Energetics of lactation in domestic dog (Canis familiaris) breeds of two sizes

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Abstract

The energetics of lactation was measured in two breeds of domestic dog during peak lactation. Labrador Retrievers (30 kg) had larger litter sizes than Miniature Schnauzers (6 kg). During the 7-day experimental period, Labrador pups increased more in mass than Schnauzer pups, both absolutely and relatively. Consequently, the energy demands of the litter, relative to maternal metabolism, were higher in Labradors than Schnauzers. Milk composition and gross efficiency of milk production were not significantly different between breeds and the costs of lactation were fuelled by increases in food intake. Metabolisable energy intake was higher than predicted in Labradors, but lower than predicted in Schnauzers. These patterns differ from interspecific expectations, which would predict larger animals to reproduce more slowly, have smaller litter sizes, and invest less energy in reproduction. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Domestic dog; Lactation; Energetics; Deuterium; Isotope; Water turnover; Allometry; Body composition; Milk composition

1. Introduction

The functional significance of differences in body size across mammals has received considerable attention in the literature (Kleiber et al., 1961; Bonner et al., 1965; Linzell et al., 1972; Harvey et al., 1985; Kozlowski et al., 1997). In the context of reproduction, larger animals generally exhibit reduced reproductive rates (Bonner et al., 1965; Krzyzanowski et al., 1975; Clutton-Brock et al., 1983), increased lengths of gestation, increased weaning times and take longer to reach maturity (Sacher et al., 1959; Millar, 1977; Western, 1979; Sacher and Staffeldt, 1999). Other variables, such as food intake, milk production and offspring growth rate tend to be greater in smaller mammals when expressed relative to adult mass (Kleiber et al., 1961; Ofteal and Gittleman, 1989; Calder, 1996). As relatively less milk is produced in larger animals, less food is required to produce the milk, as a proportion of adult body mass.

The effects of body mass on reproductive energetics are normally compared between groups of higher taxa (Millar, 1983; Millar and Zammuto, 1983; Harvey et al., 1985) because intraspecific variation in body mass is small compared with interspecific differences (Harvey et al., 1989). However, the effects of mass in these comparisons are confounded by phylogenetic and ecological
covariation. An intraspecific study, however, would have the advantage of being phylogenetically distinct (Felsenstein et al., 1985) and independent of ecology. It would be particularly worthwhile if a species could be found with a large variation in body mass. The domestic dog *Canis familiaris* has the largest range in adult body mass of any mammalian species (Burger and Johnson, 1991). This variation provided an opportunity to examine the differences in reproductive energetics between animals whose principal difference was body mass.

Although some energy additional to the maintenance level is required during gestation, lactation is the most energetically demanding period of mammalian reproduction (Hanwell et al., 1977; Millar, 1979; Oftedal and Iverson, 1987; Gittleman et al., 1988; Reilly et al., 1996). This period of maximal energy intake is likely to be a key determinant of life history as it defines the amount of resources available to produce offspring (Sadleir, 1984; Weiner, 1987). Consequently, observations were made during peak lactation in domestic dogs (Paragon et al., 1993).

The aims of this study were to obtain quantitative information on the mass, water and energy transfer between mothers and offspring during lactation in dog breeds of different masses, and investigate whether the intraspecific relationships with body mass were consistent with (interspecific) allometric predictions. In particular, we aimed to measure the following variables: litter size, body mass change in adults and offspring, food intake and faecal production, apparent digestibility of food, water kinetics, milk intake, milk composition, body composition of adults and offspring and the gross efficiency of lactation.

### 2. Materials and methods

#### 2.1. Animals

Twelve Labrador Retrievers (‘Labradors’) and six Miniature Schnauzers (‘Schnauzers’) plus litters (totalling 77 and 21 pups, respectively) were used in this study. Experiments took place over periods of 7 days between 24 and 30 days post partum that spanned the period of peak lactation for both breeds. Animals were bred and reared at the Waltham Centre for Pet Nutrition (WCPN), Melton Mowbray, UK.

#### 2.2. Diets and housing

Adults were fed a commercial diet (Waltham® Formula® Expert Growth) from the onset of gestation until the offspring were weaned. The diet consisted of 26.9% crude protein, 14.4% fat, 44.8% nitrogen free extract, 7.4% water and 6.5% ash. The metabolisable energy content of the diet was determined by complete faeces and urine collections and was 15.6 kJ/g (WCPN unpublished data). Food and water were supplied in deep stainless steel bowls that were too high for pups to drink or eat from. Food intake was determined by weighing (Sartorius F150 balance ± 0.1 g) the food bowl at 08:00 h each day. Mothers were housed with their litters in individual pens with constant access to outside runs and fresh water (Loveridge, 1994). Food intake and faeces collections were for 7 days. Urinary losses were calculated by subtraction of the metabolisable energy intake and apparent faecal losses from the gross energy intake.

#### 2.3. Analytical methods

Samples of food, faeces and milk were analysed for crude protein (nitrogen × 6.25 for food and faeces, nitrogen × 6.38 for milk) (McDonald et al., 1981) by the combustion method (DUMAS, LECO FP428 analyser), fat by acid hydrolysis and extraction with petroleum ether (Soxhelet, Gerhardt system), water by drying to constant weight at 105°C, and ash by baking at 550°C to constant weight. The gross energy contents of food and faeces were determined by adiabatic bomb calorimetry (Parr 1271, Auto Calorimeter). Milk energy density was derived from the proximate composition and was estimated using the equation:

\[
\text{Energy (kJ/g)} = \left[\text{fat (g)} \times 9.11 + \text{protein (g)} \times 5.86 + \text{sugar (g)} \times 3.95\right]/100 \times 4.186/100
\]

(Oftedal, 1984a,b; Perrin, 1958).

#### 2.4. Body composition analysis

Body composition was determined by dual energy X-ray absorptiometry (DXA) (Hologic QDR 1000/W Densitometer, Hologic, Inc. Waltham MA) (Mazess et al., 1990; Jebb et al., 1997).
Adults and pups were DXA scanned twice, once at the beginning and once at the end of the experimental period. Offspring and adults were scanned using the infant whole body and adult whole body programmes, respectively (Brunton et al., 1996; Picaud et al., 1996), providing measurements of bone mineral density (BMD, g/cm²), bone mineral content (BMC, g), lean tissue (g) and fat (g). Water content (g) was calculated as lean tissue $\times 0.73$ (Pace et al., 1945) in adults and lean tissue $\times 0.80$ in offspring (Sawicka-Kapusta, 1974). Individual scans took approx. 10 min during which the animals were anaesthetised (0.1 ml/kg Vetalar, 0.1 ml/kg Rompun).

2.5. Milk collection

Milk samples were collected at the same time each day to minimise any effect of circadian variation in milk composition (Oftedal, 1984a). Three milk samples were collected from each female at 1500 on days 24, 28 and 30 post partum. Pups were removed from their mothers for an hour prior to milking. Milk was collected by manual expression immediately after an injection of oxytocin (Intra-muscular, 10 IU for Labradors and 5 IU for Schnauzers). All nipples were milked and all glands were emptied as completely as possible producing 40–80 ml of milk per sample (Oftedal, 1984a). Milk samples were stored in rubber-sealed glass containers at $-80^\circ$C until analysis. Milking took approx. 20 min after which the pups were returned to their mother.

2.6. Drinking water

Drinking water of adults was measured from the drop in the water level of a header tank supplying the water bowl, accurate to 0.1 l. This provided an upper limit of the amount of free water drunk as it included any water that was spilled. Evaporation was discounted as a possible source of error because previous validation studies had shown evaporative water losses from the tank and drinking bowl were less than 100 ml/week (mean 80 ml/week, S.D. 10.1, $n = 4$). Pups had no access to water and only consumed milk.

2.7. Evaporative water loss

Adults were placed in whole body gas analysis (WBGA) chambers [internal dimensions were $1.5 \times 1 \times 1$ m for Labradors and $1 \times 0.75 \times 0.75$ metres for Schnauzers] for 1 h at some point during the experimental week. Animals were habituated to these chambers prior to entry. The chambers had been purpose built for analysis of dietary gasses such as methane and hydrogen sulphide (Papasouliotis et al., 1993), and consisted of an airtight transparent glass door with plywood sides to which an open circuit airflow was supplied. The air inlet was cooled ($20^\circ$C) and the humidity controlled (40–50% RH) prior to entering the chamber by a water trap. Airflow was adjusted so that increases in temperature and humidity were approximately the same for each animal. Three internal thermohygrographs were fitted at various locations inside the chambers and in the outflow. One hour was sufficient for temperature and humidity to equilibrate at flow rates of approx. 100 l/min. Evaporative water loss of the animals was measured by the difference in water contained in the air of the outflow with and without the animal inside the chamber. Water loss was calculated using information on atmospheric humidity, temperature and pressure (Webb et al., 1995) from standard Smithsonian tables (Weast et al., 1964).

2.8. Total body water turnover

Deuterated water (99.9% D₂O, MSD Isotopes Inc) was used to determine body water space ($N_d$) and water turnover rates ($r_{H_2O}$) in adults and offspring (Nagy et al., 1980). Doses, administered IV, were 7.0 ml for adult Labradors ($n = 12$), 2.5 ml for adult Schnauzers ($n = 6$) (approx. 0.1 g/kg) 1.0 ml for Labrador pups ($n = 36$) and 0.9 ml for Schnauzer pups ($n = 10$) (approx. 0.5g/kg). Six of the 12 Labrador litters were used for maternal body water measurements (litter sizes 5, 7, 7, 6, 7, 5) and six were used to measure offspring (litter sizes 6, 6, 6, 7, 8, 7). Similarly, four Schnauzer litters were used for maternal body water measurements (litter sizes 2, 3, 2, 4) and three litters for measurements of offspring (litter sizes 4, 4, 6). The calculations assume that all substances entering the animal are labelled at background levels and there is no recycling or re-entry of deuterium (Lifson et al., 1966; Nagy, 1980; Oftedal, 1984a; Oftedal et al., 1993b). Adults and offspring were labelled in separate experiments and only half the offspring of any litter were labelled so that the effects of isotope recycling could be corrected for.
We have previously shown that recycling of isotopes is unlikely to cause significant errors in maternal DEE calculations, but may cause an underestimate of pup water turnover by up to 10.9% (Scantlebury et al., 2000). We took seven blood samples per mother and seven samples per pup (1 per day). Blood samples were vacuum distilled into Pasteur pipettes (Nagy, 1983). Hydrogen, obtained by reacting the resulting distillate with LiAlH₄ (Speakman, 1997; Ward et al., 2000), was used for the determination of ²H:¹H ratio using a mass spectrometer (Optima, Micromass IRMS, Manchester, UK). Water turnover was calculated using a dedicated computer package, which took into account the effects of small deviations from 24 h for the sampling intervals and the effects of mass changes on body water pool size over the experimental period. Total body water efflux (Lifson et al., 1966) in adults and pups was calculated according to the equation:

\[
rH_2O = k_d N_d \times F \tag{2}
\]

where \( F \) is the fractionation factor of the isotope and determined by:

\[
F = \left[ pE \times (0.941) + (1 - pE) \times (1.0) \right] \tag{3}
\]

where \( pE \) is the proportion of evaporation of the isotope. Zero \( pE \) would imply a fractionation factor of 1.00, whereas 100% \( pE \) would imply a value of 0.941 (Speakman, 1997). There is uncertainty about the magnitude of in vivo fractionation in humans and animals (Schoeller et al., 1986; Speakman, 1997). However, based on our measurements of evaporative water loss in dogs (Figs. 1 and 2), a value of 25% \( pE \) was used. Eq. (2) reduces to:

\[
rH_2O = k_d N_d (0.941 + 3) \times 0.25. \tag{4}
\]

2.9. Calculation of milk intake from deuterium elimination in labelled pups

Milk intake (MI) (g/day) per offspring was calculated as:

\[
MI = 100 \times (\text{TWI} + 1.07 F_D + 0.42 P_D) \\
(\%W_M + 1.07\%F_M + 0.42\%P_M \\
+ 0.58\%S_M) \tag{5}
\]

(Ofstedal et al., 1987; Ofstedal and Iverson, 1987; Perrin, 1958) (Abbreviations according to Ofstedal et al., 1993a) in which total water intake (TWI) = (rH₂O + G); where rH₂O is the daily water turnover calculated by deuterium elimination and G is the daily increase in body water as a result of offspring growth. F_D and P_D are fat and protein deposition (g/d) and %W_M, %F_M, %P_M and %S_M are percentages of water, fat, protein and total sugar in the ingested milk respectively. Milk composition data for each female was averaged over the time period. Rates of fat and protein deposition in pups were measured by changes in the DXA estimates of body composition. Gross efficiency of lactation was calculated as:

\[
\text{Efficiency} = \frac{E_{\text{MILK}}}{(E_{\text{FIL}} + E_{\text{M}}) - E_{\text{FIP}}} \tag{6}
\]

in which \( E_{\text{FIL}} \) was the net energy intake during lactation, \( E_{\text{M}} \) was the energy obtained from mass loss during lactation, \( E_{\text{FIP}} \) was the net energy intake post lactation and \( E_{\text{MILK}} \) is the milk energy during lactation (English et al., 1985).

2.10. Statistical methods

Means are displayed with standard deviations. Paired t-tests were used to analyse changes over the experimental period in the same individuals for body mass, BMC, lean and fat tissue. Two sample t-tests were used to examine differences between breeds in food intake, faecal output, apparent absorption and dry matter digestibility. Analysis of covariance (ANCOVA) was used to test for differences between pups; body mass and litter size were entered as co-variates with breed as
a categorical factor. One pup per litter was selected at random for inclusion in the analysis to prevent pseudoreplication. Statistical analyses were performed using Minitab 5.2 software (Ryan et al., 1985).

3. Results

3.1. Animals

Adult Labradors were significantly heavier than Schnauzers (2-sample \( t_{16} = 22.85, P < 0.001 \)) (Table 1). Within a breed, there was no significant change in mean body mass over the 7 day period (paired \( t_{11} = -2.21, P = 0.054 \)) for Labradors and \( t_6 = 1.58, P = 0.19 \) for Schnauzers. Labradors had significantly larger litter sizes than Schnauzers (2-sample \( t_{16} = 4.83, P = 0.0013 \)) (Table 1). Litter size varied in Labradors from four to nine pups and in Schnauzers from two to six pups. We created divisions within breeds of ‘large’ and ‘small’ litter sizes corresponding to four to six and seven to nine pups in Labradors and one to three and four to six pups in Schnauzers. There was no significant difference in body mass for either breed between females with large and small litter sizes (2-sample \( t_{10} = 0.77, P = 0.47 \) for Labradors and \( t_4 = 0.82, P = 0.47 \) for Schnauzers).

Labrador pups were heavier than Schnauzer pups at 24 days (2-sample \( t_{16} = 18.28, P < 0.001 \)) and pups of both breeds increased significantly in body mass from 24–30-days-old (paired \( t_{20} = 19.86, P < 0.001 \)) in Labradors and \( t_{16} = 6.13, P < 0.001 \) in Schnauzers) (Table 2). These increases were significantly different between breeds when the effect of body mass was not taken into account (two-sample \( t_{66} = 10.71, P < 0.001 \), data pooled across all pups), but mass change was not significantly correlated with pup body mass (ANCOVA: \( F_{1,9} = 1.40, P = 0.267 \), breed \( F_{1,9} = 0.18, P = 0.679 \) or litter size \( F_{1,9} = 1.07, P = 0.327 \) when mass was included as a co-variate.

3.2. Food intake

Although Labradors consumed more food than Schnauzers (2-sample \( t_{16} = 7.11, P < 0.001 \)) (Table 1), there was no difference in food intake between breeds when body mass was included as a co-variate (ANCOVA: \( F_{1,15} = 3.87, P = 0.068 \)). The gross energy intakes of Labradors and Schnauzers during lactation (27367 kJ/day and 6505 kJ/day, respectively) (Table 1) were greater than that measured during the period pre-breeding (7430 (S.D. 873) kJ/day and 2506 (S.D. 336) kJ/day, respectively). Labradors with larger litters consumed significantly more food than those with smaller litters (1074 g/day, S.D. 461 for 4–6 pups, 1611 g/day, S.D. 264, for 7–9 pups, 2-sample \( t_{11} = 2.56, P = 0.038 \)). There was no significant difference in food intake between the Schnauzers that had small and large litters.

3.3. Faecal production

Labradors produced more faeces than Schnauzers (2-sample \( t_{16} = 7.65, P < 0.001 \)) (Table 1) and the dry matter had a greater energy content (2-sample \( t_{16} = 3.27, P = 0.004 \)) (Table 3). Mean faecal energy losses represented 6.1 and 5.4% of the gross energy intake of Labradors and Schnauzers respectively. Labradors with large litter sizes produced more faeces than those with small litter sizes (mean 244 g/day, S.D. 65 for large littered individuals and mean 159 g/day S.D. 23 for small littered individuals, 2-sample \( t_{11} = 3.01 P = 0.024 \)).

3.4. Apparent dry matter digestibility

There was no significant difference in apparent dry matter digestibility between breeds (2-sample \( t_{16} = 0.66, P = 0.54 \)) or within breeds between individuals with large and small litter sizes (2-sample \( t_{10} = 1.13, P = 0.30 \) in Labradors and \( t_4 = 0.43, P = 0.71 \) in Schnauzers) (Table 1).

3.5. Adult body composition

Although Labradors contained significantly more BMC, lean and fat tissue than Schnauzers because they were larger, there was no significant difference in percent BMC, lean tissue or fat between Labradors and Schnauzers (Table 3). Equally, there were no significant differences in body composition for either breed between individuals with large and small litter sizes. The only significant change in body composition over the experimental period, was a decrease in fat content of the Labradors (paired \( t_{11} = -2.42, P = 0.039 \)) (Table 3). However, BMC in the lactating moth-
Table 1
Mean and standard deviations (S.D.) of energy balance taken for adult dogs

<table>
<thead>
<tr>
<th>Energy balance</th>
<th>Labradors (n = 12)</th>
<th>Schnauzers (n = 6)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>30.26</td>
<td>3.30</td>
<td>6.47</td>
</tr>
<tr>
<td>Litter size</td>
<td>6.42</td>
<td>0.90</td>
<td>3.57</td>
</tr>
<tr>
<td>Chg (body mass) (kg)</td>
<td>-0.525</td>
<td>0.751</td>
<td>0.111</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>1439*</td>
<td>241</td>
<td>345</td>
</tr>
<tr>
<td>Gross energy intake (kJ/day)</td>
<td>27367</td>
<td>4170</td>
<td>6505</td>
</tr>
<tr>
<td>Metabolised energy intake (kJ/day)</td>
<td>22448</td>
<td>3760</td>
<td>5382</td>
</tr>
<tr>
<td>Faeces produced (g/day)</td>
<td>202*</td>
<td>65</td>
<td>45</td>
</tr>
<tr>
<td>Faecal energy output (kJ/day)</td>
<td>3437*</td>
<td>1097</td>
<td>739</td>
</tr>
<tr>
<td>Urine energy output (kJ/day)</td>
<td>1485</td>
<td>474</td>
<td>394</td>
</tr>
<tr>
<td>Apparent digestibility</td>
<td>0.81</td>
<td>0.10</td>
<td>0.82</td>
</tr>
<tr>
<td>Milk produced (g/day)</td>
<td>1303</td>
<td>815</td>
<td>389</td>
</tr>
<tr>
<td>Milk energy output (kJ/day)</td>
<td>7715</td>
<td>4346</td>
<td>2304</td>
</tr>
<tr>
<td>% Lactation efficiency</td>
<td>63</td>
<td></td>
<td>61</td>
</tr>
</tbody>
</table>

* P values denote a significant difference between Labradors and Schnauzers.
* Denotes a significant difference between large and small litter sizes.

Data for body mass (kg), litter size, change in body mass Chg (body mass) (kg), food intake (g/day), gross energy intake (kJ/day), metabolised energy intake (kJ/day), faeces produced (g/day), faecal energy output (kJ/day), urine energy output (kJ/day), apparent dry matter digestibility, milk produced (g/day), milk energy output (kJ/day), lactation efficiency (%).

Fig. 2. Flow diagram showing mass, water and energy transfer in a lactating Labrador and offspring. Arrow sizes are proportional to flow rates. Unknown quantities are denoted by dotted arrows.
Table 2
Mean and standard deviations (S.D.) of kinetic measurements taken for adult dogs

<table>
<thead>
<tr>
<th>Kinetic measurements</th>
<th>Labradors (n = 12)</th>
<th>Schnauzers (n = 6)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>Drinking water (l/day)</td>
<td>5.32</td>
<td>1.54</td>
<td>1.26</td>
</tr>
<tr>
<td>Evaporated water (l/day)</td>
<td>1.11</td>
<td>0.40</td>
<td>0.38</td>
</tr>
<tr>
<td>k (per h)</td>
<td>0.01193</td>
<td>0.00306</td>
<td>0.00783</td>
</tr>
<tr>
<td>N (l)</td>
<td>18.80</td>
<td>2.59</td>
<td>3.69</td>
</tr>
<tr>
<td>N (DXA) (l)</td>
<td>18.0</td>
<td>1.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Total water turnover (l/day)</td>
<td>5.40</td>
<td>2.80</td>
<td>0.68</td>
</tr>
<tr>
<td>% Body water</td>
<td>62</td>
<td>6</td>
<td>57</td>
</tr>
</tbody>
</table>

* P values denote a significant difference between Labradors and Schnauzers. Data for drinking water (l/day), evaporated water (l/day), k (deuterium elimination rate, h⁻¹), N (body water space estimated by deuterium dilution, litres), N(DXA) (body water space estimated by DXA, litres), total water turnover estimated by deuterium elimination (l/day) % body water calculated by deuterium dilution.

ners was significantly lower than that measured in non-breeding individuals (unpublished data, ANCOVA: F₁,₁₇ = 38.11, P < 0.001, with body mass as a co-variante, Fig. 1).

3.6. Pup body composition

There was no significant difference in percent BMC between Labrador and Schnauzer pups. However, Labrador pups contained relatively less fat and more lean tissue than Schnauzer pups (Tables 4 and 5). There were no differences in body composition between individuals of either breed that came from large and small litter sizes. All pups increased significantly in BMC and lean tissue during the experimental period, although only the Labrador pups increased significantly in fat (Table 5).

3.7. Water kinetics and milk intake

3.7.1. Drinking water

Adult Labradors drank more water than Schnauzers (2-sample t₁₆ = 8.54, P < 0.001) (Table 2). However, these differences were not different when mass was entered as a co-variante (ANCOVA: F₁,₁₆ = 1.80, P = 0.198). There were no differences in the amount of water used between mothers that raised large and small litters (t₁₁ = 1.62, P = 0.16 for Labradors and t₆ = 0.46, P = 0.69 for Schnauzers).

3.8. Evaporative water loss

Evaporative water loss was greater in Labradorors than Schnauzers (2-sample t₁₆ = 4.24, P = 0.0017) (Table 2). These differences were not significant, when mass was entered as a co-variante (ANCOVA: F₁,₁₀ = 0.37, P = 0.557). There was no difference in evaporative water loss between indi-
Table 3
Mean and S.D. of body composition taken for adult dogs.

<table>
<thead>
<tr>
<th>Body composition</th>
<th>Labradors (n = 12)</th>
<th>Schnauzers (n = 6)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>% BMC</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>% LEAN</td>
<td>79</td>
<td>4</td>
<td>82</td>
</tr>
<tr>
<td>% FAT</td>
<td>19</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>652</td>
<td>80</td>
<td>129</td>
</tr>
<tr>
<td>LEAN (g)</td>
<td>22474</td>
<td>1516</td>
<td>4191</td>
</tr>
<tr>
<td>FAT (g)</td>
<td>5335</td>
<td>1460</td>
<td>999</td>
</tr>
<tr>
<td>Chg (BMC) (g)</td>
<td>0.27</td>
<td>12.5</td>
<td>3.98</td>
</tr>
<tr>
<td>Chg (LEAN) (g)</td>
<td>-50</td>
<td>716</td>
<td>-202</td>
</tr>
<tr>
<td>Chg (Fat) (g)</td>
<td>-224**</td>
<td>293</td>
<td>-16</td>
</tr>
</tbody>
</table>

* P values denote a significant difference between Labradors and Schnauzers.

** Denotes a significant change over time (24–30 days post partum). Data for % BMC (percent bone mineral content), % LEAN (percent lean tissue), % FAT (percent fat tissue), BMC (bone mineral content, g), LEAN (lean tissue content, g), FAT (fat tissue content, g), Chg (BMC) (change in bone mineral content from 24 to 30 days of lactation), Chg (LEAN) (change in lean tissue content), Chg (Fat) (change in fat tissue content).

Table 4
Mean and S.D. of faeces composition taken for adult dogs

<table>
<thead>
<tr>
<th>Faeces composition</th>
<th>Labradors (n = 12)</th>
<th>Schnauzers (n = 6)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>% Water</td>
<td>72.7</td>
<td>2.1</td>
<td>66.2</td>
</tr>
<tr>
<td>% Ash</td>
<td>6.0</td>
<td>1.1</td>
<td>8.2</td>
</tr>
<tr>
<td>% Crude protein</td>
<td>9.6</td>
<td>1.3</td>
<td>10.7</td>
</tr>
<tr>
<td>% Crude fat</td>
<td>1.7</td>
<td>0.3</td>
<td>2.0</td>
</tr>
<tr>
<td>% Nitrogen free extract</td>
<td>10.1</td>
<td>1.2</td>
<td>12.9</td>
</tr>
<tr>
<td>Dry matter energy (kJ/g)</td>
<td>17.0</td>
<td>0.7</td>
<td>16.2</td>
</tr>
</tbody>
</table>

* P values denote a significant difference between Labradors and Schnauzers. Data for percent water, percent ash, percent crude protein, percent crude fat, percent nitrogen free extract, dry matter energy (kJ/g).

…individuals that raised large litters and small litters (2-sample t11 = 0.23. P = 0.83, data for Labradors only).

3.9. Total body water turnover

In all cases the log converted enrichments of 2hydrogen above background decreased linearly and were correlated with time over the experimental period. The r² values averaged 99.5% in both breeds. Adult Labradors had larger deuterium dilution spaces (Nd) than Schnauzers (2-sample t16 = 13.47, P < 0.001), greater deuterium elimination rates (kd) (2-sample t16 = 3.05, P = 0.022) and higher water turnover rates (rH2O) (2-sample t16 = 5.26, P = 0.0033) (Table 2). Independent body water spaces measured by DXA in Labradors and Schnauzers (Table 2) were 4 and 7%, respectively less than deuterium dilution spaces. These differences are consistent with isotope based calculations of body water producing overestimates of between 5 and 10%, because of additional isotope pools (Speakman, 1997).

Deuterium dilution spaces in Labrador pups were greater than Schnauzer pups (2-sample t66 = 9.55, P < 0.001) (Table 5). In contrast to adults, deuterium elimination rates were not significantly different between breeds (2-sample t66 = 0.11, P = 0.91). Water turnover rates were greater in Labrador than Schnauzer pups (2-sample t66 = 5.99, P < 0.001). DXA estimates
Table 5
Mean and S.D. of measurements taken for offspring

<table>
<thead>
<tr>
<th></th>
<th>Labradors (n = 51)</th>
<th></th>
<th>Schnauzers (n = 17)</th>
<th></th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
<td></td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>2350</td>
<td>520</td>
<td>860</td>
<td>160</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chg (body mass) (g)</td>
<td>241**</td>
<td>87</td>
<td>69**</td>
<td>45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total litter mass (g)</td>
<td>15060</td>
<td>2300</td>
<td>310</td>
<td>810</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body compositionb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%BMC</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0.067</td>
</tr>
<tr>
<td>%LEAN</td>
<td>84</td>
<td>2</td>
<td>80</td>
<td>4</td>
<td>0.0001</td>
</tr>
<tr>
<td>%FAT</td>
<td>14</td>
<td>2</td>
<td>18</td>
<td>4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>46</td>
<td>13</td>
<td>13</td>
<td>4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LEAN (g)</td>
<td>1971</td>
<td>405</td>
<td>674</td>
<td>109</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FAT (g)</td>
<td>345</td>
<td>117</td>
<td>166</td>
<td>59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chg (BMC) (g)</td>
<td>6.89**</td>
<td>4</td>
<td>3.03**</td>
<td>4</td>
<td>0.0015</td>
</tr>
<tr>
<td>Chg (LEAN) (g)</td>
<td>221**</td>
<td>91</td>
<td>54.2**</td>
<td>91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chg (FAT) (g)</td>
<td>15.3**</td>
<td>30</td>
<td>12</td>
<td>26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water kinetics and energy balancec</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k(d) (per h)</td>
<td>0.005379</td>
<td>0.00095</td>
<td>0.005613</td>
<td>0.00211</td>
<td>0.91</td>
</tr>
<tr>
<td>N(d) (ml)</td>
<td>1710</td>
<td>319</td>
<td>660</td>
<td>106</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N (DXA) (ml)</td>
<td>1665</td>
<td>327</td>
<td>561</td>
<td>95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water turnover (ml/day)</td>
<td>195</td>
<td>47</td>
<td>84</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Milk consumption (g/day)</td>
<td>203</td>
<td>127</td>
<td>109</td>
<td>60</td>
<td>0.0024</td>
</tr>
<tr>
<td>Milk energy intake (kJ/day)</td>
<td>1201</td>
<td>752</td>
<td>645</td>
<td>352</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Body water</td>
<td>73</td>
<td>9</td>
<td>77</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

* Body mass (kg), change in body mass Chg (body mass) (g), total litter mass (g).

b Body composition; % BMC (percent bone mineral content), % LEAN (percent lean tissue), % FAT (percent fat tissue), BMC (bone mineral, g), LEAN (lean tissue content, g), FAT (fat tissue content, g), Chg (BMC) (change in bone mineral content from 24 to 30 days of lactation), Chg (LEAN) (change in lean tissue content), Chg (FAT) (change in fat tissue content).

c Water kinetics and energy balance: k_d (deuterium elimination rate, h⁻¹), N_d (body water space estimated by deuterium dilution, ml), N(DXA) (body water space estimated by DXA, ml), water turnover calculated by isotope elimination (ml/day), milk consumption (g/day), milk energy intake (kJ/day), % body water calculated by deuterium dilution.

* P values denote a significant difference between Labradors and Schnauzers.

** Denotes a significant change over time during peak lactation (24–30 days post partum).

of body water space in Labrador and Schnauzer pups were 4 and 10%, respectively less than the deuterium dilution spaces.

3.10. Milk intake, milk composition and milk energy output

Milk intake was greater in Labrador than Schnauzer pups (2-sample t_{66} = 3.70, P = 0.0024) (Table 5). There were no differences in k_d, N_d or milk intake between those individuals that came from large or small litters. In addition, there were no differences in milk composition between breeds, within breeds with different litter sizes, or between day 24 and day 30 of lactation within individuals. Milk contained on average 21.2 (S.D. 1.33)% dry matter, 8.23 (S.D. 0.66)% crude protein, 8.11 (S.D. 1.91)% fat and 4.86 (S.D.1.42)% sugar. Milk energy averaged 5.92 (S.D. 0.60) kJ gross per gram.

4. Discussion

4.1. Animals

There is a positive relationship between litter size and body mass across dog breeds (Robinson et al., 1973), in contrast to the general mammalian pattern (Blueweiss et al., 1978). However, gestation length (Krzyzanowski et al., 1975), oestrus cycle length, weaning times (Evans and White, 1988) and age at first breeding are remarkably uniform across dog breeds. This is surprising
given the convincing allometric patterns for these traits across mammals of similar size ranges, with larger animals generally reproducing more slowly and investing less overall resources into reproduction (Hanwell et al., 1977; Calder, 1996). Hence, one might expect the (intraspecific) patterns of energy allocation observed across dogs of different masses to differ from expected (interspecific) allometric variation. Figs. 2 and 3 show the mass, water and energy transfer in lactating Labradors and Schnauzers, respectively (24–28 days post partum).

4.2. Food intake

Metabolisable energy intakes (MEI) during peak lactation were similar to the maximum predicted rates of energy assimilation. MEI’s of Labradors were 29% higher than the Weiner (1989) prediction and 13% higher than the Kirkwood (1983) prediction. In contrast, Schnauzers were 14% lower than the Weiner prediction and 18% lower than the Kirkwood prediction (Fig. 4). Hence, Labradors appeared to be working harder during peak lactation than Schnauzers, independently of their mass. These increases are consistent with Labradors having the larger litters. The fact that differences in food intake between large and small litters were only observed in the Labradors may reflect the larger litter sizes of Labradors. The extra cost of lactation was met by increases in food intake of 300–400% in Labradors and 200–300% in Schnauzers, in agreement with other studies on small mammals (Lonnerdal et al., 1981; Lochmiller et al., 1982; Glazier, 1985; Hammond et al., 1992). In contrast, when food supply is intermittent, or when lactation is necessarily brief, some large carnivores such as species of pinniped and bear cover some or all of the costs of lactation by utilising body reserves (e.g. Arnould and Ramsay, 1994; Lydersen et al., 1996, 1997; Oftedal, 1993; 1993b; Lydersen et al., 1995, 1996, 1997).

4.3. Body mass and body composition

There was no change in mass for adults of either breed during peak lactation. This is consistent with previous studies in which the costs of lactation are met by increases in food intake (Meyer et al., 1984; Oftedal, 1984a; Kienzle et al., 1985). Nevertheless, there was a significant decrease in the body fat of the Labradors. The loss in the larger breed is consistent with the idea that larger mothers may be better able to utilise body reserves during lactation (Bowen et al., 1992; Fedak et al., 1996), and it is tempting to regard this fat loss as a possible limit to energy assimilation. However, the large amount of variability in the data and the relatively minor loss of fat (0.74% reduction in body fat of the Labradors over the 7 day interval) indicate this loss is unlikely to be biologically significant. Although there was no loss of BMC during the week of peak lactation, lactating mothers had significantly less BMC than non-breeding individuals (Fig. 1). Maternal BMC was therefore likely to have been used to support offspring skeletal growth (Meyer et al., 1984; Fukuda et al., 1993). In contrast to adults, pups of both breeds increased in body mass during the experimental period. Lean tissue growth accounted for 90% of the mass increase in Labradors and 80% in Schnauzers (Table 5, Figs. 2 and 3). By comparison, offspring of some species of pinniped, with a necessarily short duration of lactation, are able to grow rapidly and 50–80% of the increase in mass is fat (Meyer et al., 1984; Oftedal et al., 1993b; Lydersen et al., 1995, 1996, 1997).

![Fig. 4. Log-converted metabolisable energy intake (MEI) against log-converted body mass (kg) for 12 Labrador Retrievers (closed symbols) and five Miniature Schnauzers (open symbols). Predicted relationships are given as lines. The solid line is the Kirkwood (1983) prediction and the dotted line is the Weiner (1989) prediction. Labradors had higher and Schnauzers lower MEI's than predicted.](image-url)
4.4. Milk composition

Milk production estimates in the current study were similar to the 1690 g/milk per day in a German Shepherd (66) and 1060 g/milk per day in Beagles at day 26 of lactation (Oftedal, 1984a). Although milk composition varies greatly between species (Jenness et al., 1970; Linzell et al., 1972), there appears to be little difference between dog breeds (Russe, 1961). Milk composition changes with period of lactation (Thomee, 1978; Lonnerald et al., 1981; Oftedal et al., 1993a; Lydersen et al., 1995), which teat and how completely a gland is emptied (Oftedal, 1984b). In the current study, milk composition was similar to that measured in beagles at a corresponding stage of lactation (Oftedal, 1984a). We found no difference in milk composition between Labradors and Schnauzers measured during the same period of lactation or any evidence of more dilute milk being produced by mothers of larger litters (e.g. in cotton rats, *Sigmodon hispidus*) (Rogowitz et al., 1995). Therefore, milk composition is unlikely to be a source of disparity in lactation energetics between these two breeds.

4.5. Milk energy output

In mammals, the magnitude of milk energy output roughly parallels body mass, with larger individuals producing more energy per day in milk (Oftedal, 1984b). Typically canids have high milk energy outputs, of which the domestic dog has one of the highest (Oftedal and Gittleman, 1989). Communal rearing of offspring might have allowed the evolution of large litter sizes which subsequently induced high milk energy outputs (Moechlem, 1989; Oftedal and Gittleman, 1989). These milk energy demands might be expected to differ, following selection for variations in body mass, with resulting litter size changes. Expression of milk energy output relative to maternal metabolic mass (MMM) ([M^{0.75}]: M = maternal body mass, 0.75 is the allometric exponent) is one way of correcting for size-related differences in energy yield (Oftedal, 1984b). Relative energy outputs calculated in this way were 598 kJ/kg^{0.75} in Labradors and 567 kJ/kg^{0.75} in Schnauzers. These values are similar, but Labradors maintained slightly higher milk energy outputs independent of their mass. Milk energy output can also be expressed in terms of litter metabolic mass (LMM) (allometric exponent is 0.83), which provides an estimate of the relative energy demands of the litter (Gittleman et al., 1987; Oftedal and Gittleman, 1989). Energy output per LMM was 591 kJ/kg^{0.83} in Labradors and 731 kJ/kg^{0.83} in Schnauzers. The ratio LMM/MMM indicates the demands of the litter relative to maternal metabolism. It explains approx. 97% of the variance in maternal energy production per kg^{0.75} and is valid for species that vary greatly in body mass (Oftedal, 1984b). LMM/MMM, was 1.01 in Labradors and 0.78 in Schnauzers, which indicates Labradors have a higher demand of the litter relative to maternal metabolism than Schnauzers (Fig. 5).

In summary, the pattern of energy allocation during reproduction in Labradors and Schnauzers was different from the predicted interspecific pattern, which predicts larger animals to reproduce more slowly, have smaller litter sizes and invest less energy in reproduction. In the current observations, although milk composition and gross efficiency of milk production were not significantly different between breeds, Labradors had a relatively higher milk energy output than the Schnauzers and invested a greater amount of resources in reproduction. The costs of lactation were met by increases in metabolisable energy intake, which was higher than predicted in Labradors and lower than predicted in Schnauzers.
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