

Low Level of Supplemental Dietary L-carnitine Increases Energy Expenditure in Overweight, But Not Lean, Cats Fed a Moderate Energy Density Diet to Maintain Body Weight

Shoveller, A.K.^{1,2}

Minikhiem D.L.¹

Carnagey K.¹

Brewer J.¹

Westendorf R.¹

DiGennaro J.¹

Gooding M.A.¹

¹Procter & Gamble Pet Care, 8700 Mason-Montgomery Rd, Mason, OH 45040, United States of America, ²Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada

Corresponding author: Anna K. Shoveller, Procter & Gamble Pet Care, 8700 Mason Montgomery Road, Mason, Ohio, 45040, United States of America.

E-mail: shoveller.ak@pg.com

KEY WORDS: Feline, overweight, L-carnitine, energy expenditure, respiratory quotient, play motivation

ABSTRACT

L-carnitine (LC) has been included in feline diets to enhance weight loss and reduce risk of hepatic lipidosis. However, many overweight cats are fed maintenance diets and are not undergoing weight loss. The objective of this study was to investigate how feeding lean and overweight adult cats dietary LC (100 mg/kg) during weight maintenance affected resting energy expenditure (EE), respiratory quotient (RQ), and play motivation. Twenty healthy adult cats were stratified by gender and body condition score (BCS) and randomly assigned to receive either a control food (CON) or

the same food supplemented with 100 mg/kg LC (LC+) for 42 days. EE was assessed using indirect calorimetry at 0, 21, and 42 days and play motivation was assessed at 0 and 42 days. Body weight did not differ between treatment groups at baseline and throughout the study ($P>0.05$), as expected. On days 21 and 42, area under the curve (AUC) for EE (kcal/ kg BW*d) and RQ did not differ between groups ($P>0.05$) for lean cats. However, overweight cats fed LC+ had greater ($P<0.05$) AUC for EE for at fasting and after receiving a meal on d 21 and 42 and a lower AUC for RQ from 0 - 210 minutes post feeding on d 42 than overweight cats fed CON. Overweight cats, but not lean cats, fed LC+ spent less time both in the start box and overall test and pushed more

weight in the obstruction test than cats fed CON ($P < 0.05$). These results suggest that dietary LC fed at a low level of supplementation results in greater EE, lower RQ, and greater motivation to play in overweight, but not lean, cats fed to maintain weight. Future research should investigate whether a similar mechanism is present in cats fed ad libitum, the feeding management strategy commonly used.

INTRODUCTION

L-carnitine (LC) is a vitamin-like, water-soluble molecule that is found in all cells of the body and is concentrated in cardiac and skeletal muscle.¹ The primary function of LC is to facilitate the transport of long-chain fatty acids (LCFA) across the inner mitochondrial membrane for beta-oxidation within the mitochondrial matrix. LC also serves as a cofactor for the transport of acetyl-CoA out of mitochondria, which further enhances the rate of fatty acid beta-oxidation and energy generation.¹ Together these actions regulate intra-mitochondrial acetyl-CoA concentrations, releasing free CoA and acetyl-carnitine that favor pyruvate oxidation. In addition to its demonstrated role in fat oxidation and energy generation, there is some evidence that LC may affect behavior.² Studies of this role have focused primarily on mood or affective behavior in aging patients, developmental disorders such as autism, or the therapeutic use of LC for chronic diseases such as celiac or neoplastic disease.^{3,4,5} Reported benefits of LC supplementation manifest as behavioral changes that include improved subjective energy level, enhanced motivation to learn, and a reduction in mental fatigue.

Healthy animals do not typically require a dietary source of LC because it is produced in sufficient amounts by the body and is also well conserved via renal reabsorption. However, LC is classified as a conditionally essential nutrient because endogenous synthesis may not be sufficient in conditions during which energy production relies largely upon beta-oxidation of fatty acids or in certain disease conditions.

For example, diminished carnitine status, as assessed by tissue concentrations of carnitine and its acyl derivatives, may play a role in hepatic lipidosis in cats, and a carnitine-responsive dilated cardiomyopathy has been described in certain genetic lines of dogs.^{6,7,8} Therefore, although the body produces LC, a dietary source may be required during periods of high demand, reduced synthesis, or increased excretion.

L-carnitine status in pet cats has been studied because of its potential role in either the pathogenesis or the treatment of hepatic lipidosis.⁹ Although not conclusive, there is evidence that liver LC homeostasis may be impaired in cats with hepatic lipidosis, and that dietary supplementation improves fatty acid oxidation in susceptible animals.^{10,11} The metabolic effects of LC have also been studied in healthy, overweight cats undergoing weight loss, a class of cats typically considered to be at risk for developing hepatic lipidosis. In a recent study, LC-supplemented (+100 ppm and +150 ppm) cats that were undergoing weight loss had higher rates of fatty acid oxidation, higher resting energy expenditure (REE) to lean body mass ratio, and lower respiratory quotients (RQ) when compared with unsupplemented cats.¹² Similarly, diet-induced hyperlipidemia in rats is associated with impaired fuel metabolism and these changes were reversed during short term LC supplementation.¹³ These studies suggest that dietary LC can support fatty acid oxidation and energy expenditure during periods of weight loss or physiological stress in overweight animals.

Most studies of LC have involved the use of animal models with certain physiological irregularities, such as an LC deficiency, obesity, hyperlipidemia, or hepatic lipidosis. There are few studies that focus on the effects of LC in healthy animals (and none with cats) with respect to energy and macronutrient metabolism. The objectives of the present study were to investigate both the metabolic and behavioral effects of 100 mg/kg of supplemental dietary LC when fed to healthy cats that were not undergoing

Table 1: Analyzed nutrient content (%*, as fed*) of Control and Test diets

Nutrient	Control Diet	Test Diet	Difference
Moisture (%)	5.16	5.29	-0.13
Fat (%)	16.8	17.77	-0.97
Ash (%)	6.3	6.32	-0.02
Crude Fiber (%)	1.27	1.48	-0.21
Protein (%)	34.75	33.83	0.92
L-carnitine (mg/kg)	29.3	120	-90.7

dietary energy restriction and were being fed to maintain current body weight and condition.

MATERIALS AND METHODS

Animals

A group of 10 neutered males (average age = 3.25 +/- 0.169 years), and 10 spayed females (average age = 3.02 +/- 0.158 years) were used in this study. All cats resided at the Procter & Gamble Pet Health and Nutrition Center (Lewisburg, OH), and were group-housed in an indoor environment with access to an outdoor enclosure during daylight hours. The interior room included resting perches, scratching posts, climbing apparatuses, and a variety of toys that were rotated regularly. Cats were socialized daily for at least 20 minutes and for 60 minutes the day following indirect calorimetry measurement, with a familiar human caretaker. Room temperature was maintained at 22° C with relative humidity of 50 to 60 %. Water was provided ad libitum via automatic watering devices. The cats were group-housed throughout the study, except during each of three 15-hour calorimetry periods, when cats were housed individually in a respiration chamber. All of the cats had been previously acclimated to respiration chambers for up to 24 hours of temporary housing.¹⁴ A standard veterinary evaluation that included physical examinations and chemical and complete blood count (CBC) blood analyses was completed on all cats prior to the initiation of the study. All cats were healthy at the start of the study period and had a mean body weight of 4.32 ± 1.07 kg and a body condition score (BCS) between 2.5 and

4.5, which was measured using a five-point scale. The animal care and handling procedures used in the study were approved by Institutional Animal Care and Use Committee at Procter & Gamble Pet Care (Mason, OH, USA).

Study Design and Diets

Cats were fed the control diet, an adult maintenance, dry extruded cat food,^a for an initial 21-day washout period. Cats were then stratified by gender and BCS and randomly assigned to one of two treatment groups. The control group (CON) was fed the adult maintenance food and the test group (LC+) was fed the control diet formulated and verified to contain supplemental LC at a concentration of 100 mg/kg of food (Table 1). Daily food allotments were calculated to maintain each cat's starting body weight using a standard equation based upon body weight and found to be applicable to this population of cats for weight maintenance. Females were fed 40.5 kcal ME/kg BW*d and males were fed 45 kcal ME/kg BW*d. Cats were individually fed once daily at 7:00 a.m. and allowed 60-minutes to complete eating. Remaining food (orts) was collected and weighed to calculate total daily intake for each cat throughout the study period; however, all cats had complete food consumption throughout the experimental period. Cats were fed their assigned diet over the 42-day study period and body weight was recorded weekly. Calorimetry was performed at the start of the study (baseline), on Day 21 (midpoint), and again on Day 42 (end of study).

Resting Energy Expenditure

Measurement

Whole body indirect calorimetry was measured on Day -1, Day 21, and Day 42 using a respiratory gas exchange measurement method. Chambers were an open-circuit flow-through design with room air pulled into chambers at a rate of 5 to 10 L/min and described previously.¹⁴ Chamber air was dried by columns of Drierite™ and again prior to being presented to the gas analyzers with magnesium perchlorate. Calorimetry data were collected using Qubit calorimetry software (Qubit C950-MCGES, Qubit Systems Inc., Kingston, Ontario Canada). Cats were fasted overnight and placed in the respiration chambers the following morning. Following a 30-minute gas equilibration period, two fasting respiratory gas measurements were taken to establish fasting VCO₂ and VO₂. Cats were fed their entire daily ration immediately after the second fasting measurement and respiration calorimetry was continued for 15 hours post-feeding. Recalibration of the CO₂ and O₂ analyzers was conducted approximately 7 to 8 hours after the start of the procedure. At the end of the 15-hour collection period, cats were removed from their chambers and returned to the group-housing room. Energy expenditure (EE) was calculated using the abbreviated Weir equation.¹⁵

The area under the curve (AUC) for RQ and EE were calculated and compared at 0 to 210, 210 to 420, 420 to 630, and 630 to 870 minutes post-feeding, and are expressed on a per kg BW basis: $EE (kcal/d) = [3.9 VO_2 + 1.11 VCO_2]$. Time periods were chosen to represent fed, post prandial, post absorptive, and return to fasting estimations.

Assessment of Play Motivation

An obstruction test was

used to measure motivation to play using testing procedures previously described.^{14,16} Briefly, cats were placed in a “start” box that included a swing door connected to a second “goal” box that contained a desirable cat toy. Cats could push on the swing door and enter the goal box to obtain access to the toy. Motivation to play was quantified by incrementally increasing the weight of the door making it progressively more difficult to open (100 g increments; max 500 g).

Cats were allowed up to 5 minutes to move into the goal box from the start box. If unsuccessful (ie, the cat did not cross the door within the allotted time), the door weight was decreased by 50 g and testing repeated for a single trial before being terminated. A cat’s threshold of motivation to play was assessed by identifying the maximum door weight that a cat successfully overcame to enter the goal box to obtain the toy, the time spent in the start box, and the total time taken to perform the entire task (defined as touching the toy in the goal box). Play motivation was assessed on Day 0 (baseline) and Day 42 of the study period at approximately 6 hrs post feeding.¹⁷

Statistical Analyses

All data were analyzed using SAS version 9.2 (SAS Institute, Cary, NC), and results

Figure 1: Energy expenditure (kcal/kg BW *day) at fasting and during the post prandial period (0-870 minutes) on Day 42 in overweight cats

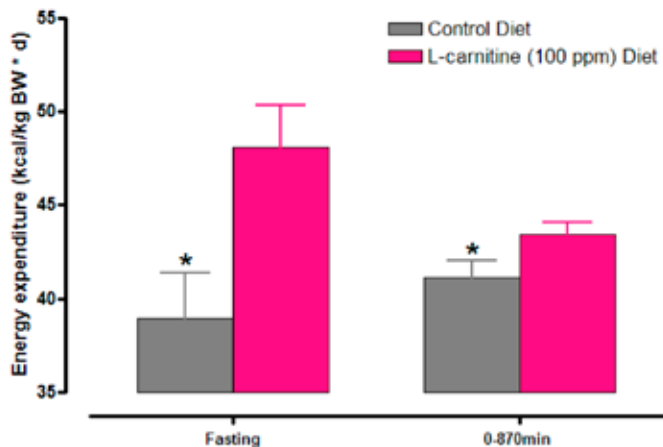


Table 2: Mean change in body weight (kg) from Day 0 of cats fed Test and Control diets on Day 21 and 42 of feeding

Dietary treatment	Change from Base (kg)			
	Day	Mean	SEM	P-value
Control	Day 21	-0.024	0.009	0.022
	Day 42	-0.022	0.021	0.304
Test	Day 21	-0.025	0.009	0.017
	Day 42	-0.026	0.021	0.235

are reported as least square means \pm pooled SEM. All diet comparisons were assessed at a significance level of 0.05. BW and calorimetry data were analyzed using a repeated measures mixed model that included diet and study week as main effects and baseline as a covariate. Also, the diet-by-study week and diet-by-covariate interaction terms were included in the model. The diet-by-covariate interaction was not significant and was removed from the final model. To assess the diet effect on lean and overweight cats separately, body condition type and its interaction with diet and diet-by-study week were added to the final model. Play motivation data were analyzed using a t-test to compare treatment groups for lean and overweight cats separately.

RESULTS

Health and Body Weight

All 20 cats remained healthy and active throughout the study. Mean body weight

between treatment groups did not differ significantly at the start, midpoint, or end of the study period ($P > 0.05$). Within treatment groups, on Day 21 cats lost weight in both treatment groups when compared to initial BW, although the physiological relevance of this small level of weight loss is likely not significant. However, at the end of the study (Day 42), there was no difference between final and initial BW in either treatment group (Table 2). Body condition score did not differ between treatment groups and did not change significantly during the study period (data not shown).

Energy Expenditure and RQ

There was no difference between the two treatment groups in either fasting or entire post-prandial energy expenditure (kcal/kg BW*d) at baseline, when all cats were analyzed or when lean and overweight cats were analyzed separately ($P > 0.05$, Table 3). RQ values at baseline were not significantly different between lean cats allocated to CON

Table 3: Energy expenditure (kcal/kg*day) or respiratory quotient on Day 0 (baseline) for lean and overweight cats fed test and control diets

BCS TYPE	Variable	Test			Control		
		Time	Mean	SEM	Mean	SEM	P value
Lean/Ideal	Respiratory quotient	fasted	0.736	0.006	0.741	0.007	NS
		0-870 min	0.832	0.005	0.828	0.003	NS
	Energy Expenditure	fasted	55.84	5.86	49.69	3.43	NS
		0-870 min	46.30	1.90	45.76	1.33	NS
Moderately Overweight	Respiratory quotient	fasted	0.747	0.010	0.780	0.012	NS
		0-870 min	0.820	0.002	0.833	0.004	0.033
	Energy Expenditure	fasted	43.71	1.67	50.49	4.46	NS
		0-870 min	38.93	3.02	34.90	1.20	NS

Table 4: Change from baseline in energy expenditure (kcal/kg BW *day) at fasting and during the post prandial period (0-870 minutes) on Day 21 and Day 41 in lean and overweight cats allocated to test and control diets.

BCS TYPE	Day	Test			Control		
		Time	Mean	SEM	Mean	SEM	P value
Lean/Ideal	Day 21	fasted	-4.45	1.80	1.10	1.60	0.033
		0-870 min	-1.30	0.54	-0.18	0.49	0.114
	Day 42	fasted	-3.83	1.80	-0.15	1.60	NS
		0-870 min	-1.85	0.54	-1.44	0.49	NS
Moderately Overweight	Day 21	fasted	-2.15	2.21	-10.98	2.44	0.014
		0-870 min	1.24	0.66	-1.12	0.87	0.023
	Day 42	fasted	-2.36	2.21	-11.56	2.44	0.011
		0-870 min	0.48	0.66	-1.80	0.87	0.028

Table 5: Change from baseline in measurements taken during the motivation test on Day 41 in lean and overweight cats allocated to test and control diets.

BCS TYPE	Play Variable (Change from Baseline)	Test		Control		P value
		Mean	SEM	Mean	SEM	
Lean/Ideal (n=13)	Time in start box (min)	3.7	65.8	-45.9	61.1	NS
	Maximum weight pushed (g)	-41.7	120.0	-14.3	106.2	NS
	Time spent (min)	15.6	53.7	-7.3	43.4	NS
Moderately	Time in start box (min)	-19.0	11.0	97.0	25.7	0.01
	Overweight (n=7)	-25.0	25.0	-250.0	132.3	0.11
	Time spent (min)	5.2	5.7	72.6	15.2	0.01

versus lean cats allocated to LC+ when fasted or during the entire post-prandial period ($P > 0.05$, Table 3). However, overweight cats allocated to LC+ had a significantly lower mean RQ during the post-prandial period than overweight cats allocated to CON (0.820 vs. 0.833, $P = 0.033$) at baseline.

There were no significant differences in energy expenditure (kcal/kg*d) between lean cats fed CON and lean cats fed LC+, with the exception that cats in LC+ had a greater decrease from baseline ($P < 0.05$) in the fasting REE when compared with lean cats fed CON on Day 21 (-4.45 vs. 1.10, $P = 0.033$, Table 4). Energy expenditure in overweight cats (kcal/kg BW*d) fed LC+ was significantly greater during fasting and over

the 870 minute post-prandial period on both Day 21 and Day 42 when compared with overweight cats fed CON (Table 4 and Figure 1, $P < 0.05$). There were no differences ($P > 0.05$) in RQ for lean or overweight cats fed CON versus LC+ during fasting or over the entire post-prandial period, at either Day 21 or Day 42. However, overweight cats fed LC+ had a significantly lower change from baseline in RQ at Day 42 in the 0-210 minute post prandial period when compared with overweight cats fed CON (0.0045 vs. 0.0172, $P = 0.026$) suggesting greater fat oxidation.

Play Motivation

There were no differences between lean cats fed CON and those fed LC+ for any

measures of motivation to play at baseline, Day 42, or change from baseline at Day 42 (Table 5). Overweight cats allocated to CON did not differ ($P>0.05$) from cats fed LC+ for any measures of motivation at baseline, but significant differences were observed at 42 days. Overweight cats fed LC+ spent significantly less time in the start box and in the overall test when compared with overweight cats fed CON (Table 5, $P = 0.01$). Furthermore, cats fed LC+ tended to push more weight than cats fed CON (Table 5, $P=0.11$).

DISCUSSION

LC is a conditionally essential nutrient that is needed for the transport of fatty acids across the inner mitochondrial membrane for oxidation. Although animals produce LC endogenously, there is evidence that providing a dietary source can enhance fat metabolism and may be beneficial to overweight animals that are undergoing weight loss. For example, when overweight cats were fed a commercial weight reduction food restricted to 60% of estimated ME requirements, cats that were supplemented with an additional 250 mg LC per day had a higher rate of weight loss when compared with unsupplemented cats.¹¹ Supplemented cats also had higher levels of circulating acetyl-carnitine, which is suggestive of an enhanced rate of fatty acid oxidation. Metabolic changes associated with LC supplementation were examined further in overweight cats fed a weight reduction diet containing either 0, 50, 100, or 150 mg/kg additional LC over a 12-week weight loss period.¹² Cats in all four groups lost similar amounts of weight and body fat. However, LC-supplemented cats had a higher ratio of REE to lean body mass and lower RQ values in comparison to unsupplemented cats, indicating that cats supplemented with LC were better able to maintain REE during weight loss and were metabolizing a higher proportion of fatty acids.

It was theorized that LC supplementation may function to protect against the decrease in REE that is typically observed during prolonged periods of dietary energy

restriction and help to sustain a normal or elevated rate of fatty acid metabolism. Similar results have been reported in dogs. In an early study with dogs, a group of overweight dogs were fed a reduced-calorie food containing 0, 50, or 100 mg/kg of LC for a 12-week restricted feeding period.¹⁸ Dogs in all three groups lost weight, but those that were fed diets containing supplemental LC lost more body fat and more weight than dogs fed the control diet. An interesting finding from this study was that both levels of supplementation provided weight loss and lean body tissue benefits, with no additional advantage accrued when L-carnitine was increased from 50 to 100 mg/kg.

The present study sought to feed lean and overweight cats to maintain their initial body weight for a period of 42 days (6 weeks). Feeding 100 mg/kg supplemental LC to overweight cats influenced fasting and post feeding REE, similar to previous results reported in overweight dogs and cats that were fed for weight loss. Overweight cats fed a diet that was supplemented with LC (100 mg/kg) had significantly higher energy expenditure after fasting and when measured over a 15-hour post-feeding period when compared with overweight cats that were fed the control diet. This difference was not observed in lean cats. Similar to the aforementioned study, RQ values were also lower in overweight cats fed diets containing LC. This effect was observed in the early, post-prandial phase (0 to 210 minutes) after 42 days of supplementation.

These results suggest that overweight cats fed supplemental LC were metabolizing a higher proportion of fat than were overweight cats that were not supplemented, during the first 4 hours following a meal. These results in the early post-prandial phase are consistent with evidence that lipid absorption predominates over carbohydrate absorption and use during the early post-prandial period in cats, which is unlike omnivores where the reverse is true. These data also suggest that LC supplementation promotes increased fat oxidation in overweight, but

not lean cats.

Several mechanisms of action may contribute to the enhanced fat metabolism that is observed in overweight cats, but not in lean cats, in response to supplementation with LC. Differences in muscle fat stores and in the types and amount of metabolic fuel used by working muscles may contribute to differences between lean and overweight animals.^{19,20} It is possible that the muscle fibers of overweight cats are not as metabolically active as those of lean cats because they are more predisposed to store lipids in skeletal and adipose tissue rather than to metabolize fat for energy. Numerically, EE is greater in lean cats as compared to overweight cats, although a correction for quantitatively measured lean body mass may eliminate this difference. As such, this difference may be influenced by an absolute or relative deficiency of LC in the muscle in overweight cats, which in turn is responsive to dietary supplementation.

A second contributing factor may be changes in adaptive thermogenesis in overweight animals. There is evidence that stressors such as reduced food intake or insulin resistance may lead to a reduction in adaptive thermogenesis, which leads to a metabolic shift towards lipid storage and away from lipid metabolism.^{21,22} Insulin resistance is common in overweight cats and may affect the availability and use of LC at the cellular level. However, we did not measure insulin sensitivity in the present study. L-carnitine enters muscle cells via the sodium/potassium pump and insulin influences both the functioning of this transport mechanism and electrolyte balance. In the normal healthy state, insulin depletes intracellular Na stores, which supports the co-transport of carnitine into muscle cells. However, because insulin-resistant tissues are less sensitive to the effects of insulin, this may also limit the amount of carnitine entering the cells of overweight cats. Further support for this effect comes from evidence that two key enzymes, CPT₁ and muscle carnitine translocase, are inhibited in insulin-

resistant individuals.²³ These enzymes aid in the transport of fatty acids across mitochondrial membranes. Providing supplemental carnitine may increase activation of these enzymes and enhance fat oxidation.

Similar to overweight human subjects, overweight cats exhibit increases in plasma triglycerides, very-low-density-lipoproteins (VLDL), and free (non-esterified) fatty acids when compared with lean cats.^{24,25} Overweight cats also tend to have lower energy expenditure on a weight (per kg) basis, when compared with lean cats.²⁶ This may be caused in part by a higher relative proportion of body fat to lean tissue and its effects upon basal metabolic rate as well as changes in adaptive thermogenesis.

Feeding additional LC to overweight cats may facilitate energy expenditure via both enhanced fat oxidation and reversing stress-induced reductions in adaptive thermogenesis, an effect that appears to be most pronounced when circulating lipids are above normal. Studies with human subjects have shown similar results to those observed in the present study. Individuals receiving supplemental LC had lower respiratory quotients suggestive of increased use of lipids for energy and enhanced fatty acid oxidation.^{27,28} Cumulatively, these results suggest a role for LC to increase fatty acid oxidation and enhance energy metabolism in overweight cats, during periods of weight maintenance, as well as during periods of weight loss.

In addition to differences in fasting and post feeding REE, the study reported here also found differences in play motivation between overweight and lean cats supplemented with LC. Feeding supplemental LC significantly enhanced play motivation in overweight cats, but not in lean cats. After 42 days of feeding, overweight cats fed a supplemented diet spent significantly less time in the start box and less time obtaining access to the toy (total time), when compared with overweight cats that were fed the control diet. Supplemented cats also pushed on average about 225 grams more than con-

trol cats to move from the start box into the goal box ($P=0.11$). This difference is likely physiologically relevant when considered in totality with the rest of the play results. Possible underlying mechanisms that may have affected these behaviors include metabolic benefits of LC such as: improved fat metabolism and energy expenditure, systemic effects such as reduced feelings of fatigue and increased alertness, or direct effects on the neurophysiology of the brain.

The motor patterns of play are similar to those of the appetitive and consummatory patterns of predation as object play is hypothesized to allow the animal to learn to perfect, organize and practice predation.^{29,30} While play and predation appear to be similar in activity pattern, there also appears to be a common underlying control system. In cats, level of hunger has been shown to reduce latency to approach prey and increase likelihood of prey directed attack.³¹ Similarly, cats demonstrate increased frequencies of play when subjected to acute calorie restriction and greater time (0 vs. 16 hr) since feeding.^{32,33,34,35}

We measured play motivation at about 6 hrs post feeding for all cats, in both treatment groups and cats were at weight maintenance; therefore, the increased play observed in the present study is not tied to variables related to food availability. While hunger appears to increase the likelihood that cats will demonstrate both predation and play behaviors, hunger is not necessary for the performance of these activities if the appropriate internal and external stimuli are available.^{36,37} Another possible explanation for a similar control system for play and predation is that both systems are controlled by separate but interacting brain regions.^{38,39,40}

Stimulation of the hypothalamus, a control region involved in energy sensing and regulation, can contribute to increased demonstration of predatory behaviors without an observed increase in food consumption.^{41,42} It is hypothesized that hypothalamic activation, regardless of hunger level, may act to increase play demonstrations by altering the

capacity of a particular toy or similar object (external cue) to induce play.⁴³ Overall, play and predatory behaviors appear to be influenced by the central regulation of energy homeostasis. Since LC supplementation in overweight cats influences metabolic regulation through a concurrent increase in fat oxidation and energy expenditure, it may be hypothesized that the increased demonstrations of play are a consequence of a change in the central energy sensing system.

Effects of LC on mental and physical fatigue may be related to its metabolic effects on fuel use and energy expenditure. It is common for aged individuals and patients with kidney disease, diabetes, or cancer to report symptoms of chronic fatigue.³ L-carnitine levels decline with age and LC deficiencies have been reported in patients with the aforementioned disease states.⁴⁴ Recent research indicates that LC supplementation may reduce age-related signs of physical and/or mental fatigue. In a study of 66 centenarians, the oral administration of LC reduced mental and physical fatigue and improved cognitive activity and mental alertness.³ Similarly, intravenous LC administration to patients with end-stage renal disease significantly improved patient-assessed fatigue.⁴⁵ LC supplementation in human patients with celiac disease and cancer resulted in improved fatigue symptoms, along with increased lean body mass.^{4,5}

Cumulatively, these results suggest that LC's effects on muscle metabolism may translate into improved subjective feelings of energy and higher activity levels. Indeed, obesity is related to both physical and mental fatigue in humans.⁴⁶ Mental alertness may contribute to an enhanced reactivity to external stimuli (toy mouse) and provide greater feelings of energy to enhance motivational drives contributing to the observed increase in demonstration of play in overweight cats. Future research should additionally investigate whether satiety hormone concentrations, genes associated with cellular energy metabolism, and voluntary activity change due to LC supplementation

to further understand the possible mechanisms of this result.

The metabolic benefits of supplemental LC in overweight animals may also directly influence the brain. Insulin resistance not only affects peripheral tissues, but can also affect brain function. Insulin is able to cross the blood brain barrier and interact with receptors in the hippocampus, the area of the brain that is involved with memory and cognitive processing. Brain insulin receptors in patients with type II diabetes mellitus are desensitized, which contributes to disrupted energy metabolism.⁴⁷ Supplemental LC may affect the brain in a manner similar to its effects on muscle cells by optimizing fat oxidation and enhancing energy metabolism and availability. Furthermore, LC may exhibit neuroprotective effects during aging and in certain diseases that lead to metabolic changes in the brain, such as increased ammonia levels observed in patients with hepatic encephalopathy.⁴⁸ The metabolic causes for these improvements are not fully understood, but it is theorized that increased glucose availability for non-insulin dependent tissues, such as the brain, along with improved fatty acid utilization by heart and muscle tissues may be contributing factors. In the present study, while the underlying mechanism of the play benefits to overweight cats were not studied, these are interesting and relevant results and warrant continued investigation.

CONCLUSIONS

Supplemental LC has been fed to cats to enhance weight loss and to potentially reduce risk of hepatic lipidosis. However, many overweight cats that live as pets in homes are fed maintenance diets and are not subjected to weight loss programs. The results of this study showed that when overweight cats were fed an adult maintenance diet that was enriched with LC (100 mg/kg) and fed to maintain body weight, they experienced increased resting and post-prandial energy expenditure, reduced RQ, and increased motivation to play. Future research should investigate whether a similar mechanism is

present in cats fed ad libitum and in-home cats.

a. Iams ProActive Health Original with Chicken

REFERENCES

1. Steiber A, Kerner J, Hoppel C. Carnitine: A nutritional, biosynthetic, and functional perspective. *Mol Aspects Med* 2004; 25:455-473.
2. Kobayashi S, Iwamoto M, Kon K, et al. Acetyl-L-carnitine improves aged brain function. *Geriatrics Gerontology Internat* 2010; 10:S99-S106.
3. Malaguarnera M, Cammalleri L, and Gargante MP. L-carnitine treatment reduces severity of physical and mental fatigue and increases cognitive functions in centenarians: a randomized and controlled clinical trial. *Am J Clin Nutr* 2007; 86:1738-1744.
4. Ciacci C, Peluso G, Iannoni E, et al. L-Carnitine in the treatment of fatigue in adult celiac disease patients: A pilot study. *Digestive Liver Dis* 2007; 39:922-928.
5. Gramignano G, Lusso MR, Madeddu C. Efficacy of L-carnitine administration on fatigue, nutritional status, oxidative stress, and related quality of life in 12 advanced cancer patients undergoing anticancer therapy. *J Nutr* 2006; 22:136-145.
6. Carroll MC and Cote E. Carnitine: A review. *Compend Cont Educ Pract Vet* 2001; 23:45-52.
7. Keene BW. L-carnitine deficiency in canine dilated cardiomyopathy. In *Current Veterinary Therapy XI*. Edited by Kirk RW, Bonagrua JD. Philadelphia, PA: WB Saunders, p 780, 1992.
8. Keene BW, Panciera DP, Atkins CE, et al. Myocardial L-carnitine deficiency in a family of dogs with cardiomyopathy. *J Am Vet Med Assoc* 1991; 198:647-650.
9. Center SA, Crawford MA, Guida L, et al. A retrospective study of 77 cats with severe hepatic lipidosis: 1975-1990. *J Vet Intern Med* 1993; 7:349-359.
10. Blanchard G, Pargon BM, Milliat F and Lutton C. Dietary l-carnitine supplementation in obese cats alters carnitine metabolism and decreases ketosis during fasting and induced hepatic lipidosis. *J Nutr* 2002; 132:204-210.
11. Center SA, et al: The clinical and metabolic effects of rapid weight loss in obese pet cats and the influence of supplemental oral L-carnitine, *J Vet Intern Med* 2000; 14:598-608.
12. Center SA, Warner KL, Randolph JF, et al. Influence of dietary supplementation with L-carnitine on metabolic rate, fatty acid oxidation, body condition, and weight loss in overweight cats. *Amer J Vet Res* 2012; 73:1002-1015.
13. Noland RC, Koves TR, Seiler SE, et al. Carnitine insufficiency caused by aging and over-nutrition compromises mitochondrial performance and metabolic control. *JBC*, 2009; 284: 22840-22852.
14. Gooding MA, Duncan IJH, Atkinson JL, Shoveller AK. Development and validation of a behavioural acclimation protocol for cats to respiration chambers used for indirect calorimetry studies. *J Anim Welfare Sci* 2012; 15:144-162.

15. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949; 109:1-9.
16. Widowski TM, Duncan IJH. Working for a dustbath: are hens increasing pleasure rather than reducing suffering? *Appl Anim Behav Sci* 2000; 68:39-53.
17. Gooding M. The effects of diet matrices on feline bioenergetics and behaviour. PhD Thesis, University of Guelph, 2012.
18. Sunvold GD. Carnitine supplementation promotes weight loss and decreased adiposity in the canine, Proceedings of the XXIII World Small Animal Veterinary Association. 1998; 746.
19. Reda E, D'Iddio S, Nicolai R, et al. The carnitine system and body composition. *Acta Diabetol* 2003; 40:S106-S113.
20. Stephens FB, Constantin-Teodosiu D, Greenhaff PL. New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *J Physiol* 2007; 581:431-444.
21. Major GC, Doucet E, Trayhurn P, et al. Review: Clinical significance of adaptive thermogenesis. *Internat J Obesity* 2007; 31:204-212.
22. Wijers SL, Saris WH, Van Marken Lichtenbelt WD. Recent advances in adaptive thermogenesis: potential implications for the treatment of obesity. *Obesity Reviews* 2009; 10:218-226.
23. Peluso G, Petillo O, Margarucci S, et al. Decreased mitochondrial carnitine translocase in skeletal muscles impairs utilization of fatty acids in insulin-resistant patients. *Frontiers Biosci* 2002; 7:109-116.
24. Hoenig M, Wilkins C, Holson JC, et al. Effects of obesity on lipid profiles in neutered male and female cats. *Amer J Vet Res* 2003; 64:299-303.
25. Jordan E, Kley S, Le AN, et al. Dyslipidemia in obese cats. *Domest Anim Endocrinol* 2008; 35:290-299.
26. Center SA, Warner KL, Randolph JF, et al: Resting energy expenditure per lean body mass determined by indirect calorimetry and bioelectrical impedance analysis in cats. *J Vet Intern Med* 2011; 25:1341-1350.
27. Gorostiaga EM, Maurer CA, Eclache JP. Decrease in respiratory quotient during exercise following L-carnitine supplementation. *Int J Sport Med* 1989; 10:169-175.
28. Müller DM, Seim H, Kiess W, et al. Effects of oral L-carnitine supplementation on in vivo long-chain fatty acid oxidation in healthy adults. *Metabolism* 2002; 51:1389-1391.
29. Leyhausen P. Cat Behaviour: The predatory and social behaviour of domestic and wild cats. Garland STPM Press, New York, NY, 1979.
30. Ewer RF. Some observations on the killing and the eating of prey by two dasyurid marsupials: The Mulgara, *Dasyurus cristicauda*, and the Tasmanian devil, *Sarcophilus harris* *Z Tierpsychol* 1969; 26:23-38.
31. Adamec RE, Stark-Adamec C, Livingston KE. The development of predatory aggression and defense in the domestic cat (*Felis catus*). I. Effects of early experience on adult patterns of aggression and defense. *Behav Neural Biol* 1980; 30:389-409.
32. Bateson P, Young M. Separation from the mother and the development of play in cats. *Anim Behav* 1981; 29:173-180.
33. Bateson P, Martin P, Young M. Effects of interrupting cat mothers' lactation with bromocriptine on the subsequent play of their kittens. *Physiol Behav* 1981; 27:841-845.
34. Bateson P, Mendl M, Feaver J. Play in the domestic cat is enhanced by rationing of the mother during lactation. *Anim Behav* 1990; 40:514-525.
35. Hall SL, Bradshaw JWS. The influence of hunger on object play by the adult domestic cat. *Appl Anim Behav Sci* 1998; 58:143-150.
36. Adamec RE. The interaction of hunger and preying in the domestic cat (*Felis catus*): An adaptive hierarchy? *Behav Bio* 1976; 18:263-272.
37. Biben M. Predation and predatory play behavior of domestic cats. *Anim Behav* 1979; 27:81-94.
38. Flynn JP, Vanegas H, Foote W, Edwards S. Neural mechanisms involved in a cat's attack upon a rat. In: E. R. Whalen, R. F. Thompson, M. Verzeano, and N. Weinberger (Eds.), *The Neural Control of Behavior*, New York: Academic Press, New York, NY, 1970; pp135-173.
39. Fonberg E, Flynn JP. Patterns of aggressive behavior evoked by hypothalamic and amygdalar stimulation. *Ann Aead Med Lodz* 1978 19:132 (Abstract).
40. Fonberg E, Serduchenko V. Predatory behaviour after hypothalamic lesions in cats. *Physiol Behav* 1980; 24:225-230.
41. Flynn JP. The neural basis of aggression in cats. In D. C. Glass (Ed.), *Neurophysiology and Emotion*, Rockefeller Univ. Press and Russel Sage, New York, NY, pp. 40-60,1967.
42. Katz RJ, Thomas E. Effect of food deprivation upon electrically elicited predation in the cat. *Behav Biol* 1977; 19:135-140.
43. Berntson GG, Hughes HC, Beattie MS. A comparison of hypothalamically induced biting attack with natural predatory behavior in the cat. *J Comp Phys Psych* 1976; 90, 167-178.
44. Cave MC, Hurt RT, Frazier TH, et al. Obesity, inflammation, and the potential application of pharmacnutrition. *Nutr Clin Pract* 2008; 23:16-34.
45. Brass EP, Adler S, Sietsema KE, et al. Intravenous L-Carnitine increases plasma carnitine, reduces fatigue, and may preserve exercise capacity in hemodialysis patients. *Am J Kidney Dis* 2001; 37:1018-1028.
46. Lim W, Hong S, Neleson R, et al. The association of obesity, cytokine levels, and depressive symptoms with diverse measures of fatigue in healthy subjects. *Arch Intern Med* 2005; 165:910-915.
47. Saltiel AR. New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. *Cell* 2001; 104:517-529.
48. Malaguarrera M, Pistone G, Elvira R. Effects of L-carnitine in patients with hepatic encephalopathy. *World J Gastroenterol* 2005; 11:7197-7202.