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Dose-response relationship between dietary choline and serum lipid profile, energy expenditure, and respiratory quotient in overweight adult cats fed at maintenance energy requirements

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Abstract

Choline is an essential nutrient linked to hepatic lipid metabolism in many animal species, including cats. The current study investigated the serum lipid profiles, serum liver enzymes, respiratory quotients, and energy expenditures of overweight cats fed maintenance diets, in response to graded doses of supplemental dietary choline. Overweight (body condition score [BCS]: ≥6/9) adult male neutered cats (n = 14) were supplemented with five choline chloride doses for 3-wk periods, in a 5 × 5 Latin square design. Doses were based on individual body weight (BW) and the daily recommended allowance (RA) for choline (63 mg/kg $BW^{0.67}$) according to the National Research Council. Doses were control (no additional choline: 1.2 × RA, 77 mg/kg $BW^{0.67}$), 2 × RA (126 mg/kg $BW^{0.67}$), 4 × RA (252 mg/kg $BW^{0.67}$), 6 × RA (378 mg/kg $BW^{0.67}$), and 8 × RA (504 mg/kg $BW^{0.67}$). Choline was top-dressed over the commercial extruded cat food (3,620 mg choline/kg diet), fed once a day at maintenance energy requirements (130 kcal/kg$BW^{0.4}$). Body weight and BCS were assessed weekly. Fasted blood samples were taken and indirect calorimetry was performed at the end of each 3-wk period. Serum was analyzed for cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, non-esterified fatty acids, glucose, creatinine, blood urea nitrogen (BUN), alkaline phosphatase (ALP), and alanine aminotransferase. Very low-density lipoprotein cholesterol (VLDL) and low-density lipoprotein cholesterol were calculated. Data were analyzed via SAS using proc GLIMMIX, with group and period as the random effects, and treatment as the fixed effect. Statistical significance was considered at $P < 0.05$. Body weight and BCS did not change ($P > 0.05$). Serum cholesterol, HDL-C, triglycerides, and VLDL increased with 6 × RA ($P < 0.05$). Serum ALP decreased with 8 × RA ($P = 0.004$). Choline at 4 × and 6 × RA decreased serum BUN ($P = 0.006$). Fed or fasted respiratory quotient and energy expenditure did not differ among dietary choline doses ($P > 0.05$). These results suggest that dietary choline supplementation at 6 × RA may increase hepatic fat mobilization through increased lipoprotein transport and beneficially support hepatic health in overweight cats. Future studies that combine these results with existing knowledge of feline weight loss and hepatic lipidosis are warranted.

Lay Summary

Choline is an essential nutrient important for lipid metabolism in the liver of many mammals. In the present study, fourteen overweight cats had their commercial extruded cat food top-dressed with different amounts of choline chloride supplement. The amounts of choline were based on the individual body weights and the published recommended allowance (RA) for dietary choline intake in adult cats. The choline treatments were control (no additional choline added, 1.2 × RA), 2 × RA, 4 × RA, 6 × RA, and 8 × RA. The cats were separated into five groups. Each group received the choline treatments once daily for 3 wk per treatment. Choline at 6 × RA increased serum cholesterol, triglycerides, and lipoproteins. There were no significant differences in respiratory quotient or energy expenditure with choline intake. The results of this study suggest that choline at 6 × RA increases the transport of lipids from the liver. This may be beneficial in supporting liver health in overweight cats. Future studies should investigate supplementing choline to cats undergoing weight loss and those at risk of developing fatty liver.

Key words: feline nutrition, feline obesity, indirect calorimetry, lipoproteins, methyl donor, one carbon metabolism

Abbreviations: ALP, alkaline phosphatase;ALT, alanine aminotransferase;AUC, area under the curve;BCS, body condition score;BUN, blood urea nitrogen;BW, body weight;CBC, complete blood count;CHOL, cholesterol;CREAT, creatinine;DE, digestible energy;DMB, dry matter basis;DSH, domestic shorthair;EE, energy expenditure;EG, ethylene glycol;FHL, feline hepatic lipidosis;GE, gross energy;GGT, gamma-glutamyl transferase;GLUC, glucose;GOT, glutamate-oxaloacetate transaminase;GPT, glutamate-pyruvate transaminase;HDL-C, high-density lipoprotein cholesterol;LDL-C, low-density lipoprotein cholesterol;LSM, least square means;ME, metabolizable energy;MR, minimum requirement;NEFA, non-esterified fatty acids;PC, phosphatidylcholine;PEMT, phosphatidylethanolamine N-methyltransferase;RA, recommended allowance;RQ, respiratory quotient;TAG, triglycerides;TMA, trimethylamine;TMAO, trimethylamine oxide;UPLC, ultra-performance liquid chromatography;VLDL, very low-density lipoprotein cholesterol

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Introduction

Domestic cats are one of the most popular pets in North America (Perrin, 2009; American Pet Products Association, 2021; Canadian Animal Health Institute, 2021), with the latest reports estimating that 35% of households in the United States and 38% in Canada own at least one cat (American Pet Products Association, 2021; Canadian Animal Health Institute, 2021). Of this large domestic cat population, 25%–35% are estimated to be overweight or obese in North America (Scarlett et al., 1994; Lund and Armstrong, 2005). Elsewhere in the world, the prevalence of overweight and obese domestic cats is estimated to be 12%–63%, depending on the country and criteria used to assess obesity (Robertson, 1999; Colliard et al., 2009; Courcier et al., 2010; Cave et al., 2012; Courcier et al., 2012; Diez et al., 2015; Teng et al., 2017; Vandendriessche et al., 2017; Öhlund et al., 2018). Obesity is estimated to be one of the most common health conditions affecting domestic cats in developed countries. Obesity is a concern as it predisposes cats to numerous devastating health conditions such as dyslipidemia, insulin resistance, diabetes mellitus, osteoarthritis, and lower urinary tract disease (Scarlett et al., 1994; Clark et al., 2013; Öhlund et al., 2018; Teng et al., 2018), which may reduce their quality of life (Christmann et al., 2016).

Weight loss plans in cats tend to be long and unsuccessful, and are associated with increased health risks for the animal (Deagle et al., 2014; Villaverde et al., 2018). A period of drastic dietary energy reduction in overweight and obese cats, who are known to have reduced insulin sensitivity and elevated concentrations of hepatic triglycerides (TAG), leads to fat mobilization and accumulation in the liver, resulting in feline hepatic lipidosis (FHL) (Barsanti et al., 1977; Center et al., 1993; Biourge et al., 1994). Feline hepatic lipidosis is estimated to be the most common liver disease affecting cats and can be fatal if left untreated (Gagne et al., 1996; Armstrong and Blanchard, 2009).

Choline is an essential nutrient for cats (da Silva et al., 1959; Anderson et al., 1979; Schaeffer et al., 1982). Of importance to FHL is the role that choline has in fat mobilization and metabolism. Choline is a precursor for the phospholipid phosphatidylcholine (PC), an essential component of very low-density lipoproteins (VLDL) (Yao and Vance, 1988). The VLDL are essential for exporting TAG and cholesterol (CHOL) out of the liver and into circulation (Yao and Vance, 1988). Research in numerous animal species, including cats, has found that a deficiency in dietary choline will result in an accumulation of hepatic lipids, leading to fatty liver and even death (da Silva et al., 1959; Schaeffer et al., 1982). Similarly, fatty livers that developed in rats who consumed high fat diets were reversed within 12 d of choline supplementation (Best and Hunstman, 1935). The supplementation of choline or its derivative betaine has also proven useful in lowering fat mass gain and increasing lean mass in livestock species (Matthews et al., 2001; Zhan et al., 2006), although the exact metabolic mechanisms are still unknown.

Recent research in cats has found that the supplementation of additional dietary choline, above the published dietary recommendations, may offer similar lipotropic benefits. A pilot study by Verbrugghe et al., (2021) found that healthy obese cats receiving choline at five times the recommended allowance (RA) published by the National Research Council (NRC) (NRC, 2006), at maintenance energy requirements, had increased levels of serum CHOL, TAG, and lipoproteins, suggesting that choline assisted in mobilizing hepatic lipids (Verbrugghe et al., 2021). Additionally, research investigating choline supplementation administered to growing kittens post-gonadectomy found that increased supplementation, at three times the NRC RA, decreased body weight gain and fat mass gains when these kittens were fed ad libitum (Godfrey et al., 2022).

Choline supplementation has potential as a novel dietary strategy for overweight and obese cats. However, minimal research has been performed investigating the use of supplemental dietary choline in cats. The choline doses administered to the cats in the published literature are based on research in rodents and growing pigs (Young et al., 1956; Southern et al., 1986; Schenkel et al., 2015), and have not been demonstrated to be the optimal dose for lipotropic activity in cats. Before additional research can be undertaken to investigate the use and efficacy of choline for both feline weight loss and FHL, it is necessary to establish a dose response relationship. Therefore, the purpose of this research was to investigate the dose response relationship between graded doses of dietary choline and its lipotropic effects in overweight cats fed to maintain an overweight body condition, with focus on serum lipid and lipoprotein profiles, energy expenditure (EE) and respiratory quotient (RQ). We hypothesized that serum lipids, lipoproteins, and EE would increase, and RQ would decrease, in positive correlation with choline dose, in overweight cats fed at maintenance energy requirements.

Materials and Methods

All procedures were reviewed and approved by the University of Guelph Animal Care Committee (AUP#4118), in accordance with provincial and national animal care and use guidelines.

Animals and housing

Fifteen domestic shorthair (DSH) male neutered adult cats (Marshall's Bio Resources, Waverly, NY) were used for this trial. All cats were 1 yr of age and deemed healthy prior to the trial, based on complete blood count (CBC), serum biochemistry, medical history, and physical examination. Fourteen of the 15 cats were considered overweight with a body condition score (BCS) of 6 or greater at the start of the trial; one cat had a BCS of 5 and was therefore excluded from statistical analysis (mean ± SEM = 6.87 ± 0.22; range = 5–8) (Laflamme, 1997). Cats had a mean body weight (BW) of 4.95 ± 0.15 kg at the start of the trial (range: 4.36–6.24 kg).

Cats were housed indoors as a group in a free-living environment (23 × 19 ft) at the Animal Biosciences Cattery at the Ontario Agricultural College of the University of Guelph (Guelph, ON, Canada). Temperature and humidity in the room were maintained at 23 °C and 40%, respectively. The room was controlled with a 12 h light 12 h dark cycle, with the lights turning on at 0700 h and off at 1900 h. Distilled water was provided ad libitum, both as still water (in bowls) and flowing water (open tap).

Litterboxes (n + 1) were provided along the perimeter of the room. The room was cleaned daily, consisting of sweeping, mopping, cleaning all surfaces, cleaning litterboxes twice daily, and changing water in bowls. Cats had access to various environmental enrichment sources within the room, including scratching posts, cat trees of varying heights, boxes for hiding, toys, and perches. Additionally, all cats received a maximum

of 2 h daily of human interaction with familiar people, 5 d a week. This interaction included petting, brushing, nail trimming, and voluntary play with high-value toys.

Diet

All cats were transitioned onto the same commercial extruded diet (Nutram Total Grain-Free Chicken and Turkey Recipe, Elmira Pet Products, Elmira, ON, Canada), formulated for adult maintenance according to the Association of American Feed Control Officials (AAFCO), 4 wk prior to the start of the trial (adaptation period) and for the 15-wk dose-response trial. Cats were fed to maintain their current BWs throughout the trial period. The daily quantity of food for each cat was calculated using the equation by the NRC (2006) for overweight cats at maintenance (130 kcal/kg $BW^{0.4}$). All cats were weighed, and body condition scored weekly during both the adaptation and trial periods. The quantity of food was adjusted on an individual basis as needed. Throughout both the adaptation and trial periods, cats were separated into individual cages once daily at 08:00 h for 1 h, in order to be fed individually.

Nutrient analysis of the diet (Table 1), including proximate analysis (moisture, crude protein, crude fat, ash, and crude fiber), total dietary fiber, choline, cobalamin (B12), pyridoxine (B6), and folate (B9), was performed according to methods outlined by the Association of Official Analytical Chemists (AOAC) and the American Oil Chemist Society (AOCS) (Bureau Veritas, Mississauga, ON, Canada) (Horwitz et al., 1970; American Oil Chemists' Society and Firestone, 1994). Moisture was assessed by gravimetric analysis (AOAC 935.29), crude protein by combustion (AOAC 990.03), crude fat by ether extraction (AOAC 920.39), ash by gravimetric analysis (AOAC 942.05), and crude fiber using the filter bag technique (AOCS Ba6a-05). Total dietary fiber was determined by enzymatic-gravimetric method (AOAC 991.43, 985.29), choline by enzymatic colorimetric method (AOAC 999.14), cobalamin by Turbidimetric Method (AOAC 986.23), pyridoxine by microbiological method [AOAC 985.32 (modified)], and folate by triple enzyme microbiological method (AOAC 2004.5). Nitrogen free extract (NFE) was calculated by difference (NFE % = 100–moisture–protein–fat-crude fiber-ash), and metabolizable energy (ME) was estimated using the Modified Atwater equation [ME = 10 × (3.5 × %crude protein) + (8.5 × %fat) + (3.5 × %NFE)] (NRC, 2006). Dietary amino acids were analyzed via ultra-performance liquid chromatography (UPLC), as previously described by Cargo-Froom et al. (2019).

Dietary choline supplementation

Choline chloride supplementation (Pet Shure, 97% choline chloride, 72.3% choline ion; Balchem Corporation, New Hampton, NY) was provided in five separate doses. These doses were based on the cat's metabolic BW and the RA for dietary choline set by the NRC, equivalent to 63 mg/kg $BW^{0.67}$ (NRC, 2006). The doses were control (no additional choline supplementation added to the cat food), 2 × NRC RA (126 mg/kg $BW^{0.67}$), 4 × NRC RA (252 mg/kg $BW^{0.67}$), 6 × NRC RA (378 mg/kg $BW^{0.67}$), and 8 × NRC RA (504 mg/kg $BW^{0.67}$).

The supplement quantities were measured by first mixing the choline chloride supplement with distilled water to create a solution (760.19 ± 22.50 mg choline chloride/mL distilled water; 549.62 ± 16.23 mg choline ion/mL distilled water). The choline doses for each cat were aliquoted and stored in separate 1.5 mL cryovials (Fisherbrand Premium Microcentrifuge Tubes; Thermo Fisher Scientific, Mississauga, ON, Canada). Each cat's daily food quantity was provided once per day and separated into two allotments per feeding. The first allotment was equivalent to ¼ of the cat's daily food intake, and the second allotment consisted of the remaining ¾. The choline chloride dose for each cat was pipetted onto

Table 1. Proximate analysis and analysis of choline, selected B-vitamins (B12, B6, B9) and amino acids of a commercial extruded adult cat food fed at maintenance energy requirements with additional dietary choline chloride supplementation to overweight adult cats (*n* = 14)

Moisture	% as fed	7.2
Protein	% DM	40.2
Fat	% DM	17.6
Ash	% DM	9.2
Crude fiber	% DM	2.3
Total dietary fiber	% DM	6.8
NFE[1]	% DM	30.7
ME[2]	kcal/kg	3801.5
Choline	mg/100 g	390.1
Cobalamin (B12)	µg/100g	7.5
Pyridoxine (B6)	mg/100g	1.9
Folate (B9)	mg/100g	0.4
Alanine	% DM	2.4
Arginine	% DM	2.7
Aspartate	% DM	3.6
Glutamine	% DM	5.3
Glycine	% DM	3.3
Histidine	% DM	0.9
Isoleucine	% DM	1.6
Leucine	% DM	2.8
Lysine	% DM	2.4
Phenylalanine	% DM	1.8
Proline	% DM	2.5
Serine	% DM	1.9
Taurine	% DM	0.2
Threonine	% DM	1.5
Tyrosine	% DM	1.3
Valine	% DM	1.8
Methionine	% DM	0.9
Cysteine	% DM	1.5
Tryptophan	% DM	0.2

[1]Metabolizable Energy (ME) calculated using the Modified Atwater Equation: ME, 10 x [(3.5 x %crude protein) + (8.5 x %fat) + (3.5 x %NFE)] (NRC, 2006);
[2]Nitrogen Free Extract (NFE) %, 100–moisture–protein–fat-crude fiber-ash (NRC, 2006); Ingredients: Deboned Chicken, Deboned Turkey, Chicken Meal, Whole Eggs, Turkey Meal, Lentils, Peas, Chickpeas, Chicken Fat (preserved with Mixed Tocopherols), Split Peas, Flaxseed, Natural Chicken Flavor, Pumpkin, Broccoli, Quinoa Seed, Dried Cranberries, Choline Chloride, Pomegranate, Raspberries, Kale, Salt, Chicory Root Extract, Vitamins & Minerals (Vitamin E Supplement, Niacin (source of Vitamin B3), Vitamin A Supplement, Thiamine Mononitrate (source of Vitamin B1), d-Calcium Pantothenate (source of Vitamin B5), Pyridoxine Hydrochloride (source of Vitamin B6), Riboflavin (source of Vitamin B2), Beta-Carotene, Vitamin D3 Supplement, Folic Acid, Biotin, Vitamin B12 Supplement, Zinc Proteinate, Ferrous Sulfate, Zinc Oxide, Iron Proteinate, Copper Sulfate, Copper Proteinate, Manganese Proteinate, Manganous Oxide, Calcium Iodate, Sodium Selenite, DL-Methionine, Taurine, Yucca schidigera Extract, Spinach, Celery Seeds, Peppermint, Chamomile, Turmeric, Ginger, Rosemary.

the first allotment of food (¼ daily food intake) and left to soak for 20 min prior to feeding. Once cats finished this first allotment of food with the choline supplement, they were provided the remaining amount (¾ daily food intake). Each cat was given 1 h to consume all of their food. Orts from each cat were measured and recorded daily.

Study design

All cats received each of the five choline doses in a 5 × 5 Latin square design. The 15 cats were separated into five groups of three prior to the trial. All groups were balanced for BW (mean group BW: 4.95 ± 0.03 kg), as well as temperament. The lean cat was included to balance BW between the groups. Each choline dose was provided for a period of three weeks at a time.

Indirect calorimetry

On the last day of each 3-wk period, indirect calorimetry was performed to assess EE and RQ. Each calorimetry session lasted 24 h at a time. Each session consisted of a 30-min gas equilibrium period, 2 h of fasted state measurement (pre-prandial), followed by measurement of the fed and extended postprandial states. All cats were previously acclimated to the calorimetry chambers, following the methods outlined by Gooding et al. (2012). Prior to each session, calibration of the gas analysers and mass flow meters was performed using two standard gas mixtures (99.98% nitrogen and 1.01% carbon dioxide; Praxair, Guelph, ON, Canada). The system was re-calibrated during the sessions whenever a drift of more than 1% was observed. Protocol followed the methods previously outlined by Godfrey et al. (2022) and Camara et al. (2020).

Plexiglass chambers (length × width × height: 146 × 60 × 89 cm) were designed as an open circuit, ventilated system (Qubit C950 Multi Channel Gas Exchange, Qubit Systems Inc., Kingston, ON, Canada), with room air being pulled through the chambers at a flow rate of 4.0–6.2 L/min, to maintain CO_2 levels in the chamber between 0.4% and 0.7%. Within each chamber were two boxes made of 2″ × 2″ wooden frames and covered in polyethylene plastic wrap. The two boxes, measuring 145 × 59 × 36 cm and 76 × 57 × 50 cm, were stacked to minimize the volume of area that the cats had within the chamber. The area of space that each cat had in the chamber was reduced in size and measured 61 × 60 × 53 cm (volume: 193.98 L). All cats had access to a litter box, a water bowl, and a blanket within the chamber. Water was filled as needed. When in the chambers, cats continued to receive their food in the same manner outlined above, with ¼ of the food being offered with choline, followed by the remaining ¾.

Respiratory quotient and EE were calculated by the C950-Multi Channel Gas Exchange system (Qubit Systems Inc, Kingston, ON, Canada), using the following calculations (Weir, 1949):

$$Respiratory\ \ Quotient\ \ (RQ) = CO_2\ produced\ \ (L) \ / \ O_2\ consumed\ \ \ (L), \quad (1)$$

$$Energy\ Expenditure\ \ (kcal) = 3.94 \times\ O_2\ consumed\ (L) + 1.11\ \times CO_2\ produced\ (L). \quad (2)$$

Blood collection and laboratory analyses

Following the 24-h calorimetry, fasted blood samples were taken from each cat under sedation (5 mL). Cats were sedated using dexmedetomidine hydrochloride (Dexdomitor, Zoetis, Kirkland, QC, Canada) (0.5 mg/mL) at a dose of 0.01 mg/kg BW, given intramuscularly (Plumb, 2011). Blood collection occurred 20 min after sedation was administered (Bouillon et al., 2020). Whole blood was sampled via venipuncture from the jugular vein on all cats, with the exception of one that was consistently sampled from the medial saphenous vein, due to the adiposity of this particular cat. Whole blood was collected and immediately syringed into serum separating tubes and tubes were stored in a fridge (5 °C) until centrifugation. Sedation was reversed by atipamezole (Antisedan, Zoetis, Kirkland, QC, Canada) (5 mg/mL), given intramuscularly at a dose 0.1 mg/kg BW (Plumb, 2011).

Samples were centrifuged within 2 h of collection at 2,500 × *g* × 15 min at 4 °C (LegendRT, Kendro Laboratory Products 2002, Germany). Once centrifuged, serum was separated and aliquoted into separate cryovials. Serum (0.5 mL) was submitted to the Animal Health Laboratory at the University of Guelph (Guelph, ON, Canada) for analysis of serum CHOL, non-esterified fatty acids (NEFA), high-density lipoprotein CHOL (HDL-C), TAG, alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (CREAT), and glucose (GLUC), via photometry using a Roche Cobas 6000 c501 analyzer (Roche Diagnostics, Basel, Switzerland). Very low-density lipoprotein CHOL (VLDL) and low-density lipoprotein CHOL (LDL-C) were calculated using the Friedewald equation [VLDL (mmol/L) = TAG (mmol/L)/2.2; LDL-C (mmol/L) = total CHOL (mmol/L) – HDL-C (mmol/L) – VLDL (mmol/L)] (Friedewald et al., 1972).

Statistical analyses

Area under the curve (AUC) for RQ (AUC_{RQ}) was calculated using the trapezoidal method in GraphPad Prism (9.3.1, GraphPad Software, San Diego, CA) for fasted RQ (pre-prandial) and fed RQ (0–125 min, 125–475 min, 475–825 min, and 825–1260 min). Statistical analyses were performed using SAS (SAS Studio 3.8, SAS Institute, Cary, NC). Concentrations of serum ALT, ALP, BUN, CREAT, GLUC, lipoprotein profile (CHOL, NEFA, HDL-C, LDL-C, VLDL, and TAG), EE, RQ, and AUC_{RQ} were analyzed as repeated measures using the proc GLIMMIX procedure within SAS, with dose as a fixed effect, period and group as the random effects, and cat as the subject. Differences in fed and fasted RQ and EE were also assessed with BW and food intake during calorimetry as covariates. The covariance matrix that had the smallest Akaike information criterion value was used.

Differences in BW, BCS, food intake (grams), ME intake (kcal/day), and choline intake (mg/day and $mg/kg^{0.67}$) throughout the trial were similarly assessed through the Proc GLIMMIX procedure, with dose as a fixed effect, period and group as the random effects, experiment week as the repeated term, and cat as the subject. Baseline BCS and BW were used as covariates when assessing change in BW and BCS.

Residuals of serum biochemistry and lipoprotein profile, indirect calorimetry, BW and BCS, and intake data were all assessed for normality with the Shapiro–Wilk test. Food intake, ME intake, and choline intake (both mg/day and $mg/kg^{0.67}$)

Table 2. BW, BCS and food, energy, and choline intake of overweight cats (n = 14) receiving choline at control (no additional choline supplementation added to the cat food: 1.2 × NRC RA, 77 mg/kg $BW^{0.67}$), 2 × NRC RA (126 mg/kg $BW^{0.67}$), 4 × NRC RA (252 mg/kg $BW^{0.67}$), 6 × NRC RA (378 mg/kg $BW^{0.67}$), and 8 × NRC RA (504 mg/kg $BW^{0.67}$), in a 5 × 5 Latin square design for 3-wk periods[1]

	Control	2 × NRC RA	4 × NRC RA	6 × NRC RA	8 × NRC RA	P_{Dose}	P_{Week}	$P_{Week*Dose}$
BW, kg	5.04 ± 0.02	5.04 ± 0.02	5.06 ± 0.02	5.07 ± 0.02	5.07 ± 0.02	0.479	0.533	0.122
BCS (1-9)	6.69 ± 0.10	6.73 ± 0.10	6.82 ± 0.10	6.77 ± 0.10	6.75 ± 0.10	0.644	0.817	0.370
Food intake, g/day	62.71 ± 1.65	62.69 ± 1.65	62.62 ± 1.65	62.85 ± 1.65	62.79 ± 1.65	0.494	**0.012**	0.637
ME intake, kcal/day	233.63 ± 6.13	233.55 ± 6.13	233.27 ± 6.13	234.15 ± 6.13	233.94 ± 6.13	0.494	**0.012**	0.637
Choline intake, mg/day	227.02 ± 21.54^{e}	371.46 ± 21.54^{d}	742.96 ± 21.54^{c}	1121.27 ± 21.54^{b}	1481.32 ± 21.54^{a}	**<0.001**	**<0.001**	**<0.001**
Choline intake, mg/kg $BW^{0.67}$	76.64 ± 2.03^{b}	125.99 ± 2.03^{c}	251.50 ± 2.03^{d}	377.89 ± 2.03^{e}	497.49 ± 2.03^{a}	**<0.001**	**<0.001**	**<0.001**

[1]Values expressed as LSM ± SEM; Values in a row with superscripts without a common letter differ; $P < 0.05$, repeated measures ANOVA with Tukey post hoc test. BW, body weight; BCS, body condition score; ME, metabolizable energy; NRC, National Research Council; RA, Recommended Allowance.

were not normally distributed, and underwent a log transformation as a result.

Least square means (LSM) were calculated using the LSMEANS statement. Tukey's post hoc test was performed for all multiple comparisons where a significant dose effect was present. Results are expressed as LSM ± standard error of mean (SEM). A P-value < 0.05 was considered significant, and a P-value of < 0.10 was considered a trend.

Results

Body weight, ME intake, and choline intake

Body weight did not change with dose, week, or dose x week interaction (Table 2; P_{Dose} = 0.479, P_{Week} = 0.533, $P_{Week*Dose}$ = 0.122). Similarly, BCS was not affected (P_{Dose} = 0.644, P_{Week} = 0.817, $P_{Week*Dose}$ = 0.370). Feed and ME intake did not change with dose or dose x week interaction (P_{Dose} = 0.494, $P_{Dose*Week}$ = 0.637). Variability in intake was noted between weeks (P_{Week} = 0.012); however, weeks were not significantly different from each other following a Tukey's post hoc. As expected, choline intake, both as total choline consumed per day (mg) and on a metabolic BW basis (mg/$kg^{0.67}$), was affected by dose, week, and dose x week interaction ($P_{Dose} \leq 0.001$, $P_{Week} \leq 0.001$, $P_{Week*Dose} \leq 0.001$). Choline intake increased with increasing dose.

Serum biochemistry and lipoprotein profile

Mean serum biochemistry and lipoprotein data are presented in Table 3. Concentrations of serum lipids TAG, CHOL, and lipoproteins VLDL, HDL-C differed between choline doses (P_{Dose} = 0.027, 0.027, 0.012, and 0.026, respectively), i.e., serum TAG, VLDL, and HDL-C were greater when cats were supplemented with choline at 6 × NRC RA, as compared to control. Similarly, 6 × NRC RA resulted in greater concentrations of serum CHOL as compared to control and 2 × NRC RA. Serum LDL-C and NEFA concentrations did not change with dose (P_{Dose} = 0.066 and 0.071, respectively).

Concentrations of serum ALP decreased when choline was supplemented at 8 × NRC RA, as compared to control, 2 × NRC RA, and 4 × NRC RA (P_{Dose} = 0.004). Serum BUN decreased with 4 × NRC RA and 8 × NRC RA, as compared to control (P_{Dose} = 0.006). Concentrations of serum ALT, CREAT, and GLUC were not affected by choline dose (P_{Dose} = 0.134, 0.702, and 0.373, respectively).

Indirect calorimetry

Fasted and fed EE did not differ between choline doses (Table 4; P_{Dose} = 0.130 and 0.835, respectively). These parameters remained insignificant when adjusted for individual cat BW (P_{Dose} = 0.153 and 0.820, respectively). Similarly, fed EE did not change when adjusted for food intake (P_{Dose} = 0.831). Fasted RQ tended to decrease with dose (P_{Dose} = 0.064) and became significant when adjusted for individual BW (P_{Dose} = 0.033). No differences occurred between doses when a Tukey's post hoc was used. Fed RQ was not affected by dose (P_{Dose} = 0.104). However, there was a trend for fed RQ to decrease with choline dose when adjusted for BW and food intake (P_{Dose} = 0.078 and 0.066, respectively).

Area under the curve for RQ changed with dose prior to feeding, along with 0–125 and 125-–75 min postprandial (P_{Dose} < 0.0001, 0.010, and 0.001, respectively). Choline at 2 × NRC RA and 4 × NRC had a larger fasted AUC, as compared to control and 8 × NRC RA. Similarly, choline at 2 × NRC RA produced a larger AUC 0–125 min postprandial, as compared to control, 6 × NRC RA, and 8 × NRC RA. Choline at 2 × NRC RA also had a larger AUC 125–475 min postprandial, as compared to control and the three higher doses.

Discussion

To the authors' knowledge, this is the first dose-response study evaluating dietary choline intake in adult cats fed at maintenance energy requirements. Presently, the NRC (2006) suggests a minimum requirement (MR) of 50 mg/kg $BW^{0.67}$ and a RA of 63 mg/kg $BW^{0.67}$ dietary choline daily for adult cats, respectively (NRC, 2006). The MR by the NRC is based on dose-response research in growing kittens, investigating various levels of dietary choline on growth, liver lipid content, and plasma liver enzyme values [glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), gamma-glutamyl transferase (GGT)], after 30 d of consumption (Schaeffer et al., 1982). It is apparent that the current published recommendations for dietary choline for adult cats are based on an intake that prevents fatty liver in growing kittens. To the authors' knowledge, similar research

Table 3. Fasted serum biochemistry and lipoprotein profile values (mmol/L) of overweight cats (n = 14) receiving control (no additional choline supplementation: 1.2 × NRC RA, 77 mg/kg $BW^{0.67}$), choline at 2 × NRC RA (126 mg/kg $BW^{0.67}$), 4 × NRC RA (252 mg/kg $BW^{0.67}$), 6 × NRC RA (378 mg/kg $BW^{0.67}$), and 8 × NRC RA (504 mg/kg $BW^{0.67}$), in a 5 × 5 Latin square design for 3-wk periods[1]

Analyte	Units	Control	2 × NRC RA	4 × NRC RA	6 × NRC RA	8 × NRC RA	P_{Dose}
TAG	mmol/L	0.37 ± 0.04^{b}	0.39 ± 0.04^{ab}	0.42 ± 0.04^{ab}	0.46 ± 0.04^{a}	0.38 ± 0.04^{ab}	**0.027**
CHOL	mmol/L	6.65 ± 0.35^{b}	6.58 ± 0.35^{b}	6.83 ± 0.35^{ab}	7.15 ± 0.35^{a}	6.90 ± 0.35^{ab}	**0.012**
HDL-C	mmol/L	5.20 ± 0.20^{b}	5.28 ± 0.20^{ab}	5.40 ± 0.20^{ab}	5.54 ± 0.20^{a}	5.42 ± 0.20^{ab}	**0.026**
LDL-C	mmol/L	1.29 ± 0.18	1.13 ± 0.18	1.24 ± 0.18	1.40 ± 0.18	1.31 ± 0.18	0.066
VLDL	mmol/L	0.17 ± 0.02^{b}	0.18 ± 0.02^{ab}	0.19 ± 0.02^{ab}	0.21 ± 0.02^{a}	0.17 ± 0.02^{ab}	**0.027**
NEFA	mmol/L	0.22 ± 0.02	0.23 ± 0.02	0.21 ± 0.02	0.20 ± 0.02	0.17 ± 0.02	0.071
ALP	U/L	26.88 ± 2.98^{a}	26.37 ± 2.98^{a}	26.35 ± 2.98^{a}	24.55 ± 2.98^{ab}	23.20 ± 2.98^{b}	**0.004**
ALT	U/L	51.23 ± 4.88	45.50 ± 4.88	54.36 ± 4.88	48.88 ± 4.88	60.18 ± 4.88	0.134
CREAT	mmol/L	114.06 ± 2.92	114.63 ± 2.92	111.44 ± 2.92	112.79 ± 2.92	114.08 ± 2.92	0.702
GLUC	mmol/L	9.73 ± 0.82	9.06 ± 0.82	9.45 ± 0.82	8.35 ± 0.82	9.37 ± 0.82	0.373
BUN	mmol/L	8.16 ± 0.23^{a}	8.00 ± 0.23^{ab}	7.75 ± 0.23^{b}	7.86 ± 0.23^{ab}	7.82 ± 0.23^{b}	**0.006**

[1]Values expressed as LSM ± SEM; values in a row with superscripts without a common letter differ; $P < 0.05$, repeated measures ANOVA with Tukey post hoc test. NRC, National Research Council; RA, recommended allowance; BW, body weight; TAG, triglycerides; CHOL, cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL, very low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CREAT, creatinine; GLUC, glucose; BUN, blood urea nitrogen.

Table 4. EE and RQ of overweight cats (n = 14) receiving control (no additional choline supplementation: 1.2 × NRC RA (77 mg/kg $BW^{0.67}$), choline at 2 × NRC RA (126 mg/kg $BW^{0.67}$), 4 × NRC RA (252 mg/kg $BW^{0.67}$), 6 × NRC RA (378 mg/kg $BW^{0.67}$), and 8 × NRC RA (504 mg/kg $BW^{0.67}$), in a 5 × 5 Latin square design for 3-wk periods[1]

	Control	2 × NRC RA	4 × NRC RA	6 × NRC RA	8 × NRC RA	P_{Dose}
EE Fasted, kcal/kg BW/day	26.31 ± 1.36	27.25 ± 1.37	24.97 ± 1.33	25.13 ± 1.33	23.01 ± 1.36	0.130
Adjusted for BW[2]	26.30 ± 1.36	27.25 ± 1.37	24.95 ± 1.34	25.15 ± 1.33	23.10 ± 1.36	0.153
EE Fed, kcal/kg BW/day	34.78 ± 0.98	34.06 ± 0.98	33.48 ± 0.98	34.21 ± 0.98	34.25 ± 0.98	0.835
Adjusted for Food Intake[2]	34.79 ± 0.98	34.06 ± 0.98	33.46 ± 0.99	34.23 ± 0.99	34.25 ± 0.98	0.831
Adjusted for BW[2]	34.73 ± 0.90	34.01 ± 0.90	33.41 ± 0.90	34.24 ± 0.90	34.41 ± 0.90	0.820
RQ Fasted	0.77 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	0.78 ± 0.01	0.77 ± 0.01	0.064
Adjusted for BW[2]	0.77 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	0.78 ± 0.01	0.77 ± 0.01	**0.033**
RQ Fed	0.84 ± 0.01	0.85 ± 0.01	0.85 ± 0.01	0.84 ± 0.01	0.83 ± 0.01	0.104
Adjusted for Food Intake[3]	0.84 ± 0.01	0.85 ± 0.01	0.85 ± 0.01	0.84 ± 0.01	0.83 ± 0.01	0.066
Adjusted for BW[2]	0.84 ± 0.01	0.85 ± 0.01	0.85 ± 0.01	0.84 ± 0.01	0.83 ± 0.01	0.078
AUC_{RQ} Fasted, RQ*min	38.73 ± 0.49^{b}	40.43 ± 0.50^{a}	40.08 ± 0.49^{a}	39.52 ± 0.49^{ab}	38.76 ± 0.49^{b}	**<0.0001**
AUC_{RQ} 0-125, RQ*min	96.93 ± 1.35^{b}	100.36 ± 1.35^{a}	97.29 ± 1.35^{ab}	96.87 ± 1.35^{b}	96.79 ± 1.35^{b}	**0.010**
AUC_{RQ} 125-475, RQ*min	292.12 ± 3.28^{b}	298.89 ± 3.28^{a}	292.43 ± 3.28^{b}	289.87 ± 3.28^{b}	288.72 ± 3.28^{b}	**0.001**
AUC_{RQ} 475-825, RQ*min	305.83 ± 3.44	301.52 ± 3.44	303.66 ± 3.44	303.33 ± 3.44	299.44 ± 3.44	0.136
AUC_{RQ} 825-1260, RQ*min	320.08 ± 5.67	317.95 ± 5.67	316.52 ± 5.67	322.06 ± 5.67	315.88 ± 5.67	0.582

[1]Values expressed as LSM ± SEM; Values in a row with superscripts without a common letter differ; $P < 0.05$, Repeated measures ANOVA with Tukey post hoc test. EE, energy expenditure; RQ, respiratory quotient; NRC, National Research Council; RA, recommended allowance; BW, body weight.
[2]Individual BW at time of calorimetry used as a covariate.
[3]Individual food intake during calorimetry used as a covariate.

has not been conducted in lean and/or obese adult cats. Cats in different life and/or metabolic stages may have different requirements, or benefit from different intakes of dietary choline. This may include adult cats, those that are overweight or obese, and those undergoing weight loss. Dose-response studies such as the present study are important to help us quantify and understand the health benefits, and potential risks, that may come from increasing choline intake beyond the published recommendations. Previous research by the authors investigating the lipotropic effects of dietary choline consumption at five times the NRC RA by obese cats has been published (Verbrugghe et al., 2021). Although the research provided promising results, a choline dose of five times was based on previous work done in rodents (Schenkel et al., 2015). A dose-response relationship between choline intake and its lipotropic effects had yet to be assessed in overweight and/or obese adult cats. The present study provided dietary choline at 1.2 (control), 2, 4, 6, and 8 times the published RA by the NRC to overweight adult cats for periods of 3 wk per treatment to fill this void of information.

Choline is considered an essential nutrient for cats. It is necessary for numerous metabolic processes within the body, including one-carbon metabolism, neurotransmission, and lipid and cholesterol transport (Zeisel, 1981). Limited quantities

of choline can be synthesized endogenously through the phosphatidylethanolamine N-methyltransferase (PEMT) pathway. However, the addition of dietary choline is important for the prevention of health conditions, such as fatty liver (Schaeffer et al., 1982). The majority of research surrounding dietary choline supplementation in animals has focused on its effects on hepatic health and body composition. However, limited research exists on these topics specifically in cats. Choline is required to synthesize PC, which is a necessary phospholipid for the formation and secretion of both HDL-C and VLDL from the liver into circulation (Yao and Vance, 1988). For this reason, choline may play a role in the pathogenesis of FHL (Biourge et al., 1991; Verbrugghe and Bakovic, 2013).

The present study observed that choline consumed at 6 × NRC RA resulted in a 24% increase of serum TAG concentrations, 7.5% increase of CHOL, 24% higher VLDL, and 6.5% higher HDL-C concentrations, as compared to control. These findings are unsurprising due to the requirement of PC for the synthesis of these lipoproteins, and their roles in transporting TAG and CHOL in circulation (Yao and Vance, 1988). The results presented herein mirror similar results reported by Verbrugghe et al., (2021), investigating choline supplementation at 5 × NRC RA in obese cats. After 5 wk of consumption, the cats similarly had elevated concentrations of serum TAG, CHOL, VLDL, HDL-C, and LDL-C as compared to the control group. The increases in serum CHOL, TAG, and lipoproteins observed with increasing dietary choline supplementation in cats align with similar increases observed in rodents, ducks, and lambs (Tinoco et al., 1964; Lombardi et al., 1968; Yao and Vance, 1990; Lien and Jan, 1999; Li et al., 2015). Although an increase was noted, serum CHOL remained within the laboratory reference range (2–12 mmol/L) for all choline treatments in the present study. To the authors' knowledge, there are no published reference ranges in cats for serum TAG, VLDL, and HDL-C. The serum TAG concentrations of all treatments within the present study were similar to those previously reported in healthy adult cats (Pazak et al., 1998; Templeman et al., 2021; Verbrugghe et al., 2021). The noted increase in these serum parameters may suggest that increasing choline intake may allow for increased export of hepatic TAG and CHOL via VLDL. Decreases in hepatic TAG content, in conjunction with increases in plasma phospholipid concentrations, have been reported in choline-supplemented rats (Lombardi et al., 1966). However, without investigating hepatic lipid concentrations, it is difficult to assess the biological relevance of the observed increases in these serum analytes. It is unclear if the increased serum HDL observed in the present study was due to the normal metabolism of VLDL, or if production of nascent HDL was increased. The synthesis of nascent HDL is similarly reliant on PC (Forte et al., 1995). These HDL particles are important for transporting excess free cholesterol from peripheral tissues back to the liver (Dominiczak and Caslake, 2011). Future research in cats should include liver biopsies to assess hepatic lipid distribution and its relationship with serum or plasma lipid concentrations. Obese cats have been reported to have increased concentrations of hepatic TAG when compared with lean cats (Clark et al., 2013). In the present study, only two of the cats were considered to be obese (BCS 8 ≥ 9 [Laflamme, 1997]). It is unknown how the hepatic TAG concentrations of overweight cats compare to lean cats.

There were no significant differences in the serum concentrations of TAG, CHOL, and VLDL at the highest dose of 8 × NRC RA, as compared to control. Increased choline concentrations (1 vs. 40 μM) in a medium of isolated rat hepatocytes resulted in decreased choline phosphorylation (58% vs. 33%) and increased choline oxidation (35% vs. 57%) (Pritchard and Vance, 1981). It is possible that when choline at 8× was supplemented, a larger proportion was oxidized to betaine. However, without further scrutinizing one carbon metabolism, this cannot be determined with certainty. Additionally, research by Diluzio and Zilversmit (1959) investigated acute vs. chronic choline supplementation in conjunction with a choline-deficient diet in dogs. In dogs, phospholipid activity in the liver was 108% greater with administration of a single dose of choline (300 mg/kg BW) after 14 d of a choline-deficient diet, as compared to 14-day supplementation of choline (150 mg/kg BW). The researchers proposed that the lipotropic benefits of choline supplementation may not be observed when the liver is not rich in TAG (Di Luzio and Zilversmit, 1959). Due to the Latin square design used in present study, the 15 cats enrolled were separated into five different groups. Only one of the five groups received choline at 8 × NRC RA during their first treatment period. The other four groups consumed 6 × NRC RA prior to 8 × NRC RA. As a result, it is possible that any potential lipotropic benefits of this higher dose were not observed due to the consumption of the dose prior. Future research could consider a parallel design to investigate the lipotropic benefits of choline supplementation. However, a larger sample size may be required to do so. Adding a washout period between doses may be another consideration.

Although serum ALT remained unchanged, choline at 8 × NRC RA decreased serum ALP by 14% when compared with control, and 12% when compared with 2 × NRC RA and 4 × NRC RA. Similar decreases in ALP with choline supplementation have been observed in obese cats, humans, and livestock species (Buchman et al., 2001; Rahmani et al., 2012; Getty and Dilger, 2015; Verbrugghe et al., 2021). Concentrations of ALP have been known to increase in cats with diagnosed hepatic diseases, including FHL (Everett et al., 1977; Center et al., 1993). However, similar to Verbrugghe et al. (2021), serum ALP concentrations remained within normal reference range (12-60 U/L) for all cats with all five doses of choline, indicating absence of hepatobiliary disease. Liver enzyme values alone are not enough to assess hepatic health, and/or function. Future studies should consider including pre- and postprandial bile acid profiles, liver biopsies, and abdominal ultrasonography to better determine hepatic health.

Similar to previous studies on choline supplementation in cats (Verbrugghe et al., 2021) and betaine supplementation in piglets (Huang et al., 2007), serum BUN decreased in the present study by 5% with 4 × NRC RA and 4% with 8 × NRC RA, as compared to control. This reduction may be representative of normal interindividual variability, as the serum BUN concentrations remained within the laboratory reference range (6–12 mmol/L) for all treatments. Alternatively, it may represent reduced amino acid oxidation and potentially improved protein synthesis. Improvement of growth performance was demonstrated in piglets with supplementation of betaine (Huang et al., 2007). Increased lean soft tissue mass was also observed during growth in many other animal species following choline or betaine supplementation (Daily et al., 1998; Fernández et al., 2000; Matthews et al., 2001). The

effects of choline on protein synthesis and body composition were not assessed in the present study and should be considered in future research.

Body composition assessment would also be useful to assess body fat mass. Choline and betaine supplementation studies in various animal species noted reduced fat deposition during growth (Daily et al., 1998; Fernández et al., 2000; Matthews et al., 2001). Lower fat mass gains were also observed in a previous study in growing kittens supplemented with additional choline for 12 wk (Godfrey et al., 2022). Body weight and BCS of overweight cats remained stable during the present study, similar to previous results in obese cats (Verbrugghe et al., 2021). This is unsurprising as in both studies cats were fed to maintenance energy requirements in order to maintain their current BW, and cats were only supplemented with choline for three weeks at a time in the present study. Hence, food intake was also similar between doses. Lower food intakes were reported in growing kittens supplemented with additional choline, when provided food ad libitum, resulting in lower fat mass gains (Godfrey et al., 2022). However, intake of these kittens was still above their calculated daily energy requirements for growth. The exact mechanism by which choline reduced food intake in kittens is unclear (Godfrey et al., 2022).

Even with unchanged food intake, it could be hypothesized that supplementation of choline may increase EE and subsequently affect body fat mass. This is because choline supplementation may increase lean soft tissue mass (Daily et al., 1998; Matthews et al., 2001), thus affecting EE as well. However, neither the present study nor the previous kitten study observed changes in fasted or fed EE with choline (Godfrey et al., 2022). Mirroring previous feline trials (Shoveller et al., 2014; Asaro et al., 2018; Camara et al., 2020), fed EE increased as compared to fasted EE when cats were fed once per day. The postprandial EE reported in the present study falls within the range of previously published EE values for overweight and/or obese cats (Nguyen et al., 2002; Center et al., 2012; Shoveller et al., 2014). Energy expenditure in overweight and obese cats has been reported to be lower than that of lean cats or cats of normal body condition score (Center et al., 2011; Shoveller et al., 2014). This is because EE largely depends on the surface area of the animal (Heymsfield et al., 2012). As a result, it is unsurprising that there were no differences in EE in the present study, as the surface area of the cats did not change with treatment. Energy expenditure is similarly related to body composition and more specifically lean tissue mass (Schutz, 1995). The present results were obtained from cats with varying BCS and therefore varying body compositions. This increased individual variability of EE, making differences more difficult to determine.

It has also been suggested that dietary choline supplementation may increase fatty acid oxidation through an increased production of carnitine (Corredor et al., 1967), by increasing the availability of methyl group donors for one carbon metabolism (Vance and Ridgway, 1988). Previous research in overweight cats has noted a decrease in postprandial RQ (0–870 min) with L-carnitine supplementation, suggesting a shift towards fatty acid oxidation (Shoveller et al., 2014). In the present study, there were no significant differences in overall RQ in the fed or fasted state. However, there was a tendency for RQ in the fasted state to change with choline dose. This difference became significant when individual BWs of the cats were taken into account. Control and choline at 8 × NRC RA had the lowest RQs of 0.77, suggesting greater fat oxidation (Richardson, 1929). Unsurprisingly, AUC for fasted RQ was also lower for these two treatments. However, as there were no differences between doses following a post hoc test, it is unclear what the biological significance of this lower RQ value is. It is also unclear why control would have resulted in a lower RQ as compared to the remaining choline doses. This may have again been due to the Latin square design used in the present study and the lack of a washout period signalling potential carry over effects among groups. Only one group of cats (n = 3) consumed the control dose prior to 8 × NRC RA. The remaining cats consumed 8 × NRC RA prior for 3 wk, before immediately starting the control treatment for 3 wk. Similarly, choline at 8 × NRC RA tended to have a lower fed RQ value of 0.83, as compared to the other treatments. Choline at 2 × NRC RA and 4 × NRC RA demonstrated the greatest increases in RQ in the immediate postprandial (0–125 min) and fed states (125–475 min), and unsurprisingly had the highest fed RQ valued of 0.85. The increase in RQ from fasted to fed suggests a greater reliance on carbohydrates to meet energy requirements in the postprandial state (Richardson, 1929). Similar findings have been reported in both lean and overweight cats receiving supplemental L-carnitine (Shoveller et al., 2014).

A limitation of the present study was that the trial was paused for 2 mo due to the COVID-19 pandemic and related institutional restrictions on research. Periods 1 and 2 occurred during the months of February and March 2020, respectively. Periods 3 through 5 occurred during the months of July and August 2020. It has been recently published that ME intake of cats decreased until 16 mo of age and that gonadectomized male cats reach mature BW at 16 mo of age (Merenda et al., 2021). In the present study, enrolled cats reached 16 mo of age during period 3, though food intake was controlled throughout the study to maintain their BWs. In the aforementioned study, cats were gonadectomized at 12 mo of age (Merenda et al., 2021), as compared to 6 mo of age in the cats used in the present trial. As sex steroids modulate growth hormone secretion in mammals (Meinhardt and Ho, 2006), age of gonadectomy may influence growth rates and age of maturation in these cats. Previous research in male cats has found altered growth dependent on age of gonadectomy (7 wk vs. 7 mo) (Root et al., 1997). It is therefore unclear if any changes in metabolism may have occurred with age of the cats that could have affected the findings of the present study.

Additionally, it is unclear if there may have been a seasonal effect. Food intake in a colony of research cats was found to be 15% lower in the summer (June–August) as compared to winter (October–February), although there were no changes in BW (Serisier et al., 2014). However, the cats in said study all had access to natural light, and the majority were housed in indoor–outdoor runs with unlimited outdoor access. In the present study, cats did not have access to the outdoors, nor did they have access to natural light. As a result, room temperature and light cycle in the present study were more tightly regulated. An effect of season seems unlikely but cannot be excluded as a confounding variable in the present study.

Although there have been no published studies investigating the safe upper limit of choline intake in cats, Verbrugghe et al. (2021) and Godfrey et al. (2022) reported safe consumption of dietary choline at intakes of 5 × NRC RA by obese adult cats for a period of 5 wk and 3 × NRC RA in growing kittens for 12 wk, respectively. A commercial canned cat food was

recalled by the US Food and Drug Administration (FDA) in July 2020, citing "elevated levels of choline chloride" as the reason for recall (US Food and Drug Administration, 2020). The initial recall stated that ingestion of said cat food could result in hypersalivation, diarrhea, vomiting, shaking, tremors and death, amongst others (US Food and Drug Administration, 2020). A case report later reported that the average analyzed choline concentration of the food was 165,316 mg/kg diet dry matter basis (DMB) (Peloquin et al., 2021). Four cats from the same household were admitted displaying hypersalivation, muscle twitching, and/or ataxia, after consuming approximately one tablespoon of said recalled food, as reported by the owner (Peloquin et al., 2021). Ethylene glycol was detected on blood work; however, it remains unclear if and how this is related to high choline chloride intake. Dietary ethylene glycol contamination was ruled out and cats did not have access to anti-freeze according to the owners. In the current study, there were no adverse health outcomes or abnormalities noted in the cats following consumption of choline at 8 × NRC RA for the 3-wk periods. Specific research on potential adverse health effects of the by-products of bacterial degradation of choline in the digestive tracts of cats is needed.

Based on the results of the present study, choline supplemented at six times the current RA published by the NRC could provide protective benefits for liver health in overweight cats. Only choline at this dose increased serum lipid and lipoprotein concentrations, as compared to control. Although no differences in BW or BCS were noted, choline at eight times NRC RA had a tendency to decrease RQ, suggesting that fatty acid oxidation may have been increased. As both obesity and weight loss are risk factors towards the development of FHL (Blanchard et al., 2004; Valtolina et al., 2005), future studies should assess the efficacy of choline supplementation for longer periods, including assessment of body composition, in overweight and/or obese cats undergoing weight loss. The inclusion of metabolomic analyses to better understand the biochemical changes that may be occurring with dietary choline supplementation, would be of value. Additionally, future studies would benefit from including liver biopsies to better assess histology, and ultrasonography to determine liver size.

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Conflict of Interest Statement

A.R. declares that they have participated in paid internships and engagements with pet food companies within Canada. H.G. declares that they have paid engagements with pet food companies within Canada. C.G. holds the Nestle Purina Professorship in Companion Animal Nutrition. A.K.S. is the Champion Petfoods Chair in Canine and Feline Nutrition, Physiology and Metabolism, consults for Champion Petfood, was previously employed by P&G and Mars Pet Care, serves on the Scientific Advisory Board for Trouw Nutrition, and has received honoraria and research funding from various commodity groups, pet food manufacturers, and ingredient suppliers. A.V. is the Royal Canin Veterinary Diets Endowed Chair in Canine and Feline Clinical Nutrition and declares that they serve on the Health and Nutrition Advisory Board for Vetdiet. A.V. has received honoraria and research funding from various pet food manufacturers and ingredient suppliers. At the time of the study, the Ontario Veterinary College received funding from Nestlé Purina Proplan Veterinary Diets to support a Registered Veterinary Technician in Clinical Nutrition, who helped perform the described animal trial.

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