Compared to omnivorous species cats have reduced activities of hepatic glucokinase (Pilkis et al., 1968), pancreatic amylase, and intestinal disaccharidases (Meyer and Kienzle, 1991). Another, more speculative theory linking HC diets to obesity is that prolonged insulin release from excess dietary CHO diverts fat from oxidation to fatty acid synthesis and storage in adipose tissue (Richard et al., 1989). The prolonged insulin release with HC diets is compounded by the persistently high level of gluconeogenesis in cats that further slows the removal rate of glucose from plasma (Meyer and Kienzle, 1991).

Conversely, several studies have shown that cats fed a high fat (HF), rather than HC diets are more susceptible to weight gain and metabolic disorders (Thiess et al., 2004; Backus et al., 2007; Slingerland et al., 2007b; Coradini et al., 2011). In humans and cats, HF diets have been shown to increase energy intake, decrease energy expenditure (via a reduction in thermic effect of feeding), and increase body fat accumulation (due to impaired autoregulation between fat intake and oxidation) (Schwartz et al., 1985; Treuth et al., 2003). Several studies have shown that cats fed HF diets are more susceptible to metabolic
changes that produce weight gain and perturbations in glucose handling (Thiess et al., 2004; Backus et al., 2007). Higher dietary fat inclusion in premium or specialty dry cat foods has a stronger association with weight gain than dry “grocery type” diets, which are typically higher in CHO and lower in fat content (Scarlett et al., 1994; Nguyen et al., 2004; Coradini et al., 2011). However, this observation has not been consistently replicated. In other epidemiological studies, obesity rates had a stronger association in cats fed commercially available dry type diets higher in CHO versus commercially available canned, higher fat diets (Oscai et al., 1984; West et al., 1994).

Although several studies have investigated the effects of HC and HF diets on body weight, glycemia, and insulinemia, it remains unclear as to which type of diet, HC or HF, poses to be the greatest risk for the development of obesity and diabetes in cats. Furthermore, there is a paucity of research in cats examining the dynamic response of macronutrient oxidation after immediate and chronic exposure to HC and HF diets. To our knowledge, there have been no studies that have measured the effect of HF vs. HC diets on blood biomarkers and substrate oxidation simultaneously. Therefore, the current study was designed to investigate the time course effects of feeding a HF versus a HC diet on blood biomarkers of metabolism and on energy and macronutrient oxidation in the fasted, fed, and extended post-prandial states.

Materials and Methods

All procedures were reviewed and approved by The Institutional Animal Care and Use Committee established at The Iams Company, Procter and Gamble - Pet Care and was in accordance with The Iams International Animal Welfare Advisory Board standards.

Animals: Fourteen domestic shorthaired gonadectomized cats (5 females, 5 males) of similar age (10 ± 2 months), body condition (BCS=3 ± 0.5; 5 point BCS scale) and weight (3.6 kg ± 0.3) were selected from the Pet Health and Nutrition Center (PHNC) at Procter and Gamble Pet Care, Lewisburg, Ohio. Standard veterinarian evaluation (physical exam, chemical and CBC blood analysis) of overall health was completed prior to the initiation of the study and all cats entered the study healthy.

All cats were previously acclimated to respiration chambers and associated environment (Gooding et al., 2012). Acclimation success was assessed using the Cat-Stress-Score (CSS; Kessler and Turner, 1997), feed intake, fearfulness (response to novel stimuli) and elimination behaviours as indices. Cats were considered successfully acclimated when they demonstrated behaviours similar to those observed in a free
living environment where they are permanently housed, as well as behaviours indicative of low stress and fear response.

Housing: Cats were housed in a free-living environment with indoor/outdoor access during the day (0800-1500 h) and indoor-only access at night (1500-0800 h). Room environmental enrichment included perches, beds, toy houses, scratching posts, toys and climbing apparatus. All cats were socialized daily for a minimum of 60 min. Cats were maintained on a 12 hour lighting schedule with the lights turning on at 0630 h and turning off at 1830 h. The room temperature was maintained at 22°C and relative humidity was 50%-60%, outdoor temperature averaged 25°C with a relative humidity of 70%. Room surfaces were cleaned daily, and disinfected weekly with Nolvasan disinfectant (Pfizer, New York, USA). Water was provided ad libitum from automatic waterers.

Respiration calorimetry chambers (Qubit Systems®, Kingston, Ontario, Canada) were made of Plexiglass and measured 53.3 x 53.3 x 76.2 cm. Each chamber contained a shelf, feeder, water bowl, hammock, litter box, toy and a free area with a fleece bed. Water was provided ad libitum from water bowls. The chamber was designed to allow sufficient separation of feeding, sleeping and elimination areas. Chambers and water bowls were disinfected, and litter, litter boxes, toys, hammocks and fleece beds were removed, cleaned and replaced daily.

Diets: Two experimental diets were produced at the Procter & Gamble Pet Care pilot plant facility. Fat and carbohydrate energy content of the diets were 10% and 46.3% as-fed for the HC diet and 30.0% and 18.9% as-fed for the HF diet and contained common ingredients used in the pet food industry since the diets were chicken and corn based (Table 2). Iams® Original Chicken Adult Dry formula was fed during the washout periods to normalize cats to a diet moderate in fat and CHO content (15 & 33% as-fed, respectfully). Cats were individually fed in cages at approximately 0730 h daily. Cats were offered 239 kJ ME/kg/d to maintain weight (Wichert et al., 2007). Cats were permitted 60 min once daily to consume their food and all remaining food was removed and ORTs were recorded.
Table 2: Nutrient (%) and metabolizable energy content of washout and test diets on an as-fed basis.

<table>
<thead>
<tr>
<th></th>
<th>Iams® Original Chicken</th>
<th>HF</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Protein</td>
<td>35.0</td>
<td>35.0</td>
<td>26.8</td>
</tr>
<tr>
<td>Fat</td>
<td>15</td>
<td>30.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Ash</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>NFE</td>
<td>33</td>
<td>18.9</td>
<td>46.3</td>
</tr>
<tr>
<td>Predicted ME (Kcal/kg)**</td>
<td>3550</td>
<td>4416</td>
<td>3396</td>
</tr>
<tr>
<td>Protein (g/kg): ME (Kcal/kg)*</td>
<td>0.0099</td>
<td>0.0079</td>
<td>0.0079</td>
</tr>
</tbody>
</table>

*Diets were formulated to contain the same Protein: Energy ratio.

**ME Calculated using Modified Atwater Equation (ME (kcal/kg) = (3.5*kg NFE)+(8.5*kg fat)+(3.5*kg protein)

Experimental Design: For two weeks prior to the initiation of the study, cats were fed Iams® Original Chicken Adult Dry diet. At the end of the first washout period, cats were allocated to either a HC or HF diet with each dietary group being balanced for gender. Each cat was fed the assigned test diet (HF vs. HC) for a total of 14 d. After the first 14 d dietary treatment, all cats were returned to Iams® Original Chicken for 14 d. Following the washout period, each group of cats were fed the alternate test diet for an additional 14 d period. Therefore, all cats received each dietary treatment for a 14 d period in a crossover design. The study lasted a total of 56 d including two washout periods. Feed intake was measured daily and body weight on a weekly basis in the fasted state.

Indirect Calorimetry: To assess the effect of length of adaptation on energy metabolism when cats were fed a HC or HF diet, three separate indirect calorimetry analyses were conducted. Oxidation studies occurred on d 0 (acute exposure) directly following the introduction (first feeding) of the test (HC or HF) diet, and on d 4 (semi-chronic exposure) and d 13 (chronic exposure). To determine whether these effects changed based on fed vs. fasted state, oxidation studies were 24 h in length to include fasted, fed, post prandial, and return to fasting states.
Indirect calorimetry was conducted by measuring respiratory gases for 5 minutes every half-hour. Concentrations of O$_2$ and CO$_2$ present in the respiratory chambers were measured with O$_2$ and CO$_2$ gas analyzers and VO$_2$ consumed and CO$_2$ produced were measured (Qubit Systems®, Kingston, Ontario, Canada). The calorimeter is an open circuit, ventilated calorimeter with the room air being drawn through at a rate of ~5L/min. Airflow was set at 5 L/min and actual rate was measured with the use of a mass flow meter to enable total volume calculation. Gas analyzers and mass flow meters were calibrated prior to each individual oxidation study and at least every 6 h during a study, or when a drift of more than 1% was observed. Calibration was performed using standard gas mixtures at 2 concentrations. Respiratory quotient, fat and CHO oxidation, and EE were calculated as follows (Ferrannini, 1988):

Respiratory Quotient (RQ) = litres CO$_2$ produced/ litres of O$_2$ consumed  

[Eq. 1]

EE (kcal) = 3.82 x litres O$_2$ consumed + 1.15 x litres CO$_2$ produced  

[Eq.2]

Carbohydrate oxidation:

$$C_nH_{2n}O_n + nO_2 \rightarrow nCO_2 + nH_2O$$  

[Eq. 3]

Fat oxidation:

$$(CH_2O)_3(CH_2)_n(CO_2H)_3 + nO_2 \rightarrow nCO_2 + nH_2O$$  

[Eq. 4]

For analysis of RQ and energy expenditure (EE) in the fasted state, three respiration samples were obtained prior to feeding and mean ± SEM for the three samples was calculated. Twenty-four hour energy expenditure, fat and CHO oxidation post feeding was calculated as mean ± SEM multiplied by 24 for each dietary treatment and exposure.

**Blood Analyses:** All Blood samples (2.5 mL) were collected via the Jugular vein using the Vacutainer ® method. This method encompasses the use of a plastic device attached to a needle which allows a predetermined volume of blood to flow into the tube by vacuum. By using the Vacutainer® method the blood cells are not subject to damage as the vacuum is equilibrated to apply the appropriate amount of pressure to avoid damage of red cells. Blood was sampled to measure changes in glucose, insulin, triglycerides, β-hydroxybutyrate, C-reactive protein and cholesterol (LDL and HDL) in response to HF or
HC diet feeding. Blood samples were taken in the fasted state following completion of oxidation measurements on d 1, 5 and 14. Then the samples were placed on ice for no more than 1 h. After clotting, samples were centrifuged at 300 rpm for 15 min at -4°C and stored at -20°C. Plasma was removed via a pipette and analysed.

Statistical Analyses: A 2x3 crossover design with repeated measures was used for this experiment. There were two dietary factors tested, HF and HC, and three lengths of dietary exposure (acute, semi-chronic and chronic) to each treatment. All statistical analyses were performed using Statistical Analysis System (SAS, version 9.1; SAS Institute Inc., 2002-2003, Cary, NC). Repeated measures ANOVA and the Tukey-Kramer test were used to measure differences between dietary treatments and exposure. Data was further analyzed using the PROC MIXED function. The model used was: \( Y_{ij} = \beta_i + \epsilon_{ij} \); in which \( Y_{ij} \) the dependent variable, \( \beta_i \) = dietary treatment (HF or HC), and \( \epsilon_{ij} \) = random residual error. Diet (HF or HC) was considered a fixed effect. Treatment least square means were compared using the pdiff multiple comparison procedure. Differences were considered significant when \( P<0.05 \) and all data are expressed as least squared means ± SEM.

Results

All results are presented on a metabolic body weight basis (kg\(^{0.67}\)).

Body Weight and Intake: There were no significant effects of the HF or HC diet on mean body weight and metabolic body weight; however, body weight was numerically higher with the consumption of the HC diet (3.78 vs. 3.82 ± 0.05, \( P=0.12 \) and 2.42 vs. 2.44 ± 0.02 kg, \( P=0.13 \)). Both body weight and metabolic body weight increased significantly with exposure to the HF and HC diet as weight during chronic dietary exposure was significantly higher than weight during acute and semi-chronic exposures (Figure 7; \( P<0.05 \)). Energy intake was not significantly different between the HF and HC diets (92.7 kcal/kg\(^{0.67}\)/d ± 1.8 vs. 87.8 kcal/kg\(^{0.67}\)/d ± 1.8, \( P>0.05 \)) despite greater ORTs recorded during the consumption of the HC diet. As expected, due to composition differences between diets fat and CHO intake differed between the HF and HC diet (\( P<0.001 \)). Dietary energy, fat and CHO intake (Table 3) did not differ due to length of dietary exposure (\( P<0.05 \)).
Figure 7: The effects of high fat or high carbohydrate diets on mean body weight (kg) ± SEM, n=10, after acute (Day 0), semi-chronic (Day 4) and chronic (Day 13) exposure. Means without a common superscript differ due to exposure within a dietary treatment, $P<0.05$. NS, $P\geq 0.05$. Means not sharing a common single or double asterisk are significantly different between groups; "*" represents the highest value and "**" the lowest value ($P<0.05$). P-value refers to the ANOVA for diet*exposure effect of treatment.

Table 3: Energy, fat and carbohydrate intake of cats consuming a high fat and high carbohydrate diet during acute (Day 0), semi-chronic (Day 4) and chronic (Day 13) dietary exposure.\(^1\)

<table>
<thead>
<tr>
<th>DIET</th>
<th>HF</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exposure</strong></td>
<td><strong>Acute</strong></td>
<td><strong>Semi-Chronic</strong></td>
</tr>
<tr>
<td>Energy Intake(^2)</td>
<td>92.6 ± 1.7</td>
<td>92.5 ± 1.7</td>
</tr>
<tr>
<td>Fat Intake(^2)</td>
<td>56.6 ± 0.54(^a)</td>
<td>56.5 ± 0.54(^a)</td>
</tr>
<tr>
<td>CHO Intake(^2)</td>
<td>15.9 ± 1.06(^b)</td>
<td>15.8 ± 1.06(^b)</td>
</tr>
</tbody>
</table>

\(^1\)Values are means ± SEM, n=10. Means without a common superscript letter differ with exposure, $P<0.05$. NS, $P\geq 0.05$. Means without a common superscript symbol (*) differ due to diet, $P<0.05$. NS, $P\geq 0.05$. P-value refers to the ANOVA for diet*exposure effect of treatment.

\(^2\)Energy, fat and carbohydrate intakes are presented on a kcal/kg\(^{0.67}\)/d basis.
**Fasted Oxidation:** Mean fasted respiratory quotient was significantly higher in cats consuming the HC diet versus the HF diet (Table 4; P<0.05). Fasted RQ was not different between exposure in cats fed the HF diet but, fasted RQ increased significantly from acute to semi-chronic exposure in cats consuming the HC diet (Table 4; P<0.05). There was no effect of diet on fasted energy expenditure (P>0.05); however, fasted energy expenditure differed significantly with exposure (P<0.05). Cats consuming the HF diet exhibited fat oxidation rates similar to that observed in the fasted state after 245 ± 74 min (4 ± 1 hrs) post feeding. Conversely, cats consuming the HC diet exhibited fat oxidation rates similar to those observed in the fasted state at 145 ± 40 min (2.5 ± 0.7 hrs) post feeding. For cats consuming the HC and HF diet, CHO oxidation did not return to fasted levels even after 20 h post feeding.

**Table 4:** The effects of high fat or high carbohydrate diets on macronutrient metabolism after acute (Day 0), semi-chronic (Day 4) and chronic (Day 13) exposure.¹

<table>
<thead>
<tr>
<th>DIET</th>
<th>HF</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasted RQ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure</td>
<td>Acute</td>
<td>Semi-Chronic</td>
</tr>
<tr>
<td>Fasted RQ</td>
<td>0.77 ± 0.01</td>
<td>0.74 ± 0.008</td>
</tr>
<tr>
<td>Fasting EE²</td>
<td>78.5 ± 3.02</td>
<td>80.3 ± 3.02</td>
</tr>
<tr>
<td>EE²</td>
<td>83.0 ± 1.5</td>
<td>79.4 ± 1.5</td>
</tr>
<tr>
<td>Fat Oxidation²</td>
<td>55.3 ± 1.3</td>
<td>55.7 ± 1.3</td>
</tr>
<tr>
<td>CHO Oxidation²</td>
<td>26.6 ± 1.1</td>
<td>22.6 ± 1.1</td>
</tr>
<tr>
<td>EE as a % Intake</td>
<td>89.6 ± 2.1</td>
<td>85.9 ± 2.4</td>
</tr>
</tbody>
</table>

¹Values are means ± SEM, n=10. Means without a common superscript letter differ with exposure, P<0.05. NS, P≥0.05. Means without a common superscript (*) differ diet, P<0.05. NS, P≥0.05. P-value refers to the ANOVA for diet*exposure effect of treatment.
²Fasting EE, EE, CHO and Fat Oxidation are presented on a kcal/kg⁰.⁶⁷/d basis

**Fed Oxidation:** There was no effect of diet on energy expenditure post feeding though energy expenditure post feeding differed significantly with dietary exposure as expected (P<0.05). Mean maximum energy expenditure (EE) of cats consuming the HF and HC diet were 106.9 ± 3.5 kcal/kg⁰.⁶⁷/d and 101.5 ± 2.2

---

82
Mean energy expenditure as a percent of intake was higher for cats consuming the HC versus the HF diet (P<0.05). There were no significant effects of exposure on energy expenditure as a percent of intake for both the HF and HC diets; however, energy expenditure as a percent of intake tended to decrease with semi-chronic exposure for both diet groups (Table 4; P<0.05). Diet significantly affected both fat and CHO oxidation, with the HF diet being significantly higher in fat oxidation than CHO. The alternative response was observed in cats consuming the HC diet. In addition, oxidation of the restricted macronutrient (CHO or fat) differed between the two diets (Table 4; P>0.05). Maximal fat oxidation (0.992 ± 0.03 g/h) for cats consuming the HF diet occurred at 35 ± 9 min post feeding and 0.799 ± 0.02 g/h for cats on the HC diet occurred 22 ± 10 min (0.4 hrs) post feeding (Figure 8; A). The maximal CHO oxidation rate was 1.90 ± 0.11 g/h, which occurred at 685 ± 76 min (11 ± 1 hrs) post feeding for cats consuming the HC diet and was 0.941 ± 0.13 g/h which occurred at 567 ± 185 min (9 ± 3 hrs) post feeding in cats fed the HF diet (Figure 8; B).
Figure 8: Mean (N=10) fat and CHO oxidation measured in grams oxidized/hour during the fasted, fed, post-prandial and return to fasted states over a 20 hr period in cats consuming a HF (A; 30% fat) and HC (B; 10% fat) diet during chronic (Day 13) dietary exposure.

*Fasting Blood Metabolites:* Serum cholesterol and glucose was significantly higher in cats consuming the HF versus the HC diet (Table 5; P<0.05). There were no significant effects of diet on serum CRP, triglycerides and LDL: HDL (P>0.05). There were no differences in serum insulin due to dietary treatment (P>0.05).
## Table 5: The effects of high fat or high carbohydrate diets on fasted blood plasma metabolites after acute (Day 1), semi-chronic (Day 5) and chronic (Day 14) exposure.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Exposure</th>
<th>Acute</th>
<th>Semi-Chronic</th>
<th>Chronic</th>
<th>Mean</th>
<th>Acute</th>
<th>Semi-Chronic</th>
<th>Chronic</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td></td>
<td>213.1 ± 20.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>235.6 ± 20.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>259 ± 21.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>236.0 ± 20.4&lt;sup&gt;*&lt;/sup&gt;</td>
<td>194.6 ± 20.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>188.1 ± 20.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>195.2 ± 21.4&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>192.6 ± 20.4**</td>
</tr>
<tr>
<td>HC</td>
<td></td>
<td>0.069 ± 0.0045&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.083 ± 0.0068&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.098 ± 0.0082&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.083 ± 0.0064&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.085 ± 0.0045&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.103 ± 0.0068&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.084 ± 0.0082&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.006</td>
</tr>
<tr>
<td>Glucose&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>86.8 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.8 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.2 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.4 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.3 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.2 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.0 ± 1.4</td>
</tr>
<tr>
<td>CRP&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>148.67 ± 8.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>154.22 ± 9.7&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>172.52 ± 9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>158.47 ± 8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.92 ± 8.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>162.92 ± 9.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>163.49 ± 9.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>157.44 ± 8.7</td>
</tr>
<tr>
<td>Insulin&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>160.90 ± 34.6</td>
<td>173.26 ± 45.2</td>
<td>180.60 ± 37.3</td>
<td>171.59 ± 23.2</td>
<td>198.54 ± 34.6</td>
<td>172.86 ± 45.2</td>
<td>238.67 ± 37.3</td>
<td>203.35 ± 23.2</td>
</tr>
<tr>
<td>Triglycerides&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>30.4 ± 3.7</td>
<td>26.7 ± 2.5</td>
<td>29.2 ± 3.4</td>
<td>28.8 ± 2.8</td>
<td>28.8 ± 2.8</td>
<td>26.4 ± 2.5</td>
<td>28.1 ± 3.4</td>
<td>27.8 ± 2.8</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means ± SEM, n=10. Means without a common superscript letter differ across exposure and diet, *P*<0.05. NS, *P*≥0.05. Mean metabolite values without a common symbol (*) differ between diets, *P*<0.05. NS, *P*≥0.05. P-value refers to the ANOVA for diet*exposure effect of treatment. <sup>2</sup>Glucose, triglycerides, CRP, insulin and cholesterol are presented on an mg/dl basis.

### Discussion

Cats were capable of adapting macronutrient oxidation to intake on HF and HC diets; however, this adaptation appeared incomplete over the 14 d feeding period. Diet did not significantly influence energy balance nor weight gain; although, cats consuming the HC diet showed a numerical increase in weight versus the cats consuming the HF diet. Cats consuming the HF diet had higher fasting glucose and cholesterol levels; however, CRP, Triglycerids, LDL:HDL ration, β-hydroxybutyrate and insulin did not change with dietary exposure.

Exposure to a HF or HC diet for 14 d did not significantly affect body weight or metabolic body weight of cats when fed to energy requirements. However, mean body weight of cats exposed to the HF and HC diet increased by 0.06 kg and 0.417 kg ± 0.05, respectively. This difference in body weight gain is interesting as cats were fed isocalorically to maintain weight and to meet their daily energy requirement.
Although not statistically significant, the increase in body weight of cats consuming the HC diet supports previous theories that HC diets may contribute to obesity possibly as a result of increased lipogenesis and fatty acid storage in adipose tissue, which may be related to unused CHO leading to hyperglycaemia (Reviewed in: Zoran, 2002). Lester et al., (1999) also observed an approximately 2% total body weight reduction in cats consuming a HF diet versus a low fat diet over 8 days that was not statistically significant. However, a 2% body weight reduction over an 8 day period may lead to significant weight loss with greater feeding durations. Humans exposed to a HF diet for 6 days lost a significant amount of weight versus humans consuming a HC diet (Schrauwen et al., 1997a, b); the rapid reduction in weight was hypothesized to be a consequence of a decline in body glycogen stores (Schrauwen et al., 1997a, b).

Similar to the reduced RQ observed for HF-fed cats in the present study, Lester et al. (1999) observed a similar trend in cats, as 24-hr RQ decreased with exposure to diets higher in fat content. Researchers concluded that the significant decrease in RQ was a consequence of the higher fat-to-CHO oxidation ratio. This apparent metabolic flexibility suggests that cats can adapt to increasing levels of dietary fat. The demonstration of increased RQ in response to increased dietary CHO further supports the hypothesis of feline CHO metabolic flexibility. Similarly, Hoenig et al. (2007) observed an increase in respiratory exchange rate (RER) when cats consumed a diet high in CHO versus protein. Humans respond in a similar fashion and exhibit, significantly decreased RQ values with exposure to diets higher in fat (Smith et al., 2000; Treuth et al., 2003). This further supports the idea that cats, similar to an omnivorous human, can adapt macronutrient oxidation to intake when minimum protein needs are met.

Fat oxidation declined significantly between the HF and HC diets while CHO oxidation increased significantly between HF and HC diets. This change in substrate oxidation in response to dietary macronutrient modification suggests that cats are capable of metabolically responding to manipulations of dietary fat and CHO content. The reciprocal relationship between fat and CHO oxidation is expected as CHO oxidation appears to occur at the expense of fat oxidation through fatty acid (FA) oxidation inhibition (Acheson et al., 1984; Chow, 1992). It has been proposed that inhibition of fat oxidation after HC diets may be caused by 1) the inhibition of NEFA availability since NEFA are the major source for fat oxidation in the resting state (Schrauwen et al., 2000) and 2) by alterations in intracellular mechanisms (Sidossis et al., 1996; Koutsari and Sidossis, 2003). The latter is more likely the explanation for the observed inhibition of fat oxidation with high CHO intake as brief hyperglycemia and hyperinsulinaemia decreases whole body fat oxidation regardless of NEFA plasma concentrations (Sidossis and Wolfe, 1996; Sidossis et al., 1996, 1999; Koutsari and Sidossis, 2003). The reduction of fat oxidation is associated to
the corresponding increase in glycolytic flux that blocks long chain fatty acid entry into the mitochondria reducing the level of FA available for oxidation (Sidossis et al., 1996).

Energy expenditure did not differ between diets in either the fasted or over the total 20-hr postprandial state. This observation is consistent with previous studies in cats and humans (Lester et al., 1999; Koutsari and Sidossis, 2003). Overall, energy intake was closely matched to EE. However, EE as a percent of intake differed between diets as cats consuming the HC diet demonstrated an EE that more closely matched intake (~92% versus 87% of energy intake for cats fed the HF diet). In humans exposed to a high fat diet (37 to 50% of energy), energy intake as a percent of EE ranged from ~84 to 87% (Smith et al., 2000), and in humans exposed to a HC diet energy expenditure matched intake at 82%; however, these subjects were fed ad libitum. Overall, results observed in humans are similar to the results observed in our study. Meal-induced thermogenesis may also help explain the observed difference in energy balance of cats consuming the HF versus the HC diet, as fat has the lowest thermic effect of feeding; 2% versus 8% and 20-30% for CHO and protein, respectively (Schutz et al., 2004; Jequier et al., 1987).

The increased EE in cats consuming the HC diet may be partially explained by weight gain (Lester et al., 1999). However, the increase in weight gain and EE in cats also could have been a consequence of the metabolic, behavioural and/or physiological effects of consuming diets high in CHO. In newborn parenterally fed infants, increased CHO intake was correlated to increased metabolic rate, which in turn was thought to be a consequence of increased lipogenesis (Sauer et al., 1986). Increased lipogenesis in cats may help explain the observed increase in weight gain and metabolic rate with exposure to a HC diet. Koutsari and Sidossis (2003) observed a lower rate of appearance of non-esterfied fatty acid (NEFA) with HC vs HF feeding in humans and concluded was attributed to decreased peripheral lipolysis and(or) elevated FA re-esterification in the adipose tissue. Small differences in plasma insulin concentrations influence lipolysis (Nurjhan et al., 1986; Jensen et al., 1989). Fasting insulin was not significantly different due to diet in the present study. However, fasting insulin was numerically higher in cats consuming the HC diet. This small increase in plasma insulin may have reduced NEFA concentrations and triggered increased lipogenesis (Koutsari and Sidossis, 2003). Even at low non-significant levels, small changes over the long term may impact body composition.

Another explanation for the observed weight gain, though non-significant over the short term (14 d) study, may be due to the effects of CHO consumption on behaviour. Rats injected with glucose and insulin caused an increase in rapid-eye-movement (REM) sleep and sleep duration over 24 hours (Danguir and
Nicolaidis, 1980). Also, an increased latency to sleep, greater amounts of sleep during the dark phase and elevated disturbed sleep have been observed in rats immunized against peripheral insulin (de Saint Hilaire et al., 1995). However, others have found that insulin has the contrary effect and ultimately caused a reduction in REM sleep in rats (Schubert et al., 1993). In humans, evidence to support the conclusion that HC diets promote increased lethargy is also contradictory. High carbohydrate and low protein diets can influence mood by promoting the production of the neurotransmitter serotonin which is associated to sleepiness, depression and anxiety (Fernstrom, 1983). In humans, HC diets have also been hypothesized to affect latency to onset of sleep, duration of sleep and type of sleep via the action of increased insulin that acts to elevate levels of serotonin in the brain through the selective entry of blood tryptophan (Fernstrom and Wurtman, 1971; Porter and Horne, 1981). Alternatively, some researchers have found that diets high in CHO do not promote lassitude but facilitate the converse effect and promote feelings of less fatigue and depression (De Castro, 1987; Wells et al., 1997) and several research groups have concluded that HF diets, not HC diets, promote greater feelings of lethargy (Wells et al., 1997; Lloyd et al., 1994; Wells et al., 1995). Physiological effects of HC diets may have influenced the behaviour of cats as stimulation of the small intestinal mucosa induces sleepiness and sleep (Kukorelli and Juhasz, 1977). Since bulk intake of the HC diet was higher than that of the HF diet and cats appear to digest CHOs relatively slowly the relative amount of physical stimulation of the small intestine may have been greater with the HC diets and contributed to greater levels of lethargy. Furthermore, if the HC diet promoted lethargic behaviours then the increased weight gain of cats consuming the HC diet may be attributed to reduced physical activity and increased resting that led to a more positive energy balance outside of the chambers.

The rate at which cats and humans adapt substrate oxidation, specifically, fat oxidation to intakes appears to differ. Human subjects transitioned from a HF diet to a HC diet demonstrate an immediate reduction in fat oxidation paralleled by a rapid increase in CHO utilization (Sauer et al., 1986; Smith et al., 2000; Bisschop et al., 2002; Treuth et al., 2003). Alternatively, fat balance, in humans, is not as immediate and is hypothesized to take several days (~7 days) (Schrauwen et al., 1997b, c; Smith et al., 2000). On the eighth day of diet exposure Lester et al., 1999, observed that cats adapted fat oxidation with a mean slope of 0.91g fat oxidized/g fat intake to incremental fat content (53 to 71% of energy). As Lester et al., 1999, did not assess fat oxidation on the first day of HF dietary exposure; it is unclear if they too would have observed immediate fat balance via a rapid increase in fat oxidation. Our results indicate that cats adapt to HF diets with acute exposure (day 0) as cats appeared to immediately match fat oxidation to intake (98%) after a single HF meal. Furthermore, cats ability to match fat oxidation with intake did not differ with
length of exposure. Lean and obese humans are capable of rapidly (within 24 hours) adjusting fat oxidation to match intake when glycogen stores were lowered through exhaustive exercise (Schrauwen et al., 1997c; Schwaren et al., 1998). Fat balance can be obtained by 1) maintaining reduced glycogen stores or 2) by increasing fat mass (Flatt, 1987). As cats did not increase body weight on the HF diet it is likely that glycogen stores were low enough not to impede fat oxidation. Cats may differ from humans as they maintain a high level of gluconeogenesis and have a slower incorporation rate of glucose into glycogen (Ballard 1965; Rogers et al., 1977). In both cats and humans the body glycogen stores are significantly less than fat reserves; however, due to the differences between cats and humans on glucose and glycogen it is expected that cats respond differently than humans to dietary fat and CHO (Flatt, 1995). The cats rapid response to dietary fat is further demonstrated when fat and CHO oxidation pattern was measured half hourly in the postpradial state during 20-hr indirect calorimetry measures. Fat was oxidized more rapidly than CHO postpradially, regardless of diet, as maximum fat oxidation (g/hr) was obtained at approximately 30 minutes post feeding. The cats’ slow response to dietary CHO is likely a consequence of 1) lack of glucokinase (GK) (Tanaka et al., 2005), 2) the slow rate of incorporation of glucose into glycogen (Ballard 1965; Kienzle, 1989) and, 3) slower time to peak insulin and lower peak insulin value (Hewson-Hughes et al., 2011). As opposed to dogs cats have increased activities of fructokinase (FK), pyruvate kinase (PK) and glucose-6-phosphate dehydrogenase all of which suggest high activities of fatty acid synthesis and may be the reason for the observed responsiveness of fat oxidation in cats (Tanaka et al., 2005). It would be expected that cats would be capable of adapting fat oxidation to intake and oxidizing fat rapidly since cats have evolved to consume diets high in fat (>50% of energy) and low in CHO versus humans who have a heavier reliance on CHO/glucose for energy.

During the consumption of the HF diet CHO oxidation exceeded intake leading to a negative CHO balance and during the consumption of the HC diet fat oxidation exceeded intake leading to a negative fat balance. Similar results have been observed in studies conducted on both cats (Lester et al., 1999) and humans (Smith et al., 2000; Abbott et al., 1990; Hill et al., 1991; Treuth et al., 2003; Schrauwen et al., 1997b). Negative CHO balance observed with HF feeding was attributed to the high rate of gluconeogenesis that occurs in cats and results in increased productivity of glucose from glycerol, lactate, and gluconeogenic amino acids which, in turn, raises the availability of glucose for oxidation and exceeds dietary carbohydrate intake. Labayen et al., 1999, argued that the negative CHO balance observed with HF feeding could be a consequence of reduced glycogen levels that facilitates high levels of fat oxidation. The negative fat balance observed during the consumption of the HC diet may have been a consequence of
the reliance on FA oxidation, primarily in the fed state following feeding, as an immediate energy source. Furthermore, the machinery involved in the oxidation of fat is highly active in cats and this enhanced activity may have lead to the greater level of oxidation over intake. Therefore, cats followed similar patterns to humans as oxidation of a macronutrient offered in a limited quantity exceeds intake with short term (<14 d) dietary exposures.

There was a significant effect of diet on fasting serum glucose, and cholesterol concentration; however, diet did not influence fasting concentrations of CRP, triglycerides, β-hydroxybutyrate, LDL: HDL ratio and insulin concentrations. While an elevation of fasting glucose due to a HF diet is not widely supported in the literature, others have demonstrated a reduced glucose clearance rate and elongated glucose elimination curve when using IV glucose tolerance tests in cats fed HF vs. HC diets (Backus et al., 2007; Thiess et al., 2004). Thiess et al., 2004 observed a small, though not significant, increase in plasma glucose with HF feeding in the post prandial state in cats. The elevated plasma glucose was clinically significant was likely a consequence of reduced insulin secretion as plasma insulin did not differ between diets. Observed increases in plasma insulin are common with HC feeding and are correlated to a reduction in oxidation of fat and elevation in glucose oxidation (Smith et al., 2000). Backus et al., 2007, also did not observe a change in plasma insulin concentration between HF and HC feeding in cats. Perhaps if we had measured fed glucose and insulin we would have observed differences as Hewson-Hughes et al., 2011, noted that a high startch diet, versus medium and low startch diets, led to an increase in glucose and insulin post feeding. HF feeding has been known to impair insulin responsiveness through reduced glucose stimulatory effectiveness on insulin secretion and possibly β-cell function in progressive cases (Randle, 1988). Mean plasma cholesterol significantly differed between HC and HF diets with plasma cholesterol levels increasing linearly with HF dietary exposure. The increase in cholesterol with HF feeding was likely a consequence of increased intestinal absorption of cholesterol associated with the consumption of the HF diets. An increase in plasma cholesterol with HF diets has been observed previously in the cat and human (Thiess et al., 2004; Dobenecker et al., 1998). The change in total cholesterol level with HF feeding was a consequence of significant increases in LDL and HDL plasma levels without modifications to the LDL: HDL ratio. There was no effect of diet on serum fasted TAG in cats, an effect that has previously been observed in both cats and humans (Backus et al., 2007; Schwauren et al., 2000; Dobenecker et al., 1998; Treuth et al., 2003). Alternatively, some researchers have observed elevated TAG levels in cats consuming HF diets (Thiess et al., 2004). Elevated TAG levels are often associated to a decline in insulin sensitivity of adipose tissue and weight gain; however, the cats used for this study were young, healthy adults of lean body mass and therefore it is expected that insulin sensitivity
remained high (Reavan, 2005). In addition, we did not observe an increase in β-hydroxybutyrate and CRP with HC or HF feeding. Increases in β-hydroxybutyrate are often associated with enhanced free fatty acid oxidation leading to the production of ketone bodies (Thiess et al., 2004). Therefore, the increase in oxidation must have not been large enough to facilitate an increase in ketone body production or inflammatory responses. Overall, differences in findings between our study and others may be attributed to time of blood sampling (fasted vs. fed), length of dietary adaptation, diet type (liquid/wet versus dry type) and fat and CHO content of the test diets. Furthermore, our HF and HC diets had equivalent protein to energy ratios (Table 2); thus, the metabolic responses observed in our study were exclusively reflections of fat and CHO dietary intake.

To our knowledge, this is the first study of its kind to combine indirect calorimetry measurements with blood plasma metabolites to investigate adaptation to HF and HC diets in cats. In conclusion, our results indicate that cats exposed to HF or HC diets and fed to energy requirements demonstrate characteristics of metabolic flexibility and adaptation to high fat and CHO content; however, this adaptation is incomplete as negative fat and CHO balances were observed with HC and HF feeding, respectively, after chronic (14 d) dietary exposure. Therefore, studies that exceed 14 days may be required for cats to completely adapt to restricted macronutrient intakes. HC diets may result in weight gain during long term exposure while HF diets caused greater perturbations in blood metabolite concentrations; therefore, studies of similar design conducted over a longer period of time are required to determine optimal dietary fat and CHO inclusion for feline diets, metabolic responses to different CHO and fat sources, and risk factors for the development of obesity and associated diseases as related to the dietary composition. Further, an understanding of the effects of ad libitum feed intake of HC and HF diets further acts to modify energy and macronutrient balance.

Acknowledgements

The authors would like to thank The Procter and Gamble Co. for their financial support of the study and Sally Perea for reading the manuscript.
References


Dobenecker, B.; Kienzle, E.; Sallmann, H.P.; Fuhrmann, H., 1998: Effect of diet on plasma triglycerides,


Slingerland, L.I.; Vasilova, V.V.; Plantinga, E.A.; Kooistra, H.S.; Beynen, A.C., 2007a: Indoor confinement and physical inactivity rather than the proportion of dry food are risk factors for the development of feline type 2 diabetes mellitus. Vet J. In Press.


CHAPTER 3: Effects of high fat and high carbohydrate diets on energy metabolism and behaviour in healthy cats

Margaret A. Gooding, Jim L. Atkinson, Ian J.H. Duncan, Lee Neil and Anna K. Shoveller

Authors Last Name for PubMed indexing: Gooding, Atkinson, Duncan, Neil, Shoveller

Addresses:
1 University of Guelph, Animal and Poultry Science, Guelph, Ontario, Canada N1G 2W1
2 Procter and Gamble Pet Care, Mason, Ohio, USA 45040

*To whom correspondence should be addressed: shoveller.ak@pg.com

Running Title: Energy metabolism and behaviour in cats

Keywords: indirect calorimetry, energy expenditure, activity, play motivation, cognition glucose, insulin.

Funding: This work was supported by Procter and Gamble Pet Care, Mason, Ohio, USA 45040

Conflict of Interest and Funding Disclosure: I.J.H.D., L.N. and J.L.A have no conflicts of interest. A.K.S has financial and personal interest in The Procter and Gamble Co. due to employment with the funding company and M.A.G is a PhD. intern.

Authors’ Contributions: M.A.G. and A.K.S. designed research with major contributions from J.L.A., L.N. and I.J.H.D. M.A.G conducted research, analyzed data, wrote the paper and had primary responsibility for final content. All authors read and approved the final manuscript.

Abbreviations used: CHO, carbohydrate, HF, high fat, HC, high carbohydrate, EE, energy expenditure, RQ, respiratory quotient, TAG, triglycerides.
Abstract

The effects of feeding diets high fat (HF; N=10) or high carbohydrate (HC; N=10) diets (34% fat, 26% carbohydrate; HF and 11% fat, 47% carbohydrate; HC) for 84 days on energy metabolism and behaviour were investigated in cats (Felis catus; ~3.5 yrs, 4 ± 1.5 kg BW). The study was a parallel design with the control (35% fat, 33% carbohydrate) diet being fed for 3 weeks prior to facilitate collection of baseline measures. Three 22-h indirect calorimetry measures were conducted on days -7, 35 and 76 and fasted blood samples were collected 18 hrs post meal feeding on days -5, 36 and 77. Play (d -21, 64), physical activity (d -14, 22, 70) and cognition (d -1, 42 84) were used to measure the behavioural effects of diet. Body weight did not differ between groups (P>0.05); however, the HF group gained body fat (0.25 kg; P<0.05) despite the two groups receiving similar dietary energy per kg body weight. Fasted and fed energy expenditure were similar between groups (P>0.05) while RQ declined and increased with exposure to the HF (RQ= 0.862 ± 0.005) and HC (RQ= 0.782 ± 0.005) diet, respectfully. HC dietary treatment appeared to impair insulin sensitivity (G:I =0.68, HC vs. G:I= 0.87, HF, P= 0.2656) and cause greater reductions in activity; although, these differences were only numerical. Cognitive performance declined with HF feeding and improved with HC feeding (p<0.05). There was no effect of diet on play motivation (p>0.05). The changes observed in energy metabolism suggest that cats adapt whole-body nutrient metabolism in response to dietary macronutrient content, but the HC diet presents a greater risk for the development of weight gain due to the reduced physical activity and declined insulin sensitivity. Future research should investigate these potential effects and compare high fat vs. carbohydrates diets in an ad libitum, rather than maintenance, feeding design as ad libitum feeding may exacerbate these effects.
Introduction

Approximately 35% of cats (*Felis Silvestris Catus*) are classified as overweight or obese in the United States (Lund et al., 2005) and body weight is associated with increased risk for the development of diabetes mellitus. Along with age, physical activity, and indoor confinement, type of diet, namely diets high in fat and carbohydrate (CHO), as a percent of energy, have been identified as risk factors for the development of weight gain and associated metabolic disorders (Panciera et al., 1990; Lund et al., 2005; McCann et al., 2007; Slingerland et al., 2007a). Although several studies have investigated the effects of high carbohydrate (HC) and high fat (HF) diets on body weight, glycemia, and insulinemia, it remains unclear as to which type of diet, HC or HF, poses the greatest risk for the development of obesity and diabetes in cats (Thiess et al., 2004; Rand et al., 2004; Backus et al., 2007; Slingerland et al., 2007b).

It has been hypothesized that prolonged consumption of high carbohydrate (CHO) diets may contribute to the development of feline diabetes mellitus by promoting obesity through exaggerating postprandial glycemia and insulinemia. This overstimulation of the pancreatic β cells may cause β cell exhaustion and eventually dysfunction (Brand Miller and Colagiuri, 1994; Rand et al., 2004). On the contrary, several studies have shown that cats fed HF, rather than HC diets, are more susceptible to weight gain and metabolic disorders as a consequence of perturbations in plasma glucose and insulin (Backus et al., 2007; Thiess et al., 2004). Lastly, Slingerland et al., 2007b, noted that exchanging dietary protein with fat or CHO improved insulin sensitivity (fasted state) over a 9 mo feeding period; however, fed state and long term effects of this impaired insulin sensitivity, known to cause β cell exhaustion/dysfunction, warrants further investigation. It is difficult to directly compare findings since some studies (Backus et al., 2007) permitted *ad libitum* feeding of the test diets where others (Thiess et al., 2004; Slingerland et al., 2007b) fed a single meal/day to weight maintenance. *Ad libitum* feeding may lead to variations in energy intake and consequently confound the effects of macronutrient composition (Mehta et al., 1977). In addition, fasted and fed state (time since feeding) would be different between the two feeding methods impacting glucose and insulin values and response. Furthermore, dietary protein: ME ratio should be controlled since protein content can impact energy metabolism (Slingerland et al., 2007b). Thus, to understand the true effects of each macronutrient (fat vs. CHO), energy and protein intake need to be consistent between test diets.

A short 14 d crossover study, measuring the effects of a HF (30% fat, 18.9% CHO) and HC diets (10% fat, 46.3% CHO) on energy metabolism, detailed a non-significant increase in body weight (BW; +0.04
kg) in lean, healthy cats consuming the HC diet and fed to energy requirements (Gooding et al., 2012). We hypothesized that the HC diet influenced daily energy expenditure (EE) via a behavioural effect ultimately contributing to weight gain since EE during calorimetry measures did not differ between groups. This hypothesis is consistent with anecdotal evidence that suggests that high levels of plasma glucose cause cats to become lethargic; a phenomenon further supported in the rat and human literature as diets high in CHO may act to reduce energy level (Pivonka & Grunewald, 1990; Kapas et al., 1993; Wells et al., 1997; Nabb and Benton, 2006). In addition to physical activity, behavioural affects of diet hypothesized to impact daily activities and the propensity to gain weight include play motivation and cognition. In the domestic cat, play motivation is influenced, but not exclusive to, level of hunger (Hall et al., 1998) and since diets high in fat and CHO content appear to have differing, but inconclusive effects on satiety (Stubbs et al., 1993) we utilized play motivation as a behavioural indicator of level of hunger since cats were fed to energy requirements. Certain diets, obesity and diabetes have been shown to cause cognitive deficits as these factors may act to modify insulin and glucose concentration and function (Riby 2008; Gonzales, 2010; Gerozissis, 2010). Glucose and insulin interact with hormones in the central nervous system (CNS) known to influence cognition as represented through memory, learning and dementia (Gerozissis, 2010; Lustman and Clouse 2005). However, the relative level of cognitive impairment or improvement with HF vs. HC feeding remains unclear in the human and rodent literature (Bellise, 2004) and has, to our knowledge, never been investigated in cats.

It remains unclear as to which type of diet, HC or HF, poses the greatest risk for the development of obesity and diabetes in cats with the behavioural impacts of diet often being overlooked as contributing factors to weight gain or the weight gain continuum. Furthermore, previous studies investigating the effects of fat and CHO on energy metabolism in the cat have mostly fed test HF or HC diets with different protein or energy intakes, with the exception of Thiess et al., 2004, that confounds outcomes through the introduction of additional variables known to impact energy metabolism (Mehta et al., 1977). Therefore, the aim of the present investigation was to measure the effects of an isonitrogenous and isocaloric feeding of HF and HC diets on blood biomarkers of metabolism, energy and macronutrient oxidation in the fasted, fed, and extended post-prandial states. We also examined these dietary treatments on behavioural indices that may compound the metabolic effects diet including play motivation, physical activity and cognition. We hypothesized an improvement in cognitive function, play motivation and physical activity with dietary HF treatment.
Materials and Methods

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Procter and Gamble Pet Care and in accordance with IACUC guidelines.

Twenty cats (N=20) of similar age (~3.5 years), and divided 10 female (5 lean and 5 moderately overweight) and 10 male (5 lean and 5 moderately overweight), were randomly separated into four groups of five. Cats were considered lean at a body weight of <3.6 kg and a body fat mass of <0.5 kg or 17% body mass and cats were considered moderately overweight at a body weight of >3.5 kg and body fat mass of >17%. The allocation to dietary treatments were balanced by body condition, weight and play motivation. Cats were provided from Pet Health and Nutrition Center (PHNC) at Procter and Gamble Pet Care, Lewisburg, Ohio. Standard veterinarian evaluation (physical exam, chemical and CBC blood analysis) of overall health was completed prior to the initiation of the study and all cats entered the study healthy.

All cats were previously acclimated to respiration chambers and the associated micro-environment. Acclimation success was assessed using the Cat-Stress-Score (CSS; Kessler and Turner, 1997), feed intake, fearfulness (response to novel stimuli) and elimination behaviour as indices (Gooding et al., 2012). Cats were considered successfully acclimated when they demonstrated behaviours similar to those observed in a free living environment where they are permanently housed, as well as behaviours indicative of low stress and fear response.

Housing: Cats were housed in a free-living group environment with indoor/outdoor access during the day (0800-1500 h) and indoor-only access at night (1500-0800 h). Room environmental enrichment included perches, beds, toy houses, scratching posts, toys and climbing apparatus. All cats were socialized daily for a minimum of 60 min and by the same person throughout the study. Cats were maintained on a 12 hour lighting schedule with the lights turning on at 0630 h and turning off at 1830 h. The room temperature was maintained at 22°C and relative humidity was 50%-60%, outdoor temperature averaged 25°C with a relative humidity of 70%. Room surfaces were cleaned daily, and disinfected weekly with Nolvasan disinfectant (Allivet®, St. Hialeah, Florida). Water was provided ad libitum from automatic waterers.

Respiration calorimetry chambers (Qubit Systems®, Kingston, Ontario) were made of Plexiglass and measured 53.3 x 53.3 x 76.2 cm. Each chamber contained a shelf, feeder, water bowl, hammock, litter
box, toy and a free area with a fleece bed. Water was provided ad libitum from water bowls. The chamber was designed to allow sufficient separation of feeding, sleeping and elimination areas. Chambers and water bowls were disinfected, and litter, litter boxes, toys, hammocks and fleece beds were removed, cleaned and replaced daily.

**Diet:** To effectively test the effects of fat and CHO on energy metabolism, provision of dietary calories intended to maintain weight and promote full feed consumption were provided equally between animals on a body weight basis. Each cat was fed to 95% energy requirement; therefore, females were fed 45 kcal ME/kg BW/d and males were fed 50 kcal ME/kg BW/d. Diets were presented in kibble form and cats were fed individually at 7:00 am and permitted 60 minutes to eat during food offerings. All remaining feed was collected and weighed to account for total (grams) feed refusal. The control diet was Iams® Original Chicken and the test diets represented HF (34% fat and 26% CHO of energy) or HC (11% fat and 47% CHO of energy) diets (Table 6).

**Table 6:** Nutrient (%) and metabolizable energy content of washout and test diets on an as-fed basis.

<table>
<thead>
<tr>
<th></th>
<th>Iams® Original Chicken</th>
<th>HF</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Protein</td>
<td>35</td>
<td>34</td>
<td>30</td>
</tr>
<tr>
<td>Fat</td>
<td>15</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>Ash</td>
<td>7</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>NFE</td>
<td>33</td>
<td>26</td>
<td>47</td>
</tr>
<tr>
<td>Predicted ME (Kcal/kg)**</td>
<td>3550</td>
<td>4758</td>
<td>3736</td>
</tr>
<tr>
<td>Protein (g/kg): ME (Kcal/kg)*</td>
<td>0.099</td>
<td>0.071</td>
<td>0.080</td>
</tr>
</tbody>
</table>

*Diets were formulated to contain similar Protein: Energy ratios.

**ME Calculated using Modified Atwater Equation (ME (kcal/kg) = (3.5*kg NFE) + (8.5*kg fat) + (3.5*kg protein)

**Experimental Design:** This study was a parallel design and the treatment period was 84 days in length. For three weeks prior to feeding the test diets, cats were fed Iams® Original Chicken and subjected to a baseline physical activity, indirect calorimetry, fasted blood and cognition measurement. At the end of the
washout period, cats were randomly allocated to either the HF or HC group. Each cat was fed their respective diet for a total of 84 d. On days -7, 35 and 76 cats were subjected to 22 hr indirect calorimetry measurements. Upon removal from the chambers fasted blood measures were obtained via jugular vena puncture. On days -14, 28 and 70 physical activity was measured and play motivation was measured on days -20 and 64 and cognition on days -1, 42 and 84. Body weight was measured weekly and feed intake measured daily. Body composition was measured via Dual Energy X-ray Absorptimetry (DXA) and BCS analysis prior to the initiation of the washout diet and on d 85 for all cats. Dual energy x-ray absorptiometry (DXA). Animals were anesthetized according to the protocol of an (IM) injection of Dexdomitor (Orion Pharma, Finland; distributed by Pfizer Corp, NY, USA) in combination with Hydromorphone (Baxter Healthcare Corp, Deerfield, IL, USA). Three DXA scans using infant software provided by Hologic Inc. (Model Delphi A with QDR® for Windows®, Hologic Inc. 35 Crosby Drive Bedford, MA, USA) were completed to measure body composition after an adequate plane of anesthesia was reached. Cats were placed in a sternal position and cranial aspect of ante brachium on the table with the phalanges facing caudally, hind limbs were bent slightly upward towards the abdomen while the tail curved just below the left rear. Whole body composition is the sum of the regions and segmented by: bone mineral content (kg), fat (kg), lean (kg), lean+ bone mineral content (kg), total mass (kg), and fat (%). Scans were reviewed while the cat was still on the DXA scanner to ensure that the scan acquisition was acquired properly. Once the scans were completed, cats were removed from the unit and placed in a recovery area and an IM injection of Antisedan (Orion Pharma, Finland; distributed by Pfizer Corp, NY, NY) was administered in order to reverse the pharmacological effects of Dexdomitor. All three scans were combined to obtain an average.

Metabolic Measurements

Indirect Calorimetry: To assess the effects of dietary treatments, indirect calorimetry was utilized to measure energy expenditure (kcal/kg^{0.67/d} and respiratory quotient. Breath was analyzed for a 5 minute period every half-hour interval. Levels of O2 and CO2 in the respiratory chambers were measured with infrared, O2 and CO2 analyzers (Qubit Systems®, Kingston, Ontario, Canada). The calorimeter is an open circuit, ventilated calorimeter with the room air being drawn through at a rate of 5-10 L/min depending on the body weight of the cat and to maintain CO2 between 0.4 – 0.9%. The rate of airflow was measured with the use of mass flow meters to enable total volume to be calculated. Calibration of the analyzers and mass flow meters was performed prior to each oxidation study and every 6 hours or sooner when a drift of >5% was observed in half hourly reference gas determination. Calibration was performed using standard
gas mixtures against known calibration standards. Indirect calorimetry measures occurred on days -7, 35 and 76.

The following calculations were used:

\[ \text{RQ} = \frac{\text{litres CO}_2 \text{ produced}}{\text{litres of O}_2 \text{ consumed}} \]  
[Eq. 1]

Heat Production (kcal) = 3.82 x litres O\(_2\) consumed + 1.15 x litres CO\(_2\) produced [Eq.2]

Carbohydrate oxidation:
\[ C_nH_{2n}O_n + nO_2 \rightarrow nCO_2 + nH_2O \]  
[Eq. 3]

Fat oxidation:
\[ (CH_2O)_{3n}(CH_2)_{3n}(CO_2H)_{3n} + nO_2 \rightarrow nCO_2 + nH_2O \]  
[Eq. 4]

(Ferrannini, 1988)

Blood Metabolites: Blood samples were obtained, via venipuncture (cephalic or jugular), to measure changes in biomarkers of metabolism including: glucose, insulin, triglycerides (TAG), and cholesterol (LDL and HDL). Blood samples for all metabolites were taken in the fasted state following the removal of the cats from the respiratory calorimetry chambers on days -6, 36 and 77. Approximately 2.5 ml of blood was taken during fasting per sample for all metabolites. Then the samples were placed on ice for 1 h. After clotting, samples were centrifuged at 300 rpm for 15 min at -4°C, and serum was decanted and stored at -20°C for later analyses. Analysis of glucose, TAG and cholesterol was completed using the Beckman Coulter AU480 automated chemistry analyzer that uses colorimetric measurements (UV/vis spectrometry; Indianapolis, IN, USA). Analysis of insulin was completed using a feline ELISA kit (Winston Salem, NC, USA). Glucose and insulin ratios were calculated using the following formula:

\[ \text{G: I} = \frac{\text{Glucose (Mg/dL)}}{\text{Insulin (mg/dL)}} \]
Behavioural Assessments

Physical Activity: Voluntary physical activity was measured using Actical activity monitors (Mini Mitter, Bend, OR, USA), which were worn parallel to the ribs and attached via a harness for 24 hrs during each physical activity measurement on days -14, 28 and 70. Cats were previously acclimated, over three months, to wearing the harness and monitors for 24 hrs to avoid significant behavioural changes associated to wearing the harnesses. Sufficient acclimation to novel research practices has been shown to reduce variability between and within animals (Meunier, 2006) allowing us to minimize animal handling and reduce duration of exposure to the harnesses to facilitate 24 hour collections. The collars, validated internally for short term measurable differences in activity, contain omnidirectional sensors capable of accurately measuring both intensity and duration of movements. Once the monitors were removed, the Actical software analyzed and converted the data into arbitrary numbers referred to as activity counts. Actical software allowed for the determination of average activity counts per designated time period (15 sec) during the light and dark phases without human interference.

Play Motivation: Play motivation was measured using an obstruction test (Widowski and Duncan, 2000). Two walled stalls (each measuring: 100 cm W X 100 cm L X 75 cm H; Quenn City polymers, Dayton, Ohio, USA), one classified as the start box and the other the goal box, each with a plexi-glass roof containing a 1 cm diameter hole in the center, were placed next to each other and connected via a swing door (23 cm W x 18 cm H). The swing door was made of 1/16” plexi-glass and attached to the top of the door frame. To assess play motivation, the swing door was made progressively more difficult to open through the addition of weights (50 g; maximum 600 g) placed into a trough at the bottom of the door. When the cat pushed at the weighted door with sufficient force it swung away from the frame, allowing the cat to pass underneath the door to enter the goal box where it was permitted to interact with a small toy mouse. Cats were assessed once per dietary treatment on days -20 and 64 at approximately 6 hrs post feeding. Cats were not permitted to interact with plush toys for four days prior to the initiation of the study. Data analyzed included: 1) maximum door weight, defined as the highest successful door weight (grams) in which the cat crossed from the start to goal box, 2) mean time to cross the door, defined as the sum of all latencies to successfully cross the door divided by the number of successful crosses and 3) mean box time, defined as the sum of all durations spent in the start box divided by the total number of trials (non-successful and successful).
Cognition: Maze tests are often utilized in studies on animal learning and behaviour; specifically, for the study of discrimination of spatial and sensory cues, habit formation, place and response learning, alternation and memory. The maze resembled a letter “T” as it was composed of a stem (including a start box) and two arms (stem measuring approximately: 7 ft L x 1.5 ft W x 1.5 ft H with each arm measuring: 3.25 ft L x 1.5 ft W x 1.5 ft H).

Maze acclimation consisted of four consecutive group (~5 cats) exposure periods for 10 min without any food rewards. Following, cats were individually acclimated to moving down the stem to obtain a 1g food reward. Over two separate exposure periods (10 min each) cats were required to successfully move down the runway (from the start box) three times per exposure. Lastly, cats were individually acclimated to moving down the stem and each arm to obtain a food reward. Successful acclimation was defined as either 10 successful arm entries in any order during a maximum number of 15 trials and for each trial cats were permitted 5 minutes to enter an arm before they were returned to the start box. All arm entries were recorded as left (L) or right (R). If a cat failed to meet the criteria as outlined additional training sessions were completed to ensure that each cat was completely acclimated to the T-maze. If the cat failed to completely acclimate following additional training the cat was removed from cognitive analysis.

Cognitive testing was conducted on days -1, 42 and 84. A visual cue was randomly assigned as a positive and negative cue for each cat and balanced for diet. The two spatial cues were a circle (●) and the letter x (X). Therefore, half of the cats consuming the HC and HF diet had the ● assigned as the positive cue and half had the X assigned as the positive cue. Evidence of maze learning was recorded via number of correct arm entries. Cues were randomly assigned to an arm for each trial. For instance, the ●, which may or may not be the positive cue for a certain animal, was assigned the left arm for trial 1, the right for trial 2 and the right for trial 3 etc. The cat was placed in the start box for 5 sec at which time the door was opened. Cats then travelled down the stem towards the “T” junction and made a selection. Following entry into the goal box of the selected arm, either incorrect or correct, the cat was restricted and permitted 30 sec to consume the food. Both arms were baited with 1 g of food to ensure that olfactory cues did not influence performance; however, food was only accessible to cats if they entered the correct arm.

Ten trials on d -1, 42, and 84 were used to measure learning. Cats were considered to have learned the task when they scored a minimum of 80% correct. However, learning was not necessary for these tests as differences in performance were compared between cats consuming the HF and HC diets.
Statistical Analysis: Unless otherwise stated, all data were analyzed using the proc mixed model of SAS Version 8.3 (SAS Institute), and differences were considered significant at $P < 0.05$. For metabolic and behavioural measures a trend was defined at $P < 0.1 – P > 0.05$. The data was analyzed using repeated-measures analysis, where the fixed effect was diet and the random variables were cat nested in diet and day, the Kenward-Roger option was used to estimate the denominator degrees of freedom. The variance-covariance matrix was chosen for each analysis based on Schwarz's Bayesian Criterion. When the effects were significant ($P < 0.05$), predetermined comparisons of least-squares means were made using the p-diff test, which is the two-tailed pair wise comparison test used by the mixed procedure. All data is represented as least squared means ± SEM.

Results

Body weight and body fat percent: There were no differences between groups in body composition during baseline measures ($p>0.05$). Further, diet and duration of exposure to dietary treatments did not impact body weight and metabolic body weight ($p>0.05$). There were no differences in body fat ($p=0.686$) and lean body mass (LBM, $p=0.682$) between treatments. However, for cats consuming the HF diet, body fat increased from baseline ($p=0.0006$) and there was a trend towards a decline in LBM ($p=0.0710$; Table 7); although changes of this magnitude may not represent a physiologically significant change.

Table 7: Body weight, total body fat and lean body mass and bone mineral content in adult domestic short hair cats after baseline (Day -7) exposure to a control diet and after chronic (Day 76) exposure to either a HF or HC diet.\(^1\)

<table>
<thead>
<tr>
<th>Day</th>
<th>Body Weight (kg)</th>
<th>Total Body Fat (kg)</th>
<th>Lean Body Mass + Bone Mineral Content (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>HF</td>
<td>Pooled</td>
</tr>
<tr>
<td></td>
<td>Pooled SEM</td>
<td>HC</td>
<td>HF</td>
</tr>
<tr>
<td>-7</td>
<td>4.392</td>
<td>4.351</td>
<td>0.379</td>
</tr>
<tr>
<td>76</td>
<td>4.348</td>
<td>4.375</td>
<td>0.428</td>
</tr>
</tbody>
</table>

\(^1\)Values are least-square means ± SEM, n = 10. Means in a row with superscripts without a common symbol (*) differ, $P < 0.05$. Means in a column with superscripts without a common letter differ, $P<0.05$. NS, $P \geq 0.05$. P-value refers to the ANOVA for diet*exposure effect of treatment. Bone mineral density is excluded from analysis of body compartments.

Energy, Fat and CHO Intakes: Energy, CHO and fat intake did not differ between groups at baseline ($p>0.05$). Energy intake did not differ from baseline for cats consuming the HF and HC diet ($p>0.05$).
Furthermore, energy intake did not differ between diets with acute (67 ± 3 kcal/kg\(^{0.67}\)/d HC vs. 66 ± 3 kcal/kg\(^{0.67}\)/d HF) and chronic (69 ± 2 kcal/kg\(^{0.67}\)/d HC vs. 66 ± 2 kcal/kg\(^{0.67}\)/d HF) exposure. As expected, due to composition differences between diets, fat (16.5 ± 1 kcal/kg\(^{0.67}\)/d HC vs. 38.5 ± 1 kcal/kg\(^{0.67}\)/d HF) and CHO (34.5 ± 1 kcal/kg\(^{0.67}\)/d HC vs. 15 ± 1 kcal/kg\(^{0.67}\)/d HF) intake differed (P<0.001).

**Fasted macronutrient metabolism:** Fasted RQ increased and decreased for cats consuming the HC and HF diet, respectfully (p<0.05). Duration of exposure to the HC (p=1.00) and HF (p= 0.314) diet had no impact on fasted RQ (Table 8; A). Fasted EE increased equally between treatments at day 35 (p<0.05); however, after day 76 fasted EE did not differ from baseline (p>0.05; Table 8; B).

Table 8: Fasted and fed respiratory quotient (A), energy expenditure and energy expenditure as a percent (%) of energy intake (B) in adult domestic short hair cats after baseline (Day -7) exposure to a control diet and after acute (Day 35) and chronic (Day 76) exposure to either a HF or HC diet \(^1\,^2\)

<table>
<thead>
<tr>
<th>Day</th>
<th>RQ</th>
<th>HC</th>
<th>HF</th>
<th>Pooled SEM</th>
<th>HC</th>
<th>HF</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7</td>
<td></td>
<td>0.791(^b)</td>
<td>0.791(^a)</td>
<td>0.01</td>
<td>0.834(^b)</td>
<td>0.832(^a)</td>
<td>0.002</td>
</tr>
<tr>
<td>35</td>
<td></td>
<td>0.814(^*)</td>
<td>0.763(^*)</td>
<td>0.01</td>
<td>0.860(^*)</td>
<td>0.774(^\ast)</td>
<td>0.004</td>
</tr>
<tr>
<td>76</td>
<td></td>
<td>0.814(^*)</td>
<td>0.755(^*)</td>
<td>0.01</td>
<td>0.863(^*)</td>
<td>0.791(^*)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>EE</th>
<th>HC</th>
<th>HF</th>
<th>Pooled SEM</th>
<th>HC</th>
<th>HF</th>
<th>Pooled SEM</th>
<th>EE as % Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7</td>
<td></td>
<td>70.7(^b)</td>
<td>66.4(^b)</td>
<td>3.7</td>
<td>67.9</td>
<td>71.0(^a)</td>
<td>1.5</td>
<td>113.4</td>
</tr>
<tr>
<td>35</td>
<td></td>
<td>76.4(^a)</td>
<td>72.6(^a)</td>
<td>4.1</td>
<td>68.3</td>
<td>72.2(^a)</td>
<td>1.5</td>
<td>102.5</td>
</tr>
<tr>
<td>76</td>
<td></td>
<td>74.3(^b)</td>
<td>67.9(^b)</td>
<td>3.6</td>
<td>65.4</td>
<td>66.7(^b)</td>
<td>1.2</td>
<td>105.5</td>
</tr>
</tbody>
</table>

\(^1\)Values are least-square means ± SEM, n = 10. Means in a row with superscripts without a common symbol differ, P < 0.05. Means in a column with superscripts without a common letter differ, P<0.05. NS, P≥0.05. P-value refers to the ANOVA for diet*exposure effect of treatment.

\(^2\)Fasting EE and EE are presented on a kcal/kg\(^{0.67}\)/d basis.
Fed macronutrient metabolism: Diet significantly affected RQ post feeding (p<0.05; Table 8; A). There was an increase in RQ with HC and a decrease in RQ with HF feeding from baseline (p>0.05). Duration of exposure (acute vs. chronic) to the HC diet had no impact on fed RQ (p=0.662); however, for cats consuming the HF diet RQ increased from acute to chronic exposure (p=0.022; Table 8; A). Maximum and minimum RQ at baseline was 0.87 ± 0.02 and 0.745 ± 0.02 and occurred at 6 and 1 hr post feeding, respectfully. Maximum RQ for the HC and HF was 0.900 ± 0.02 and 0.797 ± 0.02 and occurred at 10.5 and 14.5 hrs post feeding, respectfully. Minimum RQ for the HC and HF was 0.76 ± 0.02 and 0.73 ± 0.02 and occurred at 1 and 1.5 hrs post feeding, respectfully (Figure 9). EE post feeding was not different between diets (p<0.05; Table 8; B). In both diets there was a transient increase from baseline to day 35 (P<0.05), but no differences from baseline existed on day 76 (P>0.05) for both dietary treatments. There were no effects of diet or exposure on AUC for EE (p>0.05; Table 9). Furthermore, time to maximum (30 min) and minimum (17 hrs) AUC EE post feeding was similar across all dietary treatments (p>0.05).

Table 9: AUC for total EE (kcal/d) represented as a change from baseline as affected by diet (HF vs. HC), day and time post feeding.

<table>
<thead>
<tr>
<th>Day Post Feeding</th>
<th>Time</th>
<th>Baseline</th>
<th>SEM</th>
<th>Change from Baseline</th>
<th>SEM</th>
<th>Baseline</th>
<th>SEM</th>
<th>Change from Baseline</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>0-3 hr</td>
<td>193.3</td>
<td>10.6</td>
<td>5.2</td>
<td>4</td>
<td>192.1</td>
<td>10.6</td>
<td>7.9</td>
<td>4</td>
<td>0.611</td>
</tr>
<tr>
<td>3-9 hr</td>
<td>180.3</td>
<td>9.1</td>
<td>3.1</td>
<td>4</td>
<td>188.0</td>
<td>9.1</td>
<td>9.9</td>
<td>4</td>
<td>0.193</td>
<td></td>
</tr>
<tr>
<td>9-15 hr</td>
<td>174.8</td>
<td>10.3</td>
<td>-0.7</td>
<td>4</td>
<td>180.2</td>
<td>10.3</td>
<td>-4.9</td>
<td>4</td>
<td>0.422</td>
<td></td>
</tr>
<tr>
<td>15-22hr</td>
<td>165.6</td>
<td>12.5</td>
<td>1.6</td>
<td>6</td>
<td>178.6</td>
<td>12.5</td>
<td>-4.3</td>
<td>6</td>
<td>0.504</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>0-3 hr</td>
<td>193.3</td>
<td>10.6</td>
<td>0.2</td>
<td>4</td>
<td>192.1</td>
<td>10.6</td>
<td>-1.6</td>
<td>4</td>
<td>0.773</td>
</tr>
<tr>
<td>3-9 hr</td>
<td>180.3</td>
<td>9.1</td>
<td>-2.1</td>
<td>5</td>
<td>188.0</td>
<td>9.1</td>
<td>-5.6</td>
<td>5</td>
<td>0.618</td>
<td></td>
</tr>
<tr>
<td>9-15 hr</td>
<td>174.8</td>
<td>10.3</td>
<td>-9.7</td>
<td>7</td>
<td>180.2</td>
<td>10.3</td>
<td>-16.4</td>
<td>7</td>
<td>0.491</td>
<td></td>
</tr>
<tr>
<td>15-22hr</td>
<td>165.6</td>
<td>12.5</td>
<td>-4.9</td>
<td>6</td>
<td>178.6</td>
<td>12.5</td>
<td>-9.9</td>
<td>6</td>
<td>0.532</td>
<td></td>
</tr>
</tbody>
</table>

1Values are least-square means ± SEM, n = 10. Means in a row with superscripts without a common symbol differ, P < 0.05. Means in a column with superscripts without a common letter differ, P<0.05. NS, P≥0.05. P-value presented refers to the ANOVA for diet*exposure effect for change from baseline using baseline as a covariate.
Figure 9: Mean (n=10) RQ measured half-hourly during the fasted, fed, post-prandial and return to fasted states over a 22 hr period in adult domestic cats consuming a control diet at baseline (Day -7) and either a HF or HC diet after chronic (Day 76) dietary exposure.

**Fasted Blood Metabolites:** Insulin, glucose, TAG, cholesterol and blood urea nitrogen concentrations did not differ between groups at baseline (p>0.05) (Table 10). There was no effect of dietary treatment on insulin (p>0.05) (Table 10); however, plasma insulin significantly declined (P<0.05) from baseline on days 36 and 77 in cats fed HF. Plasma glucose was significantly greater in cats fed HF on day 36 (P<0.05), but no differences between treatments existed at day 77 (P>0.05). Plasma glucose significantly increased from baseline at day 36 (p=0.007) in cats fed HF; although, these differences were absent at day 77 (p=0.124). There was no effect of dietary treatment on glucose to insulin ratio; however, there was a trend towards a higher ratio with HF feeding on day 36 (p=0.068). Furthermore, the G:I increased from baseline on days 36 and 77 in cats fed HF (P<0.05). Plasma triglycerides (TAG) decreased from baseline in cats fed HC on day 35 (P<0.05); but were similar to baseline on day 77 (P>0.05). There were no differences in triglyceride concentrations between dietary treatments. Dietary treatment did not influence plasma cholesterol (p>0.05); however, plasma cholesterol concentrations in cats consuming HF resulted in an increase from baseline at day 36 and 77 (p<0.05). Further, there was no effect of diet on total protein (data not shown); alternatively, blood urea nitrogen (BUN) concentrations were significantly greater in cats fed HF at days 36 and 77 (P<0.05) and suggests greater amino acid oxidation that is likely correlated to the observed decline in LBM. BUN declined with exposure the HC diet (p<0.05) and there was a trend to increase with exposure to the HF diet (p=0.058). All blood metabolites are similar to those previously reported (Appleton et al., 2001; 2004; Coradini et al., 2011; Hoenig et al., 2011).
Table 10: Fasted blood plasma metabolites in adult domestic cats with baseline (Day -6) exposure to a control diet and after acute (Day 36) and chronic (Day 77) exposure to either the HF or HC diet.

<table>
<thead>
<tr>
<th>Day</th>
<th>Insulin (ng/L)</th>
<th>Glucose (Mg/dL)</th>
<th>G: I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>HF</td>
<td>Pooled</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>SEM</td>
<td>SEM</td>
</tr>
<tr>
<td>-6</td>
<td>158.4</td>
<td>163.8</td>
<td>19.1</td>
</tr>
<tr>
<td></td>
<td>19.1</td>
<td>86.4</td>
<td>86.8</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>145.1</td>
<td>130.6</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>17.5</td>
<td>83.9**</td>
<td>93.0*</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>0.62</td>
<td>0.84a</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>245.7</td>
<td>113.2</td>
<td>57.0</td>
</tr>
<tr>
<td></td>
<td>57.0</td>
<td>89.9</td>
<td>92.5ab</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>0.73</td>
<td>0.90a</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Triglycerides (Mg/dL)  
Cholesterol (Mg/dL)  
Blood Urea Nitrogen (Mg/dL)

<table>
<thead>
<tr>
<th>Day</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
<th>Blood Urea Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>HF</td>
<td>Pooled</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>SEM</td>
<td>SEM</td>
</tr>
<tr>
<td>-7</td>
<td>34.4a</td>
<td>27.3</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>196.6</td>
<td>168.3b</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>19.0</td>
<td>19.8a</td>
<td>19.0</td>
</tr>
<tr>
<td>-36</td>
<td>27.4b</td>
<td>22.2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>180.1</td>
<td>211.5a</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>16.7**ab</td>
<td>19.3*</td>
<td>0.7</td>
</tr>
<tr>
<td>-77</td>
<td>27.3ab</td>
<td>25.9</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>189.7</td>
<td>215.2a</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>17.8**ab</td>
<td>20.1*</td>
<td>0.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
<th>Blood Urea Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>HF</td>
<td>Pooled</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>SEM</td>
<td>SEM</td>
</tr>
<tr>
<td></td>
<td>34.4a</td>
<td>27.3</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>196.6</td>
<td>168.3b</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>19.0</td>
<td>19.8a</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are least-square means ± SEM, n = 10. Means in a row with superscripts without a common symbol differ, P < 0.05. Means in a column with superscripts without a common letter differ, P < 0.05. NS, P ≥ 0.05. P-value refers to the ANOVA for diet*exposure effect of treatment.

Physical Activity: Baseline (d -14) activity was similar between groups (p>0.05). There was no effect of dietary treatment on activity when compared within acute (day 28; p= 0.599) and chronic (day 70; p=0.176) exposures between the HF and HC diet (p>0.05; Figure 10). There was a significant decline in activity on day 70, versus day -14 and day 28, in cats consuming HC diet (p<0.05). There was a trend for physical activity to decline from baseline with acute (day 28) exposure to the HF diet (p=0.061); this decline in activity continued and was significantly different from baseline after chronic (day 70) exposure (p=0.01). Change from baseline in activity count with HC and HF feeding during acute exposure was -1.361 and -1.771 and during chronic exposure was -2.882 and -2.653, respectively. Activity as measured as a change from baseline did not differ between treatments (p>0.05).
Figure 10: Mean ± SEM activity count after baseline (Day -14; control diet), acute (Day 28) and chronic (Day 70) dietary exposure. Values are least-square means ± SEM, n = 10. Means within an exposure with superscripts without a common symbol differ, P < 0.05. Means across exposures with superscripts without a common letter differ, \( P < 0.05 \). NS, \( P \geq 0.05 \). P-value refers to the ANOVA for diet*exposure effect of treatment.

Play Behaviour: There were no differences between groups at baseline (d -20) or after chronic (d 64) exposure to either the HF or HC diet for any of the play motivation measures (p>0.05; Table 11).

Table 11: Change in measures of the adult domestic cats willingness to work to obtain access to a stuffed toy mouse at varying door weights (0-600 g) from baseline (Day -20) exposure to a control diet and after chronic (Day 64) exposure to either a HC or HF diet.

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>HF</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Door Weight (g)</td>
<td>250 ± 86</td>
<td>120 ± 56</td>
<td>380 ± 86</td>
</tr>
<tr>
<td>Mean Time to Cross Door (s)</td>
<td>69.5 ± 17</td>
<td>-18.2 ± 17</td>
<td>72.4 ± 17</td>
</tr>
<tr>
<td>Mean Box Time (s)</td>
<td>178.4 ± 36</td>
<td>-54.4 ± 24</td>
<td>148.7 ± 36</td>
</tr>
</tbody>
</table>

1Values are least-square means ± SEM, n = 10. Means in a row with superscripts without a common symbol differ, P < 0.05. Means in a column with superscripts without a common letter differ, \( P < 0.05 \). NS, \( P \geq 0.05 \). P-value presented refers to the ANOVA for diet*exposure effect of treatment for change from baseline using baseline as a covariate.
**Cognition:** There was an effect of group on number of correct responses during baseline (d -1) cognition testing (p<0.05; Table 12), therefore, we used change from baseline to compare dietary treatments. Performance during cognition testing after d 84 was lower for cats consuming the HF diet versus the HC diet as measured via a change in performance from baseline (p<0.05). While performance was consistent over acute (d 42) and chronic (d 84) exposure for cats consuming the HF diet (p=0.894), there was a trend for performance to improve with increased exposure to the HC diet (p=0.097).

Table 12: Change in performance (number of correct per ten trials) during T-maze testing in adult domestic cats from baseline (Day -1) exposure to a control diet and after acute (day 42) and chronic (Day 84) exposure to either a HF or HC diet.

<table>
<thead>
<tr>
<th></th>
<th>HC Baseline</th>
<th>HF Baseline</th>
<th>Change from Baseline</th>
<th>HF Change from Baseline</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 42</td>
<td>4.2 ± 0.4**</td>
<td>5.8 ± 0.4*</td>
<td>0.2 ± 0.5</td>
<td>-0.8 ± 0.5</td>
<td>0.202</td>
</tr>
<tr>
<td>Day 84</td>
<td>1.5 ± 0.8*</td>
<td>0.040</td>
<td>-0.9 ± 0.8**</td>
<td>0.040</td>
<td></td>
</tr>
</tbody>
</table>

1Values are least-square means ± SEM, n = 10, for number of correct responses over ten trials. Means in a row with superscripts without a common symbol (*) differ compared within each exposure (i.e. baseline vs. baseline; HC Day 42 vs. HF Day 42; HC Day 84 vs. HF Day 84), P < 0.05. Means in a column with superscripts without a common letter differ, P<0.05. NS, P≥ 0.05. P-value presented refers to the ANOVA for diet*exposure effect for change from baseline of treatment using baseline as a covariate.

**Discussion**

Cats were capable of modifying macronutrient oxidation according to intake during the consumption of commercially available HF and HC that provided similar protein: energy ratios diets fed to energy requirements. The balance of protein: energy ratio is important to eliminate the effects of protein intake on our metabolic parameters. Dietary treatments did not influence energy balance or body weight; although, cats consuming the HF diet gained a significant amount of body fat and had a trend to decrease LBM. This shift is interesting in light of the fact that cats fed HF have greater BUN, which suggests greater amino acid oxidation. This increase BUN was not due to dietary influences, as protein intake (g/kg BW) was similar between treatments; therefore, the increased BUN may have been due to increased protein degradation and subsequent AA oxidation. The mechanism of this relation cannot be explained by the information that we have on these diets, but in the future, an understanding of the total long chain fatty acid intake may be warranted. From the fasted data we can speculate that there was a trend towards an
increase in insulin required for effective glucose disposal with HC feeding; a relationship primarily driven by an increase in fasted plasma glucose observed during the consumption of the HF diet with no associated change in fasted plasma insulin. Play motivation was not significantly affected by diet suggesting that the HF and HC diet were equally satiating. Further, the HF diet may have a positive effect on physical activity as hourly activity counts were numerically, but not significantly, greater for cats consuming the HF diet. Lastly, cognitive function improved with the consumption of the HC diet with a corresponding decline in cognitive function in cats consuming the HF diet.

Exposure to a HF or HC diet for 76 d did not significantly affect body weight or metabolic body weight of cats. Several studies have previously shown that cats fed HF, rather than HC diets, are more susceptible to weight gain with ad libitum feeding (Backus et al., 2007; Coradini et al., 2011) and feeding to weight maintenance (Thiess et al., 2004; Slingerland et al., 2007b). The present findings are similar to those of a previous study conducted by our group (Gooding et al. 2012) where body weight was not affected by diet when cats were fed to energy requirement; However, our previous results suggested that cats consuming a HC diet tended to gain weight although we commented that it was likely not physiologically significant. These data suggests that when healthy cats are fed to weight maintenance, level of dietary fat and CHO, with consistent protein:ME ratio, does not significantly impact BW over a three month feeding period.

Absolute body fat did not differ between groups at baseline or after chronic exposure to the test diet; however, cats within the HF dietary treatment gained a significant amount of body fat, congruent with a trend for lean body mass to decline after 85 d of dietary exposure. The decline in LBM can be partly explained with the increase in BUN over time in the cats fed HF, suggesting increased amino acid oxidation from increased protein breakdown. In humans and cats, HF diets have been shown to increase risk for an accumulation of body fat similar to the present observations (Schwartz et al., 1985; Treuth et al., 2003; Nguyen et al., 2004; reviewed in Shikany et al., 2010). To our knowledge, only Backus et al., 2007, have studied the long term feeding (17 week) effects of HF and HC diets with similar protein:ME ratios on body composition in cats and found that LBM was not different between groups where fat mass increased with HF feeding. Alternatively, some researchers have suggested that HC diets contribute to obesity and apoptosis (Brand Miller and Colagiuri, 1994; Rand et al., 2004). For instance, cats fed a HC exhibited a higher level of non-esterified fatty acid (NEFA) suppression that was hypothesized to be a consequence of increased lipogenesis contributing to fat deposition in muscle (Hoenig et al., 2007). It can be concluded that the changes in body composition in our study are attributable to the modifications in fat and CHO intake as protein, know to influence LBM (Nguyen et al., 2004), was consistent between the HF
and HC diets on a ME basis. We should point out however, that we did not do a specific analysis on the amino acids or fatty acids and differences in the ratio of indispensable amino acids, the indispensable to dispensable amino acid ratio, the total intake of long chain fatty acids or the ratio of n6: n3 fatty acids all may influence macronutrient metabolism and body composition. Therefore, further investigation is required to determine if dietary fat increases body fat content when fed to maintain BW in healthy, adult cats.

Respiratory quotient in the fasted and fed state increased with exposure to the HC and decreased with exposure to the HF diet. Gooding et al., 2012, observed similar results as fasted/fed RQ was lower during the consumption of a HF diet versus a HC diet. Other researchers have also observed a similar decline in fed RQ from about 0.87 with HC feeding to approximately 0.79 with HF feeding in both cats and humans (Lester et al., 1999; Hoenig et al., 2007; Smith et al., 2000; Treuth et al., 2003). The reciprocal relationship between fat and CHO oxidation, reflected in RQ, is expected as CHO oxidation may happen at the expense of fat oxidation via inhibited fatty acid (FA) catabolism (Acheson et al., 1984; Chow, 1992). Inhibition of fat oxidation after HC diets may be caused by 1) the inhibition of NEFA availability since NEFA are the major source for fat oxidation in the resting state (Schrauwen et al., 2000) and 2) by alterations in intracellular mechanisms (Sidossis et al., 1996; Koutsari and Sidossis, 2003). The latter is more likely the explanation for the observed inhibition of fat oxidation with high CHO intake as transient elevations of plasma glucose and insulin decrease whole body fat oxidation, by blocking FA entry into the mitochondria, despite elevations in plasma NEFA concentrations (Sidossis and Wolfe, 1996; Sidossis et al., 1996, 1999; Koutsari and Sidossis, 2003). The ability to modify CHO and fat oxidation according to macronutrient intake demonstrates that cats exhibit characteristics metabolic flexibility.

Fat was oxidized more rapidly than CHO post pradially, regardless of diet as indicated by an immediate drop in RQ after feeding with a gradual increase over time. These results differ from previous data in humans where RQ increases immediately after feeding peaking around 30 min to 2 hrs when consuming an HC and HF diet, respectively (Labayen et al., 1999). Hewson-Hughes et al., 2011, also reported that cats, as compared to dogs, have lower peak insulin and delayed insulin release versus the dog. The slow response to dietary CHO in cats is likely a consequence of 1) lack of glucokinase (GK) (Tanaka et al., 2005), and 2) the slow rate of incorporation of glucose into glycogen (Ballard 1965; Kienzle, 1989). As opposed to dogs cats have increased activities of fructokinase (FK), pyruvate kinase (PK) and glucose-6-phosphate dehydrogenase (G6PD) all of which suggest high activities of fatty acid synthesis and may be the reason for the observed responsiveness of fat oxidation in cats (Tanaka et al., 2005). Cats may be
capable of adapting fat oxidation to intake and oxidizing fat rapidly since cats have evolved to consume
diets high in fat (>50% of energy) and low in CHO versus humans who have a heavier reliance on
CHO/glucose for energy. Overall, cats appear to prioritize fat oxidation over glucose oxidation in contrast
to omnivorous species where glucose is the preferred energy substrate.

There was no effect of diet on EE in the fasted state and over the 22 hours post feeding. Similarly,
previous studies have demonstrated that EE in humans and cats fed HF and HC diets meet, but not exceed
energy requirements/intake (Lester et al., 1999; Koutsari and Sidossis, 2003). EE as a percent of energy
intake was similar at baseline and throughout the study period for cats fed both HF and HC diets which
support the observed weight maintenance. EE slightly exceeded intake; however, this was expected as
cats were fed to 95% of their energy requirement. HF diets are often hypothesized to contribute to weight
gain due to their low heat increment of feeding (HIF) with the HIF being influenced by the energetic costs
of digestion and assimilation (Baldwin, 1995). The heat increment of feeding for protein, fat and CHO is
estimated to be 30, 7 and 10 percent of total energy, respectfully. HIF did not appear to influence EE as
was suggested in Gooding et al., 2012, as EE as a % intake and AUC for TEE did not differ between
dietary treatment groups. Gooding et al., submitted, found that EE as a percent of intake differed between
HF and HC diets as cats consuming the HC diet demonstrated an EE that more closely matched intake
(~92% versus 87% of energy intake for cats fed the HF diet), but this may not have been physiologically
significant. Overall, cats appear capable of adapting EE to energy intake to maintain BW during
controlled feeding despite macronutrient differences between diets.

The consumption of HC appeared to reduced peripheral insulin sensitivity as evidenced by a difference
between diets from baseline in G:I ratios suggesting impairment in the insulin mediated glucose disposal.
This effect was largely driven by the observed increase in glucose release with HF feeding correlated to a
decline in plasma insulin. While plasma insulin levels were greater for cats consuming the HC diet the
differences observed were not significant. Thus, with the consumption of the HF diet less insulin was
required to normalize plasma glucose; therefore, insulin sensitivity was, in fact, improved for cats fed HF
versus HC. Slingerland et al, 2007b, also observed an improvement in glucose disposal rates with the
consumption of a HF diet versus the HC diet over a 9 mo ad libitum feeding period. However, this
difference was driven by an increase in insulin secretion that may, over time, lead to insulin insensitivity.
The apparent difference in insulin sensitivity between HF and HC diets may be a risk for the development
of feline diabetes mellitus during long term feeding. Overstimulation of the pancreatic β cells may lead to
β cell exhaustion and ultimately dysfunction (Rand et al., 2004), but is seen diets have already resulted in
other risk factors for insulin insensitivity, such as weight gain. The variability in insulin sensitivity that is
often observed between individual cats may have not allowed significant differences in insulin sensitivity
to be measured and warrants a more detailed investigation. There was no effect of dietary treatment on
fasted serum TAG in cats, an effect that has previously been documented in cats (Backus et al., 2007).
Alternatively, some researchers have observed elevated TAG levels in cats consuming HF diets (Thiess et
al., 2004). Differences may be attributable to feeding practices since Backus et al., 2007 fed ad libitum
and Thiess et al., 2004, fed to maintain weight and total fat load may impact TAG concentration. Elevated
TAG concentrations are often associated to a decline in insulin sensitivity of adipose tissue and weight
gain; however, insulin sensitivity remained high for cats on the HF diet and therefore it is not surprising
that no differences in plasma TAG were observed (Reavan, 2005). Total plasma cholesterol increased
with exposure to the HF diet from baseline; an effect that was likely a consequence of increased intestinal
absorption of cholesterol associated with the consumption of diets high in fat (Dobenecker et al., 1998;
Thiess et al., 2004). Blood urea nitrogen increased with exposure to the HF diet and may be indicative of
protein catabolism since there was an associated numerical decline in lean body mass with HF feeding.
Though diets were fed to an equivalent protein: ME ratio the protein catabolised may have been utilized
for gluconeogenesis as reflected through the increased plasma glucose with HF feeding (Nguyen et al.,
2004). Overall, long term (>85 d) consumption of the HC diet contributed to a decline in insulin
sensitivity in cats. Reduced insulin sensitivity may contribute to β cell malfunction and potentially
diabetes with prolonged HC consumption. However, the consumption of an HF diet may contribute to
lean tissue loss and the development of obesity, but the mechanism for this is unclear and deserves further
investigation.

Physical activity declined with exposure to the HF and HC diet from baseline; however, the decline in
activity was more pronounced for cats consuming the HC diet. These results support our previous
hypothesis that HC diets may have contributed to an observed weight gain by impacting daily physical
activity patterns (Gooding et al., 2012). Similar results have been observed in humans and rats, where
diets high CHO have been shown to adversely impact plasma insulin and glucose levels, and have been
shown to influence mood by reducing energy level (Pivonka & Grunewald, 1990; Kapas et al., 1993;
Wells et al., 1997; Nabb and Benton, 2006). The release of tryptophan and serotonin, that modify mood
and sleepiness, are influenced by glucose metabolism as peripheral serotonin contributes to the regulation
of insulin release from pancreatic β cells (Watanabe et al., 2011). The modified glucose metabolism from
baseline for both test diets may have influenced serotonin release and function contributing to the
observed reduction in activity. Since diets high in CHO appear to have greater effects on serotonin the
outcomes may be exaggerated during HC feeding (Fernstrom, 1983). Further, insulin may reduce the expression of brain noradrenaline transporters decreasing the release of epinephrine and resulting in a general calmness (Figlewicz et al., 1993). Reduced physical activity may also impact insulin sensitivity as the skeletal muscle is responsible for ~75% of whole body insulin stimulated glucose uptake in humans (Shulman et al., 1990). Further, physical activity increases the lipid turnover in muscle tissue and this turnover can have a positive effect on insulin sensitivity (Corcoran et al., 2007). Alternatively, some researchers conclude that HF diets promote greater feelings of lethargy and feebleness (Wells et al., 1995, 1997; Lloyd et al., 1994, 1996). Dietary lipids act to the greatest extent over proteins and carbohydrates to stimulate the release of cholecystokinin (CCK) and somatostatin from the duodenum (Kapas et al., 1991). CCK, a peptide hormone responsible for the stimulation of digestion of fats and proteins, has been strongly implicated in the mediation of postranidal sleepiness as infusion of CCK produces sedative like effects in humans and animals (Stacher et al., 1979). In conclusion, both HF and HC diets may contribute to lethargy in cats; however, in the present study, where caloric intake was equal between treatments, these effects were greater for cats consuming the HC diet and suggest that HC diets may lead to a greater positive energy balance over time due to lower daily voluntary activity.

Dietary treatment had minimal impact on play motivation. We hypothesized that play motivation, indicative of level of hunger, would be lower in the cats exposed to the HC diet as the cat is often cited as being incapable of digesting and utilizing CHO. Cats do not express of the TAS1r2I gene, which is essential to taste dietary sugars (Li et al., 2005), they also lack salivary amylase, which aids in initiating CHO digestion (Meyer and Kienzle, 1991) and compared to omnivorous species cats have reduced activities of pancreatic amylase, and intestinal disaccharidases (Meyer and Kienzle, 1991). A reduced capacity to handle CHO may lead to altered passage rates and consequently increased gut fill which contributes to satiety and lethargy as stimulation of the small intestinal mucosa induces sleepiness and sleep in cats (Wells et al., 1998). Despite these limitations some researchers have shown that cats appear capable of digesting HC diets effectively (Thiess et al., 2003) and perhaps, differences in play motivation were not as apparent as hypothesized since cats were able to utilize the energy as provided by carbohydrates in the HC dietary treatment as evidenced by the calorimetry data. In addition, cats do not always exhibit hyperphagia with HF diets (Bradshaw et al., 1996). Backus et al., 2007, found that plasma ghrelin, an orexigenic hormone, was negatively correlated to level of dietary fat in cats suggesting that fat is more satiating than initially hypothesized. Perhaps, if we had measured play motivation at different time points the effect of diet may have been more apparent; for instance, we may have tested one hour post feeding and again 16 hours post feeding similarly to Hall et al., 1998. Lastly, Hewson-Hughes et al.,
2011, concluded that cats have a “ceiling” for carbohydrate intake where fat intake appears to be more flexible to allow for balance of nutrients (namely protein). This “ceiling” of CHO intake significantly impacted total energy intake and thus, cats may be less satiated with HC diets than initially hypothesized because intake was below predicted values (Bermingham et al., 2010). To address some of the unknowns regarding level of hunger during the consumption of a HF or HC diet, future studies correlating play motivation to feed intake and concentration of satiety/hunger signals may be necessary. In short, there appears to be no difference between HC and HF diets on level of hunger in the adult domestic cat 6 hrs post feeding. Thus, overeating may be less likely to occur with HF feeding, and HC diets may not contribute to enhanced gut fill and satiety, as was originally hypothesized.

In general, cats performed poorly during t-maze testing with only 2 cats scoring 80% during a single test. The poor performance may have been improved if exposure to the t-maze and cues were increased from three exposures of ten trials. In previous studies using T-mazes for studies of feline cognition significantly more trials were used (Irle and Markowitsch, 1982). For instance, Olmstead et al., 1976, observed that it took a median number of 170 trials for cats to meet the criterion of 80% correct during a spatial discrimination task. The HF and HC diets had inverse effects on cognitive function. Cats consuming the HF diet displayed a decline in performance during t-maze testing from baseline measures. Alternatively, cats consuming the HC diet demonstrated an improvement in performance in spatial learning with repeated testing from baseline. Inefficient glucoregulation causes cognitive declines (Gradman et al., 1993; Meneilly et al., 1993; Hewer et al., 2003; Riby et al., 2008) and since cats consuming the HC diet displayed reduced insulin sensitivities it would not have been surprising to observe a decline in cognitive function. However, cats consuming the HF diet demonstrated more impaired cognitive function. Certain cognitive functions are sensitive to short term variations in glucose availability since glucose is required for utilization as a fuel source for energy requirements and brain function (Bellise, 2004). Reduced glucose availability, as indicated by reduced glucose oxidation over 24-hrs and excess glucose oxidation as a percent of intake, may have negatively impacted cognitive function during the consumption of a HF diet. Conversely, some researchers have found that higher blood glucose levels prior to starting a cognitively demanding task demonstrate better performance (Benton and Owens, 1993; Benton et al., 1994; Martin and Benton, 1999). Cats consuming the HF diet had higher fasted blood glucose levels; however, the blood glucose levels at the time of testing, 6 hrs after feeding, were likely higher in the cats consuming the HC diet (Coradini et al., 2011). Overall, the cats consuming the HC diet demonstrated superior cognitive performance as measured from baseline. The enhanced performance may have been a direct effect of increased glucose availability for cognitive function; however, since cats did
not learn the task, further research is warranted.

To our knowledge, this is the first study of its kind to combine indirect calorimetry measurements, blood plasma metabolites and behavioural outcomes to investigate adaptation to HF and HC diets with equal provision of dietary protein (g/\text{kw BW}) fed to ER in adult domestic cats. Our results indicate that cats are capable of adapting energy metabolism to differing macronutrient intakes; however, both HF and HC diets present specific risk factors for the development of adiposity. Without positive energy balance, increased body fat and plasma cholesterol, cognitive impairment and a decline in physical activity were observed with HF feeding. Conversely, glucose responsiveness, insulin sensitivity and activity (numerically greater than cats consuming the HF diet) declined with HC feeding. Similar to previous data, it is likely that prolonged consumption of the HC diet may be the greatest risk factor for the development of obesity and diabetes due to a greater decline in physical activity (Slingerland et al., 2007b) and glucose disposal leading to β-cell exhaustion and ultimately apoptosis and diabetes (Appleton et al., 2001); two factors most often cited as the greatest risk factors for the development of obesity. However, the increased adiposity observed with HF feeding may also increase the cats risk for the development of obesity and warrants further investigation. For instance, total fat mass may not be of concern but rather relative distribution of fat should be measured. More studies on the effects of long term (>3 months) \textit{ad libitum} intake of HC and HF diets on energy and macronutrient balance may address some of our hypotheses and contribute to our understanding of feline energy metabolism and associated behavioural effects that contribute to obesity.
References


behavioral acclimation protocol for cats to respiration chambers used for indirect calorimetry studies. 
*JAAWS.* 15(2), 144-162.


regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. *J Clin Invest.* 98, 2244–2250.


Slingerland, L.I.; Vasilova, V.V.; Plantinga, E.A.; Kooistra, H.S.; Beynen, A.C., 2007a: Indoor confinement and physical inactivity rather than the proportion of dry food are risk factors for the development of feline type 2 diabetes mellitus. *Vet J.* In Press.


Thiess, S.; Becskei, C.; Tomsa, K.; Lutz, T.A.; Wanner, M., 2004: Effects of high carbohydrate and high fat diet on plasma metabolite levels and on iv glucose tolerance test in intact and neutered male cats. *J*


CHAPTER 4: Dietary mannoheptulose (MH) results in increased energy expenditure after a 28 d feeding trial in cats.¹²

Gooding, M.A¹; Shoveller, A.K.²; Zhang, J²; Davenport, G.²; Atkinson, J.L.¹; Duncan, I.J.H¹;

Authors Last Name for PubMed Indexing: Gooding, Shoveller, Zhang, Davenport, Atkinson, Duncan

Addresses:
¹Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario
²Pet Care, Procter and Gamble, Mason, Ohio

*To whom correspondence should be addressed: shoveller.ak@pg.com

Running Title: Influence of Dietary Mannoheptulose on Energy Metabolism.

Key Words: Mannoheptulose, energy expenditure, oxidation, calorimetry, glucose, insulin

Funding: This work was supported by The Procter and Gamble Co., Pet Care, Mason, Ohio, USA 45040

Abbreviations: MH, mannoheptulose, CR, calorie restriction, CRM, calorie restriction mimetic, FA, fatty acid, NEFA, non-esterified fatty acids, CHO, carbohydrate, IVGTT, intravenous glucose tolerance test, BW, body weight.
Abstract

The effect of dietary mannoheptulose (MH; 8 mg/kg BW) treatment was investigated in 20 lean (N=10) and moderately overweight (N=10) cats (Felis catus; 3 ± 5 mo, 4 ± 2 kg). Cats randomly received a control (-MH) and test (+MH) dietary treatment for 28 d in a crossover design with a 14 d washout period between each treatment leg. Two 22-h indirect calorimetry studies were conducted after cats began to receive dietary treatment, acute (d 0) and chronic (d 21). Blood samples were collected after a 24 hr fast on d 1 and 22. On d 28 cats were subjected to an intravenous glucose tolerance test (IVGTT) following an oral dose of water or dissolved MH (8 mg/kg BW) depending on dietary treatment. Cats were capable of digesting dietary MH as evidenced by significant effects of diet on fasted plasma MH and MH area under the curve during the IVGTT (p<0.05). There were no effects of MH on body weight, respiratory quotient in the fasted and post-prandial states (p>0.05). There was no effect of MH on fasted or total 22-hr energy expenditure (EE; kcal/hr); however, there was a trend for area under the curve (AUC) for EE to be greater during the 15-22 hr post feeding state (fasted) for cats subjected to the MH dietary treatment (p=0.0613). There were no significant effects of MH on fasted blood parameters including: glucose, insulin, triglycerides, free fatty acids and total cholesterol and C-reactive protein (p>0.05). Furthermore, an oral MH dose during an IVGTT did not impact glucose and insulin as there were no time point differences (p>0.05). Overall, the cat, an obligate carnivore, demonstrated some metabolic response to dietary MH treatment. Further investigation on whether certain physiological parameters and dietary parameters change the effects of dietary MH are warranted to better understand the potential effects of this purported CRM.
Introduction

Calorie restriction (CR) is the most vigorous and reproducible intervention to inhibit the physiological effects of aging, to delay the commencement of most pathologies (including cancer and diabetes) and to extend mean and maximum lifespan by 20 to 40% (McCay et al., 1935; Weindruch and Sohal, 1997; Weindruch and Walford, 1988). However, there are concerns associated with the feasibility of implementing such CR regimes for long periods of time because of the difficulty to maintain and additionally because CR is related with negative behavioural effects, such as increased aggression. Calorie restriction mimetics (CRMs) have been studied as an alternative to CR and to avoid some of the negative effects associated with the implementation of CR regimens (Ingram et al., 2004). The objectives of CRM strategies are to produce the same pro-longevity effects that CR provides without reducing caloric intake. Since the prolongevity strategies of CR influence systems involved in energy sensing, and regulation of metabolism, the initial targets of CRMs focused on metabolites that modify glucose metabolism. Glucose anti-metabolites, such as mannoheptulose (MH), are believed to inhibit the glycolytic pathway. Specifically, MH, a seven carbon sugar found in avocados, acts as a hexokinase inhibitor that prevents the phosphorylation of glucose therefore blocking flux through the glycolytic pathway. Glucose anti-metabolites mimic some of the beneficial physiological effects of CR including: reducing body weight, plasma insulin, body temperature, delayed tumour growth, and elevation of circulating glucocorticoid hormones (Roth et al., 2001).

Reducing body weight with CRM strategies has large potential application as approximately 35% of cats (*Felis Silvestris Catus*) are classified as overweight or obese in the United States (Lund et al., 2005). Consequentially, diabetes mellitus is increasing in prevalence in the domestic cat population with an estimated incidence of 2.45 cases/1000 cat years-of-risk (Panciera et al., 1990; Scarlett and Donoghue, 1998). The high incidence rate of obesity and diabetes exemplifies the need for a strategy to control obesity and the associated metabolic effects.

The objectives of the present study were to measure the influence of MH supplementation at 8mg/kg BW/d in a moderately high fat diet and to measure the effects of dietary MH treatment on the physiology and of young adult, lean, and moderately overweight cats. Measures included: fasting and fed mannoheptulose, indirect calorimetry measures of energy expenditure, fat and carbohydrate oxidation, fasted blood samples and an intravenous glucose tolerance test (IVGTT). We hypothesize that dietary MH
treatment will cause: 1) an increase in serum MH concentrations, 2) greater energy expenditure (EE) due to a shift in reliance on carbohydrate oxidation to fat oxidation, 3) lower fasted plasma glucose, insulin and cholesterol concentrations, and 3) improved insulin sensitivity.

Materials and Methods

All procedures were reviewed and approved by Procter and Gamble’s Institutional Animal Care and Use Committee in accordance with IACUC guidelines.

Animals: Twenty cats (N=20) of similar age (3 ± 5 mo), and split 10 females (5 lean and 5 moderately overweight) and 10 males (5 lean and 5 moderately overweight), were randomly separated into four groups of five. Cats were considered lean at a body weight of <3.6 kg and a body fat mass of <0.5 kg or 17% body mass and cats were considered moderately overweight at a body weight of >3.5 kg and body fat mass of >17%. Diets were balanced by both sex and body condition. Cats were provided from Pet Health and Nutrition Center (PHNC) at Procter and Gamble-Pet Care, Mason, Ohio. Standard veterinarian evaluation (physical exam, chemical and CBC blood analysis) of overall health was completed prior to the initiation of the study and all cats entered the study healthy.

All cats were previously acclimated to respiration chambers and associated environment. Acclimation success was assessed using the Cat-Stress-Score (CSS; Kessler and Turner, 1997), feed intake, fearfulness (response to novel stimuli) and elimination behaviours as indices (Gooding et al., 2012). Cats were considered successfully acclimated when they demonstrated behaviours similar to those observed in a free living environment where they are permanently housed, as well as behaviours indicative of low stress and fear response.

Housing: Cats were housed in a free-living group environment with indoor/outdoor access during the day (0800-1500 h) and indoor-only access at night (1500-0800 h). Room environmental enrichment included perches, beds, toy houses, scratching posts, toys and climbing apparatus. All cats were socialized daily for a minimum of 60 min. Cats were maintained on a 12 hour lighting schedule with the lights turning on at 0630 h and turning off at 1830 h. The room temperature was maintained at 22°C and relative humidity was 50%-60%, outdoor temperature averaged 25°C with a relative humidity of 70%. Room surfaces were cleaned daily, and disinfected weekly with Nolvasan disinfectant (Allivet®, St. Hialeah, Florida). Water
was provided ad libitum from automatic waterers.

Respiration calorimetry chambers (Qubit Systems®, Kingston, Ontario) were made of Plexiglass and measured 53.3 x 53.3 x 76.2 cm. Each chamber contained a shelf, feeder, water bowl, hammock, litter box, toy and a free area with a fleece bed. Water was provided ad libitum from water bowls. The chamber was designed to allow sufficient separation of feeding, sleeping and elimination areas. Chambers and water bowls were disinfected, and litter, litter boxes, toys, hammocks and fleece beds were removed, cleaned and replaced daily.

**Diet:** To effectively test the effects of MH on energy metabolism, food intake, intended to maintain weight, were provided equally between animals on a body weight basis; therefore, each cat was provided 45 kcal ME/kg BW/d (females) and 50 kcal ME/kg BW/d (males). Diets were presented in kibble form and cats were fed individually at 7:00 am and permitted 60 minutes to eat during food offerings. All remaining feed was collected and weighed to account for total (grams) feed refusal. The control diet was Iams® Original Chicken and the test diet was Iams® Original Chicken + MH (Table 13).

Table 13: Analyzed nutrient (%) and metabolizable energy content of control (-MH) and test (+MH) diets on an as-fed basis.

<table>
<thead>
<tr>
<th></th>
<th>Iams® Original- Control (-MH)</th>
<th>Iams® Original- Test (+MH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>6.04</td>
<td>6.3</td>
</tr>
<tr>
<td>Protein %</td>
<td>32.9</td>
<td>33.4</td>
</tr>
<tr>
<td>Fat %</td>
<td>15.96</td>
<td>15.9</td>
</tr>
<tr>
<td>Ash %</td>
<td>6.57</td>
<td>6.79</td>
</tr>
<tr>
<td>NFE %</td>
<td>34.26</td>
<td>35.25</td>
</tr>
<tr>
<td>MH</td>
<td>0 ppm</td>
<td>787 ppm</td>
</tr>
<tr>
<td>Predicted ME (Kcal/kg)*</td>
<td>3775</td>
<td>3751</td>
</tr>
</tbody>
</table>

*ME Calculated using Modified Atwater Equation (ME (kcal/kg) = (3.5*kg NFE)+(8.5*kg fat)+(3.5*kg protein)

**Experimental Design:** For two weeks prior to the initiation of the study cats were fed Iams® Original Chicken; the control diet. At the end of the first washout period, cats were randomly allocated to either the control group (-MH) or test (+ MH) group. On the first day of the study (Day 0) half the cats
continued to receive the control diet without MH treatment (0 mg/kg BW) and the remaining half were fed the control diet with MH treatment (8 mg/kg BW). Each cat was fed their respective diet for a total of 28d. For 14 days (washout), after the first 28d dietary treatment, all cats were returned to the control diet without MH treatment. Following the second washout period cats were fed the alternate diet for an additional 28d period. Feed intake was measured daily and body weight was measured weekly throughout both feeding periods.

Body Weight and Composition: Body weight was measured weekly and feed intake measured daily. Body composition was measured via Dual Energy X-ray Absorptimetry (DXA) and BCS analysis prior to the initiation of the washout diet and on d 28 for all cats. Dual energy x-ray absorptiometry (DXA). Animals were anesthetized according to the protocol of an (IM) injection of Dexdomitor (Orion Pharma, Finland; distributed by Pfizer Corp, NY, USA) in combination with Hydromorphone (Baxter Healthcare Corp, Deerfield, IL, USA). Three DXA scans using infant software provided by Hologic Inc. (Model Delphi A with QDR® for Windows®, Hologic Inc. 35 Crosby Drive Bedford, MA, USA) were completed to measure body composition after an adequate plane of anesthesia was reached. Cats were placed in a sternal position and cranial aspect of ante brachium on the table with the phalanges facing caudally, hind limbs were bent slightly upward towards the abdomen while the tail curved just below the left rear. Whole body composition is the sum of the regions and segmented by: bone mineral content (kg), fat (kg), lean (kg), lean+ bone mineral content (kg), total mass (kg), and fat (%). Scans were reviewed while the cat was still on the DXA scanner to ensure that the scan acquisition was acquired properly. Once the scans were completed, cats were removed from the unit and placed in a recovery area and an IM injection of Antisedan (Orion Pharma, Finland; distributed by Pfizer Corp, NY, NY) was administered in order to reverse the pharmacological effects of Dexdomitor. All three scans were combined to obtain an average.

Indirect Calorimetry: To assess the effect of length of adaptation to MH on energy metabolism when cats were fed a control or test (MH) diet, four separate indirect calorimetry analyses were conducted. Oxidation studies occurred on d 0 (acute exposure) and d 21 (chronic exposure). To determine whether these effects changed during the fed and fasted state, oxidation studies were 22 h in length and included fasted, fed, post prandial, and return to fasting state measurements.

Indirect calorimetry was conducted by measuring respiratory gases for 5 minutes every half-hour. Concentrations of O$_2$ and CO$_2$ in the respiratory chambers were measured with O$_2$ and CO$_2$ gas analyzers (Qubit Systems®, Kingston, Ontario, Canada). The calorimeter is an open circuit, ventilated calorimeter
with the room air being drawn through at a rate of ~5-8 L/min. Airflow was set at 5 or 8 L/min, depending on cat BW, and actual rate was measured with the use of a mass flow meter to enable total volume calculation. Gas analyzers and mass flow meters were calibrated prior to each individual oxidation study and at least every 6 h during a study, or when a drift of greater than 1% was observed. Calibration was performed using standard gas mixtures at two concentrations. Respiratory quotient, fat and CHO oxidation, and EE were calculated as follows (Jequier et al., 1987):

\[
\text{Respiratory Quotient (RQ) = litres CO}_2 \text{ produced/ litres of O}_2 \text{ consumed} \quad [\text{Eq. 1}]
\]

\[
\text{EE (kcal) = 3.82 x litres O}_2 \text{ consumed + 1.15 x litres CO}_2 \text{ produced} \quad [\text{Eq.2}]
\]

Carbohydrate oxidation:
\[
C_nH_{2n}O_n + nO_2 \rightarrow nCO_2 + nH_2O \quad [\text{Eq. 3}]
\]

Fat oxidation:
\[
(CH_2O)_3(CH_2)n(CO_2H)_3 + nO_2 \rightarrow nCO_2 + nH_2O \quad [\text{Eq. 4}]
\]

For analysis of RQ and energy expenditure (EE) in the fasted state, three respiration samples were obtained prior to feeding and least square mean ± SEM for the three samples was calculated. Twenty hour energy expenditure, fat and CHO oxidation post feeding was calculated as least square mean ± SEM multiplied by 20 for each dietary treatment and exposure.

**Blood Analyses:** All blood samples (2.5 mL) were collected via jugular venipuncture using a vacutainer and sampling from the left or right jugular vein. Blood samples were taken in the fasted state following completion of oxidation measurements on d 1 and 22. Samples were then placed on ice for 1 h. After clotting, blood samples were centrifuged at 300 rpm for 15 min at -4°C, and serum was decanted and stored at -20°C or -70°C for later analyses. Serum was measured for: glucose, insulin, triglycerides, β-hydroxybutyrate, C-reactive protein, non-esterfied fatty acids (NEFA) and total cholesterol. Analysis of glucose, TAG and cholesterol was completed using the Beckman Coulter AU480 automated chemistry analyzer which uses colorimetric measurements (UV/vis spectrometry; Indianapolis, IN, USA). Analysis of insulin was completed using a feline ELISA kit (Winston Salem, NC, USA).  

**Intravenous Glucose Tolerance Test:** An intravenous glucose tolerance test (IVGTT), based on the
assumption that glucose concentration decreases exponentially with time following a loading dose, was conducted on day 28 of the study. Day 28 was selected to test the effects of MH on rate of removal of plasma glucose. Cats were anesthetized and catheters were implanted to permit frequent blood sampling. The anesthetic protocol included induction through the administration of Dexdomitor (0.013 mg/Kg; Pfizer; Orion Corp. Espoo, Finland) and Butorphanol (0.25 mg/Kg IM; Fort Dodge Animal Health, Iowa, USA) with Propofol (1-4.4 mg/Kg IV; Hospira Inc, Illinois, USA) if needed. Anesthetic reversal was achieved with the administration of Antisedan (Pfizer; Orion Corp. Espoo, Finland), at an equal to volume of Dexdomitor IM. Once full recovery had occurred (5 hrs- based on internal unpublished data), cats in the treatment group were provided MH dose dissolved in water orally using a syringe. The control group was provided 0 mg/kg MH and only water was given orally via a syringe. This ensures that all handling practices were consistent between groups. Two hours following MH administration cats were injected intravenously with 800 mg/kg BW glucose (50% w/v) provided by Butler Schein Animal Health (Dublin, Ohio, USA). Blood samples were drawn after MH administration at times of -10, -5, -1, and after MH/glucose administration at 2, 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 min post injection of glucose. Following the last blood sample catheters were removed and cats will be fed their daily food ration. Samples were analyzed for plasma glucose, insulin and mannoheptulose.

Statistical Analyses: A crossover design with repeated measures was used for this experiment. There were two dietary factors tested (+MH or –MH) and additionally, length of dietary exposure (acute, semi-chronic and chronic) to each treatment was included in the model. A mixed linear model with cat as a random variable using PROC MIXED was used (SAS, version 9.1; SAS Institute Inc., 2002-2003, Cary, NC). The model used was: Yi = Wi + et, in which Yi = the dependent variable, Wi = dietary treatment (control (-MH) or test (+MH)), and et = random residual error. Diet (control (-MH) or test (+MH)) was considered a fixed effect. Treatment least square means were compared using the pdiff multiple comparison procedure. Differences were considered significant when P<0.05 and all data are expressed as least square mean ± standard deviation. For all measures a trend was observed at P < 0.1 – P >0.05.
Results

Body Weight and Composition: There was no effect of MH supplementation on body weight, body fat and lean body mass (Table 14; P>0.05).

Table 14: Body weight, total body fat and lean body mass in adult domestic short hair cats during the consumption of the washout/control diet (-MH, Day -2) and after chronic exposure (Day 28) to a test (+MH) or control (-MH) diet1,2.

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day -2</td>
<td>Day 28</td>
<td>Day -2</td>
</tr>
<tr>
<td>Body Weight2</td>
<td>4.22 ± 0.2</td>
<td>4.26 ± 0.2</td>
<td>4.25 ± 0.2</td>
</tr>
<tr>
<td>Body Fat2</td>
<td>0.560 ± 0.1</td>
<td>0.603 ± 0.1</td>
<td>0.596 ± 0.1</td>
</tr>
<tr>
<td>Lean Body Mass2</td>
<td>3.81 ± 0.1</td>
<td>3.74 ± 0.1</td>
<td>3.76 ± 0.1</td>
</tr>
</tbody>
</table>

1Values are least-square means ± SEM, n = 20. Means compared across day (Day -2 vs. Day 28) within diet without a common superscript (*) differ, P < 0.05. Means compared between diet (Control vs. Test) within day without a common superscript (letter) differ, P<0.05. NS, P≥0.05. P-value presented refers to the ANOVA for diet*exposure effect of treatment. 2Body weight, body fat and lean body mass are presented on a kg basis.

Indirect Calorimetry: There were no effects of dietary MH treatment or duration of exposure on fed or fasted RQ (Table 15; P>0.05). There was no effect of diet on fasted EE (kcal/kg0.67/d) compared within day 0 and day 21; however, there was a trend towards an effect of diet on EE in the fed state on d 21 (chronic exposure) as cats consuming the test diet (+MH) had elevated EE (Table 15; p=0.08). Furthermore, there was an effect of exposure on EE in the fasted and fed states for cats consuming the test (+MH) diet as EE was higher on day 21 versus day 0 (p=0.02). There were no individual time point differences over the 22 hr respiratory gas sampling period for RQ and EE on day 21 (Figure 11; A, B; P>0.05). When AUC for EE was compared between dietary treatment on day 0 (Figure 12; A) and day 21 (Figure 12; B) during the post-prandial (0-3 hrs), fed (3-9 hrs), return to fasting (9-15 hrs) and fasted (15-22 hrs) states, EE for the test (+MH) group as compared to the control (-MH) group during the 15 to 22 hour period post feeding was significantly greater on day 21 (Figure 12, B; P=0.02) when body fat was used as a covariate in the analysis. Total body fat and order of dietary exposure did not have a significant affect on fasted and fed RQ and EE and thus, was removed as a fixed effect from the model (p>0.05).
Table 15: Energy metabolism in adult domestic cats after acute (Day 0) and chronic (Day 21) exposure to a control (-MH) and test (+MH) diet.

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 21</td>
<td>Day 0</td>
</tr>
<tr>
<td>Fasting RQ</td>
<td>0.77 ± 0.005</td>
<td>0.79 ± 0.005</td>
<td>0.78 ± 0.005</td>
</tr>
<tr>
<td>RQ</td>
<td>0.84 ± 0.003</td>
<td>0.83 ± 0.005</td>
<td>0.84 ± 0.003</td>
</tr>
<tr>
<td>Fasting EE(^2)</td>
<td>76.5 ± 4.8(^*)</td>
<td>82.7 ± 4.2(^**)</td>
<td>77.8 ± 4.8</td>
</tr>
<tr>
<td>EE(^2)</td>
<td>62.6 ± 2.2(^*)</td>
<td>66.1 ± 1.6(^**)</td>
<td>62.8 ± 2.2</td>
</tr>
</tbody>
</table>

\(^1\)Values are least-square means ± SEM, n = 20. Means compared across day (Day 0 vs. Day 21) within diet without a common superscript (*) differ, \(P < 0.05\). Means compared between diet (Control vs. Test) within day without a common superscript (letter) differ, \(P < 0.05\). NS, \(P \geq 0.05\). P-value presented refers to the ANOVA for diet*exposure effect of treatment at Day 21.\(^2\)Fasting EE, EE, are represented on a kcal/kg\(^{0.67}\)/d.
Figure 11: EE (A) and RQ (B) in cats consuming the control (-MH) and test (+MH) diet during chronic (day 21) dietary exposure. Values are expressed as means, means within time points having different superscripts are significantly different (P<0.05).
Figure 12: Least square mean ± SEM for area under the curve (AUC) for energy expenditure during the post-prandial (0-3), fed (3-9), return to fasted (9-15) and fasted (15-22) after acute (day 0; A) and chronic (day 21; B) exposure to a control (-MH) and test (+MH) diet.

Asterisks represents a difference (p=0.1) between test and control diets during the fasted (15-22) state on Day 21. Post-prandial (0-3), fed (3-9), and return to fasting (9-15) groups without asterisk are not significantly different (p>0.05). Body composition was used as a covariate during analysis.

**Fasted and Fed Blood:** There were no effects of dietary MH treatment on fasted and fed cholesterol, C-reactive protein, triglycerides, insulin and glucose to insulin ratio (G:I). NEFA increased with prolonged exposure to the control diet (P<0.05) and there was a trend for NEFA to be lower with the consumption of the test (+MH) diet after chronic exposure (Day 22; P=0.095; Table 16). In addition, fed glucose increased significantly from day 1 to day 22 during exposure to the test (+MH) diet (P<0.05; Table 16). There were, however, no differences in fasted and fed glucose between diets after chronic (day 22) dietary exposure (P>0.05). Serum MH concentrations were different between control and MH test groups in both
the fasted and fed state (P<0.05; Table 16). Total body fat did not have a significant affect any blood plasma metabolite measured in the fasted and fed state and thus, was removed as a fixed effect from the model (p>0.05).

Table 16: The effects of MH and length of exposure on blood plasma metabolites in adult domestic cats consuming a control (-MH) and test (+MH) diet after acute (Day 1) and chronic (Day 22) exposure.  

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 22</td>
<td>Day 1</td>
</tr>
<tr>
<td>Fasted Glucose</td>
<td>76.3 ± 3</td>
<td>82.0 ± 3</td>
<td>79.5 ± 2</td>
</tr>
<tr>
<td>Glucose 4hr Fed</td>
<td>87.7 ± 4*</td>
<td>100.4 ± 4**</td>
<td>89.7 ± 4</td>
</tr>
<tr>
<td>Fasted Insulin</td>
<td>5.4 ± 0.8</td>
<td>5.5 ± 0.8</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>Insulin 4hr Fed</td>
<td>9.3 ± 2</td>
<td>9.3 ± 2</td>
<td>9.1 ± 2</td>
</tr>
<tr>
<td>Fasted G: I</td>
<td>0.43 ± 0.05</td>
<td>0.51 ± 0.06</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>Fed G: I</td>
<td>0.40 ± 0.06</td>
<td>0.46 ± 0.07</td>
<td>0.32 ± 0.06</td>
</tr>
<tr>
<td>Fasted NEFA</td>
<td>0.44 ± 0.2</td>
<td>0.45 ± 0.2</td>
<td>0.48 ± 0.3</td>
</tr>
<tr>
<td>NEFA 4hr Fed</td>
<td>0.22 ± 0.02</td>
<td>0.23 ± 0.02*</td>
<td>0.21 ± 0.02*</td>
</tr>
<tr>
<td>Fasted MH</td>
<td>175.2 ± 19</td>
<td>198.1 ± 19</td>
<td>NS</td>
</tr>
<tr>
<td>MH 4hr Fed</td>
<td>2719.5 ± 126</td>
<td>2915.0 ± 126</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Values are least-square means ± SEM, n = 20. Means compared across day (Day 1 vs. Day 22) within diet without a common superscript (*) differ, P < 0.05. Means compared between diet (Control vs. Test) within day (Day 1 or Day 22) without a common superscript (letter) differ, P<0.05. NS, P≥ 0.05. P-value presented refers to the ANOVA for diet*exposure effect of treatment at Day 22. 2 Glucose (mg/dL), insulin (uU/mL), NEFA (mEq/L) and MH (mg/dL).

Intravenous Glucose Tolerance Test: There were no differences in time course effects between treatments on plasma glucose and insulin during the IVGTT following a glucose bolus at time 0 (Figure 13; A, B; P>0.05). However, cats receiving an oral dose of MH (8 mg/kg BW) had greater plasma concentrations of MH at all time points when compared to cats that received an oral dose of saline during the IVGTT (Figure 13c; P<0.05).
Figure 13: Plasma glucose (A), insulin (B) and MH (C) in adult domestic cats following the consumption of either a control (-MH) or test (+MH) diet during an IVGTT on day 28 with and without MH treatment. Values are least-square means (n=20), means within time points having different superscripts (*) are significantly different (P<0.05).
Discussion

Overall, MH is digested and absorbed, as demonstrated by plasma MH changes with dietary intake in the adult domestic short hair cat. There were no significant effects of dietary MH treatment on body weight, body fat or lean body mass. Further, there were no effects of MH on RQ and thus, relative amounts of fat and carbohydrate oxidation. However, MH caused an increase in fasted and fed EE, likely due to the trend toward an increase in EE observed during the return to fasted state at 15-22 hrs post-feeding. There were minimal effects of MH on blood plasma metabolites; although, we did observe an increase in plasma glucose in the fed state after 22 d of exposure to the test (+MH) diet and an increase in NEFA on day 22 during exposure to the control (-MH) diet. To our knowledge, no one else has sought to understand the practical application of diets containing a CRM on feline metabolism, which is markedly different than other mammals due to the greater need for protein vs. carbohydrate.

All blood metabolites are similar to those previously reported in healthy, adult domestic short hair cats (Appleton et al., 2001; 2004; Coradini et al., 2011; Hoenig et al., 2011). Plasma MH concentration was greater in cats fed dietary MH treatment or when MH was orally delivered MH during the IVGTT. The presence of MH in the serum indicates that cats can absorb dietary/orally administrated MH. MH has also been demonstrated to be available to other species including rabbits, humans, rats and dogs (Roe and Hudson 1936; Blatherwick et al., 1940; Koh and Berdanier, 1974; Issekutz et al., 1977). Cats are obligate carnivores and possess lower digestive and absorptive capacity for dietary carbohydrates relative to other mammals due to evolutionary adaptations, including the lack of production or sufficient production of salivary amylase, pancreatic amylase and intestinal disaccharides (Meyer and Kienzle, 1991). Despite these limitations, previous studies have suggested that cats are capable of digesting dietary sugars; however, their capacity to cope with large amounts dietary carbohydrates may be limited. Kienzle, 1993, tested the effects of glucose, galactose, sucrose, and lactose on protein and fat digestibility, and intestinal metabolism and found that dietary monosaccharides are not well tolerated by cats due to a reduction in protein digestibility of 4 to 5 percent when sucrose or lactose diets were fed. Kienzle, 1994, tested several diets containing high levels of different dietary sugars including: glucose (40% dry matter), galactose (39% dry matter), starch (29-37% dry matter), sucrose (36% dry matter), and lactose (11 and 28% dry matter) on protein and fat digestibility and on intestinal metabolism and observed signs of toxicity in cats at relatively low intakes of galactose (5.6 g/kg BW/day). Furthermore, intake of these mono and disaccharides caused glucosuria and hyperglycemia (Kienzle, 1994). In the present study, cats fed MH
did not exhibit any signs of MH indigestibility and MH had no untoward effects on serum biochemistry and plasma insulin. Furthermore, the results from the present study suggest that cats can indeed digest dietary sugars in moderation as Kienzle 1993, 1994 has previously demonstrated. The present study adds the seven carbon sugar, mannoheptulose, to our understanding of tolerable and digestible carbohydrates for cats.

We did not observe any change in body weight or body composition in the present study; however, we imposed no change in maintenance caloric intake. Furthermore, the cats used in this study were lean or moderately overweight and all considered young. True CR regimes often cause significant reductions in BW and fat mass. Colman et al., 1998, examined the effects of a 20-30% CR on body composition in macaques and found that total body fat, as analyzed with duel energy x-ray absorptiometry, declined with CR. Cefalu et al., 1997, also found that a CR of 30% caused a significant reduction in body weight and intra-abdominal fat in restricted non-human primates. Though some CRM strategies impact energy sensing pathways, dietary CRM supplementation does not necessarily lead to a reduction in body weight or body fat as subjects are fed to energy requirements or ad libitum (Mamczarz et al., 2005). However, Lane et al., 1998, who tested three doses of 2-deoxy-glucose (0.2%, 0.4% and 0.6%), a glycolytic inhibitor similar to MH in function but toxic at high levels, observed an initial decline in feed intake and body weight in rats fed all three doses. After several weeks of feeding, body weight of rats fed dosed with 0.2 and 0.4% 2-deoxy-glucose were no different from controls; however, the 0.6% supplementation caused rats to maintain the lower weight, although it should be noted that the 0.6% dose was found to be toxic, causing death, if fed at this level on a daily basis for an extended period of time. Wan et al., 2003, also found that rats fed 2-deoxy-glucose had similar body weights to control rats; however, the rats subjected to true CR had a significant reduction in body weight. Koh and Berdanier, 1974, fed diets of different protein, carbohydrate and sucrose amounts with half of the rats being dosed with 20 mg of MH. MH treatment did not impact body weight of rats consuming the high sucrose (65% energy) diet; however, MH treatment caused rats on the carbohydrate and protein diets to gain weight (~18 grams or 24%). In the present study, cats fed 8 mg/kg BW did not differ in BW from cats fed a control diet. Because of the equal caloric intake, we did not expect a change in BW.

There were significant effects of dietary MH treatment on EE (kcal/hr) in the fasted and post-prandial states. However, there were no specific time point differences over the 22-hr respiratory sampling period, though the cats consuming the test (+MH) diet had greater energy expenditure than cats fed control. When AUC EE was compared between treatments over specified time periods, representing the post-prandial,
fed, return to fasting and fasted states, there was a significant increase in EE with MH dietary treatment in the 15-22 hour time period post feeding. The greater EE in cats represents an additional daily calorie expenditure of 9.6 kcal or approximately 4% of an average cat’s (BW= 4 kg) daily energy intake. The excess caloric intake, via a reduced EE, in control cats is noteworthy as it may lead to a body weight increase of 0.45 kg/year therefore, over the lifetime a cat (15 years average) this may lead to a body weight difference of 6.75 kg. There remains to be a consensus on whether CR and CRM’s act to decrease or increase respiration rates in mammals. Some researchers (reviewed in: Speakman et al., 2002) have concluded that CR and some CRM’s cause a reduction in metabolism and thus a decline in energy expenditure; however, these conclusions are invalid when EE is corrected for differences in body weight and body composition between animals subjected to CR and controls because CR animals tend to decline in BW and percent body fat. Blanc et al., 2003, studied the effects of a 30% CR over 11 years in rhesus monkeys and found that CR reduced resting EE by 13% when adjusted for fat free mass (20% when not adjusted). Similarly other researchers have found that EE adjusted for body weight and fat mass declines with CR (Dulloo and Girardier, 1993; Gonzalez-Pachero et al., 1993; Rothwell and Stock, 1982; Santos-Pinto et al., 2001). On the contrary, some researchers have found no change in EE with CR particularly if adjusted for body composition (Keese and Corbet, 1990; Even and Nicolaides, 1993; Ballor, 1991; Masoro et al., 1982; McCarter et al., 1985; McCarter and Palmer, 1992; Ramsey et al., 2000) and others have found that EE increases with changes in body composition at a 40% CR versus ad libitum in rats (Selman et al., 2005). There is limited data regarding the effect of CRM’s on EE and the data available is contradictory. Dark et al., 1994, reported that hamsters subjected to a 1500 mg of MH/kg caused them to enter into a state of torpor which was hypothesized to be a consequence of reduced glucose availability. IGF-1 knockout mice, designed to have reduced signaling through the IGF-1 and insulin pathways, have been shown to have an increased lifespan and improved insulin sensitivity with no other changes in energy metabolism, including EE, compared to controls (Shimokawa et al., 2002). In our study, cats had greater EE when fed diets containing MH in both the fasted and fed state and the increase in EE was associated to the significant increase that was observed during the 15-22 hrs (fasted) post feeding. The additional energy expenditure may have been attributable to the decreased availability of glucose for energy and a shift to fat oxidation to supply energy; however, a shift in RQ was not observed. An alternative hypothesis may be that there is an up regulation in SIRT1, a regulator of mitochondrial biogenesis, gene expression as influenced by the metabolic changes associated to MH consumption that may lead to an increase in mitochondrial function and thus EE (Guarente, 2006). Differences in results of the effects of CR on EE may be attributable to methods used to determine EE; for instance, doubly-labeled water versus calorimetry, the state in which the measures of EE were taken (resting versus active) and whether or not
changes in body size and composition were accounted for. Further research is warranted to determine if there is indeed a modification in metabolic rate when animals are fed CRMs; however, the enhanced EE observed with MH treatment after 21 days presents a novel opportunity for such CRMs to be used for weight control in cats.

There were no effects of dietary MH on RQ or relative amount of fat and CHO oxidation in either the fasted or fed states. This result was unexpected since MH supplementation typically causes a shift in macronutrient oxidation from a principle reliance on glucose to an increased emphasis on fatty acid oxidation to meet energy demands (Bruss et al., 2010). Increased fatty acid (FA) oxidation and decreased FA synthesis and glucose oxidation are hypothesized to be the underlying metabolic adaptations to CR (Bruss et al., 2010). Similar results are observed in CRM strategies, like MH, designed to impact energy sensing pathways and competitively inhibit cellular usage of glucose. Koh and Berdanier, 1974, studied the effects of a 20 mg dose of MH on the hepatic synthesis of FA in rats and found that FA oxidation increased with MH treatment. The additional FA oxidation is likely a consequence of increased release of NEFA into plasma by adipose tissue and enhanced hepatic uptake of FA for oxidation as has been observed in MH treated rats (Simon et al., 1972; Mitzkat and Meyer, 1970). However, our results indicate that there was a trend for NEFA to be lower for cats consuming the test (+MH) diet. Several other groups have also observed that dietary MH supplementation causes a decline in glucose oxidation in isolated islet cells of the mouse pancreas (Hedeskov et al., 1972; Ashcroft et al., 1970; Matschinsky et al., 1971). The inhibitory effect of MH on glucose oxidation has further been supported by Sener et al., 1998, who noted that islet cells of the pancreas incubated with mannoheptulose at 1.0 mmol/l on decreased glucose utilization in addition to glucose oxidation. Scruel et al., 1998, noted that other organs (liver and parotid cells) were less impacted than pancreatic islet cells on functional responses to glucose. Overall, more research is needed to elucidate the effects of MH on fat and carbohydrate oxidation in cats; perhaps, the collection of fecal and urine nitrogen is warranted to would provide a more accurate assessment of the relative amounts of fat and carbohydrate oxidation.

There were no significant effects of MH on fasted and fed cholesterol, C-reactive protein, triglycerides, insulin and G:I ratio. In addition, there were no time course effects of MH on plasma insulin and glucose during an IVGTT following a glucose bolus. Of note is that these cats ranged from lean to moderately overweight and are all young with plasma metabolites within healthy ranges; therefore, we did not expect any of these cats to have significant changes in plasma metabolites and perturbations in glucose and insulin metabolism. However, treatment with dietary MH caused an increase in blood glucose 4 hrs post
feeding. Previous research has indicated that intramuscular, subcutaneous and oral administration of MH causes a decline of serum insulin leading to a hyperglycemic state similar to that observed in diabetics (Viktora et al., 1969). These findings were supported by in vitro studies where MH was shown to inhibit glucose-stimulated release of insulin in slices of pancreatic tissues (Coore et al., 1963; Paulsen et al., 1967). Furthermore, elevated plasma glucose failed to suppress the release of glucagon when rats were fed MH (Simon and Frenkel, 1972) causing plasma concentrations of glucagon and hepatic cyclic AMP to increase (Klain et al., 1976). The elevated glucose often observed with MH treatment may be caused by two factors: 1) a reduction in glucose clearance due to inhibited insulin release and insulin insensitivity, and 2) an increase in gluconeogenesis due to the stimulatory effects of MH on the activity of hepatic fructose-1,6-bisphosphate, phosphoenolpyruvate carboxykinase and incorporation of alanine into blood glucose and hepatic glycogen (Issekutz et al., 1977; Klain et al., 1976). Increased glycogenolysis is likely not one of the reasons for the observed elevation in plasma glucose concentration since an increase in liver glycogen has been measured without a decline in muscle glycogen content in rats fed MH (Simon and Kraicer, 1957). As cats are hypothesized to be in a constant state of gluconeogenesis with a slow glucose clearance it may be concluded that they would be less sensitive to MH as the pathways impacted by MH increasing plasma glucose and decreasing insulin output are already functioning at a higher capacity. However, our results are similar to those previously published as fed glucose increased with MH treatment suggesting that healthy cats can, in fact, respond in a predictable manner to the metabolic effects of MH. Lastly, we observed no change in plasma NEFA concentration with MH treatment; however, plasma NEFA concentration increased with prolonged exposure to the control (-MH) diet. While reduced levels of plasma NEFA are indicative of a healthy body weight and composition, plasma NEFA concentrations typically decline with increased glucose oxidation and since we expected an increase in fat oxidation with (+MH) treatment we were expecting a correlated increase in NEFA availability for oxidation. Elevated insulin also decreases plasma NEFA concentration (Jensen, 1998); although, we did not see a significant change in plasma insulin there was a small numerical increase in insulin during the consumption of the control (-MH) diet (Jensen, 1998) and thus, we would have further expected NEFA concentration to be lower during the consumption of the control (-MH) diet versus the test (+MH) especially because MH causes hyperglycemia via reduced insulin output (Viktora et al., 1969). Overall, the cat demonstrated some reactivity to MH treatment via elevations in plasma glucose in the fed state; however, the elevated NEFA concentrations observed during control feeding are unexpected and further analysis of fatty acid metabolism during MH treatment is warranted to gain additional understanding.

As previously discussed, the cat, an obligate carnivore, appears to be responsive in the short term to the
purported physiological effects of dietary MH as evidenced by the increase EE and fed plasma glucose in response to dietary MH. However, cats do not appear to shift fuel selection towards fatty acid oxidation as is previously observed with MH feeding in more omnivorous animals. MH acts in a competitive manner to block D-glucose phosphorylation impacting the functional and metabolic effects of glucose in the pancreatic islet B-cells by inhibiting the activity of glucokinase and hexokinase (Volskey and Dimant, 1978; Scruel et al., 1998). Cats, like other carnivores, have unique metabolic processes because they evolved to a diet high in protein and fat with low carbohydrate, thus glucokinase (GK) activity and GK gene expression are minimal or absent in the feline liver (Kley et al., 2009). GK, one of the four isoenzymes of the mammalian hexokinase (HK) group, is considered to be one of the rate limiting enzymes of glycolysis as GK is responsible for catalyzing the first reaction of glycolysis where phosphate is added to glucose to form glucose-6-phosphate. However, the activities of hexokinase, fructokinase (FK), pyruvate kinase (PK), glucose-6-phosphate dehydrogenase (G6PD), fructose-1,6-bisphosphatase (FBPase), and glucose-6-phosphatase (G6Pase) are significantly higher in cats as compared to dogs, who are thought to have normal GK activity (Tanaka et al., 2005). Therefore, the higher activity of these alternative enzymes may compensate for the lack of GK activity in cats and modify their responsiveness to MH since there are already compensatory mechanisms in place to adapt to limited glucose phosphorylation capacity. However, Picton and Malaisse, 1999, demonstrated that cells with minimal GK activity (starved) were still capable of responding to MH. Therefore, there are likely additional factors influencing responsiveness to dietary MH in cats and may explain the elevated glucose and energy expenditure observed during the return to fasting (15-22) hours post feeding state with MH treatment. Cats are also unique to many other species as they appear to prioritize fatty acid oxidation versus glucose oxidation. This is evidenced through indirect calorimetry measures and fat/CHO oxidation curves and is also supported by the elevated activities of FK, PK and G6PD in the feline liver that promote higher activities of fatty acid synthesis as compared to the canine liver (Tanaka et al., 2005). Furthermore, cats appear to be in a constant state of gluconeogenesis (MacDonald et al., 1984); enzymatic support for the elevated rate of gluconeogenesis in the cat liver is the observed increase of FBPase and G6Pase activity - two key enzymes of gluconeogenesis (Rogers et al., 1997). Several researchers have shown that the effectiveness of the inhibitory action of MH on glucose metabolism can be influenced by the cellular environment namely: 1) the capacity for intracellular transport of MH and 2) the extracellular (or in vitro medium) glucose concentration (Mailaisse et al., 1968; Scruel et al., 1998; Picton and Malaisse, 1999). Therefore, the unique cellular environment created by a constant state of gluconeogenesis and prioritized FA oxidation in the cat may influence the cellular glucose environment modifying MH inhibition of glucose phosphorylation by impacting the cellular transport of glucose and MH.
In conclusion, MH appears to affect some biomarkers of glucose metabolism and resulted in greater EE in the late post-prandial, return to fast period and glucose as measure 4 hrs post feeding. The differences in EE and glucose may impact body weight, composition and glucose/insulin profiles in longer term feeding and warrants further investigation. In addition, the overall responsiveness of cats to MH, a CRM with promising metabolic impacts, may be utilized to provide additional understanding of the idiosyncrasies of the feline metabolism once the mechanisms of function of MH have been elucidated.

Acknowledgements

The authors would like to thank Procter and Gamble for their financial support.
References:


Dulloo, A.G.; Girardier, L., 1993: 24 hour energy expenditure several months after weight loss in the underfed rat: evidence for a chronic increase in whole-body metabolic efficiency. Int J Obes Relat Metab Disord 17:115–123


Rogers, Q.R.; Morris, J.G.; Freedland, R.A., 1977: Lack of hepatic enzymatic adaption to low and high


CHAPTER 5: Dietary mannoheptulose (MH) enhanced play motivation after a 28 d feeding trial in cats.\textsuperscript{1,2}

Gooding, M.A\textsuperscript{1}; Davenport G.M\textsuperscript{2}; Zhang, J\textsuperscript{2}; Duncan, I.J.H\textsuperscript{1}; Atkinson, J.L.\textsuperscript{1}, Shoveller, A.K.\textsuperscript{1,2}

Authors Last Name for PubMed Indexing: Gooding, Shoveller, Davenport, Zhang, Duncan, Atkinson

Addresses:  
\textsuperscript{1}Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario  
\textsuperscript{2}Pet Care, Procter and Gamble, Lewisburg, Ohio  
*To whom correspondence should be addressed: shoveller.ak@pg.com

Running Title: Influence of Dietary Mannoheptulose on Play Motivation.

Key Words: Mannoheptulose, play, motivation, predatory, hunger

Funding: This work was supported by The Procter and Gamble Co., Pet Care, Lewisburg, Ohio, USA 45338

Abbreviations: MH, mannoheptulose, CR, calorie restriction, CRM, calorie restriction mimetic
Abstract

The effect of dietary mannoheptulose (MH; 8 mg/kg BW) treatment on play motivation was investigated in 20 lean (N=10) and moderately overweight (N=10) cats (3 ± 5 mo, 4 ± 2 kg). Cats randomly received a control (-MH) and test (+MH) dietary treatment for 28 d in a crossover design with a 14 d washout period between each treatment leg. Two tests were conducted to measure play motivation. The “observation test”, conducted on day 15, measured predatory play patterns and non-play patterns during a 2-min interaction with a toy. The “weighted door test”, conducted on day 23, measured the willingness to work (represented by increasing weights added to a swing door separating the cat and toy) to gain access to a toy. Cats subjected to MH did not differ in their performance of predatory and non-predatory play behaviours (P>0.05). Cats consuming the test (+MH) diet worked harder to gain access to a toy mouse. Mean number of successful trials, speed to cross the door and mean maximum weight of the door (grams) was greater for cats consuming the test +MH diet (P>0.01). Eight cats that did not cross the door on the control (-MH) diet, crossed the door on the test (+MH) diet and four cats, that opened the door on both diets, pushed more weight on the test (+MH) diet than the control (-MH) diet. In conclusion, MH has differing affects on the appetitive and consummatory components of play in the domestic cat as motivation to gain access to a toy was increased with MH treatment while there was a negligible impact on play once the toy was acquired.
Introduction

Object play is infrequently observed in adults of wild species; however, play is regularly observed in adult domesticated species including the dog (Canis familiaris) and cat (Felis Silvestris Catus) (Fox and Bekoff, 1975; Leyheusen, 1979). Unlike other mammals, when cats are on a low plane of nutrition they do not decrease performance of play, but have been observed to increase demonstrations of play (Bateson et al., 1990). Kittens subjected to weaning at 5 weeks of age compared to kittens that remained with their mother demonstrated higher frequencies of play (Bateson and Young, 1981). The elevated play performance was likely due to the energy restriction that often correlates to weaning. When milk provision was restricted, kittens (6-8 wks of age) developed play behaviours earlier than control kittens not subjected to lactation interruption (Bateson et al., 1981). In addition, a calorie restriction (CR) of 20% for the first 18 days after birth also caused increased demonstrations of play of kittens 78-84 days after birth (Bateson et al., 1990). Play motivation increased with greater time since feeding; therefore, authors concluded that hunger was likely the underlying motivational system for play behaviour in adult cats (Hall and Bradshaw, 1998). Consequently, underlying systems associated to CR and hunger may drive play motivation in cats.

CR is of interest to nutritionists as it remains the most vigorous and reproducible intervention to inhibit the physiological effects of aging, to delay the commencement of most pathologies (including cancer and diabetes) and to extend mean and maximum lifespan by 20 to 40% (McCay et al., 1935; Weindruch and Sohal, 1997; Weindruch and Walford, 1988). However, there are concerns associated with the feasibility of implementing such CR regimens for long periods of time as they are difficult to maintain and are associated with negative behavioural effects (Ingram et al., 2004). Calorie restriction mimetics (CRMs) have been studied as an alternative to CR and to avoid some of the negative effects associated with the implementation of CR regimens (Ingram et al., 2004). The objective of CRM strategies are to produce the same prolongevity effects that CR provides, but without reducing caloric intake. Since the prolongevity strategies of CR influence systems involved in energy sensing, regulation and metabolism, the initial targets of CRMs focused on metabolites that modify glucose metabolism. Glucose anti-metabolites, such as mannoheptulose (MH), are believed to act by competitively inhibiting some aspects of the glycolytic pathway essential for glucose metabolism (Roth et al., 2001). Resultantly, MH causes a decline of serum insulin leading to a hyperglycemic state similar to that observed in diabetics (Viktora et al., 1969).

The lateral hypothalamus, referred to as the “feeding center” is responsive to plasma glucose levels
Increased glucose oxidation and electrical activity of the lateral hypothalamus (Anand et al., 1964) and a brief transient decline in plasma glucose all precede feeding in humans and rats and are correlated to feelings of hunger (Campfield and Smith, 2003). The induction of a diabetogenic state with MH consumption may reduce glucose responsiveness in the lateral hypothalamus, thus impacting satiety/hunger signals associated to glucose metabolism. Therefore, hunger, that affects the motivational system underlying play behaviour in the domestic cat, is also influenced by glucose metabolism. Since MH inhibits glucose metabolism and reduces glucose responsiveness then perhaps the metabolic effect of MH would be indicated by observed increase in activity of the cat through enhanced demonstrations of play.

The objective of our study was to evaluate the effects of dietary MH, on the motivational system of play, which is also sensitive to energy balance, in the domestic cat. We hypothesized that a CRM strategy with MH may influence the demonstrations of play in adult domestic cats.

Materials and Methods

All procedures were reviewed and approved by the Procter and Gamble Institutional Animal Care and Use Committee in accordance with IACUC guidelines.

Animals: Twenty cats (N=20) of similar age (3 ± 5 mo), and split 10 females (5 lean and 5 moderately overweight) and 10 males (5 lean and 5 moderately overweight), were randomly separated into four groups of five. Cats were considered lean at a body weight of <3.6 kg and a body fat mass of <0.5 kg or 17% body mass and cats were considered moderately overweight at a body weight of >3.5 kg and body fat mass of >17%. Diets were balanced by both sex and body condition. Cats were provided from the Pet Health and Nutrition Center at Procter and Gamble Pet Care, Lewisburg, Ohio. Standard veterinarian evaluation (physical exam, chemical and CBC blood analysis) of overall health was completed prior to the initiation of the study and all cats entered the study healthy.

Housing: Cats were housed in a free-living environment with indoor/outdoor access during the day (0800-1500 h) and indoor-only access at night (1500-0800 h). Environmental enrichment included perches, beds, toy houses, scratching posts, toys and climbing apparatus provided in the rooms at all times of day. All cats were socialized daily for a minimum of 60 min. Cats were maintained on a 12 hour lighting schedule with the lights turning on at 0630 h and turning off at 1830 h. The room temperature was...
maintained at 22°C and relative humidity was 50%-60%, outdoor temperature averaged 25°C with a relative humidity of 70%. Room surfaces were cleaned topically daily and disinfected weekly with Nolvasan disinfectant (Pfizer, New York, USA). Water was provided ad libitum from automatic waterers.

**Diet:** To effectively test the effects of MH on play each cat was provided 40.5 kcal ME/kg BW/d (females) and 45 kcal ME/kg BW/d (males) which is the amount needed to maintain body weight in this cohort. Caloric intake was ~95% of total energy requirements and cats were fed 95% of their estimated maintenance energy requirement to ensure full feed consumption and not result in any significant metabolic or body weight changes. Diets were presented in kibble form and cats were fed individually at 7:00 am and permitted 60 minutes to eat during food offerings. The control diet was Iams® Original Chicken and the test diet was Iams® Original Chicken + 8mg/kg BW (Table 17).

Table 17: Nutrient (%) and metabolizable energy content of control (-MH) and test (+MH) diets on an as-fed basis.

<table>
<thead>
<tr>
<th></th>
<th>Iams® Original- Control (-MH)</th>
<th>Iams® Original- Test (+MH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>6.04</td>
<td>6.3</td>
</tr>
<tr>
<td>Protein %</td>
<td>32.9</td>
<td>33.4</td>
</tr>
<tr>
<td>Fat %</td>
<td>15.96</td>
<td>15.9</td>
</tr>
<tr>
<td>Ash %</td>
<td>6.57</td>
<td>6.79</td>
</tr>
<tr>
<td>NFE %</td>
<td>34.26</td>
<td>35.25</td>
</tr>
<tr>
<td>MH</td>
<td>0 ppm</td>
<td>787 ppm</td>
</tr>
<tr>
<td>Predicted ME (Kcal/kg)</td>
<td>3775</td>
<td>3751</td>
</tr>
</tbody>
</table>

**ME Calculated using Modified Atwater Equation (ME (kcal/kg) = (3.5*kg NFE) + (8.5*kg fat) + (3.5*kg protein))**

**Experimental Design:** For two weeks prior to the initiation of the study cats were fed the control diet as a washout. At the end of the washout period cats were randomly allocated to either control or control + MH group (test). On the first day of the study (Day 0) half the cats continued to receive the control diet without MH treatment (0 mg/kg BW) and the remaining half were fed the control diet with MH treatment (8 mg/kg BW). Each cat was fed their respective diet for a total of 28d. After this test period, cats were placed on control diet a second time for a second washout period. Following the second washout period cats were fed the alternate diet for a second 28d period.
Behavioural Analysis: Two separate tests were conducted to measure play motivation in cats subject to MH treatment and compared to controls. Toys used for both play tests were similar in design and size; however, color of the toy was varied for each test to maintain novelty and prevent habituation (Hall et al., 2002).

**Play Test #1- 2 Minute Observation Test:** A walled stall measuring 100 cm W X 100 cm L X 100 cm H (Queen City polymers, Ohio, USA) with a plexi-glass roof containing a 10 cm diameter hole in the center was equipped with a closed-circuit video camera to record all behaviours. The stall was placed in a room in which all cats had been previously acclimated. Cats were assessed one time per dietary treatment on day 15 for a two minute time period at 5 hrs post feed removal, with feed being offered once daily. Cats were not permitted to interact with plush toys for 4 days prior to the initiation of the study. All procedures and measurements for assessing play motivation during behavioural analysis were adapted from Hall and Bradshaw, 1998. Hall and Bradshaw, 1998, tested cats at 0 hr and 16 hr post feeding at 0900. Cats were placed in a walled cubicle (135 cm x 155 cm) and allowed to play with either a small or large ellipsoidal toy. The experimenter remained present during testing and swung the toy in a standard arc during moments where the cat stopped playing to further entice the animal to play. All trials were video recorded and analyzed for the behaviour patterns described in Table 18 (with the addition of a play behaviour describing a “kick”) (Hall and Bradshaw, 1998).
Table 18: Descriptions of behaviour patterns recorded during play motivation tests (Hall et al., 1998)

<table>
<thead>
<tr>
<th>Behaviour Pattern</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Play Patterns</strong></td>
<td></td>
</tr>
<tr>
<td>Sniff (f)</td>
<td>Cat sniffs object</td>
</tr>
<tr>
<td>Bat (f)</td>
<td>Cat touches object with a front paw, claws retracted</td>
</tr>
<tr>
<td>Hit (f)</td>
<td>Cat touches object with front paw, claws extended, with more force that “bat”. Object may be thrown aside.</td>
</tr>
<tr>
<td>Rear (f)</td>
<td>Cat stands on hind legs to reach object</td>
</tr>
<tr>
<td>Grasp (f)</td>
<td>Cat uses both front paws to take hold of the object, claws either extended or retracted</td>
</tr>
<tr>
<td>Clutch</td>
<td>Object is held close to the body with one or both front paws</td>
</tr>
<tr>
<td>Killbite (f)</td>
<td>Cat delivers a strong bite to the object</td>
</tr>
<tr>
<td>Holdmouth</td>
<td>Object is held firmly and retained in the mouth, for a greater duration than is necessary for a single bite.</td>
</tr>
<tr>
<td>Chew (f)</td>
<td>Cat holds object in mouth and chews it with rapidly repeated, small bites. Bites are less forceful than killbites.</td>
</tr>
<tr>
<td>Lick (f)</td>
<td>Cat licks object.</td>
</tr>
<tr>
<td><strong>Non-Play Patterns</strong></td>
<td></td>
</tr>
<tr>
<td>Walk¹</td>
<td>Cat moves with no more than two paws off the ground at any time</td>
</tr>
<tr>
<td>Sit¹</td>
<td>Cat is stationary with front legs straight and vertical, and rump on the ground</td>
</tr>
<tr>
<td>Stand¹</td>
<td>Cat is stationary with just its four paws in contact with the ground</td>
</tr>
<tr>
<td>Recline¹</td>
<td>Cat lies on the floor, on one side or its front or back, paws extended to one side or folded</td>
</tr>
<tr>
<td>Crouch¹</td>
<td>Cat is stationary with ventrum and legs in contact with the ground, paws unfolded</td>
</tr>
<tr>
<td>Watch²</td>
<td>Cat pays close attention to object, tracking its movement. Eyes are wide open, ears are directed at the object</td>
</tr>
<tr>
<td>Avoid²</td>
<td>Cat ignores the object, paying no attention to it</td>
</tr>
<tr>
<td>Groom²</td>
<td>Cat either licks its fur or a front paw and wipes it over its head</td>
</tr>
</tbody>
</table>

All patterns recorded as duration, except (f) as frequencies. Type 1 non-play patterns were recorded as combinations with type 2 non-play patterns, for example ‘walk watch’, ‘stand avoid’.

Training Procedure: Cats underwent a two week acclimation prior to the initiation of the study to become acclimated to the walled stall for a 3 minute time period. In the stall, individual cats were required to interact with toys and handlers and were offered treats as a positive reward. Acclimation was determined through the demonstration of play and postures/behaviours indicative of low stress as outlined in Kessler and Turner, 1997 during exposure times to the walled stall.

Testing Procedure: Each cat was individually removed from the group living room and placed in the stall. A toy resembling a mouse, known to elicit intense play (Hall et al, 2002), was attached to a string and hung from the cut-out hole in the center of the plexi-glass roof. Once the cat was placed in the stall the researcher flicked the string once to elicit movement of the toy. All behaviour patterns identified in Table
18 were recorded during trial sessions. Each trial session lasted 2 minutes. Behaviours recorded included predatory play patterns and non-play patterns such as indicators of orientation and locomotion. The duration of every behaviour pattern was recorded except for patterns such as ‘hit’, ‘killbite’ and ‘sniff’, which have a fixed duration and were recorded as frequencies.

**Play Test #2- Weighted Door Test:** Procedures and measurements for this behavioural assessment were similar to those described in Widowski and Duncan, 2000, where chickens were increasingly challenged through the incremental addition of 100 g weights to a swing door required to be pushed open to gain access to a nest box. Two walled stalls (each measuring: 100 cm W X 100 cm L X 100 cm H; Queen City polymers, Ohio, USA), one classified as the start box and the other as the goal box, each with a plexi-glass roof containing a 10 cm diameter hole in the center, were placed next to each other and connected via a swing door (23 cm W x 18 cm H). The swing door was made of 1/16” Plexiglass and was attached to the top of the door frame. The door was similar to the type of door that cats are acclimated to using in their group living rooms. To assess play motivation, the swing door was made to be progressively more difficult to open by the addition of weight. Weights were placed into a trough at the bottom of the door. When the cat pushed at the weighted door with sufficient force it swung away from the frame, allowing the cat to pass underneath the door to enter the goal box where the cat was permitted to interact with the toy for 30 sec. Cats were assessed one time per dietary treatment on day 23 at 5 hrs post feed removal, with feed being offered for one hour in the AM. Cats were not permitted to interact with plush toys for 4 days prior to the initiation of the study.

**Training Procedure:** Cats underwent a two week acclimation prior to the initiation of the study to ensure complete adaptation to utilizing the swing door. Cats were first trained to walk between chambers while the door was propped open. Following, cats were enticed to push through the swing door in order to move from the start stall to the goal stall with food rewards. The procedure was repeated on different days until all of the cats learned to consistently leave the start box and push through the swinging door to obtain a treat. No weight was added to the door during the training procedure.

**Testing Procedure:** Each cat was individually removed from the group living room and placed in the start stall. A toy resembling a stuffed mouse was attached to a string and hung from the cut-out hole in the center of the plexi-glass roof of the goal stall only. After being released into the start stall, the cat was allowed 5 min to push through the swing door to gain access to the toy. Each testing series began with zero weight added to the door. If the cat successfully opened the door and entered the goal box, the cat
was permitted 30 sec in contact with the toy. After 30 s, the cat was returned to the start box, and additional weights were added to the door. With each successful door crossing the weight of the door was increased by 100 grams. However, if the cat was unsuccessful (i.e. the cat did not cross the door after 5 min in the start box at any weight excluding the first door weight of 0 grams) the door weight was decreased by 50 grams and the testing repeated for a single trial at the reduced weight before terminating testing for the day. If the cat was repeatedly successful then the testing was repeated up to a maximum weight of 500g. In addition to obtaining the maximum door weights each cat would push to enter the goal box, other behaviour patterns were measured to assess motivation: the latency to open the door, number of unsuccessful attempts to open the door, and the latencies from opening the door to first intentional contact with the toy were measured. Data analyzed included: 1) maximum door weight, defined as the highest successful door weight (grams) in which the cat crossed from the start to goal box, 2) mean box time, defined as the sum of all durations spent in the start box divided by the total number of trials (non-successful + successful), 3) successful trials, are defined as trials in which a cat crossed the door, 4) non-successful trials, are defined as trials in which cats did not cross the door and stayed in the start box for 300 s.

Statistical Analyses: A 2x3 crossover design with repeated measures was used for this experiment. Dietary treatment (control or control + MH) and length of dietary exposure (acute, semi-chronic and chronic) were the factors tested. All statistical analyses were performed using Statistical Analysis System (SAS, version 9.1; SAS Institute Inc., 2002-2003, Cary, NC). Data was further analyzed using the PROC MIXED function. The final model used was: \( Y_{ij} = W_i + e_i \); in which \( Y_{ij} \) = the dependent variable, \( W_i \) = dietary treatment (control (-MH) and test (+MH)), and \( e_i \) = random residual error. Diet (control or test) was considered a fixed effect. The dependent variables body weight and body condition were initially included within the model but were later removed as there was no significant effects of these variable on play motivation. Treatment least square means were compared using the pdiff multiple comparison procedure. Differences were considered significant when \( P<0.05 \) and all data are expressed as mean ± standard deviation.

Results

Play Test #1- 2 Minute Observation Test: Behaviour of the cats subject to test (+MH) diet treatment did not differ in their performance of predatory and non-predatory play behaviours as observed during the two minute interaction with a toy compared to cats subjected to control (–MH) diets (Table 19; \( P>0.05 \)).
Table 19: Mean frequency (A) and duration (B) of performance of predatory and non-predatory play behaviours of cats consuming control (-MH) and test (+MH) diets.

### A

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Test</th>
<th>Control</th>
<th>Standard Error</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sniff</td>
<td>3.1</td>
<td>3.9</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Bat</td>
<td>8.9</td>
<td>10.8</td>
<td>1.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Rear</td>
<td>2.2</td>
<td>1.6</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Grasp</td>
<td>5.4</td>
<td>4.4</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Clutch</td>
<td>6.8</td>
<td>5.6</td>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Killbite</td>
<td>9.5</td>
<td>7.6</td>
<td>1.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Chew</td>
<td>10.3</td>
<td>11.1</td>
<td>1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Softbite</td>
<td>9.4</td>
<td>8.7</td>
<td>1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Lick</td>
<td>1.7</td>
<td>2.3</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

### B

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Test</th>
<th>Control</th>
<th>Standard Error</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interaction Duration</td>
<td>8.7</td>
<td>8.6</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Walk-Watch</td>
<td>0.37</td>
<td>0.51</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Sit-Watch</td>
<td>2.5</td>
<td>2.2</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Sit-Avoid</td>
<td>2.0</td>
<td>2.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sit-Groom</td>
<td>0.44</td>
<td>0.37</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Stand-Watch</td>
<td>0.34</td>
<td>0.16</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Stand-Avoid</td>
<td>1.1</td>
<td>1.3</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Total Time Watch</td>
<td>3.0</td>
<td>2.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Total Time Avoid</td>
<td>4.6</td>
<td>4.9</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Non-Interaction Duration</td>
<td>6.1</td>
<td>5.9</td>
<td>0.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n=20). Means without common superscript (*) differ with dietary treatment (P<0.05).
**Play Test #2- Weighted Door Test:** Behaviour tested using the weighted door motivation test of the cats consuming the control (-MH) and test (+MH) diets were different. Specifically, mean duration in the start box on the maximum door weight was lower for the cats consuming the MH than those consuming the control (P=0.01). Second, mean number of successful trials were greater and therefore the mean number of non-successful trials were less for cats consuming the test (+MH) diet (P=0.01). Mean maximum weight of the door (grams) was greater for cats consuming the test +MH diet (Table 20; P=0.01).

Individual cats also showed differences with dietary treatment (Figure 14). For instance, 8 of the 20 cats did not cross the door on the control diet (-MH), but many crossed the door on the test (+MH) diet, with four reaching the maximum weight of 500 g. Four of the 20 cats crossed the weighted door while consuming both diets; however, cats consuming the test (+MH) diet pushed more weight. Six of the cats crossed the maximum door weight and two cats never crossed the door for all trials on both test and control diets.

Table 20: Measures of the cats willingness work to obtain access to a stuffed toy mouse at varying door weights during test (+MH) and control (-MH) dietary treatments\(^1\).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Test</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean duration (s) in start box</td>
<td>100.8 ± 25</td>
<td>185.39 ± 26*</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean number of successful trials</td>
<td>5.15 ± 0.5*</td>
<td>2.90 ± 0.6</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean number of non-successful trials</td>
<td>0.25 ± 0.1</td>
<td>0.68 ± 0.1*</td>
<td>0.007</td>
</tr>
<tr>
<td>Mean Maximum Weight (g)</td>
<td>407.5 ± 46.1*</td>
<td>227.8 ± 47.4</td>
<td>0.003</td>
</tr>
</tbody>
</table>

\(^1\)Values are means ± SEM (n=20). Means without common superscript (\*) differ with dietary treatment (P<0.05).
Figure 14: Maximum weight cats would push in order to obtain access to a toy when exposed to the test (+MH; solid) and control (-MH; checker) diets. Values are maximum door weights (grams) each cat was willing to push open to gain access to a toy during the test and control dietary treatment.

Discussion

The results of this study suggest that object play in the domestic, adult cat is impacted by dietary consumption of MH, a CRM. MH significantly affected the motivation of the cat to acquire a toy mouse, but had no influence on the pattern of play or motor patterns during free access to the toy. Therefore, MH appears to have differential effects on the appetitive (behaviour that will increase likeliness of satisfying a need or want) and consummatory (behaviour that occurs in response to acquiring a desired stimuli) components of play. The differential effects of MH are likely impacted by metabolites of energy metabolism and their influence on the brain and motivational systems that drive motor patterns of object play in the domestic cat.

In the present study, there was no effect of dietary MH on pattern of object play. This may be due to: 1) the inability of a toy to permit the cat to follow all consummatory behaviour patterns associated with predation, since the motor patterns of object play are similar to those of appetitive and consummatory behaviours of predation, (Leyhausen, 1979), and 2) a CRM, like MH, may not cause feelings of hunger, thought to impact object play and predatory behaviour patterns, since there is no true calorie restriction.
With an inanimate object, such as a toy mouse, all consummatory patterns of predation cannot be performed. Furthermore, the prey response to predation (escape, defense) is absent and therefore the complete predatory behavioural repertoire cannot be displayed during object play. However, Hall and Bradshaw, 1998, found that time since feeding was associated with greater intensity of play and reduced time spent avoiding the toy and that this suggested that predatory and play behaviour share a common underlying motivational system. Cats in conflict, less hungry and presented with a challenging prey item, demonstrate higher amounts of play versus predation as displayed by increased play time and longer latencies to kill (Biben, 1979). Further, hunger reduced the latency of approach of a cat to contact a toy object similar in size and shape to a small squirrel (Katz and Thomas, 1979). These results are supported by those of Adamec et al., 1980, who concluded that hunger enhanced likeliness for lethal biting and pawing attacks on prey. It remains unclear as to whether or not CRM strategies induce feelings of hunger; however, since there is no calorie restriction associated to the CRM, satiety signals (such as ghrelin and cholecystokinin) would presumably not be impacted by MH (Roth et al., 2001). Therefore, if MH has no impact on feelings of satiety there may be no difference in play pattern, as observed in the present study. Further, hyperglycaemic states, produced with MH feeding, act to increase the electrical activity of the “satiety” centers of the hypothalamus and generate a fall in voltage in activity of the “feeding” centers; however, it is unknown how MH impacts glucose uptake/sensing in the brain (Anand et al., 1961). An additional reason for the difference in results may be attributed to the effect of diet type and time of feeding on activity level of the cat. Anecdotal evidence that suggests that prolonged/high levels of plasma glucose and insulin and gut fill perpetuated by the consumption of many commercial dry cat diets, cause cats to become lethargic following consumption; a phenomenon further supported by data in rats and humans (Wells, et al., 1997; Lloyd et al., 1994, 1996; De Castro, 1987). Further, calorie restriction in cats has been shown to cause an elevation in total physical activity level (Bateson and Young, 1981). Though we did not observe an effect of MH on fasting plasma glucose and insulin (Gooding et al., 2012) we tested all of our cats at five hours post feeding where we have previously demonstrated that MH reaches peak concentrations in the plasma; therefore, we likely removed any effects of diet on lethargy similar to the procedures described in Biben, 1979, but different from other researchers (Adamec, 1976, Adamec et al., 1980; Hall and Bradshaw, 1998). It may be concluded that MH does not impact consummatory motor patterns of object play, as has been shown to be significantly affected by hunger in the domestic cat, and from these results one may conclude that MH does not cause feelings of hunger although MH works to modify the energy-sensing pathway associated to glucose metabolism.

In contrast to the null object play results, cats fed MH demonstrated increased motivation to obtain access
to a toy. These results may be a consequence of the fact that appetitive and consummatory play behaviours appear to have separate but interacting control systems (Leyhausen, 1965). Where hunger impacts performance of play behaviours, dietary MH may influence internal energy sensing systems and enhance responsiveness to external stimuli increasing motivation to approach prey or a toy with similar characteristics. These results support those of internal, unpublished data, where there was a significant impact of dietary MH on elderly dog’s (> 8 years) responsiveness to environmental cues where dogs receiving dietary MH demonstrated enhanced reactivity to owners and external stimuli. Hunger is important for predation; however, it is not necessary and this may or may not be true for play. Adamec, 1976, concluded that predatory stimuli took precedence over gustatory stimuli or feeding since cats still killed a live rat while eating a highly palatable food. Therefore, Adamec, 1976, concluded that full consummatory behaviour (eating) does not completely negate food-getting response or appetitive predatory behaviour. From these data it may be further concluded that the priority of predatory behaviour over feeding may function to increase food input through multiple killings and ensure maintenance of adequate food supply in “storage” (Adamec, 1976). If the cats consuming MH were more reactive to stimuli, the innate drive to predate presented with the appropriate cues would likely be enhanced. Enhanced responsiveness to prey stimuli would consequently increase the cat’s motivation to gain access to the toy mouse, an object with comparable characteristics to a prey item, due to the similar underlying motivational systems driving predatory approach and play. Lastly, some animals show enhanced play prior to feeding, such as meerkats (Sharpe and Cherry, 2003) as well as species that hunt communally, including lions (Graham, 2010). Perhaps, domestic cats demonstrate similar appetitive patterns of play prior to hunting and this may have represented itself by an enhanced motivation to gain access to a toy. Briefly, cats use a systematic hunting approach to locate prey. Once located, the cat will use sit and wait stalking techniques to capture prey (Layheusen, 1956). The cat will pounce only once the prey has moved away from cover. Play will occur if the prey item is captured or cornered; this type of play is thought to act to “prepare” the cat for a kill and is likely similar to the play observed in meerkats and lions (Ewer, 1969). MH may act to enhance appetitive behaviours of object play by not increasing feelings of hunger but by increasing the cat’s reactivity to environmental stimuli. This ultimately creates a conflict between drives to capture and “kill” the prey (toy) and this conflict is represented through enhanced motivation to approach a prey item and play prior to a kill without any impact on consummatory behaviours of object play.

The differences in appetitive and consummatory behaviours of cats fed MH is further supported by brain stimulation studies that have identified anatomically different brain regions and substrates that control
feeding and predatory behaviours in domestic cats (Wasman and Flynn, 1962; Flynn et al., 1970). Roberts and Kiess, 1964, found that hypothalamic brain stimulation caused cats that previously demonstrated low levels of predatory behaviour to rapidly learn a Y maze that allowed access to a live rat they could kill. Performance during the Y maze diminished once the hypothalamic stimulation was stopped. Furthermore, these researchers noted that hypothalamic stimulation caused the cats to kill multiple rats regardless of state of hunger; thus, enhancing motivation to kill without full consummatory processes. Hypothalamic stimulation does not trigger a fixed behavioural pattern of predatory (and likely play) responses since specific behaviours are not necessarily correlated in a sequential manner. Rather, there are multiple sensorimotor mechanisms controlling separate components of a complete behaviour pattern (Berntson et al., 1976). Fonberg and Serduchenko, 1980, found that hypothalamic lesions caused a decline in feed intake without significantly impacting predatory behaviour. Stimulation of the hypothalamic area known to elicit attack caused cats to aggressively bite food and begin prowling around the cage; this behaviour was hypothesized to be appetitive predatory behaviour without food consumption (Flynn, 1967). Lesions of the ventromedial amygdala in cats reduce predatory behaviour without significantly reducing food intake (Panksepp and Trowill, 1969). Therefore, it was concluded that predatory behaviour is mediated by a motivation different from alimentary behaviour and that the crucial area for the cat's predatory motivation is in the ventromedial amygdale (Panksepp and Trowill, 1969). Fonberg and Flynn, 1978, stimulated the medial central amygdaloid nucleus of the cat brain and observed motor patterns of predatory behaviour without any goal directed attacks on rats; therefore, it was concluded that the amygdala may influence the hypothalamus and overt predatory behaviours. Comparative studies conducted in primates suggest that the neocortex, cerebellum, amygdala, and hypothalamus all act to influence performance of play (Lewis and Barton, 2006). The amygdala is important for play because it is a target of androgens, is associated to emotion and social and gender-related behaviours and damage to the associated structures causes reductions in play (Panksepp 1998; Vanderschuren 2010). Overall, there appears to be associations in neurological control systems of predation, play and hunger with some anatomical and motivational differences controlling hunger and predation. Although predation and play appear to have similar underlying motivational systems this has yet to be directly studied and requires further research. The present study suggests that MH, and the correlated impacts on glucose metabolism, act positively on the brain regions controlling appetitive patterns of object play (responsiveness to stimuli) with no effect on the brain regions controlling the consummatory effects of object play (primarily impacted by hunger). However, it should be noted that more research needs to be completed to determine if MH truly has an impact on brain glucose metabolism or an associated metabolic signaling system. The results of the current study suggest that a physiological
effect of MH may exist by observing a behavioural effect via enhanced motivation to obtain access to a toy mouse.

In conclusion, dietary MH, a CRM impacting cellular energy sensing, influenced cats’ motivation to access a toy (appetitive/approach behaviour); however, there was no apparent impact of MH on pattern of play once the toy was accessible (consummatory behaviour). MH may enhance cats’ internal motivational system by enhancing the level of responsiveness to external prey like cues promoting behaviours required to acquire prey which may be presented as play.
References


Bateson, P.; Mendl, M.; Feaver, J. 1990: Play in the domestic cat is enhanced by rationing of the mother during lactation. Anim. Behav. 40, 514-525.


de Castro JM. 1987: Macronutrient relationships with meal patterns and mood in the spontaneous feeding


Lloyd, H.M.; Green, M.W.; Rogers, P.J. 1994: Mood and cognitive performance effects of isocaloric lunches differing in fat and carbohydrate content. Physiol Behav 56:51–56


Slingerland, L.I.; Vasilova, V.V.; Plantinga, E.A.; Kooistra, H.S.; Beynen, A.C. 2007: Indoor confinement and physical inactivity rather than the proportion of dry food are risk factors for the development of feline type 2 diabetes mellitus. *Vet J.* In Press.


Conclusion

Many owners consider their cats to be conscious beings that are capable of experiencing a variety of emotions (Burghardt, 2009). Consequently, the relationship between cats and their owners can be profound and deep. With the evolution of the human-cat relationship, pet owners express concerns about the quality of life experienced by their pet (Tannenbaum, 1991). While many pets live in “pampered” environments (as perceived by their owners) there is a clear disconnect between owner perception of their animal’s welfare and the true welfare status of the animal as is related to obesity. As a means of strengthening the relationship with their pet, owners often use food and feeding time to reinforce the bond. Around feeding time, cats typically display a predictable pattern of social behaviour directed towards the owner. The level of affection that the owner receives from the cat around feeding may, in turn, drive an owner to continue to feed their pet aiming at a mutually beneficial relationship (Bradshaw and Cook, 1996). Consequently, the prevalence of obesity is increasing greatly in feline populations, is the most commonly cited nutritional disorder, and is of concern since obesity contributes to the aetiology of many diseases, including: diabetes and cardiovascular disease, negatively impacting the welfare of their pet cat (Hoenig et al., 2011). This issue is compounded by two factors: 1) that many owners of obese cats do not readily recognize that their pet is obese and 2) compliance to weight loss regimes as suggested by a veterinarian is low, regardless of the fact that owners appear concerned with balanced nutrition (Kienzle and Bergler, 2006). The apparent “joy” that feeding time brings both pet and human may be the reason for the non-compliance to recommended weight loss regimes. Therefore, while pet owners feel that they are fulfilling their ethical obligation to their pets by providing excess food and environments free of suffering, some owners are unconsciously negatively impacting the health and welfare of their pets by contributing to obesity. Thus, this presents a unique challenge for nutritionist to overcome.

The majority of research for my Ph.D. attempted to expand our understanding of the effects of diet composition as a relative risk for development of adiposity and ultimately obesity in the domestic cat. Although the prevalence of obesity is rising, weight loss formulas remain popular, likely because they are recommended by veterinarians and often do not require a reduction in quantity (gram basis) of food due to their large, low calorie carbohydrate content. However, high carbohydrate diets are often hypothesized as contributing to the development of weight gain in the cat via perturbations in energy metabolism (Thiess et al., 2004). Thus, it is apparent that methods to prevent changes in metabolism facilitating weight gain, without active owner intervention, are imperative. My research attempted to address some of these issues; however, strategies like calorie restriction mimetics that improve metabolism and enhance physical
activity, require further investigation to fully understand long term benefits and methods of action in the feline. In addition, through increased physical activity and play a cat may become more engaging; improving the human-pet bond and benefiting both human and animal welfare.

Throughout my PhD the behavioural consequences of feeding certain diets were studied; a relationship that is often neglected in the area of pet nutrition. Understanding not only the metabolic effects of diets but also the related impacts on behaviour is vital (Kienzle and Bergler, 2006). Slingerland et al., 2007, concluded that it was not, in fact, diet that contributed to weight gain but indoor housing and, thus, a reduction in level of physical activity. Since it appears to be a combination of the effects of diet and activity that contribute to weight gain, both factors necessitate investigation as individual and combined entities. In addition, we have only begun to understand some the effects of diet, body composition and activity on cognitive function of cats. This is an especially important area to study since owners are becoming more sensitive to their pets behaviour and cognitive function throughout all life stages (especially senior and geriatric years). Techniques designed to improve cognitive function and prevent the onset of dementia associated to aging can enhance the quality of life of the cat, reduce number of relinquishments and increase the human-pet bond (Serpell, 1996).

Since we observed some effects of high fat (HF) and high carbohydrate (HC) diets on cognition and activity it would be interesting to investigate these areas in more detail. Combining accelerometer data with global positioning system (GPS) and/or video data of household or research cats on different diets may allow us to understand the impacts of diet on activities of daily living. Specifically, we can measure percentage of time spent sleeping, eating, playing and interacting with other pets or humans. To enhance our understanding of the effect of diet on cognition the amount of T-maze testing should be increased to a minimum exposure of 100 trials to ensure that the majority of cats truly learn the association between a symbol and a reward. Since the T-maze test appears difficult for cats to learn, studies designed to mimic the natural hunting behaviour of cats, as they use environmental cues to systematically search particular areas, may offer more insight as to the effects of diet on ability to use spatial cues. To complement behavioural data, an additional blood biomarker that may offer insight on the relative propensity of a particular diet to contribute to obesity and diabetes is amylin. Lutz and Rand, 1996, concluded that elevated amylin concentration may contribute to feline diabetes mellitus by contributing improved insulin sensitivity. However, the effects of typical diet matrices (HF vs HC) on amylin concentrations have not been investigated. In addition, amylin appears to have a significant effect on energy homeostasis since the peptide plays a role in the regulation of hunger, feed intake and gastric emptying rate (Lutz, 2010). The
play motivation test may be used to measure level of hunger in cats with significantly different amylin concentrations and also plasma amylin may be used as a physiological measure of satiety as influenced by relative intake of fat and carbohydrate.

It remains unclear as to whether or not calorie restriction mimetics (namely mannoheptulose) truly impact hunger levels (Ingram et al., 2004). To measure this effect the same obstruction test could be repeated and in addition to providing a toy mouse in the goal box a food reward can be offered. Similar motivation measures can be assessed in addition to frequency of selection of food versus the toy. Further, Roberts and Kiess, 1964, found that hypothalamic brain stimulation caused cats that previously demonstrated low levels of predatory behaviour to rapidly learn a Y maze that allowed access to a live rat they could kill. Performance during the Y maze diminished once the hypothalamus stimulation was stopped. Since we hypothesize that dietary MH treatment may activate the predatory/play centres of the feline brain it would be interesting to compare the performance of cats during T-maze testing with and without MH treatment. In addition, since we are not clear on the mechanisms of MH action, the use of positron emission tomography (PET) may allow us to track uptake and metabolic activity of glucose in tissue (the brain and liver are of particular interest) in cats with and without MH treatment. The particular time points that would be of interest are the 4 hrs after feeding when MH is at its peak plasma concentration and again at 15-22 hours after feeding when we observed significant increases in energy expenditure.

In conclusion, the research that was completed throughout my PhD has contributed to the understanding of dietary composition, namely fat and carbohydrate inclusion, and the relative risk for weight gain. The data presented regarding calorie restriction mimetics is a novel approach in the area of feline nutrition and presents a unique opportunity, through dietary intervention, to promote healthy energy metabolism, body composition and motivation to play. Diets that improve health and positively impact behaviour as represented through cognition, activity and play will create pets that are more engaging and energetic. However, our level of understanding of the relationship between nutrition and behaviour in the domestic cat is minor and yields a plethora of opportunity for further investigation. With the design of optimal diets, nutritionists present an opportunity for pet owners to improve the quality of life of their pet; fulfilling our ethical obligation to animals and, in turn, enhancing owner attachment levels.
References


Slingerland, L.I.; Vasilova, V.V.; Plantinga, E.A.; Kooistra, H.S.; Beynen, A.C., 2007: Indoor
confinement and physical inactivity rather than the proportion of dry food are risk factors for the development of feline type 2 diabetes mellitus. *Veterinary Journal*. In Press.
