

Peak energy turnover in lactating European hares: the role of fat reserves

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SUMMARY

European hares (*Lepus europaeus*) in central Europe have high energetic costs of reproduction, mainly due to precocial, rapidly growing young that rely largely on energy-rich milk. Thus, hares in this climate build up large fat stores during winter that are then gradually depleted during the spring-to-autumn breeding season. We hypothesized that the diminishing fat stores of females over the breeding season might affect resource allocation, peak energy assimilation during lactation or the total investment in offspring. Therefore, we measured energy intake, milk quality and milk production throughout lactation in spring, summer and autumn in females raising (size-manipulated) litters with three young each, under a natural photoperiod but at buffered ambient temperatures inside our facility. Over the course of the breeding season, the amount of milk production remained constant, but the fat content of the milk decreased. Hence, total energy transfer to young decreased significantly in autumn. By using undecanoic acid as a tracer of body fat mobilization, we were able to show that milk fat partially originated from maternal fat stores, particularly in spring. The peak sustained energy assimilation rates of lactating females were significantly higher in autumn, due to increased rates of food intake. We conclude that fat stores allow female hares to downregulate energy intake and expenditure early in the breeding season, whereas late breeding forces them to reach peak energy intake levels. Accordingly, we suggest that in hares, peak energy turnover during lactation varies with the availability of fat reserves. Limits to the sustained metabolic rate serve as variable constraints on reproductive investment. Thus, there might be a trade-off in energetic costs to mothers rearing early *versus* late litters in the year.

Key words: energy allocation, parental investment, energetic cost, reproduction, reproductive effort, seasonal shift, allocation strategy, fat depletion, *Lepus europaeus*.

INTRODUCTION

Reproduction in European hares (*Lepus europaeus*) is characterized by the production of precocial, self-sustained young, which lack protective burrows and stay above ground from birth. The fully-furred young (or 'leverets') grow very rapidly, have high energy demands and are capable of thermoregulation, (Hackländer et al., 2002a; Hackländer et al., 2002b). Because young hares start to consume substantial amounts of solid food only around their third week of life, the major part of their energy intake until weaning after approximately 4 weeks is obtained from milk, which causes a high energetic burden for reproducing females (Hackländer et al., 2002b).

There are several observations indicating that female hares, similar to other mammals, might indeed reach an upper limit of energy turnover – i.e. maximum sustained metabolic rate (SusMR) – during lactation. Food intake and milk production in hares increase rapidly but then level off after the second week of lactation. Peak rates of energy assimilation at a high litter size of three young have been estimated to range around five times resting metabolic rate (RMR), which is comparable to SusMR in other small mammals (Hackländer et al., 2002b; Hammond and Diamond, 1997). Furthermore, as total litter mass increases, energy intake and milk production of lactating females do not increase proportionally, and consequently body masses of young at weaning decrease markedly with litter size (Hackländer et al., 2002a; Hackländer et al., 2002b). Together, these findings suggest that female hares, like other mammals, might face a physiological limit of energy throughput during peak lactation. These limits have been related to the capacity of 'central' alimentary organs (Koteja, 1996), to peak metabolism

in peripheral tissues such as the mammary glands (Hammond et al., 1996) or, more recently, to the maximum ability of females to dissipate excess heat that is generated as a by-product of increased metabolism (Krol et al., 2007).

Most previous studies on SusMR during peak lactation have used small rodents, such as laboratory mice, as animal models. Arguably, in these species, reproductive performance is determined mainly by the maximum rate of energy assimilation from food and its conversion to milk, because they lack sufficient body energy reserves that could be used to supplement milk production. European hares, however, are well known to build up considerable fat deposits in autumn and winter (their non-breeding season in central Europe) (Zörner, 1996) and might even partly restore fat deposits during the non-reproductive period or during gestation in the breeding season. Until now, the role of these body fat reserves for the lactation performance of European hares was unclear, however. As presented in Drent and Daan (Drent and Daan, 1980), breeding females in general might shift between two different strategies of resource allocation. The 'capital breeder' strategy refers to females using their own body fat reserves to cover the high energetic costs of reproduction, whereas 'income breeders' are considered to raise their energy or food intake when facing high energy demands. We hypothesized that fat deposits accumulated over winter could serve lactating hares as an additional milk energy supply, in particular for the first litters of the year. If that were the case, a gradual shift from a predominantly 'capital' to an 'income' strategy over the breeding season (February to October in central Europe) would give rise to several possible scenarios of maternal investment during peak lactation: (1) females could use body fat reserves to supplement

milk fat while keeping peak food intake and energy assimilation (i.e. SusMR) constant; (2) females could maintain constant energy transfer to young by adjusting/reducing food energy intake when fat deposits are available; (3) females could adjust both milk energy transfer and energy assimilation during the breeding season. In all of these scenarios, it is of interest to determine whether possible seasonal changes in milk fat content are compensated by adjustments of milk production (i.e. quantity).

To address these issues, we determined energy intake, milk transfer and milk energy content throughout lactation in laboratory-housed European hares at three times during the breeding season: in spring, summer and autumn. To keep the energetic costs of reproduction comparable between females, litter sizes were kept constant at three leverets. To minimize external limitations, all experimental females were given a high-fat diet *ad libitum* throughout the experiments. In the wild, hares are very selective on their diet and pick food plants rich in fat (Reichlin et al., 2006). Our laboratory diet, which was arranged to match average stomach contents of carbohydrate, protein, fat and fibre in free-ranging hares (Hackländer et al., 2002b), but with the addition of 10% oil, simulated optimal provisioning with fat even better than in natural set-asides, where hares can select preferred food plants. We also determined the growth and solid food intake of young to assess the possible effects of changes in lactation performance on the offspring.

MATERIALS AND METHODS

Animals and housing

European hares (*Lepus europaeus*, Linnaeus), often called 'Brown hares', were born and kept in our outbred breeding colony at the Research Institute of Wildlife Ecology, University of Veterinary Medicine Vienna, Austria. Hares were housed individually in cages, as outlined by Hackländer and colleagues (Hackländer et al., 2002b). All animals (females and young) were provided with water and food *ad libitum*. Food pellets (Raiffeisen, Salzburg, Austria) were enriched with sunflower oil (12.5 kg oil per 100 kg pellets). The mean gross energy content of the diet over the whole study period was 19.09 ± 0.03 kJ g⁻¹ with 15.3 ± 0.04% protein, 16.1 ± 0.04% fibre and 13.4 ± 0.07% fat. To ensure that the energy content and particularly the fat content were stable throughout the study period, we analysed dietary fat content every time the pellets were mixed with oil. The analyses of the diet were performed as described by Hackländer and colleagues (Hackländer et al., 2002b).

Data were sampled between February 2004 and October 2007 on a total of 28 mothers and their 50 litters. All experimental animals were aged between 1 and 5 years and were in good health and condition. Hares were exposed to a natural photoperiod and to indoor temperatures in an unheated housing facility that varied between 8°C and 25°C over the study period, but mean ambient temperature varied by less than 2.5°C between the three seasons (see below). During the 9-month yearly reproductive period, the body mass of all animals was determined weekly to the nearest gramme. Food intake was determined over bi-weekly feeding trials (over 3- and 4-day intervals) by weighing offered and uneaten food in all females, resulting in eight data points for each female per 28 d lactation period. Food items spilled from the racks were dried, weighed and subtracted from food consumption. To minimize effects of changes in humidity, food pellets were stored next to the cages before usage.

Total faeces produced by the animals were collected biweekly over 3- and 4-day intervals, dried at 60°C in a drying oven (Heraeus, Germany) for 48 h and then the mass determined to the nearest 0.1 g

(Ohaus, Germany). It was impossible for us to distinguish between old and new faeces, and similarly we were unable to assess the amount of re-ingested faeces because hares perform coprophagy by taking up faeces right from the anus and re-digesting it. Gross energy content was determined for faecal samples by near-infrared spectroscopy (NIRS). Samples were analyzed using a FT-NIR spectrometer MPA (Bruker Optik GmbH, Ettlingen, Germany) with an integrating sphere in diffuse reflection. The samples were measured in a rotating cup with a diameter of 50 mm three to six times each using a resolution of 8 cm⁻¹ and 64 scans. The spectrometer was equipped with software OPUS 5.5 with the additional packages OPUS/LAB and OPUS/QUANT (2005, Bruker, Ettlingen, Germany). The following parameters were determined: dry matter, protein, fat, ash, acid detergent fiber (ADF) and lignin. Nitrogen free extracts (NFE) were computed by subtracting lignin from ADF. For calibration of the NIRS analysis, 80 samples were chemically analyzed using standardized methods for crude protein, crude fat, crude ash and dry matter (Nehring, 1960). ADF and lignin were determined by Van Soest detergent analyses (Otzelberger, 1983). The NIR calibration results were evaluated by cross validation. Coefficients of determination for fat, protein, ash, lignin and dry matter were 0.93, 0.93, 0.83, 0.87, 0.87 and 0.96, respectively.

Females were paired with males for two days three times per year – i.e. in February–March (spring), May–June (summer) and late-July–August (autumn). To allow litter size manipulations, matings took place synchronously each time. Immediately after birth of the young (40.8 ± 0.13 days after mating), litter sizes were manipulated to achieve a litter size of three for all females investigated. Mean litter size amounted to 1.3, 1.7 and 1.2 for spring, summer and autumn, respectively, and did not differ significantly between seasons ($F_{2,71} = 2.45$, $P = 0.09$). These litter sizes are also comfortably in the range of litter sizes in free-ranging hares (1–5, average 2–3) (Zörner, 1996). We did not fully cross-foster litters, but in most cases we added one or two pups from another female, which was then left without pups until the next mating. All females readily accepted and nursed additional young, independent of whether they were crossfostered. Note that long-term data from our breeding colony show that only 10.1% of all females have litters larger than three leverets ($N = 813$ litters) and very few females are able to wean successfully more than three young. Thus, by raising three leverets, all females in our experiment had high (see also Hackländer et al., 2002b) and approximately equal energy requirements.

Females were kept separately from their young, except for a short nursing period in the morning (8–9 am), which simulates the short daily nursing bouts of free-living hares (Broekhuizen and Maaskamp, 1980). The milk intake of young was measured daily by weighing the leverets before and after the 1 h suckling period, with the mass determined to the nearest 0.1 g. Initial trials showed that mass losses during the nursing period due to faeces and urine losses in juveniles were negligible (<2 g per juvenile) in comparison with the milk intake (~60 g). Therefore, faeces and urine losses during these periods were not determined. During this period, leverets had no access to other food sources. Otherwise, the leverets had *ad libitum* access to the same food as adults, and the food intake of each litter was determined at weekly intervals. During each season, small milk samples (<3 ml) from a subsample of females were collected and chemically analyzed as outlined by Hackländer and colleagues (Hackländer et al., 2002b). In one year of our study (2005), we added a saturated fatty acid, undecanoic acid (C 11:0; Sigma Aldrich, Germany), which does not naturally occur in the

diet of hares, to the pellets (17.66 g undecanoic acid per litre sunflower oil) and fed it to experimental mothers each time they were gestating, but not during lactation. This was to see whether undecanoic acid would be incorporated into fat reserves and later transferred to milk during lactation. The concentration of undecanoic acid in milk samples was determined by gas-liquid chromatography (Perkin Elmer Autosystem XL with Autosampler and FID; Norwalk, CT, USA) using a capillary column (HP INNOWax, 30m×0.25 mm; Hewlett Packard, USA). Fatty acid methyl esters from milk were identified by comparing retention times with those of fatty acid methyl ester standards (Sigma Aldrich, St Louis, MO, USA). Peaks were integrated using Total Chrom 6.3. software (Perkin Elmer, Norwalk, CT, USA).

For reasons of management of our breeding colony, we had to kill some females at the end of each breeding season in the autumn. From this subsample of animals that were either non-reproducing ($N=78$), or post-lactating ($N=19$; on the day of weaning of young), we determined the fresh masses of completely dissected peri-renal white adipose tissue deposits, with masses determined to the nearest 0.1 g.

Computation of energy contents and statistical analyses

The energy content of solid food and faeces was calculated by using energetic values given in Livesey (Livesey, 1984) and Livesey and Marinos (Livesey and Marinos, 1988). Thus, the gross energy content of protein, fat and fibre/nitrogen-free extract (NFE) was 23.3 kJ g^{-1} , 39.6 kJ g^{-1} and 17.5 kJ g^{-1} , respectively. Gross energy intake (GEI) was computed from the amount of food consumed per day multiplied by its energy content. Metabolizable energy intake (MEI) was calculated by (i) correcting GEI for urinary energy losses due to nitrogen excretion by using a metabolizable protein energy content of 19.3 kJ g^{-1} (Livesey, 1984) and (ii) computing the difference between this corrected, utilizable GEI and the energy content of the daily amount of faeces excreted. The estimated average urinary energy loss was 3.3% of GEI. Energy assimilation rates were computed as: $(\text{MEI}/\text{GEI}) \times 100$. The conversion factors above (using 19.3 kJ g^{-1} for protein), as well as an energetic value of 16.5 kJ g^{-1} of lactose (Stubbs et al., 1997), were also used to compute milk energy content. To allow comparison with published data from other species, multiples of mass-specific resting metabolic rate (RMR) were computed by dividing both mass-specific GEI and mass-specific MEI by $172.3 \text{ kJ kg}^{-1} \text{ day}^{-1}$, the RMR of non-reproducing hares at thermoneutrality (20°C) measured for 4 h with open-flow respirometry, as outlined elsewhere (Hackländer et al., 2002a).

Data on female food intake, GEI, MEI, milk production, milk energy transfer, mass of fat deposits in females, as well as growth of young (weaning, birth masses) and solid-food intake of litters were analysed with a repeated measures design, as data within and partly between study years were sampled from the same animals. We fit linear mixed-effect models, with separate intercepts for each female included as the random factor. In models involving juvenile growth and juvenile food intake, we used 'litter' as the random factor. Fixed effects in these multiple-regression models were lactation week (fitted by a quadratic term owing to the nonlinear time-course of all variables tested) (e.g. Fig. 1), season (spring, summer, autumn), ambient temperature, female age (1–5 years) and mean ambient temperature over the measurement interval. The body masses of females differed slightly between seasons ($3480 \pm 42 \text{ g}$, $3402 \pm 26 \text{ g}$ and $3510 \pm 32 \text{ g}$ in spring, summer and autumn respectively). However, the possibly confounding effect of individual body mass was eliminated by inserting body mass as a covariate in all models for data obtained from females. In some

cases, regression models and ANOVAs were also computed for the second half of the lactation period, which is considered to reflect peak lactation (intervals 5–8). Full models indicated that none of the response variables showed a further significant increase during this last phase. Residuals from all models were normally distributed and showed no evidence for heterogeneity of variances. Only for one response variable – milk undecanoic acid content – were data non-normally distributed owing to the fact that all values in autumn were below the detection level and set to zero. Therefore, we used a Kruskal–Wallis H-test to compare seasonal differences in undecanoic acid content. We tested for all possible two-way interactions, but none of them reached significance. All statistical analyses were computed in R (2.7.0.) (R Development Core Team, 2008), using the package nlme (Pinheiro et al., 2008). The data are presented as means \pm s.e.m.

RESULTS

Gross and metabolizable energy intake

Both GEI and MEI increased with body mass (GEI: $F_{1,314}=84.62$, $P<0.0001$; MEI: $F_{1,314}=53.84$, $P<0.0001$) but were independent from fluctuations in ambient temperature (GEI: $F_{1,314}=0.31$, $P=0.57$; MEI: $F_{1,314}=0.24$, $P=0.62$). Over the 4-week lactation period, GEI and MEI increased by 68% and 54%, respectively, comparing the maximum of each curve with interval 1 (Fig. 1). Both GEI and MEI reached a stable plateau after interval 4 (i.e. day 14), which was maintained until the end of lactation. The mean levels of GEI and MEI during peak lactation corresponded to an energy intake of 8.7 and 5.7 times the RMR of European hares, respectively (Fig. 1). As indicated by the increasing difference between GEI and MEI, the energy assimilation rate continuously decreased from $73.0 \pm 1.09\%$ during interval 1 to $65.7 \pm 0.53\%$ during interval 8 ($F_{1,317}=38.47$, $P<0.0001$).

Determinants of MEI

Surprisingly, MEI was not constant over the breeding season but increased significantly in autumn (Fig. 2). This was the case whether the entire lactation period was considered ($F_{2,314}=7.60$, $P<0.001$) or peak lactation only ($F_{2,178}=7.30$, $P<0.001$). This difference between seasons was not due to differences in mean ambient temperature, which varied only slightly between spring ($17.14 \pm 0.38^\circ\text{C}$), summer ($19.24 \pm 0.15^\circ\text{C}$) and autumn ($17.91 \pm 0.29^\circ\text{C}$). Also, MEI was only slightly affected by the age of females ($F_{4,314}=2.25$, $P=0.06$), owing to an 8.2% lower MEI in young females (age 1).

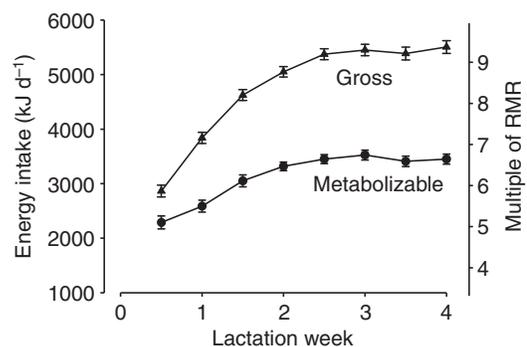


Fig. 1. Gross (triangles) and metabolizable (filled circles) energy intake in the course of lactation. The right axis shows energy intake as a multiple of resting metabolic rate (RMR). Means over pooled data from all three seasons \pm s.e.m. from a total of 50 lactation periods in 28 females.

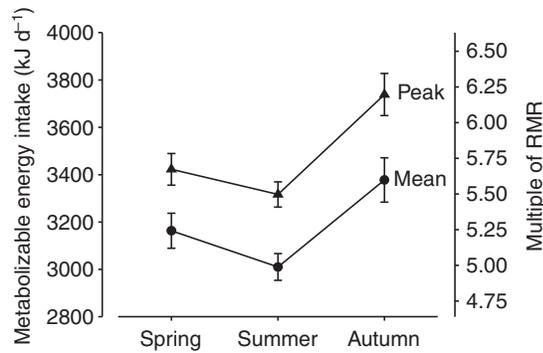


Fig. 2. Metabolizable energy intake throughout lactation (means) and at peak lactation (weeks 2–4) at three time-points within the breeding season. Means \pm s.e.m. from 50 lactation periods and 28 females.

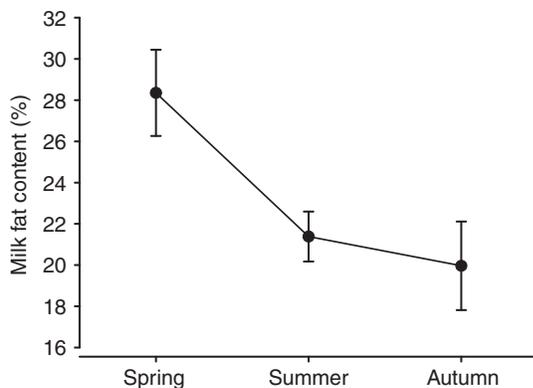


Fig. 3. Milk fat content (of dry-matter content) in the course of the year. Means \pm s.e.m. from 22 milk samples from all study years (six from spring, 11 from summer, five from autumn).

Milk production and fat mobilization

The milk output of mothers increased with lactation week ($F_{2,314}=182.36$, $P<0.0001$), and, similar to MEI, levelled off after interval 4. As expected, milk output increased with the female body mass ($F_{1,314}=70.69$, $P<0.0001$). We observed no significant change of total milk output over the reproductive season ($F_{2,314}=2.24$, $P=0.11$). There was, however, a decrease in milk quality in the course of the year. Milk energy content decreased significantly from 14.5 kJ g^{-1} in spring to 11.03 kJ g^{-1} in autumn ($F_{2,15}=7.7$, $P=0.005$). This was mainly owing to a decrease in milk fat content (Fig. 3) ($F_{2,15}=5.2$, $P=0.019$). As a consequence, mean daily energy transfer to the young through milk significantly decreased over the breeding season both when the entire lactation period was considered ($F_{2,314}=13.32$, $P<0.0001$) and peak lactation (interval 5–8) only (Fig. 4) ($F_{2,127}=11.66$, $P<0.0001$). This seasonal change of milk energy transfer was independent of variation in ambient temperature ($F_{1,314}=1.8$, $P=0.19$). There was a slight effect of age on mean milk energy transfer, mainly due to an 18.7% lower transfer in young (age 1), compared with other females ($F_{4,314}=2.7$, $P=0.03$). Age, however, did not affect milk energy transfer at peak lactation (interval 5–8, $F_{4,127}=1.92$, $P=0.112$).

Supplementary feeding of undecanoic acid before the birth of young indicated that lactating females were mobilising body fat reserves that had accumulated during the preceding gestation period (Fig. 5). The concentration of the marker in the milk decreased

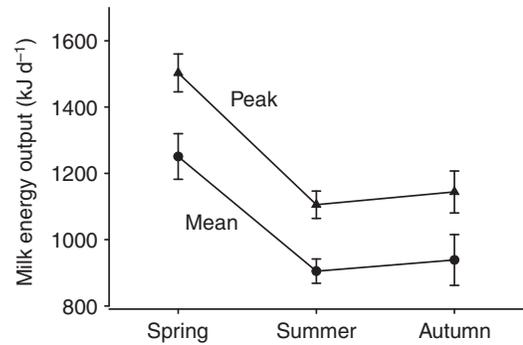


Fig. 4. Total milk energy transfer (in kJ day^{-1}) to young over the entire lactation period (means shown as filled circles) and at peak lactation (weeks 2–4, shown as triangles) in the course of the reproductive season. Means \pm s.e.m. from 50 lactation periods in 28 females.

significantly, however, over the breeding season (Kruskal–Wallis $\chi^2=9.5337$, d.f.=2, $P=0.008$). Fat mobilisation during lactation was indeed restricted to the first and second litters in the year because undecanoic acid could not be detected in autumn milk samples. In autumn, females had significantly less retroperitoneal white adipose tissue immediately after the weaning of young than non-reproducing hares that were sacrificed at the same time (Fig. 6) ($F_{1,32}=13.03$, $P=0.001$).

Energy budgets

The energy budgets of females during the three breeding seasons are given in Table 1. The transfer of body energy reserves to milk in spring (see above) was estimated from the difference between mean milk energy transfer during the first (spring) and later litters (summer and autumn). These budgets indicate that the amount of energy allocated to milk derived from food consumption was approximately equal ($\sim 270 \text{ kJ kg}^{-1} \text{ day}^{-1}$) at all seasons. Also, the resulting amount of energy from MEI used for maintenance and thermoregulation (MEI–milk energy from food) was not different between spring and later litters ($F_{1,321}=2.18$, $P=0.14$).

Growth and energy consumption of young

During all seasons, the costs of growth were predominantly covered by milk energy. The gross energy used from milk per gramme of body mass increase (day 1–28) was $21.9 \pm 0.62 \text{ kJ day}^{-1}$, $15.1 \pm 0.36 \text{ kJ day}^{-1}$ and $16.6 \pm 0.60 \text{ kJ day}^{-1}$ in spring, summer and autumn, respectively. Gross energy consumption from solid food amounted to $1.04 \pm 0.12 \text{ kJ day}^{-1}$ (spring), $1.32 \pm \text{kJ day}^{-1}$ (summer) and $1.47 \pm 0.07 \text{ kJ day}^{-1}$ (autumn).

The mean birth masses of young were similar at all seasons ($124.4 \pm 2.89 \text{ g}$ in spring, $123.2 \pm 2.37 \text{ g}$ in summer and $127.4 \pm 3.19 \text{ g}$ in autumn). Mean weaning masses at day 28 were $627.7 \pm 22.8 \text{ g}$ in spring, $690.3 \pm 20.5 \text{ g}$ in summer and $734.0 \pm 30.6 \text{ g}$ in autumn. The corresponding slight differences in gain in mass were statistically not significant ($F_{2,16}=2.516$, $P=0.112$). The fact that weaning masses remained stable indicates that leverets apparently compensated for the seasonally decreasing milk quality by consuming more solid food and thereby increasing GEI during late lactation in summer and autumn ($F_{6,151}=3.83$, $P=0.0014$; week \times season interaction). This effect was most pronounced in week four of lactation, during which mean solid food intake per litter was $236.4 \pm 39.7 \text{ kJ day}^{-1}$ per litter in spring, $277.3 \pm 26.8 \text{ kJ day}^{-1}$ in summer and $422.7 \pm 37.7 \text{ kJ day}^{-1}$ in autumn.

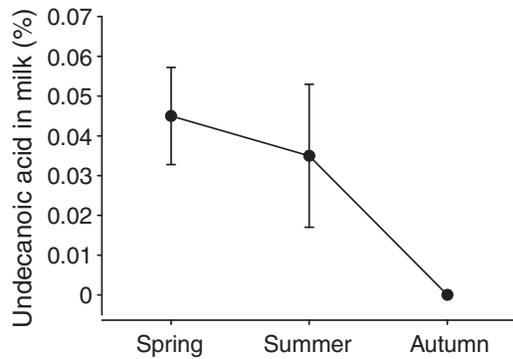


Fig. 5. The percentage of undecanoic acid (C11:0) in the milk of lactating females over the reproductive season, related to all fatty acids determined, means \pm s.e.m., $N=16$ from the year 2005 only.

DISCUSSION

Our results confirm previous studies indicating that European hares have relatively high energetic costs during lactation (Hackländer et al., 2002a; Hackländer et al., 2002b). Other precocial mammals, such as the Guinea pig, elevate rates of energy turnover to only ~ 3.9 times RMR. This is mainly because juvenile Guinea pigs start to feed on solid food (in addition to milk) even on their first day of life (Künkele and Trillmich, 1997; Künkele, 2000). Juvenile hares, however, which start to consume substantial amounts of solid food only in week three, depend to a much higher degree on their mother's milk, so their early life development is determined by maternal nutrition and energy allocation (c.f. Rogowitz and McClure, 1995). Hence, sustained MEI and GEI in female hares reached up to 6.2 and 9.3 times RMR, respectively, suggesting that they might reach a metabolic ceiling during lactation. This is well in the range of SusMR in altricial small rodents where sustained MEI and GEI were reported to amount to six and eight times RMR, respectively (Hammond and Diamond, 1997; Johnson et al., 2001). Contrary to mice, for which assimilation efficiency was reported to remain constant (at $\sim 80\%$) throughout lactation (Johnson et al., 2001), hares showed a 7.5% decrease in digestible energy. Similar changes in digestible energy have been observed in small rodents when food intake increased in response to cold exposure (Liu et al., 2002; Song and Wang, 2006). In hares, which have generally low mean retention times of digesta in their gastrointestinal tract (Stott, 2008), the observed drop in assimilation efficiency during lactation might have resulted from a further decrease of mean retention time, along with the simultaneous increase in food intake.

The very high levels of energy intake could suggest that female hares approached an upper physiological limit during peak lactation, particularly in autumn. This hypothesis is also corroborated by the strong decrease in weaning mass with increasing litter size (Hackländer et al., 2002a; Hackländer et al., 2002b). Furthermore, the time-course of increasing GEI and MEI during lactation (Fig. 1) and the associated energy assimilation rates indicate that increased food intake was accompanied by a decline in the efficiency of digestion caused, for instance, by an upper limit in the capacity of alimentary organs. Thus, based on these observations alone – without discriminating between seasons – we would conclude that the observed peak levels of sustained energy turnover in hares were probably due to physiological constraints, whether they act 'centrally' on energy intake, 'peripherally' on energy output or otherwise (for a review, see Speakman, 2008). However, our comparison of seasons shows that females in autumn were able to

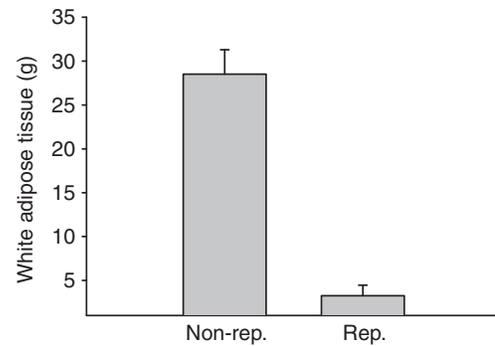


Fig. 6. Peri-renal white adipose tissue in non-reproducing (Non-rep.) and reproducing (Rep.) European hares, immediately after weaning of young. Means \pm s.e.m. from 78 non-reproducing and 19 reproducing individuals.

further increase MEI significantly, compared with spring and summer (Fig. 2). Our data give no evidence for seasonal changes in juvenile energy demands that could have caused these adjustments of MEI in females. Birth and weaning masses of juveniles did not differ throughout the seasons. Also, mean ambient temperature, which affects the thermoregulatory costs of juveniles, varied little between seasons and had no detectable effect on female energy turnover.

Therefore, it seems that seasonal changes in reproductive investments were driven by the availability of fat stores that allowed females to downregulate energy intake and expenditure in the early breeding season. Late-season breeding then forced females to elevate their energy intake in order to compensate for the lack of body fat reserves at that time of the year. It remains to be seen whether this peak rate of energy turnover in autumn actually represents a physiologically constrained upper limit of SusMR in hares or whether still-higher levels might be reached under other conditions – i.e. additional cold load. However, our findings that females (i) downregulated energy turnover in spring and summer and (ii) did not fully compensate in terms of total milk energy output for the lack of body reserves in autumn both point to adverse effects of very high energy turnover, and hence to trade-offs between current and future reproduction. Generally, there appears to be a trade-off between reproduction and survival and between current and future reproduction (Williams, 1966; Stearns, 1989). Thus, limiting the upper level of energy turnover early might well represent a 'prudent parent' strategy (Drent and Daan, 1980) in hares that serves to maximise lifetime reproductive success.

Currently, we can only speculate about the nature of the potential fitness costs associated with intense levels of energy throughput in hares. Two mutually compatible consequences of high rates of SusMR could be: (i) increased rates of aging and (ii) increased predation risk. High levels of metabolic rate are known to cause mitochondrial lipid peroxidation that leads to deleterious products such as reactive aldehydes, which cause damage to membranes as well as enzymes and inhibits DNA and protein synthesis (Hulbert, 2005). Although there is probably no simple relationship between metabolism and longevity (Speakman, 2005), it has been argued that rapid changes between lower and peak rates of energy turnover, as occur in the time-course of lactation, are particularly damaging and might impair life span. This could be because such fluctuations lead to a temporal imbalance between deleterious metabolic products and anti-oxidant defence systems (Demetrius, 2004; Demetrius, 2005).

A second, simpler explanation for the costs of high SusMR is that increased food intake and hence foraging is probably associated with increased locomotion, conspicuousness and thus predation risk (Jönsson, 1997; Kraus et al., 2008). Consequently, limiting risky foraging, whenever body energy reserves allow this strategy, should increase longevity and fitness. A prerequisite for this tactic is resource allocation to energy stores before, and their use during, the breeding season (Speakman, 2008). This was clearly the case in the females studied here. Our feeding trials using undecanoic acid as a marker for mobilization of fat reserves demonstrated that, early in the breeding season, mothers transferred fatty acids to milk that had been stored previously. Also, the observed decrease of milk fat and energy content over the reproductive season (Fig. 3) suggests that body energy reserves stored during the previous winter served as an important resource for provisioning the first litters of the year. Thus, lactating hares might use stored fat reserves to reduce predation risk caused by high energy needs.

During the peak of their seasonal body fat cycle (approximately 2 months before the onset of the breeding season), captive female hares can have total body lipid stores of 8% of body mass, amounting to 280 g in a 3.5 kg animal (F. Tataruch, unpublished observations from total carcass analysis). The difference in milk fat content between spring and later litters in the present study showed that, during the spring lactation period, mothers on average transferred ~195 g of body fat reserves to milk. Thus, it seems at least possible that females during spring could mobilize further – albeit limited – fat reserves to also sustain their own maintenance costs and metabolic rate, in addition to the transfer of fat to milk. If this were the case, it should be reflected by a significantly lower proportion of MEI allocated to maintenance costs in spring. However, our estimates of energy budgets (Table 1) indicated that the amount of energy from MEI used for maintenance remained almost constant throughout all seasons. Thus, there was no evidence for a sizeable role of energy reserves in supporting metabolic rate, which also means that the seasonal time-course of sustained metabolizable energy intake (SusMEI) most probably mirrored that of SusMR.

The seasonal decline of milk fat content (Fig. 3) and the extremely low mass of fat deposits after lactation in autumn (Fig. 6) indicate that fat reserves were gradually depleted over the breeding season. Females in autumn increased energy intake apparently to raise successfully their last litters of the season. Thus, in the course of the breeding season, females gradually switched from a ‘capital breeding’ to an ‘income breeding’ strategy of resource allocation (Drent and Daan, 1980; Jönsson, 1997). Again, the major benefit of the early ‘capital breeding’ phase might be the reduction of

predation risk by avoiding intensive foraging (Jönsson, 1997). The compensatory increase of energy intake during the ‘income breeding’ phase in autumn was not accompanied, however, by an increase in milk volume transferred to young. One might be tempted to attribute this constancy of milk volume to the peculiar, once-a-day, nursing bouts in hares, during which young consume the entire daily milk supply (Broekhuizen and Maaskamp, 1980). Milk intake of free-living young takes place during a daily period shorter than 5 min (Broekhuizen and Maaskamp, 1980; Hackländer et al., 2002a; Hackländer et al., 2002b) and can amount to up to 200 g per mother. Conceivably then, there might be an upper limit to milk intake due to the capacity of the gastrointestinal tract of juveniles. However, this was certainly not the limiting factor in the present experiments in which litter size was kept constant at three leverets because it has been shown that juveniles in smaller litters, when competition among leverets is lower, have a significantly (>50%) higher milk intake (Hackländer et al., 2002a).

As a result of constant milk volume but declining milk energy content, the energy intake of litters obtained from milk significantly decreased in summer and autumn. In our experimental setting (with high caloric food available *ad libitum*), this did not result in an actual impairment of the growth of young during lactation. Juveniles in autumn clearly compensated for lower milk quality by increasing solid food intake in the last phase of lactation (week 3–4). In free-living leverets, this compensatory increase in energy intake would arguably be limited by the quality or quantity of natural food sources, as well as increased predation risk. Therefore, in natural populations, litters born late in the season do in fact experience disadvantages due to impaired milk energy uptake. *Vice versa*, these observations support previous views that leverets born early in the year have a higher reproductive value (Marboutin et al., 2003). However, while this conclusion was based on the time of birth only, which might allow early-born young to start reproducing already in the same year, our present results show that these early litters also benefit from the higher energy reserves of females and increased total maternal investments. Together, these data point to important ecological consequences of the accumulation of body energy reserves in females before the breeding season. Compared with the wild, where stomach contents of free-ranging hares have been reported to contain 11 kJ g⁻¹ (Hackländer et al., 2002b), our lab diet with an energy content of 19 kJ g⁻¹ certainly enhanced the capability of hares to deposit fat stores. However, our females were incapable of building up considerable fat reserves during periods of pregnancy but instead used fat reserves accumulated during the preceding late-autumn period. Previous studies have focused largely on the digestibility and energy content of natural diets of hares during the breeding season alone (Hackländer et al., 2002b; Smith et al., 2004; Reichlin et al., 2006) but paid little attention to previously accumulated fat reserves. Future programmes for conservation measures in this species, which has been severely declining across Europe over the past few decades (Mitchell-Jones et al., 1999), should therefore focus more on environmental conditions during the late autumn/early winter fattening phase of European hares.

Conclusions

Our study demonstrates that SusMR during peak lactation varies within the breeding season according to the availability of body fat reserves (c.f. Speakman and Krol, 2005). Also, our data indicate that SusMR in lactating hares, when energy reserves are high, is downregulated below physiologically possible levels, which points to a trade-off between the cost and benefits of maximum energy turnover. We argue that this view of limitations on energy throughput

Table 1. Energy budgets of lactating female European hares (*Lepus europaeus*)

	Spring	Summer	Autumn
MEI ¹	918.1±22.7	884.8±15.4	961.9±25.5
Milk energy total output ²	356.8±14.7	266.0±8.2	272.6±13.0
Milk energy from reserves ³	88.9	0	0
Milk energy from food ⁴	267.9±14.7	266.0±8.2	272.6±13.0
Maintenance costs ⁵	650.2±16.8	618.8±13.2	689.2±16.1

Data are given as means ± s.e.m. (kJ kg⁻¹ day⁻¹). Metabolizable energy intake is allocated to either milk or maintenance costs. In spring, milk energy content was augmented by transfer of body energy reserves.

¹Metabolizable energy intake. ²Milk energy content × milk mass. ³Difference between milk energy content in spring and later in the year. ⁴Milk energy total output – milk energy from reserves. ⁵MEI – milk energy from food.

during lactation as a variable of reproductive life-history tactics has received too little attention in the past. Research on SusMR over the past few decades has largely focused on various physiological constraints that might explain peak energy throughput (Weiner, 1992; Hammond and Diamond, 1997; Bacigalupe and Bozinovic, 2002; Speakman and Krol, 2005; Speakman, 2008). This focus was probably due to a bias towards models utilising very small mammals in which limitation by physiological capacities seems more likely and has, in fact, been convincingly demonstrated (e.g. Krol et al., 2007). However, it seems that more studies, especially on medium-sized and larger mammals that are able to build-up fat reserves during gestation or the non-reproductive period, are needed to see how frequently peak energy turnover during lactation is dominated by these physiological ceilings, rather than by an active restriction of reproductive investments.

LIST OF ABBREVIATIONS

ADF	acid detergent fibre
FID	flame ionization detector
FT-NIR	Fourier-transformed near infrared
GEI	gross energy intake
MEI	metabolizable energy intake
NFE	nitrogen free extracts
NIRS	near-infrared spectroscopy
RMR	resting metabolic rate
SusMEI	sustained metabolizable energy intake
SusMR	sustained metabolic rate

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