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EFFECTS OF WEIGHT LOSS WHILE FEEDING A MODERATE-PROTEIN, HIGH-FIBER DIET  
ON BODY COMPOSITION, VOLUNTARY PHYSICAL ACTIVITY, AND  
BLOOD METABOLITE PROFILES IN OVERWEIGHT CATS

BY

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THESIS

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## ABSTRACT

Obesity is a major nutritional disorder in cats and is associated with several comorbidities and reduced life span. The primary objectives of this study were to determine the effects of feeding a moderate-protein, high-fiber diet on BW loss, voluntary physical activity levels, body composition, and blood metabolite profiles in overweight cats. During a 4-wk baseline period, 8 adult neutered male domestic shorthair cats (mean BW =  $7.7 \pm 0.4$  kg, mean BCS = 7.6/9) were fed to maintain BW. For 18 wk following baseline, food intake was adjusted to allow cats to lose weight at a rate of  $\sim 1.5\%$  BW/wk. Cats were group-housed for 20 h/d and individually housed for two, 2-h periods each day for feedings. Daily food intake, twice-weekly BW, and weekly BCS were recorded throughout the study. Voluntary physical activity was measured over a 7-d period at wk 0, 6, 12, and 18 using Actical® activity monitors. Dual energy X-ray absorptiometry (DEXA) scans were taken at wk 0, 4, 8, 12, and 16 to estimate body composition. Overnight fasted blood samples were collected at wk 0, 1, 2, 4, 8, 12, and 16. The University of Illinois Institutional Animal Care and Use Committee approved all procedures. As expected, mean BW ( $7.7 \pm 0.4$  vs.  $6.2 \pm 0.4$  kg) and mean BCS (7.6 vs. 6.0) decreased ( $P < 0.05$ ) from wk 0 to wk 18. The NRC (2006) maintenance energy requirement (MER) for overweight cats is  $130(\text{BW}_{\text{kg}}^{0.40})$ . In comparison, the mean MER during baseline in our study was  $113(\text{BW}_{\text{kg}}^{0.40})$ . Throughout wk 1-4, 5-8, and 9-18, the energy levels to sustain weight loss were 76, 64, and 57% of baseline MER, respectively, demonstrating how restrictive feeding must be for consistent weight loss. Mean fat mass was decreased ( $P < 0.001$ ) at wk 8, 12, and 16 (2417, 2097, and 1810 g, respectively) versus wk 0 (2924 g); therefore, body fat percentage also was decreased ( $P < 0.05$ ) at wk 8, 12, and 16 (36.8, 34.0, 30.7%, respectively) versus wk 0

(40.9%). Mean lean body mass was lower ( $P < 0.01$ ) at wk 12 and 16 (3671 and 3664 g, respectively) versus wk 0 (3865 g). Importantly, lean body mass percentage was increased ( $P < 0.05$ ) at wk 8, 12, and 16 (61.5, 64.4, and 67.6%) versus wk 0 (57.6%). Mean daily activity tended to be higher ( $P = 0.061$ ) at wk 12 vs. wk 0. The mean light:dark ratio of activity was increased ( $P < 0.05$ ) at wk 18 vs wk 0, 6, and 12. Except for elevated mean creatinine ( $P < 0.05$ ) during weight loss, all blood metabolites remained within reference ranges. Mean triglyceride concentrations were decreased ( $P < 0.05$ ) throughout the weight loss phase. Using non-targeted gas chromatography, liquid chromatography, and mass spectrometry, a total of 535 named biochemicals were identified, with up to 269 metabolites altered ( $P$ - and  $q$ - values  $< 0.05$ ) at any time point. Principal component analysis showed a continual shift in metabolite profile as weight loss progressed. Components 1 and 2 explained 14.3% and 10.3% of the variability, respectively. There was a significant and dramatic reduction of bile acids (cholate; taurocholate; deoxycholate) with weight loss. A reduction in numerous non-esterified fatty acids (NEFA) and an increase in ketones (acetoacetate; 3-hydroxybutyrate) and monoglycerides suggested a shift toward lipolysis and hepatic NEFA oxidation. Decreased markers of inflammation and oxidative stress were indicated by reduced pro-inflammatory oxylipids, eicosanoids, and oxidized biomarkers following weight loss. Mevalonate was decreased ( $P < 0.05$ ) after wk 8 compared to baseline, which agrees with the reduced bile acids without altering cholesterol concentration. In conclusion, restricted feeding of a moderate-protein, high-fiber diet is a safe and effective means for weight loss in cats, leading to increased physical activity and reduced blood triglycerides. Global metabolomics identified biomarkers of reduced food intake, weight loss, and/or altered metabolism. Based on our data, the

current NRC (2006) MER estimates for cats appear to be too high and should be reconsidered.

*For my mom and dad.*

*Thank you for your endless  
support and encouragement, and  
most of all, love.*

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# **Chapter 1**

## **Introduction**

Pets are becoming more integral members of the family, with 68% of US households owning a pet and 37.3% of US households owning a cat in 2014 (American Pet Products Association, 2014). Unfortunately, there is a continually increasing incidence of obesity in companion animals in the US and obesity is now considered the most common nutritional disorder in pets (German, 2006). Recent data from a 2014 survey conducted by the Association for Pet Obesity Prevention (APOP) found that 57.9% of US cats, or approximately 55 million, are overweight (29.8%) or obese (28.1%). To further complicate the issue, there is a 'fat gap' inhibiting owners from recognizing the degree by which their pets are overweight (Colliard et al., 2009; Courcier et al., 2012). It has been reported that approximately 45% of owners considered their pet as having a normal body weight (BW) when the veterinarian assessed them as overweight (Calabash, 2013). A general classification defines an overweight cat as weighing 10-20% over their ideal BW and an obese cat weighing >20% above their ideal BW (Toll et al., 2010). Each unit increase of body condition score (BCS) above ideal (BCS=5) is roughly 10 to 15% over ideal BW (Mawby et al., 2004; Laflamme, 2012).

Feline obesity is associated with comorbidities that have detrimental effects on health. These include, but are not limited to, diabetes mellitus (DM), hepatic lipidosis, urinary tract diseases, hypertension, dystocia, respiratory disorders, neoplasia, feline lower urinary tract disease (FLUTD), lameness, dermatosis, and decreased life span (Scarlett and Donoghue, 1998; Lund et al., 2005; German, 2006; Brooks et al., 2014). The traditional development of obesity is due to a positive imbalance between energy intake and energy

expenditure (German, 2006). Aspects of domestication and humanization of pets also attribute to obesity. These risk factors include neutering (Colliard et al., 2009; Cave et al., 2012; Courcier et al., 2012), decreased physical activity, increased food intake, and access to highly palatable high-fat and/or high-carbohydrate diets (Nguyen et al., 2004; Backus et al., 2007; Farrow et al., 2013).

While obesity prevention would ideally avoid these conditions, it is necessary to develop effective and safe obesity treatment methods. The recommendation for safe and reasonable weight loss for cats is 1% to 1.5% of BW loss per week (Burkholder and Bauer, 1998). To safely avoid inducing hepatic lipidosis during weight loss, cats should eat at least 50% of their maintenance energy requirement (MER) (Toll et al., 2010). Previous experimental and clinical trials have used caloric restrictions between 59 and 80% of MER without evidence of hepatic lipidosis (Markwell et al., 1996). Therefore, the objective of this study was to determine effects of weight loss while feeding a moderate-protein, high-fiber diet on body composition, voluntary physical activity, and blood metabolite profiles in overweight cats.

## **Chapter 2**

### **Literature Review**

#### **CAT POPULATION AND OBESITY RATES**

Pet ownership and spending is at an all-time high. A 2013-2014 National Pet Owners Survey reported that 68% of all US households own a pet (APPA, 2014). With cat ownership reaching 45.3 million households (37.3%), there are 95.6 million cats in the US. Along with a growing pet population, the human-animal bond is becoming increasingly strong. The American Pet Products Association (APPA) reported that the total US pet industry expenditures were \$55.72 billion in 2013 and \$58.04 billion in 2014, with an estimated expenditure of \$60.59 billion in 2015. As pets are more often referred to as members of the family, owners are seeking ways to provide their pets the highest quality of life and are inclined to spend more on pet products. In fact, the top spending in 2014 was on pet food (\$22.26 billion) and veterinarian care (\$15.04 billion). The pet food industry utilizes this desire of owners to their advantage, marketing diets that target owner preferences.

There is a continually increasing incidence of obesity in companion animals in the US. Obesity is now considered the most common nutritional disorder in pets (German, 2006). Recent data from a 2014 survey conducted by the Association for Pet Obesity Prevention (APOP) found that 57.9% of US cats, or approximately 55 million, are overweight (29.8%) or obese (28.1%). Often referred to as the 'fat gap', owners often are unable to assess the degree to which their pets are overweight (Colliard et al., 2009; Courcier et al., 2012). This troubling separation between a pet's physical state and owner perception of health makes it difficult for veterinarians and pet food professionals to

initiate companion animal weight loss regimens. While obesity prevention is ideal, it is important to develop effective and safe obesity treatment methods.

## DEFINING OBESITY

Obesity is defined as an accumulation of excessive amounts of adipose tissue in the body, which results in impairment of health or body function (Laflamme, 2005; Toll et al., 2010). A general classification defines an overweight cat as weighing 10-20% over their ideal BW, with an obese cat weighing >20% above their ideal BW (Toll et al., 2010). It is inconclusive how much excess fat and adiposity is needed to diagnose using these parameters, however. Given the genetic variability that exists in cats and because an ideal BW often is unknown, BW alone is not sufficient for obesity assessment. To determine the extent of obesity, it is important to distinguish fat mass (FM) from lean body mass (LBM) (Burkholder, 2001). There are many methods for body composition analysis in dogs and cats. However, none of these methods are without criticism. Many tests are considered precise, referring to the ability to be repeated, but not accurate, referring to the correctness of a value. There are also considerations of cost, ease of use, and acceptance by clients due to perceptions of invasiveness. Common techniques include chemical analysis, densitometry, total body water measurement, absorptiometry, ultrasonography, and advanced imaging techniques (German et al., 2006).

Dual-energy X-ray absorptiometry (DEXA), also referred to as quantitative density radiograph (QDR), is considered the standard for estimating body composition of cats (Speakman et al., 2001; German et al., 2006; Vasconcellos et al., 2009; Bjornvad et al., 2011). It is typically utilized in research and clinical trials because it provides accurate

measurements of body fat mass, lean mass, and bone mass compared to total mass, with no subject instruction necessary.

Veterinarians in clinical settings and pet owners at home require an economical and quick, yet reliable, method of body condition assessment. Morphometry, meaning shape or form, is a quantitative analysis. Those approaches include measurements of skinfold thickness and dimensional evaluations (i.e., leg length or girth compared with weight to calculate body mass index [BMI]). Body condition scoring (BCS) methods are a qualitative analysis (German, 2006). Due to ease, accuracy, and repeatability, the most commonly used is the 9-point BCS system developed by researchers at Nestle Purina in 1997. The validation of this system reported a significant correlation between DEXA values and BCS ( $r = 0.86-0.91$ ) (Laflamme, 1997). This technique utilizes visual and palpable assessments to assign a numeric score. Cats store most of their fat subcutaneously along their ventral abdomen, intra-abdominally, and in their faces (Toll et al., 2010). The evaluator scores the amount of subcutaneous fat, abdominal fat, and musculature of the ribcage, waist, and dorsal spinous processes. Each unit increase of BCS above ideal (BCS=5) is roughly 10 to 15% over ideal BW (Mawby et al., 2004; Laflamme, 2012).

Despite its high use, BCS is a subjective method requiring a learned skill for best accuracy. What one may judge as an excessive amount of fat covering the ribs or surrounding the waist may seem appropriate to another. Still, German et al. (2006) demonstrated excellent correlation between experienced operator scores for cats ( $r = 0.99$ ,  $P < 0.0001$ ), along with a strong correlation ( $r = 0.83$ ,  $P < 0.0001$ ) between the assigned score and body fat determined by DEXA. More importantly, that study showed that the scores of owners presented good agreement with those of experienced operators ( $r = 0.86$ ,

$P < 0.0001$ ). That relationship provides a certain level of confidence that with simple training an owner can assess the body condition of their pet without special equipment. Veterinarians can encourage pet owners to utilize this practical tool to monitor their pets' health and hopefully prevent overweight or obese body conditions from developing.

### OBESITY-RELATED COMORBIDITIES

Similar to human obesity, which is a characteristic of metabolic syndrome, feline obesity is associated with comorbidities that have detrimental effects on health. These include, but are not limited to, diabetes mellitus (DM), hepatic lipidosis (HL), urinary tract diseases, hypertension, dystocia, respiratory disorders, neoplasia, feline lower urinary tract disease (FLUTD), lameness, and decreased life span (Lund et al., 2005; German, 2006; Brooks et al., 2014). In general, clinical care of obese patients is more difficult and comes with higher risk. Routine procedures such as physical exams, abdominal palpation, blood sampling, diagnostic imaging, and anesthetization are more problematic. In a study by Lund et al. (2005), overweight cats were often pre-diagnosed with oral or urinary tract disease. Similarly, obese cats were more likely to be concurrently diagnosed with DM, oral disease, dermatopathy, or neoplasia.

Overweight cats have more limited mobility and as a consequence are less able to sufficiently groom themselves. This is associated with many types of dermatoses such as feline acne, alopecia, scale development, and pressure sores. Obese cats are 2.3 times more likely to develop non-allergic skin disease (Scarlett and Donoghue, 1998). Obese cats are also five times more likely to develop lameness as an effect of mechanical overload, joint stress, and chronic low grade inflammation (Scarlett and Donoghue, 1998).

## Diabetes Mellitus

Obese cats are up to four times more likely to develop DM (Scarlett and Donoghue, 1998; Lund et al., 2005). The frequency of feline DM ranges from 0.5 – 2% (Panciera et al., 1990; Rand et al., 2004). This range may be an underestimation due to diagnostic measures. In cats, obesity is a well-known risk factor for DM (Nelson et al., 1990; Biourge et al., 1997; Scarlett and Donoghue, 1998; Appleton et al., 2001; Hoenig et al., 2002; Lund et al., 2005). Resembling that of humans, type II DM accounts for 80-95% of feline diabetes cases (Rand et al., 2004). Diabetic cats have been shown to have significantly impaired insulin secretion and action (Scarlett and Donoghue, 1998; Feldhahn et al., 1999; Hoenig, 2002; Hoenig et al., 2006;). In a study by Appleton et al. (2001), 52% of obese cats had decreased tissue sensitivity to insulin and diminished glucose effectiveness. Insulin sensitivity refers to the decrease in blood glucose in response to insulin secretion, while insulin resistance indicates significantly decreased insulin sensitivity by tissues (Porte, 1990). Even a slight change in BW can have an effect on insulin sensitivity in cats. Previous research has shown that each kg BW increase in adult cats contributed to a 30% decrease in insulin sensitivity (Hoenig et al., 2007).

Some clinical signs of DM are increased thirst (polydipsia), increased urination (polyuria), increased appetite (polyphagia), signs of depression or weakness, and rapid gain or loss in BW. In humans, DM is diagnosed when fasting plasma glucose exceeds 125 mg/dL or when hemoglobin A1c is 6.5% or greater. Diagnosis for cats is less clear and is usually only diagnosed when overt clinical signs are present. This is when blood glucose concentrations are greater than 290 mg/dL and exceed the renal threshold (Rand et al., 2004). Because DM is a slowly developed disease, most cats are already insulin-dependent

by the time they are diagnosed and treatment is more challenging (Nelson, 2000).

Therefore, the rate of diagnosis is expected to continually increase due to awareness of predisposing factors such as increased obesity rates and decreased physical activity (Prahl et al., 2007). Changes in glucose homeostasis due to obesity are reversible and it is estimated that weight loss may eliminate 13-18% of DM cases (Scarlett and Donoghue, 1998; Prahl et al., 2007).

### Hepatic Lipidosis

Hepatic lipidosis is the most commonly diagnosed liver disease in cats in North America (Armstrong and Blanchard, 2009). Secondary HL can result from anorexia due to a primary disease such as pancreatitis, neoplasia, small intestinal disease, kidney disease, and DM (Center, 2005). Primary HL develops during times of inadequate intake such as rapid weight loss, food refusal, and stress. The most rapid onset of HL appears to occur congruently with rapid weight loss from a very high BCS (Armstrong and Blanchard, 2009). Obese yet healthy cats that have lost 30-40% of their BW through caloric restriction are at risk of primary HL (Biourge et al., 1994a; Biourge et al., 1994b). This may develop as quickly as 2 to 7 days (Center, 2005) so safe weight loss in cats is important.

### BLOOD METABOLITE PROFILES AND HORMONES

Fluctuations in the concentrations of hormones, especially in relation to neutering and aging, are linked to body composition changes and obesity. Adipose tissue is a highly active tissue that produces adipokines, which are hormones and cytokines involved in cell signaling and may contribute to diseases related to obesity (Kershaw and Flier, 2004;



Trayhurn and Wood, 2005). In obese humans, there is a positive correlation between cytokine concentrations with impaired glucose intolerance and insulin resistance (Bastard et al., 2002; Shoelson et al., 2006). Two well-studied adipokines are leptin and adiponectin. Leptin, a member of the interleukin-6 (IL-6) family of cytokines, positively correlates with increased adiposity and insulin resistance. After leptin crosses the blood-brain barrier via leptin receptors, it further activates receptors on the hypothalamus. As BW increases, leptin concentrations increase to regulate appetite and energy expenditure to counteract weight gain. Leptin responsiveness is diminished in DM and in overweight and obese cats, indicating the development of leptin resistance.

The association between obesity and serum adiponectin concentrations are inconsistent. Total adiponectin often has been shown to be negatively correlated with adiposity and insulin concentration, but rises with weight loss (Fruebis et al., 2001; Hoenig et al., 2007; Hoenig et al., 2013). Others have found no correlation of body fat and total adiponectin, but a decrease in high-molecular-weight adiponectin (the most common isoform) with obesity (Bjornvad et al., 2014).

Ghrelin is also important for the control of food intake. Often referred to as the “hunger hormone”, plasma ghrelin concentrations usually increase preprandial and decrease postprandial. When the upper gastrointestinal tract is empty of calories, ghrelin is produced from endocrine cells found mainly in the fundic mucosa of the stomach (Holst and Schwartz, 2004). This surge of ghrelin signals hunger by either stimulating neuropeptide and agouti-related protein-containing neurons in the arcuate nucleus of the hypothalamus, or indirectly acting through the dorsal vagal complex in the nucleus tractus solitarius in the brain stem (Holst and Schwartz, 2004).

## Dyslipidemia

In obese humans, altered lipid metabolism is displayed by elevated triglycerides, cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), apolipoprotein B concentrations, and lowered high-density lipoprotein (HDL) concentrations (Despres et al., 1990; Griffin, 1999). While most other species display one HDL band, the cat is similar to the human in that the HDL fraction is subdivided into two minor components, HDL<sub>2</sub> and HDL<sub>3</sub>, with similar density ranges (Terpstra et al., 1982; Demacker et al., 1987). Thus, the cat is suggested to be an appropriate model for the metabolism of HDL subclasses. Serum HDL cholesterol in cats is higher than many other species, including humans, in the lean state. In man, LDL is the main cholesterol carrier and comprises 63% of the total serum cholesterol, which is much higher than the 13-14% reported in cats (Demacker et al., 1987). Furthermore, cats increase concentrations of HDL<sub>2</sub> and HDL<sub>3</sub> ( $45.0 \pm 9.3$  to  $59 \pm 16.1$  and  $44.3 \pm 14.0$  to  $62.5 \pm 9.0$ , respectively) at the onset of obesity (Hoenig et al., 2003). Given HDL's beneficial role in reverse cholesterol transport, such high concentrations likely provide a mechanism to protect cats against the development of cardiovascular disease.

Nonesterified fatty acids (NEFA), or free fatty acids, are the major components of triglycerides. Under conditions of insulin resistance, such as DM, inhibition of hormone sensitive lipase (HSL) is lost. This imbalance causes an increase in fat mobilization from adipose tissue to hepatic tissue, which leads to an elevation of NEFAs in blood. Two studies by Hoenig et al. (2002; 2003) have shown that baseline serum NEFA concentrations do not differ between lean ( $0.49 \pm 0.15$  mEq/L) and obese ( $0.46 \pm 0.18$  mEq/L) cats, but concentrations were higher ( $P < 0.05$ ) in lean vs. obese males ( $0.38 \pm 0.11$  versus  $0.54 \pm$

0.08 mEq/L). Other studies have reported that obese cats have higher serum NEFA concentrations ( $P < 0.005$ ) than lean cats (0.33 – 0.40 versus 0.49 – 0.56 mEq/L, respectively) (Ferguson et al., 2007; Jordan et al., 2008).

Data prove that obese cats display insulin resistance, dyslipidemia, elevated cholesterol and triglycerides, and DM. While insulin resistance is associated with atherosclerosis in humans, cats do not suffer from atherosclerosis, hypertension, and cardiovascular disease in the same manner (Hoenig et al., 2013). Hypothyroidism is also extremely rare in cats (German, 2006). More research is needed to determine what anti-atherogenic factors protect obese cats from these conditions.

### Thyroid Hormones

Hyperthyroidism is the most common endocrine disease in cats and becomes more prevalent with age. Thyroid hormones have great impact on the control of thermogenesis and energy balance (Silva, 1995). Hyperthyroidism results from a decreased basal metabolic rate and is diagnosed when plasma thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) are below normal ranges. Oral  $T_3$  supplementation increases metabolic rate and NEFA in both lean and obese cats, while also preventing the increase in  $T_4$ .  $T_3$  administration also caused obese cats to reduce feed intake by 5.8% and lose BW (Hoenig et al., 2007).

Hoenig et al. (2002) reported that free  $T_4$  concentrations were only higher ( $P = 0.022$ ) in females than males before neutering ( $2.5 \pm 0.7$  versus  $1.8 \pm 0.7$  pmol/L), but reported no significant difference between males and females after neutering. Ferguson et al. (2007) reported higher ( $P < 0.0001$ ) free  $T_4$  serum concentrations in obese vs. lean cats, which were positively correlated with BW ( $P < 0.002$ ), body fat percentage ( $P < 0.0001$ ),

girth ( $P < 0.0001$ ), and BMI ( $P < 0.02$ ). In that study, the free  $T_4$  fraction (FFT<sub>4</sub>) serum total  $T_4$  tended to be higher ( $P < 0.08$ ) in obese vs. lean cats and also positively correlated with BW ( $P < 0.001$ ), body fat percentage ( $P < 0.02$ ), girth ( $P < 0.003$ ), and BMI ( $P < 0.03$ ). These data suggest that obesity may cause thyroid hormone resistance.

### Inflammatory cytokines

Inflammation is a critical immune response for surviving infection and injury. Chronic, low-grade inflammation is a characteristic of obesity that increases concentrations of circulating inflammatory markers with increasing adiposity. White adipose tissue (WAT) is now considered an active endocrine organ that secretes hormones and protein factors that are collectively termed adipokines, which influence many biological systems such as glucose homeostasis, inflammation and immunity, vascular biology, cell proliferation, angiogenesis, and neurotrophic functions (Radin et al., 2009; German et al., 2010). Obese humans have been shown to have elevated tissue expression and circulating concentrations of C-reactive protein (CRP), IL-6 and IL-18, haptoglobin, monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor alpha (TNF- $\alpha$ ), along with a general reversal with weight loss (Esposito et al., 2003; Trayhurn and Wood, 2004; Manco et al., 2007).

In cats, there are no significant differences in IL-6, IL-1, or TNF- $\alpha$  plasma concentrations with increasing BW (Hoenig et al., 2012; Van de Velde et al., 2013). Previous research by Hoenig et al. (2007) reported an increased mRNA expression of TNF- $\alpha$  in subcutaneous adipose tissue of obese cats in association with decreased activity of lipoprotein lipase (LPL). Additionally, skeletal muscle tissue expressed higher LPL and HSL

mRNA expression, indicating that obesity results in a redistribution of fatty acids from adipose to muscle for storage.

A study by Van de Velde et al. (2013) evaluated mRNA expression of adipose tissue in obese vs lean cats. Obese cats had more ( $P = 0.039$ ) T-lymphocytes present than lean cats, while B-lymphocytes and macrophages were not different. TNF- $\alpha$  and interferon- $\gamma$  mRNA expression tended to be higher ( $P = 0.059$  and  $0.052$ , respectively), but the expression of IL-6 and IL-10 did not differ. Elevated concentrations of chemokine-5 ( $P = 0.004$ ) and MCP-1 ( $P = 0.042$ ) also were reported in obese cats. These data support the need for further research to identify the involvement of inflammation in the development of insulin resistance in obese cats.

### Global Metabolomic Profiling

Although there are heritable traits of obesity, the worldwide prevalence of obesity is increasing within all age groups, especially in developed countries (Aranceta, 2002). Therefore, it is agreed that obesity is a complex and multifactorial disease not only involving genetics, but also environmental and lifestyle factors (von Deneen et al., 2011). Metabolomics, similar to genomics and proteomics, is now used to analyze samples to gain insight on global metabolite responses to stimuli in order to link phenotype with genotype (Xie et al., 2012). Obesity is a disease that affects whole body function and, therefore, elicits a plethora of metabolic responses during both weight gain and loss, yet the specific alterations of metabolism and dysfunction of organs are not entirely understood (Kusmann et al., 2006).

Metabolomic assays are newly used bioanalytical techniques to identify low molecular weight compounds as biomarkers of disease progression and evaluate the effects of nutritional intervention (Zhang et al., 2012). Using a multiplatform approach (e.g., nuclear magnetic resonance spectroscopy, mass spectroscopy, liquid and gas chromatography) with high sensitivity allows for the untargeted determination or targeted quantification of biological components (Swanson and de Godoy, 2015). These biomarkers increase the understanding of metabolism and physiological influence. For example, biomarkers of high meat intake have been associated with diseases such as hypertension, cardiovascular disease, and cancer (Verhoef et al., 2005; Cross et al., 2006, 2007; Stolzenberg-Solomon et al., 2007; Koeth et al., 2013). While the field of metabolomics is developing in human health, less is known about the global metabolite profile of the domestic cat (Colyer et al., 2011; Allaway et al., 2013; Deng et al., 2014). Although two recent studies have evaluated nutritional interventions in cats and dogs, only one unpublished study has researched metabolomic changes in canine obesity (de Godoy et al., 2015).

## DEVELOPMENT AND TREATMENT OF OBESITY

### Energy Imbalance

The main reason for developing obesity is a positive imbalance between energy intake and energy expenditure (German, 2006). To emphasize this issue, it is estimated that a 4 kg cat consuming just 10 kcal/d in excess can gain nearly 0.5 kg of adipose tissue (energy content = 7920 kcal/kg adipose tissue) or 12% of its BW in just one year (Michel and Scherk, 2012). Many factors are contributors, including genetics, extent of physical

activity, excessive dietary intake, and energy content of the diet (Burkholder, 2001). There are two mechanisms by which body fat may increase with weight gain. Hypertrophy is an increase in the size of the adipocytes, while hyperplasia is an increase in the number of adipocytes. Hyperplastic obesity typically occurs during times of growth and development and is considered more difficult to treat in the future, as the body has limited capacity to decrease adipocyte numbers. Early research in rats has shown that overfeeding during growth leads to hyperplasia and an increase in total body fat during adulthood (Faust et al., 1980). Spalding et al. (2008) used environmental  $^{14}\text{C}$  as a tracer to estimate that the average half-life of an adipocyte is 8.3 years, with 10% of adipocytes renewed annually to replace lost ones, keeping the total number of adipocytes controlled over the long term. This supports the hypothesis that adipocyte cell number is relatively fixed by early adulthood and obesity is attributed to hypertrophy.

### Lifestyle

Domestication has provided pets the benefits of protection from natural predators and proper veterinary care to prevent and treat infectious diseases. As a result, the lifespan of domesticated cats has increased to nearly 15 years, which is dramatically longer than the estimated 4.7 years of a feral cat (Kraft, 1998; Levy et al., 2003). However, many aspects of domestication may be attributed to the etiology of feline obesity. As carnivores in the wild, cats must hunt for frequent, small meals throughout the day. They must be physically fit to survive and reproduce in order to preserve their species. As pets, the majority of cats live indoors and no longer have reproductive purposes. As discussed later in this review, neutering is also an influential result of domestication and predisposes cats to becoming

obese. There is no demand of physical activity for cats to readily and consistently consume a highly palatable and energy-dense diet, which often is fed ad libitum by owners. As society spends more time on leisure activities, so do its pets. This combination of decreased energy expenditure and increased energy intake leads to an energy imbalance.

As a result of the anthropomorphism of pets, the human-animal bond is suggested to be a link to obesity, finding that owners of overweight pets use food to communicate and interact with their pet (Kienzle and Bergler, 2006). Further examples of this bond are that obese dogs were more likely to sleep in their owners' beds (Kienzle et al., 1998) and owners of obese cats were more likely to talk about their cat, feel less lonely because of their cat, and enjoy watching their cat eat (Kienzle and Bergler, 2006). Owners of obese versus lean cats (32 versus 12%) more often stated that they were not as happy before acquiring their cat, and the cat consoled and encouraged them (Kienzle and Bergler, 2006).

Treats are an integral part of the pet-owner bond and are, therefore, more difficult to entirely remove from a weight loss program (Kienzle et al., 2001). A limited amount of treats can be allowed if calculated into the daily caloric allowance. To avoid manifesting the relationship in food, treats, and over-humanization, owners should be encouraged to bond with their cats through physical activity and play. This should decrease excess energy from food and increase energy expenditure, leading to the negative energy balance necessary for weight loss. Physical activity promotes fat loss and may help prevent a drastic decrease in lean tissue during weight loss. A study performed by Butterwick and Markwell (1996) reported that 18 wk of weight loss via controlled caloric restriction resulted in no significant loss of absolute lean body tissue (90.5% lost as body fat, 8.2% lost as lean body



mass, and 1.3% lost as bone mineral mass), but an increase ( $P < 0.001$ ) as a percentage of total body tissue.

Physical activity is more difficult to encourage in cats than in dogs. Methods to increase physical activity in cats utilizes environmental enrichment, including the placement of food and water bowls in various locations around the house, adding climbing towers, window perches, and scratching posts, or using interactive feeders that require the cat to work to receive its meal. Environmental enrichment and weight loss has been briefly studied (Trippany et al., 2003), with reports of cats with environmental enrichment having increased ( $P < 0.05$ ) physical activity and tending to have increased ( $P < 0.10$ ) weight loss. Cats also enjoy toys that promote their natural inclination to stalk and pounce. Interestingly, a cat will submit to predatory behaviors and abandon a highly palatable diet to kill a rat and then leave the kill to finish the diet (Adamec, 1976). This emphasizes that playtime may be an important tool to encourage physical activity and assist weight loss.

Dietary water content may be an alternate method to influence voluntary physical activity. Research by Cameron et al. (2004) used Actical® physical activity monitors to show that cats eating a low energy dense diet containing 40% added water had higher ( $P = 0.03$ ) activity levels than cats fed an energy dense diet (0% hydrated). Deng et al. (2014) also reported that voluntary physical activity tended to increase ( $P = 0.06$ ), especially during the dark period ( $P = 0.007$ ), in cats fed a diet containing 70% additional water content vs. a dry diet containing 12% moisture.

## Physiological Factors

Endogenous factors that present cats with a predisposition to obesity include age, sex, neuter status, and genetics. In efforts to control the feline population, owners are encouraged to spay their cats just before maturity, which is as young as 8 wk of age. The majority of household cats, over 80% in the US, are neutered (Scarlett et al., 1994; Chu et al., 2009). Unfortunately, neutering status is a significant risk factor for obesity. With reported odds ratio (OR) of 3.55-9.3, neutering is associated with a higher odds of becoming obese (Colliard et al., 2009; Cave et al., 2012; Courcier et al., 2012). Over 40% of cats between the ages of 5 and 11, and more than 41% of all neutered male cats, have been reported to be overweight or obese (Russell et al., 2000; Lund et al., 2005). Previous studies have attributed this to a decrease in resting metabolic rate (RMR) after neutering, which resulted in an increased ( $P < 0.05$ ) accumulation of fat tissue (Root, 1995; Fettman et al., 1997; Harper et al., 2001). When comparing RMR using respiratory indirect calorimetry, it can be interpreted that neutered male and female cats require 28 and 33% fewer calories than intact controls, respectively (Root, 1995). Furthermore, voluntary food intake increases ( $P < 0.05$ ) after neutering (Fettman et al., 1997; Martin et al., 2001; Nguyen et al., 2004; Belsito et al., 2009). A failure to recognize this decreased energy need, paired with the tendency to eat more, may easily lead to overweight or obesity.

Male cats have been shown to be at greater risk for developing obesity (Scarlett et al., 1994; Lund et al., 2005; Cave et al., 2012; Courcier et al., 2012; Corbee, 2013). Lund et al. (2005) used a cross-sectional study design to determine that 35% of adult cats seen by US veterinarians were overweight or obese. Of these, neutered (41%) and intact (31.6%) males were more overweight or obese than spayed (32.3%) and intact (25.2%) females.

Research also has shown a high prevalence of obesity in show cats, with 45.5% having a BCS > 5 and 4.5% having a BCS > 7 (Corbee, 2013). These data also reported a higher rate of BCS > 5 in intact (43.6%) and neutered (90.2%) males than in intact (28.7%) and spayed (81.8%) females, supporting the notion that male cats are at a greater risk of obesity. Breed is a less established risk factor in cats than in dogs, but breeds most commonly represented with an overweight or obese body condition are mixed breeds, Manx, Domestic Shorthair (DSH), Domestic Mediumhair (DMH), or Domestic Longhair (DLH) (Lund et al., 2005; Colliard et al., 2009).

### Diet Characteristics

Just as it is a substantial factor in the prevalence of human obesity, highly palatable foods also have a direct influence on feline obesity. Feeding premium and therapeutic (veterinarian) foods has been associated with increased risk of overweight and obesity (Lund et al., 2005). Premium diets are developed for specific breeds and life stages (i.e., growing, lactation, maintenance, performance, etc.) and generally contain highly digestible, high quality ingredients (Case et al., 2010). In laboratories, it has been long proven that rats offered highly palatable diets become obese (Scalafani and Springer, 1976). The same outcome has been observed with high-fat and high-calorie diets (Slattery and Potter, 1985).

High palatability causes both people and animals to eat in excess of need or hunger. Pet food companies use palatability as a major marketing force. Again, with such anthropomorphism of pets, owners select diets that also appeal to their own preferences. Characteristics such as smell, texture, consistency, moisture, and even temperature are methods to influence an inclination to buy a diet. Simple sugars and high fat content are

often used to increase palatability and caloric density of foods. Interestingly, sweet receptors in cats function differently than in other mammals, causing them to show neither attraction nor avoidance of sweet tastes (Carpenter, 1956; Bartoshuk et al., 1975; Beauchamp et al., 1977). Xia et al. (2006) identified two receptor genes in cats, *Tas1r3* and *Tas1r2*, which encode the sweet taste heteromer T1R2/T1R3 in other mammals. It was found that the *Tas1r3* gene is similar to those of dogs, humans, mice, and rats. But, *Tas1r2* contains a microdeletion of 247 base pair in exon 3. This causes a frame shift resulting in premature stop codons, rendering the gene non-functional.

Nutrient composition is an important consideration at any life stage of an animal. As obligate carnivores, cats rely on nutrients in animal tissues to meet their nutritional needs. Therefore, they are best equipped for higher protein metabolism and lower carbohydrate utilization, which is evident through their physiological adaptations (Zoran, 2002). In fact, carnivores are more similar to ruminants in the use of gluconeogenic amino acids to regulate blood glucose (Morris and Rogers, 1982). Cats lack salivary amylase and have low activity of intestinal and pancreatic amylases to metabolize disaccharides (Kienzle, 1993a; 1993b). Glucose that is absorbed must be converted to glucose-6-phosphate before being further metabolized, which is catalyzed by glucokinase and hexokinase. Although cats have hexokinase, they have very minimal levels of hepatic glucokinase that cannot be upregulated in response to a glucose load (i.e., after a meal) (Ballard, 1965; Ureta, 1982). Cats also have low activity of hepatic glycogen synthetase, which converts glucose to glycogen for storage in the liver (Zoran, 2002).

Despite no physiological requirement, carbohydrates are present in most commercial feline diets. Due to the physiological uniqueness of cats, high carbohydrates

have been blamed for obesity in these carnivorous pets. It is possible that the low capacity for glucose disposal increases the amount of carbohydrates for fatty acid synthesis and storage (Zoran, 2002), or that prolonged insulin release causes fatty acids to be stored rather than oxidized (Richard et al., 2004). In healthy cats, those fed a high-carbohydrate diet (25% ME protein; 26% fat; 47% carbohydrate) had a 20-25% higher ( $P < 0.012$ ) postprandial glucose response than cats fed a high-protein (46% protein; 26% fat; 27% carbohydrate) or high-fat (26% protein; 47% fat; 26% carbohydrate) diet, which produced similar results (Farrow et al., 2013). Cats fed a high-fat diet are at a greater risk for increasing body fat, especially after neutering (Nguyen et al., 2004; Backus et al., 2007). For safe and effective weight loss in cats, there are three main dietary goals that include the following: (1) feed a high-protein diet ( $>40\%$  ME,  $>45\%$  DM, or  $>10$  g/100 kcal) to prevent lean muscle tissue loss; (2) feed a reduced energy diet, restricted in fat ( $< 4$  g/100 kcal) and carbohydrates ( $< 3$  g/100 kcal), to stimulate fat mobilization; and (3) monitor and adjust energy intake as needed (Zoran and Rand, 2013).

The minimum protein requirement for cats is 16% of ME, being double that of dogs (NRC, 2006). Cats are less able to adjust to a dramatic decrease in protein intake (Center, 1998) and are at a greater risk to develop HL when protein is restricted (Biourge et al., 1994). Therefore, weight loss diets should contain at least 30% of ME from protein (Case et al., 2010). The thermic effect of protein (20-30%) is higher than both carbohydrates (5-15%) and fat (0-3%), contributing to diet-induced thermogenesis with high-protein diets (Westerterp et al., 1999). Although significant weight loss almost always results in a loss of lean body tissue along with fat (Forbes, 1987), increased dietary protein from 35 to 45% of energy has resulted in more than 10% greater fat loss in overweight cats, with conserved

lean body mass (Laflamme and Hannah, 2008). Other research agrees that high-protein diets are an effective means to maintain lean body mass while increasing fat loss in cats (Nguyen et al., 2004; Vasconcellos et al., 2009; des Courtis et al., 2014).

There are multiple methods to reduce energy density in diets, such as reduced fat and carbohydrates, and increased fiber or water content. However, there are manufacturing challenges with extruded dry kibbles, as low-fat diets tend to require higher carbohydrate content to maintain shape and texture. Although many commercial diets have relatively low dietary fiber concentrations (NRC, 2006), addition of fiber may help alleviate this processing issue and is a major nutritional consideration for weight loss diets. Fiber is not digested by the host and may or may not be fermented by the gut microbiota, providing little dietary energy in comparison to other dietary macronutrients. When replacing fat, caloric density of food is reduced. Support for feeding fiber-supplemented foods comes from data showing that fiber may slow gastrointestinal transit time (Burrows et al., 1982; Fahey et al., 1990), increase fecal volume (Lewis et al., 1994; Wichert et al., 2002), increase gastric emptying (Armburst and Milliken, 2003), increase short-chain fatty acid (SCFA) production (Muir et al., 1996; Bednar et al., 2000; Swanson et al., 2001), and alter insulin sensitivity in peripheral tissues (Nelson et al., 2000).

Crude fiber (CF) is a required value on the guaranteed analysis of a pet food label. Yet CF analysis, developed in 1806, offers an unreliable fiber analysis because it recovers variable and incomplete fractions of cellulose, hemicelluloses, and lignin (Van Soest and McQueen, 1973; de-Oliveira et al., 2012). Fibers used for weight control may differ in solubility and fermentability. Insoluble, non-fermentable fibers increase fecal bulk, decrease transit time, reduce nutrient digestibility, and dilute caloric density. Soluble,

fermentable fibers increase transit time, increase satiety, assist in glycemic control, lower blood cholesterol concentrations, and modify SCFA production to promote gut microbial growth (de Godoy et al., 2013). While little information is known in regards to SCFA production and the physiological implications in the feline colon, research in other species has shown that increased SCFA production may improve colon mucosa metabolism and function.

Nelson et al. (2000) reported diabetic cats fed a canned high-fiber diet containing 12% cellulose (dry matter basis) had a decreased ( $P < 0.001$ ) 12-hr mean serum glucose concentration compared to cats fed a low-fiber diet (1% cellulose, dry matter) containing higher digestible carbohydrate. Mean daily insulin dosage was lower ( $P = 0.05$ ) and mean glycated hemoglobin concentration tended to be lower ( $P = 0.07$ ) in cats fed the 12% cellulose diet. Fischer et al. (2012) examined the effects of diets containing beet pulp (BP; 26% fiber), wheat bran (WB; 24% fiber), and sugarcane fiber (SF; 28% fiber) compared to a control diet (CO; 11.5% fiber). Cats fed the SF-containing diet had the lowest ( $P < 0.05$ ) dietary fiber digestibility and the greatest ( $P < 0.05$ ) reduction of dietary energy digestibility, and the greatest ( $P < 0.05$ ) reduction in postprandial mean glucose concentration. Cats fed the BP-containing diet had improved indices of gut health and the greatest fiber fermentability, having reduced ( $P < 0.05$ ) fecal DM and pH, increased ( $P < 0.05$ ) wet fecal output, and increased ( $P < 0.05$ ) fecal concentrations of acetate, propionate, and lactate.

Increased dietary water content, as in canned foods, increases gut volume and hydration status while decreasing energy density. Wei et al. (2011) reported that cats fed a commercially available canned food (80% moisture) had lower ( $P < 0.05$ ) energy intake

(1,053 ± 274.9 kJ/d) compared with the energy intake of cats fed the same canned food (10% moisture) with water removed via freeze drying (1,413.8 ± 345.8 kJ/d). Cameron et al. (2011) fed cats a 20% hydrated diet at 80% of baseline energy intake for 6 wk of weight loss. Following weight loss, ad libitum feeding of a 0% hydrated diet produced higher ( $P = 0.026$ ) mean body mass regain (330.2 ± 164.3 g) than a 40% hydrated diet (266.6 ± 134.9 g). This was true despite the fact that mean dry matter intake was greater ( $P > 0.001$ ) for the 40% hydrated diet (93.7 ± 19.4 g/d; 1493 ± 306 kJ/d) versus the 0% hydrated diet (86.7 ± 18.4 g /d; 1381 ± 292 kJ/d). These data suggest that body mass regain may be limited by decreasing the caloric density of the diet, with water being a viable option.

Apart from the diet itself, feeding frequency and regimen may have great impact. Ad libitum feeding is a common strategy with cats. Some animals may be able to self-regulate their feed intake to consistently maintain an optimal BW so that ad libitum feeding presents no issues. This is not the case with most cats, however, because those with free access to food are more commonly obese (Kienzle et al., 2001) and have higher BCS than those fed meals (Russell et al, 2000). It is estimated that caloric intake exceeds requirements by 30-40% in cats fed ad libitum (NRC, 2006). Appleton et al. (2001) reported that cats given free access to a highly palatable diet increased their BW by a mean of 44.2% over a ten-month period. A similar issue is uncontrolled allowance of treats and table scraps. Russell et al. (2000) reported that cats receiving treats two to three times a week had a higher ( $P < 0.01$ ) mean BCS (6.18 ± 0.91) than those who received treats less than once a week (5.56 ± 1.11). The social environment during meals also influences intake. Cats in households with four or more cats were further subject to competition for food and



had a higher ( $P = 0.02$ ) body condition than cats in households containing fewer companions ( $6.29 \pm 1.12$  versus  $5.69 \pm 1.03$ ) (Russell et al., 2000).

Rather than a single meal per day, cats on weight loss regimens should be fed multiple small meals throughout the day (German and Martin, 2008; Toll et al., 2010). This may not only reduce feelings of hunger, but may also increase physical activity. Deng et al. (2014) reported that the average daily activity level for cats fed 1 meal per day was lower than cats fed 4 meals per day ( $P = 0.004$ ) and cats fed a random number of meals ( $P = 0.02$ ) per day. The activity level of cats during the dark period was greater in cats fed 1 meal per day compared with cats fed 2 meals ( $P = 0.008$ ) or 4 meals ( $P = 0.007$ ) daily. Cats fed 1 meal per day showed lower ( $P < 0.001$ ) two-hour food anticipatory activity (FAA) before scheduled meal times than the cats fed multiple meals per day.

## MAINTENANCE OF WEIGHT LOSS

### Owner Education

The basis to initiate a successful weight loss intervention is to educate the owner and establish their commitment to help their pet lose weight. Body weight loss results from creating a negative energy balance. Dietary intervention appears to be the foundation to achieve and maintain a healthy weight. Along with this, increased physical activity and behavioral changes are also important. Owners need to understand what an ideal and healthy weight is for their animal and accept that weight loss is necessary. To put this into perspective, an excess of 0.5 kg for a cat is estimated to be similar to an increase of 6.8 kg for an average height (5'4") woman or 7.7 kg for an average height (5'9") man (Michel and Scherk, 2012). The risk of long-term obesity must be discussed along with its associations

with reduced quality of life and shortened lifespan. Weight loss programs require careful attention to the rate in BW loss by frequently weighing the cat and making adjustments as necessary, as well as maintaining routine check-ups with a veterinarian to confirm healthy progress. To develop a successful weight loss program, the owner must consider habits that may have initiated the weight gain. Diet, feeding method and amount, treat allowance, and physical activity are all factors that contribute to weight management. Each plan must be tailored to the specific cat, depending on the starting weight and determined target weight.

### Energy Requirements

Dietary intervention is a necessary and effective component of weight loss programs and is most successful when customized to the individual patient and is closely monitored. While energy is not a nutrient, it is determined by the composition of fat, digestible carbohydrate, and protein in the diet. Energy requirements change throughout the life stage of the animal. Cats have different requirements depending on their age, state of growth and development, maintenance requirements, reproductive stage, and physical activity (NRC, 2006). Multiple exponents and metabolic constants for estimating energy requirements have been suggested. A study by Nguyen et al. (2001) assessed the accuracy of estimating energy requirements of adult cats using metabolic BW. For metabolizable energy intake alone, an exponent of 0.4 was established. Using the double-labeled water method, total energy expenditure reflected an exponent of 0.64. The relationship between resting energy expenditure and BW was expressed by an exponent of 0.65.

Therefore, the cat is believed to have a justified allometric coefficient of 0.67 when lean or 0.40 when overweight (Earle and Smith, 1991; Nguyen et al., 2001). The resting energy requirement (RER) is estimated by the equation  $100 \times BW_{kg}^{0.67}$  for lean cats (BCS=5) or  $130 \times BW_{kg}^{0.40}$  for overweight cats (BCS>5) (NRC, 2006). The RER recommendation by the Association of American Feed Control Officials (AAFCO) is  $70 \times BW_{kg}^{0.75}$  (Toll et al., 2010). As is the focus of this review, a majority of cats are overweight or obese. As straightforward and convenient as these equations may appear, they are only estimations for the entire feline population, with energy requirements of individual cats differing greatly. In fact, the NRC (2006) states that these calculations may over- or underestimate energy needs by more than 50% for an individual cat.

There are different methods for a veterinarian or owner to estimate the caloric requirements for sufficient weight loss in cats. Three common methods include the use of the guaranteed analysis values listed on the product label, calculations based on estimated ideal weight, and calculations based on current food intake (Toll et al., 2010). With a growing market for weight loss diets, many product labels provide feeding guidelines for weight loss. For all methods, it is important to determine an ideal BW. If medical records are available, an ideal body condition and BW may be reported or the BW at one year of age may be a good indicator of ideal weight. If an ideal weight is estimated, it can be used to calculate RER. The RER of a cat at an ideal body condition represents about 70% of their maintenance energy requirement (MER) or daily energy requirement (DER). Therefore, the RER calculated with the ideal weight could be an initial estimate of the calories required for weight loss with about 30% caloric restriction. Another method is based off of the current amount of calories the overweight cat is consuming. This is difficult because many

overweight cats are fed ad libitum and are given additional treats. If an accurate caloric intake can be estimated, feeding 70% of the caloric intake is an appropriate starting point for a weight loss program. This level of restriction is most conservative to prevent a risk of HL (Biourge et al., 1994). However, additional restriction is often necessary. Experimental and clinical trials have used caloric restrictions between 59 and 80% of RER without evidence of HL (Markwell et al., 1996). To safely avoid inducing HL, cats should be eating at least 50% of their RER (Toll et al., 2010).

### Setting Realistic Goals

It is important to emphasize that weight loss must be closely monitored. While this is easy to monitor in an experimental setting, pet owners need to consider how they will track progress. A successful weight loss program may take up to twelve months or longer. Therefore, veterinary check-ups are an integral component to any weight loss program. The recommendation for safe and reasonable weight loss for cats is 1 to 1.5% of BW loss per week (Burkholder and Bauer, 1998). Slow weight loss not only protects that animal from HL, but may better preserve lean body mass and maintain metabolically active tissues (Hoenig et al., 2007).

## Chapter 3

### **Effects of Weight Loss While Feeding a Moderate-Protein, High-Fiber Diet on Body Composition, Voluntary Physical Activity, and Blood Metabolite Profiles in Overweight Cats**

#### ABSTRACT

Obesity is a major nutritional disorder in cats and is associated with several comorbidities and reduced life span. The primary objectives of this study were to determine the effects of feeding a moderate-protein, high-fiber diet on BW loss, voluntary physical activity levels, body composition, and blood metabolite profiles in overweight cats. During a 4-wk baseline period, 8 adult neutered male domestic shorthair cats (mean BW =  $7.7 \pm 0.4$  kg, mean BCS = 7.6/9) were fed to maintain BW. For 18 wk following baseline, food intake was adjusted to allow cats to lose weight at a rate of  $\sim 1.5\%$  BW/wk. Cats were group-housed for 20 h/d and individually housed for two, 2-h periods each day for feedings. Daily food intake, twice-weekly BW, and weekly BCS were recorded throughout the study. Voluntary physical activity was measured over a 7-d period at wk 0, 6, 12, and 18 using Actical® activity monitors. Dual energy X-ray absorptiometry (DEXA) scans were taken at wk 0, 4, 8, 12, and 16 to estimate body composition. Overnight fasted blood samples were collected at wk 0, 1, 2, 4, 8, 12, and 16. The University of Illinois Institutional Animal Care and Use Committee approved all procedures. As expected, mean BW ( $7.7 \pm 0.4$  vs.  $6.2 \pm 0.4$  kg) and mean BCS (7.6 vs. 6.0) decreased ( $P < 0.05$ ) from wk 0 to wk 18. The NRC (2006) maintenance energy requirement (MER) for overweight cats is  $130(BW_{\text{kg}}^{0.40})$ . In comparison, the mean MER during baseline in our study was  $113(BW_{\text{kg}}^{0.40})$ . Throughout wk 1-4, 5-8, and 9-18, the energy levels to sustain weight loss were 76, 64, and 57% of baseline MER, respectively, demonstrating how restrictive feeding must be for consistent

weight loss. . Mean fat mass was decreased ( $P < 0.001$ ) at wk 8, 12, and 16 (2417 , 2097 , and 1810 g, respectively) versus wk 0 (2924 g); therefore, body fat percentage also was decreased ( $P < 0.05$ ) at wk 8, 12, and 16 (36.8, 34.0, and 30.7%, respectively) versus wk 0 (40.9%). Mean lean body mass was lower ( $P < 0.01$ ) at wk 12 and 16 (3671 and 3664 g, respectively) versus wk 0 (3865 g). Importantly, lean body mass percentage was increased ( $P < 0.05$ ) at wk 8, 12, and 16 (61.5, 64.4, and 67.6%) versus wk 0 (57.6%). Mean daily activity tended to be higher ( $P = 0.061$ ) at wk 12 vs. wk 0. The mean light:dark ratio of activity was increased ( $P < 0.05$ ) at wk 18 vs wk 0, 6, and 12. Except for elevated mean creatinine ( $P < 0.05$ ) during weight loss, all blood metabolites remained within reference ranges. Mean triglyceride concentrations were decreased ( $P < 0.05$ ) throughout the weight loss phase. Using non-targeted gas chromatography, liquid chromatography, and mass spectrometry, a total of 535 named biochemicals were identified, with up to 269 metabolites altered ( $P$ - and  $q$ - values  $< 0.05$ ) at any time point. Principal component analysis showed a continual shift in metabolite profile as weight loss progressed. Components 1 and 2 explained 14.3% and 10.3% of the variability, respectively. There was a significant and dramatic reduction of bile acids (cholate; taurocholate; deoxycholate) with weight loss. A reduction in numerous non-esterfied fatty acids (NEFA) and an increase in ketones (acetoacetate; 3-hydroxybutyrate) and monoglycerides suggested a shift toward lipolysis and hepatic NEFA oxidation. Decreased markers of inflammation and oxidative stress were indicated by reduced pro-inflammatory oxylipids, eicosanoids, and oxidized biomarkers following weight loss. Mevalonate was decreased ( $P < 0.05$ ) after wk 8 compared to baseline, which agrees with the reduced bile acids without altering cholesterol. In conclusion, restricted feeding of a moderate-protein, high-fiber diet is a safe

and effective means for weight loss in cats, leading to increased physical activity and reduced blood triglycerides. Global metabolomics identified biomarkers of reduced food intake, weight loss, and/or altered metabolism. Based on our data, the current NRC (2006) MER estimates for cats appear to be too high and should be reconsidered.

## INTRODUCTION

Pets are becoming more integral members of the family, with 68% of US households owning a pet and 37.3% of US households owning a cat in 2014 (APPA, 2014). Unfortunately, there is a continually increasing incidence of obesity in companion animals in the US and obesity is now considered the most common nutritional disorder in pets (German, 2006). Recent data from a 2014 survey conducted by the Association for Pet Obesity Prevention (APOP) demonstrated that 57.9% of US cats, or approximately 55 million, are overweight (29.8%) or obese (28.1%). To further complicate the issue, there is a 'fat gap' inhibiting owners from recognizing to degree by which their pets are overweight (Colliard et al., 2009; Courcier et al., 2012). It has been reported that approximately 45% of owners considered their pet as having a normal BW when the veterinarian assessed them as overweight (Calabash, 2013). A general classification defines an overweight cat as weighing 10-20% over their ideal BW and an obese cat weighing >20% above their ideal BW (Toll et al., 2010). Each unit increase of body condition score (BCS) above ideal (BCS=5) is roughly 10 to 15% over ideal BW (Mawby et al., 2004; Laflamme, 2012).

Feline obesity is associated with comorbidities that have detrimental effects on health. These include, but are not limited to, diabetes mellitus (DM), hepatic lipidosis, urinary tract diseases, hypertension, dystocia, respiratory disorders, neoplasia, feline lower

urinary tract disease (FLUTD), lameness, dermatosis, and decreased life span (Scarlett and Donoghue, 1998; Lund et al., 2005; German, 2006; Brooks et al., 2014). The traditional development of obesity is due to a positive imbalance between energy intake and energy expenditure (German, 2006). Aspects of domestication and humanization of pets also contribute to obesity. These risk factors include neutering (Colliard et al., 2009; Cave et al., 2012; Courcier et al., 2012), decreased physical activity, increased food intake, and access to highly palatable high-fat and/or high-carbohydrate diets (Nguyen et al., 2004; Backus et al., 2007; Farrow et al., 2013).

While obesity prevention would ideally avoid these conditions, it is necessary to develop effective and safe obesity treatment methods. The recommendation for safe and reasonable weight loss for cats is 1 to 1.5% of BW loss per week (Burkholder and Bauer, 1998). To safely avoid inducing hepatic lipidosis during weight loss, cats should eat at least 50% of their maintenance energy requirement (MER) (Toll et al., 2010). Previous experimental and clinical trials have used caloric restrictions between 59 and 80% of MER without evidence of hepatic lipidosis (Markwell et al., 1996). Therefore, the objective of this study was to determine the effects of weight loss while feeding a moderate-protein, high-fiber diet on body composition, voluntary physical activity, and blood metabolite profiles in overweight cats.

## MATERIALS AND METHODS

### Animals and Diet

Eight neutered adult male domestic shorthair cats (mean BW =  $7.7 \pm 0.42$  kg; mean BCS =  $7.6 \pm 0.38$  on a 9-point scale (Laflamme et al., 1997) were used. Mean age at the start



of the study was  $7.78 \pm 0.03$  years. Cats were housed in a temperature- ( $20^{\circ}\text{C}$ ) and light-controlled (16 h light: 8 h dark cycle) room at the University of Illinois. Cats were individually housed in stainless steel cages (0.61m x 0.61m x 0.61m) for two, 2-h periods each day during feedings to allow for individual food intake records. During the other 20 h/d, cats were group housed and allowed to socialize with one another and exercise outside of their cages in the room (2.84m x 5.97m). All animal procedures were approved by the University of Illinois Animal Care and Use Committee (IACUC) prior to animal experimentation. All cats were fed a dry commercial diet (NATURAL CHOICE® WEIGHT LOSS Adult Cat Chicken & Whole Brown Rice Formula; The Nutro Company, Franklin, TN) throughout the duration of the study, which is formulated to meet nutrient recommendations for adult domestic cats (AAFCO, 2013). Protein sources in this diet include chicken, chicken meal, pea protein, potato protein, and salmon meal. The high fiber content of this diet is due to ingredients such as whole brown rice, dehydrated alfalfa meal, oat fiber, dried plain beet pulp, and flaxseed. Water was available *ad libitum* at all times.

### Experimental Design

This experiment was performed as a repeated measures design. The first 4 wk of the study represented the baseline period, when all cats were fed the same diet in quantities to maintain their starting BW. After baseline (wk 0), BW was measured twice a week and food intake was adjusted to target weight loss at approximately 1.5% BW/wk. Because this colony of cats has previously been fed to maintain a healthy weight and BCS, appropriate estimation of ideal BW and ME to maintain BW was known. Cats were fed individually twice a day from 8:30 am-10:30 am and 3:00 pm-5:00 pm in their assigned cages. Any

uneaten food was weighed and recorded at the end of the feeding period. Daily food intake, twice-weekly BW, and weekly BCS were recorded throughout the study. The following outcome variables were analyzed: BW, voluntary physical activity, fasted blood samples for global metabolite profiling, and body composition analysis via DEXA.

## SAMPLE COLLECTION AND ANALYSIS

### Diet

A subsample of diet was collected weekly and stored at 4°C until further analysis (Table 3.1). Sub-samples were composited and ground through a 2-mm screen with dry ice using a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) in preparation for chemical analysis. The diet was analyzed for dry matter (DM) and organic matter (OM) according to AOAC (2006, Methods 934.01 and 942.05); fat concentration by acid hydrolysis according to AACC (1983) followed by ether extraction according to Budde (1952) and AOAC (2006, Method 922.06); crude protein (CP) according to AOAC (2006, Method 992.15) using a Leco Nitrogen/Protein Determinator (model FP-2000, Leco Corporation, St. Joseph, MI); gross energy (GE) by bomb calorimeter (Model 1261, Parr Instruments Co., Moline, IL); and total dietary fiber (TDF) content according to Prosky et al. (1992) and AOAC (2006, Method 985.29).

### Voluntary Physical Activity

Voluntary physical activity was measured at wk 0, 6, 12, and 18 by use of Actical® Physical Activity Monitors (Mini Mitter Co., Bend, OR), which were worn on a collar around the neck for a 7-d measurement period at each time point. Human interference was limited

to the feeding schedule only to ensure the physical activity was voluntary. To summarize, the monitors contain an omnidirectional sensor that integrates the amplitude and frequency of motion and produces an electrical current that varies in magnitude. As intensity of motion increases, voltage also increases. Once removed, Actical® software analyzed the data compiled by the collar and converted them into arbitrary numbers referred to as activity counts. In this study, average activity was represented as activity counts per epoch (one epoch length = 15 sec, or 0.25 min). Actical® software reported average activity counts per epoch during the entire day (daily), light cycle (7:00 am-11:00 pm), and dark cycle (11:00 pm-7:00 am). The light:dark ratio of activity counts were also analyzed.

#### Body Composition Analysis

DEXA scans were taken at wk 0, 4, 8, 12, and 16 to estimate body composition. This technique has been validated for use in dogs and cats (Speakman et al., 2001) and is used in a variety of clinical and research applications at the University of Illinois Veterinary Teaching Hospital. Prior to the DEXA scans, cats were sedated by an intramuscular injection of a cocktail of butorphanol (0.3 mg/kg BW), dexmedetomidine (0.02 mg/kg BW), and atropine (0.04 mg/kg BW). This caused immobilization and sedation while scans were performed. The reversal to sedation was an intramuscular injection of atipamezole at 0.2 mg/kg BW, which was ten times the dose of dexmedetomidine. To perform the scan, cats were placed in ventral recumbency and body composition was analyzed using a Hologic model QDR-4500 Fan Beam x-ray Bone Densitometer and software (Hologic Inc., Waltham, MA). The four legs, trunk, and head of each cat was scanned and analyzed individually.

Measurements of fat content, lean non-bone tissue, and bone mineral content were taken in each body region. Body fat and lean tissue percentage also were calculated for each region and for the entire body. Cats were monitored until fully recovered from anesthesia.

### Serum Chemistry and Global Metabolite Profiles

Overnight fasted (at least 12 h) blood samples (5 mL) were collected via radial, femoral, or jugular venipuncture at wk 0, 1, 2, 4, 8, 12, and 16. Animals were restrained, but sedation was not necessary because procedures were familiar to the cats and stress was minimal. Blood was collected into BD Vacutainer serum separator tubes (Becton, Dickinson, and Company, Franklin Lakes, NJ) and allowed to clot at room temperature. All tubes were centrifuged at  $13,000 \times g$  for 15 min at 4°C. The supernatant (serum) then was pipetted into cryogenic vials. Samples were stored at -80°C until further analysis. Serum chemistry profiles were analyzed at the University of Illinois Clinical Diagnostic Laboratory, (Urbana).

Serum was analyzed by Metabolon (Metabolon, Inc., Durham, NC) to evaluate changes in global metabolite profiles and to identify markers of weight loss. Samples were shipped on dry ice and immediately stored at -80°C upon arrival. Each sample was inventoried into the Metabolon Laboratory Information Management System (LIMS) system and assigned a unique identifier to track all handling, tasks, and results. Samples were prepared using the automated MicroLab STAR® system (Hamilton Company, Salt Lake City, UT). For quality control purposes, a recovery standard was added prior to the first step of the extraction process. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse

metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into fractions for analysis by liquid chromatography-mass spectroscopy (LC-MS) and gas chromatography-mass spectroscopy (GC-MS), and a fraction was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. For LC, the samples were stored overnight under nitrogen before preparation for analysis. For GC, each sample was dried under vacuum overnight before preparation for analysis.

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS): The LC/MS portion of the platform was based on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a high resolution/accurate mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). The sample extract was dried, then reconstituted, in acidic or basic LC-compatible solvents. One aliquot was analyzed using acidic positive ion optimized conditions and the other using basic negative ion optimized conditions in two independent injections using separate dedicated columns (Waters UPLC BEH C18-2.1x100 mm, 1.7  $\mu$ m). Extracts reconstituted in acidic conditions were gradient eluted from a C18 column using water and methanol containing 0.1% formic acid. The basic extracts were similarly eluted from C18 using methanol and water, however with 6.5 mM ammonium bicarbonate. The third aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7  $\mu$ m) using a gradient consisting of water and acetonitrile with 10 mM ammonium formate. The MS analysis alternated between MS and data-dependent MS-MS scans using dynamic exclusion (scan range = 80-1000  $m/z$ ).

Gas Chromatography-Mass Spectroscopy (GC-MS): The samples destined for analysis by GC-MS were dried under vacuum for a minimum of 18 h prior to being derivatized under dried nitrogen using bistrimethyl-silyltrifluoroacetamide (BSTFA). Derivatized samples were separated on a 5% diphenyl/95% dimethyl polysiloxane fused silica column (20 m x 0.18 mm ID; 0.18  $\mu$ m film thickness) with helium as carrier gas and a temperature ramp from 60° to 340°C in a 17.5 min period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization (EI) and operated at unit mass resolving power (scan range = 50–750  $m/z$ ).

Quality Control (QC): Three types of controls were analyzed in concert with the experimental samples: a pooled matrix sample generated from a small volume of each sample served as a technical replicate throughout the data set; extracted water samples served as process blanks; and a cocktail of QC standards spiked into every analyzed sample for instrument performance monitoring and chromatographic alignment. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers. The RSD for this study was 4%. Overall process variability for this study was 11% and was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled matrix samples. Experimental samples were randomized across the platform run with QC samples spaced evenly among the injections.

Metabolite Identification: Raw data were extracted, peak-identified, and QC processed with Metabolon's hardware and software. Metabolites were identified by

comparison to library entries of purified standards or recurrent unknown entities. Identification of known chemical entities was based on comparison to Metabolon's reference library entries of purified standards. Biochemical identifications were based on three criteria: retention index within a narrow RI window of the proposed identification, accurate mass match to the library  $\pm 0.005$  amu, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. Currently, more than 3,300 commercially available purified standard compounds have been acquired and registered into Metabolon LIMS for distribution to both the LC and GC platforms for the determination of their analytical characteristics. Additional mass spectral entries have been created for structurally unnamed biochemicals, which have been identified by virtue of their recurrent nature (both chromatographic and mass spectral).

**Data Curation and Normalization:** Curation procedures using Metabolon proprietary visualization and interpretation software were carried out to ensure that a high quality data set was made available for statistical analysis and data interpretation. The QC and curation processes were designed to ensure accurate and consistent identification of true chemical entities, and to remove those representing system artifacts, mis-assignments, and background noise. Library matches for each compound were checked for each sample and corrected if necessary. Peaks were quantified using area-under-the-curve. For studies spanning multiple days, a data normalization step was performed to correct variation resulting from instrument inter-day tuning differences. Each compound was corrected in run-day blocks by registering the medians to equal one (1.00) and normalizing each data

point proportionately (termed the “block correction”). For studies that did not require more than one day of analysis, no normalization was necessary, other than for purposes of data visualization.

### Statistical Analysis

All data other than those from Metabolon were analyzed using the Mixed Models procedure of SAS® (SAS Institute, Cary, NC). The experimental design consisted of a single factor (week) experiment with repeated measures, with cat and week as fixed effects. Differences among treatments were determined using a Fisher-protected LSD with a Tukey adjustment to control for experiment-wise error. A probability of  $P \leq 0.05$  was accepted as statistically significant, while  $P \leq 0.10$  was considered to be a trend.

To evaluate changes in global metabolic profiles due to weight loss, a heat map was made using 535 normalized known metabolites. Hierarchical clustering was used to show large-scale differences in metabolic patterns, and determination of distinct clusters was done using Array Studio with complete linkage and distance correlation settings. Principal component analysis (PCA) was performed using all named metabolites to provide a simultaneous comparison of metabolic alterations that accompanied weight loss. Random forest (RF) analyses were performed to provide an estimate of how well individuals may be classified in the dataset. For a given decision tree, a random subset of data are selected to build a tree (“bootstrap sample”), and the remaining data, the “out-of-bag” (OOB) variables, are passed through the tree to obtain a class prediction for each sample. After the process is repeated thousands of times, a forest is produced. The final classification of each sample is determined by computing the class prediction frequency for the OOB variables over the



whole forest; therefore, the OOB error rate is a measure of prediction accuracy. A total of 21 comparisons were made over time (0, 1, 2, 4, 8, 12, or 16 wk), with two groups being compared at a time. To determine which variables (metabolites) made the largest contribution to the classification, the mean decrease accuracy (MDA) was determined by randomly permuting a variable, running the observed values through the trees, and then reassessing the prediction accuracy. If a variable was important to the classification, the prediction accuracy dropped after such a permutation. Thus, the RF analysis provided an importance rank ordering of metabolites. The top 30 metabolites were reported for each comparison.

One-way ANOVA with repeated measures identified metabolites that changed with weight loss. An estimate of a false discovery rate (q-value) was calculated to take into account multiple comparisons. A combination of P- and q-value  $\leq 0.05$  was used to declare statistical significance. Statistical analyses were performed using the program “R” (<http://cran.r-project.org/>) and JMP (SAS Inst. Inc., Cary, NC: <http://www.jmp.com>).

## RESULTS

### Food Intake

During the 4 wk baseline period, there was no change in BW, BCS, or food intake. Mean baseline food intake was 79.5 g/d (255.3 kcal ME/d). At wk 1, weight loss was initiated by reducing mean baseline caloric intake by 20%. Food intake was lower ( $P < 0.0001$ ) at all wk vs. baseline. Food intake was not different from wk 8 to 18 (47.7 to 44.7 g/d; 153.2 to 143.7 kcal ME/d), but cats continued to lose weight. These results are summarized in Table 3.2.

### Body Weight and Composition

All cats lost weight from caloric restriction. Mean BW (7.7 versus 6.2 kg) and mean BCS (7.6 versus 6.0) decreased ( $P < 0.0001$ ) from wk 0 to wk 16. Mean fat mass was decreased ( $P < 0.001$ ) at wk 8, 12, and 16 (2417 , 2097 , and 1810 g, respectively) versus wk 0 (2924 g); therefore, body fat percentage also was decreased ( $P < 0.05$ ) at wk 8, 12, and 16 (36.8, 34.0, 30.7, respectively) versus wk 0 (40.9%). Mean lean body mass was lower ( $P < 0.01$ ) at wk 12 and 16 (3671 and 3664 g, respectively) versus wk 0 (3865 g). Importantly, lean body mass percentage was increased ( $P < 0.05$ ) at wk 8, 12, and 16 (61.5, 64.4, and 67.6%) versus wk 0 (57.6%). Mean bone mineral content was decreased ( $P < 0.05$ ) at wk 12 and 16 (92.7 and 92.4 g, respectively) versus wk 0 (108.2 g). These results are summarized in Table 3.3.

### Voluntary Physical Activity

Mean daily voluntary physical activity levels tended to be higher ( $P = 0.061$ ) at wk 18 vs. wk 0 (14.15 versus 10.73), but not at other time points. Although mean light activity numerically increased with weight loss, there were no significant changes in mean light or dark hours of activity. The mean ratio of light:dark activity was greater ( $P < 0.05$ ) at wk 18 than at wk 0, 6, and 12. These results are summarized in Table 3.4.

### Serum Chemistry

With the exception of elevated ( $P < 0.0001$ ) creatinine concentrations from wk 1 to wk 16 versus wk 0, all serum chemistry results remained within their respective reference ranges throughout the duration of the study, as determined by University of Illinois Clinical

Diagnostic Laboratory (Urbana, IL). Creatinine was increased ( $P < 0.05$ ) at all wk versus baseline (1.59 mg/dL), with wk 12 and 16 (1.94 and 1.91 mg/dL) being higher ( $P < 0.05$ ) than all other wk. Total protein temporarily decreased ( $P < 0.05$ ) at wk 1, 2, and 4 (7.01, 7.04, and 7.05 g/dL, respectively) versus wk 0 (7.33 g/dL), and then numerically increased at wk 8, 12, and 16 so that they were no longer different than baseline. Compared to baseline (22.0 mg/dL), blood urea nitrogen (BUN) was higher ( $P < 0.05$ ) at wk 1 (23.6 mg/dL), but then lower ( $P < 0.05$ ) at wk 8, 12, and 16 (19.4, 18.8, and 19.4 mg/dL). Cholesterol was only lower ( $P < 0.05$ ) at wk 8 and 12 (112.9 and 113.0 mg/dL, respectively) versus wk 0 (125.6 mg/dL). Interestingly, mean triglyceride concentrations were decreased ( $P < 0.001$ ) at all wk versus baseline (56.0 mg/dL) and were lowest at wk 12 (36.4 mg/dL). Globulin was lower ( $P < 0.05$ ) at wk 1, 2, 4, and 12 (3.8, 3.8, 3.7, and 3.8 g/dL, respectively) versus wk 0 (4.1 g/dL). The albumin/globulin ratio was higher ( $P < 0.05$ ) at wk 4 versus all other wk. Compared to baseline (9.5 mg/dL), calcium was higher ( $P < 0.05$ ) at wk 2 (9.7 mg/dL) and lower ( $P < 0.05$ ) at wk 8 (9.3 mg/dL). Phosphorus was lower ( $P < 0.05$ ) at wk 12 and 16 (4.4 and 4.4 mg/dL, respectively) versus wk 0 (4.7 mg/dL). Potassium was lower ( $P < 0.05$ ) at wk 1, 8, 12, and 16 (4.9, 4.9, 4.9, and 4.9 mmol/L, respectively) versus wk 0 (5.2 mmol/L). The sodium/potassium ratio was higher ( $P < 0.05$ ) at wk 1, 8, and 16 versus baseline. Total alkaline phosphatase was higher ( $P < 0.05$ ) at wk 4, 8, 12, and 16 (26.9, 27.9, 27.8, and 30.9 U/L, respectively) versus wk 0 (21.0 U/L). Total bilirubin was lower ( $P < 0.05$ ) at wk 4, 8, and 16 (0.10, 0.10, and 0.10 mg/dL, respectively) versus wk 1 (0.15 mg/dL). Bicarbonate was only higher ( $P < 0.05$ ) at wk 8 vs. baseline (18.5 vs. 17.0 mmol/L). Anion gap values were lower at wk 12 (18.4) versus wk 0, 1, 2, and 16 (20.1, 20.3, 20.4, and 20.1, respectively). There were no changes ( $P > 0.05$ ) in

serum glucose, albumin, sodium, chloride, or alanine amino transferase (ALT). These results are summarized in Table 3.5.

### Global Blood Metabolites

A total of 535 named biochemicals were identified, with up to 269 metabolites altered (P- and q- values < 0.05) at any time point. PCA (Figure 3.1) showed a continual shift in metabolite profile as weight loss progressed. Components 1 and 2 explained 14.3% and 10.3% of the variability, respectively. Although distinct clusters did not form, a possible biphasic relationship may distinguish an early (wk 1 – 4) and late (wk 8 – 16) response to weight loss. Broadly, wk 1 and 2 appeared similar to baseline, wk 8, 12, and 16 were more differentiated than baseline, and wk 4 was more variable. Random forest analysis was performed to rank the top 30 metabolites by order of influence on predictive accuracy (Figures 3.2 – 3.7). Individual comparisons made for wk 0 versus the other time points (i.e., wk 1, 2, 4, 8, 12, and 16) are presented. The metabolites N-acetylglycine (AA metabolism) and choline phosphate (lipid metabolism) reported consistently high MDA values at all wk versus 0. To connect the possibly biphasic relationship, certain metabolites were consistently reported throughout wk 1 – 4 versus wk 0 and wk 8 – 16 versus wk 0. Metabolites with the highest MDA in early weight loss (wk 1 – 4 vs. 0) were typically of lipid (propionylcarnitine and phosphoethanolamine) metabolism. Other metabolites with consistently high MDA values were 2-hydroxy-3-methylvalerate (AA metabolism), ethyl glucuronide (xenobiotic metabolism), lactate (carbohydrate metabolism), and uracil (nucleotide metabolism). Late weight loss (wk 8 – 16 versus wk 0) resulted in metabolites with a high MDA from lipid (10-undecenoate [11:1n1]), AA (ophthalmate and 1-

methylhistidine), and xenobiotic (thymol sulfate and 2-hydroxyisobutyrate) metabolism. Although the only metabolite of peptide metabolism, gamma-glutamyl-2-aminobutyrate, had a consistently high MDA in wk 8 – 16 versus wk 0. All of the following discussed changes were considered to be significant with a combined P- and q- value  $\leq 0.05$ .

Lipid Metabolism: 144 of the metabolites altered with weight loss were related to lipid metabolism (Table 3.6). Metabolites of inositol (myo-inositol, scyllo-inositol, and inositol –phosphate) and phospholipid metabolism (choline phosphate, phosphoethanolamine, and glycerophosphoethanolamine) immediately decreased by wk 1 of weight loss and remained lower at all wk versus wk 0. Monoacylglycerols, mainly 2-palmitoylglycerol (wk 1 fold change = 3.05), were increased at wk 1 of weight loss and remained increased throughout weight loss. Long chain fatty acids (FA) and polyunsaturated FA (PUFA) were decreased at wk 8, 12, and 16 versus wk 0. The medium-chain FA 10-undecanoate (11: 1n1) was increased at wk 1 (fold change = 1.11) and remained increased at all wk versus wk 0. Markers of primary bile acid metabolism, cholate and taurocholate, dramatically and immediately decreased with weight loss, with a fold change of 0.03 and 0.14, respectively, at wk 1. Markers of secondary bile acid metabolism, deoxycholate and ursodeoxycholate, also decreased with weight loss. Markers of glycerolipid metabolism, glycerol 3-phosphate (G3P) and glycerophosphoglycerol, decreased at wk 1 versus wk 0 (fold change = 0.59 and 0.72, respectively). Monohydroxy FA (i.e., 3-hydroxysebacate, 5-hydroxydextanoate, and 13-HODE + 9-HODE) and dihydroxy FA (12, 13-DiHOME and 9, 10-DiHOME) decreased with weight loss. Some lysolipids decreased with weight loss, with many changes occurring at wk 4 or later. Many dicarboxylate FA (i.e., 2-hydroxyadipate, azelate, and 1,11-undecanedicarboxylate)

decreased with weight loss. While markers of BCAA metabolism (butyrylcarnitine and propionylcarnitine) decreased, those of acyl glycine and acyl carnitine metabolism increased throughout weight loss. The ketone bodies acetoacetate and 3-hydroxybutyrate also increased throughout the weight loss period.

**Amino Acid (AA) and Peptide Metabolism:** Of the altered metabolites, 100 were related to AA metabolism and 14 to peptide metabolism (Table 3.7). The sub-pathways of AA metabolism had variable results, containing metabolites that both increased and decreased with weight loss. Most metabolites of lysine metabolism (i.e., N-6-trimethyllysine, glutaryl carnitine, and 3-methylglutaryl carnitine) were increased at wk 1 or 2 versus wk 0 and remained increased. Conversely, glutarate was decreased at wk 1 versus wk 0 (fold change = 0.75) and remained decreased at all wk versus wk 0. Metabolites of glycine, serine, and threonine metabolism had differing results, with wk 1 versus wk 0 being increased for N-acetylglycine (fold change = 1.37) and decreased for sarcosine (fold change = 0.62). Glutamate was lower at wk 1, 2 and 16 versus wk 0 (fold change = 0.71, 0.73, and 0.67, respectively), while glutamine was only higher at wk 12 versus wk 0 (fold change = 1.19). 1-methylhistidine was increased by wk 1 versus wk 0 (fold change = 1.2) and remained increased at all wk versus baseline. Most metabolites of phenylalanine and tyrosine metabolism were decreased with weight loss, with N-acetylphenylalanine being decreased at wk 1 versus wk 0 (fold change = 0.87), o-cresol sulfate was decreased at wk 2 versus wk 0 (fold change = 0.49), and others were decreased from wk 4 versus wk 0. Metabolites of BCAA (leucine, isoleucine, and valine) metabolism were inconsistently changed by weight loss, with 2-hydroxy-3-methylvalerate being the only metabolite to decrease at wk 1 versus wk 0 (fold change = 0.57) and remain decreased

at all wk. Metabolites of methionine, cysteine, S-adenosylmethionine (SAM), and taurine metabolism were mostly decreased, with methionine sulfoxide, S-adenosylhomocysteine (SAH), taurine, and hypotaurine all being decreased by wk 1 versus wk 0 (fold change = 0.73, 0.63, 0.31, and 0.72, respectively) and remaining lower throughout weight loss. Urea cycle metabolites such as urea and citrulline were decreased at wk 8, 12, and 16 versus wk 0, while pro-hydroxy-pro was increased at wk 2, 4, 8, 12, and 16 versus wk 0. Markers of creatine metabolism gave opposing results, with both creatine and creatine phosphate being decreased at wk 1 and 2 versus baseline, and creatinine and its precursor guanidinoacetate being decreased from wk 1 to 4 versus baseline, respectively. Ophthalmate, a metabolite of glutathione metabolism, was doubled at wk 4 (fold change = 2.02) and remained increased with weight loss. In general, most peptide-related metabolites were increased with weight loss. Of note are gamma-glutamylisoleucine, gamma-glutamyl-2-aminobutyrate, N-acetylcarnosine, and prolylglycine, which were increased at wk 1 versus wk 0 (fold change = 1.23, 1.41, 1.24, and 1.27 respectively) and remained increased at all weeks.

**Carbohydrate and Energy Metabolism:** There were 9 and 7 metabolites related to carbohydrate and energy metabolism, respectively, that were altered by weight loss (Table 3.8). Lactate and glycerate were decreased at wk 1 (fold change = 0.68 and 0.88, respectively) and remained low with weight loss, while fructose and mannose were increased at wk 1 (fold change = 1.15 and 1.27, respectively) and remained higher with weight loss. Citrate was only higher at wk 16 versus wk 0 (fold change = 1.09) and alpha-ketoglutarate was only lower at wk 2 versus wk 0 (fold change = 0.81). Fumarate and phosphate were lower at all weeks versus wk 0.

Nucleotide, Xenobiotic, and Cofactor and Vitamin Metabolism: The remaining altered metabolites were related to nucleotide (26 metabolites), xenobiotic (38 metabolites), and cofactor and vitamin (19 metabolites) metabolism (Table 3.9). Markers of xanthine- orinosine-containing purine metabolism were decreased, with xanthine, 2'-deoxyinosine, and urate quickly decreasing by wk 1 versus wk 0 (fold change = 0.48, 0.46, and 0.74, respectively). Some markers of pyrimidine metabolism such as uracil, 2'-deoxyuridine, and cytidine were decreased, while others such as orotate were increased. Most xenobiotics decreased with weight loss. Markers of benzoate metabolism such as 4-ethylphenylsulfate and 4-vinylphenol, were lower at wk 1 versus wk 0 (fold change = 0.41 and 0.49) and continued to decrease with weight loss. Similar results were observed in xenobiotics related to food and plant components (i.e., ergothioneine and pyrrolaline), drugs (i.e., 4-acetylphenol sulfate and hydroquinone sulfate), and chemicals (i.e., O-sulfo-L-tyrosine and ethyl glucuronide), with all being decreased with weight loss. Conversely, the chemical related xenobiotic 2-hydroxyisobutyrate increased with weight loss (fold change = 1.87 at wk 16 versus wk 0).

## DISCUSSION

Obesity is the most common nutritional disorder in cats, with reports of 57.9% of US cats being overweight or obese (APOF, 2014). Clearly, efforts to prevent and treat obesity have become a challenge for veterinarians and owners, making this issue a major area of research. A successful weight loss program can correct malnutrition, reduce prevalence or risk of disease, and improve livelihood. This success weighs heavily on diet selection as



well as owner compliance to carefully track weight and modify food intake allowance, while attempting to increase physical activity.

The first step to a weight loss program is identifying the body condition of the cat. On the 9-point BCS system, each unit increase above ideal (BCS=5) is roughly 10 to 15% over ideal BW (Mawby et al., 2004; Laflamme, 2012). A general classification defines an overweight cat as weighing 10-20% over their ideal BW and an obese cat weighing >20% above their ideal BW (Toll et al., 2010). Therefore, the mean baseline BCS of  $7.6 \pm 0.38$  and body fat percentage of  $40.9 \pm 4.03\%$  for cats in this study both indicate obesity. There was no change in BW or food intake during the 4 wk baseline period (mean BW =  $7.7 \pm 0.42$  kg; mean food intake =  $79.5 \pm 0.9$  g/d). Weight records from April 2013 were used to determine the appropriate ideal target weight for each cat (mean target BW = 4.60 kg). At that weight, mean body fat percentage was 10.72% and mean lean tissue percentage was 87.10%.

As expected, caloric restriction of this diet was a successful means for weight loss. Cats were weighed twice weekly and individual food intake was adjusted to target weight loss at approximately 1.5% BW/wk. Mean baseline intake was  $235.4 \pm 2.74$  kcal ME/d or  $79.5 \pm 0.93$  g/d. Initial caloric intake at wk 1 was established to be a 20% reduction of baseline intake. Additional caloric restriction was required to maintain weight loss. Food intake at wk 5 ( $53.42 \pm 1.37$  g/d) was lower ( $P < 0.0001$ ) than wk 1, with calories being restricted by 33% of baseline. Additionally, food intake at wk 10 ( $47.45 \pm 1.37$  g/d) was lower ( $P = 0.045$ ) than wk 5, with calories being restricted by 40% of baseline. Food intake was not different from wk 8 to 18 (47.7 to 44.7 g/d), but cats continued to lose weight with mean caloric intake being restricted by 42% of baseline. Compared to baseline, wk 18 BW

was a  $19.5 \pm 3.35\%$  reduction (final mean BW =  $6.2 \pm 0.4$  kg, mean BCS = 6/9), with average weekly weight loss at  $1.26 \pm 0.16\%$ . These results are comparable to those from a study conducted by Butterwick and Markwell (1996), where cats lost  $18.1 \pm 3.53\%$  of their starting BW over 18 wk of caloric restriction.

Maintenance of lean body tissue is an essential goal of safe weight loss, as it is directly related to total energy expenditure (Nguyen et al., 2001) and may prevent weight regain, and is an important reservoir to support protein turnover (Sève, 1997). Yet some lean body tissue loss is almost always a result of significant weight loss, however, and may even be considered physiologically necessary to maintain a ratio of lean tissue to fat mass (Forbes, 1987). Our data agree with previous research (Forbes, 1987; Butterwick and Markwell, 1996) that those with higher initial fat content have a higher proportion of weight loss from fat, while other data positively correlate lean tissue loss with overall weight loss (German et al., 2008). Butterwick and Markwell (1996) reported that after 18 wk of caloric restriction, 90.5% of the weight loss was from fat, 8.2% from lean tissue, and 1.3% from bone mineral content via DEXA results. A case of weight loss in client-owned cats reported that 86% of the weight loss was from fat, 13% from lean tissue, and 0.9% from bone mineral content over an average of 40 wk (German et al., 2008). Those data are very similar to the DEXA results of the current study where weight loss by wk 16 was 84.03% from fat, 14.75% from lean tissue, and 1.21% from bone mineral content. The current study also confirms the notion that weight loss in a research setting (Butterwick and Markwell, 1996; Laflamme and Hannah, 2005) often is accomplished at a faster rate than that in a clinical setting (German et al., 2008). This is likely due to owner non-compliance with the dietary plan.

Ideally, weight gain is prevented and a healthy weight is maintained. The cats in this study were fed to maintain their BW for 4 wk prior to weight loss. During this 4 wk baseline period, the average energy intake was 255.3 kcal ME/d. The NRC (2006) MER for overweight cats is estimated to be determined using the following equation:  $130 \times BW_{\text{kg}}^{0.40}$ . The average weight at baseline was 7.7 kg. Therefore, the NRC (2006) equation estimated that the baseline MER would have been 293.9 kcal ME/d, which is a 14.1% overestimation of the food intake required to maintain BW in our colony of cats. While an additional 38.4 kcal ME/d may not seem like a significant number of calories, it is estimated that a 4 kg cat consuming 10 kcal/day in excess can gain nearly 0.5 kg of adipose tissues (energy content = 7920 kcal/kg adipose tissue) or 12% of its BW in one year (Michel and Scherk, 2012). Using mean food intake and metabolic BW ( $\text{kg}^{0.40}$ ), the MER during baseline in this study was calculated and represented by the following equation:  $113 \times BW_{\text{kg}}^{0.40}$ . To initiate weight loss, cats were fed 80% of their baseline MER, which was 203.0 kcal ME/d. In comparison, this is only 69% of the NRC (2006) MER calculation. Throughout wk 1-4, 5-8, and 9-18, the energy content needed to sustain weight loss was 76, 64, and 57% of MER =  $113 \times BW_{\text{kg}}^{0.40}$ , respectively. If calculated from the NRC (2006) estimate, energy content was 66, 56, and 50% of MER =  $130 \times BW_{\text{kg}}^{0.40}$ , respectively. These data suggest that the NRC (2006) equation overestimates energy needs. Therefore, the NRC (2006) MER equation should be reconsidered to better prevent the overconsumption of calories and accurately calculate needs for a successful weight loss program.

A combination of decreased energy expenditure and increased energy intake leads to an energy imbalance that promotes weight gain. Physical activity can be difficult to encourage in cats, but when successful can help promote fat loss and may prevent a drastic

decrease in lean tissue during weight loss. Environmental enrichment such as climbing towers, window perches, or scratching posts have been shown to be effective in increasing ( $P < 0.05$ ) physical activity (Trippany et al., 2003). Rather than feeding 1 meal per day, voluntary physical activity was increased when cats were fed 4 meals ( $P = 0.004$ ) or a random number of meals ( $P = 0.02$ ) per day (Deng et al., 2014). The cats in the current study had access to environmental enrichment such as scratching posts and towers and were fed twice daily. Daily voluntary physical activity tended ( $P = 0.061$ ) to increase at wk 12 versus wk 0, while the ratio of light:dark voluntary physical activity was increased ( $P = 0.008$ ) by wk 18 versus wk 0. Physical activity during the light cycle numerically increased over time with weight loss, but no time points were statistically significant from baseline. Rather than examining how physical activity promotes weight loss, we conclude that weight loss promotes voluntary physical activity, which thereby assists in achieving the negative energy balance necessary to sustain weight loss.

Creatinine was the only serum metabolite that was elevated above the reference range (0.4 – 1.6 mg/dL), as determined by the University of Illinois Clinical Diagnostic Laboratory. Serum creatinine is used as an indicator of renal function and elevated concentrations often are used to diagnose chronic renal failure (Polzin, 2013). However, by the time concentrations rise above the reference range, glomerular filtration rate (GFR) is already decreased by 75% (Finco et al., 1995). Decreased GFR can identify early signs of kidney disease, but is an expensive and uncommon diagnostic test. The International Renal Interest Society (IRIS) Study Group categorizes renal disease by creatinine concentration (mg/dL) as follows: stage I =  $< 1.6$ , stage II =  $1.6 - 2.8$ , stage III =  $2.8 - 5.0$ , and stage IV =  $> 5.0$  (Syme et al., 2006). Elevated blood urea nitrogen (BUN), potassium, calcium, and

phosphorus are also considerations in the diagnosis of renal disease, yet all remained within their reference range throughout the study. Furthermore, a recent study determined healthy geriatric cats to be within a serum creatinine reference range of 0.7 – 2.1 mg/dL, established by the American Association of Feline Practitioners (2005), which no longer considers results of this study elevated (Hall et al., 2014). Due to these reasons, we have little reason to be concerned with declined renal function in the current study.

It has been shown that hypertriglyceridemia is present in obese and type II diabetic cats (Hoenig et al., 2003; Jordan et al., 2008; Belsito et al., 2009), yet development of hypertension and atherosclerosis has not been observed. Results of this study agree with those of Jordan et al. (2008), which reported that cholesterol concentrations were not different ( $P > 0.05$ ) in lean versus obese ( $132 \pm 36$  versus  $139 \pm 28$  mg/dL) cats, but triglyceride concentrations were lower ( $P < 0.001$ ) in lean versus obese cats ( $21 \pm 8$  versus  $48 \pm 19$  mg/dL). We also observed no change in cholesterol concentrations at wk 16 versus wk 0 ( $125.5$  versus  $125.6 \pm 7.99$  mg/dL), but a decrease ( $P = 0.0002$ ) in triglyceride concentrations at wk 16 versus wk 0 ( $37$  versus  $56 \pm 3.99$  mg/dL). Phospholipids, NEFAs, plasma protein subclasses, and particle size were not determined in this study, but may be helpful in determining overall improvements in dyslipidemia with weight loss.

Previous research has demonstrated that subcutaneous fat is a source of lactate (Jansson et al., 1994) and that a high-fat diet (HFD) induced obese mice or obese Zucker rats lacking the leptin receptor have higher concentration of lactate in the urine, blood, and liver tissue (Serkova et al., 2006; Kim et al., 2009; Rull et al., 2009; Waldram et al., 2009; Duggan et al., 2011). Because lactate is a precursor of gluconeogenesis, increased plasma lactate in obese models may reflect alterations in hepatic glucose and lipid metabolism.

Pyruvate enters the tricarboxylic acid cycle (TCA) via citrate, which is regulated in the plasma by insulin, glucose, fatty acid utilization, cholesterol synthesis, and liver clearance and excretion (Shearer et al., 2008). Plasma citrate has been reported to be increased in diabetic rats (DeVilliers et al., 1966) and HFD-fed obese mice (Shearer et al., 2008), and decreased with insulin administration in children (Natelson et al., 1963). Alternately, lower serum citrate has been reported in humans with type-2 DM (Zhang et al., 2009). Results of the current study demonstrate a decrease in serum lactate and glycerate, which may indicate a beneficial shift in glucose metabolism with weight loss. Citrate remained unchanged for the majority of weight loss, with an increase occurring only at wk 16 versus wk 0 (fold change = 1.09). It has been shown that uridine infusion induced insulin resistance in rats (Buse, 2006) and has been correlated with insulin resistance in hypertensive patients (Hamada et al., 2007). Results of the current study show decreased uridine at wk 2, 4, 12, and 16 versus wk 0 (fold change = 0.69, 0.68, 0.7, and 0.61, respectively). Uracil forms uridine when it is combined with a sugar ribose by a glycosidic linkage. Uracil was decreased at all weeks versus wk 0, with a fold change of 0.53 at wk 1 versus wk 0. Fructose is converted to glycerol and acyl groups for synthesis of triglycerides in the liver (Basciano et al., 2005). Increased fructose at all weeks versus wk 0 agrees with the previously discussed decreases in fasting serum triglyceride concentrations. Literature suggests that alpha-ketoglutarate is a positive predictor of obesity (Rodriguez-Gallego et al., 2014), yet alpha-ketoglutarate was only decreased at wk 2 versus wk 0 (fold change = 0.81) in the current study.

Lipids in the blood are derived from the diet or from adipose tissue and liver. They are an important source of energy for the host and are stored primarily as triglycerides in

adipose tissue (Kim et al., 2011). Obesity is generally associated with elevated plasma, serum, and liver concentrations of free fatty acids (FFA), especially saturated fatty acids (SFA) (Wang et al., 2003). Furthermore, higher concentrations of stearic acid ( $P = 0.035$ ), total SFA ( $P = 0.051$ ), and palmitoleic acid ( $P = 0.068$ ) along with lower linoleic acid ( $P = 0.084$ ) concentrations were detected in obese men (Kim et al., 2010). Most long-chain FA, including palmitate and stearate, and PUFA were decreased later in weight loss (wk 8, 12, and 16 versus 0). Carnitine transports fatty acids into the mitochondrion in order to produce energy via  $\beta$ -oxidation; therefore, carnitine often is used to promote weight loss (Yoo et al., 2009). Furthermore, obese mice (Kim et al., 2011) and humans (Kim et al., 2010) have displayed a depletion of carnitine in liver tissue. It may be that decreased carnitine with obesity leads to insufficient  $\beta$ -oxidation of NEFA, resulting to NEFA stored as triglycerides in adipose tissue and ultimately an accumulation of fat (Xie et al., 2012). Choline plays a role in cell membrane structure, methyl metabolism, and lipid metabolism. The majority (>95%) of choline is used to synthesize phosphatidylcholine (PC) (Gibellini and Smith, 2010), which has shown to be increased in HFD-fed obese mice (Kim et al., 2011).

High fasted concentrations of BCAA and aromatic AA have been documented in obese persons (Felig et al., 1974) and are thought to contribute to obesity-related comorbidities such as insulin resistance and glucose intolerance (Newgard et al., 2009). Reportedly, obese men had plasma valine and leucine concentrations that were 23 and 14%, respectively, higher than lean men (Kim et al., 2010). Valine was not changed with weight loss in the current study, but leucine was decreased at wk 12 versus wk 0. BCAA catabolism may be inhibited with obesity, as obese *ob/ob* mice and Zucker rats had

reportedly depressed activity of BCAA aminotransferase and branched chain  $\alpha$ -ketoacid dehydrogenase enzyme complex (She et al., 2007). Conversely, HFD-fed mice have been shown to have decreased concentrations of serum BCAA (Shearer et al., 2008; Duggan et al., 2011).

C3 acylcarnitine is a byproduct of isoleucine and valine catabolism, while C5 acylcarnitines are intermediates of mitochondrial isoleucine and leucine catabolism. Both C3 and C5 acylcarnitines have been observed to increase with obesity (Newgard et al., 2009). Furthermore, propionylcarnitine, butyrylcarnitine, and hexanoylcarnitines have been identified as being increased in obese men (Kim et al., 2010). Butyrylcarnitine was decreased from wk 2-16 versus wk 0 and propionylcarnitine was decreased at all wk versus wk 0 in the current study. Glutamine is the most abundant AA in plasma and glycine is generated from serine, which is derived from pyruvate. Both of these AA, which are precursors of urea biosynthesis and glucose metabolism (Parimi et al., 2004), were previously reported to be decreased ( $P < 0.05$ ) in obese individuals (Backman et al., 1975; Oberbach et al., 2011) and were observed to be increased at wk 12 versus wk 0 in the current study. Arginine and glycine synthesize creatine, which is broken down in skeletal muscle to produce creatinine (Walker et al., 1961). Previous research has shown an increase of creatinine in the urine of obese individuals ( $P < 0.01$ ) (Konishi, 1964) and serum of HFD-fed mice ( $P < 0.05$ ) (Duggan et al., 2011). While creatine and creatine phosphate were decreased at wk 1 and 2 versus wk 0, creatinine and guanidinoacetate were increased with weight loss in the current study.

Taurine plays a role in conjugation of cholesterol and bile acids and has been thought to play a role in obesity (Xie et al., 2012). Taurine, hypotaurine, and N-



acetyltaurine were decreased at all weeks versus wk 0, with hypotaurine having the greatest reduction with a fold change (0.31 at wk 1 versus wk 0). Mevalonate, the product of rate-limiting HMG-CoA reductase (HMGR), was significantly decreased after wk 8 versus wk 0 (fold change = 0.65). Cholesterol is the major product of HMGR, yet remained unchanged. The cholesterol-derived primary and secondary bile acids were greatly decreased with weight loss. Cholate, in particular, was reduced by a fold change of 0.03 and 0.01 at wk 1 and wk 16 versus wk 0, respectively. Taurocholate and deoxycholate had similar reductions. The bile acid precursor 7-Hoca was essentially unchanged, with an increase only at wk 8 versus wk 0 (fold change = 1.1), suggesting a reduced need for emulsifying bile acids to aid gut absorption.

A recent study by Schmedes et al. (2015) reported an elevated ( $P < 0.001$ ) concentration of the ketone bodies, 3-hydroxybutyrate (BHBA) and acetoacetate, and a decreased ( $P < 0.001$ ) concentration of choline, glucose, tyrosine, and lactate in serum of overweight female subjects after a 6-wk very low-calorie diet (average energy = 617 kcal/d) weight loss regimen. These results indicate that lean subjects use ketone bodies and fatty acids for energy production more than obese subjects (Schmedes et al., 2015). During energy restriction, NEFA in the liver are preferentially used for  $\beta$ -oxidation to produce acetyl-coA and, therefore, ketone bodies rather than being esterified to triglycerides (Volek et al., 2005). This would be reflected by an increase in ketone bodies and a decrease in triglycerides in fasting serum samples. Our results agree, with acetoacetate and 3-hydroxybutyrate increasing up to 1.93 and 2.09 fold, respectively, and triglycerides concentrations were decreased ( $P < 0.001$ ) at all wk vs. baseline (56.0 mg/dL). Another study by Perez-Cornago et al. (2014) reported that total SFA (palmitic

acid [C16:0] and stearic acid [C18:0]) and monounsaturated fatty acids were decreased ( $P < 0.005$ ) after 8 wk of energy restriction (-15% MER) in obese adults. In agreement, most long chain fatty acids in this study were decreased at wk 8, 12, and 16 versus wk 0.

Interestingly, monoacylglycerols, which are intermediates of lipolysis, were increased with weight loss.

Eicosanoids are oxygenated bioactive metabolites derived from the FA, arachidonic acid, including prostaglandins, thromboxanes, leukotrienes, and lipoxins (Haeggstrom et al., 2010). They are mediators of acute inflammation, fever, and diseases such as cancer, atherosclerosis, thrombosis, and cancer. Therefore, preventing eicosanoid synthesis and action is the aim of many drugs. Eicosanoid-related metabolites were decreased with weight loss in the current study. Thromboxane B2 was lower at wk 16 versus wk 0 (fold change = 0.31) and 12- hydroxyeicosatetraenoic acid (HETE) was more quickly decreased with a fold change of 0.56 and 0.25 by wk 1 and wk 16 versus wk 0, respectively.

Triglyceride-rich lipoprotein (TGRL) lipolysis products cause inflammatory stimuli that possibly alter endothelial barrier function and have pro-atherogenic and pro-inflammatory properties (Wang et al., 2009). Linoleic acid derived 13- hydroxyl ocatadecadienoic acid (HODE) and 9-HODE are the major oxidized components of LDL and VLDL, respectively (Lenz et al., 1990; Newman et al., 2007). Other linoleic acid oxidation products include 12,13 dihydroxyoctadecanoic acid (DiHOME), 9,10 DiHOME and epoxy octadecenoic acid (EpOME). Research by Wang et al. (2009) reported that significant amounts of these oxidized lipids are released during TGRL lipolysis. During weight loss in the current study, 12,13 DiHOME was primarily decreased, with a fold change of 0.74 and 0.24 at wk 1 and wk 16 versus wk 0, respectively. 9,10 DiHOME was also decreased at wk 8, 12, and 16 versus

wk 0. 13-HODE and 9-HODE were decreased from wk 4 to 16 versus wk 0 of weight loss. These results agree with the literature that obesity is a state of low grade inflammation and that weight loss can reduce this state.

In conclusion, monitored restriction feeding of a moderate-protein, high-fiber diet was a safe and effective means for weight loss in overweight adult domestic cats, resulting in a mean decrease of body fat percentage and increase in lean mass percentage. Mean daily voluntary physical activity tended to increase and the light:dark ratio of activity increased. Except for mean creatinine, all blood metabolites remained within reference ranges, with mean triglycerides decreasing throughout weight loss. Global metabolite profiling indicates a decreased abundance of metabolites related to inflammation and oxidative stress and a shift in metabolism, with a preference for lipolysis and FA oxidation rather than glycolysis for energy during weight loss.

## TABLES AND FIGURES

**Table 3.1.** Proximate analysis of diet

Dry matter	93.0 %
-----% DM Basis -----	
Organic matter	92.3
Crude protein	35.9
Acid hydrolyzed fat	8.9
Total dietary fiber	16.8
Insoluble	14.9
Soluble	1.9
ME, kcal/kg	3,207

Ingredients in diet: Chicken, Pea Protein, Brewers Rice, Chicken Meal, Whole Brown Rice, Split Peas, Dehydrated Alfalfa Meal, Oat Fiber, Potato Protein, Dried Plain Beet Pulp, Flaxseed, Chicken Fat (preserved with mixed Tocopherols), Natural Flavors, Rice Bran, Salmon Meal, Potassium Chloride, Choline Chloride, DL-Methionine, Salt, Taurine, Vitamin E Supplement, Zinc Sulfate, L-Ascorbyl-2-Polyphosphate (source of Vitamin C), Iron Proteinate, Yucca Schidigera Extract, Vitamin B12 Supplement, Copper Proteinate, Niacin Supplement, Selenium Yeast, Manganese Proteinate, Biotin, Riboflavin Supplement (Vitamin B2), Calcium Pantothenate, Potassium Iodide, Thiamine Mononitrate (Vitamin B1), Vitamin A Supplement, Pyridoxine Hydrochloride (Vitamin B6), Vitamin D3 Supplement, Folic Acid, Rosemary Extract, Decaffeinated Green Tea Extract, Spearmint Extract

**Table 3.2.** Summary of food intake and weight loss

Week	Average Weight, kg	Average Intake, g	Average Intake, kcal/day	%MER Consumed (x)(MER)	% BW Loss/Week	Total % BW lost
1	7.64	63.25	203.03	0.80	1.27	1.26
2	7.58	62.77	201.48	0.79	1.20	2.08
3	7.52	59.01	189.43	0.74	1.04	2.73
4	7.41	57.16	183.47	0.72	0.87	4.20
5	7.35	53.42	171.47	0.67	1.11	5.01
6	7.24	51.54	165.43	0.65	1.45	6.23
7	7.13	51.11	164.06	0.64	1.11	7.76
8	7.06	47.73	153.22	0.60	1.61	8.73
9	6.95	48.05	154.23	0.61	0.97	10.12
10	6.88	47.45	152.30	0.60	1.57	11.10
11	6.80	47.02	150.93	0.59	0.82	12.08
12	6.67	44.88	144.05	0.57	1.65	13.69
13	6.58	44.88	144.05	0.57	1.42	14.91
14	6.53	44.38	142.44	0.56	1.17	15.57
15	6.43	43.88	140.84	0.55	1.51	16.81
16	6.34	44.30	142.22	0.56	1.45	17.92
17	6.28	44.88	144.05	0.57	0.96	18.78
18	6.15	44.75	143.65	0.56	1.51	20.36

**Table 3.3.** Body composition during weight loss in adult cats

	Week 0	Week 4	Week 8	Week 12	Week 16	SEM
Mean BCS	7.6 <sup>d</sup>	7.6 <sup>cd</sup>	7.3 <sup>bc</sup>	7.0 <sup>b</sup>	6.0 <sup>a</sup>	0.38
Mean BMC, g	108.2 <sup>c</sup>	105.4 <sup>c</sup>	104.4 <sup>bc</sup>	92.7 <sup>ab</sup>	92.4 <sup>a</sup>	4.44
Total fat, g	2924 <sup>d</sup>	2782 <sup>d</sup>	2417 <sup>c</sup>	2097 <sup>b</sup>	1810 <sup>a</sup>	55.1
Total lean, g	3865 <sup>b</sup>	3751 <sup>ab</sup>	3780 <sup>ab</sup>	3672 <sup>a</sup>	3665 <sup>a</sup>	45.6
Fat, %	40.9 <sup>d</sup>	40.4 <sup>d</sup>	36.8 <sup>c</sup>	34.0 <sup>b</sup>	30.7 <sup>a</sup>	0.90
Lean, %	57.6 <sup>a</sup>	58.0 <sup>a</sup>	61.5 <sup>b</sup>	64.4 <sup>c</sup>	67.6 <sup>d</sup>	0.95

BCS = body condition score; BMC = bone mineral content

<sup>a-d</sup> Means without a common superscript letter within a row differ ( $P < 0.05$ );  $n=8$ .

**Table 3.4.** Physical activity during weight loss in adult cats

	Week 0	Week 6	Week 12	Week 18	SEM
Daily	10.73 <sup>x</sup>	13.42 <sup>xy</sup>	14.56 <sup>xy</sup>	14.15 <sup>y</sup>	1.38
Light	10.77	13.28	15.36	16.27	1.42
Dark	10.65	12.87	12.96	9.82	1.57
Light:Dark	1.02 <sup>a</sup>	1.18 <sup>a</sup>	1.27 <sup>a</sup>	1.74 <sup>b</sup>	0.15

Data are presented as activity counts per epoch (epoch length = 15 sec.).

<sup>a-b</sup> Means without a common superscript letter within an activity period differ ( $P < 0.05$ );  $n=8$ ; light:dark ratio SEM = 0.15.

<sup>x-y</sup> Means without a common superscript differ ( $P < 0.10$ );  $n=8$ ; daily SEM = 1.38; light SEM = 1.42; dark SEM = 1.57.

**Table 3.5.** Fasted serum chemistry profiles during weight loss in adult cats

	Reference	Week							SEM
	Range	0	1	2	4	8	12	16	
Creatinine, mg/dL	0.4-1.6	1.59 <sup>a</sup>	1.74 <sup>b</sup>	1.76 <sup>b</sup>	1.75 <sup>b</sup>	1.86 <sup>bc</sup>	1.94 <sup>c</sup>	1.91 <sup>c</sup>	0.08
BUN, mg/dL	18-38	22.0 <sup>c</sup>	23.6 <sup>d</sup>	22.1 <sup>cd</sup>	21.0 <sup>bc</sup>	19.4 <sup>a</sup>	18.8 <sup>a</sup>	19.4 <sup>ab</sup>	0.87
Total Protein, g/dL	5.8-8.0	7.3 <sup>b</sup>	7.0 <sup>a</sup>	7.0 <sup>a</sup>	7.1 <sup>a</sup>	7.2 <sup>ab</sup>	7.1 <sup>ab</sup>	7.2 <sup>ab</sup>	0.12
Glucose, mg/dL	60-122	79.4	74.9	77.5	82.1	83.4	82.1	84.1	3.92
Cholesterol, mg/dL	66-160	125.6 <sup>c</sup>	122.3 <sup>abc</sup>	124.6 <sup>c</sup>	122.4 <sup>bc</sup>	112.9 <sup>a</sup>	113.0 <sup>ab</sup>	125.5 <sup>c</sup>	7.99
Triglycerides, mg/dL	21-166	56.0 <sup>b</sup>	42.5 <sup>a</sup>	38.5 <sup>a</sup>	39.0 <sup>a</sup>	37.3 <sup>a</sup>	36.4 <sup>a</sup>	37.0 <sup>a</sup>	3.99
Albumin, g/dL	2.8-4.1	3.26	3.21	3.24	3.31	3.28	3.26	3.23	0.04
Globulin, g/dL	2.6-5.1	4.06 <sup>c</sup>	3.8 <sup>ab</sup>	3.8 <sup>ab</sup>	3.74 <sup>a</sup>	3.9 <sup>abc</sup>	3.83 <sup>ab</sup>	3.96 <sup>bc</sup>	0.11
Albumin:Globulin	0.6-1.1	0.8 <sup>a</sup>	0.85 <sup>a</sup>	0.84 <sup>a</sup>	0.89 <sup>b</sup>	0.84 <sup>a</sup>	0.86 <sup>a</sup>	0.83 <sup>a</sup>	0.03
Calcium, mg/dL	8.8-10.2	9.54 <sup>a</sup>	9.7 <sup>ab</sup>	9.76 <sup>bc</sup>	9.48 <sup>a</sup>	9.28 <sup>d</sup>	9.5 <sup>a</sup>	9.7 <sup>ac</sup>	0.10
Phosphorus, mg/dL	3.2-5.3	4.66 <sup>b</sup>	4.51 <sup>ab</sup>	4.56 <sup>ab</sup>	4.59 <sup>ab</sup>	4.73 <sup>b</sup>	4.38 <sup>a</sup>	4.35 <sup>a</sup>	0.14
Sodium, mmol/L	145-157	149.8	149.9	151.3	149.3	149.6	149.4	150.4	0.50
Potassium, mmol/L	3.6-5.3	5.21 <sup>b</sup>	4.91 <sup>a</sup>	5.05 <sup>ab</sup>	4.96 <sup>ab</sup>	4.89 <sup>a</sup>	4.94 <sup>a</sup>	4.89 <sup>a</sup>	0.09
Sodium:Potassium	28-36	28.8 <sup>a</sup>	30.6 <sup>b</sup>	30.0 <sup>ab</sup>	30.3 <sup>ab</sup>	30.5 <sup>b</sup>	30.4 <sup>ab</sup>	30.6 <sup>b</sup>	0.58
Chloride, mmol/L	109-126	117.9	117.3	118.6	117.1	117.3	118.0	117.9	0.58
Alkaline Phosphatase Total, U/L	10-85	21.0 <sup>a</sup>	23.1 <sup>ab</sup>	23.6 <sup>abc</sup>	26.8 <sup>bcd</sup>	27.9 <sup>cd</sup>	30.9 <sup>d</sup>	27.8 <sup>d</sup>	2.28
ALT (SGPT), U/L	14-71	42.6	41.8	36.8	37.0	40.0	39.4	39.4	1.95
Total Bilirubin, mg/dL	0.0-0.3	0.14 <sup>ab</sup>	0.15 <sup>b</sup>	0.14 <sup>ab</sup>	0.10 <sup>a</sup>	0.10 <sup>a</sup>	0.10 <sup>ab</sup>	0.10 <sup>a</sup>	0.01
Bicarbonate, mmol/L	12-21	17.0 <sup>a</sup>	17.3 <sup>ab</sup>	17.4 <sup>ab</sup>	17.6 <sup>ab</sup>	18.5 <sup>b</sup>	18.0 <sup>ab</sup>	17.4 <sup>ab</sup>	0.61
Anion Gap	10-27	20.1 <sup>b</sup>	20.3 <sup>b</sup>	20.4 <sup>b</sup>	19.5 <sup>ab</sup>	18.9 <sup>ab</sup>	18.4 <sup>a</sup>	20.1 <sup>b</sup>	0.45

<sup>a-d</sup> Means without a common superscript letter within a row differ ( $P < 0.05$ ); n=8.



**Table 3.6.** Serum metabolite alterations in lipid metabolism of cats during weight loss

Metabolic Pathway	Metabolite	Fold change					
		wk 1	wk 2	wk 4	wk 8	wk 12	wk 16
		wk 0	wk 0	wk 0	wk 0	wk 0	wk 0
Lipids							
Medium Chain Fatty Acid	heptanoate (7:0)	0.89	0.86	0.94	0.9	0.85	0.84
	pelargonate (9:0)	0.83	0.75	0.84	0.72	0.67	1.26
	caprate (10:0)	0.91	0.88	0.92	0.88	0.79	0.96
	undecanoate (11:0)	0.86	0.83	0.86	0.94	0.73	0.99
	10-undecenoate (11:1n1)	1.11	1.21	1.4	1.52	1.48	1.6
	laurate (12:0)	0.95	0.85	0.88	0.89	0.73	0.74
Long Chain Fatty Acid	pentadecanoate (15:0)	0.93	0.9	0.85	0.8	0.8	0.81
	palmitate (16:0)	1	0.99	0.96	0.87	0.87	0.9
	palmitoleate (16:1n7)	0.98	0.94	0.9	0.88	0.86	0.82
	margarate (17:0)	0.97	0.98	0.88	0.76	0.81	0.77
	10-heptadecenoate (17:1n7)	0.99	0.92	0.85	0.81	0.82	0.74
	stearate (18:0)	1.02	1.03	0.96	0.85	0.88	0.87
	oleate (18:1n9)	0.88	0.95	0.86	0.8	0.89	0.82
	cis-vaccenate (18:1n7)	0.93	1.06	0.89	0.85	0.91	0.86
	arachidate (20:0)	1.04	1.06	0.97	0.82	0.86	0.86
Polyunsaturated Fatty Acid (n3 and n6)	stearidonate (18:4n3)	0.91	0.87	0.81	0.66	0.55	0.49
	eicosapentaenoate (EPA; 20:5n3)	0.95	0.94	0.86	0.7	0.65	0.74
	docosahexaenoate (DHA; 22:6n3)	1.15	1.16	1.14	1.08	0.94	0.98
	linoleate (18:2n6)	0.96	0.96	0.88	0.83	0.82	0.85
	linolenate (18:3n3 or 6)	0.88	0.88	0.79	0.71	0.69	0.64
	dihomo-linolenate (20:3n3 or n6)	1	0.97	0.93	0.83	0.75	0.74
	arachidonate (20:4n6)	1.03	1.13	1.13	1.01	0.84	0.81
	docosapentaenoate (n6 DPA; 22:5n6)	1.28	1.3	1.29	1.25	1.13	1.05
Fatty Acid, Branched	15-methylpalmitate (isobar with 2-methylpalmitate)	0.99	1.02	0.88	0.81	0.84	0.64
	pristanate	0.96	0.88	0.97	0.88	0.8	0.68
Fatty Acid, Dicarboxylate	adipate	0.77	0.71	0.8	0.67	0.71	0.74
	2-hydroxyadipate	0.79	0.7	0.65	0.6	0.57	0.71
	maleate (cis-Butenedioate)	0.93	0.93	0.83	0.78	0.92	0.85
	pimelate (heptanedioate)	0.82	0.73	0.76	0.66	0.63	0.83
	suberate (octanedioate)	0.81	0.7	0.77	0.65	0.6	0.79
	azelate (nonanedioate)	0.81	0.7	0.78	0.65	0.6	0.84
	sebacate (decanedioate)	0.82	0.73	0.79	0.65	0.62	0.76
	undecanedioate	0.83	0.73	0.79	0.68	0.65	0.83
	1,11-undecanedicarboxylate	0.77	0.69	0.75	0.68	0.66	0.91
	dodecanedioate	0.84	0.75	0.78	0.69	0.67	0.84
	tetradecanedioate	0.9	0.81	0.83	0.8	0.75	0.84
	hexadecanedioate	0.77	0.69	0.72	0.7	0.69	0.74

**Table 3.6 (cont.)**

	octadecanedioate	0.9	0.9	0.98	0.91	0.98	0.97
	eicosanodioate	1.02	1.03	1.12	1.09	1.16	1.09
	docosadioate	0.84	0.81	0.83	0.7	0.76	0.72
	3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	0.82	0.81	0.72	0.56	0.63	0.82
Fatty Acid, Amino	2-aminoheptanoate	0.96	1.05	1.09	1.14	1.13	1.26
	2-aminooctanoate	1.16	1.12	1.21	1.07	0.95	0.82
Fatty Acid Synthesis	malonylcarnitine	1.21	1.35	1.57	1.77	1.84	1.58
	malonate (propanedioate)	1.36	1.44	1.38	1.3	1.37	1.36
	2-methylmalonyl carnitine	1.22	1.4	1.68	1.96	2.14	1.86
Fatty Acid Metabolism (also BCAA Metabolism)	butyrylcarnitine	0.85	0.78	0.8	0.81	0.8	0.69
	propionylcarnitine	0.65	0.63	0.59	0.67	0.63	0.62
	propionylglycine	0.82	0.85	0.84	0.74	0.65	1.61
FA Metabolism (Acyl Glycine)	hexanoylglycine	1.55	1.56	1.98	2.22	2.33	2.11
	N-octanoylglycine	1.22	1.3	1.55	3.04	2.78	1.7
FA Metabolism (Acyl Carnitine)	hydroxybutyrylcarnitine*	0.94	1.13	1.32	1.85	2	1.63
Ketone Bodies	acetoacetate	1.54	1.59	1.93	1.91	1.54	1.08
	3-hydroxybutyrate (BHBA)	1.51	1.72	1.95	2.09	2	1.74
Fatty Acid, Monohydroxy	2-hydroxyoctanoate	0.81	0.79	0.87	0.71	0.55	0.48
	2-hydroxydecanoate	0.86	0.77	0.86	0.67	0.47	0.41
	3-hydroxyoctanoate	1.02	1.06	1.27	1.31	1.36	1.32
	3-hydroxydecanoate	0.86	0.81	0.94	0.86	0.83	0.86
	3-hydroxysebacate	0.58	0.6	0.49	0.46	0.47	0.64
	3-hydroxylaurate	0.74	0.71	0.75	0.64	0.64	0.64
	3-hydroxymyristate	0.86	0.82	0.85	0.69	0.77	0.7
	5-hydroxyhexanoate	0.94	0.82	0.78	0.71	0.64	0.83
	5-hydroxydecanoate	0.84	0.69	0.76	0.67	0.58	0.78
	8-hydroxyoctanoate	0.8	0.78	0.83	0.7	0.67	0.81
	16-hydroxypalmitate	0.9	0.78	0.81	0.8	0.73	0.69
	13-HODE + 9-HODE	0.81	0.78	0.62	0.59	0.47	0.35
Fatty Acid, Dihydroxy	12,13-DiHOME	0.74	0.62	0.6	0.44	0.3	0.24
	9,10-DiHOME	0.86	0.74	0.73	0.48	0.42	0.38
Eicosanoid	thromboxane B2	0.68	1.23	0.81	0.82	0.48	0.31
	12-HETE	0.56	0.82	0.61	0.54	0.4	0.25
Endocannabinoid	oleic ethanolamide	1.18	1.31	1.19	1.05	1.01	1.03
	palmitoyl ethanolamide	1.17	1.21	1.23	1.22	1.15	1.28
	N-stearoyltaurine	1.11	1.05	0.99	0.91	0.77	0.7
	N-palmitoyltaurine	1.02	0.96	0.96	0.82	0.75	0.76
	N-linolenoyltaurine *	0.8	1.2	0.99	0.77	0.82	0.58
Inositol Metabolism	myo-inositol	0.66	0.66	0.76	0.77	0.81	0.74
	scyllo-inositol	0.6	0.59	0.7	0.79	0.78	0.65
	inositol 1-phosphate (I1P)	0.73	0.82	0.71	0.75	0.68	0.58

**Table 3.6 (cont.)**

Phospholipid Metabolism	choline	0.73	0.69	0.71	0.69	0.7	0.59
	choline phosphate	0.63	0.64	0.63	0.64	0.59	0.53
	glycerophosphorylcholine (GPC)	0.72	0.73	0.64	0.62	0.66	0.7
	phosphoethanolamine	0.51	0.54	0.64	0.64	0.61	0.51
	glycerophosphoethanolamine	0.67	0.64	0.57	0.59	0.65	0.59
Lysolipid	1-palmitoylglycerophosphocholine	1.19	0.7	0.68	0.99	0.89	0.38
	1-palmitoleoylglycerophosphocholine	1.46	0.75	0.7	1.57	1	0.35
	2-palmitoleoylglycerophosphocholine	0.83	0.78	0.61	0.61	0.38	0.54
	1-stearoylglycerophosphocholine	1.01	0.55	0.56	0.91	0.75	0.32
	2-stearoylglycerophosphocholine	1.42	0.62	0.53	1.11	0.59	0.3
	1-oleoylglycerophosphocholine	1.18	0.66	0.55	0.97	0.72	0.31
	1-linoleoylglycerophosphocholine	1.16	0.73	0.62	0.98	0.82	0.35
	1-linolenoylglycerophosphocholine	1.18	0.6	0.47	0.78	0.58	0.17
	1-palmitoylplasménylethanolamine	0.66	0.72	0.65	0.67	0.54	0.5
	1-stearoylplasménylethanolamine	0.85	1.3	1.26	0.96	0.98	0.56
	1-oleoylplasménylethanolamine	0.57	0.74	0.62	0.52	0.35	0.32
	1-oleoylglycerophosphoethanolamine	0.74	0.65	0.53	0.47	0.54	0.6
	1-linoleoylglycerophosphoethanolamine	0.78	0.75	0.65	0.6	0.7	0.7
	1-arachidonoylglycerophosphoethanolamine	0.86	0.82	0.75	0.69	0.76	0.73
	1-palmitoylglycerophosphoinositol	1.56	1.73	1.37	1.07	0.32	0.82
	1-stearoylglycerophosphoinositol	1.1	1.19	1.05	0.73	0.6	0.6
	1-oleoylglycerophosphoinositol	0.93	1.24	1.05	0.3	0.31	0.26
	1-linoleoylglycerophosphoinositol	1.13	1.17	0.88	0.65	0.65	0.72
	1-arachidonoylglycerophosphoinositol	1.29	1.4	1.12	0.95	0.83	0.98
	1-linoleoylglycerophosphoserine	0.76	0.8	0.28	0.22	0.23	0.12
	1-arachidonoylglycerophosphate	1.11	0.84	0.41	0.61	0.36	0.37
	oleoyl-linoleoyl-glycerophosphoinositol	0.83	0.84	0.8	0.58	0.59	0.56
	palmitoyl-arachidonoyl-glycerophosphocholine	1.3	1.33	1.06	1.17	1.08	1.36
	palmitoyl-linoleoyl-glycerophosphoinositol	0.82	0.88	0.84	0.63	0.59	0.58
	stearoyl-arachidonoyl-glycerophosphocholine	1.01	1.14	1.15	1.04	0.96	0.89
	stearoyl-arachidonoyl-glycerophosphoinositol	0.98	0.96	1	0.85	0.78	0.75
	stearoyl-linoleoyl-glycerophosphocholine	0.97	0.95	0.85	0.8	0.81	0.94
	stearoyl-arachidonoyl-glycerophosphoinositol	1.79	1.14	1.47	2.03	1.76	2.78
Glycerolipid Metabolism	glycerol	0.9	0.78	1	1.08	1	0.99
	glycerol 3-phosphate (G3P)	0.59	0.52	0.7	0.67	0.77	0.68
	glycerophosphoglycerol	0.72	0.62	0.6	0.61	0.6	0.6
Monoacylglycerol	1-palmitoylglycerol (1-monopalmitin)	1.41	1.5	1.69	1.85	1.68	1.74
	2-palmitoylglycerol (2-monopalmitin)	3.05	1.81	2.18	2.74	2.59	1.92
	1-stearoylglycerol (1-monostearin)	0.99	1.07	1.19	1.1	1.22	1.06
	1-linoleoylglycerol (1-monolinolein)	1.37	1.65	1.81	1.59	1.45	1.65
	2-linoleoylglycerol (2-monolinolein)	1.48	1.76	2.86	2.17	1.47	2.05
	1-arachidonoylglycerol	1.79	2.35	2.73	2.45	2.18	2.41

**Table 3.6 (cont.)**

	2-arachidonoyl glycerol	1.48	2.91	3.07	2.61	2.33	2.19
	1-docosahexaenoylglycerol	1.52	2.31	2.53	2.15	1.76	2.26
	2-docosahexaenoylglycerol*	1.71	1.67	2.71	1.87	1.32	1.34
Sphingolipid Metabolism	stearoyl sphingomyelin	1.07	1.09	1.19	1.35	1.24	1.37
	oleoyl sphingomyelin	1.11	1.13	1.33	1.32	1.24	1.35
	sphingosine	0.53	0.41	0.34	0.23	0.36	0.16
	palmitoleoyl sphingomyelin*	0.99	1.07	1.19	1.07	1.11	1.12
	erucoyl sphingomyelin*	1.17	1.17	1.57	1.52	1.35	1.96
	arachidoyl sphingomyelin*	2.21	0.9	2	1.81	2.56	2.09
Mevalonate Metabolism	mevalonate	1.11	0.95	0.84	0.68	0.65	0.59
Sterol	cholesterol	0.96	0.99	0.95	0.94	0.93	0.95
	7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	0.97	1	1.09	1.1	1.07	1.04
	cholestanol	0.9	0.91	0.87	0.86	0.79	0.81
	beta-sitosterol	0.88	0.94	0.8	0.74	0.79	0.79
	campesterol	0.85	0.89	0.81	0.8	0.77	0.8
	fucosterol	0.94	0.91	0.81	0.65	0.72	0.61
Steroid	5alpha-pregnan-3beta,20beta-diol monosulfate (1)	1.18	1.11	1.18	1.21	1.12	1.05
	cortisol	0.38	0.33	0.44	0.84	0.67	0.69
	cortisone	0.6	0.48	0.73	0.93	0.94	0.89
Primary Bile Acid Metabolism	cholate	0.03	0.01	0.01	0	0.01	0.01
	taurocholate	0.14	0.19	0.17	0.19	0.19	0.19
Secondary Bile Acid Metabolism	deoxycholate	0.38	0.41	0.35	0.26	0.32	0.29
	tauroolithocholate 3-sulfate	0.97	0.83	0.88	0.91	0.94	0.91
	ursodeoxycholate	0.55	0.6	0.51	0.24	0.31	0.36

<sup>a</sup> For each metabolite, mean value is the group mean of re-scaled data to have median equal to 1.

<sup>b</sup> Mean values in green were decreased, whereas in red were increased with *P*- and *q*- values < 0.05. *P* values were calculated from one way Anova; *q*- values were used to estimate the false discovery rate (FDR) in multiple comparisons.

**Table 3.7.** Serum metabolite alterations in amino acid and peptide metabolism of cats during weight loss

Metabolic Pathway	Metabolite	Fold change					
		wk 1	wk 2	wk 4	wk 8	wk 12	wk 16
		wk 0	wk 0	wk 0	wk 0	wk 0	wk 0
Amino Acids							
Glycine, Serine and Threonine Metabolism	glycine	1.1	1.09	1.18	1.22	1.25	1.08
	N-acetylglycine	1.37	1.41	1.51	1.61	1.66	1.63
	sarcosine (N-Methylglycine)	0.62	0.72	0.61	0.5	0.57	0.53
	threonine	0.95	0.92	0.87	0.82	0.78	0.8
	N-acetylthreonine	1.07	1.1	1.11	1.13	1.16	1.03
Alanine and Aspartate Metabolism	aspartate	0.76	1	1.51	1.72	2.32	1.71
Glutamate Metabolism	glutamate	0.71	0.73	0.82	0.81	0.95	0.67
	glutamine	1.05	1.1	1.09	1.1	1.19	1.06
Histidine Metabolism	histidine	0.98	0.94	0.95	0.94	0.95	0.91
	1-methylhistidine	1.2	1.2	1.17	1.28	1.34	1.28
	3-methylhistidine	1.14	1.12	1.13	1.12	1.31	1.15
	trans-urocanate	0.99	0.81	1.06	1.85	1.25	1.14
	imidazole lactate	1.15	1.2	1.33	1.33	1.37	1.18
	N-acetylhistamine	0.57	0.75	0.76	0.74	0.47	0.48
Lysine Metabolism	lysine	1.04	1.04	1.06	1.1	1.07	1.04
	N6-acetyllysine	1.01	1.08	1.15	1.19	1.15	1.17
	N-6-trimethyllysine	1.16	1.21	1.19	1.25	1.22	1.24
	2-aminoadipate	0.89	0.92	1.06	1.19	1.15	1.12
	glutarate (pentanedioate)	0.75	0.64	0.67	0.61	0.63	0.71
	glutaryl carnitine (C5)	1.19	1.32	1.43	1.46	1.53	1.47
	3-methylglutaryl carnitine (1)	1.41	1.43	1.68	1.95	2.2	1.74
	N-acetyl-cadaverine	1.14	1.21	1.01	0.98	0.94	0.82
Phenylalanine and Tyrosine Metabolism	phenylalanine	1.01	0.99	1	0.94	0.89	0.92
	N-acetylphenylalanine	0.87	0.81	0.81	0.8	0.77	0.8
	phenylpyruvate	0.92	1.02	0.98	1.08	0.8	1.09
	phenyllactate (PLA)	0.7	0.68	0.72	0.74	0.74	0.69
	4-hydroxyphenylacetate	0.55	0.84	0.89	0.72	0.41	0.21
	phenylacetylglutamine	0.76	1.12	0.8	0.93	0.72	0.55
	tyrosine	1	0.95	1	0.95	0.88	0.87
	tyramine	0.54	0.95	0.7	0.87	0.47	0.43
	3-(4-hydroxyphenyl)lactate	1.09	1.09	1.15	1.16	1.17	1.09
	phenol sulfate	1.06	0.93	1.46	1.51	1.22	1.27
	o-cresol sulfate	0.68	0.49	0.26	0.27	0.36	0.47
	3-methoxytyrosine	1	0.94	1.13	1.09	1.16	1.3
	Gentisate	0.79	0.85	0.58	0.72	0.45	0.59

**Table 3.7 (cont.)**

	3-[3-(sulfooxy)phenyl]propanoic acid	0.79	0.86	0.47	0.56	0.47	0.47
	3-(3-hydroxyphenyl)propionate	0.68	0.75	0.45	0.52	0.45	0.48
	3-(4-hydroxyphenyl)propionate	0.45	0.57	0.37	0.32	0.31	0.28
	4-hydroxyphenylacetyl glycine	0.67	0.79	0.79	0.63	0.76	0.71
	2-hydroxyphenylacetate	0.78	0.93	0.86	0.8	1	0.82
	4-hydroxycinnamate sulfate	0.66	0.69	0.43	0.38	0.31	0.28
Tryptophan Metabolism	tryptophan	1.06	1.03	1.1	1.03	0.99	1.02
	N-acetyltryptophan	0.85	0.78	0.76	0.65	0.68	0.7
	indolelactate	0.87	0.87	0.91	0.89	0.87	0.83
	indoleacetate	0.9	0.85	0.93	0.86	0.73	0.59
	indolepropionate	0.71	0.66	0.64	0.59	0.61	0.56
	3-indoxyl sulfate	0.92	0.87	0.7	0.98	0.75	0.71
	kynurenine	1.04	1.05	1.16	1.18	1.19	1.19
	kynurenate	1.03	1.04	1.1	1.08	1.21	1.12
	picolinate	0.95	0.89	0.72	0.58	0.58	0.48
	5-hydroxyindoleacetate	0.87	0.84	0.81	0.77	0.94	0.95
	tryptophan betaine	1.03	1.02	1.14	1.29	1.77	2.25
	indole-3-carboxylic acid	0.82	0.79	0.78	0.53	0.49	0.49
	C-glycosyltryptophan	0.94	0.86	0.96	0.96	1.01	0.91
Leucine, Isoleucine and Valine Metabolism	leucine	1.02	1	0.98	0.93	0.89	0.95
	N-acetylleucine	0.89	0.83	0.82	0.81	0.75	0.85
	isovalerate	1.02	1.01	0.97	0.91	0.8	0.67
	isovaleryl glycine	1.21	1.08	1.18	1.28	1.41	1.38
	isovalerylcarnitine	0.79	0.71	0.9	0.77	0.86	0.77
	alpha-hydroxyisovaleroyl carnitine*	0.86	0.84	0.73	0.76	0.64	0.63
	alpha-hydroxyisovalerate	0.96	0.9	0.96	1.02	1.11	1.04
	methylsuccinate	1.28	1.15	1.04	1.13	1.08	0.95
	allo-isoleucine	0.97	1.26	1.22	1.55	1.42	1.18
	3-methyl-2-oxovalerate	1.1	1.04	1.11	1.14	1.1	1.16
	2-hydroxy-3-methylvalerate	0.57	0.55	0.6	0.65	0.7	0.66
	3-hydroxy-2-ethylpropionate	1.32	1.19	1.3	1.63	1.53	1.48
	ethylmalonate	1.14	1.09	1.06	0.98	0.93	0.79
	isobutyryl glycine	1.24	1.06	0.84	1.24	1.25	1.51
	alpha-hydroxyisocaproate	0.94	0.99	1.01	1.06	1.19	1.18
	6-hydroxynorleucine	1.1	1.14	1.18	1.24	1.25	1.15
Methionine, Cysteine, SAM and Taurine Metabolism	methionine	0.91	0.88	0.85	0.76	0.71	0.79
	N-acetylmethionine	0.85	0.83	0.79	0.83	0.8	0.8
	N-formylmethionine	1.04	1.06	1.07	1.1	1.11	1.1
	methionine sulfoxide	0.73	0.74	0.71	0.6	0.6	0.69
	S-adenosylhomocysteine (SAH)	0.63	0.56	0.63	0.72	0.46	0.5
	Cystathionine	0.8	0.8	0.79	0.68	0.76	0.76

**Table 3.7 (cont.)**

	2-aminobutyrate	1.23	1.2	1.29	1.5	1.41	1.28
	S-methylcysteine	1.05	1.14	1.1	1.08	1.18	1.17
	hypotaurine	0.31	0.31	0.35	0.41	0.36	0.28
	taurine	0.72	0.72	0.72	0.72	0.68	0.62
	N-acetyltaurine	0.86	0.86	0.82	0.81	0.84	0.74
Urea cycle; Arginine and Proline Metabolism	urea	1.05	0.95	0.93	0.83	0.83	0.79
	proline	0.91	0.93	0.95	0.92	0.91	0.97
	citrulline	0.96	0.94	0.98	0.91	0.87	0.85
	homocitrulline	1.15	1.11	1.11	1.12	1.07	0.98
	dimethylarginine (SDMA + ADMA)	1.14	1.18	1.25	1.3	1.39	1.13
	N-delta-acetylornithine	0.94	0.89	0.86	0.81	0.77	0.74
	N-methylproline	0.86	0.88	0.8	0.8	0.74	0.8
	trans-4-hydroxyproline	0.91	1.03	0.97	0.86	1.01	1.09
	pro-hydroxy-pro	1.16	1.47	1.63	1.55	1.5	1.92
Creatine Metabolism	creatine	0.68	0.66	0.8	0.84	0.85	0.71
	creatinine	1.1	1.1	1.13	1.18	1.19	1.17
	creatine phosphate	0.46	0.55	0.68	0.72	0.75	0.64
	guanidinoacetate	0.94	1.07	1.29	1.35	1.34	1.24
Glutathione Metabolism	5-methylthioadenosine (MTA)	0.66	0.6	0.82	0.8	0.67	0.58
	N-acetylputrescine	0.97	1.02	1.06	0.98	0.94	0.9
	cysteine-glutathione disulfide	0.88	0.91	1	1.11	1.07	0.99
	ophthalmate	0.99	1.2	2.02	3.68	3.09	2.64
Feline Metabolism	felinine	1.07	1	1.04	1.06	1.11	1.08
	N-acetylfelinine*	1.16	1.08	1.03	1.04	0.98	0.98
<i>Peptides</i>							
Gamma-glutamyl Amino Acid	gamma-glutamylalanine	1.07	1.03	1.24	1.36	1.37	1.28
	gamma-glutamylglutamate	0.73	0.78	0.95	1.03	0.98	0.88
	gamma-glutamylglutamine	1.12	1.12	1.16	1.25	1.2	1.18
	gamma-glutamylisoleucine*	1.23	1.22	1.23	1.35	1.22	1.25
	gamma-glutamylleucine	1.16	1.14	1.17	1.19	1.14	1.15
	gamma-glutamylmethionine	1.02	0.99	0.98	0.92	0.88	0.87
	gamma-glutamylvaline	1.2	1.12	1.15	1.24	1.19	1.14
	gamma-glutamyl-2-aminobutyrate	1.41	1.56	1.77	2.11	1.99	1.91
Dipeptide Derivative	carnosine	1.03	0.99	0.95	0.93	0.95	0.85
	N-acetylcarnosine	1.24	1.33	1.37	1.37	1.27	1.36
	anserine	1.08	1.09	1.11	1.16	1.22	1.17
Dipeptide	glycylleucine	0.73	0.73	0.81	0.73	0.52	0.79
	prolylglycine	1.27	1.35	1.34	1.49	1.38	1.33
	valylglycine	0.8	1.18	0.96	0.86	0.85	0.67

<sup>a</sup> For each metabolite, mean value is the group mean of re-scaled data to have median equal to 1.

<sup>b</sup> Mean values in green were decreased, whereas in red were increased with *P*- and *q*- values < 0.05. *P* values were calculated from one way Anova; *q*- values were used to estimate the false discovery rate (FDR) in multiple comparisons.

**Table 3.8.** Serum metabolite alterations in carbohydrate and energy metabolism of cats during weight loss

Metabolic Pathway	Metabolite	Fold change <sup>a, b</sup>					
		wk 1	wk 2	wk 4	wk 8	wk 12	wk 16
		wk 0	wk 0	wk 0	wk 0	wk 0	wk 0
Carbohydrates							
Glycolysis, GNG, and Pyruvate Metabolism	lactate	0.68	0.68	0.68	0.8	0.84	0.74
	glycerate	0.88	0.81	0.85	0.85	0.84	0.84
Pentose Metabolism	ribose	0.68	0.52	0.48	0.59	0.73	0.59
	arabitol	0.98	0.96	0.8	0.81	0.92	0.8
Fructose, Mannose and Galactose Metabolism	fructose	1.15	1.22	1.18	1.21	1.29	1.25
	mannose	1.27	1.23	1.19	1.21	1.18	1.11
Aminosugar Metabolism	glucuronate	1.03	0.98	0.93	0.9	0.91	0.85
	N-acetylneuraminate	0.74	0.72	0.61	0.62	0.56	0.54
	erythronate*	1.12	1.11	1.08	1.07	1.14	1.07
Energy							
TCA Cycle	citrate	1.09	1.07	0.99	0.98	1.05	1.09
	alpha-ketoglutarate	0.83	0.81	0.85	0.94	0.92	0.95
	succinylcarnitine	1.18	1.23	1.33	1.51	1.65	1.53
	succinate	0.81	0.83	0.84	0.91	0.91	0.83
	fumarate	0.56	0.54	0.59	0.75	0.7	0.69
	tricarballylate	1.04	0.91	1.6	0.7	0.54	0.48
Oxidative Phosphorylation	phosphate	0.93	0.93	0.91	0.92	0.9	0.88

<sup>a</sup> For each metabolite, mean value is the group mean of re-scaled data to have median equal to 1.

<sup>b</sup> Mean values in green were decreased, whereas in red were increased with *P*- and *q*- values < 0.05. *P* values were calculated from one way Anova; *q*- values were used to estimate the false discovery rate (FDR) in multiple comparisons.



**Table 3.9.** Serum metabolite alterations in nucleotide, xenobiotic, and cofactor and vitamin metabolism of cats during weight loss

Metabolic Pathway	Metabolite	Fold change					
		wk 1	wk 2	wk 4	wk 8	wk 12	wk 16
		wk 0	wk 0	wk 0	wk 0	wk 0	wk 0
Nucleotide							
Purine Metabolism, (Hypo)Xanthine/ Inosine containing	inosine	1.02	0.96	1.01	0.97	0.97	0.89
	hypoxanthine	0.81	0.74	0.72	0.78	0.8	0.7
	xanthine	0.48	0.44	0.51	0.66	0.63	0.7
	2'-deoxyinosine	0.46	0.51	0.45	0.55	0.74	0.63
	urate	0.74	0.7	0.77	0.84	0.74	0.84
	allantoic acid	1.21	1.25	1.26	0.81	1.26	1.05
Purine Metabolism, Adenine containing	N6-methyladenosine	1.1	1.33	1.6	1.66	1.62	1.63
	N6-carbamoylthreonyladenosine	1.02	1.12	1.27	1.36	1.3	1.22
Purine Metabolism, Guanine containing	guanosine	0.98	0.83	0.78	0.9	0.91	0.76
	guanine	0.68	0.58	0.49	0.65	0.62	0.61
	7-methylguanine	1.08	1.06	1.07	1.06	1.11	1.12
Pyrimidine Metabolism, Orotate containing	orotate	1.1	1.08	1.17	1.26	1.38	1.2
	orotidine	0.96	1.15	1.29	1.46	1.5	1.11
Pyrimidine Metabolism, Uracil containing	uridine	0.82	0.69	0.68	0.77	0.7	0.61
	uracil	0.53	0.46	0.6	0.65	0.69	0.62
	pseudouridine	1.08	1.07	1.06	1.05	1.09	1.03
	2'-deoxyuridine	0.63	0.6	0.68	0.86	0.79	0.75
	3-ureidopropionate	1.08	1.11	1.2	1.21	1.25	1.12
	N-acetyl-beta-alanine	0.88	0.91	0.85	0.91	0.93	0.87
Pyrimidine Metabolism, Cytidine containing	cytidine 5'-monophosphate (5'-CMP)	0.63	0.72	0.39	0.66	0.52	0.4
	cytidine	0.89	0.86	0.8	0.79	0.67	0.68
	5-methylcytidine	0.91	0.9	0.84	0.76	1	1.16
	N4-acetylcytidine	1.13	1.11	1.27	1.31	1.21	1.22
	2'-deoxycytidine	1.02	1.02	1.06	1.05	1.11	1.12
	5-methyl-2'-deoxycytidine	0.95	0.98	0.99	0.84	0.93	1.02
Pyrimidine Metabolism, Thymine containing	3-aminoisobutyrate	1.11	1.25	1.13	1.29	1.19	1.18
Xenobiotics							
Benzoate Metabolism	2-hydroxyhippurate (salicylurate)	0.89	0.93	0.88	0.73	0.64	0.62
	3-hydroxyhippurate	0.95	0.85	0.52	0.5	0.49	0.43
	4-hydroxyhippurate	0.87	0.87	0.58	0.58	0.58	0.5
	mandelate	1.08	0.99	0.86	0.76	1.3	1.27
	3-methyl catechol sulfate (1)	0.8	0.94	0.68	0.61	0.92	1.04
	4-methylcatechol sulfate	0.95	1.13	0.63	1	0.64	0.82
	4-ethylphenylsulfate	0.41	0.46	0.24	0.34	0.26	0.22
	4-vinylphenol sulfate	0.49	0.36	0.31	0.24	0.19	0.19
	3-(2-hydroxyphenyl)propionate	0.82	0.75	0.63	0.58	0.46	0.66

**Table 3.9 (cont.)**

	3-methoxycatechol sulfate (2)	0.45	0.67	0.38	0.55	0.46	0.46
	methyl-4-hydroxybenzoate sulfate	0.35	0.22	0.14	0.18	0.24	0.49
Food Component/ Plant	2-piperidinone	0.83	0.81	0.74	0.81	0.85	0.76
	gluconate	0.64	0.63	0.76	0.71	0.37	0.34
	cinnamoylglycine	1.58	1.73	1.09	2.27	1.32	2
	equol sulfate	0.67	0.84	0.47	0.41	0.17	0.07
	ergothioneine	0.85	0.8	0.79	0.74	0.64	0.57
	ferulic acid 4-sulfate	0.37	0.65	0.35	0.08	0.14	0.23
	indoleacrylate	0.81	0.85	0.84	0.7	0.72	0.73
	thymol sulfate	0.72	0.57	0.41	0.22	0.14	0.1
	4-allylphenol sulfate	1.05	1.06	1.14	1.28	1.32	1.44
	methyl glucopyranoside (alpha + beta)	0.93	0.92	0.8	0.63	0.55	0.39
	4-vinylguaiacol sulfate	1.8	1.34	1.2	1	1	0.81
	pyrraline	0.82	0.83	0.72	0.6	0.72	0.78
	eugenol sulfate	0.82	0.94	0.92	0.83	0.65	0.51
Drug	4-acetylphenol sulfate	0.4	0.44	0.44	0.26	0.89	0.29
	6-oxopiperidine-2-carboxylic acid	1.13	1.11	1.04	1.04	1.16	1
	hydroquinone sulfate	0.7	0.78	0.46	0.47	0.44	0.51
	salicylate	0.73	0.71	0.65	0.5	0.34	0.31
Chemical	1,2-propanediol	0.83	0.67	0.83	0.76	0.78	0.78
	2-pyrrolidinone	0.97	0.89	0.94	0.92	1.01	1.64
	O-sulfo-L-tyrosine	0.84	0.8	0.76	0.77	0.8	0.69
	ethyl glucuronide	0.17	0.24	0.31	0.28	0.91	1.36
	2-aminophenol sulfate	0.83	0.91	0.67	0.63	0.54	0.6
	2-ethylhexanoate	1.05	1.05	1.1	1.1	1.04	1.09
	2-hydroxyisobutyrate	1.18	1.27	1.34	1.63	1.9	1.87
	dimethyl sulfone	1.4	1.31	1.29	1.2	0.94	0.94
	ectoine	1.05	1.03	1.14	1.22	1.23	1.12
	3-hydroxypyridine sulfate	0.69	0.74	0.54	0.45	0.65	0.78
<i>Vitamins and Cofactors</i>							
Nicotinate and Nicotinamide Metabolism	nicotinamide	0.43	0.44	0.61	0.62	0.57	0.55
	1-methylnicotinamide	0.83	0.7	0.7	0.58	0.7	0.37
	trigonelline (N'-methylnicotinate)	0.99	0.92	0.93	0.83	0.81	0.9
	N1-Methyl-2-pyridone-5-carboxamide	1.06	0.99	0.95	0.89	0.83	0.59
Riboflavin Metabolism	riboflavin (Vitamin B2)	0.95	0.81	0.75	0.69	0.61	0.77
Pantothenate and CoA Metabolism	pantothenate	0.98	0.94	0.95	0.96	0.92	0.86
Ascorbate and Aldarate Metabolism	threonate	0.98	0.99	0.98	0.96	0.97	0.91
	oxalate (ethanedioate)	0.92	0.9	0.97	0.89	0.92	0.88
	gulonic acid*	1.14	1.07	1.01	0.92	0.95	0.88
Tocopherol Metabolism	alpha-tocopherol	0.94	0.97	0.92	0.89	0.91	0.94

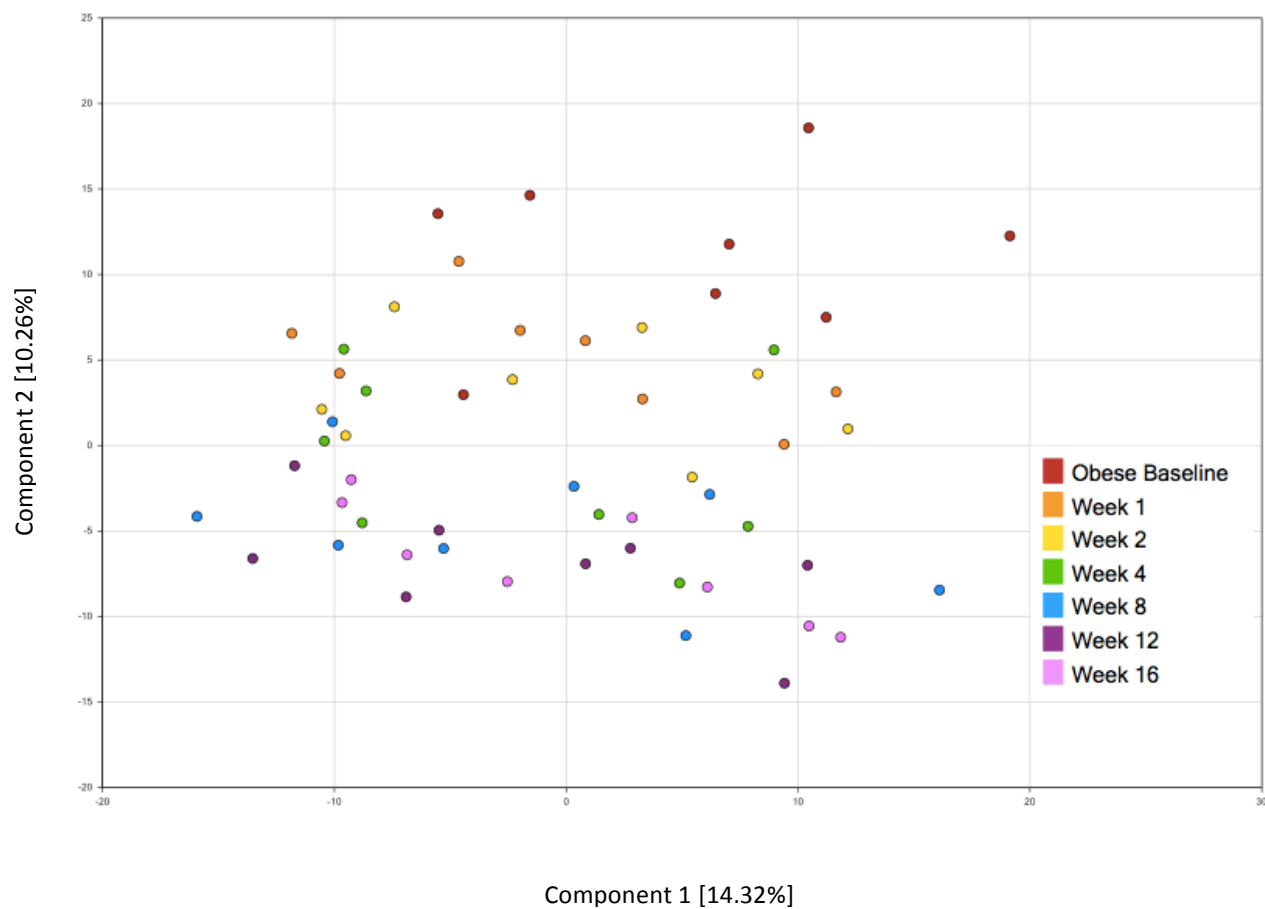
**Table 3.9 (cont.)**

	delta-tocopherol	0.68	0.69	0.59	0.71	0.55	0.63
	gamma-tocopherol	0.82	0.89	0.76	0.64	0.55	0.56
	alpha-CEHC sulfate	1.07	1.06	1.09	1.44	1.44	1.53
Hemoglobin and Porphyrin Metabolism	bilirubin (Z,Z)	1.04	0.74	0.87	0.58	0.87	0.71
	bilirubin (E,E)*	2.06	1.58	1.18	0.85	1.63	1.91
	biliverdin	1.12	0.86	0.94	0.42	0.83	0.66
Vitamin B6 Metabolism	pyridoxine (Vitamin B6)	0.72	0.74	0.69	0.74	0.54	0.46
	pyridoxal	1.39	1.42	1.55	1.39	1.12	1.42
	pyridoxate	1.19	1.21	1.16	1.06	1.01	0.96

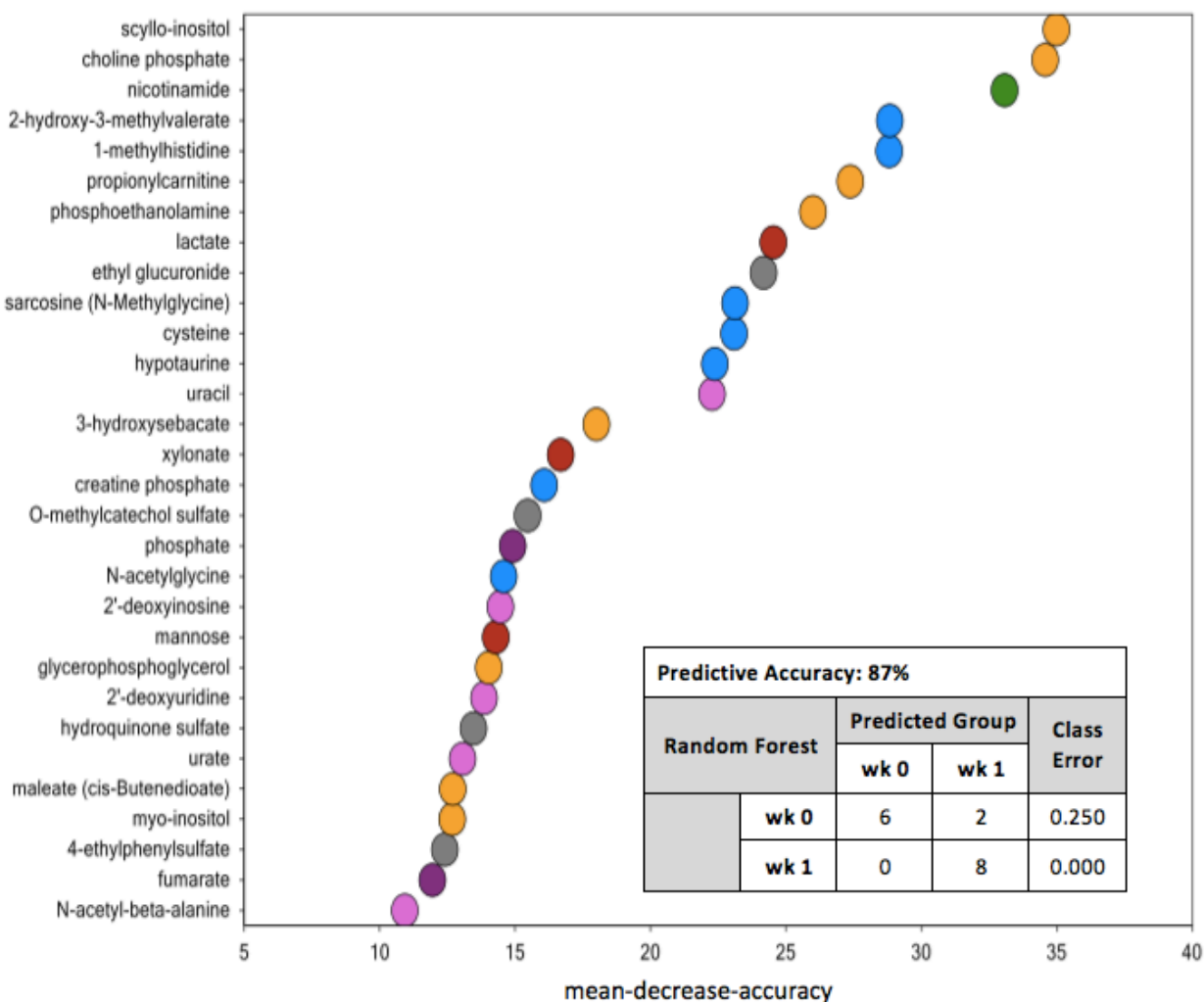
<sup>a</sup> For each metabolite, mean value is the group mean of re-scaled data to have median equal to 1.

<sup>b</sup> Mean values in green were decreased, whereas in red were increased with *P*- and *q*- values < 0.05. *P* values were calculated from one way Anova; *q*- values were used to estimate the false discovery rate (FDR) in multiple comparisons.

**Figure 3.1.** Principal component analysis (PCA) representing the metabolite profiles of cats during weight loss.



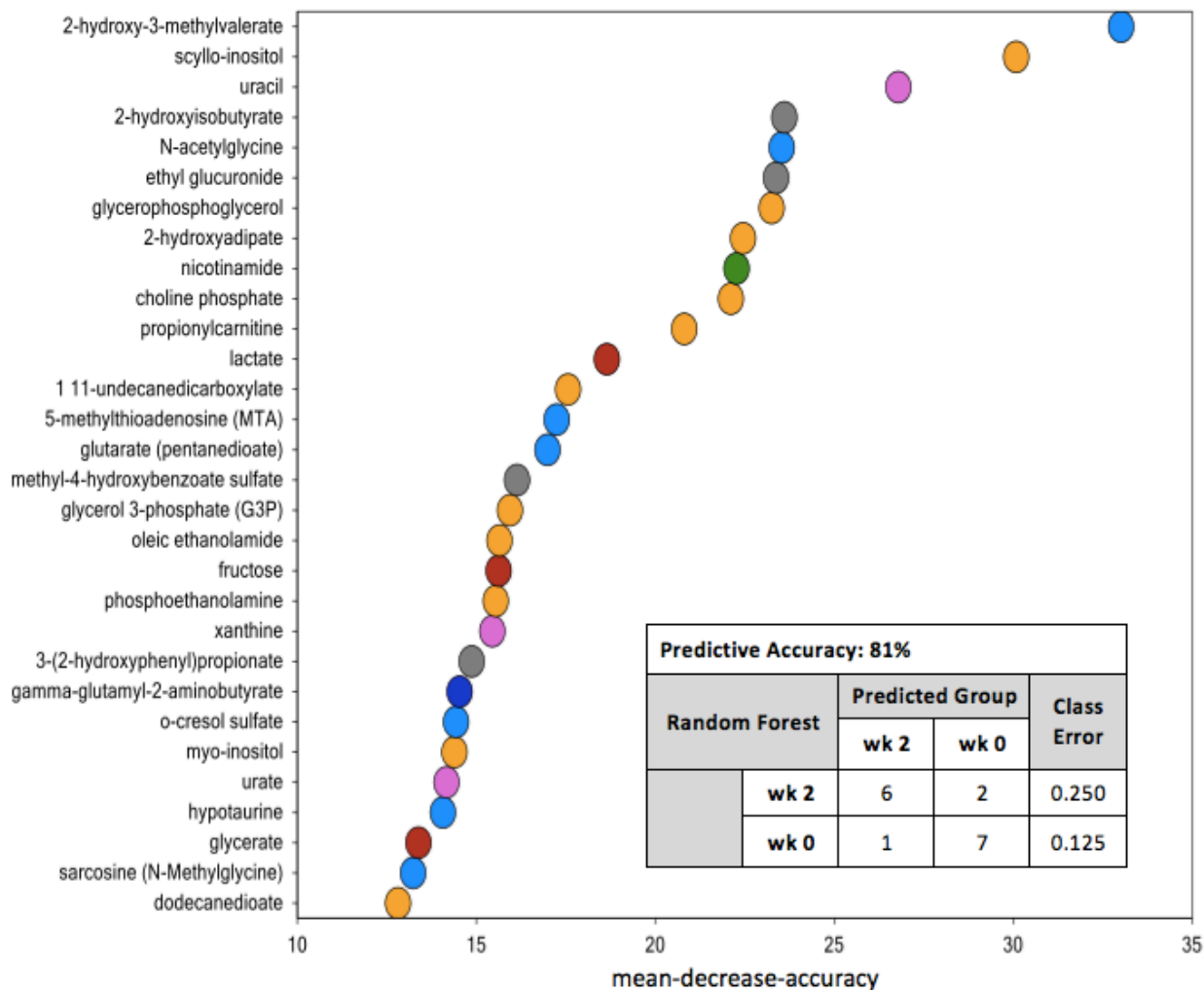
**Figure 3.2.** Random forest analysis: Metabolites having greatest predictability for cats at baseline (fed to maintain ideal BW) or during weight loss for a 16 wk-period using random forest analysis. The metabolites are ranked according to their contribution to the predictive accuracy. The top 30 metabolites are listed on the y-axis by importance. The mean decrease in accuracy for each metabolite is plotted on the x-axis. Insets present the prediction accuracy of the separation of week. Classification of cats at wk 0 versus wk 1 out of bag (OOB) error rate is 13.0%



Color by Super Pathway

- Amino Acid
- Carbohydrate
- Cofactors and Vitamins
- Complex Lipids
- Energy
- Lipid
- Nucleotide
- Peptide
- Xenobiotics
- Unknown

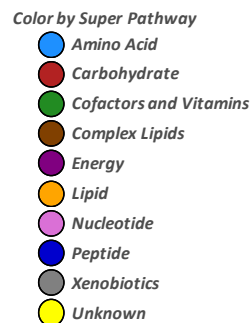
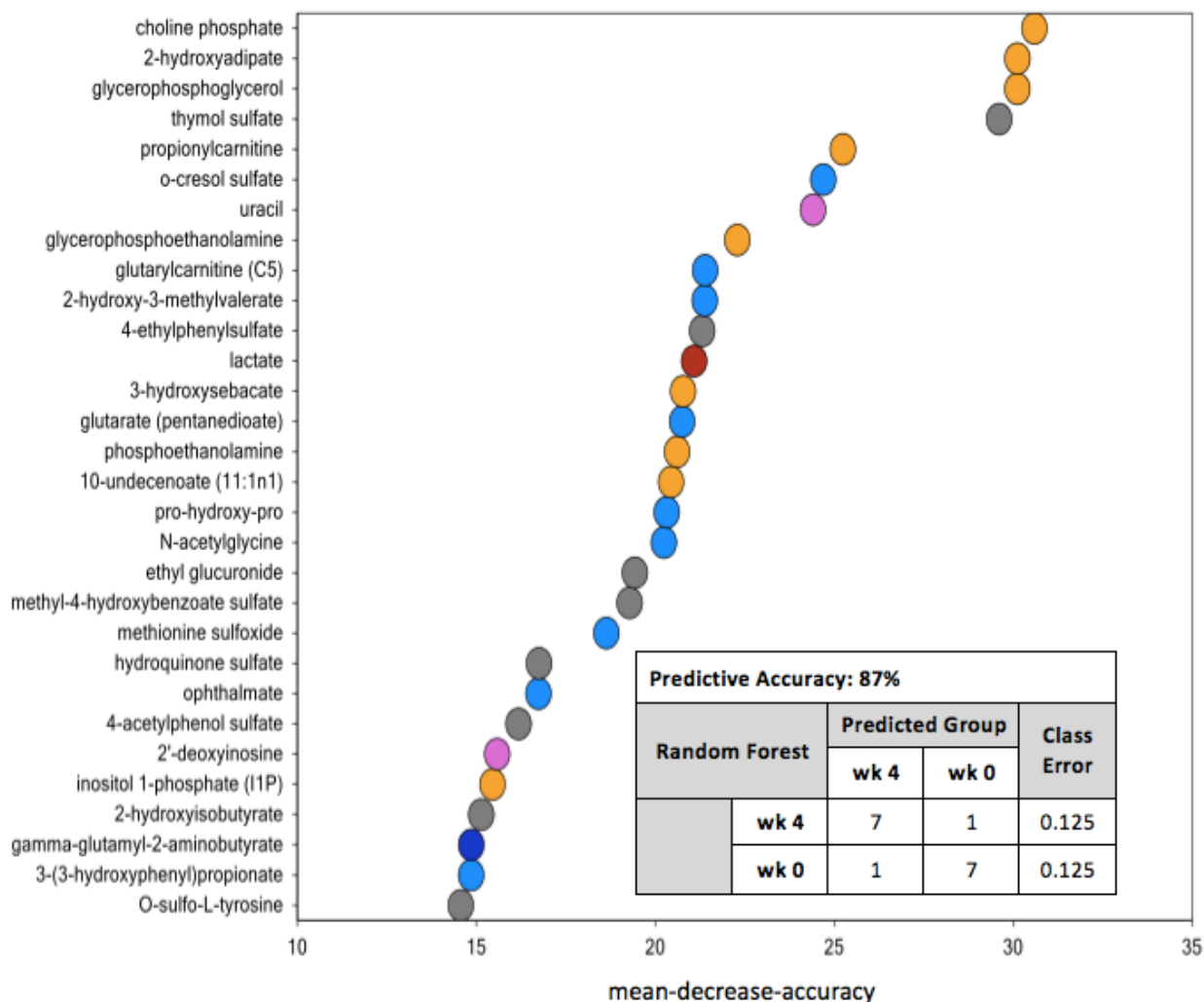
**Figure 3.3.** Random forest analysis: Metabolites having greatest predictability for cats at baseline (fed to maintain ideal BW) or during weight loss for a 16 wk-period using random forest analysis. The metabolites are ranked according to their contribution to the predictive accuracy. The top 30 metabolites are listed on the y-axis by importance. The mean decrease in accuracy for each metabolite is plotted on the x-axis. Insets present the prediction accuracy of the separation of week. Classification of cats at wk 0 versus wk 2 out of bag (OOB) error rate is 19.0%



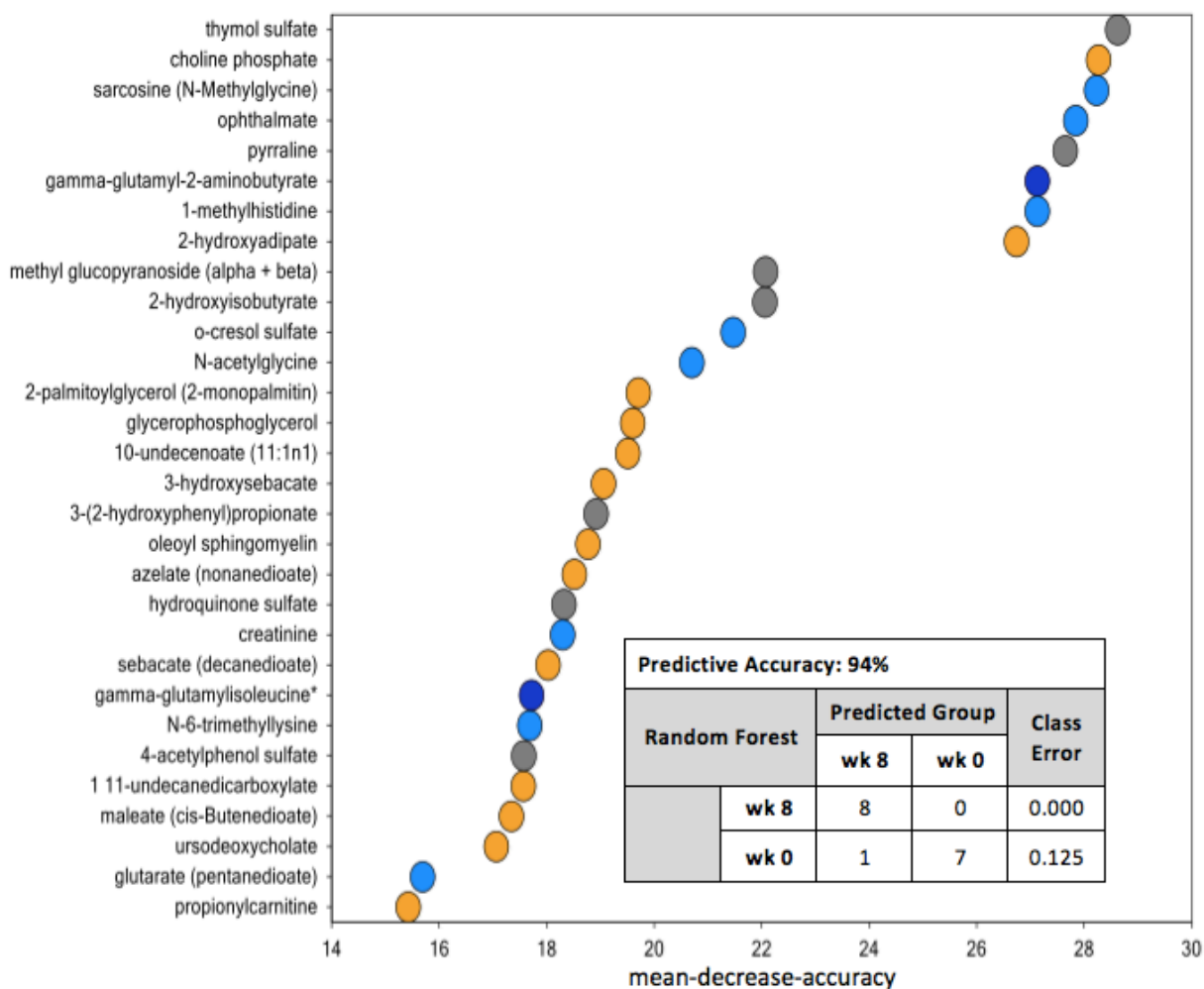
Color by Super Pathway

- Amino Acid
- Carbohydrate
- Cofactors and Vitamins
- Complex Lipids
- Energy
- Lipid
- Nucleotide
- Peptide
- Xenobiotics
- Unknown

**Figure 3.4.** Random forest analysis: Metabolites having greatest predictability for cats at baseline (fed to maintain ideal BW) or during weight loss for a 16 wk-period using random forest analysis. The metabolites are ranked according to their contribution to the predictive accuracy. The top 30 metabolites are listed on the y-axis by importance. The mean decrease in accuracy for each metabolite is plotted on the x-axis. Insets present the prediction accuracy of the separation of week. Classification of cats at wk 0 versus wk 4 out of bag (OOB) error rate is 13.0%

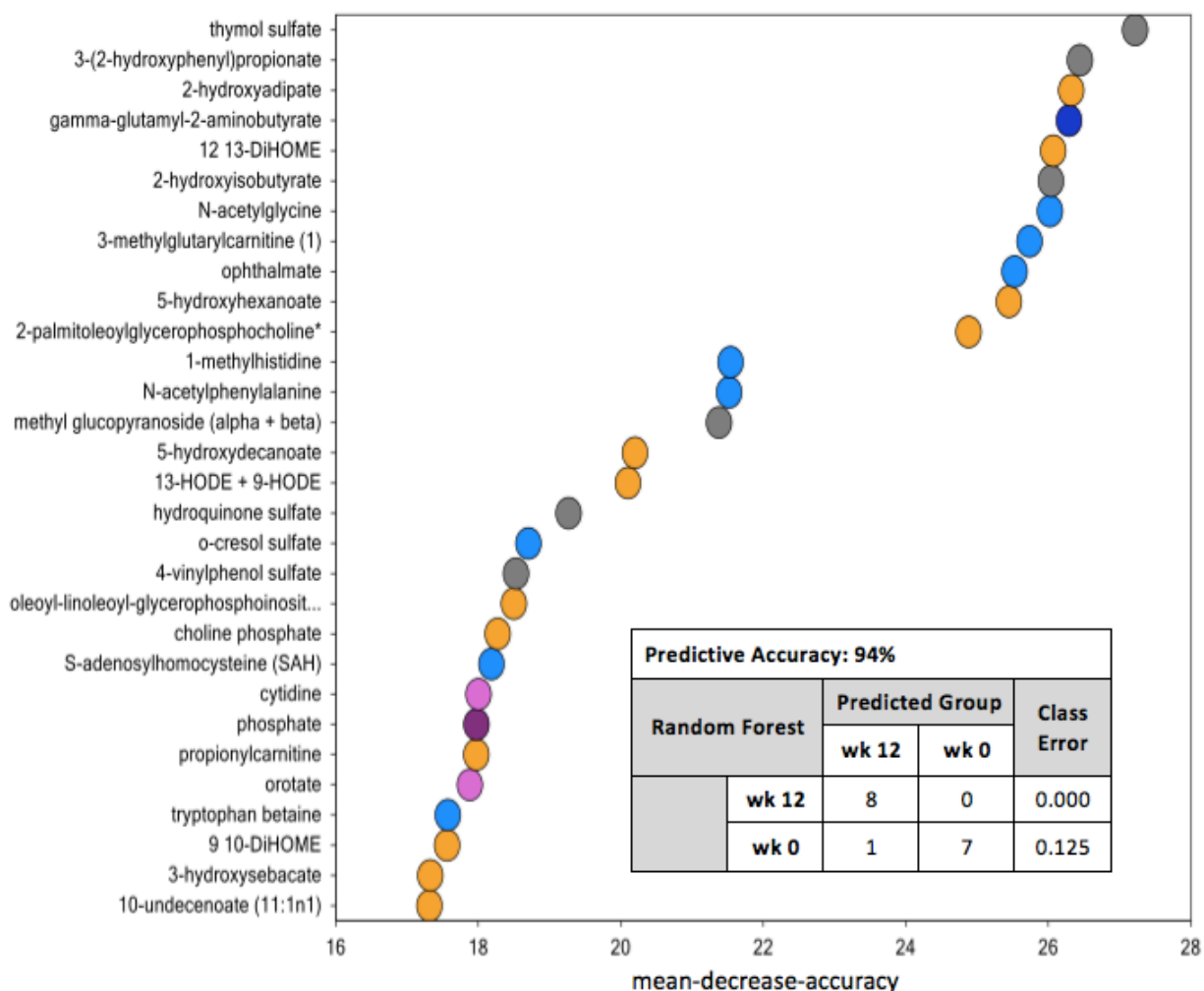


**Figure 3.5.** Random forest analysis: Metabolites having greatest predictability for cats at baseline (fed to maintain ideal BW) or during weight loss for a 16 wk-period using random forest analysis. The metabolites are ranked according to their contribution to the predictive accuracy. The top 30 metabolites are listed on the y-axis by importance. The mean decrease in accuracy for each metabolite is plotted on the x-axis. Insets present the prediction accuracy of the separation of week. Classification of cats at wk 0 versus wk 8 out of bag (OOB) error rate is 6.0%





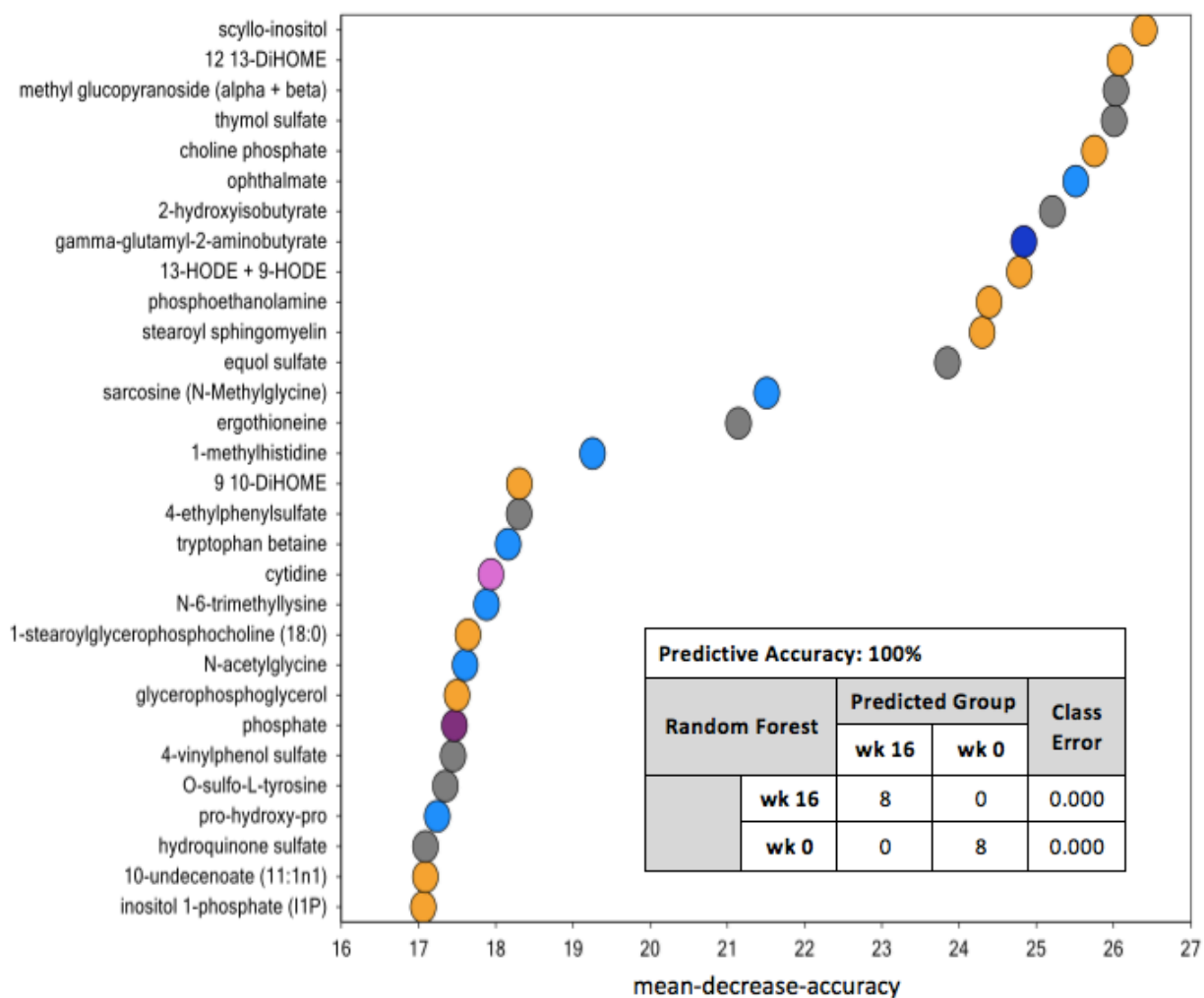
**Figure 3.6.** Random forest analysis: Metabolites having greatest predictability for cats at baseline (fed to maintain ideal BW) or during weight loss for a 16 wk-period using random forest analysis. The metabolites are ranked according to their contribution to the predictive accuracy. The top 30 metabolites are listed on the y-axis by importance. The mean decrease in accuracy for each metabolite is plotted on the x-axis. Insets present the prediction accuracy of the separation of week. Classification of cats at wk 0 versus wk 12 out of bag (OOB) error rate is 6.0%



Color by Super Pathway

- Amino Acid
- Carbohydrate
- Cofactors and Vitamins
- Complex Lipids
- Energy
- Lipid
- Nucleotide
- Peptide
- Xenobiotics
- Unknown

**Figure 3.7.** Random forest analysis: Metabolites having greatest predictability for cats at baseline (fed to maintain ideal BW) or during weight loss for a 16 wk-period using random forest analysis. The metabolites are ranked according to their contribution to the predictive accuracy. The top 30 metabolites are listed on the y-axis by importance. The mean decrease in accuracy for each metabolite is plotted on the x-axis. Insets present the prediction accuracy of the separation of week. Classification of cats at wk 0 versus wk 16 out of bag (OOB) error rate is 0.0%



Color by Super Pathway

- Amino Acid
- Carbohydrate
- Cofactors and Vitamins
- Complex Lipids
- Energy
- Lipid
- Nucleotide
- Peptide
- Xenobiotics
- Unknown

## Chapter 4

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