Ecology, 86(11), 2005, pp. 2881–2888

NUTRITION, PHYSIOLOGY, AND STABLE ISOTOPES: NEW INFORMATION FROM FASTING AND MOLTING PENGUINS

YVES CHEREL,[1,4] KEITH A. HOBSON,[2] FRÉDÉRIC BAILLEUL,[1] AND RENÉ GROSCOLAS[3]

[1]*Centre d'Etudes Biologiques de Chizé, UPR 1934 du CNRS, BP 14, 79360 Villiers-en-Bois, France*
[2]*Prairie and Northern Wildlife Research Centre, Environment Canada, Saskatchewan S7N 0X4 Canada*
[3]*Centre d'Ecologie et Physiologie Energétiques, CNRS, 67087 Strasbourg Cedex 02, France*

Abstract. Stable isotopes are increasingly used in animal ecology, but little attention has been paid to the underlying physiological processes accounting for changes in $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios, for example, the influence of protein balance on $\delta^{15}N$ values. We investigated a ''professional'' faster, the King Penguin (*Aptenodytes patagonicus*), to test the effect of long-term food deprivation on the isotopic signature of tissues that can be nondestructively sampled, i.e., blood and feathers. Fasting for 25 days induced a tissue ^{15}N enrichment, thus leading to a moderate increase in the apparent trophic levels of penguins. As expected, ^{15}N enrichment was higher in tissues with high protein turnover rates (e.g., plasma, 0.70‰) than in those with low turnover rates (e.g., blood cells, 0.24‰). Fasting decreased the $\delta^{13}C$ value of plasma, which was due to an increase in its lipid content, as indicated by a concomitant rise in plasma C/N ratio. Finally, food deprivation induced a ^{15}N enrichment in keratin (1.68‰), as indicated by the lower nitrogen signature for portions of new feathers that were synthesized at sea than for those parts grown on land, thus illustrating the different pathways for resource allocation (dietary vs. endogenous reserves) in molting birds. The study also emphasized the usefulness of collecting whole blood (or blood cells) in the field to overcome both the fasting and lipid effects observed in plasma.

Key words: Aptenodytes patagonicus; *blood cells; carbon isotopes; dietary vs. endogenous reserves; enrichment factors; fasting; feather; King Penguin; nitrogen isotopes, plasma; resource allocation; whole blood.*

INTRODUCTION

The measurement of ratios of stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) is a powerful tool in ecology and is increasingly used to explore ecosystem-level energy flows and to describe diet reconstruction and animal movement patterns (Gannes et al. 1998, Hobson 1999, Kelly 2000). For example, nitrogen in the protein of consumers is typically enriched in ^{15}N relative to dietary nitrogen; consequently, $\delta^{15}N$ values of animals increase with their trophic position. In this context, interpretation of $\delta^{13}C$ and $\delta^{15}N$ values relies on the premise that these measurements are primarily determined by the diet of consumers. Application of the methodology, however, is dependent on physiological and biochemical assumptions that are rarely taken into account and tested; hence, the call for more ecophysiological works in controlled conditions to investigate them (Gannes et al. 1997, 1998, Rubenstein and Hobson 2004).

Many wild animals fast in connection with breeding, molting, or migration (Mrosovsky and Sherry 1980). Using their endogenous nutrient stores, fasting animals literally ''feed on themselves.'' Because ^{14}N is excreted preferentially, proteins, in theory, must be progressively enriched in ^{15}N without N equilibrium with food inputs (Gannes et al. 1997). A main consequence of this is that $\delta^{15}N$ values of fasting animals are dependent not only on their food sources and trophic levels, but also on their nutritional status. On one hand, the method shows considerable promise as a diagnostic tool to estimate animals' body condition (Gannes et al. 1998); on the other hand, the fasting effect can lead to erroneous interpretations of $\delta^{15}N$ values within the context of feeding ecology. Although many studies have discussed a possible fasting effect on the interpretation of data collected in the field (Best and Schell 1996, Polischuk et al. 2001), only one work has investigated this topic in endotherms (Hobson et al. 1993).

Our purpose was to quantify ^{15}N enrichment in tissues of a well-known ''professional'' faster, the King Penguin *Aptenodytes patagonicus*, a good model animal with which to describe the fasting metabolic adaptations in endotherms (Cherel and Le Maho 1985, Cherel et al. 1988*a*, *b*). Due to spatial segregation between their foraging grounds at sea and breeding habitats on land, seabirds have a feasting and fasting way of life, and, among seabirds, penguins are the best suited to fast during the weeks and months when they breed and molt (see Plate 1). We focused on two tissues, blood and feathers, because they can be sampled easily and nondestructively in the field. Moreover, due to different turnover times, they integrate different periods of in-

Manuscript received 8 April 2005; revised 13 May 2005; accepted 18 May 2005. Corresponding Editor: T. D. Williams.
[4] E-mail: cherel@cebc.cnrs.fr

PLATE 1. A pair of King Penguins defending their breeding territory in the Crozet Islands, southern Indian Ocean. Photo credit: Y. Cherel.

formation. Blood cells and whole blood represent a period of a few weeks in small endotherms and a few months in larger bodied animals, whereas plasma typically represents information over a period of a few days to a week (Hobson and Clark 1992*a*, Hobson and Clark 1993, Hilderbrand et al. 1996, Haramis et al. 2001, Bearhop et al. 2002). On the other hand, feathers reflect the diet of birds at the time they were grown, because keratin is inert after synthesis (Hobson and Clark 1992*a*, Bearhop et al. 2002, Pearson et al. 2003).

We compared in detail the isotopic signature of various blood components (whole blood, plasma, and blood cells) to critically assess which tissue is more practically appropriate to collect in the field for investigating animals' trophic relationships. We considered the effect of fasting on $\delta^{15}N$ values, the effect of lipids (as measured through the C/N ratio) on the $\delta^{13}C$ value of blood, and also the need to separate blood into its cellular and plasma constituents.

We predicted that ^{15}N enrichment due to fasting should be higher in tissues with high protein turnover rates (e.g., plasma) than in those with low turnover rates (e.g., blood cells and whole blood). During the natural incubating fast of Ross's Goose *Chen rossii*, Hobson et al. (1993) found a higher ^{15}N enrichment in the liver than in muscle, tissues that are known to have a high and low rate of protein turnover, respectively (Cherel et al. 1991). Secondly, we expected that $\delta^{15}N$ values should be lower in the distal (earliest grown) region of feathers than in the remaining part of the feathers grown on land during the fast. Penguins fast when molting, but synthesis of new feathers begins at sea (Cherel et al. 1994). We consequently hypothesized that distal regions of new feathers were built up using exogenous (at sea) food sources with comparatively lower $\delta^{15}N$ values, whereas proximal regions of feathers were grown with endogenous sources of N, resulting in a relative ^{15}N enrichment due to fasting. The stable isotope methodology for molting penguins thus has the potential to investigate resource allocation during feather synthesis to shed new light on the old problem of quantifying to what extent nutrient needs are met by relative contributions from food and endogenous reserves during molt in birds (Murphy 1996).

MATERIALS AND METHODS

Fieldwork was carried out on the Ile de la Possession, Crozet Archipelago (46°25′ S, 51°45′ E) during the austral spring 2001 and winter 2002 in the colony of La Baie du Marin. We randomly chose 10–12 King Penguins for each of the following groups.

1) *Fasting breeding adults (longitudinal study).* Male birds were captured just after their arrival in the colony to breed. They were kept in an outdoor fenced

TABLE 1. Body mass and plasma metabolite concentrations (mean ± SD) in King Penguins.

Group	*n*	Body mass (kg)	Plasma metabolites (mmol/L)		
			Uric acid	β-hydroxybutyrate	Glucose
Fasting breeding adults					
Day 0	12	14.48 ± 0.59	0.48 ± 0.28	0.69 ± 0.26	12.87 ± 0.90
Day 25	12	10.37 ± 0.51	0.28 ± 0.12	1.36 ± 0.41	13.20 ± 1.18
Fasting molting adults					
Beginning	10	16.34 ± 0.97	0.39 ± 0.14	0.60 ± 0.13	11.40 ± 0.83
End	10	9.43 ± 0.53	0.16 ± 0.06	1.85 ± 0.52	11.36 ± 0.72
Underfed winter chicks					
Beginning	12	9.93 ± 0.70	1.06 ± 0.24	0.34 ± 0.10	16.00 ± 1.14
End	11	4.66 ± 0.73	0.34 ± 0.29	1.78 ± 0.66	10.89 ± 0.74
Molting chicks	10	11.06 ± 1.21	0.55 ± 0.19	1.44 ± 0.67	11.99 ± 0.85
Chick-rearing adults	10	13.56 ± 1.28	0.60 ± 0.21	0.92 ± 0.35	12.04 ± 2.06

area for 25 days, and then released. In the colony, males fast for 5–6 weeks at the beginning of the breeding cycle and they are able to fast >50 days in captivity (Cherel et al. 1988*b*).

2) *Fasting molting adults (transversal study).* One group of birds was caught on the beach as soon as they arrived on land to molt. In penguins, new and old feathers remain physically attached until mid-molt. Consequently, we collected 10–15 old body feathers (synthesized one year earlier), together with the distal region of new feathers that was synthesized at sea. This region of the feather also includes an ephemeral and protective sheath. A second group of birds was caught at the end of molt, when new feathers had reached their full length out of the skin and old feathers had been lost. We also collected 10–15 new body feathers on those birds. In King Penguins, the whole molting process lasts about three weeks, and birds can fast ~40 days at that time (Cherel et al. 1988*a*).

3) *Chick-rearing adults.* Birds were caught on arrival from the sea, before feeding their chicks in spring. Stomach contents were collected and analyzed following Cherel and Ridoux (1992).

4) *Molting chicks*. In spring, King Penguin chicks synthesize a waterproof adult-like plumage before going to sea. We collected old down (synthesized in summer 2001) and new feathers from those animals.

5) *Underfed winter chicks (transversal study).* Two groups of chicks were caught, at the beginning (early May) and end (early September) of the austral winter 2002. During winter, chicks are rarely fed by their parents and can fast for five months (Cherel et al. 1987).

In each group, birds were weighed and a blood sample was taken from a flipper vein with heparinized syringes (at days 0 and 25 in captive males). Most of the blood was subsequently centrifuged in order to obtain plasma and blood cells, together with whole blood, for each sample. Tissues (blood, feathers, and stomach contents) were kept at −20°C until isotopic and biochemical analysis. Nutritional status of penguins was assessed through measurement of their body mass and the determination of plasma uric acid (the main nitrogen excretory product in birds) and β-hydroxybutyrate (a ketone body), which are good indices of protein and lipid utilization, respectively (Cherel and Le Maho 1985, Cherel et al. 1988*a*, *b*). Determination of plasma metabolites (uric acid, β-hydroxybutyrate, and glucose) followed the methods described in Cherel and Le Maho (1985).

For isotopic analysis, blood components and a subsample of each stomach content were freeze-dried and powdered. Due to the high lipid content of King Penguin food (Cherel and Ridoux 1992), lipids were removed from dietary samples using a Soxhlet apparatus with chloroform solvent for 4–6 hours. Feathers were cleaned of surface contaminants using a 2:1 chloroform : methanol rinse, air-dried, and cut with stainless steel scissors into small fragments.

Relative abundance of stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in homogenized samples were determined by continuous-flow isotope-ratio mass spectrometry. Results are presented in the usual notation relative to PDB belemnite and atmospheric N_2 (Air) for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards indicate measurement errors of ±0.1‰ and ±0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Values are means ± SD. Data were analyzed using SYSTAT 9 for WINDOWS (Wilkinson 1999).

RESULTS

Nutritional status

During the 25-day male breeding fast, adult molting fast, and the long winter period of undernutrition of the chicks, the body mass of King Penguins decreased by 28%, 42%, and 53%, respectively (Table 1). During these periods, plasma uric acid decreased (for breeding birds, paired-samples *t* test, $t = 3.09$, $P = 0.010$; for molting birds and chicks, two-sample *t* tests, $t = 4.89$ and 6.55, respectively, both $P < 0.0001$) and plasma β-hydroxybutyrate increased ($t = 6.17$, 7.32 and 7.52, respectively, all $P < 0.0001$). Plasma glucose did not change during the adult breeding ($t = 1.33$, $P = 0.209$)

TABLE 2. Stable isotopic signature and C/N ratio of plasma, blood cells, and whole blood of King Penguins (mean ± SD).

Group	*n*	$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)
		Plasma	Blood cells	Whole blood	Plasma
Fasting breeding adults					
Day 0	12	10.33 ± 0.39	9.91 ± 0.20	9.95 ± 0.24	−23.55 ± 0.56
Day 25	12	11.03 ± 0.29	10.15 ± 0.20	10.25 ± 0.16	−24.26 ± 0.50
Fasting molting adults					
Beginning	10	10.05 ± 0.22	9.50 ± 0.27	9.65 ± 0.23	−23.11 ± 0.28
End	10	9.67 ± 0.44	9.81 ± 0.41	9.82 ± 0.44	−23.61 ± 0.67
Underfed winter chicks					
Beginning	12	10.97 ± 0.46	10.49 ± 0.24	10.64 ± 0.29	−22.83 ± 0.43
End	11	10.75 ± 0.38	10.73 ± 0.32	10.71 ± 0.37	−23.83 ± 0.48
Molting chicks	10	9.88 ± 0.31	9.79 ± 0.23	9.76 ± 0.19	−24.03 ± 0.53
Chick-rearing adults	10	10.10 ± 0.27	9.66 ± 0.28	9.66 ± 0.31	−23.33 ± 0.60

and molting ($t = 0.13$, $P = 0.901$) fasts, but it was lower at the end than at the beginning of the chick winter fast ($t = 12.60$, $P < 0.0001$). Molting chicks and chick-rearing adults had moderate levels of plasma uric acid and moderate (adults) to high (chicks) levels of plasma β-hydroxybutyrate (Table 1).

Isotopic signature and C/N ratio of blood components

Overall, the mean value (and variance) in C/N ratio was higher in plasma than in blood cells ($n = 87$; 4.71 ± 0.42, range 3.95–6.04 for plasma, vs. 3.36 ± 0.02, range 3.32–3.45 for blood cells; paired samples *t* test, $t = 29.97$, $P < 0.0001$). Plasma was also consistently depleted in ^{13}C when compared to blood cells ($t = 30.71$, $P < 0.0001$), the difference averaging −1.91‰ ± 0.58‰ (Table 2). Interestingly, and as illustrated for the group of birds with the largest range in plasma $\delta^{13}C$ values and C/N ratios (winter chicks), the former parameter is linearly inversely related to the latter in the three groups of fasting birds (Fig. 1).

The isotopic signatures of plasma, blood cells, and whole blood and their C/N ratio differed significantly ($n = 87$, all $P \leq 0.002$; Table 2). The isotopic signature and C/N ratio of whole blood were intermediate between those of plasma and of blood cells, but they were much closer to those of blood cells than to those of plasma, as illustrated in Fig. 1 for winter chicks. Consequently, the average absolute differences in $\delta^{13}C$ and $\delta^{15}N$ values and C/N ratio were smaller between whole blood and cells than between whole blood and plasma (for $\delta^{13}C$, 0.28‰ ± 0.17‰ vs. 1.63‰ ± 0.52‰; for $\delta^{15}N$, 0.05‰ ± 0.15‰ vs. 0.30‰ ± 0.37‰; for C/N ratio: 0.15 ± 0.06 vs. 1.19 ± 0.40; $t = 23.21$, 5.86 and 24.19, respectively, all $P < 0.0001$).

Fasting and blood isotopic signature

Fasting for 25 days increased $\delta^{15}N$ values of both plasma and blood cells of adult King Penguins (paired-samples *t* tests, $t = 6.15$ and 7.17, respectively, both $P < 0.0001$), but the increase was higher in plasma than in blood cells (0.70‰ ± 0.39‰ vs. 0.24‰ ± 0.12‰, $t = 3.53$, $P = 0.005$; Table 2). However, the increase in blood cell (and in whole blood) $\delta^{15}N$ was minimal and within the measurement precision of the method. Food deprivation for 25 days also decreased $\delta^{13}C$ values in plasma and increased the plasma C/N ratio ($t = 6.77$ and 4.83, $P < 0.0001$ and $P = 0.001$, respectively), whereas values of blood cells remained unaffected ($t = 0.91$ and 0.13, $P = 0.381$ and 0.903).

The adult molting fast and the long period of undernutrition of chicks during winter did not significantly increase the ^{15}N content of plasma and blood cells. On the other hand, they induced a decrease in plasma $\delta^{13}C$ values (two-sample *t* tests, $t = 2.20$ and 5.26, $P = 0.041$ and $P < 0.0001$, respectively) and an increase in plasma C/N ratio ($t = 4.81$ and 5.46, both $P < 0.0001$). There was, however, no impoverishment of blood cells in ^{13}C during either the molting fast or the winter fast, and the C/N ratio of blood cells remained essentially unchanged at that time (Table 2).

Feather isotopic signature

Distal regions of new adult feathers that were synthesized at sea had a different isotopic signature than old feathers of the same individuals (paired-samples *t* tests, $t = 13.29$ and 6.18 for $\delta^{15}N$ and $\delta^{13}C$, respectively, both $P < 0.0001$), with the distal region being depleted in both ^{15}N (−1.68‰ ± 0.40‰) and ^{13}C (−1.13‰ ± 0.58‰) (Table 3). The distal region of feathers differed in the same way with adult new feathers and chick new feathers that were synthesized in spring 2001, and the signature of this region was also different from that of chick down from the previous summer (two-sample *t* tests, all $P < 0.0001$). On the other hand, little variation occurred in the isotopic signature of 2000 and 2001 adult feathers, and between adult and chick feathers. The three groups had identical $\delta^{13}C$ values (ANOVA, $F_{2,27} = 0.15$, $P = 0.859$), and almost identical $\delta^{15}N$ values (ANOVA, $F_{2,27} = 4.53$, $P = 0.020$), the only significant difference being between chick feathers and adult old feathers (Tukey post hoc test, $P = 0.016$).

TABLE 2. Extended.

δ^{13}C (‰)		C/N mass ratio		
Blood cells	Whole blood	Plasma	Blood cells	Whole blood
−21.92 ± 0.33	−22.08 ± 0.34	4.58 ± 0.21	3.36 ± 0.02	3.47 ± 0.03
−21.96 ± 0.39	−22.26 ± 0.40	4.86 ± 0.18	3.36 ± 0.01	3.47 ± 0.03
−21.70 ± 0.33	−21.87 ± 0.33	4.19 ± 0.11	3.34 ± 0.01	3.46 ± 0.03
−21.64 ± 0.42	−21.92 ± 0.42	4.77 ± 0.37	3.36 ± 0.01	3.57 ± 0.06
−21.42 ± 0.20	−21.78 ± 0.22	4.60 ± 0.25	3.37 ± 0.03	3.55 ± 0.05
−21.10 ± 0.39	−21.62 ± 0.36	5.42 ± 0.45	3.36 ± 0.02	3.58 ± 0.06
−22.18 ± 0.25	−22.48 ± 0.29	4.52 ± 0.29	3.38 ± 0.02	3.57 ± 0.08
−21.33 ± 0.40	−21.46 ± 0.38	4.68 ± 0.19	3.36 ± 0.01	3.47 ± 0.01

Food and enrichment factors

In agreement with previous findings (Cherel et al. 1993), the main food of King Penguin chicks in spring 2001 was fish (98.2% by mass), with three main prey, the myctophids *Krefftichthys anderssoni* (50.1% by number), *Protomyctophum tenisoni* (37.3%), and *Electrona carlsbergi* (6.5%). As expected, the δ^{15}N value of the food was lower than that of blood components and feathers of King Penguin chicks and adults (statistics are not detailed here; all $P < 0.0001$). This allowed us to calculate isotopic enrichment factors in ^{15}N between food and whole blood (2.2–2.5‰), food and feathers (3.3–3.6‰), and food and the distal region of new feathers (2.1‰).

DISCUSSION

Fasting and blood δ^{15}N signature

Changes in King Penguin body mass and plasma metabolites due to fasting are in agreement with the metabolic adaptations to long-term food deprivation previously described in birds, including the King Penguin (Cherel and Le Maho 1985, Cherel et al. 1988*a*, *b*). High plasma levels of β-hydroxybutyrate and low levels of uric acid after 25 days of fasting and at the end of adult molting and chick winter fasts indicate that penguins were still in the main period of fasting (the so-called phase II), marked by both lipid utilization and protein sparing. However, when they arrived ashore, adult birds, like molting chicks, were in the transition period between the fed and fasted states (phase I), whereas chicks at the beginning of winter were in the fed state marked by high plasma uric acid and low plasma β-hydroxybutyrate concentrations.

To our knowledge, this study is the first to investigate the influence of fasting on the stable isotope signatures of blood of vertebrates. Our longitudinal sampling showed that 25 days of food deprivation induced ^{15}N enrichment in all of the blood components of adult King Penguins. These results validate the hypothesis that, during periods of food deprivation, animals ''feed on themselves,'' thus increasing their apparent trophic level. Although fasting is marked by a decrease in both protein synthesis and degradation rates in the whole body (Cherel et al. 1991), this low level of protein turnover is still sufficient to induce, in the long term, an increase in tissue δ^{15}N values through extensive recycling of endogenous amino acids.

As predicted, the increase in δ^{15}N values was higher in plasma (0.70‰), a tissue with an elevated protein turnover rate, than in blood cells (0.24‰), which have

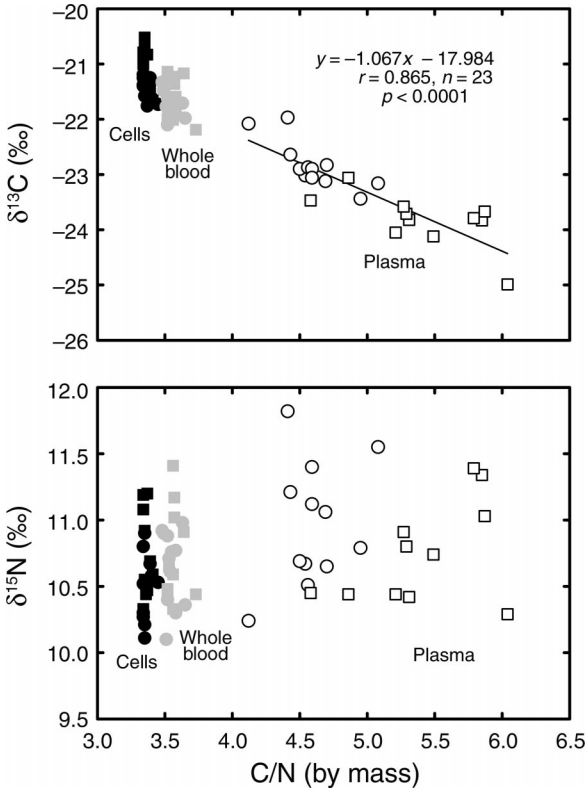

FIG. 1. Relationships between δ^{13}C (upper panel) and δ^{15}N (lower panel) and C/N ratio in plasma, blood cells, and whole blood of King Penguin chicks at the beginning (circles) and the end (squares) of winter. The regression line for δ^{15}N vs. C/N is not significant ($P = 0.886$).

TABLE 3. Stable isotopic signature and C/N ratio of feathers and food of King Penguins (mean ± SD).

Group	Molt characteristics	*n*	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	C/N mass ratio
Fasting molting adults/beginning					
Old feathers without distal regions	spring 2000 molt, on land, fasting	10	11.21 ± 0.32	−21.16 ± 0.60	3.30 ± 0.06
Distal regions of new feathers	spring 2001 molt, at sea, feeding	10	9.53 ± 0.20	−22.28 ± 0.29	3.67 ± 0.05
Fasting molting adults/end					
New feathers without distal regions	spring 2001 molt, on land, fasting	10	11.03 ± 0.41	−21.10 ± 0.41	3.37 ± 0.06
Molting chicks					
Down	summer 2001 growth, on land, fed	10	10.98 ± 0.30	−20.31 ± 0.31	3.31 ± 0.04
New feathers	spring 2001 molt, on land, underfed	10	10.75 ± 0.30	−21.21 ± 0.24	3.26 ± 0.03
Spring chick food		10	7.43 ± 0.36	−22.68 ± 0.50	3.73 ± 0.15

a lower protein turnover. These results are in agreement with those previously found in wild Ross's Geese that showed a higher fasting effect in liver than in muscle (Hobson et al. 1993). Physiological investigations have shown that the protein synthesis rate is higher in liver than in muscles and that fasting does not change protein synthesis in the liver but induces a major decline in muscle protein synthesis (Cherel et al. 1991). Consequently, the fasting effect on $\delta^{15}N$ values is tissue dependent at two different levels, the absolute rate of protein synthesis and its time response to the length of food deprivation.

It has been hypothesized recently that, because nitrogen wastes are isotopically impoverished in ^{15}N, changes in plasma uric acid have to be taken into account to interpret the nitrogen signature of avian plasma (Bearhop et al. 2000). However, the effect is likely to be minimal because the amount of protein in plasma is three orders of magnitude higher than that of uric acid in both fed and fasted birds (grams vs. milligrams per liter; Garcia-Rodriguez et al. 1987, Boismenu et al. 1992).

Unlike the longitudinal study on captive male King Penguins, the transversal work on fasting molting adults and underfed winter chicks in the colony surprisingly did not show significant ^{15}N enrichments either in plasma or in blood cells. Our explanation is that, even for specialist consumers like King Penguins, differences in feeding ecology among individuals promote interindividual differences in $\delta^{15}N$ values (see, e.g., their range in Fig. 1) that overcome the moderate ^{15}N enrichment due to fasting. Consequently, it should be increasingly difficult to detect a fasting/nutritional effect in transversal investigations as consumers present an increasing number of specialized individuals leading to an increasing variance in $\delta^{15}N$ values at the population level.

The effect of fasting on the nitrogen signature of penguin tissues is likely to be generalized to most species of fasted and underfed endotherms, because birds and mammals adapt to food deprivation through identical metabolic changes (Cherel and Groscolas 1999). However, a higher effect of food deprivation can be expected in at least two physiological situations marked either by a high level of protein utilization (in lean animals with almost no lipid reserves), or by a high level of energy expenditure (during long-distance migration flight), and this requires further investigation.

Methodological comments and practical consequences

In both longitudinal and transverse investigations, fasting and undernutrition in adults and chicks of King Penguins induced a decrease in plasma $\delta^{13}C$ values, while there was a concomitant increase in plasma C/N ratio. Moreover, plasma $\delta^{13}C$ values, but not $\delta^{15}N$ values, were inversely related to their C/N ratio (Fig. 1). In animal tissues, C/N is positively related to lipid content (Schmidt et al. 2003, Matthews and Mazumder 2005), and lipids are depleted in ^{13}C when compared to proteins (Kelly 2000). Consequently, the fasting effect on $\delta^{13}C$ values was probably due to an increase in the lipid content of plasma. Indeed, ''normalizing'' $\delta^{13}C$ values to a constant C/N ratio abolishes the observed decrease in $\delta^{13}C$ values after 25 days of fasting (data not shown). In other tissues, fasting induces either little or no change in $\delta^{13}C$ values and C/N ratios (blood cells of King Penguins, this study; liver and muscle of Ross's Goose; Hobson et al. 1993).

Relative to blood cells, the plasma of each individual King Penguin was systematically depleted in ^{13}C and had a higher C/N ratio, again suggesting a lipid effect on plasma $\delta^{13}C$ values. A similar influence of lipids on $\delta^{13}C$ values and the C/N ratio of plasma was recently demonstrated between adult birds and their growing chicks (Cherel et al., *in press*). Thus, plasma lipid content may vary with age and nutritional status, which affects interpretation of plasma $\delta^{13}C$ signatures and pre-

cludes accurate comparisons among groups of animals or tissues of the same individuals. Lipids must therefore be removed from plasma. Alternatively, if the main goal of the study is not to investigate short-term diets, the best way to overcome the lipid effect on $\delta^{13}C$ values as well as the nutritional effect on $\delta^{15}N$ values is to use blood cells. However, the easiest way to work in the field is to collect whole blood that does not require centrifugation. Blood cells contain more organic matter than plasma. Consequently, whole blood has an isotopic signature and C/N ratio very close to that of blood cells and, hence, is little affected by either fasting or the lipid content of plasma.

Resource allocation during molt

A main finding of this study is that the distal region of adult new feathers was, as expected, depleted in ^{15}N when compared to all other types of feathers of King Penguins. This strongly supports the hypothesis that amino acids used for keratin synthesis of the distal region came directly from dietary proteins when birds were at sea, whereas those used for feather synthesis during the molting fast were derived from endogenous protein reserves (Cherel et al. 1994). In agreement with this, the ^{15}N enrichment factor between food and tissues was lower in the distal feather region (2.1‰) than in the proximal regions (3.3–3.5‰), the former being similar to that of whole blood (Cherel et al. 2005; this study) and the latter being identical to the value (3.5‰) obtained for feathers of captive penguins feeding on a constant diet (Cherel et al. 2005).

The ^{15}N enrichment factor is tissue dependent; in birds, feathers tend to yield the highest estimates among various tissues, including whole blood (Vanderklift and Ponsard 2003, Pearson et al. 2003, Cherel et al. 2005). However, a detailed analysis of the studies in which the ^{15}N enrichment factor of whole blood and feathers were both estimated shows a different picture. In some cases, the ^{15}N enrichment factor was much higher in feathers than in blood (difference +1.4‰ to +1.8‰), whereas in others the differences were smaller or nonexistent (−0.6‰ to +0.8‰) (Hobson and Clark 1992*b*, Bearhop et al. 2002, Hobson and Bairlein 2003, Pearson et al. 2003, Cherel et al. 2005). Our results for penguin molt indicate that different patterns of resource allocation may account for these discrepancies. High ^{15}N enrichment factors in feathers, together with a large difference between ^{15}N enrichment factors of whole blood and feathers, suggest that most amino acids available for keratin synthesis are provided by endogenous reserves. Conversely, almost identical and low ^{15}N enrichment factors in whole blood and feathers suggest a dietary origin of feather amino acids.

Exogenous vs. endogenous amino acids for the synthesis of keratin have important implications for the interpretation of the $\delta^{15}N$ values of keratinaceous tissues (feathers, nails, hair, and baleens) in terms of feeding ecology. It is generally assumed that the keratin signature reflects the diet at the time of synthesis, but this assumption rarely has been investigated (Hobson and Clark 1992*a*, Pearson et al. 2003). For example, a diet-switching experiment on Great Skuas (*Catharacta skua*) showed that the feather isotopic signature stabilizes at a new value several weeks after the switch (Bearhop et al. 2002). This indicates that keratin is synthesized using amino acids from different pools, each having its own temporal integration, and is thus a mixture of dietary and endogenous amino acids. Clearly, more experiments are needed on the metabolic pathways and turnover times of various amino acid pools for keratin synthesis in relation to diet quality and nutritional status of animals (Ayliffe et al. 2004).

In conclusion, the present work illustrates how research in ecology and physiology are inextricably linked. We have demonstrated and quantified the nutritional effect of fasting on the nitrogen isotopic signature of plasma, blood cells, and feathers. We have shed new light on the relative contributions of immediate diet vs. long-term stores in the nutrient needs of molt in birds. Finally, our study has emphasized the usefulness of collecting whole blood (or blood cells) in the field to overcome both the fasting and lipid effects observed in plasma.

Acknowledgments

The authors thank D. Verrier for her help in the field and P. Healy for preparing samples for stable isotope analysis. The present work was supported financially and logistically by the Institut Polaire Français Paul Emile Victor (IPEV, Programme N°109), the Terres Australes et Antarctiques Françaises, and the Canadian Wildlife Service.

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