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ORIGINAL ARTICLE

Resting energy expenditure and body composition of Labrador Retrievers fed high fat and low fat diets

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Summary

A high dietary fat intake may be an important environmental factor leading to obesity in some animals. The mechanism could be either an increase in caloric intake and/or a decrease in energy expenditure. To test the hypothesis that high fat diets result in decreased resting energy expenditure (REE), we measured REE using indirect calorimetry in 10-adult intact male Labrador Retrievers, eating weight-maintenance high-fat (HF, 41% energy, average daily intake: 8018 ± 1247 kJ/day, mean ± SD) and low-fat (LF, 14% energy, average daily intake: 7331 ± 771 kJ/day) diets for a 30-day period. At the end of each dietary treatment, body composition measurements were performed using dual-energy X-ray absorptiometry. The mean ± SD REE was not different between diets (4940 ± 361 vs. 4861 ± 413 kJ/day on HF and LF diets respectively). Measurements of fat-free mass (FFM) and fat mass (FM) also did not differ between diets (FFM: 26.8 ± 2.3 kg vs. 26.3 ± 2.5 kg; FM: 3.0 ± 2.3 vs. 3.1 ± 1.5 kg on HF and LF diets respectively). In summary, using a whole body calorimeter, we found no evidence of a decrease in REE or a change in body composition on a HF diet compared with LF diet.

Introduction

A considerable amount of veterinary literature has focused on the treatment of obesity in companion animals, along with the incidence and treatment of obesity related diseases in dogs (Buffington, 1994; Legrand-Defretin, 1994; Markwell et al., 1994; Parkin, 1994; Butterwick and Hawthorne, 1998). However, little is actually known regarding the aetiology of obesity. There is extensive evidence that dietary fat intake is associated with obesity in animals (Storlien et al., 1986; Rocchini et al., 1987; Kaiyala et al., 2000). These data are supported by human studies (Romieu et al., 1988; Tremblay et al., 1989). It is apparent that a high fat (HF) intake may be an important environmental factor promoting obesity in some individuals. As obesity results from a positive energy balance, there are two possible mechanisms for this association. First, HF diets may increase energy intake (EI), i.e. hyperphagia (Rocchini et al., 1987). The second possibility is that HF diets may lower energy expenditure (Storlien et al., 1986). It is also possible that a combination of both mechanisms may occur.

To determine the importance of the latter mechanism, we examined the effects of feeding diets of different fat content on resting energy expenditure (REE) and body composition in Labrador Retrievers. The information obtained from this study should help clarify the role of dietary fat in the development of obesity in dogs. In addition, this study will provide supplementary data on maintenance energy requirements, REE and body composition in Labrador Retrievers.

Materials and methods

Animals and study design

Ten purebred intact, adult (2.2 ± 0.5 years) male black Labrador Retrievers were recruited for this study from breeders throughout the USA raising AKC registered dogs. Owners were required to give written consent. All dogs were normal healthy adults as determined by an initial physical examination, evaluation of blood haematology and serum biochemistries. They were vaccinated currently and given a heartworm preventative monthly (Heartgard, 6 μg/kg ivermectin; Merial Animal Health, St. Louis, MO, USA) during the course of the experiment. Dogs were housed individually in runs measuring 2.1 × 1.2 × 3.6 m, with a 12 h light/dark cycle (lights on at 07:00 hours and off at 19:00 hours). Dogs were taken for a walk twice a day and trained to obey common commands. The experiment was conducted between 11 May 2003 and 16 June 2004. At the conclusion of the study, the dogs were adopted out to new homes. This study was approved by the Animal Care and Use Committee at the University of California in Davis.

Two diets, one containing 14% fat [low fat (LF)], the second 41% fat (HF) content on an energy basis, were fed to maintain body weight. Each diet was fed twice a day for 30 days. Water was provided *ad libitum*. Dogs were fed with the LF diet for 2 months prior to the first phase to stabilize their body weights. Body weights were recorded three times a week and food intake was adjusted weekly to maintain body weight. At the end of each phase, 8-h indirect calorimetry measurements were completed on each dog. At the conclusion of the calorimetic measurements for each dietary phase, body composition was measured using dual energy X-ray absorptiometry (DEXA) (Speakman et al., 2001).

Diet

Two dry extruded diets were used in this study: a LF and a HF diet (Table 1). Both were representative of common commercial diets and were readily accepted by all dogs. Food intake was assessed by weighing food prior to feeding. Before each feeding, a portion of the allotted amount of food was used as incentive during training, which consisted of sit, stay, lie down and chamber entry commands. The remainder of the food was fed after training. Any food remaining in the run was also measured prior to subsequent feedings. The mean proximate analysis of the LF diet was 21% protein, 5% fat, 55% nitrogen free-extract (NFE), 5% fibre and 4.6% ash with a metabolizable energy (ME) value of 12.9 kJ/g (determined using modified Atwater values of 14.6 kJ/g for carbohydrate, 35.6 kJ/g for fat and 14.6 kJ/g for protein); the HF diet contained 25% protein, 18.4% fat, 38% NFE, 4.5% fibre and 6.3% ash with a ME value of 15.8 kJ/g.

Table 1 Ingredient composition of the low and high fat diets*

Batch meal – chunk	Low fat (g/100 g)	High fat (g/100 g)
Ingredients		
Chicken by-product meal with BHA/BHT	21.00	34.00
Rice gluten 50%	6.25	6.25
Brewers rice	62.82	49.82
Cellulose powder	4.54	4.54
WVC SP Vit premix 40†	2.50	2.50
WVC SP premix V3†	2.50	2.50
PET-OX custom dry†	0.20	0.20
Naturox dry†	0.20	0.20
Total	100	100
Extrusion – chunk		
Corn oil – stabilized	4.50	7.00
Water moisture balance	−3.50	−3.50
Core – chunk	99.00	96.50
Total	100	100
Coating		
Catfish digest	5.00	5.00
Beef tallow	0.00	8.00
Extruded chunk	95.00	87.00
Total	100	100

*All ingredients are reported on a dry matter basis.
†Proprietary vitamin/mineral blend, Waltham, USA.
BHA, butylated hydroxyanisde;
BHT, butylated hydroxytoluene.

Indirect calorimetry protocol

Resting energy expenditure (REE) measurements were made by whole-body indirect calorimetry. The calorimetry chamber measured 1.3 × 0.8 × 1.0 m with a Plexiglass window in front. All dogs were introduced to the chamber 1 month prior to the first calorimetry measurements and multiple calorimetry runs were performed to acclimate each dog to the procedure throughout the adjustment period. The chamber was maintained at room temperature (23 °C) by fans blowing over condenser coils circulating chilled water (Isotemp 3006; Fisher Scientific, Pittsburgh, PA, USA). Dogs were fasted for 8 h prior to each measurement. Calorimetry measurements began at 16:00 hours and concluded at 24:00 hours. Each dog was measured at least twice at the conclusion of each dietary treatment to ensure repeatability. Resting energy expenditure was determined by averaging energy expenditure over prolonged (approximately 60 min) minimal periods from two separate runs when the dog was observed to be at rest. Dogs were under supervision for the entire measurement and their disposition (i.e. standing and prone) was recorded every 5 min. Resting disposition was defined as the dog lying prone with its head down. Water was provided *ad libitum* during measurements.

An open-circuit, flow through design was used for the calorimetry chamber. Room air was drawn through the chamber at 25 l/min by a diaphragm vacuum pump (Gast Manufacturing, Benton Harbor, MI, USA). The flow rate from the calorimetry chamber was controlled and measured with a mass flow meter (Sierra Instruments, Monterey, CA, USA). Samples of room and chamber air were dried by a peltier condenser (Sable Systems Intl., Las Vegas, NV, USA) which reduced water vapour pressure to approximately 0.75 kPa and corrected for temperature differences in gas samples by chilling to 2.0 °C. Pressure was equalized on calibration and sample channels using independent pressure gauges. Oxygen content was measured by a paramagnetic analyzer (Rosemount Analytical, Orrville, OH, USA) and carbon dioxide content was measured by an infrared analyzer (AEI Technologies, Pittsburgh, PA, USA). The calorimeter was calibrated weekly using an ethyl alcohol recovery. Sample gas was collected cyclically every minute for 10 min for the duration of each test. Calibration was checked prior to each sample cycle using two separate gas mixtures, 1.9% carbon dioxide calibration gas and room air. Sample gas, 1.9% carbon dioxide reference gas and room air were cycled using a multiposition microelectric valve actuator (Vici Valco Instruments, Houston, TX, USA) controlled by data acquisition software (National Instruments, Austin, TX, USA). Data from the mass flow meter and gas analyzers were collected using a data acquisition system (National Instruments) within a PC using software written with LabView (National Instruments). Energy expenditure was calculated using the abbreviated Weir equation (Weir, 1990),

$$EE = (16.5\text{kJ/l} \times V\text{o}_2) + (4.63\text{kJ/l} \times V\text{co}_2)$$

Dual-energy X-ray absorptiometry protocol

At the conclusion of the calorimetry runs for each diet phase, measurements of body composition were performed with a Norland Eclipse DEXA densitometer (Norland Corporation, Fort Atkinson, WI, USA). The host software used was version 3.9.4 and the scanner software used was version 2.1.0. Calibration of the scanner was verified daily by scanning a Norland calibration phantom. After food was withheld overnight, dogs were pre-medicated with 0.01 mg/kg acepromazine (Vedco, St. Joseph, MO, USA) and 0.1 mg/kg butorphanol (Torbugesic; Fort Dodge Animal Health, Fort Dodge, IA, USA) subcutaneously. Dogs were anaesthetized by IV administration of propofol (Propoflo; Abbott Animal Health, Abbott Park, IL, USA) at 10 mg/kg to effect. After intubation, anaesthesia was maintained with 1–3% isoflurane (Halocarbon Laboratories, River Edge, NJ, USA) and oxygen. Each dog was positioned in sternal recumbancy with hindlimbs in a frog leg position and fore limbs positioned cranially. To ensure repeatability, two total body scans were performed for each dog and each took approximately 20 min to complete. The parameters estimated by the software were bone mineral density (g/cm^2), lean tissue mass (g), FM (g) and bone mineral content (g).

Statistical analysis

The effect of diet (LF compared with HF) on REE, body weight (BW), fat free-mass (FFM), fat mass (FM), EI and the difference between EI and REE were compared between groups by Student's *t*-test and within groups by univariate regression analysis. The regression equations for REE and FFM were analysed separately for the data from each diet phase. These were compared by analysis of covariance (ANCOVA) to determine whether there was a difference in the slopes (Systat 11; Systat Software, Richmond, CA, USA). An additional analysis was also performed to compare REE while controlling for FFM. Unless otherwise indicated, all data are expressed as mean ± SD. The level of statistical significance was set at $p < 0.05$.

Results

Comparison between low fat and high fat diets

The average age of the dogs was 2.2 ± 0.5 years upon arrival. There were no differences in mean REE ($p = 0.351$), body weight ($p = 0.373$), or FM ($p = 0.711$) between diet phases (Table 2). In addition, mean body weights were not different at the start and end of either diet phase ($p = 0.72$ for the LF phase, $p = 0.98$ for the HF phase). However, FFM tended to be higher when the dogs were fed the HF diet (26.8 ± 2.5 compared with 26.3 ± 2.3 kg, $p = 0.094$). The difference in daily EI approached significance with EI on the HF diet

Table 2 Characteristics of the study population*

	p†	LF‡	HF‡
REE (kJ/day)	0.34	4861 ± 413	4940 ± 361
Body weight (kg)	0.37	32.0 ± 3.6	31.6 ± 3.6
FFM (kg)	0.09	26.3 ± 2.5	26.8 ± 2.3
FM (kg)	0.71	3.1 ± 1.5	3.0 ± 1.7
Energy intake (kJ/BW$^{0.75}$/day)	0.05	550.6 ± 86.2	614.6 ± 121.8

REE, resting energy expenditure; EI, energy intake; FFM, fat-free mass; FM, fat mass.
*Mean ± SD.
†Statistical significance of Student's *t*-test.
‡$n = 10$.

greater than the LF diet (8.1 ± 1.2 compared with 7.3 ± 0.8 mJ/day, p = 0.05 respectively). Accordingly, the difference between EI and REE tended to be greater when dogs were fed the HF diet compared with the LF diet (3.2 ± 1.1 compared with 2.5 ± 0.8 mJ/day, p = 0.055). The resting respiratory quotient was significantly lower with the HF diet compared with the LF diet (0.81 ± 0.02 compared with 0.87 ± 0.01, p < 0.0001 respectively).

Determinants of energy expenditure in dogs fed high and low fat diets

Univariate analysis

As no significant differences were observed between diet phases, paired values from both phases were averaged and univariate regression analysis was performed, relating REE to body weight, FFM, FM and EI (Table 3). Resting energy expenditure correlated significantly with BW ($R = 0.80$, $p < 0.01$) and FFM ($R = 0.77$, $p < 0.05$) but not with EI ($R = -0.06$, $p = 0.31$). Resting energy expenditure correlated positively with FM but not significantly ($R = 0.59$, $p = 0.07$).

Univariate regression equations for REE and FFM

Linear regression of REE by FFM was significant for both the LF and HF phases (Fig. 1). The slope of this relation was not significantly different between LF and HF phases (p = 0.78 by ANCOVA). The difference in mean REE corrected for FFM was not significant between diet phases [4.91 ± 0.09 (SEM) mJ/day compared with 4.89 ± 0.09 (SEM) mJ/day, p = 0.84 on the HF and LF diets respectively]. Therefore, linear regression was performed and found to be significant on paired averaged values from both diet phases:

$$\text{REE (kJ/day)} = 119.0(\text{kg FFM}) + 1735.0$$

where $R = 0.77$ and p = 0.01.

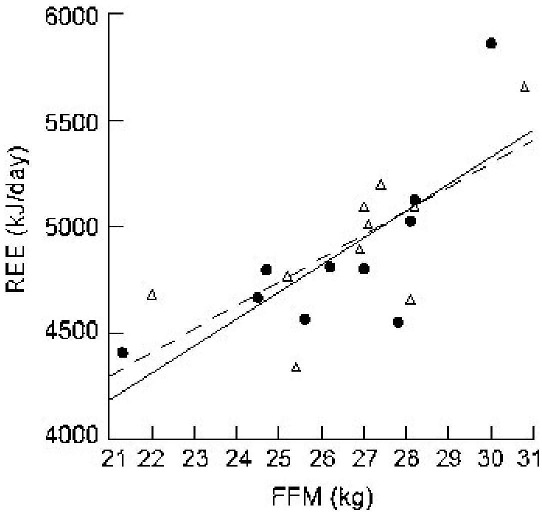

Fig. 1 Univariate regression analyses for fat-free mass (FFM) and resting energy expenditure (REE) in dogs fed with low fat diet (•, solid line; $n = 10$) and a high fat diet (△, dashed line; $n = 10$).

Table 3 Univariate correlation coefficients for averaged paired values between diets

	REE	BM	FFM	FM	EI
REE	1.000				
BM	0.799*	1.000			
FFM	0.766**	0.944***	1.000		
FM	0.588	0.797*	0.614	1.000	
EI	0.358	−0.162	−0.070	−0.337	1.000

REE, resting energy expenditure; BM, body mass; FFM, fat-free mass; FM, fat mass; EI, energy intake.
*p < 0.01, **p < 0.05, ***p < 0.001.

Discussion

The maintenance energy requirements for the dogs in this study closely matched those reported previously in Labrador Retrievers (615 ± 121 and 552 ± 88 kJ ME kg per BW$^{0.75}$ for LF and HF diets respectively) (Kienzle and Rainbird, 1991; Patil and Bisby, 2001; Speakman et al., 2003). However, REEs measured by indirect calorimetry in this study were slightly lower than those reported previously in dogs of various breeds (Ogilvie et al., 1993, 1996; Walters et al., 1993; Speakman et al., 2003). The use of different breeds of dog may contribute to this disagreement. However, this discrepancy was most likely because of the different indirect calorimetry methodologies employed to estimate REE. The four studies mentioned above used the method of attaching a sealed facemask to determine energy expenditure in dogs. O'Toole et al., (2001) reported that substantial differences in energy expenditure using facemask indirect calorimetry in conscious dogs may be attributable to the dog's level of awareness and presumably, state of anxiety. To obtain accurate REE measurements, it is important that the calorimetry procedures do not cause anxiety or agitation in the animal being studied. In our study, the dogs were gradually acclimated to spending time in the calorimetry chamber during the 4 weeks preceding the measurements. This adaptation period was used to prevent the development of any anxiety associated with exposure to a novel environment. Also, the animals were monitored for signs of agitation and results were collected only after repeatable REE measurements could be demonstrated for each dog. The methods used to adapt dogs to the facemask technique are not well described in many of the studies and it is possible that anxiety over this novel environmental condition could contribute to the results observed in at least some of these studies. Thus, the reported energy expenditures from these studies may not represent true REE.

The principal finding in this study was that REE measured by indirect calorimetry was not significantly different between Labrador Retrievers consuming a LF or a HF diet fed to maintain body weight. To our knowledge, this is the first study to demonstrate this in healthy dogs. We confirmed previous findings in dogs with lymphoma that REE remained the same when either HF or LF diets were consumed (Ogilvie et al., 1993). However, in that study, the dogs with lymphoma had significantly lower average REE kg/BW$^{0.75}$ than control dogs. Because of this disparity of REE between healthy control dogs and dogs with lymphoma, comparisons must be made with caution.

Previous studies in other animals have shown conflicting results. Studies in humans and rats have shown that REE either increases (Cooling and Blundell, 1998; Iossa et al., 1999) or remains unchanged (Corbett et al., 1986; Abbott et al., 1990) with the consumption of a HF diet compared with a LF diet. Although the reason for this discrepancy is unclear, there is evidence suggesting that stage of growth may be an important factor in determining whether REE increases in response to a HF diet. Young rats in the active stage of growth fed a HF diet showed a higher REE but did not differ in body weight gain or body fat content compared with young rats fed a LF diet, despite a significantly higher EI (Iossa et al., 2000). This was partially attributed to an elevated ability to metabolize dietary fat as energy. However, adult rats were not able to utilize the additional energy from fat as efficiently and subsequently, body fat and body weight increased compared with controls (Iossa et al., 2003). Cooling and Blundell (1998) demonstrated that REE was significantly greater in young human males (age approximately 20 years) habitually consuming a HF diet compared with a LF diet without significant differences in body weight or FM between groups. In contrast, in a separate study, REE did not differ in middle-aged human subjects (age approximately 35 years) fed a HF or LF diet (Abbott et al., 1990). These studies suggest that there is an adaptive increase in energy expenditure in response to HF intakes and that this ability declines with age. However, in these studies, it is unclear if any adaptive responses in energy expenditure were a result of HF diets *per se* or a consequence of overall excess EI. All dogs in the present study were adults (>2 years) and presumably beyond the active stage of growth. Accordingly, REE was unchanged between diets despite a slightly higher total energy requirement on the HF diet. As the dogs were fed to maintain body weight, any effect of dietary macronutrient composition on REE would have been independent of EI. In addition, there were no significant differences in FM between diets. This suggests that dietary fat content alone does not predispose to adipose tissue gain in adult dogs.

The finding that dogs on the HF diet required a slightly higher amount of total energy than the LF diet to maintain body weight was unexpected and it is not entirely clear why a HF diet should induce such a response. As dietary fat is generally absorbed and utilized at a greater efficiency than carbohydrate or protein, HF diets tend to increase the energetic efficiency of fuel utilization (Jequier, 2002). Therefore, we expected a slightly decreased total energy requirement for the dogs consuming a HF diet. The increase in energy requirement on the HF diet, given no significant differences in FM between diets, can be explained by a disproportionate increase in fat oxidation. The increase in fat oxidation on the HF diet may have been partially because of the interaction of spontaneous activity, likely independent of dietary fat content. While spontaneous activity was not measured in this study, FFM tended to be higher in the dogs when fed with the HF diet. Accounting for the unchanged REE between diets, suggests that activity was responsible for this increased energy requirement on the HF diet. However, these changes were not highly significant and it is possible that no difference would be observed if more dogs were included in the study. Furthermore, as digestibility trials were not conducted in our study, the predicted ME content represent an estimate, which may be subjected to errors. However, utilizing several different prediction equations (Laflamme, 2001; Eqs 5, 6 and 10), total daily caloric intake remained consistently higher when the HF diet was fed (data not shown). Nevertheless, it is unclear if differing digestibilities of the diets contributed to this unexpected response. In addition, we cannot exclude the possibility that either temperature or amount of time spent in the study had an effect on energy expenditure. Due to the design of the study, two separate groups of dogs were recruited at different times during the year. A cross-over design may have attenuated any confounding effects of temperature and time.

Additional studies will be needed to determine if HF diets do truly decrease energy requirements in dogs through pathways other than a decrease in REE. The duration of the phases may have been too short for any effect to become apparent. In addition, the statistical correlations found between REE and body composition are not an allometric relationship as the body weights of the dogs fell within a small range. Therefore, these results may not apply to different breeds of dog. In choosing Labrador Retrievers, we attempted to recruit Labrador Retrievers bred both for show and field trials. Anecdotally, it has been suggested that show dogs are obesity prone while field trial dogs are obesity resistant. Unfortunately, because of unforeseen circumstances, we were unable to recruit enough dogs for comparison between these sub-types within the breed, resulting in a small overall sample size. Thus, the experiment should be repeated with a larger sample size to see if any differences become clear. It is also possible that a HF diet induced decrease in energy expenditure is only evident with overfeeding compared with weight-maintenance feeding.

In conclusion, we find no evidence that a weight maintenance, HF diet reduces REE in Labrador Retrievers. In addition, body composition did not change significantly between HF and LF diets. The data suggest that if HF diets do predispose to obesity, then the mechanism is either an increase in caloric intake or a decrease in physical activity.

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