

Article

Serum Lipid, Amino Acid and Acylcarnitine Profiles of Obese Cats Supplemented with Dietary Choline and Fed to Maintenance Energy Requirements

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Simple Summary: Research has estimated that the majority of domestic cats are overweight or obese. Current weight-loss plans tend to have disappointing outcomes and are not without risk. During periods of severe energy restriction, obesity predisposes cats to developing fatty liver. Choline has been linked to fat metabolism in other animals but has not been studied in cats. Twelve obese cats were split into two groups and were fed a control diet ($n = 6$; 4587 mg choline/kg dry matter) or a high choline diet ($n = 6$; 18,957 mg choline/kg DM) for 5 weeks. Cats were fed to maintain body weight. Choline increased serum cholesterol, triacylglycerides, lipoproteins, and plasma methionine. It also decreased serum blood urea nitrogen and alkaline phosphatase as well as the ratio of plasma acylcarnitine to free carnitine. The results suggest that choline supplementation may increase fat transport out of the liver and help maintain liver health in obese cats. Choline supplementation may prove useful for safe weight loss in obese cats by minimizing the risks of fatty liver.

Abstract: Obesity is a health concern for domestic cats. Obesity and severe energy restriction predispose cats to feline hepatic lipidosis. As choline is linked to lipid metabolism, we hypothesized that dietary choline supplementation would assist in reducing hepatic fat through increased lipoprotein transport and fatty acid oxidation. Twelve obese cats (body condition score [BCS] $\geq 8/9$) were split into two groups. Cats were fed a control ($n = 6$; 4587 mg choline/kg dry matter [DM]) or a high choline diet ($n = 6$; 18,957 mg choline/kg DM) for 5 weeks, for adult maintenance. On days 0 and 35, fasted blood was collected, and the body composition was assessed. Serum lipoprotein and biochemistry profiles, plasma amino acids and plasma acylcarnitines were analyzed. The body weight, BCS and body composition were unaffected ($p > 0.05$). Choline increased the serum cholesterol, triacylglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol and plasma methionine ($p < 0.05$) and decreased the serum blood urea nitrogen and alkaline phosphatase ($p < 0.05$). Choline also reduced the plasma acylcarnitine to free carnitine ratio ($p = 0.006$). Choline may assist in eliminating hepatic fat through increased fat mobilization and enhanced methionine recycling.

Keywords: methyl donor; one carbon; obesity; methionine; fatty liver

1. Introduction

Akin to the obesity epidemic in humans, obesity is largely prevalent in domestic cats. Globally, it is estimated that 19–63% of cats are obese, depending on the country and the criteria used for classification [1–11]. This high number of obese animals is of concern, as

obesity has the potential to lead to damaging health conditions including osteoarthritis, lower urinary tract diseases and diabetes mellitus [1,3,12]; subsequently resulting in a reduced quality of life [13].

Specifically, obese cats were found to be five times more likely to develop issues with mobility and four times more likely to develop diabetes mellitus, as compared with lean cats (with an optimal body condition) [1]. Numerous risk factors have been implicated with weight gain and obesity in cats. These include environment-specific factors, such as indoor housing, lack of physical exercise and ad libitum feeding [3,7,8]. Animal-specific factors, including age, sex and gonadectomy, have also been implicated as risk factors for the development of obesity [1,3,4,7,9,10,14,15].

Although weight reduction is recommended for cats that are overweight or obese, weight-loss attempts can be disappointing and can also be associated with health risks [16–18]. A weekly weight loss at a rate of 0.5–2% of the initial body weight is clinically considered safe [19,20]. Rapid weight loss that exceeds this recommendation predisposes cats to feline hepatic lipidosis (FHL); defined as an accumulation of lipids within the liver [18]. Feline hepatic lipidosis is a common form of liver disease, affecting an estimated 0.16% of cats in North America [21].

It is most commonly diagnosed in cats that were initially overweight or obese [22]. Although the pathophysiology of FHL is not fully understood, reduced energy intake appears to be the primary factor in its development [23]. It is reported that FHL can occur within 2 days to 2 weeks in a clinical setting, depending on how overweight or obese the animal initially was, in combination with the degree of energy restriction imposed [22,24]. An estimated restriction of 50–75% of a cat's maintenance energy requirements is required in order to induce FHL [25–27]. When left untreated, FHL commonly leads to liver failure and/or death in affected cats [28].

Choline is an essential nutrient that gives rise to numerous important products within the body, including phosphatidylcholine (PC), acetylcholine and betaine [29,30]. By doing so, choline plays a critical role in numerous pathways, including neurotransmission and cell signalling. However, of interest to the topic of FHL and feline obesity, are the roles that choline plays in lipid metabolism and hepatic function. Choline has been metabolically linked to lipid metabolism in other animal models. In livestock, such as poultry and swine, the supplementation of choline or its derivative betaine, has been shown to improve lean carcass composition and decrease the deposition of fat [31–37].

Moreover, supplementation of choline was shown to reduce the accumulation of hepatic fat in obese mice, through increased lipolysis and mobilization of fat outside of the liver [38]. Choline assists in the removal of fat from the liver through synthesis of PC, which is a structural component of very low-density lipoproteins (VLDL) [39]. Moreover, the choline oxidation product, betaine, stimulates homocysteine re-methylation to methionine [40]. By doing so, S-adenosyl-methionine (SAM) is produced, resulting in increases in both PC and carnitine [41]. Carnitine is essential for the mobilization of long-chain fatty acids to the inner membrane of the mitochondria for oxidation [42].

With this in mind, we hypothesized that feeding supplemental choline to obese cats could assist in the elimination of hepatic fat through increased lipoprotein transport and enhanced fatty acid oxidation within the liver. The purpose of this study was to assess the effects of dietary choline supplementation in obese cats fed at maintenance energy requirement on serum lipid, amino acid and acylcarnitine profile. Emphasis was placed on assessing body composition, serum lipid profile, as well as plasma amino acid and acylcarnitine profiles.

2. Materials and Methods

All experimental procedures were approved by the University of Guelph Animal Care Committee (AUP#2494). All procedures were in accordance with national and institutional guidelines for the care and use of animals.

2.1. Animals and Housing

Twelve domestic shorthair cats (six neutered males and six spayed females) were included in this study. All cats were considered chronically obese, with a body condition score (BCS) of $\geq 8/9$ [43]. At the start of the study, the cats had a mean body weight (BW) of 7.4 ± 0.3 kg (mean \pm SEM; range: 5.9–8.9 kg) and a mean age of 9.0 ± 0.7 years (mean \pm SEM; range: 5–13 years). Although chronically obese, all cats were healthy based on a physical exam, complete blood count (CBC), and serum biochemical analysis. The cats were housed at a private contract research facility. Throughout the study cats were kept in their usual group housing. The cats were separated from 4 PM to 7 AM daily, in order to be fed individually. Water was available ad libitum all day for each cat.

2.2. Experimental Diets

The two experimental diets (Elmira Pet Products, Elmira, ON, Canada) used for this research were non-commercial extruded cat foods formulated according to the feline adult nutrient profile of the American Association of Feed Control Officials (AAFCO). Choline chloride (99% choline chloride, Vitacholine, Balchem, New Hampton, NY, USA) was used as the dietary choline supplement. Choline chloride was added into the diets prior to extrusion at 0.19% for the control diet and 2.86% for the high choline diet, aiming at 300 mg per 100 g dry matter (DM) and 2679 mg per 100 g DM based on formulation, respectively. Dietary analysis showed that the control diet contained 459 mg choline per 100 g DM, compared to the high choline diet, which contained 1896 mg per 100 g DM. Both diets had the same ingredient and nutrient profile (Table 1), apart from the additional choline chloride supplementation in the high choline diet.

Table 1. Proximate analysis and dietary analyses of chloride, sodium, selected B-vitamins and amino acids of two experimental diets: a control diet and a high choline diet (DMB).

		Control Diet	High Choline Diet
Moisture	% as fed	5.60	5.05
Protein	% DM	35.53	36.70
Fat	% DM	19.81	16.75
Ash	% DM	6.43	6.47
Crude Fibre	% DM	2.75	3.58
NFE ¹	% DM	35.48	36.50
ME ²	kcal/100g	414.31	400.43
Chloride	% DM	0.77	1.33
Sodium	% DM	0.55	0.59
Choline	mg/100 g	458.69	1895.73
Cobalamine (B12)	μ g/100g	8.47	10.53
Folate (B9)	mg/100g	0.32	0.42
Pyridoxine (B6)	mg/100g	1.02	1.37
Methionine	% DM	0.94	0.94
Cysteine	% DM	0.48	0.47

DMB: dry matter basis; NFE: nitrogen free extract; ME: metabolizable energy Values reported on DMB, except for moisture; ¹ NFE (%) = 100 – moisture – protein – fat – fibre – ash; ² Estimated by a four-step calculation [19]. Ingredients: chicken meal, brewer's rice, corn gluten meal, whole corn, chicken, poultry fat, whole oat flour, yellow pea hulls, whole dried egg, dried chicken, beet pulp, salmon oil, potassium chloride, sodium chloride, choline chloride, DL-methionine, taurine, vitamin and mineral premix, and yucca extract.

The diets were analysed for moisture, protein, crude fat and ash in-house by Elmira Pet Products (Elmira ON, Canada), according to the Association of Official Analytical Chemists (AOAC). Moisture was assessed by gravimetric analysis (AOAC 935.29), crude protein by combustion (AOAC 990.03), crude fat by acid hydrolysis (AOAC 954.02) and ash by gravimetric analysis (AOAC 942.05) [44]. Crude fiber was analysed by SGS Canada Inc. (Guelph, ON, Canada), in accordance with the American Oil Chemists Society (AOCS), using the filter bag technique (AOCS Ba6a-05) [45]. Metabolizable energy was estimated

using predictive equations, according to a four-step calculation based on the calculation of gross energy (GE) and digestible energy (DE) [19].

The choline, cobalamine, pyridoxine and folic acid analyses were performed by Maxxam Analytics (Mississauga, ON, Canada). Choline was assessed by the enzymatic colorimetric method (AOAC 999.14), cobalamine by the turbidimetric method (AOAC 986.23), pyridoxine by the microbiological method (AOAC 985.32 (modified)) and folic acid by the triple enzyme microbiological method (AOAC 2004.5) [44]. The dietary free amino acid (methionine, lysine and threonine) and total amino acid contents of each diet were analysed by AminoLab[®], Evonik Industries (Kennesaw, GA, USA) using high-performance liquid chromatography (HPLC) post-column derivatization (AOAC 999.13) and performic acid oxidation (AOAC 994.12), respectively.

2.3. Experimental Design

Prior to the treatment period, a 4-week adaptation period was implemented in order for the accurate determination of each cat's individual maintenance energy requirements. All cats were fed the control diet during the adaptation period. Initially, the amount of food offered was calculated based on the maintenance energy requirement for obese cats according to the National Research Council (NRC) ($130 \text{ kcal/kg BW}^{0.4}$) [19]. Leftovers from each meal were weighed daily and used to calculate the daily food intake for each cat. The cats were weighed twice weekly, and the BCS was assessed on a nine-point-scale and recorded weekly [43]. The amount of food offered was adjusted to maintain a constant BW and BCS.

At the end of the adaptation period, the cats were divided into two groups, balanced for gender, BW and age. During the 5-week treatment period, one group of cats ($n = 6$) continued to receive the control diet, while the other group ($n = 6$) was fed the high choline diet. Cats continued to receive the same amount of food that was offered at the end of the adaptation period and were shown to maintain a stable BW and BCS. Recording of the food intake, BW and BCS followed the same schedule as during the adaptation period.

2.4. Blood Collection and Analyses

Blood samples (16 mL) were taken from the jugular vein after a 12-h fast on day 0 and day 35 of the treatment period. Whole blood (10 mL) was collected in plastic serum tubes (Plus Plastic Serum Tubes, Vacutainer[®], Becton Dickinson, Franklin Lakes, NJ, USA) for analyses of serum cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), triacylglycerides (TAG), non-esterified fatty acids (NEFA), alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (CREAT), glucose, insulin and leptin.

Serum was obtained after the centrifugation of whole blood at $2500 \times g$ for 15 min at 4°C . Serum was stored at -20°C until analyzed. Whole blood (6 mL) was also collected in EDTA tubes (Spray-coated K2EDTA Tubes, Vacutainer[®], Becton Dickinson, Franklin Lakes, NJ, USA) and stored as dried blood spots (375 μL) using Whatman[™] 903[™] Protein Saver Cards (GE Healthcare Bio-Sciences Corp. Westborough, MA, USA) at -20°C until the amino acid and acylcarnitine analyses.

The serum CHOL, TAG, NEFA, HDL-C, ALP, ALT, BUN, CREAT and glucose concentrations were analysed photometrically at the Animal Health Laboratory, University of Guelph (Guelph, ON, Canada), using a Roche Cobas 6000 c501 Analyzer (Roche Diagnostics, Basel, Switzerland). Very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) were calculated using the Friedewald equation ($\text{VLDL (mmol/L)} = \text{TAG (mmol/L)} / 2.2$; $\text{LDL-C (mmol/L)} = \text{total CHOL (mmol/L)} - \text{HDL-C (mmol/L)} - \text{VLDL (mmol/L)}$). This equation has been established for use in humans and has previously been used in cats [46–48].

Serum insulin concentrations were determined by use of a commercially available ELISA kit (Feline Insulin ELISA, Mercodia AB, Uppsala, Sweden), validated for use in cats [49]. The Bennet Index (BI), the homeostasis model assessment (HOMA), insulin to

glucose ratio (I-G) and the quantitative insulin sensitivity check index (QUICKI) were calculated as previously described by Appleton et al. (2005) [50]. Serum leptin concentrations were determined at the Laboratory of Livestock Physiology, Immunology and Genetics, Department of Biosystems, K.U. Leuven (Leuven, Belgium) using a commercially available RIA kit (Multi-species Leptin RIA kit, Linco Research Inc., St Charles, MO, USA), validated for use in cats [51].

Quantitative electrospray tandem mass spectrometry was performed at the Department of Clinical Chemistry, Laboratory of Metabolic Disorders, University Hospital Ghent (Ghent, Belgium) to determine the plasma free and total carnitine, acylcarnitine and amino acid profile as described by Rizzo et al. (2003) and Vreken et al. (2002) [52,53]. Short-, medium- and long-chain acylcarnitines were calculated as derivatives with carbon chains C2–C5, C6–C12 and C14–C18, respectively. The ratio of plasma acylcarnitines to free carnitine (AC/FC) was calculated by dividing the total concentration of acylcarnitines by the free carnitine concentration.

2.5. Dual Energy X-ray Absorptiometry

The body composition was assessed by dual energy X-ray absorptiometry (DEXA), using a commercially available machine (QDR-4500 Acclaim Series Elite, Hologic Inc, Bedford, MA, USA). The cats were positioned in ventral recumbency on the scanner table for all scans [54]. Scans were performed once at each time point (days 0 and 35) for each cat. Cats were anesthetized by propofol induction at 6.5 mg/kg BW intravenously. Standard monitoring of vital signs was applied throughout anaesthesia. The area, bone mineral content (BMC), bone mineral density (BMD), fat mass (FM), lean body mass (LBM), total mass (TM) and body fat percentage (BF%) were analyzed and calculated from the resulting images of the scans using a commercially available software (Apex, Version 2.3, Hologic Inc, Bedford, MA, USA).

2.6. Statistical Analyses

Statistical analysis was performed using SPSS[®] Statistics (Version 26, IBM[®], New York, NY, USA). Prior to analysis, the data were tested for normality using the Shapiro–Wilk test. For normally distributed data, a repeated measures analysis of variance (ANOVA) model was used, with time as the within subject factor and diet as the between subject factor. The time, diet and the time \times diet interaction were assessed. When any significance occurred, a Bonferroni post-hoc test was performed to assess multiple comparisons for the fixed effects (time and diet) and their respective interaction. Data that was not normally distributed (BCS), was log transformed prior to analysis. Statistical significance was set at $p < 0.05$. The results are expressed as the mean \pm SEM.

3. Results

3.1. Energy, Food and Choline Intake

All cats ate their assigned diet and showed no signs of illness and/or maldigestion. The mean energy intake was similar during the treatment period as during the adaptation period and was also similar between diet groups ($P_{\text{time}} = 0.549$; $P_{\text{time} \times \text{diet}} = 0.994$; and $P_{\text{diet}} = 0.442$). Accordingly, the food intake did not differ between diet groups or between the testing and adaptation periods ($P_{\text{time}} = 0.588$; $P_{\text{time} \times \text{diet}} = 0.987$; and $P_{\text{diet}} = 0.093$).

Cats on the control diet consumed an average of 283.82 ± 9.41 mg choline per day during the treatment period (77.27 ± 4.60 mg choline/kg BW^{0.67}; and 40.85 ± 3.13 mg choline/kg BW). In comparison, the group on the high-choline diet consumed 1252.29 ± 34.23 mg choline per day (321.98 ± 5.71 mg choline/kg BW^{0.67}; and 165.24 ± 4.85 mg choline/kg BW).

3.2. Body Weight, Body Condition Score and Body Composition

There were no changes in the weekly BCS between the two diet groups throughout the trial ($P_{\text{time}} = 0.343$; $P_{\text{time} \times \text{diet}} = 0.453$; and $P_{\text{diet}} = 0.387$). Additionally, there were

no significant effects of time, diet or time \times diet interaction for BW or body composition; including area, BMC, BMD, FM, LBM, TM and BF% ($p > 0.05$; Table 2).

Table 2. Body weight and body composition in 12 chronically obese cats at baseline and after feeding a control food (458.7 mg choline/100g DM) ($n = 6$) or a high-choline food (1895.7 mg choline/100g DM) ($n = 6$) for 5 weeks.

	Control Diet		High Choline Diet		<i>p</i> -Value		
	Baseline	5 Weeks	Baseline	5 Weeks	Time	Diet	T \times D
BCS	8.7 \pm 0.2	8.5 \pm 0.2	9.0 \pm 0.2	8.8 \pm 0.2	0.343	0.387	0.453
BW/kg	7.1 \pm 0.5	7.1 \pm 0.5	7.6 \pm 0.4	7.6 \pm 0.3	0.915	0.491	0.409
DEXA SCAN							
Area/cm ²	578.3 \pm 28.5	574.5 \pm 23.1	600.8 \pm 20.7	586.5 \pm 19.2	0.075	0.607	0.272
BMC/g	167.7 \pm 12.4	167.6 \pm 11.2	167.4 \pm 6.6	164.6 \pm 6.4	0.180	0.901	0.191
BMD g/cm ²	167.7 \pm 12.4	167.6 \pm 11.2	167.4 \pm 6.6	164.6 \pm 6.4	0.180	0.901	0.191
FM/g	2784.8 \pm 295.5	2919.5 \pm 283.5	3034.7 \pm 292.6	2995.0 \pm 357.4	0.497	0.714	0.225
LBM/g	4427.2 \pm 327.7	4300.8 \pm 340.5	4710.1 \pm 146.0	4730.2 \pm 209.1	0.371	0.365	0.226
TM/g	7211.9 \pm 500.6	7220.3 \pm 497.0	7744.8 \pm 345.1	7725.2 \pm 347.8	0.847	0.635	0.412
BF/%	38.4 \pm 2.7	40.3 \pm 2.6	38.7 \pm 2.5	38.2 \pm 3.4	0.394	0.827	0.161

Values expressed as the mean \pm SEM; $n = 6$; values in a row with superscripts without a common letter differ; $p < 0.05$, Repeated measures ANOVA with Bonferroni post-hoc test. T \times D = time \times diet interaction; BW = body weight; DEXA = dual energy X-ray absorptiometry; BMC = bone mineral content; BMD = bone mineral density; FM = fat mass; LBM = lean body mass; TM = total mass; and BF = body fat percentage.

3.3. Serum Lipid Profile

There was a significant time \times diet interaction for serum TAG, HDL-C and VLDL ($P_{\text{time} \times \text{diet}} = 0.038$, $P_{\text{time} \times \text{diet}} = 0.029$ and $P_{\text{time} \times \text{diet}} = 0.038$, respectively), as the serum concentrations increased in the cats consuming the high choline diet after 5 weeks (Table 3; Figure 1). However, individual effects of diet or time were not observed for these serum parameters ($p > 0.05$). Serum CHOL and LDL-C were affected by time ($P_{\text{time}} = 0.017$ and $P_{\text{time}} = 0.001$, respectively), with significant increases being noted from baseline to endpoint in the high choline group ($P_{\text{time} \times \text{diet}} = 0.013$ and $P_{\text{time} \times \text{diet}} = 0.014$, respectively). An individual effect of diet on serum CHOL and LDL-C did not occur ($P_{\text{diet}} = 0.634$ and $P_{\text{diet}} = 0.683$, respectively). There were no changes in the serum NEFA between groups over the 5-week treatment period ($P_{\text{time}} = 0.110$, $P_{\text{time} \times \text{diet}} = 0.279$ and $P_{\text{diet}} = 0.090$).

Table 3. Serum lipid profiles (mmol/L) in 12 chronically obese cats at baseline and after feeding a control food (458.7 mg choline/100 g DM) ($n = 6$) or a high-choline food (1895.7 mg choline/100g DM) ($n = 6$) for 5 weeks.

	Control Diet		High Choline Diet		<i>p</i> -Value		
	Baseline	5 Weeks	Baseline	5 Weeks	Time	Diet	T \times D
CHOL	5.64 \pm 0.384	5.61 \pm 0.296	4.92 \pm 0.210 ^a	5.98 \pm 0.200 ^b	0.017	0.634	0.013
TAG	0.533 \pm 0.033	0.500 \pm 0.026	0.483 \pm 0.070 ^a	0.583 \pm 0.054 ^b	0.260	0.799	0.038
HDL-C	5.02 \pm 0.304	4.93 \pm 0.246	4.51 \pm 0.132 ^a	5.21 \pm 0.164 ^b	0.077	0.679	0.029
NEFA	0.417 \pm 0.034	0.515 \pm 0.027	0.417 \pm 0.046	0.4133 \pm 0.038	0.110	0.279	0.090
LDL-C	0.378 \pm 0.106	0.453 \pm 0.101	0.194 \pm 0.107 ^a	0.503 \pm 0.144 ^b	0.001	0.683	0.014
VLDL-C	0.242 \pm 0.015	0.227 \pm 0.012	0.220 \pm 0.032 ^a	0.265 \pm 0.025 ^b	0.260	0.799	0.038

Values expressed as the mean \pm SEM; $n = 6$; values in a row with superscripts without a common letter differ; $p < 0.05$, Repeated measures ANOVA with Bonferroni post-hoc test. T \times D = time \times diet interaction; CHOL = cholesterol; TAG = triacylglycerides; HDL-C = high-density lipoprotein cholesterol; NEFA = non-esterified fatty acids; LDL-C = low-density lipoprotein cholesterol; and VLDL = very low-density lipoprotein cholesterol.

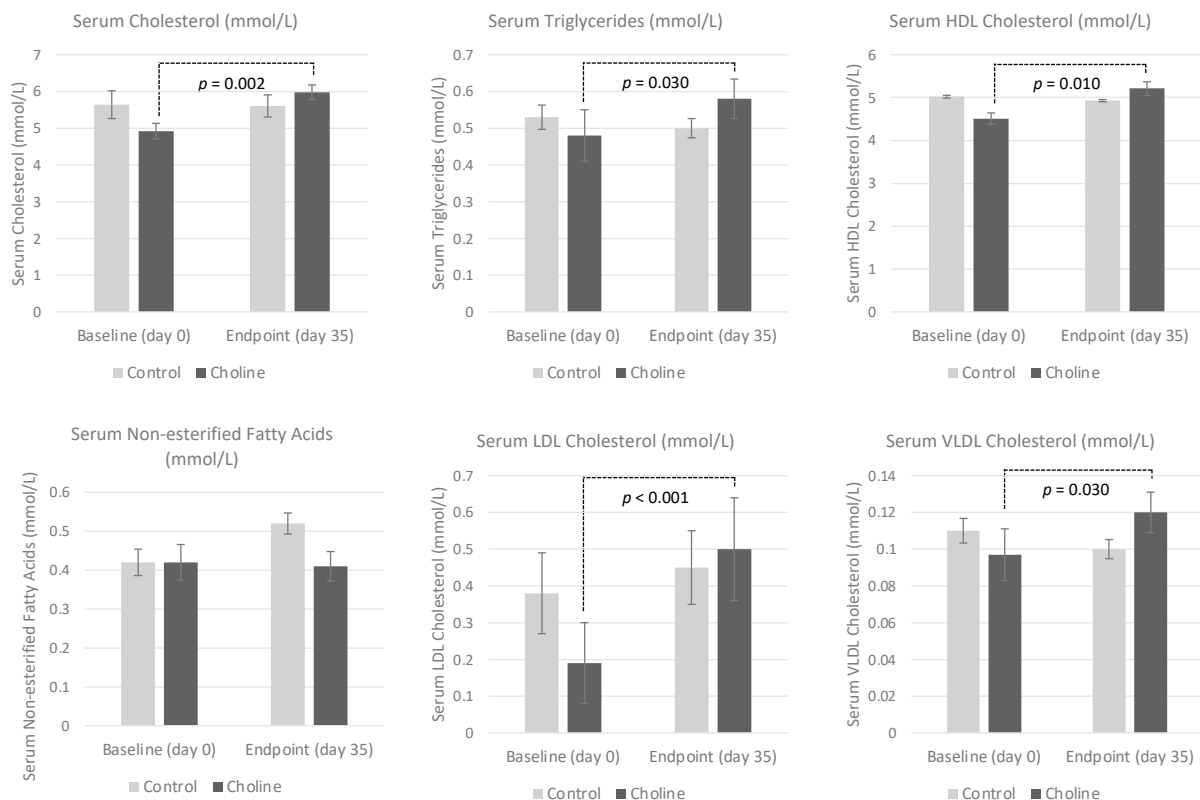


Figure 1. Serum lipoprotein concentrations (mmol/L) in 12 chronically obese cats following 5 weeks of a control extruded cat food (458.7 mg choline/100 g dry matter (DM)) ($n = 6$) or a high-choline extruded cat food (1895.7 mg choline/100 g DM) ($n = 6$). Both groups were fed at maintenance energy requirements. Values are expressed as the mean \pm SEM.

3.4. Serum Liver Enzymes

Although there was no individual effect of diet on the serum ALP ($P_{\text{Diet}} = 0.712$), the concentration of ALP decreased for both groups over the 5-week treatment period ($P_{\text{time}} = 0.012$). The decrease in serum ALP from baseline to endpoint was significant for the cats on the high choline group ($P_{\text{time} \times \text{diet}} = 0.027$). There were no changes in the serum ALT concentrations over time and between groups ($P_{\text{time}} = 0.087$, $P_{\text{time} \times \text{diet}} = 0.319$ and $P_{\text{diet}} = 0.879$).

3.5. Serum Creatinine and Blood Urea Nitrogen

Concentrations of the serum BUN were lower at the endpoint compared to the baseline in both groups ($P_{\text{time}} = 0.027$). However, there were no significant effects of diet or time \times diet interaction ($P_{\text{diet}} = 0.470$ and $P_{\text{time} \times \text{diet}} = 0.099$). The serum CREAT concentrations were not affected by diet and time ($P_{\text{time}} = 0.802$, $P_{\text{diet}} = 0.509$ and $P_{\text{time} \times \text{diet}} = 0.643$).

3.6. Serum Glucose, Insulin and Leptin

Serum glucose concentrations tended to decrease after 5 weeks in the high choline group but not in the control group ($P_{\text{time} \times \text{diet}} = 0.051$). There were no individual effects of time or diet on glucose ($P_{\text{time}} = 0.747$ and $P_{\text{diet}} = 0.467$). There were no effects of time, diet or time \times diet interaction for serum insulin, I-G, BI, HOMA or QUICKI Index ($p > 0.05$). An individual effect of time was present for serum leptin ($P_{\text{time}} = 0.039$), as concentrations decreased for both groups over the 5-week treatment period, yet the effects of diet and time \times diet interaction were not significant ($P_{\text{diet}} = 0.377$ and $P_{\text{time} \times \text{diet}} = 0.224$).

3.7. Plasma Amino Acid Profile

Of the plasma amino acids analyzed (glycine, alanine, valine, leucine, ornithine, methionine, phenylalanine, citrulline and tyrosine), significant changes were only found for methionine ($P_{\text{time}} = 0.010$; $P_{\text{diet}} = 0.190$; and $P_{\text{time} \times \text{diet}} = 0.005$; data not shown). The plasma methionine concentrations ($\mu\text{mol/L}$) increased from the beginning to the end of the treatment period in the high choline group (baseline: 71.63 ± 3.26 ; endpoint: 98.20 ± 5.78); this change did not occur in the control group (baseline: 78.49 ± 3.26 ; endpoint: 76.84 ± 5.47). No diet differences occurred at baseline, yet the plasma methionine concentration at the end of the treatment period was significantly greater for the high choline group as compared to the control group.

3.8. Plasma Acylcarnitine Profile

The plasma free carnitine, total carnitine and total acylcarnitine concentrations were not affected by diet, time or time \times diet interaction ($p > 0.05$; data not shown). An effect of diet was present for AC/FC ratio ($P_{\text{diet}} = 0.006$). The AC/FC ratio at endpoint was significantly lower in the high choline group compared to the control group. No effect of time or diet–time interaction was present ($P_{\text{time}} = 0.296$ and $P_{\text{time} \times \text{diet}} = 0.811$, respectively). No effects of diet, time or time \times diet interaction were seen in combined plasma short-, medium- or long chained acylcarnitine concentrations ($p > 0.05$). An effect of time was present for both malonylcarnitine (C3-DC) and octanoylcarnitine (C8) ($P_{\text{time}} = 0.050$ and $P_{\text{time}} = 0.015$, respectively).

The plasma concentrations of these acylcarnitines decreased in both diet groups after 5 weeks. However, the plasma concentrations of C3-DC and C8 was not affected by diet ($P_{\text{diet}} = 0.465$ and $P_{\text{diet}} = 0.200$, respectively) or time \times diet interaction ($P_{\text{time} \times \text{diet}} = 0.245$ and $P_{\text{time} \times \text{diet}} = 0.603$, respectively). Methylmalonylcarnitine (C4-DC), palmitoleic acid (C16:1) and 3OH-palmitoylcarnitine (3OH-C16) were affected only by diet ($P_{\text{diet}} = 0.002$, $P_{\text{diet}} \leq 0.001$ and $P_{\text{diet}} = 0.009$, respectively). Plasma concentrations of the aforementioned acylcarnitines were lower at both baseline and endpoint in the high choline group as compared to the control group. However, the effects of time ($P_{\text{time}} = 0.821$, $P_{\text{time}} = 0.416$ and $P_{\text{time}} = 0.267$, respectively) and time \times diet interaction ($P_{\text{time} \times \text{diet}} = 0.134$, $P_{\text{time} \times \text{diet}} = 0.212$ and $P_{\text{time} \times \text{diet}} = 0.148$, respectively) were insignificant.

A significant time \times diet interaction was present for valerylcarnitine (C5) and 3OH-dodecanoylcarnitine (3OH-C12) ($P_{\text{time} \times \text{diet}} = 0.035$ and $P_{\text{time} \times \text{diet}} = 0.040$, respectively). Plasma concentrations of C5 decreased from baseline to endpoint in the control group but conversely increased in the high choline group. However, there were no individual effects of time ($P_{\text{time}} = 0.713$) or diet ($P_{\text{diet}} = 0.311$). In comparison, plasma 3OH-C12 concentrations increased after 5 weeks for the control group but remained consistent for the high choline group. Similarly, there were no effects of time ($P_{\text{time}} = 0.097$) or diet ($P_{\text{diet}} = 0.209$) on plasma 3OH-C12.

4. Discussion

To the authors' knowledge, the current study is the first to investigate the effects of choline supplementation on serum lipid, amino acid and acylcarnitine profile in obese cats. Choline is considered an essential nutrient [55,56], and, when de novo synthesis in the liver is inadequate, small concentrations of dietary choline can prevent and/or resolve numerous health conditions in both animals and humans, including fatty liver [57]. As such, researchers have proposed that choline may be involved with the pathogenesis and treatment of FHL [26,58]. Choline has important roles for numerous metabolic pathways within the body, including being a precursor for the synthesis of PC and being an important source of methyl group donor betaine [29,30,55]. As such, modifying the intake of choline can alter lipid and methionine metabolism.

Phosphatidylcholine is essential for the structural integrity of the plasma membrane and is integral for the lipid metabolism. Approximately 95% of the choline in tissue is found as PC [59]. In relation to the lipid metabolism, PC is a necessary component of VLDL [39].

This is especially important to consider for liver health, as fatty acids are incorporated into TAG, which accumulate in the liver. Under normal circumstances, the TAG will be packed with PC into VLDL, and secreted out of the hepatocytes and into circulation. As a result, choline deficiency can diminish synthesis of PC, leading to increased TAG accumulation within the liver [60–64].

Obese cats have increased accumulation of hepatic TAG compared to lean cats [65]. Although liver tissue samples were not collected in the present study, increased serum TAG and VLDL were observed with dietary choline supplementation, suggesting increased export of TAG and VLDL from the liver into circulation (Figure 2). The concentrations of serum CHOL, HDL-C and LDL-C also increased after 5 weeks in cats receiving the high choline diet.

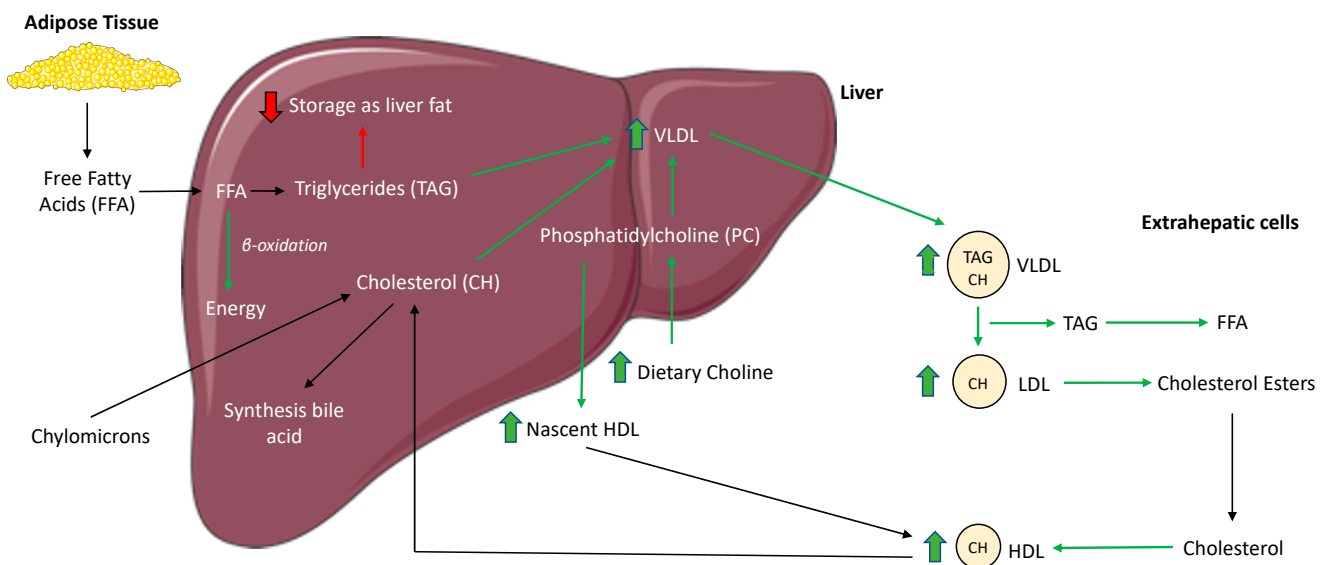


Figure 2. The proposed mechanism by which supplemental dietary choline alters the hepatic lipid and lipoprotein metabolism in obese cats. An increase in dietary choline allows for an increase in phosphatidylcholine (PC), necessary for the formation of very-low-density lipoproteins (VLDL). Hepatic triacylglycerides (TAG) and cholesterol are packed into VLDL and put into circulation for distribution within the body. The formation of nascent high-density lipoprotein (HDL) within the liver is also dependent on PC. PC: phosphatidylcholine; VLDL: very-low-density lipoprotein; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TAG: triacylglycerides; FFA: free fatty acids.

These are unsurprising findings due to the increase in VLDL, the role that HDL-C and LDL-C have in transporting cholesterol and the similar involvement of PC in the assembly of these lipoproteins [66,67]. The risks of cardiovascular disease in regards to increased serum lipids in cats are believed to be minimal, as dyslipidemia on its own does not appear to induce atherosclerosis or hypertension [68,69] as it might in other species [70].

The results of the present study align with previous findings in rats, which demonstrated that rats fed choline deficient diets had significantly lower levels of serum HDL-C, LDL-C, VLDL, TAG and CHOL as compared to those receiving choline [60,61,71,72]. Additionally, young lambs fed rumen-protected choline similarly had increased concentrations of serum HDL-C and LDL-C compared to the control group fed a diet with no rumen-protected choline [73]. Lien et al. (1998) also found that ducks consuming supplemental choline during the growing period had significantly increased serum VLDL concentrations as compared to the control. Ducks consuming higher doses during the laying period had significantly greater serum concentrations of both VLDL and HDL-C as compared to the control group [74].

In the present study, a decrease in serum ALP was observed with additional choline supplementation, which aligns with previous research in humans, dairy cows and piglets [75–78]. This decrease in serum ALP suggests that additional choline sup-

plementation improves the hepatic health and function in these obese cats, as increased ALP concentrations have previously been associated with numerous hepatobiliary diseases in cats, including FHL and bile duct occlusion [22,79,80]. However, as biopsies and ultrasonography were not performed in the present study, hepatic health could not be assessed in these cats, aside from a lack of a hepatic enzymopathy.

Choline also participates in the one-carbon cycle through its derivative betaine (trimethylglycine) [81]. In the liver, choline is first oxidized by choline dehydrogenase to betaine aldehyde [82]. Following this, in the presence of NAD⁺, betaine aldehyde is oxidized to betaine by betaine aldehyde dehydrogenase in the mitochondria [83]. Betaine is a methyl group donor in the one-carbon cycle, as it can re-methylate homocysteine to methionine through a reaction catalyzed by betaine-homocysteine-methyltransferase (BHMT) [40].

In the present study, the supplementation of additional choline resulted in higher levels of plasma methionine, likely through increased concentrations of betaine and increased re-methylation of homocysteine. Supplementation with additional dietary choline also tended to reduce the serum BUN and glucose. This could suggest increased recycling of methionine and potentially increased protein synthesis as opposed to amino acid degradation, thus, resulting in gluconeogenesis and the formation of urea [84].

The re-methylation of homocysteine through BHMT not only converts methionine and detoxifies homocysteine but also results in the production of S-adenosylmethionine (SAM) [85]. Research in mice showed that, when fed a choline-deficient diet, the concentrations of hepatic SAM were decreased by up to half in these animals [86–89]. The generation of SAM is important as it is the main methyl group donor in numerous metabolic pathways and is involved in the synthesis of proteins, hormones and phospholipids as well as in DNA methylation [90].

More specifically, SAM is an important methyl donor for the methylation and synthesis of carnitine [41]. The entry of fatty acids into the mitochondria for beta oxidation relies on carnitine for transport of the fatty acids from circulation [42]. The oxidation of fatty acids in the mitochondria is important as it is the main pathway for disposal of fatty acids under normal physiological conditions. The oxidation of fatty acids is also important as it results in the production of acetyl-CoA by the Krebs cycle, allowing for the production of ATP by ATP synthase [91].

Acylcarnitines are products of fatty acids entering the mitochondria for beta oxidation [92]. Previous research in mice found that the supplementation of methyl donors reduced plasma acylcarnitine concentrations through regulation of gene expression related to lipogenesis, lipolysis and fatty acid oxidation [38,93]. A decrease in plasma acylcarnitines may indicate that mitochondrial oxidation of fatty acids improved [93]. Increased plasma acylcarnitine concentrations reflect incomplete beta oxidation of fatty acids and have been associated with insulin resistance [92,94].

An increased concentrations of plasma acylcarnitines is commonly observed in conjunction with obesity [92,95,96]. In the present study, choline supplementation did not significantly alter the plasma acylcarnitine concentrations. However, a reduced ratio of AC/FC was observed with choline supplementation in obese cats, suggesting improvements in fatty acid utilization, which may contribute to lowering liver fat accumulation.

Methylmalonyl carnitine (C4-DC), palmitoleic acid (C16:1) and 3OH-palmitoylcarnitine (3OH-C16) were lower in the high choline group at the baseline and endpoint compared to the control group, which may be attributed to individual variations in the cats within these groups. However, it is unclear why malonylcarnitine (C3-DC) and octanoylcarnitine (C8) decreased in both groups throughout the trial. Valerylcarnitine (C5) decreased from the baseline to endpoint in the control group but increased in the high choline group.

Valerylcarnitine is a by-product of the branched chain amino acid (BCAA) catabolism. Previous research by Sivanesan et al. (2018) suggested upregulation of the amino acid catabolism in mice supplemented additional choline. Specifically, BCAA concentrations (isoleucine and valine) were reduced with supplementation in the mice [97]. However,

there were no significant changes in the BCAA analyzed in the present study (leucine and valine). An increase in the BCAA catabolism is suggested to be associated with insulin resistance and obesity.

Previous research has found that C5 is elevated in humans suffering from insulin resistance, obesity and/or steatosis [96,98]. However, to the authors' knowledge, these relationships have not been established in cats. An increase in 3OH-dodecanoylcarnitine (3OH-C12) occurred in the control group after 5 weeks, while concentrations remained consistent in the high choline group. Dodecanoylcarnitine was previously implicated as a marker of obesity-induced inflammation in rats fed nutrient poor "cafeteria diets" [99].

In the present study, cats receiving the control and high choline diets consumed an average of 284 mg and 1252 mg of choline, respectively, per day. Although some endogenous biosynthesis of choline does occur through the breakdown of PC [41], the NRC recommends that adult cats receive a minimum of 50 mg/kg BW^{0.67} choline daily. The NRC recommended allowance (RA) is 63 mg/kg BW^{0.67} daily [19]. In the present study, the cats on the high choline diet consumed more than five times the NRC RA and close to seven times the NRC minimum requirement daily.

In humans, choline deficiency can lead to numerous health conditions, including the development of non-alcoholic fatty liver and dysfunctions in muscle function [75,100–102]. Due to a lack of information investigating the choline requirements of cats, the minimum allowance established by the NRC is based on research by Schaeffer et al. (1982) investigating growth and hepatic lipid accumulation in growing kittens with choline supplementation [103]. It is unknown whether this level of choline can be considered optimal for health in adult cats, especially in overweight cats fed at maintenance energy requirement or during energy restriction and weight loss [104].

Research in cats has found that choline is often the limiting nutrient, provided below NRC's minimum requirement and/or RA for adult maintenance. A study observed that all obese cats undergoing weight loss and consuming a specific commercially available veterinary therapeutic weight loss diet (350 mg choline/100 g diet as fed) had a daily choline intake that was less than the NRC RA [105]. The daily choline intake of the majority of these cats was also below the NRC minimum requirement.

This finding is especially alarming due to the individual risks that both weight loss in cats and choline deficiency pose on hepatic health and function. Additionally, choline was the nutrient most frequently provided below NRC RA in feline homemade diet formulations intended to be complete and balanced for adult maintenance [106]. Choline was less than NRC RA in almost 90% of the homemade recipes evaluated in said study.

Currently there is evidence that providing additional dietary choline, on top of an animal's minimum requirement can enhance the lipid metabolism [31–37]. There were no changes in the BW, BCS or body composition observed between treatment groups in the present study. Additionally, there were no changes in the leptin concentration with additional choline supplementation. However, given the limited duration of testing and that the cats were fed at maintenance energy requirement, we did not expect to see significant decreases in these measures. Another possible limitation of the present study may have been its limited sample size, although similar sample sizes have previously been published in rodent research [38].

As cats in the high choline group consumed five-times the NRC RA, future studies should focus on identifying the minimum dose of choline necessary stimulate hepatic fat mobilization in overweight and obese cats. Furthermore, evaluation of the liver size by ultrasonography and biopsies of the liver should be performed in order to confidently assess the liver histology and expression of genes related to the hepatic energy metabolism, including gluconeogenesis, lipogenesis, lipolysis and VLDL secretion. Moving forward it will also be important to pair the results of this research with the already existing knowledge of weight loss.

It is known that cats are more susceptible to FHL. Although the precise pathogenesis remains a mystery, most researchers believe that multiple factors associated with the

unique pathways of protein and lipid metabolism in cats are involved. Obesity, energy restriction and essential nutrient deficiency have been put forward as major risk factors for FHL [23,107]. The degree of energy restriction needed to induce hepatic lipidosis was identified to be between 50% and 75% [25–27]. Caloric restriction paired with supplemental dietary choline could potentially offer an additive, beneficial effect with respect to weight loss and a decrease in hepatic lipids.

5. Conclusions

In conclusion, the supplementation of additional choline on top of the NRC requirements for adult maintenance could assist in eliminating hepatic fat through increased lipid mobilization, enhanced methionine recycling and fatty acid oxidation in obese cats fed at maintenance energy requirements. Further research is warranted to further elucidate the metabolic effects of dietary choline and its benefits for obesity prevention and weight loss in cats.

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Institutional Review Board Statement: The study was conducted in accordance with national and institutional guidelines for the care and use of animals, and approved by the University of Guelph Animal Care Committee (AUP#2494).

Data Availability Statement: The dataset generated and analysed during the current project is available at the Scholars Portal Dataverse server (<https://dataverse.scholarsportal.info/dataset.xhtml?persistentId=doi:10.5683/SP2/SC3TC4>, accessed on 24 July 2021).

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