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Effect of photoperiod on body mass, food intake and body composition in the field vole, *Microtus agrestis*

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Summary

Many small mammals respond to seasonal changes in photoperiod by altering body mass and adiposity. These animals may provide valuable models for understanding the regulation of energy balance. Here, we present data on the field vole (*Microtus agrestis*) – a previously uncharacterised example of photoperiod-induced changes in body mass. We examined the effect of increased day length on body mass, food intake, apparent digestive efficiency, body composition, *de novo* lipogenesis and fatty acid composition of adipose tissue in cold-acclimated (8°C) male field voles by transferring them from a short (SD, 8 h:16 h L:D) to long day photoperiod (LD, 16 h:8 h L:D). During the first 4 weeks of exposure to LD, voles underwent a substantial increase in body mass, after which the average difference between body masses of LD and SD voles stabilized at 7.5 g. This 24.8% increase in body mass reflected significant increases in absolute amounts of all body components, including dry fat mass, dry lean mass and body water mass. After correcting body composition and organ morphology data for the differences in body mass, only gonads (testes and seminal vesicles) were enlarged due to photoperiod treatment. To meet energetic demands of deposition and maintenance of extra tissue, voles adjusted their food intake to an increasing body mass and improved their apparent digestive efficiency. Consequently, although mass-corrected food intake did not differ between the photoperiod groups, the LD voles undergoing body mass increase assimilated on average 8.4 kJ day^{-1} more than animals maintained in SD. The majority (73–77%) of the fat accumulated as adipose tissue had dietary origin. The rate of *de novo* lipogenesis and fatty acid composition of adipose tissue were not affected by photoperiod. The most important characteristics of the photoperiodic regulation of energy balance in the field vole are the clear delineation between phases where animals regulate body mass at two different levels and the rate at which animals are able to switch between different levels of energy homeostasis. Our data indicate that the field vole may provide an attractive novel animal model for investigation of the regulation of body mass and energy homeostasis at both organism and molecular levels.

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Key words: photoperiod, energy balance, digestive efficiency, organ morphology, *de novo* lipogenesis, fatty acid composition, Siberian hamster, Syrian hamster, collared lemming, field vole, *Microtus agrestis.*

Introduction

Many small mammals inhabiting temperate and arctic regions exhibit annual cycles of reproduction that are accompanied by morphological, physiological and behavioural changes. These include seasonal variations in body mass, adiposity, pelage condition, thermogenesis, immunity and locomotor activity (e.g. Heldmaier and Steinlechner, 1981; Stebbins, 1984; Bartness and Wade, 1985; Klingenspor et al., 1996; Drazen et al., 2002; Bartness et al., 2002). Since many of these changes require time to develop, they must be initiated in anticipation of the forthcoming season. Some rodents (e.g. ground squirrels *Spermophilus* sp., dormice *Glis* sp. and marmots *Marmota* sp.) use an endogenous 'clock' of unknown aetiology to trigger these changes, while others (e.g. hamsters *Phodopus* sp. and *Mesocricetus* sp., voles *Microtus* sp. and lemmings *Dicrostonyx* sp.) rely on environmental cues, such as increasing or decreasing day lengths (Dark et al., 1983; Mrosovsky, 1983; Bartness et al., 2002). This day length is transduced into a biochemical signal through the nightly secretion of melatonin *via* the pineal gland (e.g. Heldmaier and Steinlechner, 1981; Bartness and Wade, 1984; Bartness et al., 2002; Morgan et al., 2003a).

The responses to changing photoperiod can be readily induced in the laboratory by simulating the natural progression of day lengths or by acutely transferring animals between summer (long) and winter (short) day lengths. This makes hamsters, voles and lemmings attractive study species for investigations in which biological rhythms may provide valuable insights into mechanisms underlying regulation of body mass (Mercer and Speakman, 2001; Bartness et al., 2002), eating disorders (Morgan et al., 2003b) or infertility (Bavister, 2002).

Apart from reproductive activity, annual cycles of body mass and adiposity are the most profound features of seasonality in mammals. Although these robust rhythms occur in a variety of mammalian species, they have been studied most extensively in the Siberian or Djungarian hamster (*Phodopus sungorus*), Syrian or golden hamster (*Mesocricetus auratus*) and collared lemming (*Dicrostonyx groenlandicus*). In the Siberian hamster both sexes defend a maximal body mass (associated also with a significant adipose tissue store) in summer and reduce their body masses in winter (Steinlechner and Heldmaier, 1982; Bartness and Goldman, 1988). The transfer of adult male hamsters (housed at room temperature) from long day photoperiod (LD, 16 h:8 h L:D) to short day photoperiod (SD, 8 h:16 h L:D) results in a gradual weight loss accompanied by reduced food intake. After 12–18 weeks, hamsters lose approximately 30–40% of their initial body mass, with more than half of this being attributed to mobilization of fat reserves (Ebling, 1994; Klingenspor et al., 2000; Mercer et al., 2000, 2001). Decreases in body fat reserves are a result of a reduction in adipocyte size, and affect intraperitoneal white adipose tissue more than subcutaneous fat depots (Bartness, 1996). It has been suggested that the seasonal changes in body mass and adiposity in Siberian hamsters are mediated by defence of a sliding set-point (e.g. Bartness et al., 1989; Mercer and Speakman, 2001; Morgan et al., 2003a). Similar seasonal changes (i.e. SD-induced decreases in body mass and adiposity) have also been demonstrated in the European hamster (*Cricetus cricetus*; Canguilhem et al., 1988), montane vole (*Microtus montanus*; Petterborg, 1978), meadow vole (*Microtus pennsylvanicus*; Dark and Zucker, 1984), tundra vole (*Microtus oeconomus*; Wang and Wang, 1996) and bank vole (*Clethrionomys glareolus*; Peacock et al., 2004).

By contrast, Syrian hamsters and collared lemmings have seasonal cycles in body mass with maxima in the winter. In Syrian hamsters, the body weight gain induced by exposure to SD reflects an increase in lipid stores and is more pronounced in females than males (Bartness and Wade, 1985). In response to SD photoperiod (10 h:14 h L:D), adult female hamsters housed at room temperature increase body mass by approximately 50–60% over an 8 week period, with no concomitant increase in food intake (Campbell and Tabor, 1983). In collared lemmings, males maintained at 18°C and weaned into SD (8 h:16 h L:D) grow to an adult body mass of 75 g, which represents an 88% increase above the control level (40 g) achieved by males weaned into LD (22 h:2 h L:D) (Reynolds and Lavigne, 1989; Hunter and Nagy, 2002). The corresponding values for females are 60 g in SD and 35 g in LD. The increase in mass associated with exposure to SD at weaning is the result of an increase in the absolute quantity of dry fat mass, dry lean mass and body water mass (Nagy and Negus, 1993). After achieving adult mass, smaller LD male lemmings exposed to SD conditions are able to increase body mass by a further 65% (from 40 g to 66 g) over a 11 week period, also with no increase in food intake (Nagy, 1993). In contrast to immature lemmings, the body mass gain of adult animals results predominantly from an increase in mass of lean tissue and body water (Nagy et al., 1994).

Here we present data on the field vole (*Microtus agrestis*) – a novel model to investigate the regulation of body mass and energy homeostasis at both organism and molecular levels. We examined the effect of increased day length on body mass, food intake, apparent digestive efficiency and body composition in cold-acclimated male voles by transferring them from short to long day photoperiod. We also estimated the proportion of fatty acids synthesized *de novo* in adipose tissue of SD and LD voles to evaluate the contribution of dietary fat to the accumulation of adipose tissue triggered by the change in photoperiod. If the changes in *de novo* lipogenesis induced by photoperiod are substantial, they may affect the fatty acid composition of adipose tissue. Thus, another aim of this study was to compare fatty acid profile of fat depots in SD and LD voles.

Materials and methods

Animals and experimental protocol

Field voles (*Microtus agrestis* L.) were obtained from a captive bred population started from approximately 50 individuals captured near Aberdeen (57°N) in 2000. The breeding colony was maintained on a 16 h:8 h L:D photoperiod (lights on 04:40 h) at an ambient temperature of 21°C (range 20 to 22°C). All voles were housed in plastic cages (23×33×18 cm) provided with sawdust and *ad libitum* water and food (CRM, Pelleted Rat and Mouse Breeder and Grower Diet, Special Diets Services, BP Nutrition, UK). The nutrient content of the diet was by weight 18.8% crude protein, 60.3% carbohydrates, 3.4% crude oil, 3.7% crude fibre and 3.8% ash (all values calculated to nominal 10% water content). Experiments were carried out in two temperature-controlled walk-in environmental chambers with similar ambient temperature and relative humidity, but different photoperiod regime (details below). All illumination was provided by fluorescent lights, and the chambers were not entered by research or husbandry staff during the dark phase.

The voles used in the experiment (83 males) were second and third generation born in captivity between 10 February and 20 April 2001. They were weaned at 3 weeks of age and kept in groups of 3–5 animals per cage. When the voles were 6–10 weeks old, they were transferred to a short day photoperiod (SD, 8 h:16 h L:D, lights on 08:40 h) at an ambient temperature of 8°C (range 7 to 9°C). We chose 8°C, since a preliminary study demonstrated that the response of

voles to photoperiodic manipulation was more pronounced at this temperature than at 21°C. The voles were allowed to acclimate to these conditions for a total of 14 weeks (10 weeks of group housing and 4 weeks of individual housing). Bedding was provided for the first 2 weeks of the acclimation period and then removed.

Following the acclimation period, all animals were kept in SD for a further 24 days to obtain baseline measurements of body mass and food intake, after which 45 voles were exposed to a long day photoperiod (LD, 16 h:8 h L:D, lights on 04:40 h) at 8°C (range 7 to 9°C), whereas 38 control voles remained in original SD conditions. Both groups were matched for body mass and food intake, and monitored for up to 70 days following the exposure. To exclude the effect of particular cold room on body mass and food intake, we swapped the LD and SD voles between rooms on three occasions (days 7, 28 and 49 of exposure to LD) and adjusted photoperiod in the rooms accordingly.

Body mass was recorded twice per week, and food intake was measured over 3 days in each week. Some of the food intake data were also used to assess apparent digestive efficiency (N=23 voles). Following exposure to LD, we randomly selected 4–6 LD and 2–4 SD voles each week and dissected them for organ morphology. The same dissection protocol was applied to nine SD voles (three animals per week) that were sacrificed prior to exposure to LD. Fat depots of six LD and three SD voles were also used to determine fatty acid composition of adipose tissue. Consequently, each of the 83 voles used in the experiment had 2–28 measurements of body mass and 1–14 measurements of food intake (depending on when the animal was dissected), 0–1 measurements of apparent digestive efficiency and 0–1 determinations of fatty acid composition of adipose tissue. All voles were dissected to evaluate organ morphology. We investigated the effect of photoperiod on the proportion of fatty acids synthesized *de novo* in adipose tissue on a separate group of male voles (N=39) maintained under similar conditions.

Body mass, food intake and apparent digestive efficiency

Measurements of body mass (±0.01 g, Sartorius top-pan balance; Epsom, Surrey, UK) were conducted between days –24 and 70 (where day 0 was the day of exposure to LD). Food intake was measured ±0.01 g, Sartorius top-pan balance) between days –14 and 70. At the same time each week, voles were weighed, placed in cages with fresh sawdust and provided with a weighed portion of dry food. Three days later the animals were re-weighed and any uneaten food was collected. Sorting through the sawdust of 23 cages (used in the digestive efficiency measurements) revealed that spillage of food from the hoppers was negligible (0.8±0.4% of the food removed from the hoppers over 3 days). The collected food was subsequently dried and weighed to calculate dry food intake. Food was dried in a convection oven at 60°C for 10 days, before it was given to the animals and after it was collected from the cages to correct for changes in food water content related to different levels of temperature and relative humidity in the food storage (21°C, 97%) and animal rooms (8°C, 42%). During 4 day intervals between the 3 day food intake measurements, all voles were fed with non-dried food.

Apparent digestive efficiency was measured in voles exposed to LD for 11–21 days (N=8) and 53–63 days (N=8). Simultaneous measurements of digestibility were also conducted on SD controls (N=7). We collected the faeces produced by these animals during the 3 day feeding measurements and dried them at 60°C to a constant mass. The gross energy content of food and faeces were measured by bomb calorimetry (Gallenkamp Autobomb Adiabatic Bomb Calorimeter; Loughborough, Leicestershire, UK). The apparent digestive efficiency was calculated as the percentage of gross energy intake that was digested. We used the food intake and digestibility data to calculate metabolizable energy intake, assuming that urinary energy loss was 3% of the digestible energy intake (Drożdż, 1975).

Body composition and organ morphology

Body composition (dry lean mass, dry fat mass, body fat and water content) was derived from organ morphology data. Voles were weighed and killed by CO_2 overdose in the middle of the light phase. Following cardiac puncture to collect blood, voles were immediately dissected. We removed the brain, interscapular brown adipose tissue, white adipose tissue (anterior subcutaneous, posterior subcutaneous, epididymal, perirenal, retroperitoneal and mesenteric fat depots), thyroid, heart, lungs, gut, liver, pancreas, spleen, kidney and gonads (testes and seminal vesicles). To ensure enough tissue for further analyses, anterior and posterior subcutaneous fat depots were pooled and are henceforth called subcutaneous fat. The gut was cut at the pyloric and cardiac sphincters, the ileocaecal junction and the anus. The excised stomach, caecum, and small and large intestines, were cut open longitudinally to remove any residual gut contents and mucous. The remaining body parts were divided into the pelage and the carcass including skeletal muscle and bone. All organs, apart from the brains, which were used for other analyses, were weighed (±0.0001 g, Ohaus Analytical Plus; Beaumont Leys, Leicester, UK) to determine wet mass, then dried in a convection oven at 60°C for 14 days and re-weighed to determine dry mass. We took sub-samples of subcutaneous fat depots (to determine fatty acid composition of adipose tissue) from nine voles, immediately after recording the wet mass of the organs, and weighed the remaining tissue. For these voles, dry mass of subcutaneous fat depots was estimated from the wet mass of the tissue (before the sub-samples were taken) and water content of the tissue that remained after sub-sampling.

De novo *lipogenesis*

We estimated *de novo* lipogenesis from the incorporation of deuterium into fatty acids of subcutaneous and epididymal fat depots in voles over days 10–24 (N=14) and 52–66 (N=13) of the exposure to LD. Simultaneous measurements were also conducted on SD voles (N=12). Animals were injected intraperitoneally with approximately 0.2 g of deuterated water

(^2H_2O, 9.4 atom%) and then maintained on drinking water enriched with deuterium (0.06 atom%) for 14 days. This protocol was designed to maintain stable ^2H enrichment in the body water at approximately 0.05% throughout the study. Blood samples were taken from the voles twice (on days 7 and 14 following the injection) to determine ^2H enrichment in the body water. Blood samples (50–70 µl) were flame sealed into pre-calibrated Vitrex pipettes (Modulohm A/S, Herlev, Denmark) immediately after collection and stored at 4°C until analysis was performed. After taking the second blood sample (day 14), voles were sacrificed and subcutaneous and epididymal fat depots removed. We also blood sampled and sacrificed nine unlabelled voles (three for each photoperiod group) to determine background levels of ^2H in the body water and adipose tissue.

All fat samples were weighed (±0.0001 g, Ohaus Analytical Plus), then dried in a convection oven at 60°C for 14 days (to evaporate inter- and intra-cellular body water containing enriched ^2H) and re-weighed to determine the mass of water lost. We replaced the evaporated body water (by soaking the dry tissue at room temperature for 48 h) in an excess of distilled water (15 times the mass of water lost). This protocol was developed in our laboratory to allow deuterium bound to labile hydrogen sites in the tissue (hydroxyl and amino groups) to re-exchange with distilled water. Consequently, the only ^2H left in the tissue was that irreversibly incorporated into lipids (covalent C–H bond) during *de novo* synthesis (Culebras and Moore, 1977; Haggarty et al., 1991). After the process of isotope equilibration, the fat samples were dried at 60°C for a further 14 days and stored at this temperature (to minimise the contamination by atmospheric hydrogen) until total lipid and fatty acid extractions were performed.

Total lipids were extracted using a modification of the method of Bligh and Dyer (1959). Dry samples of fat tissue (100-200 mg) were macerated and dissolved in 24 ml chloroform/methanol (2:1). After mixing the dissolved fat samples with water (6 ml), the aqueous and chloroform/methanol phases were separated by centrifugation (1500 ***g***, 10 min). The aqueous layer was then discarded and the lipid layer was filtered through a Whatman 1PS filter paper. The solvents were evaporated at 40°C under vacuum. The resulting total lipids (50 mg) were saponified with 1 ml 0.5 mol l^{-1} KOH in 95% ethanol for 90 min at 100°C. After adding diethyl ether (9 ml) and water (3 ml), the aqueous phase (containing saponifiable material) and ether phase (containing non-saponifiable material) were separated by centrifugation (1500 ***g***, 5 min). The aqueous layer was then acidified with 5 mol l^{-1} H_2SO_4 and free fatty acids extracted with 9 ml hexane/diethyl ether (19:1). The samples were stored at 60°C until further analysis was performed.

Blood and fatty acid samples were used for isotope-ratio mass spectrometric analysis of ^2H. Full details of the analysis of the water distilled from blood samples are given in Speakman and Król (2005). Each lipid sample was sub-sampled 5–7 times. These sub-samples (1.0–1.3 mg) were put in pressed silver capsules (4.0×3.2 mm, Elemental Microanalysis Limited, Okehampton, UK) and immediately sealed. The capsules were then placed on the carousel of a EuroEA40/2-IRMS solid autosampler (EuroVector, Milano, Italy), attached to a standard EA 3000 elemental analyser (EuroVector, Milano, Italy). The samples were flushed with helium and pyrolytically decomposed in a high-temperature pyrolysis system (1300°C), in the presence of reactive carbon (Gehre and Strauch, 2003). The resulting hydrogen gas was carried in the helium stream through a GC column to an open split sampling capillary and into the source of an isotope ratio mass spectrometer (IRMS). We measured the ^2H:^1H ratios with a single-inlet IRMS (IsoPrime, Micromass UK Ltd) containing an electrostatic energy filter that separates the helium tail from the hydrogen peak.

We used isotopically characterized hydrogen gas (CP grade gases BOC Ltd) in the reference channel of the IRMS. The reference gas was characterized every three months relative to SMOW and SLAP (Craig, 1961) supplied by the IAEA. Each batch of blood and lipid samples was run adjacent to triplicates of three laboratory standards to correct for day-to-day differences in mass spectrometer performance. All isotope enrichments were measured in δ per mil relative to the working standards and converted to p.p.m., using established ratios for these reference materials. The measures of isotope enrichment in the samples were based on analysis of 5 (blood) or 5–7 (lipids) sub-samples; all subsequent calculations were performed on the mean values.

The proportion of fatty acids synthesized *de novo* in adipose tissue (%) was calculated as $(E_{FA} - bgE_{FA}) / ((E_{BW} - bgE_{BW}) \times 0.53)$, where E_{FA} and E_{BW} are ^2H enrichments (p.p.m.) of fatty acids and body water in the labelled vole, and bgE_{FA} and bgE_{BW} are background ^2H enrichments (p.p.m.) of fatty acids and body water in unlabelled animals. The constant 0.53 is the factor accounting for the partial incorporation (18 H) of hydrogen atoms of average fatty acids (34 H) from body water (Jungas, 1968; Guo et al., 2000; Haggarty et al., 2000). Our estimate of *de novo* lipogenesis includes all fatty acids synthesized from carbohydrates that were accumulated in adipose tissue over 14 days.

Fatty acid composition

We determined fatty acid composition of subcutaneous fat depots in SD voles (N=3) and LD voles exposed to long photoperiod for 17 (N=3) and 59 days (N=3). Within 15 min after sacrificing the animals, the tissue samples were frozen in liquid nitrogen and capped under a stream of N_2 to prevent oxidation of double bonds. The tissues were then stored at –20°C until lipid extraction was performed. Total lipids were extracted from adipose tissue as described above for the measurements of ^2H incorporation. Fatty acid methyl esters (FAMEs) were prepared by reacting total lipids (10–20 mg) with dry methanol (0.5 ml) containing 2 mol l^{-1} HCL for 2 h at 100°C, and then dissolving the lipids in 1 ml hexane/diethyl ether (19:1). After mixing the dissolved FAMEs with water (0.5 ml), the aqueous and hexane/diethyl ether phases were

separated by centrifugation (1500 ***g***, 10 min) and the lipid layer dried by passing through anhydrous Na_2SO_4. The solvents were evaporated at 40°C under a stream of N_2 and the residue FAMEs were taken up in hexane containing 0.02% butylated hydroxytoluene (BHT).

FAMEs were analysed by a HP5890 gas chromatograph (Hewlett Packard, Sunnydale, CA, USA) using a DB-23 column, 30 m×0.25 mm, with a 0.25 μm film thickness (J & W Scientific, Folsum, CA, USA). Samples (1 μl) were injected into a split injection system (1:16) and carried through the GC column in a helium stream. The GC temperature program was 160°C for 1 min, increasing by 10°C min^{-1} to 180°C (held for 3 min) and then increasing by 2°C min^{-1} to 220°C (held for 15 min). A total of 37 fatty acids were identified on the basis of retention times of their methyl esters as compared to fatty acid methyl ester standards (Supelco™ 37 Component FAME Mix, Sigma-Aldrich, St Louis, MO, USA). The peaks were analysed using a HP3396A integrator (Hewlett Packard, Sunnydale, CA, USA).

Each fatty acid was expressed as a percent of all 37 fatty acids identified in the sample. Fatty acids below 1% of the total were excluded from further analysis. These included C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C15:1, C17:0, C17:1, C18:4, C19:0, C20:0, C20:1, C20:2, C21:0, C20:3, C20:4, C20:5, C22:0, C22:1, C22:2, C22:3, C22:4, C23:0, C24:0, C22:5, C22:6 and C24:1.

Statistics

Data are reported as mean ± S.D. (N = number of animals unless stated otherwise). The significance of temporal changes in body mass and food intake in LD and SD voles was assessed by one-way repeated measures analysis of variance (ANOVA). The Tukey *post hoc* test was used when differentiation between days was required. The effect of photoperiod on body mass and food intake was evaluated by two-way repeated measures ANOVA, with group (LD *versus* SD) and day of exposure as factors. We used one-way ANOVA followed by Tukey pairwise comparisons to test for differences in gross energy content of faeces, apparent digestive efficiency, body composition, organ morphology, ^2H enrichment of body water, ^2H enrichment of adipose tissue, *de novo* lipogenesis and fatty acid composition between the groups. Differences between the groups in food intake, metabolizable energy intake and dry masses of fat and gonads were also tested by analysis of covariance (ANCOVA), with body mass as a covariate. The group means were adjusted to a common body mass using the least mean squares method. For measurements conducted on the same animals (^2H enrichment of body water on days 7 and 14, ^2H enrichment of subcutaneous and epididymal fat depots, *de novo* lipogenesis in subcutaneous and epididymal fat depots) we used paired t-tests. Relationships between these data were also described using Pearson product-moment correlation coefficients. All data were tested for normality and homogeneity of variance and were transformed and reanalysed if these assumptions were not met. Arcsine-square-root transformations were performed prior to analysis for percentage data (apparent digestive efficiency, body water and fat contents, *de novo* lipogenesis and fatty acid composition). All statistical analyses were conducted using Minitab for Windows (version 13.31, Minitab Inc.) (Ryan et al., 1985), apart from two-way repeated measures ANOVAs (SigmaStat for Windows, version 3.00, SPSS Inc.). Statistical significance was determined at $P<0.05$, unless stated otherwise. All tests were two-tailed. Exact test values have been omitted in the text for simplification and clarity of the presented results.

Results

Body mass

Mean body mass and food intake of all LD and SD voles are presented in supplementary material. Since a sub-sample of voles was sacrificed each week, the number of voles measured decreased from 45 LD and 38 SD animals on day –24 to 5 LD and 4 SD voles on day 70, making statistical interpretation of the data difficult. Therefore, to elucidate the effect of photoperiod on body mass and food intake, we restricted our analyses to 12 LD and 11 SD animals, which were measured from day –24 to day 56 of exposure. We will first present temporal changes in body mass/food intake for the LD and SD voles separately, and then analyse the changes in body mass/food intake of the LD voles relative to SD controls.

Body mass of the 12 voles exposed to LD increased

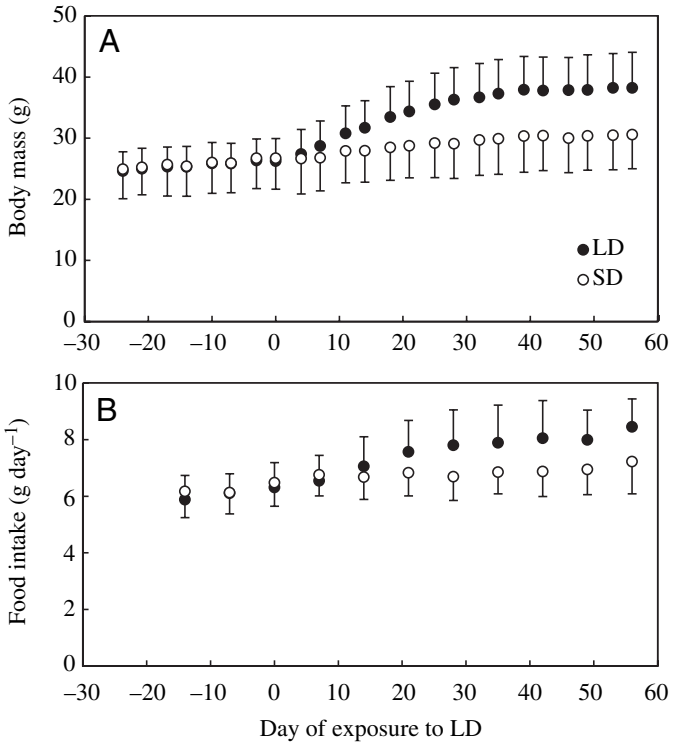

Fig. 1. Effect of exposure to long day photoperiod (LD, 16 h:8 h L:D) on mean body mass (A) and food intake (B) of 12 male field voles measured between days –24 and 56 (day 0 is the day of exposure to LD). Data for 11 voles kept in short day photoperiod (SD, 8 h:16 h L:D) are also shown. Error bars indicate 1 S.D.

significantly throughout the experiment ($P<0.001$, Fig. 1A). Prior to LD exposure (days –24 to 0), vole body mass remained stable and averaged 25.6±3.3 g. Following LD exposure, body mass increased from a mean of 26.2±3.7 g on day 0 to 36.3±5.2 g on day 28 (all comparisons between days 11 to 28 and days –24 to 0, $P<0.05$). Over the next 4 weeks, body mass remained stable and averaged 37.7±5.4 g. Hence, the body mass trajectory of the voles eventually exposed to LD could be characterised by a stable pre-exposure phase (days –24 to 0), followed by an increase phase (days 1 to 28) and a subsequent plateau phase (days 29 to 56). The analysis of body mass changes repeated on five of the 12 LD voles that were monitored to day 70 confirmed the existence of these three distinct phases and indicated no changes in body mass between days 56 and 70. Therefore, the final plateau phase can be extended to day 70. In the 11 SD voles, body mass averaged 24.9±4.8 g at the beginning of the experiment (day –24) and 30.6±5.6 g on day 56 (Fig. 1A). The increase in body mass of SD voles was gradual but significant ($P<0.001$).

Since both LD and SD voles increased their body mass over time, we evaluated the effect of photoperiod on body mass by comparing group × day interactions (reflecting group differences in the patterns of body mass change) and main group effects (reflecting body mass differences averaged over the analysis interval) prior to LD exposure (days –24 to 0), during the increase phase (days 1 to 28) and during the final plateau phase (days 29 to 56). As expected, LD and SD voles did not differ in their pattern of day-to-day body mass changes or in their mean body mass prior to exposure to LD (interaction group × day, $P=0.97$; group effect, $P=0.88$; day effect, $P<0.001$), since at this stage all voles were in the same conditions. During the first 4 weeks of LD exposure (days 1–28), the LD voles exhibited substantially greater weight increases than SD voles (interaction group × day, $P<0.001$; group effect, $P=0.09$; day effect, $P<0.001$). Between days 29 and 56 of LD exposure, the pattern of body mass changes of LD voles was no longer significantly different from that of SD voles and the significant body mass differences between LD and SD animals stabilized at 7.5±0.3 g (interaction group × day, $P=0.68$; group effect, $P=0.004$; day effect, $P<0.001$).

Food intake

Food intake of the 12 LD voles monitored between days –24 and 56 increased significantly throughout the experiment ($P<0.001$, Fig. 1B). Prior to LD exposure (days –14 to 0), food intake remained constant and averaged 6.1±0.8 g day^{-1}. Following LD exposure, voles increased their daily food intake from a mean of 6.3±0.9 g on day 0 to 7.8±1.2 g on day 28 (all comparisons between days 14 to 28 and days –14 to 0, $P<0.05$). Over the next 4 weeks, food intake stabilized at a level of 8.1±1.2 g day^{-1}. The trajectory of food intake for voles exposed to LD closely resembled the changes in body mass (Fig. 1A,B). For the 11 SD voles, daily food intake averaged 6.2±0.9 g on day –14 and 7.2±1.1 g on day 56 (Fig. 1B). This increase in food intake was gradual but significant ($P<0.001$), and also mirrored the pattern of changes in body mass.

We compared food intake of LD and SD voles using the same method as for body mass. Prior to exposure to LD, the two groups of voles did not differ in day-to-day changes in food intake or mean food intake (interaction group × day, $P=0.54$; group effect, $P=0.61$; day effect, $P=0.009$). During the first 4 weeks of exposure to LD (days 1 to 28), the increases in food intake observed in LD animals were significantly greater than in SD voles (interaction group × day, $P<0.001$; group effect, $P=0.33$; day effect, $P<0.001$). Between days 29 and 56 of LD exposure, the temporal changes in food intake did not differ between the two groups and difference in mean food intake stabilized at 1.1±0.1 g day^{-1} (interaction group × day, $P=0.87$; group effect, $P=0.016$; day effect, $P<0.001$).

To investigate the effect of photoperiod on food intake in relation to changes in body mass, we compared food intake of the 12 LD voles during the pre-exposure, increase and plateau phases, including body mass as a covariate in the analysis. After adjusting for differences in body mass, the effect of LD on food intake was not significant (interaction body mass × photoperiod, $P=0.94$; body mass effect, $P<0.001$; photoperiod effect, $P=0.95$) (Fig. 2A). Consequently, the rate at which voles ingested food during the pre-exposure, increase and plateau phases (adjusted to a common body mass of 32.7 g) averaged 7.2, 7.2 and 7.3 g day^{-1}, respectively. This suggests that LD voles responded to increases in body mass by increasing their food intake, i.e. the increase in food intake following exposure to LD was entirely attributable to the changes in body mass.

Apparent digestive efficiency and metabolizable energy intake

The mean gross energy content of food was 18.16±0.12 kJ g^{-1} dry mass ($N=3$ replicates). The gross energy content of faeces did not differ between SD voles and LD voles during the increase or plateau phases ($P=0.47$) and averaged 17.11±0.41 kJ g^{-1} dry mass ($N=23$). The apparent digestive efficiency averaged 74.2±5.5% ($N=7$) in SD voles, 81.4±5.5% ($N=8$) in voles during the increase phase and 80.3±4.1% ($N=8$) during the plateau phase. These means were significantly different ($P=0.031$), with the digestive efficiency of LD voles during the increase and plateau phases being higher than in SD animals ($P<0.05$).

We calculated metabolizable energy intake (MEI) for the 12 LD voles monitored between days –24 and 56 during the pre-exposure (days –24 to 0), increase (days 1 to 28) and final plateau (days 29 to 56) phases using their food intake data (Fig. 2A) and the mean digestive efficiencies of 74.2, 81.4 and 80.3%, respectively. After adjusting for differences in body mass, the effect of LD on MEI was significant (interaction body mass × photoperiod, $P=0.81$; body mass effect, $P<0.001$; photoperiod effect, $P=0.006$), with MEI during the increase and plateau phases being significantly higher than during the pre-exposure phase ($P<0.05$) (Fig. 2B). The rate at which voles assimilated energy during the pre-exposure, increase and plateau phases (adjusted to a common body mass of 32.7 g) averaged 95.3, 103.7 and 102.9 kJ day^{-1}, respectively.

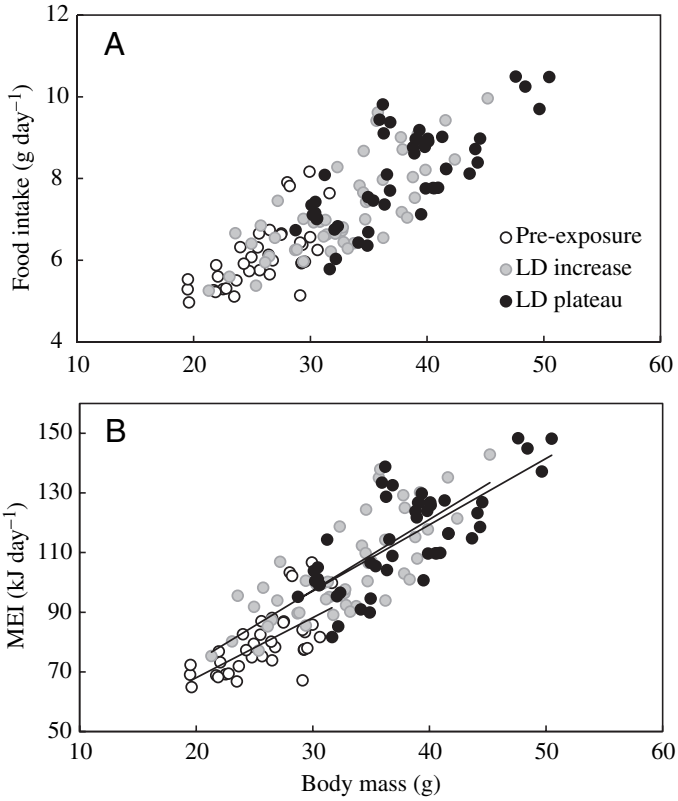

Fig. 2. Food intake (A) and metabolizable energy intake (B) as a function of body mass for 12 male field voles prior to exposure to long day photoperiod (LD, 16 h:8 h L:D), during the increase phase (days 1–28 of LD exposure) and during the plateau phase (days 29–56 of LD exposure). Prior to exposure to LD, the animals were kept in short day photoperiod (SD, 8 h:16 h L:D). The measurements of food intake were repeated three times during the pre-exposure phase and four times during the increase and plateau phases. After adjusting for differences in body mass, photoperiod had no effect on food intake, but it did have a significant effect on MEI (for statistical details, see Results). The relationships are described by y=27.7+2.0x for pre-exposure, y=26.0+2.4x for LD increase and y=30.4+2.2x for LD plateau.

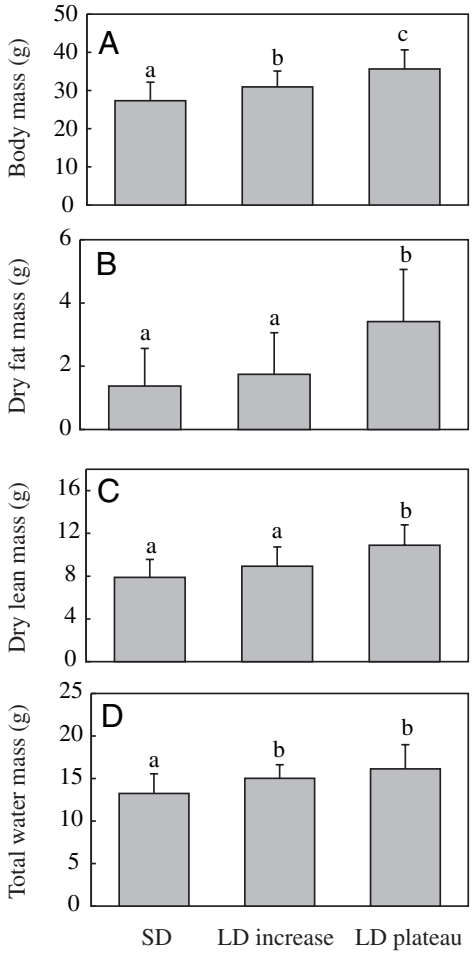

Fig. 3. Body mass (A), dry fat mass (B), dry lean mass (C) and body water mass (D) in male field voles exposed to short day photoperiod (SD, 8 h:16 h L:D, N=38) and long day photoperiod (LD, 16 h:8 h L:D) for 1–28 days (the increase phase, N=20) or 29–70 days (the plateau phase, N=25). Dry fat mass was calculated as the sum of subcutaneous, epididymal, perirenal, retroperitoneal and mesenteric fat depots. Bars are means + 1 S.D. Different letters above bars indicate significant differences between the groups, as assessed by one-way ANOVA followed by Tukey pairwise comparisons.

Body composition and organ morphology

Wet mass, dry mass and water content of organs of all voles sacrificed during the experiment are presented in supplementary material. Comparisons of body mass, body composition and organ morphology in voles exposed to SD, LD for 1–28 days (the increase phase) and LD for 29–70 days (the plateau phase) demonstrated that animals sacrificed during the final plateau phase were significantly heavier than SD controls (Table 1). This increase in body mass was associated with significant increases in absolute amounts of all body components, including dry fat mass, dry lean mass, and body water mass (Fig. 3). On average, voles exposed to LD for 29–70 days had a significantly higher body fat content (12.9%) than SD individuals (7.1%). This was associated with a significant decrease in body water content, from 59.4% in SD voles to 53.3% in LD voles. At the organ level, voles sacrificed during the plateau phase had absolute increases in dry masses of brown adipose tissue, subcutaneous, epididymal, perirenal and retroperitoneal fat depots, thyroid, lungs, caecum, liver, kidney, gonads (testes and seminal vesicles), pelage and carcass. By contrast, the differences in dry masses of mesenteric fat, heart, gut (stomach and small and large intestines), pancreas and spleen were not significant between the LD and SD voles.

Since organ size frequently correlates with body mass (e.g. Selman et al., 2001; Król et al., 2003), we repeated the analyses of body composition and organ morphology using body mass as a covariate. As expected, most of the body components (apart from spleen, pancreas, large intestine and caecum) were positively related to body mass (P<0.001). After adjusting for body mass, the differences in dry fat mass between LD and SD voles were not significant (interaction body mass ×

photoperiod, P=0.29; body mass effect, P<0.001; photoperiod effect, P=0.14) (Fig. 4). The effect of photoperiod on body composition and organ morphology was significant only for gonads (interaction body mass × photoperiod, P=0.65; body mass effect, P<0.001; photoperiod effect, P<0.001), with voles exposed to LD for 29–70 days having bigger gonads than SD controls or voles exposed to LD for 1–28 days (P<0.05) (Fig. 5). The dry mass of gonads adjusted to a mean body mass of all voles (30.7 g) averaged 0.172 g in SD animals, 0.207 g in LD voles sacrificed during the increase phase and 0.330 g in LD voles at the plateau phase.

De novo *lipogenesis*

Background levels of deuterium in body water and fatty acids extracted from subcutaneous and epididymal fat depots did not differ between SD voles and LD voles during the increase or plateau phases (Table 2). After pooling the data for all unlabelled animals (N=9), the ^{2}H content of body water, subcutaneous and epididymal fat depots averaged 144.6±0.4, 124.6±0.9 and 124.6±0.8 p.p.m., respectively. The difference between the two adipose tissues was not significant (P=0.98). However, the background level of ^{2}H in fat depots was 18–21 p.p.m. below that of the body water (P<0.001). This relative ^{2}H depletion in lipid has been reported previously (e.g. Haggarty et al., 2000) and is the result of isotope discrimination against heavy hydrogen during reductive biosynthesis of fatty acids (Jungas, 1968).

Deuterium enrichment of body water measured after 7 days of dosing with the isotope did not differ between SD voles and LD voles during the increase or plateau phases (Table 2).

Table 1. *Body composition and organ morphology in male field voles exposed to short and long day photoperiods*

	Photoperiod			ANOVA	
Trait	SD	LD increase	LD plateau	$F_{2,80}$	P
Body mass (g)	27.3[a]	30.9[b]	35.6[c]	23.0	**<0.001**
Total wet mass (g)[1]	22.512[a]	25.687[b]	30.436[c]	23.6	**<0.001**
Total dry mass (g)[2]	9.261[a]	10.672[a]	14.298[b]	21.0	**<0.001**
Body water mass (g)[3]	13.250[a]	15.014[b]	16.138[b]	13.3	**<0.001**
Body water content (%)[4]	59.4[a]	59.1[a]	53.3[b]	10.1	**<0.001**
Dry fat mass (g)[5]	1.372[a]	1.743[a]	3.414[b]	18.7	**<0.001**
Dry lean mass (g)[6]	7.890[a]	8.929[a]	10.884[b]	21.3	**<0.001**
Body fat content (%)[7]	7.1[a]	8.0[a]	12.9[b]	13.9	**<0.001**
Organ dry mass (g)					
Brown adipose tissue	0.137[a]	0.162[a]	0.296[b]	15.7	**<0.001**
Subcutaneous fat[8]	0.947[a]	1.148[a]	2.152[b]	16.6	**<0.001**
Epididymal fat	0.315[a]	0.449[a]	0.907[b]	20.2	**<0.001**
Perirenal and retroperitoneal fat	0.092[a]	0.122[a]	0.327[b]	16.7	**<0.001**
Mesenteric fat	0.017	0.023	0.027	4.8	0.011
Thyroid	0.125[a]	0.176[b]	0.179[b]	9.8	**<0.001**
Heart	0.042	0.043	0.047	5.1	0.008
Lungs	0.084[a]	0.099[a,b]	0.117[b]	9.9	**<0.001**
Stomach	0.074	0.082	0.082	1.6	0.208
Caecum	0.128[a]	0.168[b]	0.146[a,b]	8.5	**<0.001**
Small intestine	0.175	0.207	0.190	3.8	0.026
Large intestine	0.065	0.067	0.065	0.1	0.909
Liver	0.380[a]	0.446[b]	0.502[b]	16.1	**<0.001**
Pancreas	0.034	0.037	0.042	3.4	0.039
Spleen	0.022	0.031	0.021	5.0	0.009
Kidney	0.080[a]	0.093[b]	0.106[c]	25.1	**<0.001**
Gonads[9]	0.144[a]	0.204[b]	0.374[c]	69.7	**<0.001**
Pelage	2.331[a]	2.571[a]	3.434[b]	13.3	**<0.001**
Carcass	4.068[a]	4.529[a]	5.283[b]	18.8	**<0.001**

Male field voles were exposed to short day photoperiod (SD, 8 h:16 h L:D, N=38) and long day photoperiod (LD, 16 h: 8h L:D) for 1–28 days (the increase phase, N=20) or 29–70 days (the plateau phase, N=25). Values are absolute means.

For traits with significant P values (bold type), different letters indicate significant differences between the groups, as assessed by the Tukey pairwise comparisons.

The P values in bold are significant after Bonferroni corrections (0.05 divided by 27 comparisons).

[1]The sum of wet masses of all dissected organs apart from brain (the discrepancy between total wet mass and body mass is due to mass of brain, blood and gut contents); [2]the sum of dry masses of all dissected organs apart from brain; [3]the difference between total wet and dry masses; [4]body water mass expressed as a percentage of total wet mass; [5]the sum of dry masses of subcutaneous, epididymal, perirenal, retroperitoneal and mesenteric fat depots; [6]the difference between total dry mass and dry fat mass; [7]wet fat mass expressed as a percentage of total wet mass; [8]anterior and posterior subcutaneous fat depots; [9]testes and seminal vesicles.

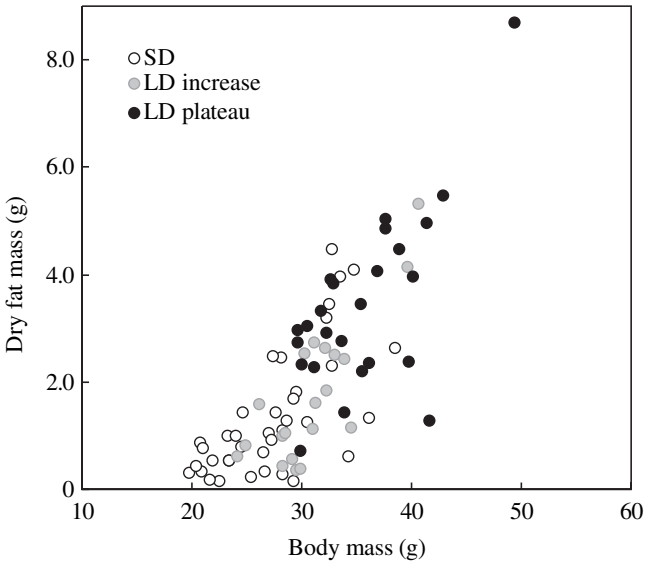

Fig. 4. Dry mass of subcutaneous, epididymal, perirenal, retroperitoneal and mesenteric fat depots as a function of body mass for male field voles exposed to short day photoperiod (SD, 8 h:16 h L:D, *N*=38) and long day photoperiod (LD, 16 h:8 h L:D) for 1–28 days (the increase phase, *N*=20) or 29–70 days (the plateau phase, *N*=25). After adjusting for the differences in body mass, the differences in dry fat mass between the three groups of voles were not significant (for statistical details, see Results).

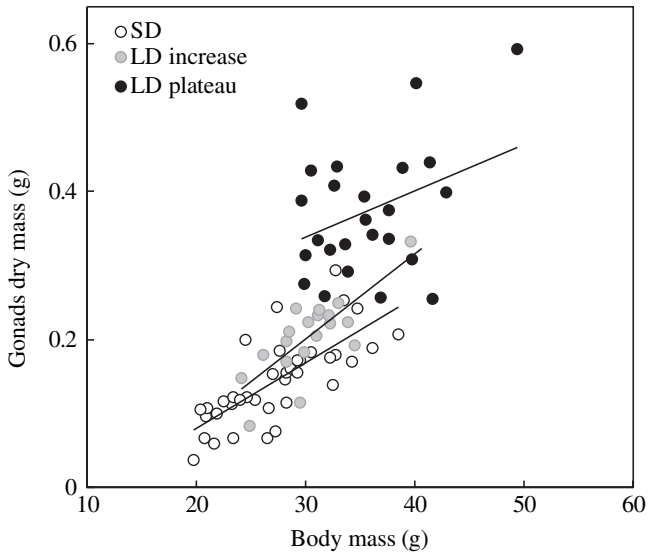

Fig. 5. Dry mass of gonads (testes and seminal vesicles) as a function of body mass for male field voles exposed to short day photoperiod (SD, 8 h:16 h L:D, *N*=38) and long day photoperiod (LD, 16 h:8 h L:D) for 1–28 days (the increase phase, *N*=20) or 29–70 days (the plateau phase, *N*=25). The relationships are described by $y=-0.097+0.009x$ for SD, $y=-0.146+0.012x$ for LD increase and $y=0.152+0.006x$ for LD plateau (for statistical details, see Results).

Similarly, the differences between the three groups in body water ^{2}H enrichment measured at the end of the labelling period (day 14) were not significant either. The deuterium content of body water across all labelled voles (*N*=39) averaged 479.4±21.7 p.p.m. on day 7 and 482.2±20.1 p.p.m. on day 14, with no significant changes between the two days (*P*=0.30). Thus, the stable plateau enrichment of ^{2}H was

Table 2. *Deuterium (^{2}H) enrichment of body water and adipose tissue in male field voles exposed to short and long day photoperiods*

	Photoperiod			ANOVA	
Trait	SD	LD increase	LD plateau	F^1	*P*
^{2}H enrichment (p.p.m.)					
Body water					
Unlabelled voles[2]	144.6±0.5	144.7±0.4	144.4±0.4	0.4	0.68
Labelled voles					
Day 7[3]	477.0±24.4	485.7±23.4	474.8±16.6	1.0	0.40
Day 14[3]	479.3±13.8	487.6±24.9	479.0±19.5	0.8	0.46
Subcutaneous fat[4]					
Unlabelled voles[2]	124.8±1.4	124.3±0.2	124.7±1.1	0.2	0.84
Labelled voles	173.0±15.8	174.1±14.9	164.4±15.2	1.6	0.22
Epididymal fat[4]					
Unlabelled voles[2]	124.7±0.8	125.0±0.7	124.1±0.7	1.1	0.39
Labelled voles	172.9±15.2	174.6±12.8	167.5±16.3	0.8	0.44
De novo lipogenesis (%)					
Subcutaneous fat	27.1±8.5	27.4±7.4	22.7±9.3	1.5	0.24
Epididymal fat	27.1±8.3	27.3±6.6	24.7±9.9	0.5	0.61

Male field voles were dosed with deuterium for 14 days in short day photoperiod (SD, 8 h:16 h L:D, *N*=12), long day photoperiod (LD, 16 h:8 h L:D) over days 10–24 (the increase phase, *N*=14), and long day photoperiod over days 52–66 (the plateau phase, *N*=13). Contribution of *de novo* lipogenesis to fatty acids of adipose tissue is also presented. Values are means ± S.D.

[1]$F_{2,6}$ for unlabelled and $F_{2,36}$ for labelled voles; [2]*N*=3 for each photoperiod group; [3]following the beginning of the dosing period; [4]measured in fatty acids extracted from the tissue.

Table 3. *Fatty acid composition of total lipids extracted from subcutaneous fat depots in male field voles exposed to short and long day photoperiods*

	Percent of all fatty acids			ANOVA	
Fatty acid	SD	LD increase	LD plateau	$F_{2,6}$	P
C16:0	18.3±2.5	18.6±2.6	18.1±2.0	0	0.97
C16:1	3.4±0.4	2.3±1.3	2.7±0.2	1.3	0.34
C18:0	1.2±0.3	1.5±0.1	1.6±0.4	1.6	0.29
C18:1	35.6±3.3	37.0±3.9	36.0±2.3	0.1	0.88
C18:2	29.7±3.4	28.7±3.6	29.7±3.3	0.1	0.92
C18:3	1.3±0.3	1.2±0.3	1.4±0.2	0.9	0.47
SFA[1]	19.5±2.8	20.1±2.6	19.7±2.2	0	0.96
MUFA[2]	39.0±3.6	39.2±5.2	38.7±2.1	0	0.99
PUFA[3]	31.0±3.4	29.9±3.7	31.1±3.3	0.1	0.90
UFA[4]	70.0±3.6	69.1±4.4	69.8±2.4	0.1	0.95

Male field voles were exposed to short day photoperiod (SD, 8 h:16 h L:D, N=3) and long day photoperiod (LD, 16 h:8 h L:D) for 17 days (the increase phase, N=3) or 59 days (the plateau phase, N=3). Only fatty acids that constitute >1% of all identified fatty acids are presented. Values are means ± S.D.

[1]Saturated fatty acids; [2]monounsaturated fatty acids; [3]polyunsaturated fatty acids; [4]unsaturated fatty acids (MUFA+PUFA).

maintained for at least the last seven days of the experiment. The data from both days were highly correlated (r=0.69, P<0.001).

Deuterium incorporation into fatty acids of subcutaneous fat depots measured at the end of the labelling period (day 14) did not differ between SD voles and LD voles during the increase or plateau phases (Table 2). The effect of photoperiod on ^{2}H enrichment of epididymal fat depots was also not significant. When data were pooled for all labelled voles (N=39), the ^{2}H enrichment of subcutaneous and epididymal fat depots averaged 170.5±15.5 p.p.m. and 171.7±14.7 p.p.m., respectively, and the differences between the two tissues were not significant (P=0.30). The ^{2}H enrichments of subcutaneous and epididymal fat depots were highly correlated (r=0.89, P<0.001).

The proportion of synthesized *de novo* fatty acids accumulated in subcutaneous fat depots over 14 days averaged 27.1±8.5% for SD voles (N=12), 27.4±7.4% for LD voles during the increase phase (N=14) and 22.7±9.3% during the plateau phase (N=13). The contribution of *de novo* lipogenesis to fatty acids of epididymal fat depots in SD voles and LD voles during the increase and plateau phases averaged 27.1±8.3%, 27.3±6.6% and 24.7±9.9%, respectively. The fractional rates of *de novo* lipogenesis in subcutaneous and epididymal fat depots were not affected by photoperiod (Table 2). After pooling the data for all voles (N=39), the proportions of fatty acids synthesized from carbohydrates in subcutaneous (25.7±8.5%) and epididymal fat depots (26.4±8.2%) were not significantly different (P=0.30) and highly correlated within individuals (r=0.89, P<0.001).

Fatty acid composition

Comparison of total lipid fatty acid profile between adipose tissue of voles exposed to different photoperiod regime was performed on fatty acids that constituted >1% of all identified fatty acids. These included palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (18:3) fatty acids.

The fatty acid composition of total lipids extracted from subcutaneous fat depots did not differ between SD voles and LD voles during the increase or plateau phases (Table 3). After pooling the data for all voles (N=9), the most abundant fatty acid in subcutaneous fat depots was oleic acid (36.2±2.9%), followed by linoleic (29.3±3.0%), palmitic (18.4±2.1%), palmitoleic (2.8±0.8%), stearic (1.4±0.3%) and linolenic (1.3±0.2%) acids.

Discussion

The field vole is an arvicoline rodent that inhabits the grassland and open forest of the Palaearctic region of Europe (Clarke, 1999). Apart from the field vole, the genus *Microtus* comprises more than 40 other species, with some distributed through North America and Asia. The diet of field voles (monocotyledonous grasses) is usually low in energy content, with peak biomass and nutrient content in summer (Corbet and Harris, 1991; Hjältén et al., 1996). The breeding season, which is synchronized with the abundance of high-quality grass and warmer ambient temperatures, lasts from March till September (Larsson and Hansson, 1977). Because species belonging to *Microtus* do not hibernate or enter torpor, they face high costs of thermoregulation in the winter. Although these costs could potentially be met by an increase in energy intake, this strategy would require prolonged foraging outside the nest, which in turn would be associated with greater thermoregulatory and activity costs as well as a potentially elevated risk of predation (Lima, 1998). Instead, *Microtus* voles have evolved the ability to minimise their winter energy requirements by reducing the absolute amount of energy spent on maintenance, including basal metabolism, thermoregulation and activity costs.

The main strategy employed by adult voles to reduce winter maintenance costs is a 20-40% reduction in body mass (e.g.

Iverson and Turner, 1974; Hansson, 1992; Aars and Ims, 2002). This reduction affects all three major carcass components – lean tissue, fat and body water (Dark et al., 1983). A similar strategy is also adopted by immature voles born late in the season, which delay growth to the adult size until the next spring (e.g. Negus et al., 1986; Boonstra, 1989; Hansson, 1990). Despite the obvious benefits of this strategy (smaller animals need less food and therefore spend more time in a warm and possible safe nest), reduced body mass also has some disadvantages. These include a higher surface-to-volume ratio and consequently a higher relative heat loss, a lower thermogenic capacity to sustain body temperature during cold periods and a lower fasting endurance to survive prolonged periods of negative energy balance (e.g. Millar and Hickling, 1990; Speakman, 1996; Jackson et al., 2001). To counteract these problems, wintering voles build larger nests, increase the insulative properties of their pelage and also rely on energetic benefits of huddling (e.g. Walsberg, 1991; Hayes et al., 1992).

In field vole populations, the mean wintering body mass may vary considerably between animals at different locations and between years within the same location (e.g. Chitty, 1952; Agrell et al., 1992). Individuals in increasing populations are usually larger than those in declining populations (Krebs and Myers, 1974). Interestingly, when voles are moved among locations that differ in mean wintering body mass, the transplanted individuals adjust their body size to the average mass at the site to which they are moved (Ergon et al., 2001). This suggests that the optimal wintering body mass depends on extrinsic factors associated with the immediate environment (e.g. ambient temperature, food quality/availability, predation or parasite load) rather than intrinsic factors such as age distribution, physiological state or genetic and maternal effects (Speakman et al., 2003; Ergon et al., 2004). Although a smaller body mass increases winter survival, a larger wintering body mass may enable an earlier onset of reproduction (Ergon et al., 2001). In response to this trade-off, voles have evolved the ability to switch between winter- and summer-appropriate body masses by undergoing rapid spring growth, which is initiated by increasing day length.

Although the rate at which field voles gained weight in our study (24.8% increase over 4 weeks) was similar to that reported in Syrian hamsters (27.5% increase over 4 weeks as recalculated from Campbell and Tabor, 1983) and collared lemmings (23.6% increase over 4 weeks as recalculated from Nagy, 1993), the mechanisms behind the deposition and maintenance of this extra tissue appear to be different. Both Syrian hamsters and collared lemmings exposed to SD increase body mass without increasing food intake. This could be achieved by increasing digestive efficiency and/or by metabolic compensation, i.e. diverting some energy from maintenance to somatic growth and fat deposition. The fact that neither Syrian hamsters nor collared lemmings increase digestive efficiency in SD (Bartness and Wade, 1985; Nagy and Negus, 1993) strongly suggests that some aspect of their energy expenditure (such as basal metabolism, thermoregulation or activity) must be reduced. Indeed, it has been demonstrated that Syrian hamsters exposed to SD reduce wheel-running activity (and presumably energy spent on locomotion) by approximately 66% (males) and 48% (females) relative to controls maintained in LD (Ellis and Turek, 1979; Widmaier and Campbell, 1980). Furthermore, energy savings generated by winter communal nesting could also contribute to an accelerated mass gain. Syrian hamsters housed in groups for 65–75 days displayed a chronic 13% reduction in mass-specific resting metabolic rate, accompanied by a 20% increase in body mass and a 53% increase in body fatness, relative to individuals housed individually (Borer et al., 1988). Decrease in maintenance metabolism has also been demonstrated in collared lemmings (Powell et al., 2002). In particular, male lemmings exposed to SD for 10 days had significantly lower total energy expenditure, resting energy expenditure and UCP-1 mRNA levels than animals maintained in LD, whereas non-resting energy expenditure (related to activity) was not affected by photoperiod. These data indicate that the increase in body mass that occurs when collared lemmings are exposed to SD may be primarily fuelled by a decrease in resting metabolic rate, related to the differences in the activity of brown adipose tissue.

In contrast to Syrian hamsters and collared lemmings, field voles responded to photoperiod-induced body mass gain by increasing their food intake (Figs 1B, 2A). Over the period of body mass increase (days 1–28 of exposure to long day photoperiod), LD voles ate 16.3 g more food than SD individuals. Over the next 4 weeks (days 29–56), the difference between food intake of LD voles (8.1 g day^{-1}) and SD controls (7.0 g day^{-1}) stabilized at 1.1 g day^{-1}. This strategy of increasing total energy intake instead of reducing energy spent on maintenance may be related to the fact that grass-eating voles have evolved a large and efficient digestive system (McNab, 1986; Koteja and Weiner, 1993), which enables them to assimilate larger amounts of energy when high-quality grass is abundant. Conversely, the ability of collared lemmings to increase food intake may be limited by the capacity of the liver to detoxify their diet, which consists of dicotyledons such as *Salix* shrubs and *Dryas* spp. (Batzli, 1993; Negus and Berger, 1998). Although dicotyledons as a food source are seasonally more stable and more nutritious than grass, they also contain more plant secondary compounds and are, therefore, potentially more toxic. Thus, the ability of field voles to adjust their food intake to an increasing body mass may also depend on the nutritional ecology of this species.

Field voles exposed to LD not only adjusted their food intake to the increasing body mass but also improved their apparent digestive efficiency, from 74.2% in SD conditions to 81.4% during the rapid body mass gain (days 1–28 of exposure to LD) and 80.3% during the final plateau phase (days 29–56 of exposure to LD). Consequently, although mass-adjusted food intake did not differ between the photoperiod groups (Fig. 2A), the LD voles undergoing body mass increase (days

1–28) assimilated on average 8.4 kJ day^{-1} more than animals maintained in SD (Fig. 2B). This surplus of energy was presumably allocated toward somatic growth and deposition of fat.

This raises the question of whether 8.4 kJ day^{-1} covers all the energetic demands associated with the body mass gain, or only part of them. If the latter is the case, some energy that is allocated to growth would have to be withdrawn from maintenance. Because we did not measure the energy costs of deposition (i.e. the sum of the energy accumulated as new tissue and costs of its biosynthesis), the answer to this question depends on which values, estimated for other species, are assumed to apply to voles. Energy costs of deposition vary from 16.5 kJ per 1 g of new tissue assimilated in rats (*Rattus norvegicus*; Farrell and Williams, 1989) to 20.9 kJ g^{-1} in hedgehogs (*Erinaceus concolor*; Król, 1994) and 35.9 kJ g^{-1} in common voles (*Microtus arvalis;* Jagosz et al., 1979). If we assume the costs of deposition in field voles to be 16.5, 20.9 or 35.9 kJ g^{-1}, LD voles that produced 7.5 g of tissue over 28 days would have to allocate 4.4, 5.6 and 9.6 kJ day^{-1} more to growth, respectively, than animals maintained in SD. Thus, depending on the assumption, the difference in metabolizable energy intake between voles exposed to LD for 1–28 days and SD controls (8.4 kJ day^{-1}) could meet 87 to 100% of the energy demands associated with the body mass gain. After the increase in body mass was complete (days 29–56), the mass-adjusted metabolizable energy intake of LD voles did not return to the SD level, but remained on average 7.6 kJ day^{-1} higher (Fig. 2B). This may reflect photoperiod-induced changes in the physiology and behaviour of male voles, related to the need for an intense competition for polygynous territories in the early spring (Ostfeld, 1985). Additional studies on the energy costs of deposition and other components of the energy budget are required to evaluate these possibilities.

During the first 4 weeks of exposure to LD, field voles produced on average 2.0 g of white adipose tissue, which in turn led to an increase in body fatness, from 7.1% in SD voles to 12.9% in voles exposed to LD for 29–70 days (Table 1). The mechanisms enabling mammals to deposit fat include elevated dietary lipid uptake and increased *de novo* lipogenesis, i.e. conversion of dietary carbohydrates to fatty acids. Therefore, if the voles accumulating adipose tissue (days 1–28 of exposure to LD) were limited by the amount of dietary fat available for synthesis of triacylglycerides, we would expect them to increase *de novo* lipogenesis. Otherwise, the rate of fatty acid synthesis should remain unchanged or decrease. The latter would suggest that although voles had enough dietary fat to accumulate adipose tissue, they were limited by the amount of energy available for somatic growth and fat deposition. Conversion of carbohydrates to fat prior to β-oxidation is thermogenically costly and approximately 28% of the energy content of carbohydrates is lost as heat (Flatt, 1978; Hellerstein, 1999).

Over the period when voles accumulated 2.0 g of adipose tissue (days 1–28 of exposure to LD), they digested on average 6.7 g of dietary fat (6.9 g daily food intake × 3.4% crude oil in the diet × 28 days). The actual amount of dietary lipid available for fat deposition depends on partitioning of dietary fat for β-oxidation *versus* storage and remains unknown. However, voles that were accumulating adipose tissue had proportions of *de novo* synthesized fatty acids in subcutaneous and epididymal fat depots (27.1 and 27.1%, respectively) similar to SD voles (27.4 and 27.3%, respectively) and LD voles during the plateau phase (22.7 and 24.7%, respectively) (Table 2). Thus, regardless of the photoperiod-induced differences in fat deposition and the type of adipose tissue, 73–77% of the stored lipids had dietary origin. This suggests that the voles accumulating adipose tissue were not limited by the amount of dietary fat available for synthesis of triacylglycerides.

We also demonstrated that fatty acid composition of subcutaneous fat depots in voles accumulating adipose tissue (days 1–28 of exposure to LD) was not significantly different from that in SD voles or LD voles during the plateau phase (Table 3). These results suggest that there was no gross shift in the source of lipids deposited in stores, which is consistent with the fact that that photoperiod had no effect on *de novo* lipogenesis. The most abundant fatty acids in vole adipose tissue were oleic (36.2%), linoleic (29.3%) and palmitic (18.4%) acids. Similar results have been reported for other mammals, including Norway rats *Rattus norvegicus* (Reidinger et al., 1985), deer mice *Peromyscus maniculatus* (Geiser, 1991), fat-tailed dwarf lemurs *Cheirogaleus medius* (Fietz et al., 2003), echidnas *Tachyglossus aculeatus* (Falkenstein et al., 2001) and polar bears *Ursus maritimus* (Grahl-Nielsen et al., 2003).

In conclusion, field voles may provide an attractive novel animal model for investigation of the regulation of body mass and energy homeostasis at both organism and molecular levels. When exposed to LD, cold-acclimated male field voles showed an increase in body mass, which reflected significant increases in absolute amounts of all body components, including dry fat mass, dry lean mass and body water mass. To meet energetic demands of deposition and maintenance of extra tissue, field voles adjusted their food intake to an increasing body mass and improved their apparent digestive efficiency. On average, 73–77% of fat accumulated as adipose tissue had dietary origin. The rate of *de novo* lipogenesis and fatty acid composition of adipose tissue were not affected by photoperiod. The most important characteristics of this new model are the clear delineation between phases where animals regulate body mass at two different levels and the rate at which animals are able to switch between different levels of energy homeostasis. Field voles maintained in SD regulated their body mass at the lowest seasonal level. After 4 weeks of exposure to LD they regulated body mass at a 24.8% higher level than prior to exposure. By comparison, Siberian hamsters, Syrian hamsters and collared lemmings restore homeostasis not earlier than 8 weeks following photoperiod manipulation (e.g. Campbell and Tabor, 1983; Nagy, 1993; Mercer et al., 2000).

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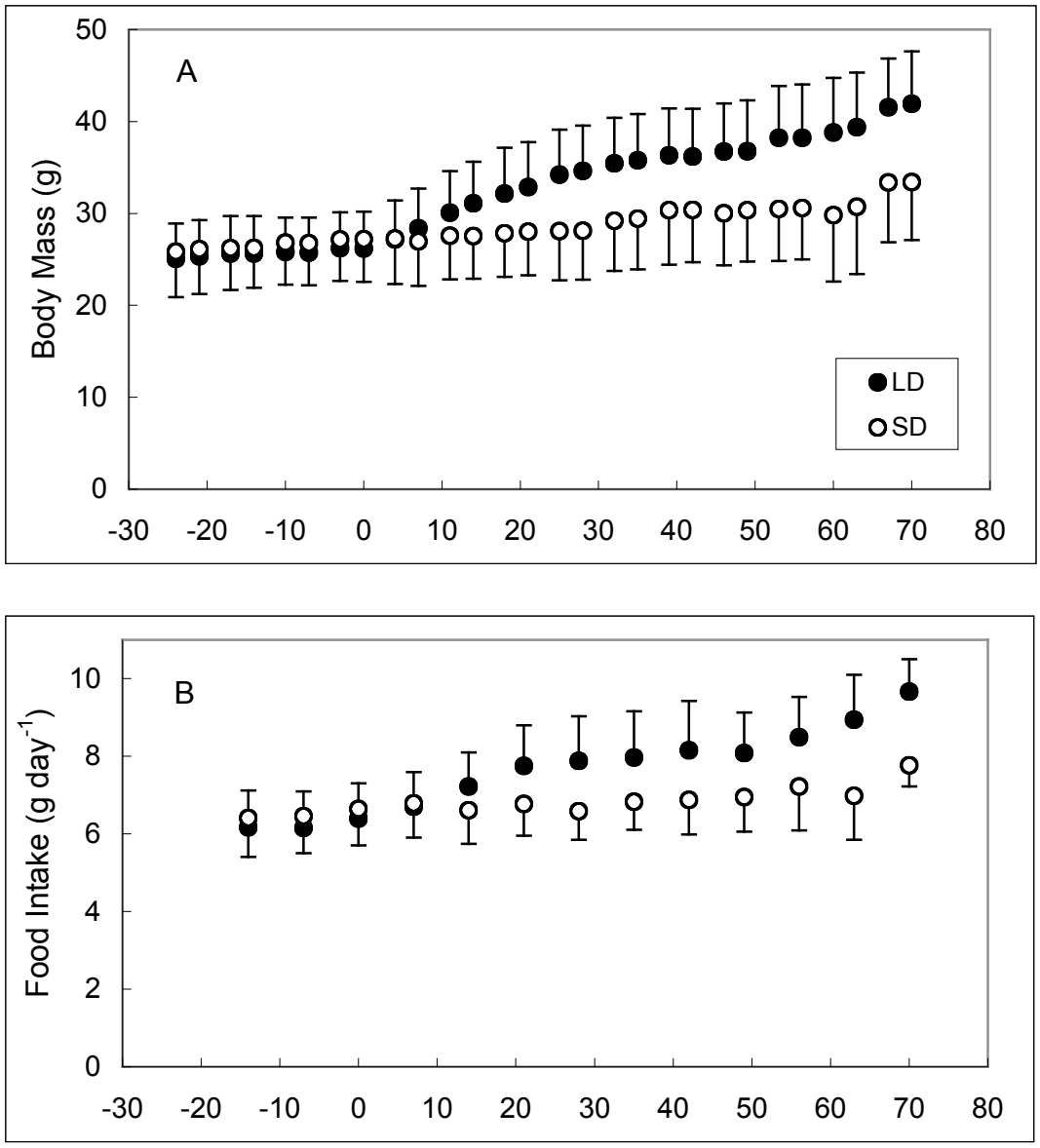

Mean body mass (A) and food intake (B) of male field voles exposed to long day (LD, 16 h:8 h L:D) and short day (SD, 8 h:16 h L:D) photoperiod. Day 0 is the day of exposure to LD. The sample size decreased throughout the experiment, from 45 LD and 38 SD animals on day –24 to 5 LD and 4 SD animals on day 70 (for details, see Materials and methods). Error bars indicate 1 S.D.

Mass and water content of organs in male field voles

	Organ mass (g)		
Organ	Wet	Dry	Water content (%)
Brown adipose tissue	0.299±0.175	0.194±0.142	38.7±12.3
Subcutaneous fat[1]	1.673±1.095	1.386±0.993	21.3±9.3
Epididymal fat	0.616±0.534	0.556±0.512	15.4±10.6
Perirenal & retroperit. fat[2]	0.246±0.259	0.182±0.221	38.2±19.0
Mesenteric fat	0.057±0.031	0.023±0.017	61.8±5.2
Thyroid	0.349±0.119	0.155±0.064	55.7±10.1
Heart	0.177±0.028	0.044±0.007	75.2±0.9
Lungs	0.396±0.375	0.108±0.098	72.7±3.4
Stomach	0.362±0.101	0.079±0.019	77.9±2.6
Caecum	0.680±0.209	0.142±0.038	78.8±1.8
Small Intestine	0.800±0.209	0.188±0.043	76.3±1.9
Large intestine	0.310±0.088	0.066±0.016	78.5±2.3
Liver	1.463±0.325	0.436±0.100	70.2±0.9
Pancreas	0.136±0.044	0.037±0.013	72.6±4.3
Spleen	0.101±0.059	0.023±0.013	76.6±1.3
Kidney	0.344±0.061	0.092±0.018	73.4±1.6
Gonads[3]	0.906±0.393	0.234±0.124	74.7±4.7
Pelage	4.345±1.148	2.749±0.970	37.8±6.9
Carcass	12.62±2.468	4.566±0.922	63.8±3.0

Data (mean ± S.D.) are pooled for all dissected animals (*N*=83). [1]Anterior and posterior subcutaneous fat depots; [2]perirenal and retroperitoneal fat depots; [3]testes and seminal vesicles.