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Bone marrow fat mobilization in relation to lipid and protein catabolism during prolonged fasting in barn owls

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Abstract To assess the role of bone marrow fat in survival during a period of negative energy balance, we investigated the relationship between the time-course of marrow fat mobilization and the metabolic states associated with body fuel utilization during a prolonged fast. In order to mimic the winter fast of the barn owl (*Tyto alba*), captive birds were subjected to fasts of various durations at 5 °C ambient temperature. Body mass and plasma metabolites were used to determine the metabolic state at the end of fasting. Skeleton composition remained unchanged throughout phase II of fasting, during which the birds essentially rely on lipid fuels. During the following phase III, characterized by an increase in net body protein breakdown, the lipid mass in skeleton marrow decreased sharply by 78%, concomitant with an increase of the bone water content. This marrow fat mobilization occurred in all parts of the skeleton. This observation supports the hypothesis that bone marrow fat is not only involved in local nutrition, but can also be used as a lipid reserve for total energy requirements. However, in contrast to other fat deposits, marrow fat is mobilized only during phase III of the fast, when the last shift from lipid to protein fuel metabolism occurs. Thus, metabolic and/or hormonal changes associated with this transition could be involved in bone marrow fat mobilization. Lastly, our results suggest that the measurement of bone marrow fat can be used as an accurate index of the nutritional status (i.e. phase II or phase III) in barn owls.

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Key words Starvation · Body reserves · Body condition · Survival · Birds

Abbreviations $dm\,m^{-1}\,dt^{-1}$ daily body mass loss per unit body mass · *FFA* free fatty acid

Introduction

Wild animals are often confronted with periods of nutritional stress that can lead to an extensive depletion of their body lipid and/or protein reserves. Among the different adipose deposits, yellow bone marrow, which contains mainly fat cells (Little 1973), is generally considered to be the last one utilized during starvation (for review, see Pond 1978). Consequently, the absence of bone marrow fat has often been used to indicate severe fasting conditions in individuals found dead, both among birds (Hutchinson and Owen 1984; Jeske et al. 1994; Raveling et al. 1978; Ringelman et al. 1992) and mammals (Chan-McLeod et al. 1995; Davis et al. 1987; LaJeunesse and Peterson 1993). However, the presence of marrow fat does not necessarily reflect a good nutritional condition and thus, the "marrow-fat index" is still limited for assessing body condition (Hutchinson and Owen 1984; Mech and Delgiudice 1985). A better understanding of marrow fat mobilization during energy depletion, particularly in relation to changes in lipid versus protein mobilization that occur during prolonged fasting (Belkhou et al. 1991; Goodman et al. 1980; Le Maho et al. 1981; Robin et al. 1988), may permit the establishment of better relationships between bone marrow fat reserves and individual nutritional status.

During a long-term fast, three physiological phases have been determined, corresponding to a differential mobilization of lipid versus protein reserves (Goodman et al. 1980; Le Maho et al. 1981; Robin et al. 1988). After a short phase of adaptation (phase I), most of the energy derives from lipid in phase II, allowing body

protein to be spared. When the fast is prolonged, a further metabolic state is reached (phase III), characterized by an increase in net protein breakdown. This last state, reversible (Handrich et al. 1993; Robin et al. 1988) but critical because proteins are no longer spared, may occur in wild animals, spontaneously during their annual cycle (Cherel et al. 1988a; Robin et al. 1988) or imposed by climatic conditions. Investigations showing relationships between the mobilization of bone lipids and whole body lipid and/or protein reserves during a severe nutritional stress are scarce. The few data available show no significant correlation between marrow fat and whole body lipid content, except for lean animals (Chan-McLeod et al. 1995; Ringelman et al. 1992), which supports a late mobilization of marrow fat. However, in these studies, the physiological phases encountered during starvation were not taken into account.

The main objectives of the present study were therefore: (1) to determine the time-course of marrow fat utilization during a long-term fast; (2) to quantify the distribution and the use of marrow fat in the different parts of the skeleton; (3) to test the hypothesis of a relationship between the utilization of this fat deposit and the physiological state reached during phase III of a prolonged fast. These objectives were achieved as part of a larger study on the changes in body condition of captive barn owl (*Tyto alba*) while fasting in a cold environment. This situation mimics the unfavourable environmental conditions encountered by the wild barn owl in winter, which can lead to an extensive depletion of its body reserves (Marti and Wagner 1985). The composition of the whole skeleton and of different bony compartments was examined in relation to the distinct metabolic phases encountered during a fast.

Materials and methods

Experimental procedure

This study was conducted during the 1994–1995 late winter, on nine captive barn owls originating from our breeding facilities. The birds were housed in individual $0.7 \times 0.8 \times 1.1$ m metabolic cages in a climatic chamber that maintained the natural winter photoperiod and a temperature of $5 \pm 1\,^{\circ}C$. They were acclimatized to the experimental conditions for at least 3 weeks, being provided with freshly killed mice *ad libitum*, at 1500 hours daily. At this time and at 0700 hours, the body mass was determined ($\pm$ 0.1 g).

After the period of acclimatization, birds were subjected to a total fast. Body mass was measured daily at 0700, 1500 and 2300 hours. This division of the 24-h cycle enables a more precise determination of the duration of the different phases of the fast (Handrich et al. 1993). For the calculation of daily body mass loss, the body mass at only one time was used, 1500 hours. The daily body mass loss per unit body mass ($d\mathrm{m}\,\mathrm{m}^{-1}\,d\mathrm{t}^{-1}$) is minimal and stable in phase II and increases during phase III (Le Maho et al. 1981). This criterion was used to determine the time of sacrifice in order to obtain birds in the different metabolic phases of a long-term fast. During phase II, birds were killed at regular 24-h intervals. For the birds fasting until a well-defined phase III, the experiment was stopped at a value of $d\mathrm{m}\,\mathrm{m}^{-1}\,d\mathrm{t}^{-1}$ corresponding to a reversible state of the fast (Handrich et al. 1993). At the end of the fast, birds were killed by cardiac puncture under gas anaesthesia and immediately plucked and frozen until analysis.

Dissection

Skin, adipose tissues and viscera were removed from the body and weighed. The "remains" (i.e. bones and muscles) were separated into six parts: left and right leg, left and right wing and two hemi-carcasses (divided by sagittal plane). For each part, muscles were separated from bones (tendons being included with bones). Thus, for skeleton and for muscles, each individual could be divided into left and right sides, each containing one anterior limb, one posterior limb and one hemi-carcass. Isolated bones and nomenclature of bone compartments used in this study are listed in Table 1. Each compartment was weighed (wet mass), freeze-dried and reweighed (dry mass). During the dissection, some water was lost, corresponding on average to 4.9% of the total wet body mass. A repeated weighing, at each step of the dissection of the separated compartments allowed an accurate correction for this water loss. Corrections were made taking account of the mass and the water content of the different compartments. For the skeleton, this corresponded to a 5% readjustment of the skeletal water mass.

Sampling and biochemical analysis

The excreta were collected daily, as described previously (Handrich et al. 1993), and their nitrogen content was determined on a liquid aliquot by the Kjeldhal method.

Ash content was determined after total combustion in a furnace at 800 °C for 24 h. To limit possible errors due to grinding and aliquot utilization, ash was measured on the whole right side of the skeleton. The lipid content was determined gravimetrically by a method adapted from Folch et al. (1957). Each tissue was extracted twice. For muscles, skin and viscera, homogeneous powder aliquots of 0.5–2 g were used. For the skeleton, the entire left side was used, the bones being directly cracked in the solvent. For the bony compartment, a third extraction was performed after grinding the nearly delipidated bones. Additionally, for the "pelvic bones" compartment (which represents more than a quarter of the skeletal lipid mass, see Results section), the phospholipid content was indirectly measured by a phosphorus determination (Rouser et al. 1969) on the total lipid extract. Phosphorus was converted to phospholipid by multiplying

Table 1 Partitioning of the skeleton

Wing		
Humerus		Pectoral bones
Coracoid	-Shoulder	
Clavicle	-Shoulder	
Scapula	-Shoulder	
Radius, ulna		Pectoral remains
Carpal bones		Pectoral remains
Digits		Pectoral remains
Leg		
Femur		Pelvic bones
Tibiotarse		Pelvic bones
Fibula		Pelvic bones
Tarsometatarse, digits		Pelvic remains
Other bones of the skeleton		Carcass bones

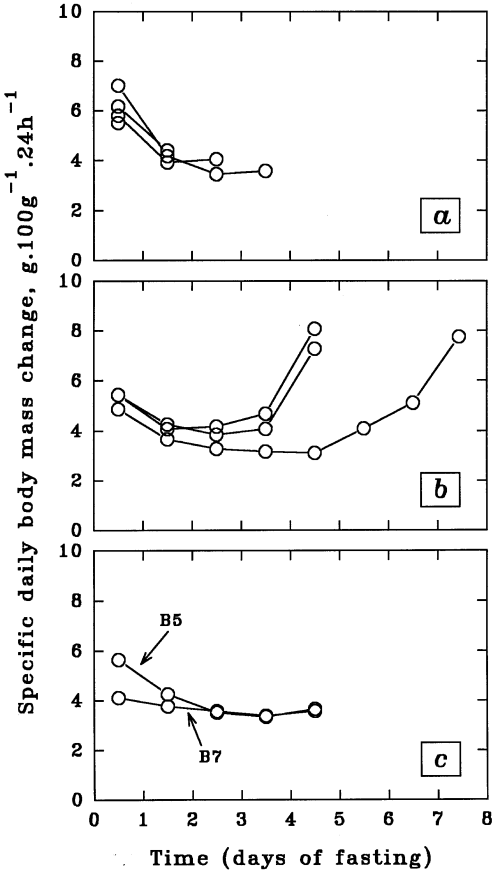

Fig. 1a–c Individual values of specific daily loss in body mass (dm m^{-1} dt^{-1}) during the fast in barn owls. Birds are pooled depending on whether they were, at the end of fasting, in phase II (**a**), phase III (**b**), or a metabolic status that could not be determined using only the body mass changes (**c**)

by 25 (mean phospholipid/phosphorus mass ratio). Protein content was calculated by subtracting ash and lipid content from dry weight.

Uric acid (Sheibe et al. 1974) and free fatty acid (FFA, using C-test Wako kit for nonesterified fatty acid) were assayed on whole plasma. These metabolites accurately reflect, respectively, protein and lipid fuel mobilization during a long-term fast (Cherel et al. 1988b), and are thus helpful indices for the determination of the fasting metabolic states (phase II and phase III). Values measured for fasting captive barn owl by Handrich et al. (1993) were used as references.

Statistical analysis

Data presented in the tables are means ± SEM. Comparisons between phases of the fast were performed using a Mann-Whitney U-test. For comparisons between more than two groups, a Kruskal-Wallis test was used, followed by a Dunn's test if differences were found among groups. For each of the three birds fasting until the well-delimited phase III (Fig. 1b), the values of dm m^{-1} dt^{-1} during phase II were compared using analysis of variance for repeated measures. No significant variation was found [F (3,4) = 0.58, P = 0.66] and an average value of dm m^{-1} dt^{-1} was therefore calculated for each bird. A mean (± SEM, n = 3) value of dm m^{-1} dt^{-1} during phase II was then determined for this group.

Results

Individual metabolic status at the end of the fast

Using the dm m^{-1} dt^{-1} criteria, the nine birds could be distributed into three groups: (1) the birds for which the fast was stopped during phase II, as reflected by the low value of the dm m^{-1} dt^{-1}; (birds B1, B4, B6 and B9; Fig. 1a); (2) the birds fasted until a well-defined phase III (birds B2, B3 and B8; Fig. 1b), in which birds exhibited an increase in dm m^{-1} dt^{-1}, characteristic of phase III (Handrich et al. 1993), rising from 3.9 ± 0.3 g 100 g^{-1} 24 h^{-1} in phase II up to 7.7 ± 0.2 g 100 g^{-1} 24 h^{-1} at the end of the fast; (3) the last two birds, B5 and B7, that had low and stable dm m^{-1} dt^{-1} values at the end of the fast, as during phase II (Fig. 1c). However, because of the relatively long duration of their fast, additional criteria were necessary to confirm the metabolic state (phase II or phase III) reached by these two birds. Measurement of plasma FFA and uric acid indicated that B5 and B7 were at the phase II/phase III transition (Table 2). The nearly total exhaustion of abdominal lipid mass (a good index of phase II/phase III transition, see legend of Table 2) indicated that for B5, the fast was stopped just at the onset of phase III. However, more than 1 g of abdominal lipid indicated that B7 was not yet in phase III at the end of the fasting period.

In summary (Table 2, last column), by using the different criteria we determined that at the end of fasting: (1) five birds (B1, B4, B6, B9 and B7) were in phase II, the last one being at the extreme end of phase II (early transition), and (2) four birds (B2, B3, B8 and B5) were in phase III, the last one being just at the onset of this phase (late transition).

Composition of the whole skeleton

The ash and protein masses and the total wet mass of the skeleton did not change significantly during the fast (Table 3). Conversely, visual analysis of individual values shows that the drop in the lipid mass of the skeleton was very sharp and coincident with the phase II/phase III transition. This drop occurred after about 1.2 g of total nitrogen loss (Fig. 2a), or after 5 days of fasting (Fig. 3a), or below an adiposity of about 4% (Fig. 3b). Lipid and water masses of the skeleton were 4.6-fold lower and 1.4-fold higher, respectively, in the well-defined phase III than in phase II ($P < 0.05$, Table 3). Thus, in phase III, lipid and water accounted for 2% and 43% of the fresh mass of the skeleton versus 10% and 34% in phase II (Table 3). During phase III the increase in water was more progressive than the decrease in lipid (Fig. 2b).

Table 2 Concentration of two plasma metabolites and abdominal lipid mass at the end of the fast for nine captive barn owls

Bird	Uric acid[a]		FFA[b]		Abdominal lipid mass[c]		Final established period of the fast[d]
	mmol l^{-1}	Predicted phase of the fast[d]	mmol l^{-1}	Predicted phase of the fast[d]	g	Predicted phase of the fast[d]	
B1	0.47	PII	1.19	PII	0.24	PII	PII
B2	1.74	PIII	0.16	PI or PIII	—	PIII	PIII
B3	1.86	PIII	0.34	PI or PIII	—	PIII	PIII
B4	0.84	?	0.74	PII or PIII	0.17	PII	PII
B5	0.89	?	1.51	Trans	0.08	Trans	TransL
B6	0.79	?	0.67	PII or PIII	0.17	PII	PII
B7	0.46	PII	2.59	Trans	1.06	PII	TransE
B8	1.67	PIII	0.42	PII or PIII	0.01	PIII	PIII
B9	0.54	PII	0.59	PII or PIII	0.16	PII	PII

[a] Reference values: phase II, 0.34 ± 0.08 (SD) mmol l^{-1}; phase III, 1.75 ± 0.13 (SD) mmol l^{-1} (from Handrich et al. 1993)
[b] Reference values: phase I, 0.18 ± 0.09 (SD) mmol l^{-1}; progressive increase during phase II with a more than 1.3 ± 0.3 (SD) mmol l^{-1} level reached at the phase II/phase III transition; sharp drop during phase III until value lower than at the beginning of the fast (from Handrich et al. 1993)
[c] Mass of abdominal fat nearly or completely exhausted in phase III (Dewasmes et al. 1980; S. Massemin, Y. Handrich, Y. Le Maho, unpublished data)
[d] Fast stopped while birds in phase II (*PII*), phase III (*PIII*) or just at the phase II/phase III transition (*Trans*). For this last period, early (*TransE*) or late (*TransL*) transition can be distinguished

Table 3 Whole skeleton composition of fasting captive barn owls before the phase II/phase III transition (*PII*, $n = 5$) and while in a well-defined phase III (*PIII*, $n = 3$)[b]

		PII	PIII
Mass, g	Fresh mass	42.92 ± 1.63	48.69 ± 2.79
	Dry mass	28.23 ± 1.11	27.50 ± 1.26
	Water	14.68 ± 0.57 [a]	21.19 ± 1.54
	Total proteins	12.01 ± 0.47	13.45 ± 0.84
	Total lipids	4.48 ± 0.20 [a]	0.98 ± 1.15
	Total ash	11.74 ± 0.45	13.07 ± 0.35
Dry content, %	Protein	42.55 ± 0.11 [a]	48.84 ± 0.99
	Lipid	15.87 ± 0.22 [a]	3.54 ± 0.39
	Ash	41.59 ± 0.27 [a]	47.62 ± 1.36

[a] $P < 0.05$ significantly different between groups
[b] Because it was just at the onset of phase III when the fast was stopped, bird B5 was not included in the calculation. Values are means ± SEM

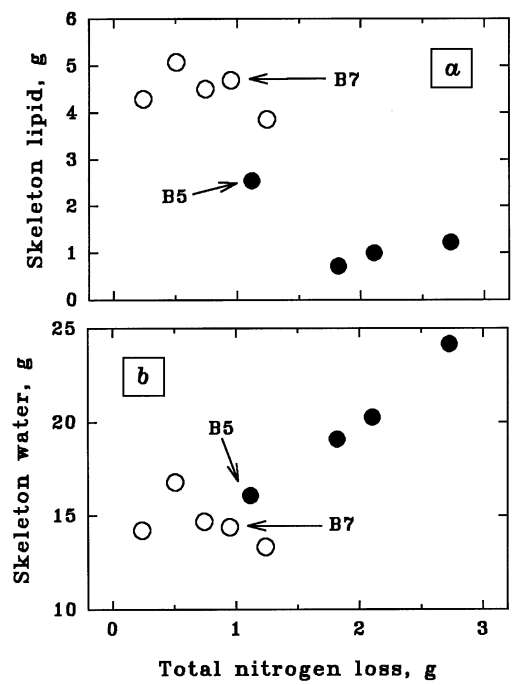

Fig. 2a, b Relationship between the skeletal lipid (**a**) or water (**b**) masses and the total nitrogen loss in barn owls at different stages of the fast. Nitrogen loss was used as a reflection of the development of the fast. *Arrows* indicate birds killed while in early (*B7*) or late (*B5*) phase II/phase III transition

Composition of the different bony compartments in phase II

Among the six bony compartments (Table 4), the humerus had the highest ash and lowest lipid content ($P < 0.05$ vs all the other bony compartments). The "pectoral remains" compartment had the highest lipid content (30%, i.e. 22 times more than the humerus). Because of their differences in mass and composition, bones contributed differently to the composition of the total skeleton. Thus, the humerus contributed less than 1% (0.70 ± 0.04%), while "pectoral remains" with 39.8 ± 2.1% made up the largest fraction of the skeletal lipid mass ($P < 0.05$).

Changes of bone composition in phase III

Consistent with the lack of change in the total protein and ash mass of the skeleton between phase II and phase III (Table 3), the protein and ash mass of the

different bony compartments did not change significantly during the fast. In contrast, the lipid mass decreased for all compartments, e.g. by up to 11-fold in the "pelvic bones" (Table 4). The fractional loss of lipids from phase II to phase III in the different bony compartments ranged from 45% (carcass bones) to 92% (pelvic bones; Table 5). Lipids were depleted more in proximal bones than in distal bones. Within proximal and distal bones, leg bones were more depleted than wing bones (Table 5). Due to its high lipid level (Table 4) the "pectoral remains" compartment contributed more than one-third to the mobilization of skeletal lipids (Table 5), although 47% of the lipids remaining in the skeleton in phase III were in this bony compartment.

In the "pelvic bones", the phospholipid mass was not significantly different between phase II and the well-defined phase III (Mann-Whitney; $U = 3$, $P > 0.1$).

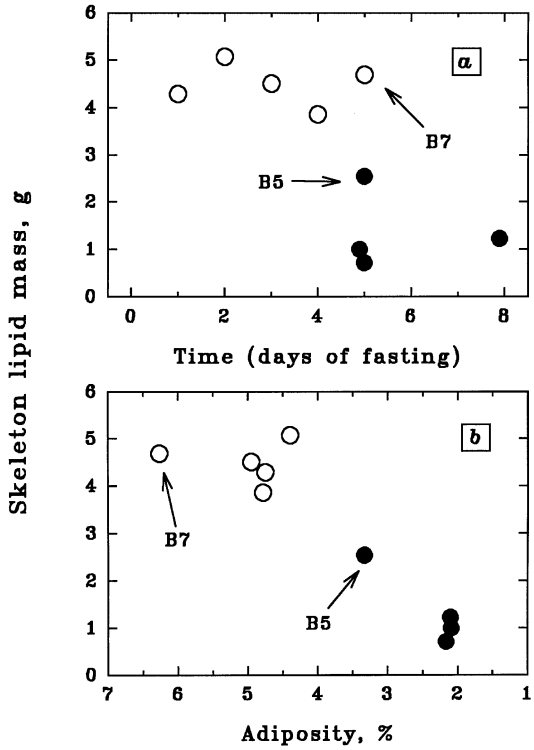

Fig. 3a, b Relationship between the skeletal lipid mass and (**a**) the duration of fasting or (**b**) the adiposity (total lipid as percentage of body mass minus digestive tract content) for barn owls at different stages of the fast. *Arrows* indicate birds killed while in early (*B7*) or late (*B5*) phase II/phase III transition

Discussion

Changes in skeletal composition during a long-term fast

This study demonstrates the constancy of lipid and water masses in the skeleton until the end of phase II of

Table 5 Loss of lipids and contribution to the total skeletal lipids loss from different bones or bony compartments in fasting captive barn owls. Paired bones or bony compartments were combined. Values were obtained from the difference between mean values in phase II ($n = 5$) and phase III ($n = 3$)

	Lipid loss, g	Fractional loss, %	Contribution to total loss, %
Humerus	0.02	61.4	0.6
Shoulder	0.23	88.5	6.6
Pectoral remains	1.28	72.3	36.6
Pelvic bones	1.09	92.3	31.2
Pelvic remains	0.71	81.9	20.3
Carcass bones	0.17	45.2	4.8
Total skeleton	3.50	78.1	–

Table 4 Dry composition of different skeletal compartments of fasting captive barn owls, during phase II (*PII*, $n = 5$) or while in a well-defined phase III (*PIII*, $n = 3$)

Skeletal compartments	Phase	Dry mass, g	Dry mass content, %		
			Protein	Lipid	Ash
Humerus	PII	2.28 ± 0.07[a]	37.27 ± 0.18	1.37 ± 0.11[a]	61.36 ± 0.20
	PIII	2.61 ± 0.11	37.40 ± 0.36	0.47 ± 0.02	62.13 ± 0.34
Shoulder	PII	1.38 ± 0.08	39.45 ± 0.77[a]	18.84 ± 0.89[a]	41.71 ± 0.20[a]
	PIII	1.25 ± 0.03	46.11 ± 0.88	2.41 ± 0.47	51.48 ± 0.41
Pectoral remains	PII	5.95 ± 0.13	33.03 ± 0.86[a]	29.78 ± 1.02[a]	37.18 ± 0.48[a]
	PIII	5.59 ± 0.56	45.78 ± 1.95	8.34 ± 2.19	45.88 ± 3.65
Pelvic bones	PII	4.78 ± 0.20[a]	34.28 ± 0.62[a]	24.71 ± 1.10[a]	41.01 ± 0.74[a]
	PIII	3.91 ± 0.10	41.94 ± 0.84	2.34 ± 0.46	55.72 ± 0.40
Pelvic remains	PII	6.11 ± 0.29	53.70 ± 0.84[a]	14.08 ± 1.00[a]	32.22 ± 0.42[a]
	PIII	5.74 ± 0.39	60.25 ± 1.02	2.76 ± 0.23	36.99 ± 0.86
Carcass bones	PII	7.74 ± 0.39	48.39 ± 0.49	4.73 ± 0.28[a]	46.88 ± 0.45
	PIII	8.40 ± 0.27	50.09 ± 1.48	2.40 ± 0.07	47.51 ± 1.55

[a] $P < 0.05$, significantly different from phase III
Paired bony compartments combined. Values are means ± SEM

fasting. These results support the suggestion of Groscolas et al. (1991), for spontaneously fasting great-winged petrels, that the skeleton does not contribute to the whole body loss of water and lipid during this phase. In contrast, beyond the phase II/phase III transition, there is a sharp decrease of skeleton lipid along with an increase of water. This is consistent with previous studies in mammals (Neiland 1970) and may be ascribed to the confining of the marrow in a rigid cavity.

Several previous investigators argued for an involvement of marrow fat in local nutrition (mainly for erythropoietic processes) rather than as an energy reserve for body requirements during a fast (Tavassoli 1974; Tavassoli et al. 1977; Tran et al. 1981). It seems clear that marrow fat plays an important part in erythropoietic processes, especially in the red marrow (see references in Rozman et al. 1990). However, the present study, as well as other reports on birds (Hutchinson and Owen 1984), mammals (Chan-McLeod et al. 1995) and fish (Phleger et al. 1995), have shown that, during a severe nutritional stress, an extensive breakdown of this lipid reserve occurs in all bones, even in those normally containing almost exclusively yellow marrow. In support of a role of marrow fat as an energy source during fasting is the loss of marrow fat without a corresponding increase in erythropoietic mass during starvation in mammals (Evans et al. 1954). Thus, our results strongly support the hypothesis that marrow fat can be mobilized like other adipose tissues during a fast, to provide an energy source for the whole body and is not used only for local nutrition.

The marrow fat breakdown is late and associated with the last shift of metabolic state encountered during a prolonged fast

Whereas body lipids have been shown to represent the main source of energy during phase II of the fast (Cherel et al. 1988a), bone marrow fat is only mobilized in phase III. This agrees with studies on other birds (Hutchinson and Owen 1984; Ringelman et al. 1992) and mammals (Chan-McLeod et al. 1995), which found no correlation between bone marrow and whole body lipids except for lean individuals. While not associated with a complete exhaustion of body lipid reserves (Belkhou et al. 1991; Robin et al. 1988), the triggering of phase III seems to depend on a threshold of lipid depletion (Cherel et al. 1992). Marrow fat mobilization, which begins at the phase II/phase III transition, appears to depend on this threshold of body lipids. The increase in net protein breakdown in phase III has been assumed to be the consequence of a diminution of fatty acid release by major fat deposits (Groscolas 1990). It is possible that bone marrow fat begins to be catabolized in order to help to maintain the fatty acid flux above a minimum level.

The delay in the breakdown of marrow fat until the beginning of phase III leads one to question the physiological and biochemical mechanisms involved in this mobilization. Our study shows the greatest decline to occur in the proximal part of the wing and the leg compared to distal ones, as already observed in other birds (Hutchinson and Owen 1984) and mammals (Davis et al. 1987; LaJeunesse and Peterson 1993; Peterson et al. 1982). Circulatory differences within capillary networks in colder extremities have been suggested to explain the pattern of fat mobilization between bones (Peterson et al. 1982). Similarly, since the rate of blood flow influences the release of fatty acid from adipose tissues (Blaak et al. 1995), an increase in blood flow from a low level in phase II to a higher level in phase III, in bones, could be proposed to explain the late lipid mobilization from the marrow compared to other adipose deposits. However, mechanisms that would be involved in such changes of blood flow at the phase II/phase III transition remain to be determined. Biochemical mechanisms associated with fatty acid release from adipose tissues may also explain the late catabolism of marrow fat compared to other adipose deposits. In the barn owl (S. Massemin, Y. Handrich, Y. Le Maho, unpublished data), as in other species (Pond 1978), individual fat deposits are not mobilized with the same time-course during food deprivation. For example, abdominal adipose tissue is utilized before the subcutaneous, while the marrow fat depot is mobilized last. Several studies have shown that, in a single species, differences exist in the control of lipolysis between adipose tissues from different localities (Östman et al. 1979; Tran et al. 1981). In mammals, these differences are mainly related to the balance between alpha-and beta-adrenoreceptors, i.e. between the antilipolytic and lipolytic sensitivity, respectively (Östman et al. 1979). A weaker response to lipolytic agents has been shown in the marrow fat deposit compared to the omental fat pad (Tran et al. 1981). Thus, a high concentration of lipolytic hormones might be necessary to stimulate the mobilization of lipids from bone marrow, explaining the late mobilization of this deposit. Actually, a rise in the plasma concentration of glucagon, the main lipolytic hormone in birds, has been observed during phase III in fasting birds (Cherel et al. 1988b; Le Ninan et al. 1988). Endocrine changes associated with the phase II/phase III transition might therefore trigger the mobilization of bone marrow fat deposits.

Marrow fat and individual survival

Our results make it clear that marrow adipose tissue is involved in the energy supply of barn owls during severe nutritional stress. Previous data show that, in this species, about 7 g of total lipids are used during the reversible phase III of the fast (C. Thouzeau unpublished work). From the present study, it appears that

the skeleton then contributes almost 50% of the lipid fuel supply during this phase. As a result, a significant part of the total energy expenditure of phase III (33%) is provided by marrow fat oxidation. Clearly, in contrast to previous suggestions (Tavassoli 1974; Tran et al. 1981), bone marrow fat appears to be an energy storage tissue, involved in the energetic balance of the fasting organism. For the 213 kJ $24h^{-1}$ daily energy expenditure previously measured in fasting captive barn owls during winter (Handrich et al. 1993), and based on a 39.3 kJ g^{-1} energy content of lipid (Schmidt-Nielsen 1990), it can be estimated that the use of bone marrow fat (3.5 g) would meet the energy requirements of these birds for less than 1 day.

The marrow fat content has often been used as an index of body condition. We agree with Mech and Delgiudice (1985), who stated that the marrow fat criterion is limited as an indicator of body condition, since it does not reflect the whole body lipid content of the individual. However, as shown in the present study, the changes in marrow fat are related to the metabolic status during a long-term fast. This establishes a precise relationship between bone lipids and body reserves: a barn owl with a still high marrow fat content, even during a fasting period, has not yet reached the critical phase III of the fast. In studies of the nutritional condition of wild birds found dead, this criterion could therefore be very helpful in assessing the nutritional status, particularly so if scavengers have partly consumed the body, making it impossible to analyse the body composition.

In conclusion, we provide strong evidence that bone marrow fat is involved in the total energy supply of a fasting individual, but this is only when the last shift from lipid to protein catabolism occurs, i.e. when phase III of the fast is reached. This allows the use of the marrow fat content as an accurate index of the phase II/phase III transition. Metabolic and/or hormonal changes associated with this transition could be involved in the mobilization of fatty acid from marrow fat deposits. Further investigations are required to test this hypothesis.

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