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Carniking™ Supplementation to Labrador Retrievers Prevents Weight Gain, Decreases Feed Intake and Increases Basal Metabolic Rate During High Calorie Intake

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Keywords: Carniking™; L-carnitine supplementation; Body weight and composition; High calorie intake; Feed intake; Basal metabolic rate.

Abbreviations: APKm: Activity Per Kilometer; BMR: Basal Metabolic Rate; DXA: Dual Energy X-Ray Absorptiometry Machine; kph: Average Moving Speed; MCT: Medium changed triglycerides; MER: Maintenance energy requirements.

Abstract

The present study aims to investigate the effect of L-carnitine on feed intake, body weight and composition, and Basal Metabolic Rate (BMR), in Labrador Retrievers fed high calorie diets. Each dog was sorted into one of three groups providing 0 mg/day, 236 mg/d or 492 mg/d of L-carnitine. Each group was further split into half receiving 110% or 120% of their Maintenance Energy Requirement (MER), assessed in a metabolic chamber at baseline. The trial consisted of a 8-week weight gain phase followed by a 7-week exercise phase. During the exercise phase, all dogs performed a twice weekly endurance run which increased incrementally in distance, and had their activity and average moving speeds recorded. Body composition and ´BMR were assessed at baseline, and at the end of each phase. Serum L-carnitine content was determined at the end of the study. Dogs fed high calorie intake (120% MER) showed a dose-dependent reduction in feed consumption and significant decrease in body weight at 236 mg and 492 mg of L-carnitine, effects independent from exercise ($p<0.01$), and driven by females ($p<0.01$). L-carnitine supplementation increased lean/fat ratio during exercise and at high calorie ($p= 0.04$). Fat mass increased significantly in the control group from baseline to the end of the weight gain phase ($p= 0.03$) while no significant increase was found with L-carnitine. BMR was increased at high calorie and during exercise ($p<0.05$). L-carnitine supplementation increased plasma total and free L-carnitine concentrations (each $p<0.01$) and decreased Ester:Free ratio ($p= 0.04$).

Dogs fed high calorie diets could benefit from supplementation with L-carnitine as weight gain is prevented most likely because of the observed decrease in food intake and increase in BMR.

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Introduction

The prevalence of overweight and obesity is increasing dramatically not only in humans but also in dogs with a clear relationship between owner and canine obesity [1,2]. It is believed that 18% to 44% of dogs worldwide were overweight or obese [2]. Part of the issue is linked to over-feeding dogs by providing them excessive treats. According to Mintel, 75% of US. consumers confirm they show love to their pets by offereing them treats daily, and only 24% give treats for health-related issues [3]. Also comparable to human, overweight or obese dogs are more likely be affected by several health concerns including metabolic and endocrine diseases, cardiorespiratory issues, urogenital dysfunction and orthopaedic disorders [4-7]. Dilated cardiomyopathy in particular is an increasing concern for dogs provided an imbalanced and incomplete diet [3]. Achieving a sustained weight loss is very difficult in obese dogs with most of the current strategies focusing on energy restriction [6,8]. There are, however, various attempts to identify ingredients with an effect on weight management in order to include into dog food. L-carnitine is considered one promising candidate. According to Dr. Collings, one way to overcome this problem is to reduce calorie intake and increase satiety by providing dogs 'active' treats, formulated with higher fiber and ingredients like L-carnitine [9]. Because of its basic mechanism of action, transporting long and medium chain fatty acids into the mitochondria, making them available for β-oxidation, L-carnitine plays an important role in energy production from fatty acids while sparing the use of branched-chain amino acids for protein synthesis [10]. Peroxisomal fatty acid α-oxidation also has reported to be reduced by L-carnitine [11,12]. These mechanisms have been proven in several animal studies where supplementation with L-carnitine preserved loss of lean body mass in dogs and cats while helping with weight loss [13-15]. L-carnitine is also necessary for normal cardiac and skeletal muscle function [16], suggesting a role in preventing some of the obesity-related issues such as dilated cardiomyopathy.

A systematic review and meta-analysis of 37 randomized controlled clinical trials in humans with dose-response analysis revealed an effect of L-carnitine supplementation on body weight, BMI and fat mass, particularly among overweight and obese adults [17]. There are also succesfull studies having investigated the effect of L-carnitine on weight management in general in dogs [13,14,18-23]. As exercise can impact body composition such as decrease in fat mass and increase in lean mass, benefits of L-carnitine on exercise performance have been studied for decades in humans and summarized in a comprehensive review [24]. While most of the studies in dogs showed benefits, in sled dogs and beagles during exercise, L-carnitine did not show an effect on parameters of performance such as heart rate and exercise-induced oxidative stress [25,26]. Racing greyhounds, however, supplemented with L-carnitine showed increased oxygen transport and reduction in markers of exercise-induced muscle damage [27]. In Labrador Retrievers, exercise-induced muscle damage was also prevented by L-carnitine supplementation and performance and recovery from exercise were improved [14]. In female Labrador Retrievers, in addition to improvement in oxygen consumption rates and energy expenditure, L-carnitine prevented muscle loss during exercise and decreased fat mass [28]. Further studies investigated L-carnitine in combination with other functional ingredients and showed positive effects on exercise related markers in the blood [29]. Although most reports showed benefits on weight management and general health, some did not reach similar conclusion most likely because of differences in the methods and testing. For instance, L-carnitine effects may vary with age, exercise regimen, metabolic status and calorie intake. In this study, we aimed to investigate the effect of L-carnitine in preventing weight gain in dogs fed a high calorie diet and based on their Maintenance Energy Requirements (MER). Two doses of L-carnitine, 236 mg and 492 mg per day were supplemented to Labrador Retrievers 5 to 11 year old and fed 110% or 120% of their MER. Feed consumption, body weight and composition, Basal Metabolic Rate (BMR) and physical activity have been recorded and correlated to plasma L-carnitine levels.

Methods

Animals and housing

Sixty-one Labrador Retrievers (31 males and 30 females) ranging from 5 to 11 years of age (on average 7 year old) and including 42 intact and 19 neutered were assessed in this trial (Table 1). All dogs were housed individually overnight in temperature-controlled kennels and were aired in outside yards for approximately six hours daily, dependent on weather. Dogs were fed their assigned diets and treatments once daily in the morning and they had free access to automatic waterers. Vaccinations were up to date and dogs received monthly prophylactic heartworm (Heartgard Plus, Merial, Duluth, GA) and parasite prevention (Simparica, Zoetis Petcare, Parsippany, NJ). Out of the 61 Labrador Retrievers one neutered and one intact male dogs were removed from the trial for reasons not related to the L-carnitine supplementation. Another neutered male dog was substituted due to tear of cranial cruciate ligament in the knee, which might be linked to the weight gain but not to the supplementation.

Experimental design

The trial was a 15-week randomized, single-blinded, placebo-controlled trial consisting of two phases (weight gain and exercise phase). During a 2-3 weeks baseline recording phase, dogs were screened based on their MER, BMR and body composition. Over the course of the study, all dogs were fed a basal diet. At the start of the weight gain phase (phase I), all dogs were sorted into six equal treatment groups of two levels of L-carnitine in addition to the control, and two levels of calorie intake based on gender, reproductive status, age, and body composition (Table 1). The feeding regime remained the same throughout the entire trial. Dogs were weighed once per week. During the exercise phase, all dogs performed twice weekly endurance runs at incrementally increasing distances (Table 2). At baseline, the middle, and the end of the trial, all dogs had body composition scans performed and basal metabolic rate determined via indirect calorimetry. At the end of the trial, all dogs had serum samples collected for L-carnitine measurements. All experimental procedures were approved by the Institute of Animal Care and Use Committee at Four Rivers Kennel.

Diets and treatment

All dogs were fed Purina Pro Plan Savor Chicken & Rice diet for the duration of the trial. This diet was selected due to low total L-carnitine content, 16.57 mg/kg as determined by LC-MS/MS. Each dog was sorted into one of three treatment groups and were further split each into half receiving 110% or 120% of maintenance energy requirement.

Dogs received either powdered Carniking™ (Lonza Consumer Health Inc. Morristown, NJ) consisting of 50% L-carni-

tine/50% silica, in addition to sucrose to assist with uptake of L-carnitine into the muscle (Table 1). Feed offered was calculated based on basal metabolic rate and historical weight maintenance by calorie consumption assessed during baseline. Once the feed and supplementation amounts were determined, they remained the same throughout the trial. All dogs were fed once daily in the morning, first receiving the powdered supplements top-dressed in 200 g of feed. After consumption of the first 200 g bowl, the dogs were fed the rest of their allotment. All feed and supplements were weighed out daily and any refusals after 30-min weighed back and recorded.

Exercise regimen

All dogs participated in a bi-weekly running regimen during the exercise portion of the second phase of the trial following the 8-week weight gain period. The distance increased incrementally over the course of the 7 weeks (Table 2). All dogs ran alongside an all-terrain vehicle and were free to run, stop, and play. All dogs wore Actical® accelerometer collars to monitor the activity intensity and Garmin® GPS collars to monitor the distance and running speed. Every effort was made to ensure that all dogs ran the minimum prescribed mileage. Due to the older age of the dogs, dogs that refused to complete the runs were allowed to return to the kennel. Activity Per Kilometer (APKm) was calculated based on activity points divided by actual GPS distance ran. Average Moving Speed (kph) was automatically generated via GPS collar based on the average moving speed of each dog, not including stopped time.

Measurements

To monitor the impact of L-carnitine supplementation on energy expenditure, basal metabolic rate was determined via indirect calorimetry at baseline, at the end of the 8-week weight gain phase, and at the end of the 7-week exercise phase. Each dog was placed in a metabolic chamber connected to an open-circuit indirect calorimetry machine (Oxymax; Columbus Ins.) to obtain measurements of oxygen consumption and carbon dioxide expiration. Each dog was placed in the chamber to settle into a resting state until readings of steady flow and heat production (kcal/hour) were reached. All testing was performed in a thermoneutral environment at the same time each day.

At baseline, at the end of weight gain and exercise phases, all dogs were scanned for body composition using a Dual Energy X-ray Absorptiometry Machine (DXA) (GE Lunar). Each dog was sedated by a licensed veterinarian using a combination dexmedetomidine hydrochloride (0.01 mg/lb), butorphanol tartrate (0.1mg/lb), and atropine sulfate (0.54 mg/lb). Each dog was placed dorsoventrally on the scanner and bone density, total tissue mass, lean mass, and fat mass were quantified. At the end of the trial, and just prior to being sedated for DXA scans, all dogs had serum samples collected via jugular venipuncture, for L-carnitine content determination.

Statistics

Statistical analyses were performed using JMP 13.1.0 (SAS Institute, Cary, NC). Data was tested for normality and a mixed model used to compare the effect of treatment by time points for feed intake, body weights, body composition, activity, moving speed, and basal metabolic rate. Tukey's multiple comparisons was then applied to compare the least square means. Low intake and high intake groups were analyzed separately. Dog was analyzed as the random effect and sex analyzed as a fixed effect based on the potential variance between male and female. Results were considered significant if a p-value of 0.05 or less was obtained. Data were presented as means with their standard errors. For the body composition outcomes, data were presented as changes between different treatment phases except for the lean/fat ratio.

Results

Feed intake

Overall and independently from exercise, dogs fed low calorie diet (110% MER) did not significantly respond to low dose of L-carnitine supplementation (Figure 1A), although a small but significant increase in feed consumption was observed with the high L-carnitine dose ($p=0.03$). In contrast, dogs at high calorie intake (120% MER), receiving low or high L-carnitine doses, consumed significantly lower amounts of feed compared to control ($p<0.01$). These effects were driven by females and are dose-dependent ($p<0.01$). During the weight gain phase, and at low calorie intake, the group supplemented with the high L-carnitine dose, consumed significantly more feed compared to the low dose group (but not compared to control and at 120% MER) ($p=0.01$) (Figure 1B). However, at high calorie intake, there was a significant decrease in feed consumption with L-carnitine in all dogs and dose-dependent in females ($p<0.01$). A similar effect was obtained during the exercise phase, with L-carnitine supplementation leading to a decrease in feed intake only at high calorie intake ($p<0.01$), an effect also driven by female dogs ($p<0.01$). In male dogs, significant differences were seen only between the control and the low L-carnitine dose group ($p<0.01$).

Body weight

Overall and independently from exercise, body weight was significantly lower in the control group compared to 2x110 group ($p<0.01$) at low calorie intake (Figure 2A). This effect was mainly driven by female dogs as shown by a dose-dependent increase in body weight ($p<0.01$). Male dogs didn't show any significant changes between treatment groups. However, at high calorie intake, all dogs and independently from exercise showed a significant dose-dependent decrease in body weight with L-carnitine supplementation compared to control. The 2x120 male group had significantly lower body weights than 0x120 or 1x120 groups ($p=0.04$), and 0x120 female group had significantly higher body weights compared to both 1x120 and 2x120 females ($p<0.01$). Similar observations were noted when data are analyzed based on the exercise status (Figure 2B). During the resting phase, body weight also significantly increased at low calorie intake with L-carnitine supplementation ($p<0.01$) and decreased at high calorie intake in overall and female dogs ($p<0.01$). These effects were not significant in males. Similar effects were observed during the exercise phase. These data suggest that both doses of L-carnitine prevented weight gain only at high calorie intake and independently from the exercise regimen.

Body composition

Total tissue mass decreased significantly during the exercise phase at high calorie intake and with the high L-carnitine dose in all dogs ($p<0.01$) and males ($p=0.02$) but not in females (Figure 3A). No significant differences were found at low calorie intake groups. Overall, fat mass was not significantly changed (Figure 3B). When percent body fat is estimated, interestingly, at low calorie intake the lower L-carnitine dose led to a significantly lower increase in percent body fat compared to the high

dose and from baseline to the end of the weight gain phase, both overall (p=0.03) and in males (p=0.01) but not in females (Figure 3C). No significant effect was seen at high calorie intake (Figure 3C). At high calorie intake, the higher dose of L-carnitine showed significant increase in lean mass compared to the lower dose during the weight gain phase (p=0.03) (Figure 3D). No significant differences were found for the low-calorie intake groups or by gender analysis for both feeding levels. Lean/fat ratio was significantly higher at the end of the exercise phase, at high calorie intake and at the high L-carnitine dose in all dogs (p=0.04) (Figure 3E). No significant differences were found for low calorie intake groups. No significant differences were shown for bone mineral density (data not shown).

Activity and moving speed

Effects on the Activity Per Kilometer (APKm) are in general small but significant (Table 3). Compared to low L-carnitine dose, high L-carnitine dose is effective in increasing the activity among male dogs at low calorie (p<0.01). At high calorie intake, However, only low L-carnitine dose led to an increase in the activity as compared to control males (p<0.01). Surprisingly, in female dogs the activity seems to decrease with supplementation (p<0.01). When average moving speed is considered (kph) (Table 4), no significant difference was noted in the low calorie intake between groups. At high calorie intake, L-carnitine supplementation showed a small but significant decrease in the average moving speed. The 0x120 group had significantly higher average moving speed compared to the 2x120 group (p<0.01). The 0x120 females had significantly higher average moving speed compared to the 1x120 and 2x120 females (p<0.01).

Basal metabolic rate (BMR)

At baseline, the BMR of the control group at low calorie intake was significantly higher compared to both dose 2 groups, an effect mainly observed among males (Figure 4). This intended baseline difference, resulting from the randomization, is corrected by the ANCOVA analysis. Supplementation with L-carnitine led to a significant increase in BMR at high calorie intake and at rest compared to baseline (p=0.02) (Figure 4). These effects are not significant during the exercise period. Interestingly, at low calorie intake and during the weight gain phase, the supplementation tended to decrease BMR among male dogs.

Plasma L-carnitine content

There was a significant and dose-dependent increase in total (p<0.01) and free (p<0.01) serum L-carnitine levels with increasing L-carnitine dosages and independently from energy intake (Figure 5). Esters:free ratio was significantly lower in 1x110 and 2x110 compared to 0x110 (p=0.04).

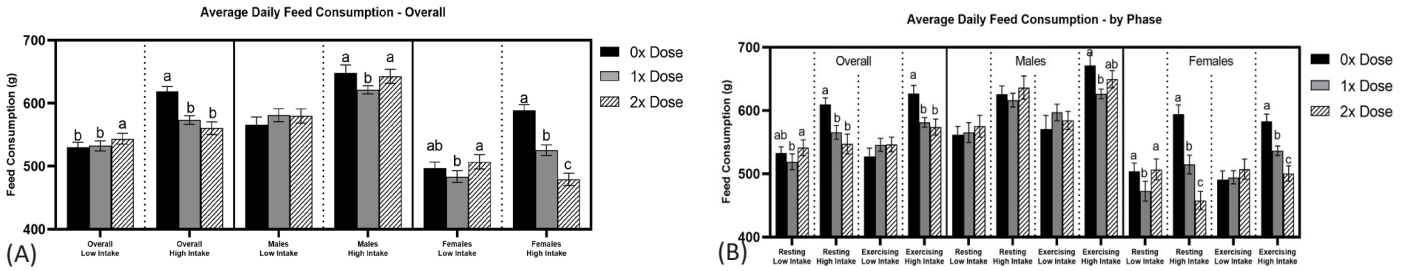

Figure 1: Average daily feed consumption overall (A) and by phase (B) in male and female Labrador Retrievers at low calorie intake (110% MER) and high calorie intake (120% MER) supplemented with 236 mg/d of elemental L-carnitine (1x dose, grey bar), 492 mg/d of elemental L-carnitine (2x dose, striped bar) and control (0x dose, black bar) at the end of the weight gain (resting) and the exercise phase. Values are means with standard errors represented by vertical bars. a, b, c indicates significant difference between dose (p<0.05).

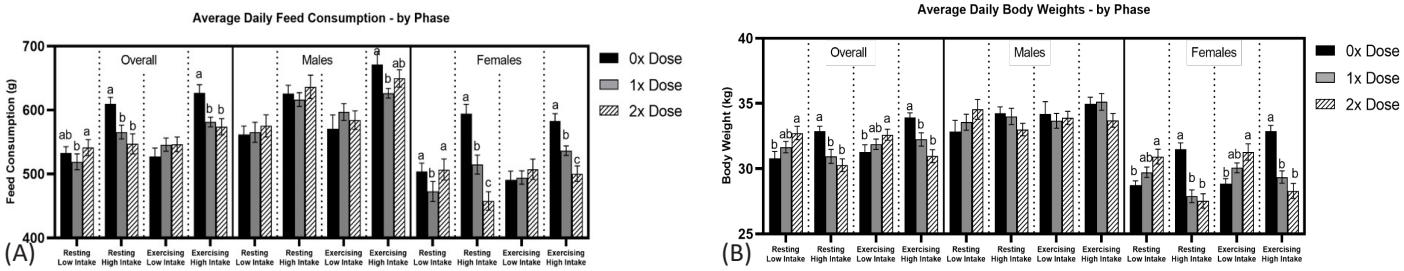

Figure 2: Average daily feed consumption overall (A) and by phase (B) in male and female Labrador Retrievers at low calorie intake (110% MER) and high calorie intake (120% MER) supplemented with 236 mg/d of elemental L-carnitine (1x dose, grey bar), 492 mg/d of elemental L-carnitine (2x dose, striped bar) and control (0x dose, black bar) at the end of the weight gain (resting) and the exercise phase. Values are means with standard errors represented by vertical bars. a, b, c indicates significant difference between dose (p<0.05).

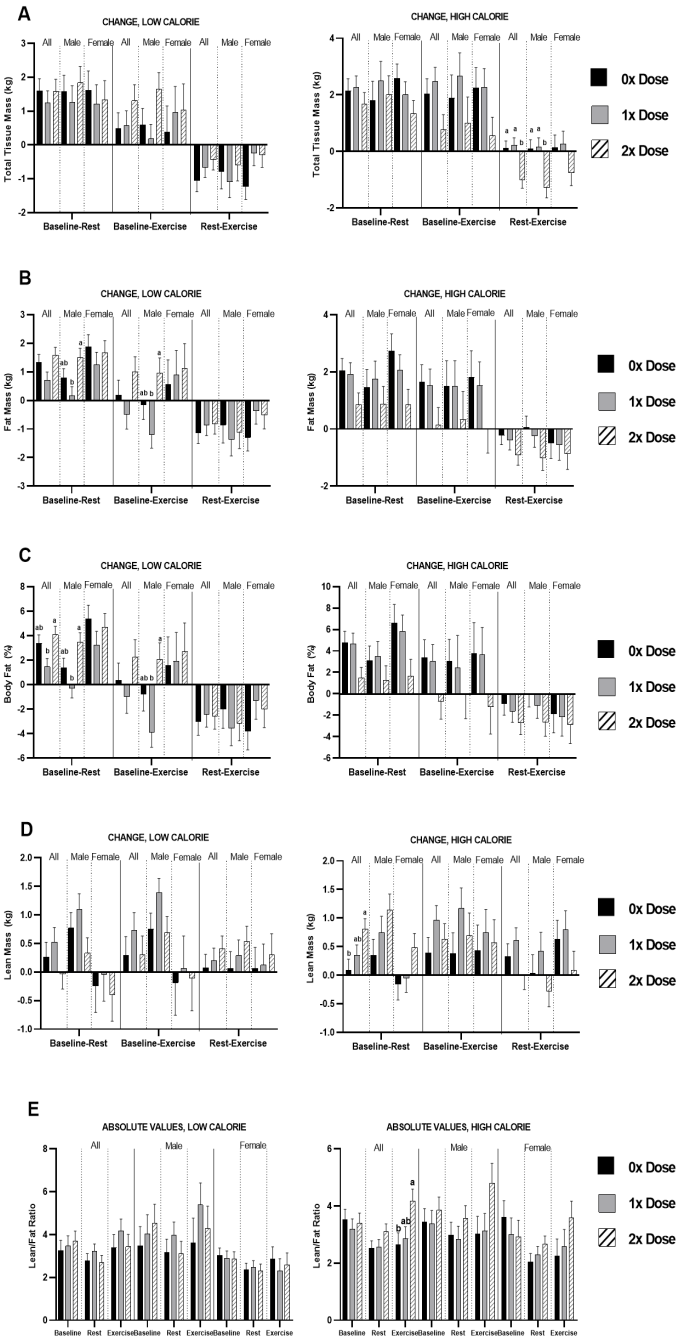

Figure 3: Changes in total tissue mass (A), fat mass (B), body fat percent (C), lean mass (D), and ratio lean/fat (E) in male and female Labrador Retrievers at low calorie intake (110% MER) and high calorie intake (120% MER) supplemented with 236 mg/d (1x dose, grey bar), 492 mg/d (2x, striped bar) and o mg/d of L-carnitine (0x, black bar) pre and post exercise. Values are mean changes with standard errors represented by vertical bars. a, b, indicates significant difference between treatment groups ($p<0.05$). Only for lean/fat ratio: a,b indicates differences within treatments and across time.

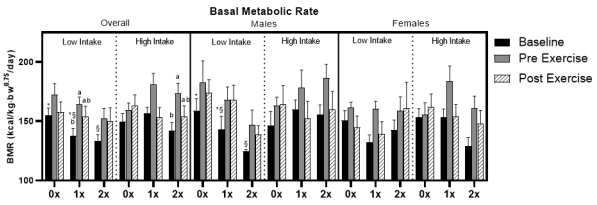

Figure 4: Basal metabolic rate in male and female Labrador Retrievers at low calorie (110% MER) and high calorie intake (120% MER) supplemented with 236 mg/d (1x), 492 mg/d of L-carnitine (2x) and control (0x) at the end of the weight gain (grey bars) and the exercise phase (stripe bars) vs. baseline (black bar). Values are means with standard errors represented by vertical bars. a, b indicates significant difference within treatment and across time ($p<0.05$); *§ Indicates significant difference within time and between treatments ($p<0.05$).

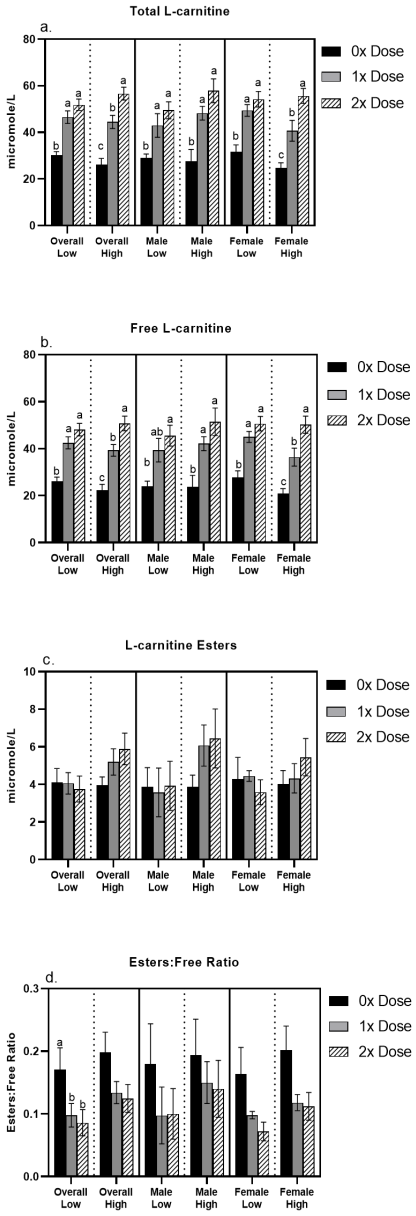

Figure 5: Plasma total L-carnitine (a), free L-carnitine (b), L-carnitine esters (c) and Ester:Free L-carnitine ratio (d) in male and female Labrador Retrievers at low calorie intake (110% MER) and high calorie intake (120% MER supplemented with 236mg/d of elemental L-carnitine (1x dose, grey bar), 492 mg/d of elemental L-carnitine (2x dose, striped bar) and control (0x dose, black bar) at the end of the study. Values are means with standard errors represented by vertical bars. a, b, c indicates significant difference between dose ($p<0.05$).

Table 1: Demographics of dogs and feeding regime.

Groups	MER* (%)	L-carnitine Average (mg/d)	L-carnitine Range (mg/d)	Sucrose (ppm)	Total	Males	Females	Intact	Altered
0x110	110	0	0	750	10	5	5	7	3
1x110	110	231	197-276	500	11	6	5	7	4
2x110	110	460	363-565	250	10	5	5	7	3
0x120	120	0	0	750	10	5	5	7	3
1x120	120	241	194-323	500	10	5	5	7	3
2x120	120	524	396-704	250	10	5	5	7	3
*MER,		Maintenance		Energy			Requirement		

Table 2: Running regimen during the exercise phase.

Week	1	2	3	4	5	6	7
Run 1	-	3.2km	4.8km	6.4km	6.4km	8km	8km
Run 2	3.2km	4.8km	4.8km	6.4km	6.4km	8km	8km

Table 3: Activity per Kilometer (APKm) over all runs in male and female Labrador Retrievers at low calorie intake (110% MER) and high calorie intake (120% MER) supplemented with 236 mg/d (1x dose), 492 mg/d of L-carnitine (2x dose) and control (0x dose)

	All dogs		Male dogs		Female dogs	
	Mean	SD	Mean	SD	Mean	SD
0x110	49008^{a}	746	43457^{b}	927	53364^{a}	1029
1x110	43410^{b}	705	42435^{b}	821	44387^{b}	1029
2x110	47019^{a}	708	48086^{a}	821	45935^{b}	1037
P-value	<0.01		<0.01		<0.01	
	All dogs		Male dogs		Female dogs	
0x120	43579^{a}	819	42162^{b}	1025	44908^{a}	1130
1x120	41375^{ab}	819	45594^{a}	1017	37287^{b}	1139
2x120	40611^{b}	822	36736^{c}	1025	44305^{a}	1139
P-value	0.03		<0.01		<0.01	

Table 4: Average moving speed (kph) over all runs in male and female Labrador Retrievers at low calorie intake (110% MER) and high calorie intake (120% MER) supplemented with 236 mg/d (1x dose), 492 mg/d L-carnitine (2x dose) and control (0x dose)

	All dogs		Male dogs		Female dogs	
	Mean	SD	Mean	SD	Mean	SD
0x110	11.12	0.09	11.26	0.13	11.00	0.13
1x110	11.09	0.09	11.21	0.12	10.98	0.13
2x110	10.96	0.09	10.96	0.12	10.97	0.13
p-value	0.41		0.16		0.98	
	All dogs		Male dogs		Female dogs	
0x120	10.97^{a}	0.06	10.77	0.10	11.17^{a}	0.08
1x120	10.80^{ab}	0.06	11.02	0.10	10.58^{b}	0.08
2x120	10.63^{b}	0.06	10.85	0.10	10.43^{b}	0.08
p-value	<0.01		0.1803		<0.01	

a, b, c indicates significant difference between dose ($p<0.05$).

Discussion

The present placebo-controlled, randomized intervention study in Labrador Retrievers fed two levels of calorie diets provides evidence that the supplementation with L-carnitine confers significant benefits on feed consumption, body weight, body composition, and BMR all correlated to increased serum L-carnitine levels. These effects were observed during the weight gain phase and mostly independent from exercise. Interestingly, BMR increased during the exercise period but tended to decrease during the weigh gain period. The observed decrease in body weight is most likely driven by an increase in satiety as shown by decreased feed consumption at the highest calorie intake. The prevention of body weight and fat mass increases during the weight gain period, suggests a benefit of L-carnitine supplementation when dogs are over-fed beyond their MER. BMR also increased at the end of the weight gain phase with L-carnitine supplementation, although a dose-dependant effect was not observed. This can be the result of the a wide age range among dogs. It is interesting to note that at low calorie intake and at rest, BMR actually tends to decrease with L-carnitine supplementation. Aging and reduced energy (fasting) have both been correlated with lower BMR [30,31]. In the present study dogs are 5 to 11 year old and fed at least 110% above their MER. During resting period and in the absence of activity, mitochondrial function is increased leading to the formation of reactive oxygen species [32,33]. As L-carnitine contribute to the mitochondrial function (increased energy production) and reported elsewhere [34] to be a powerful anti-oxidant, it is possible that L-carnitine supplementation acts as a buffer to balance the energy production and the oxidative stress formation. This can be acheived through a decrease in BMR during the resting phase as seen in this study. It has been reported otherwise that C. elegans mutants for longer life-span do have a lower BMR [35] and L-carnitine has been suggested to counter the aging process by increasing the activity of telomerase [36]. The observation that L-carnitine tends to decrease BMR among male dogs at low calorie and during rest, might offer an additional benefit. The beneficial effect of L-carnitine incorporated in weight management diets on body weight and composition has long been known. L-carnitine has been shown to help preventing muscle protein breakdown in dogs during exercise [37]. An underlying mechanism for the protective effect on lean body mass is pos-

sibly due to the effects L-carnitine on promoting the usage of fat to produce energy through its effects on the mitochondrial β-oxidation of long chain fatty acids. Decrease in adipose formation and increase in energy availability from fat lead to preserve muscle glycogen usage [4,37,38] and branched chain amino acids, sparing them for new protein synthesis [10]. In healthy lean beagles, supplementation with dietary Medium Changed Triglycerides (MCT), fish oil, and L-carnitine maintained total-lean-body weight compared to a control group [19]. In overweight and obese adult Beagles, incorporating L-carnitine, lipoic acid, lysine, leucine, and coconut oil to a low calorie, high fibre dry dog food diet reduced body weight and body fat while lean body mass was maintained over the course of the weight loss phase [13]. After post-treatment and during the weight maintenance phase, and although the feed intake increased, the dogs continued to lose body fat and recorded gains in lean body mass with constant body weight. This effect was partly attributed to the supplementation with L-carnitine and leucine contained in the food and might be also caused by an increase in BMR; an outcome not measured by Floerchinger et al., [13] but reported in our study. L-carnitine supplemented dogs fed 120% their MER and at the end of the weight gain phase increased their BMR. Further our results showed an increase in fat mass only in the control group but not in dogs supplemented with L-carnitine, indicating that the supplementation prevented the increase in fat mass and body weight. In the present study, the effects of L-carnitine supplementation on reducing feed consumption at high calorie intake, agrees with previous intervention in obese Beagles where effects of a dietary fibres plus L-carnitine supplementation on post-prandial satiety and consequently weight management has been reported [39]. Based on the latter, feed and energy intake was significantly reduced in 12 intact female dogs 3 hours after high fibre/L-carnitine diet compared to control. After the 6 weeks lasting weight loss phase, conducted with 7 intact female dogs, those fed the high fibre/L-carnitine diet lost significantly more body weight and body fat than dogs fed control diet. To what extent the effect of L-carnitine on satiety goes beyond the established effect of dietary fibre [18,40,41] remains, however, to be elucidated. L-carnitine supplementation increased total and free serum L-carnitine levels by 40 to 50% and decreased esters:free ratio, correlating the observed clinical outcomes to the supplementation. This is in line with previous studies. Adding dietary L-carnitine for six months increased serum L-carnitine levels in healthy beagles [19]. Varney et al. demonstrated higher serum but also muscle total, free and esterified L-carnitine levels in Labrador Retrievers supplemented with 125mg L-carnitine daily after 13 weeks and with exercise [28]. Very recently, Soeder et al. found lower serum L-carnitine levels in overweight as compared to lean intact male Labrador Retrievers, which might link low serum L-carnitine to increased adiposity [42]. Free L-carnitine might be reduced in overweight dogs due to increased formation of acylcarnitines [34]. It was previously reported that Labrador Retrievers supplemented with L-carnitine had higher activity, muscle recovery, and antioxidative capacity than control dogs [14]. The follow-up study demonstrated that female dogs benefited from supplementation with 125mg L-carnitine daily with regard to oxygen consumption rate and energy expenditure [28]. These results are in contrast to the present study, which did not provide a clear picture on L-carnitines effect on activity levels. In our study, dogs were fed over maintenance energy requirement, which might have an impact on activity and moving speed of these dogs. The age of the dogs also differed substantially between the present study and the studies by Varney et al., [14,28]. Gender-specific differences, with females being in general more responsive to the L-carnitine supplementation, observed for some of the outcomes, might be explained by different hormone status, being neutered or not or the level of exercise volume during the exercise period. Indeed, Varney et al., (2017) also suggested that the female responsiveness to the L-carnitine supplementation during exercise might be due to the fact that the exercise volume still too low for males as compared to females. Further experiments with higher exercise volume are needed to assess these effects in male dogs [14].

Some limitations of the study should be noted. The dogs differed in age range (large variability) and whether or not neutered. This could have affected some outcomes such as physical activity. In conclusion, the present study showed that adding L-carnitine under the form of Carniking™ to high calorie feed prevented body weight gain, improved body composition and decreased feed consumption and that's independently from exercise. Basal metabolic rate also increased during exercise. These results support addition of Carniking™ to dog food and treats in situation that might result in increased calorie intake, for example during excessive and uncontrolled treat rewarding from dog owners.

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