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Photoperiodic effects on body mass, energy balance and hypothalamic gene expression in the bank vole

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Summary

We examined the effect of increasing photoperiod, at a constant low temperature, on the body mass and energy budget of the bank vole *Clethrionomys glareolus*. Simultaneously, we determined the hypothalamic gene expression of neuropeptides and receptors known to be involved in short-term energy balance. Despite an increase in body mass (approximately 10% of initial mass), we found no significant changes in any energetic parameters (food intake, energy assimilation rate, resting metabolic rate and total daily energy expenditure by doubly-labelled water). Apparent energy assimilation efficiency was higher in voles exposed to long-days (LD) compared to short-days (SD). Surprisingly, gene expression of corticotrophin releasing factor (CRF; in the paraventricular nucleus), and the melanocortin-3 receptor (in the arcuate nucleus), both known to be involved in appetite suppression and elevation of energy expenditure in short-term energy balance, were higher in voles kept in LD compared to SD. CRF expression was also elevated in females compared to males. These paradoxical data suggest an alternative mechanism for the control of seasonal body mass changes compared to short-term body mass changes, and between male and female voles. Furthermore, they highlight the need for studies to perform simultaneous measurements at both the molecular and whole animal levels.

Key words: seasonal adaptation, resting metabolic rate (RMR), daily energy expenditure (DEE), doubly-labelled water (DLW) technique, hypothalamic neuropeptides, *Clethrionomys glareolus*, bank vole.

Introduction

It is widely believed that body fatness and hence body mass are regulated by a neuro-hormonal system, which compares actual body fat with a target that is encoded in the brain (Schwartz et al., 2000; Mercer and Speakman, 2001; Morgan and Mercer, 2001). Differences between actual fatness and the target are then corrected through compensatory adjustments in both energy intake and expenditure (Kalra et al., 1999; Schwartz et al., 2000; Jeanrenaud and Rohner-Jeanrenaud, 2001; Mercer and Speakman, 2001): the so-called lipostatic regulation system (after Kennedy, 1953). Many components of this system have been characterised in small mammals, and their importance in the aetiology of human obesity was subsequently demonstrated (Montague et al., 1997). One component of the system, however, that has proved elusive is the nature of the encoded target fat mass to which compensatory adjustments are made. Understanding the nature of this target is important because genetic polymorphism within this target system, resulting in a failure to adequately regulate body fatness, is a potential contributory factor to human obesity (Barsh et al., 2000).

A model system that has proved useful in the characterisation of the differences between compensatory adjustments in body mass, which tend to keep body mass at a fixed target level, and anticipatory adjustments, which involve changes in the target itself (Mercer, 1998; Morgan and Mercer, 2001; Mercer and Speakman, 2001), is the photoperiod-induced change in body mass of Djungarian and Siberian hamsters (*Phodopus sungorus*) (Mercer et al., 1995, 1997, 2000, 2001; Boss-Williams and Bartness, 1996; Reddy et al., 1999; Adam et al., 2000). These animals demonstrate profound (30–35%) reductions in body mass (mostly fat) when exposed to short photoperiods. The generality of this model, however, is unknown, as is its applicability in other less profound photoperiod-induced body mass changes. In the current study, we sought to characterise the patterns of change in both energetic parameters and neuropeptide gene expression during photoperiod-induced changes in the body mass of the bank vole *Clethrionomys glareolus*, a small arvicoline rodent found throughout Europe.

Like many species of small mammal from the temperate zone (Iverson and Turner, 1974; Plunkett et al., 2000), bank voles reduce their body mass during winter (Klaus et al., 1988). This strategy is suggested to potentially offset some of the negative impacts of winter on energy balance, which include

higher thermoregulation costs compounded with a reduction in food quantity and/or quality (Dark and Zucker, 1983). By reducing body mass, it is suggested that animals offset these increased energy demands by reductions in their resting metabolic rate, and the unit costs of thermoregulation and physical activity, thus reducing total daily energy requirements (Nagy and Negus, 1993). After winter, body mass increases again in response to increased day length (Steinlechner et al., 1983). Two interesting questions arise concerning energetic adjustments during the seasonal cycle of body mass. First, what aspects of energy balance does the animal modify to effect the changes in body mass in response to photoperiod changes, and how are these signalled in the brain? Second, once the transitions have occurred, what is the magnitude of the effect of changes in mass on energy demands, what energetic adjustments does the animal perform to meet these demands and thus defend its body mass at the elevated level, and what changes in the brain encode the altered mass that is defended? This paper focuses predominantly on the latter of the two questions.

If, as is widely hypothesised, the decrease in mass under short photoperiods serves to save energy by reducing resting metabolism, then the increase in body mass under long photoperiods is likely to result in elevated resting metabolism. The extent of this increase will depend on the changes in mass of specific body components and their contribution to resting metabolic rate (RMR). Most studies suggest that adipose tissue plays a relatively small part in metabolic activity, when compared with organs such as the alimentary tract and liver (Field et al., 1939; Krebs, 1950; Schmidt-Nielsen, 1984; Selman et al., 2001; but see Geluso and Hayes, 1999; Speakman and Johnson, 2000). To accommodate the predicted rise in RMR, as voles move from low to high body mass, they may modify other components of expenditure, such as that devoted to physical activity (Stebbins, 1984), to maintain a constant total energy expenditure. Alternatively, energy absorption may be increased, either through increased food intake (Dark and Zucker, 1983) or by increasing absorption efficiency (Speakman and McQueenie, 1996; Nagy and Negus, 1993). Finally, animals may utilise a combination of these approaches.

The alterations in energy balance and thus changes in body and fat mass in response to photoperiod are probably signalled predominantly in the hypothalamus, which has long been established as a site responsible for control of feeding behaviour, particularly the arcuate (ARC), ventromedial (VMN) and paraventricular (PVN) hypothalamic nuclei. Peptidergic neurons within these nuclei produce a number of neuropeptides involved in energy balance regulation (Schwartz et al., 2000), which can be divided into two categories. Anabolic neuropeptides, such as neuropeptide Y (NPY) and agouti-related protein (AGRP), increase energy intake and decrease energy expenditure (Stanley et al., 1986; Boss-Williams and Bartness, 1996; Hagan et al., 2000), and as a result, body fat stores and thus body mass increase. Catabolic neuropeptides, such as melanocortins cleaved from the pro-opiomelanocortin (POMC) precursor molecule, as well as cocaine- and amphetamine-regulated transcript (CART) and corticotrophin-releasing factor (CRF), suppress food intake and increase energy expenditure (Bray et al., 1990; Kristensen et al., 1998; Tritos et al., 1998), which results in a decrease of amount of fat stored and body mass loss.

In the present study, we examined the effect of increased photoperiod on the energy balance and body composition of the bank vole. Simultaneously, we quantified hypothalamic mRNA levels for neuropeptides and melanocortin receptors known to be involved in short-term energy regulation, to determine their role in the maintenance of seasonally different levels of body mass.

Materials and methods

Animals

Forty-two bank voles *Clethrionomys glareolus* Schreber 1780 were bred in the laboratory and maintained in a 12 h:12 h L:D photoperiod at a temperature of 20±2°C. Voles were weaned at 18–20 days of age and kept in single-sex groups of 4–6 animals. Rodent Maintenance Diet (CRM – Special Diets Services; Witham, UK) and water were provided *ad libitum*. At 7–10 months old, an age by which they had reached sexual maturity, the 20 male and 22 female voles were switched to a cold room at an ambient temperature of 6±2°C; the photoperiod remained 12 h:12 h L:D. After 10 days, voles were separated into individual cages (28 cm×11 cm×12 cm) and the photoperiod was switched to 8 h:16 h L:D. They were then matched for body mass and assigned to either the long-day (LD) or short-day group (SD). The LD group consisted of 11 males and 12 females (N=23) and the SD group 9 males and 10 females (M=19).

Experimental protocol

After 10 weeks of acclimation at 6°C in the short photoperiod, baseline measurements of all energetic parameters were obtained over the following 3 weeks. Body mass and food intake were measured daily for 7 days, during 4 days of which we obtained faecal samples for gross energy (GE) analysis. RMR and daily energy expenditure (DEE) were measured once for each animal. After baseline measurements were taken, the weight of each vole and of its food was recorded. The LD group was then placed in a room similar in all respects to the first except that the photoperiod was 16 h:8 h L:D (Day 0). Temperature remained at 6°C, as this is representative of natural conditions in Aberdeen at the same time of year. Over the next 12 weeks, all voles were subject to the following regime. Body mass and food intake were recorded every third day for the whole duration. Faeces were collected over a 4-day period in week 7, RMR was measured in week 8 and DEE in week 9, to allow any modifications due to the body mass increase to be determined.

Resting metabolic rate

RMR was measured using an open circuit respirometry

system (Servomex Ltd., Crowborough, UK). Each respirometry measurement lasted for 2 h and food and water were not denied prior to measurements. Voles were placed in a sealed Perspex chamber within an incubator (INL-401N-010, Gallenkamp, Loughborough, UK) at a temperature of 25±0.5°C, which is within the thermoneutral zone of these animals (Peacock and Speakman, 2001). Air was dried using silica gel (BDH, UK) and pumped through the system at a rate of 600–800 ml min^{-1} (Alexander Wright flowmeter DM3A, Zeal Ltd., London, UK). A 150 ml sample of dried excurrent air was subsequently passed though an oxygen analyser and the mean measurements recorded on a computer at 30 s intervals. Carbon dioxide was not absorbed prior to gas analysis to minimise error in the conversion of oxygen consumption to energy expenditure (Koteja, 1996; Speakman, 2000). RMR was calculated from the ten lowest consecutive readings, equivalent to 5 min within the respirometry chamber, using the appropriate equation from Speakman (2000), with values corrected for temperature and pressure.

Daily energy expenditure

DEE was measured using the doubly-labelled water (DLW) technique (Speakman, 1997). Individuals were weighed to ±0.01 g using a balance (Sartorius, Epsom, UK) and labelled with an intraperitoneal injection of approximately 0.2 g of water containing enriched deuterium (4.63 atom%) and oxygen-18 (9.44 atom%). The syringe was weighed before and after the injection (±0.0001 g; Ohaus Analytical Plus, Brooklyn, USA) to provide an accurate measurement of the amount of isotope injected. An initial 50–100 μl blood sample was collected by tail tipping 1 h after the injection, which was the time generally assumed to be required for the isotopes to reach equilibrium (Anbar and Lewitus, 1958; Nagy, 1983). Blood samples were immediately flame-sealed into 50 μl pipettes (Vitrex, Camlab Ltd., Cambridge, UK) until analysis. A final blood sample was collected 24 h after the initial sample. Blood samples were vacuum-distilled into glass Pasteur pipettes (Volac, John Poulten Ltd., Barking, UK; Nagy, 1983) and the distillates used for mass spectrometric analysis of stable isotopes. Mass spectrometric analysis of deuterium enrichment was performed using H_2 gas. The H_2 was produced by reacting water, distilled from the blood, with $LiAlH_4$ (Ward et al., 2000). For analysis of O^{18} enrichment in the blood samples, the water distilled from the blood was equilibrated with CO_2 gas using the small sample equilibration technique (Speakman et al., 1990). DEE was calculated using the single pool intercept method, equation 7.17 (Speakman, 1997).

Organ morphometrics

In week 12, 11 voles (five males and six females) from LD and ten voles (five males and five females) from SD were chosen at random. These animals were killed, in the middle of the light phase, by cervical dislocation and dissected into 20 components. Brains were removed, frozen on dry ice and stored at –80°C for further analysis. The contents of the stomach and intestines were removed, after which the length of the caecum, small and large intestines were measured with a ruler (±1 mm). All tissues were weighed (±0.0001 g; Ohaus Analytical Plus), dried in an oven at 60°C for 14 days, and then reweighed to determine the dry mass.

Gross energy analysis

Food and faecal samples were placed in a 60°C oven for at least 14 days to obtain dry mass and subsequently analysed for GE content using adiabatic bomb calorimetry (Gallenkamp). Dry mass digestibility (DMD) and apparent energy assimilation efficiency (AEAE) were determined using the following equations:

$$DMD = (FI_{Dry} - FM_{Dry}) / FI_{Dry} \quad (1)$$

$$AEAE = [(FI_{Dry} \times GE_{Food}) - (FM_{Dry} \times GE_{Faeces})] / (FI_{Dry} \times GE_{Food}) , \quad (2)$$

where FI is food intake in g and FM is faeces mass in g.

Hypothalamic gene expression

Levels of mRNA for neuropeptide and receptor systems were quantified by *in situ* hybridisation in coronal hypothalamic sections (Mercer et al., 1995, 1997). Antisense riboprobes complementary to rat NPY (Mercer et al., 1995), hamster POMC and AGRP (Mercer et al., 2000), rat CART (Adam et al., 2000), rat CRF (Mercer et al., 1995) and human MC3-R and MC4-R (Adam et al., 2000) were transcribed from cloned cDNA templates.

Coronal hypothalamic sections (20 μm) of bank vole brains were cut on a cryostat and collected from the caudal extent of the ARC through to the rostral extent of the PVN onto two sets of eight slides, with six or seven sections mounted on each slide. The first set of slides spanned the region from approximately bregma –2.70 mm to bregma –1.46 mm according to the atlas of the mouse brain (Franklin and Paxinos, 1997), and therefore contained the full extent of the ARC and the caudal part of the VMN. The second set of slides continued to bregma –0.58 mm, and thus contained the rostral part of the VMN, as well as the caudal and rostral extent of the PVN. Slides were fixed, acetylated (optional), and hybridised overnight at 58°C with ^{35}S-labelled riboprobes in hybridisation buffer ($1–2\times10^7$ c.p.m. ml^{-1}). For each riboprobe, the hybridisations were carried out simultaneously on 21 slides (one slide from each individual). The next day, slides were treated with ribonuclease A, desalted with a final high-stringency wash (30 min) in 0.1× SSC at 60°C, air-dried and exposed to autoradiography film (Kodak Biomax MR1, Amersham Pharmacia Biotech UK Ltd.) for 7–22 days, depending on the riboprobe used. Autoradiographic images were quantified using the Image-Pro Plus system (Media Cybernetics, Silver Spring, MD, USA), which determines the intensity and area of the hybridisation signal. Image analysis was performed on four or five sections from the ARC and the VMN, and two or three sections from the PVN. Data were analysed using a standard curve generated by simultaneous exposure of ^{14}C autoradiographic microscales (Amersham

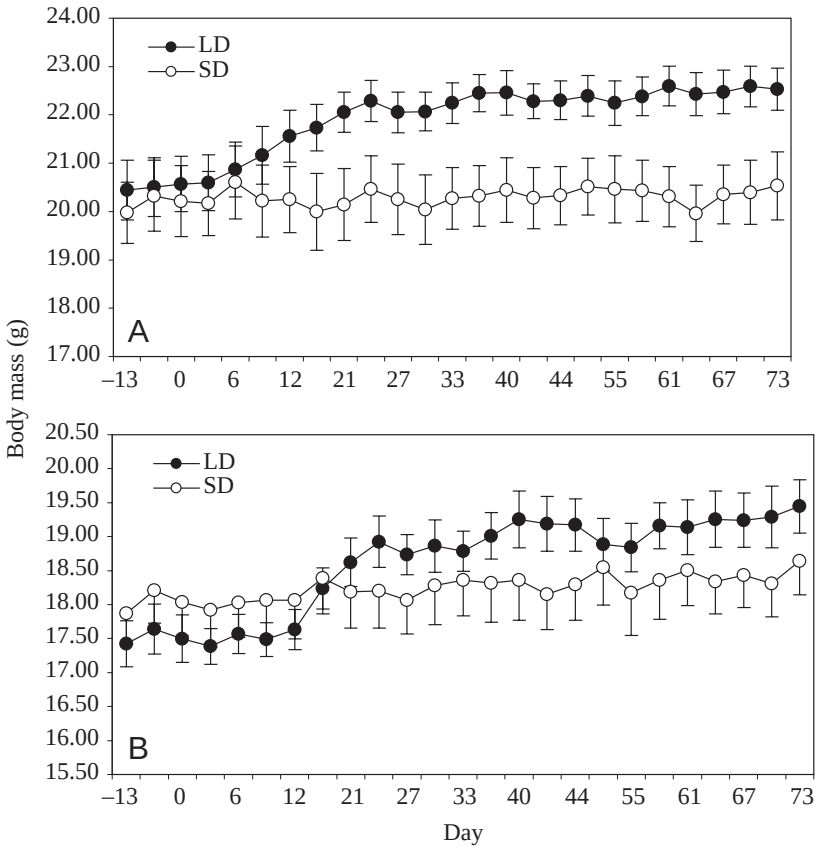

Fig. 1. Body mass of male (A) and female (B) bank voles exposed to a long-day (LD) or short-day (SD) photoperiod and measured over 10 weeks, commencing at Day 0. Values are expressed as means ± S.E.M.

Pharmacia Biotech, Amersham, UK), and the integrated intensity of the hybridisation signal was computed.

Statistical analysis

Data were analysed using Minitab version 11 (Ryan et al., 1985). All data were tested for normality using the Anderson–Darling test and non-parametric tests were used for non-normal data. Organ mass data were analysed with general linear models (GLM), following Selman et al. (2001), and body mass was used as a covariate where appropriate. Repeated one-way analysis of variance (ANOVA) was used to analyse the effect of time on body mass and food intake, followed by least-squares regression (LSR) to determine daily variation or general trends. The effect of photoperiod and sex on hypothalamic neuropeptide and receptor gene expression was determined by two-way ANOVA. Tukey's test was employed *post hoc* when required. Finally, two-sample *t*-tests, paired *t*-tests or Mann–Whitney *U* tests were used where appropriate. All results had a significance level of $P<0.05$. We aimed for sample sizes of 12 animals in both male and female treatment and control groups; however, due to losses in the course of the acclimation period and the experiments we ended up with samples of 11 and 9 males and 12 and 10 females. Analyses were restricted to animals where we had complete data. We have previously determined the repeatability of RMR

Table 1. *Energy budget of male and female bank voles exposed to long-day photoperiod*

	Response phase			
	Stable	Increase	Plateau	*P*
Males				
Change in BM (g day^{-1})	0.01±0.01	0.08±0.02	0.01±0.004	<0.001*
Food intake (g day^{-1})	6.37±0.43	6.39±0.33	6.28±0.29	0.56
Energy assimilated (kJ day^{-1})	81.8±5.50	83.0±4.35	81.6±3.70	0.55
RMR (kJ)	29.0±1.40		30.6±1.61	0.15
DEE (kJ)	86.6±6.26		88.8±5.13	0.79
Females				
Change in BM (g day^{-1})	0.003±0.01	0.10±0.02	0.01±0.01	<0.001*
Food intake (g day^{-1})	5.50±0.22	5.59±0.17	5.66±0.18	0.09
Energy assimilated (kJ day^{-1})	70.8±2.78	72.7±2.27	73.6±2.32	0.02**
RMR (kJ)	28.8±1.45		28.0±1.18	0.55
DEE (kJ)	80.7±2.75		70.9±3.95	0.06

Values are means ± S.E.M.

RMR, resting metabolic rate; DEE, daily energy expenditure.

Males (N=11): Stable-phase = Days –13 to 3; Increase-phase = Days 3–24; Plateau-phase = Days 24–73; Females (N=12): Stable-phase = Days –13 to 9; Increase-phase = Days 9–24; Plateau-phase = Days 24–73.

Photoperiod switched from SD to LD on Day 0.

*Increase-phase is greater than Stable and Plateau-phases; **Plateau-phase is greater than Stable-phase.

measurements using our respirometry system, and the day to day coefficient of variation on repeated measures is 7.7% (*N*=69 paired measurements: Król et al., 2003). With a sample size of 10 per group, and alpha set at 0.05, the power to detect a 10% difference in RMR between the control and treatment groups was 80%. For the repeated measurements comparing pre- and post-treatment samples the power for the same sample size was 95.4%. Using the estimated mean deviations in validation studies of the doubly-labelled water technique, we could detect differences of 13% between control and treatment groups with a power of 80% at alpha=0.05. Using day to day variation in food intake measures we estimated that we could detect differences of 5% in daily food intake at the same power and significance levels for *N*=9 per group. The corresponding discrimination abilities in the repeated-measures analyses were 10% for doubly-labelled water estimates and 3% for daily food intake measures.

Results

There was no significant difference in any variable during the baseline period between voles in LD and SD groups (body mass: W=519, P=0.99; FI: W=535, P=0.49; DMD: W=80, P=0.47; RMR: T_{41}=0.26, P=0.80; DEE: T_{25}=1.22, P=0.24; AEAE: T_{15}=0.56, P=0.58).

Body mass

There was a significant increase in body mass over time in both male and female bank voles switched to LD ($F_{20,482}$=2.16, P=0.003; LSR $F_{1,24}$=105.7, P<0.001), which

Table 2. *Dry masses (g) of dissected organs of male and female bank voles exposed to a long-day (LD) or short-day (SD) photoperiod*

	Male		Female	
Organ	SD (*N*=5)	LD (*N*=5)	SD (*N*=5)	LD (*N*=6)
Subcutaneous fat	0.51±0.09	0.77±0.18	0.33±0.08	0.57±0.05
Mesenteric fat	0.004±0.001	0.02±0.01	0.02±0.004	0.05±0.01
Abdominal fat	0.16±0.03	0.29±0.06	0.06±0.02	0.12±0.05
Brown adipose tissue	0.04±0.004	0.06±0.02	0.04±0.01	0.06±0.01
Stomach	0.06±0.004	0.06±0.002	0.07±0.004	0.08±0.003
Small intestine (SI)				
Mass	0.07±0.003	0.07±0.01	0.08±0.002	0.09±0.01
Length (mm)	418±15.2	421±9.3	415±5.0	415±16.0
Large intestine (LI)				
Mass	0.03±0.001	0.03±0.002	0.04±0.003	0.04±0.002
Length (mm)	155±7.1	160±3.1	167±4.8	159±2.4
Caecum (C)				
Mass	0.04±0.001	0.04±0.001	0.05±0.01	0.05±0.01
Length (mm)	123±4.1	117±4.3	127±8.3	117±3.4
LI+C				
Mass	0.07±0.002	0.07±0.003	0.08±0.01	0.08±0.01
Length (mm)	278±7.9	277±5.6	295±9.5	276±5.5
Whole gut (SI+LI+C)				
Mass	0.15±0.003	0.14±0.01	0.16±0.01	0.17±0.02
Length (mm)	696±20.8	698±10.4	710±13.7	690±21.1
Reproductive organs	0.27±0.03	0.37±0.02	0.07±0.03	0.04±0.001
Thyroid	0.13±0.01	0.13±0.01	0.10±0.01	0.10±0.02
Spleen	0.01±0.001	0.004±0.001	0.01±0.001	0.01±0.001
Pancreas	0.05±0.003	0.05±0.01	0.04±0.01	0.04±0.003
Liver	0.33±0.02	0.34±0.01	0.33±0.02	0.33±0.02
Kidneys	0.09±0.01	0.09±0.01	0.08±0.01	0.08±0.004
Heart	0.04±0.01	0.04±0.003	0.04±0.003	0.03±0.001
Lungs	0.08±0.02	0.09±0.01	0.07±0.01	0.06±0.01
Tail	0.06±0.002	0.06±0.01	0.07±0.003	0.06±0.002
Pelage	0.97±0.04	0.98±0.08	0.99±0.04	1.08±0.09
Carcass	2.08±0.13	2.22±0.07	2.03±0.16	2.20±0.09

Values are means ± S.E.M.

was not observed in the control SD groups ($F_{20,434}$=1.23, P=0.23; Fig. 1). The change in body mass following the switch from SD to LD was divided into three distinct periods. An initial stable period preceded the increase-phase, which was then followed by the plateau-phase. These distinct phases were observed in both male and females but the durations and timings were slightly different between sexes. In males, the increase-phase began on day 3 and lasted for 21 days, during which time the voles gained 1.96 g (9.6% of the initial body mass). Female bank voles exhibited a similar mass increase of 1.95 g (11.1% of the initial body mass); however, the increase-phase was slightly shorter, beginning on day 9 and lasting only 15 days. In both sexes body mass reached a plateau on day 24 following the photoperiod manipulation. The rate of change in body mass during the increase-phase was significantly higher than the changes observed during the initial stable period and the plateau-phase for both sexes (male $F_{2,32}$=10.5, P<0.001; female $F_{2,35}$=34.3, P<0.001). The components of energy intake and expenditure for male and female voles in the treatment group, divided into the different phases of the response, are presented in Table 1.

Body composition

Dry masses of the dissected organs of male and female voles, culled after 12 weeks exposure to long-day photoperiod

Table 3. *Results of GLM analysis of organ masses, including lengths of gut components, of bank voles exposed to a long-day or short-day photoperiod*

	Sex		Photoperiod		Body mass	
Organ	*F*	*P*	*F*	*P*	*F*	*P*
Subcutaneous fat	3.28	**0.09**	5.65	**0.03**		ns
Mesenteric fat	12.7	**0.002**	11.1	**0.004**		ns
Abdominal fat	0.54	0.47	5.56	**0.03**	10.3	**0.01**
Brown adipose tissue	2.46	0.14	2.27	0.15	5.16	**0.04**
Stomach	19.9	<**0.001**	0.30	0.59		ns
Small intestine (SI)						
Mass	1.74	0.20	0.17	0.69		ns
Length	6.32	**0.02**	0.48	0.50	18.8	<**0.001**
Large intestine (LI)						
Mass	15.1	**0.001**	2.88	0.11	4.74	**0.04**
Length	1.18	0.29	0.16	0.69		ns
Caecum (C)						
Mass	10.4	**0.01**	0.82	0.38	6.49	**0.02**
Length	0.20	0.66	2.51	0.13		ns
LI+C						
Mass	15.5	**0.001**	1.76	0.20	7.79	**0.01**
Length	1.03	0.32	1.88	0.19		ns
Whole gut (SI+LI+C)						
Mass	11.4	**0.004**	0.34	0.57	6.57	**0.02**
Length	7.25	0.02	1.87	0.19	14.4	**0.001**
Reproductive organs	217.9	<**0.001**	7.40	**0.02**		ns
Thyroid	0.13	0.72	0.05	0.83	5.59	**0.03**
Spleen	0.03	0.85	0.50	0.49		ns
Pancreas	3.41	0.08	0.31	0.59		ns
Liver	10.3	**0.01**	0.47	0.50	35.1	<**0.001**
Kidneys	3.40	0.08	0.24	0.63	30.4	<**0.001**
Heart	1.19	0.29	0.86	0.37	12.1	**0.003**
Lungs	3.81	0.07	0.00	0.98		ns
Tail	3.88	0.07	0.11	0.74	5.42	**0.03**
Pelage	6.51	**0.02**	0.19	0.67	6.99	**0.02**
Carcass	26.5	<**0.001**	2.55	0.13	70.5	<**0.001**

Reproductive organs were the only tissue to have a significant interaction for sex*pp, the result was F=4.94, P=0.04.
Values in bold indicate significant differences.
ns, not significant.
GLM, general linear model.

from both the treatment (LD) and control (SD) groups are presented in Table 2. The main effects of the photoperiod treatment were on the masses of the white adipose depots (subcutaneous, mesenteric and abdominal), which were all significantly larger in LD voles (N=11) compared to those in SD (N=10; Tables 2 and 3). The combined mass of white adipose depots was 0.89±0.13 g and 0.54±0.07 g in voles from LD and SD, respectively, an increase of 64.8%. Of the 15 lean tissues dissected, only the reproductive organs differed significantly in mass between voles from LD and SD groups. There was a large sex difference in the dry mass of the reproductive organs, with the organs of males being much larger than those of females (Tables 2 and 3). Sex differences were also apparent in the sizes of the components of the alimentary tract, liver, pelage and total dry mass of the empty carcass but photoperiod had no effect on any of these components (Tables 2 and 3).

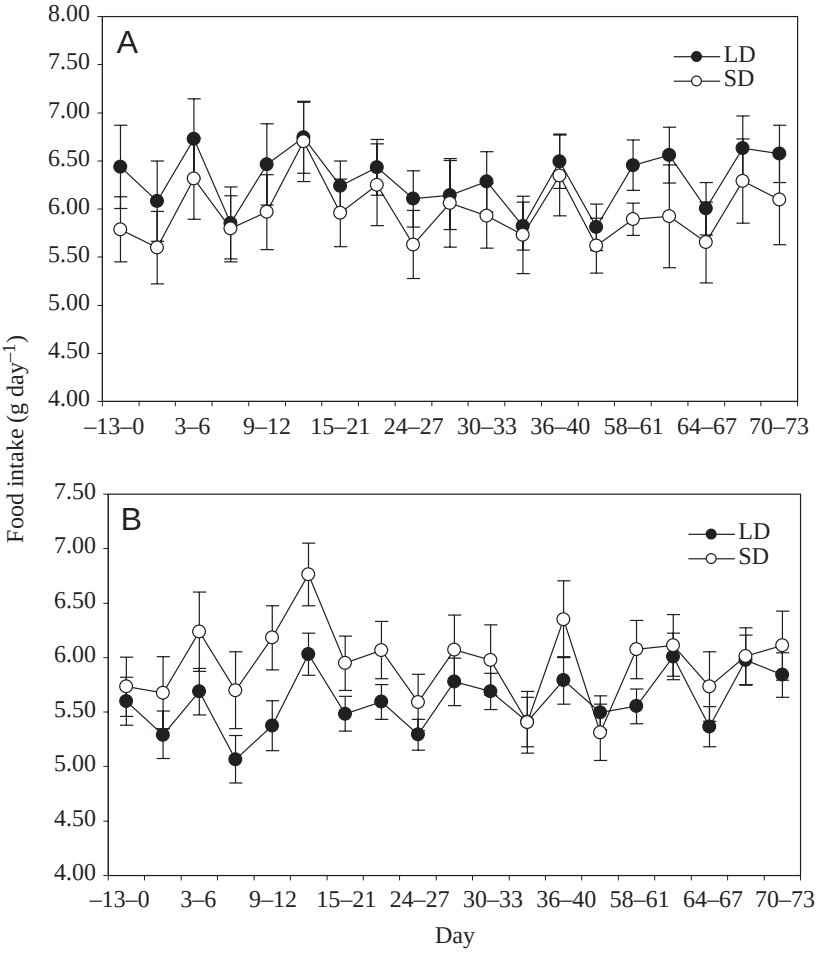

Fig. 2. Food intake (g day^{-1}) of male (A) and female (B) bank voles exposed to a long-day (LD) or short-day (SD) photoperiod and measured over 10 weeks, commencing at Day 0. Values are expressed as means ± S.E.M.

Food intake

Across all groups the mean food intake was 6.0±0.15 g per day. There were no significant effects of sex ($F_{1,42}$=0.03, P=0.87), photoperiod ($F_{1,42}$=1.78, P=0.19) or time ($F_{18,436}$=15.4, P<0.001, LSR $F_{1,18}$=0.88, P=0.36) on the level of food intake. To illustrate the constancy of food intake, the food intake of the treatment (LD) and control (SD) groups measured at 3 day intervals through the entire experiment and averaged across all individuals, are presented in Fig. 2. Total food intake over days –13 to 73, pooled across sexes, was 416.4±13.7 g in the LD group and 412.5±16.0 g in the SD group (T_{41}=0.19, P=0.85). There was also no difference in food intake between the three phases for male or female voles from the LD group (Table 1).

Digestive efficiency

Dry food had an energy content of 17.6 kJ g^{-1}. DMD and AEAE were significantly higher in the LD group (81.2±0.80 and 83.8±0.79, respectively) compared to the SD group (78.6±0.57 and 81.2±0.69, respectively) following 7 weeks in LD (DMD: T_{15}=2.85, P=0.01; AEAE: T_{15}=2.57, P=0.02). Sexes were not analysed separately due to the small sample size. Measured AEAE was used to transpose food intake into

Table 4. *Summary of doubly-labelled water analysis of voles exposed to a long-day (LD) or short-day (SD) photoperiod*

	LD				SD			
	Pre-manipulation		Week 9		Pre-manipulation		Week 9	
	Male (N=7)	Female (N=7)	Male (N=7)	Female (N=7)	Male (N=6)	Female (N=7)	Male (N=6)	Female (N=7)
k_O	0.05±0.002	0.07±0.004	0.04±0.003	0.05±0.003	0.05±0.002	0.05±0.001	0.05±0.002	0.05±0.001
k_D	0.03±0.002	0.04±0.004	0.02±0.003	0.04±0.003	0.06±0.03	0.03±0.002	0.03±0.003	0.04±0.001
k_O/k_D	1.54±0.03	1.48±0.03	1.78±0.11	1.49±0.04	1.54±0.02	1.58±0.05	1.58±0.11	1.55±0.03
No (% BM)	67.8±0.86	67.9±1.34	67.3±1.37	64.0±0.76	67.6±0.85	66.0±1.42	67.7±2.57	63.7±1.70
N_D/N_O	1.09±0.01	1.09±0.002	1.26±0.03	1.21±0.02	1.08±0.02	1.10±0.01	1.23±0.03	1.23±0.03
DEE kJ day^{-1}	86.6±6.25	80.7±2.75	88.8±5.13	70.9±3.95	82.0±4.01	74.6±4.55	78.7±7.40	73.8±2.25

k_O, elimination rate of oxygen-18; k_D, elimination rate of deuterium; N_D/N_O, dilution space ratio; N_O, oxygen dilution space; N_D, deuterium dilution space; BM, body mass; DEE, daily energy expenditure.

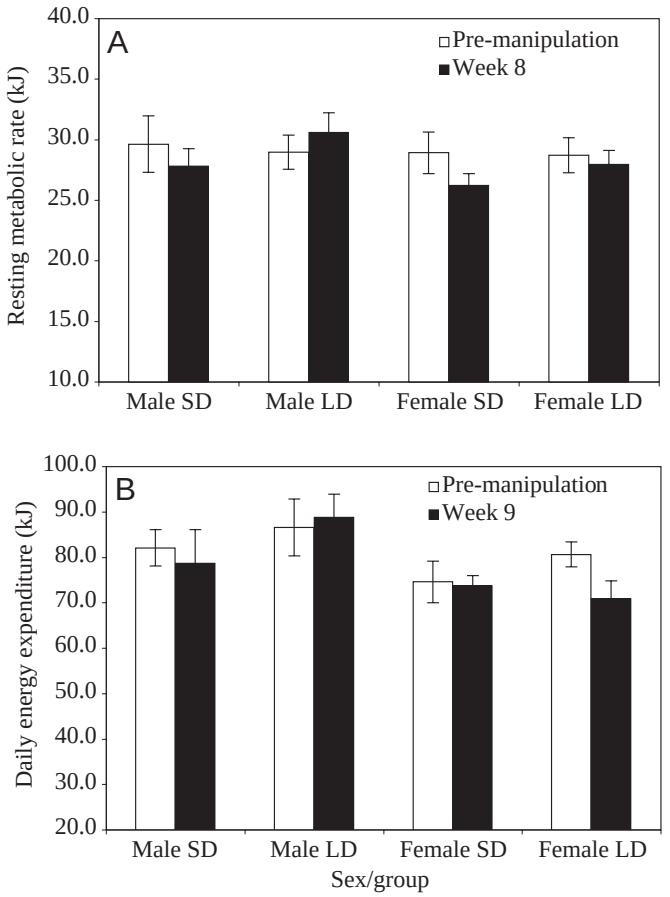

Fig. 3. Resting metabolic rate (A) and daily energy expenditures (B) of male and female bank voles exposed to a long-day (LD) or short-day (SD) photoperiod and measured before (Pre-manipulation) and 8 weeks (A) or 9 weeks (B) after the photoperiod switch. Values are expressed as means ± S.E.M.

assimilated energy (kJ). There was no significant effect of time ($F_{18,436}$=15.4, P<0.001; LSR $F_{1,18}$=1.14, P=0.30), sex ($F_{1,42}$=2.11, P=0.15) or photoperiod ($F_{1,42}$=0.26, P=0.61) on the daily energy assimilation. Summing the total energy assimilation over days –13 to 73, voles in the LD group assimilated on average 5396±178 kJ compared with 5180±192 kJ by voles in the SD group. Interestingly, female voles in the LD group assimilated significantly more energy per day during the plateau-phase compared to the stable-phase (Table 1).

Resting metabolic rate

Across all groups RMR averaged 28.6 kJ day, approximately 40% of the energy assimilation rate. Despite the fact that the LD animals increased their body mass by approximately 10%, there were no significant effects of time, sex or photoperiod on RMR (Fig. 3A; time T=0.45, P=0.66; sex $F_{1,42}$=2.66, P=0.11; photoperiod $F_{1,42}$=2.88, P=0.10).

Daily energy expenditure

Parameters used in DLW estimates of DEE are presented in Table 4. Across all eight groups [sex (2), photoperiod (2) and time (2)], the average DEE measured by DLW was 5.4% greater than the calculated energy assimilation rate. This discrepancy appeared to be greater in males than in females. Hence, the average DEE estimated by DLW across the four groups of males was 8.1% higher than the calculated energy assimilation, while in females it was only 2.7% greater. Despite the fact that the LD animals increased in body mass by approximately 10%, there were no significant effects of time or photoperiod on DEE (Fig. 3B; time T_{26}=0.73, P=0.47; photoperiod $F_{1,26}$=0.46, P=0.50). Female voles, however, exhibited a mean DEE 14% lower than male voles ($F_{1,26}$=5.66, P=0.03). The ratio of DEE to RMR varied between 2.5 and 3.0 in the groups during the baseline period (mean=2.79). During the final phase of the experiment, after the voles had increased in mass by 10%, the average was almost identical at 2.77 (range 2.5–2.9).

Hypothalamic neuropeptide and receptor gene expression

The distribution of NPY, AGRP, POMC, CART, CRF, MC3-R and MC4-R mRNAs in the bank vole hypothalamus (Fig. 4) was consistent with other rodent species. NPY, AGRP and POMC mRNAs were mainly expressed in the ARC. CART was widespread throughout hypothalamus and strongly expressed in the ARC. CRF and MC4-R gene expression were most dense in the PVN. The major sites of the gene expression for MC3-R were the ARC and the VMN.

Gene expressions of NPY, AGRP, CART, MC3-R in the VMN, POMC in the ARC and MC4-R were not affected by photoperiod or sex (Fig. 5). There was a significant effect of photoperiod ($F_{1,18}$=5.62, P=0.03) and sex ($F_{1,18}$=4.51, P=0.05) on CRF gene expression, with higher levels of expression in the PVN of voles exposed to LD, and mRNA levels higher in females than in males. Voles in the LD group also had upregulated levels of MC3-R mRNA in the ARC ($F_{1,18}$=4.66, P=0.05).

Discussion

Energetic responses to increased photoperiod

As anticipated from their seasonal responses in the wild (Klaus et al., 1988), both male and female bank voles increased their body mass following the switch from a short-day to a long-day photoperiod. This change involved significant increases in all white adipose tissue (WAT) depots, which is also consistent with previous studies of seasonal composition changes in small rodents (Dark and Zucker, 1986; Bartness et al., 1989). In males, the testes also increased in mass, indicative of a readiness for reproduction (Blank, 1992). In contrast to the changes in WAT there was no significant change in brown adipose tissue (BAT) mass. Similarly, McDevitt and Speakman (1994) found short-tailed field voles (*Microtus agrestis*) did not downregulate their BAT capacities when switched to a long photoperiod at constant low temperature. The difference in amplitude of body mass change between wild voles (Klaus et al., 1988) and our present study may be a consequence of a number of factors. In the present study only

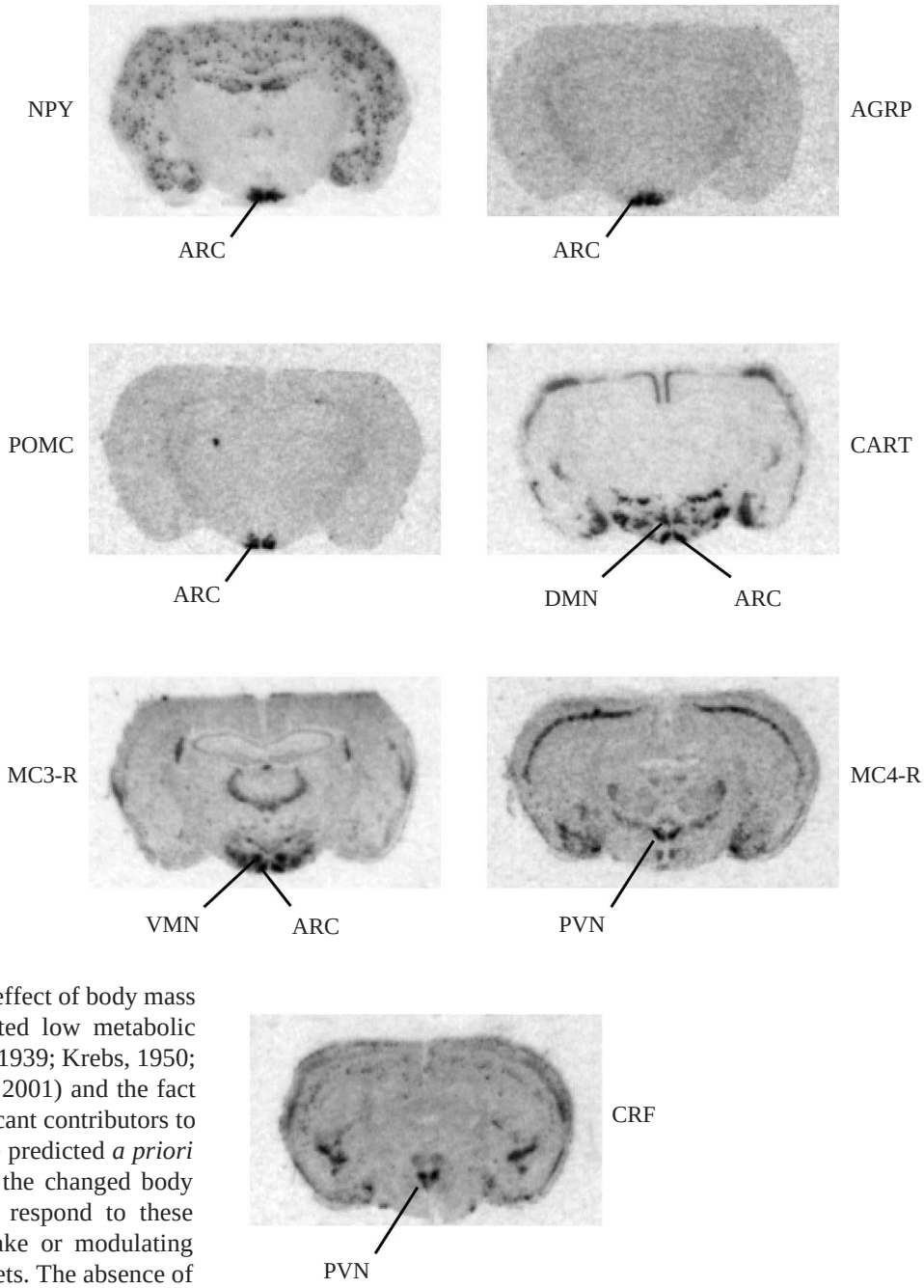

Fig. 4. Autoradiographs showing localization of neuropeptide and receptor gene expression in coronal sections of male bank vole brain, following 12-week exposure to long-day photoperiod (LD). NPY, neuropeptide Y; AGRP, agouti-related protein; POMC, pro-opiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; CRF, corticotrophin-releasing factor; MC3-R and MC4-R, melanocortin-3 and -4 receptors; ARC, arcuate nucleus; VMN, ventromedial nucleus; PVN, paraventricular nucleus.

the photoperiod was altered, whereas in the wild, ambient temperature and changes in food quality and quantity may combine with changes in photoperiod to influence weight or reproductive cycles (Clarke, 1985). In addition, we followed the same individuals over time, but Klaus et al. (1988) trapped different samples of the population each month, and sex and age differences, which were not controlled for, may thus have contributed to the greater apparent effect.

Although the voles increased in body mass under long photoperiod, there was no effect of this change on RMR in either sex. This absence of an effect of body mass change is consistent with the suggested low metabolic intensity of adipose tissue (Field et al., 1939; Krebs, 1950; Schmidt-Nielsen, 1984; Selman et al., 2001) and the fact that WAT depots were the most significant contributors to the increases in overall body mass. We predicted *a priori* that RMR would increase because of the changed body mass, and that voles would need to respond to these changes by increasing their food intake or modulating other components of their energy budgets. The absence of a change in RMR meant that changes in food intake and in total daily energy expenditure were not required to sustain the increased body mass. It was not surprising, therefore, to find that total daily energy demands also did not differ between the SD and LD animals for both sexes and, although food was available *ad libitum*, voles did not increase their food intake concurrent with body mass increase. The overall impression then was that food intake, total DEE and RMR were almost completely unaffected by the photoperiod and the consequent body mass changes.

Although the changes in body mass observed in our voles in response to photoperiod mimic those observed in the wild, in terms of direction, the negligible energetic consequences of these changes call into question the common interpretation that reduced body mass in the wild is an adaptation to reduce energy demands during winter when food is in short supply. Our data suggest that voles could sustain significantly greater masses (+10%) with no energetic penalty. An alternative explanation is that the reduction in body mass during winter may reduce the risk of predation when cover due to vegetation is relatively sparse. Lighter individuals may be more adept at escaping predators because they are not slowed by carrying large fat stores (McNamara and Houston, 1990; Witter and Cuthill, 1993; Gosler et al., 1995).

Male voles in LD had a 14% higher daily energy expenditure than females in LD, possibly reflecting higher activity levels

in males (Norrdahl and Korpimäki, 1998; Marczinski et al., 1998).

Hypothalamic neuropeptide and receptor gene expression

In bank voles, photoperiod had no effect on gene expression of the anabolic neuropeptides NPY and AGRP, or the catabolic neuropeptides CART and POMC. Gene expression of melanocortin receptors MC3-R in the VMN and MC4-R in the PVN were also not altered by photoperiod. The only changes related to LD exposure were elevated CRF in the PVN, and the melanocortin receptor MC3-R in the ARC.

The relationship between photoperiod and gene expression of hypothalamic neuropeptides and receptors has been extensively studied in male Siberian hamsters (Reddy et al., 1999; Mercer et al., 2000, 2001), a species which, similar to bank voles, exhibits its maximum body mass during long-days in summer and reduces body mass during short-days in winter. All the experiments conducted on the hamsters to date have involved a transfer of animals from LD to SD. In contrast, the bank voles in the current study were transferred from SD (8 h:16 h L:D) to LD (16 h:8 h L:D). Despite this difference in experimental protocol, comparison between bank voles and hamsters is appropriate since the changes in body mass induced by photoperiod are reversible and closely mimic the physiological changes normally expressed during the life cycle of these species; although hamsters also exhibit an increase in fat free mass as well as fat mass. To allow ready comparison of the effects of photoperiod upon hypothalamic gene expression between studies and species, we have compiled these data in Table 5, where the gene expression of LD animals is expressed relative to SDs for both bank voles and Siberian hamsters.

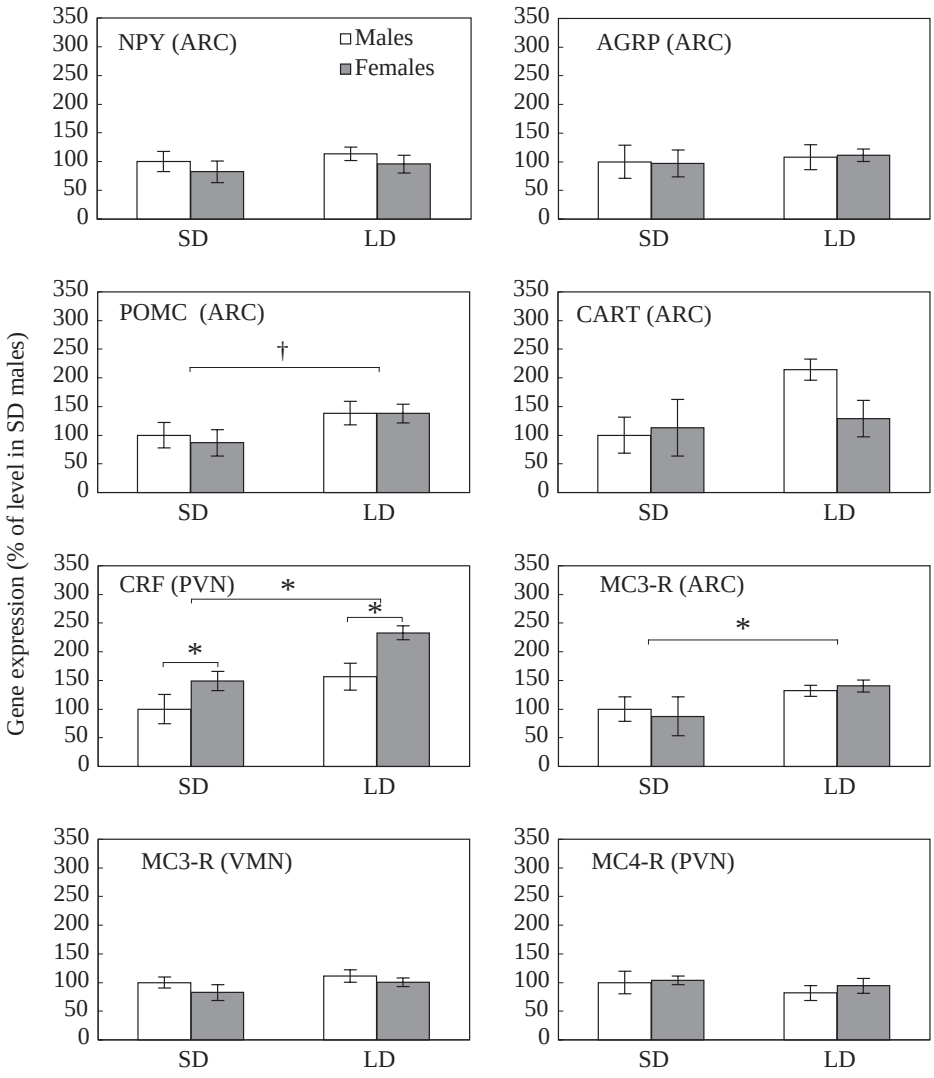

Fig. 5. Effect of 12-week exposure to long-day photoperiod (LD) on hypothalamic neuropeptide and receptor gene expression in male and female bank voles. Data for short-day photoperiod (SD) females and LD individuals are expressed as a percent of the level in SD males, and presented as means ± S.E.M. The effect of photoperiod and sex on gene expression was tested by two-way ANOVA. *Significant difference ($P<0.05$); †difference approached significance at $P=0.06$. See Fig. 4 for abbreviations.

The sensitivity of hypothalamic gene expression to photoperiod in bank voles had similarities to the changes reported for Siberian hamsters. However, three of the mRNA species studied (CART and POMC in the ARC and MC3-R in the VMN) were regulated differently in the two species. There is strong evidence that both POMC and CART are powerful physiological anorexic signals (Kristensen et al., 1998; Lambert et al., 1998). Differential regulation of these neuropeptides in bank voles and hamsters may be related to differing effects of LD on food intake. Bank voles showed no changes in food intake in response to photoperiod (Fig. 2), whereas hamsters exposed to SD had significantly lower food intake than those in LD (Knopper and Boily, 2000; Mercer et al., 2001).

Both bank voles and hamsters exposed to LD had an elevated gene expression of melanocortin receptor MC3-R in the ARC. The role of this receptor in the regulation of energy balance has been demonstrated by the genetic knockout of MC3-R (Chen et al., 2000). Mice lacking MC3-R are characterised by reduced food intake, normal body mass and elevated adiposity relative to wild-type littermates. Bank voles exposed to LD had higher levels of MC3-R mRNA than those in SD, but they had unaltered food intake. This suggests the changes in MC3-R in the ARC

Table 5. *Summary of the effect of long-day photoperiod (LD) on gene expression of hypothalamic neuropeptide and receptor systems in bank voles and Siberian hamsters*

Neuropeptide/receptor	Bank voles	Siberian hamsters[a]	Siberian hamsters[b]
NPY (ARC)	↔	↔	↔
AGRP (ARC)	↔	↔	–
POMC (ARC)	↑[c]	↑	↑
CART (ARC)	↔	↓	–
CRF (PVN)	↑	↑	–
MC3-R (ARC)	↑	↑	–
MC3-R (VMN)	↔	↓	–
MC4-R (PVN)	↔	↔	–

Arrows indicate the lack of change (↔), elevated (↑) or reduced (↓) gene expression relative to short-day photoperiod (SD) individuals. Data for Siberian hamsters were originally presented as gene expression for SD individuals relative to LD individuals.

[a]Mercer et al., 2001; [b]Reddy et al., 1999; [c]the difference approached significance at P=0.06.

NPY, neuropeptide Y; ARC, arcuate nucleus; AGRP, agouti-related protein; POMC, pro-opiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; CRF, corticotrophin-releasing factor; PVN, paraventricular nucleus; MC3-R and MC4-R, melanocortin-3 and -4 receptors; VMN, ventromedial nucleus.

may not be related to short-term energy regulation, which is consistent with recent data on hypothalamic gene expression in LD and SD food-restricted hamsters suggesting that MC3-R expressed in the ARC is not involved in short-term energy homeostasis (Mercer et al., 2001). MC3-R levels in the ARC therefore remain potentially part of a putative coding mechanism for the long-term 'appropriate' body mass. The elevated levels of CRF in the bank voles transferred to LD were probably unrelated to energy regulation but more closely connected to the function of CRF as a regulator of the hypothalamic–pituitary–adrenal axis (Bray et al., 1990). Gender-specific patterns in the neural mechanisms controlling hypothalamic–pituitary–adrenal axis activity, as indicated by sex-differences in the expression of genes encoding CRF in the hypothalamus of rats, have been reported (Patchev et al., 1995; Patchev and Almeida, 1996). In accordance, we found that female voles had higher levels of CRF gene expression in the PVN compared to males. This difference may be due to the role of CRF in the regulation of reproduction (Rivest and Rivier, 1995; Lin et al., 2001), as also indicated by the direct effect of estradiol on CRF gene expression (Vamvakopoulos and Chrousos, 1993, 1994).

Maintenance of photoperiod-induced body mass changes in bank voles were not associated with major changes in activity of hypothalamic neuropeptide and receptor systems involved in short-term energy homeostasis. This suggests that both LD and SD bank voles perceived themselves to be in energy homeostasis, despite their differences in body mass. The energy balance measurements we have made in both states confirm that this was indeed the case. The implication is that seasonal cycles of body mass in bank voles (and Siberian hamsters) reflect changes in the 'appropriate body mass level' around which homeostasis occurs, rather than changes in activity of anabolic and catabolic neuropeptides directly involved in maintaining this homeostasis. The genes involved in coding the appropriate body mass remain obscure, apart from the possible involvement of MC3-R in the ARC.

The main focus of the present paper was the energetic consequences of differences in body mass that were stimulated by a change in photoperiod and how these might relate to differences in hypothalamic gene expression for transcripts known to be linked to short-term energy balance. Our data show that these consequences are undetectable at the resolution of our existing methods for measuring energy balance, and that despite their elevated body mass and body fatness, the voles perceive themselves after a period at LD to be in energy homeostasis. Between the periods of homeostasis, prior to the change in photoperiod and after stability in body mass has been regained, there is a period of dynamic change in body mass (Fig. 1). Our data show that during this phase food intake levels remain unchanged, strongly implicating changes in expenditure (either RMR or physical activity) as the mechanism used to increase body mass. Recent studies of collared lemmings (*Dicrostonyx groenlandicus*) suggest that reductions in RMR facilitate mass gains after photoperiod changes (Powell et al., 2002), consistent with the absence of differences in food intake during this phase in our study. Our future work will focus on this dynamic phase to elucidate in more detail the whole-body energy balance and central signalling mechanisms that underpin the body mass increase.

List of abbreviations

AEAE	apparent energy assimilation efficiency
AGRP	agouti-related protein
ANOVA	analysis of variance
ARC	arcuate nucleus
BAT	brown adipose tissue
BM	body mass
CART	cocaine- and amphetamine-regulated transcript
CRF	corticotrophin-releasing factor
DEE	daily energy expenditure
DLW	doubly-labelled water
DMD	dry mass digestibility
FI	food intake
FM	faeces mass
GE	gross energy
GLM	general linear model
L:D	light:dark
LD	long day
LSR	least-squares regression
NPY	neuropeptide Y
POMC	pro-opiomelanocortin
PVN	paraventricular nucleus

RMR	resting metabolic rate
SD	short day
VMN	ventromedial nucleus
WAT	white adipose tissue

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References

Adam, C. L., Moar, K. M., Logie, T. J., Ross, A. W., Barrett, P., Morgan, P. J. and Mercer, J. G. (2000). Photoperiod regulates growth, puberty and hypothalamic neuropeptide and receptor gene expression in female Siberian hamsters. *Endocrinol.* **141**, 4349-4356.

Anbar, M. and Lewitus, Z. (1958). Rate of body-water distribution studied with triple labelled water. *Nature* **181**, 344.

Barsh, G. S., Farooqi, S. I. and O'Rahilly, S. (2000). Genetics of body-weight regulation. *Nature* **404**, 644-651.

Bartness, T. J., Hamilton, J. M., Wade, G. N. and Goldman, B. D. (1989). Regional differences in fat pad responses to short days in Siberian hamsters. *Am. J. Physiol.* **257**, R1533-R1540.

Blank, J. L. (1992). Phenotypic variation in physical response to seasonal environments. In *Mammalian Energetics: Interdisciplinary Views of Metabolism and Reproduction* (ed. T. E. Tomasi and T. H. Horton), pp. 83-104. New York: Cornell University Press.

Boss-Williams, K. A. and Bartness, T. J. (1996). NPY stimulation of food intake in Siberian hamsters is not photoperiod dependent. *Physiol. Behav.* **71**, 493-501.

Bray, G. A., Fisler, J. and York, D. A. (1990). Neuroendocrine control of the development of obesity: understanding gained from studies of experimental animal models. *Front. Neuroendocrinol.* **11**, 128-181.

Chen, A. S., Marsh, D. J., Trumbauer, M. E., Frazier, E. G., Guan, X. M., Yu, H., Rosenblum, C. I., Vongs, A., Feng, Y., Cao, L. et al. (2000). Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean mass. *Nat. Genet.* **26**, 97-102.

Clarke, J. R. (1985). The reproductive biology of the bank vole (*Clethrionomys glareolus*) and the wood mouse (*Apodemus sylvaticus*). *Symp. Zool. Soc. Lond.* **55**, 33-59.

Dark, J. and Zucker, I. (1983). Short photoperiods reduce winter energy requirements of the meadow vole, *Microtus pennsylvanicus. Physiol. Behav.* **31**, 699-702.

Dark, J. and Zucker, I. (1986). Photoperiodic regulation of body mass and fat reserves in the meadow vole. *Physiol. Behav.* **38**, 851-854.

Field, J., II, Belding, H. S. and Martin, A. W. (1939). An analysis of the relation between basal metabolism and summated tissue respiration in the rat. I. The post-pubertal albino rat. *J. Cell. Comp. Physiol.* **14**, 143-157.

Franklin, K. B. J. and Paxinos, G. (1997). *The Mouse Brain in Stereotaxic Coordinates*. London: Academic Press.

Gosler, A. G., Greenwood, J. J. D. and Perrins, C. (1995). Predation risk and the cost of being fat. *Nature* **377**, 621-623.

Geluso, K. and Hayes, J. P. (1999). Effects of dietary quality on basal metabolic rate and internal morphology of European starlings (*Sturnus vulgaris*). *Physiol. Biochem. Zool.* **72**, 189-197.

Hagan, M. M., Rushing, P. A., Pritchard, L. M., Schwartz, M. W., Strack, A. M., Van der Ploeg, L. H. T., Woods, S. C. and Seeley, R. J. (2000). Long-term orexigenic effects of AgRP-(83-132) involve mechanisms other than melanocortin receptor blockade *Am. J. Physiol.* **279**, R47-R52.

Iverson, S. L. and Turner, B. N. (1974). Winter weight dynamics in *Microtus pennsylvanicus. Ecology* **55**, 1030-1041.

Jeanrenaud, B. and Rohner-Jeanrenaud, F. (2001). Effects of neuropeptides and leptin on nutrient partitioning: dysregulations in obesity. *Annu. Rev. Med.* **52**, 339-351.

Kalra, S. P., Dube, M. G., Pu, S., Xu, B., Horvath, T. L. and Kalra, P. S. (1999). Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocrine Rev.* **20**, 68-100.

Kennedy, G. C. (1953). The role of depot fat in the hypothalamic control of food intake in the rat. *Proc. R. Soc. Lond. B* **140**, 578-592.

Klaus, S., Heldmaier, G. and Ricquier, D. (1988). Seasonal acclimation of bank voles and wood mice: nonshivering thermogenesis and thermogenic properties of brown adipose tissue mitochondria. *J. Comp. Physiol. B* **158**, 157-164.

Knopper, L. D. and Boily, P. (2000). The energy budget of captive Siberian hamsters, *Phodopus sungorus*, exposed to photoperiod changes: Mass loss caused by a voluntary decrease in food intake. *Physiol. Biochem. Zool.* **73**, 517-522.

Koteja, P. (1996). Measuring energy metabolism with open-flow respirometric systems: which design to choose? *Funct. Ecol.* **10**, 675-677.

Krebs, H. A. (1950). Body size and tissue respiration. *Biochim. Biophys. Acta* **4**, 249-269.

Kristensen, P., Judge, M. E., Thim, L., Ribel, U., Christjansen, K. N., Wulff, B. S., Clausen, J. T., Jensen, P. B., Madsen, O. D., Vrang, N. et al. (1998). Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* **393**, 72-76.

Król, E., Johnston, M. S. and Speakman, J. R. (2003). Limits to sustained intake. VIII. Resting metabolic rate and organ morphology of laboratory mice lactating at thermoneutrality. *J. Exp. Biol.* **206**, 4283-4291.

Lambert, P. D., Couceyro, P. R., McGirr, K. M., Dall Vechia, S. E., Smith, Y. and Kuhar, M. J. (1998). CART peptides in the central control of feeding and interactions with neuropeptide Y. *Synapse* **29**, 293-298.

Lin, J., Barb, C. R., Kraeling, R. R. and Rampacek, G. B. (2001). Developmental changes in the long form leptin receptor and related neuropeptides gene expression in the pig brain. *Biol. Reprod.* **64**, 1614-1618.

Marczinski, C., Perrot-Sinal, T. S., Kavaliers, M. and Ossenkopp, K.-P. (1998). Sex differences in spontaneous locomotor activity and rotational behaviour in meadow voles. *Physiol. Behav.* **65**, 387-391.

McDevitt, R. M. and Speakman, J. R. (1994). Central limits to sustainable metabolic rate have no role in cold acclimation of the short-tailed field vole (*Microtus agrestis*). *Physiol. Zool.* **67**, 1117-1139.

McNamara, J. M. and Houston, A. I. (1990). The value of fat reserves and the trade-off between starvation and predation. *Acta Biotheor.* **38**, 37-61.

Mercer, J. G. (1998). Regulation of appetite and body weight in seasonal mammals. *Comp. Biochem. Physiol. C* **119**, 295-303.

Mercer, J. G. and Speakman, J. R. (2001). Hypothalamic neuropeptide mechanisms for regulating energy balance: from rodent models to human obesity. *Neurosci. Biobehav. Rev.* **25**, 101-116.

Mercer, J. G., Lawrence, C. B., Beck, B., Burlet, A., Atkinson, T. and Barrett, P. (1995). Hypothalamic NPY and preproNPY mRNA in Djungarian hamsters: effects of food deprivation and photoperiod. *Am. J. Physiol.* **269**, R1099-R1106.

Mercer, J. G., Lawrence, C. B., Moar, K. M., Atkinson, T. and Barrett, P. (1997). Short-day weight loss and effect of food deprivation on hypothalamic NPY and CRF mRNA in Djungarian hamsters. *Am. J. Physiol.* **273**, R768-R776.

Mercer, J. G., Moar, K. M., Ross, A. W., Hoggard, N. and Morgan, P. J. (2000). Photoperiod regulates arcuate nucleus POMC, AGRP, and leptin receptor mRNA in Siberian hamster hypothalamus. *Am. J. Physiol.* **278**, R271-R281.

Mercer, J. G., Moar, K. M., Logie, T. J., Findlay, P. A., Adam, C. L. and Morgan, P. J. (2001). Seasonally inappropriate body weight induced by food restriction: effect on hypothalamic gene expression in male Siberian hamsters. *Endocrinology* **142**, 4173-4181.

Montague, C. T., Farooqi, I. S., Whitehead, J. P., Soos, M. A., Rau, H., Wareham, N. J., Sewter, C. P., Digby, J. E., Mohammed, S. N., Hurst, J. A. et al. (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **387**, 903-908.

Morgan, P. J. and Mercer, J. G. (2001). The regulation of body weight: lessons from the seasonal animal. *Proc. Nutri Soc.* **60**, 127-134.

Nagy, K. A. (1983). *The doubly labelled water ($^3HH^{18}O$) method: a guide to its use*. UCLA Publication no. 12-1417, University of California, Los Angeles, California, USA.

Nagy, T. R. and Negus, N. C. (1993). Energy acquisition and allocation in

male collared lemmings (*Dicrostonyx groenlandicus*): Effects of photoperiod, temperature and diet quality. *Physiol. Zool.* **66**, 537-560.

Norrdahl, K. and Korpimäki, E. (1998). Does mobility or sex of voles affect risk of predation by mammalian predators? *Ecology* **97**, 226-232.

Patchev, V. K. and Almeida, O. F. X. (1996). Gonadal steroids exert facilitating and 'buffering' effects on glucocorticoid-mediated transcriptional regulation of corticotrophin-releasing hormone and corticosteroid receptor genes in rat brain. *J. Neurosci.* **16**, 7077-7084.

Patchev, V. K., Hayashi, S., Orikasa, C. and Almeida, O. F. X. (1995). Implications of estrogen-dependent brain organization for gender differences in hypothalamo–pituitary–adrenal regulation. *FASEB J.* **9**, 419-423.

Peacock, W. L. and Speakman, J. R. (2001). Effect of high-fat diet on body mass and energy balance in the bank vole. *Physiol. Behav.* **74**, 65-70.

Pistole, D. H. and Cranford, J. A. (1982). Photoperiodic effects on growth in *Microtus pennsylvanicus. J. Mamm.* **63**, 547-553.

Plunkett, S. S., Fine, J. B. and Bartness, T. J. (2000). Photoperiod and gender affect adipose tissue growth and cellularity in juvenile Syrian hamsters. *Physiol. Behav.* **71**, 493-501.

Powell, C. S., Blaylock, M. L., Wang, R., Hunter, H. L., Johanning, G. L. and Nagy, T. R. (2002). Effects of energy expenditure and *Ucp*1 on photoperiod-induced weight gain in collared lemmings. *Obes. Res.* **10**, 541-550.

Reddy, A. B., Cronin, A. S., Ford, H. and Ebling, F. J. P. (1999). Seasonal regulation of food intake and body weight in the male Siberian hamster: studies of hypothalamic orexin (hypocretin), neuropeptide Y (NPY) and pro-opiomelanocortin (POMC). *E. J. Neurosci.* **11**, 3255-3264.

Rivest, S. and Rivier, C. (1995). The role of corticotropin-releasing factor and interleukin-1 in the regulation of neurons controlling reproductive function. *Endocrinol. Rev.* **16**, 177-199.

Ryan, B. F., Joiner, B. L. and Ryan, T. A. (1985). *Minitab Handbook.* Boston, MA: PWS-Kent.

Schmidt-Nielsen, K. (1984). *Scaling: Why Is Animal Size So Important?* pp. 90-98. Cambridge: Cambridge University Press.

Schwartz, M. W., Woods, S. C., Porte, D., Jr, Seeley, R. J. and Baskin, D. G. (2000). Central nervous system control of food intake. *Nature* **404**, 661-671.

Selman, C., Lumsden, S., Bünger, L., Hill, W. G. and Speakman, J. R. (2001). Resting metabolic rate and morphology in mice (*Mus musculus*) selected for high and low food intake. *J. Exp. Biol.* **204**, 777-784.

Speakman, J. R. (1997). *Doubly Labelled Water: Theory and Practice.* London: Chapman and Hall.

Speakman, J. R. (2000). The cost of living: Field metabolic rates of small mammals. *Adv. Ecol. Res.* **30**, 177-297.

Speakman, J. R. and Johnson, M. S. (2000). Relationships between resting metabolic rate and morphology in lactating mice: What tissues are the major contributors to resting metabolism? In *Life in the Cold* (ed. G. Heldmaier and M. Klingenspor), pp. 479-486. Heidelberg: Springer-Verlag.

Speakman, J. R. and McQueenie, J. (1996). Limits to sustained metabolic rate: The link between food intake, basal metabolic rate, and morphology in reproducing mice, *Mus musculus*. *Physiol. Zool.* **69**, 746-769.

Speakman, J. R., Nagy, K. A., Masman, D., Mook, W. G., Poppitt, S. D., Strathearn, G. E. and Racey, P. A. (1990). Interlaboratory comparison of different analytical techniques for determination of oxygen-18 abundance. *Anal. Chem.* **62**, 703-708.

Stanley, B. G., Kyrkouli, S. E., Lampert, S. and Leibowitz, S. F. (1986). Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. *Peptides* **7**, 1189-1192.

Stebbins, L. L. (1984). Overwintering activity of *Peromyscus maniculatus, Clethrionomys gapperi, C. rutilus, Eutamias amoenus* and *Microtus pennsylvanicus.* In *Winter Ecology of Small Mammals.* Special Publication vol. 10 (ed. J. Merritt), pp. 201-213. Pittsburgh: Carnegie Museum Natural History.

Steinlechner, S., Heldmaier, G. and Becker, H. (1983). The seasonal cycle of body weight in the Djungarian hamster: photoperiodic control and the influences of starvation and melatonin. *Oecologia* **60**, 401-405.

Tritos, N. A., Vicent, D., Gillette, J., Ludwig, D. S., Flier, E. S. and Maratos-Flier, E. (1998). Functional interactions between melanin-concentrating hormone, neuropeptide Y, and anorectic neuropeptides in the rat hypothalamus. *Diabetes* **47**, 1687-1692.

Vamvakopoulos, N. C. and Chrousos, G. P. (1993). Evidence of direct estrogenic regulation of human corticotrophin-releasing hormone gene-expression – potential implications for the sexual dimorphism of the stress-response and immune inflammatory reaction. *J. Clin. Invest.* **92**, 1896-1902.

Vamvakopoulos, N. C. and Chrousos, G. P. (1994). Hormonal regulation of human corticotrophin-releasing hormone gene expression: implications for the stress response and immune/inflammatory reaction. *Endocrinol. Rev.* **15**, 409-420.

Ward, S., Scantlebury, M., Król, E., Thomson, P. J., Sparling, C. and Speakman, J. R. (2000). Preparation of hydrogen from water by reduction with lithium aluminium hydride for the analysis of delta H-2 by isotope radio mass spectrometry. *Rapid Comm. Mass Spect.* **14**, 450-453.

Witter, M. S. and Cuthill, I. C. (1993). The ecological costs of avian fat storage. *Phil. Trans. R. Soc. Lond. B* **340**, 73-92.